

Research paper

Epigenetic control on transcription of vernalization genes and whole-genome gene expression profile induced by vernalization in common wheat

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ABSTRACT

Vernalization is necessary for winter wheat to flower. However, it is unclear whether vernalization is also required for spring wheat, which is frequently sown in fall, and what molecular mechanisms underlie the vernalization response in wheat varieties. In this study, we examined the molecular mechanisms that regulate vernalization response in winter and spring wheat varieties. For this purpose, we determined how major vernalization genes (*VRN1*, *VRN2*, and *VRN3*) respond to vernalization in these varieties and whether modifications to histones play a role in changes in gene expression. We also identified genes that are differentially regulated in response to vernalization in winter and spring wheat varieties. We found that in winter wheat, but not in spring wheat, *VRN1* expression decreases when returned to warm temperature following vernalization. This finding may be associated with differences between spring and winter wheat in the levels of tri-methylation of lysine 27 on histone H3 (H3K27me3) and tri-methylation of lysine 4 on histone H3 (H3K4me3) at the *VRN1* gene. Analysis of winter wheat transcriptomes before and after vernalization revealed that vernalization influences the expression of several genes, including those involved in leucine catabolism, cysteine biosynthesis, and flavonoid biosynthesis. These findings provide new candidates for further study on the mechanism of vernalization regulation in wheat.

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1. Introduction

Wheat is usually sown in fall and then passes through the winter to flower in warm spring. This cooling of winter wheat seeds, which accelerates flowering, is called vernalization. The genetic basis of vernalization response in winter wheat has been extensively studied. To date, four genes have been identified that directly participate in the wheat vernalization response, namely *VRN1*, *VRN2*, *VRN3* and *VRN4* (Yan et al., 2003, 2004, 2006; Kippes et al., 2015). However, it is unclear whether these same genes play a role in responding to vernalization in spring wheat varieties.

VRN1 (also known as *VRT1*), which is homologous to *APTALA1* in *Arabidopsis thaliana*, encodes a MADS-box family transcription factor (Yan et al., 2003). *VRN1* is expressed at low levels before vernalization, but after vernalization induction, *VRN1* is highly

expressed in the apex and leaves, and the expression level increases with the increasing duration under low temperature (Danyluk et al., 2003; Distelfeld et al., 2009). In hexaploid wheat, there are three homologous copies of *TaVRN1* gene (Trevaskis, 2010; Konopatskaia et al., 2016). *VRN2* is a flowering inhibitor that includes two closely located genes encoding CCT domain containing proteins, *ZCCT1* and *ZCCT2* (Yan et al., 2004). In hexaploid wheat, there are six homologous copies of *TaVRN2*. Mutations in the CCT domain of *TaVRN2* or the deletion of the entire *TaVRN2* gene can weaken the requirement of vernalization (Yan et al., 2004). In addition, *TaVRT2* has been shown to interact with *TaVRN1* and overexpression of *TaVRT2* in some winter wheat varieties (e.g., Kenong199) promotes heading even under incomplete vernalization conditions (Xie et al., 2021). *VRN3* is an ortholog of the *Flowering T locus (FT)* gene in *Arabidopsis* and the *Heading date 3a (Hd3a)* gene in rice. It encodes “florigen” in wheat. *VRN3* expression levels are higher in varieties with retrotransposon insertions in the *VRN3* promoter region. Studies have shown that *TaVRN3* interacts with *TaFDL2* and 14-3-3 proteins to form a flowering activation complex, which then binds to the ACGT cis-acting element in the *TaVRN1*

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promoter region to activate the expression of *TaVRN1*, thus promoting flowering in wheat (Li and Dubcovsky, 2008; Li et al., 2015). *VRN3* can integrate vernalization and photoperiod signals to accelerate flowering. Under long day conditions, *VRN2* suppresses the expression of *VRN3* by inhibiting the expression of *VRN1* to delay wheat flowering (Distelfeld and Dubcovsky, 2010; Chen and Dubcovsky, 2012). *VRN1* binds to the promoter regions of *VRN2* and *VRN3* to suppress the expression of *VRN2* and to promote the expression of *VRN3* (Deng et al., 2015). The photoperiod genes *PPD1* and *CO* also affect the expression of *VRN3*. *Phytochrome C (PHYC)* mediates the transcriptional activation of *PPD1* and *CO*, thereby upregulating the expression of *VRN3* (Chen et al., 2014; Pearce et al., 2016).

Changes in the expression of vernalization-responsive genes are often coupled with changes in histone modification. Studies have shown that histone modifications are extensive in genes of the flowering pathway, photoperiod, and circadian clock (Huan et al., 2018). Previous studies have also reported that the overall levels of H3K27me3 and H3K4me3 modifications in winter (Norstar) and spring wheat (Manitou) increase after vernalization (Diallo et al., 2012). Specifically, Chromatin Immunoprecipitation followed by Quantitative Real-Time PCR (ChIP-qPCR) has been used to show that the H3K4me3 modification level on *TaVRN1A* increased in these two varieties, whereas there was no significant difference in H3K27me3 modification level. Similarly, in an additional wheat variety (i.e., Kenong199), vernalization increased H3K4me3 modification levels of *TaVRN1A*, but decreased H3K27me3 modification levels of the same gene (Xie et al., 2021). Interestingly, in both a wheat variety that requires vernalization (Sonja) and one that does not (Morex), H3K4me3 modification levels on *HvVRN1* were found to increase, whereas the H3K27me3 modification levels on *HvVRN1* decreased (Oliver et al., 2009). However, more data is required to further confirm the dynamic modifications of H3K4me3 and H3K27me3 on the *VRN1* gene before and after vernalization.

In this study, we examined the molecular mechanisms that regulate vernalization response in winter and spring wheat varieties. For this purpose, we determined how major vernalization genes (*VRN1*, *VRN2*, and *VRN3*) respond to vernalization in these varieties and whether modifications to histones play a role in changes in gene expression. We also identified genes that are differentially expressed in response to vernalization in winter and spring wheat varieties.

2. Materials and methods

2.1. Plant materials and vernalization treatments

Wheat varieties involved in this study included Chinese Spring (CS), Fielder, Kenong199 (KN199), and Aikang 58 (AK58). The five vernalization treatment conditions were as follows: (a) plants were grown at 22 °C for 2 weeks (referred to as V0); (b) plants were grown at 22 °C for 2 weeks and then transferred to 4 °C for 2 weeks (referred to as V14); (c) plants were grown at 22 °C for 2 weeks and then transferred to 4 °C for 2 weeks before being returned to 22 °C for 1 week (referred to as V14N7); (d) plants were grown at 22 °C for 2 weeks and then transferred to 4 °C for 4 weeks (referred to as V28); (e) plants were grown at 22 °C for 2 weeks then transferred to 4 °C for 4 weeks before being returned to 22 °C for 1 week (referred to as V28N7). All vernalization treatments were carried out in a climate-controlled growth chamber.

2.2. RNA extraction and reverse transcription

Entire above-ground parts of wheat plants were sampled for RNA extraction by TRIzol. Reverse transcription was performed using the

TransGen Reverse Transcription kit (ET101-01). Two micrograms of RNA were used for cDNA synthesis, and after reverse transcription, the cDNA was diluted 4-fold for subsequent experiments.

2.3. RNA-seq data processing

AK58 seedlings grown under different conditions (i.e., V0, V28, and V28N7) were collected for RNA extraction and library preparation. Barcoded cDNA libraries were constructed using the VAHTS Universal V8 RNA-seq Library Prep Kit (NRM605-02) and were sequenced on MGI DNBseq-T7 platforms at 150 bp. Trimmomatic (version 0.32; Bolger et al., 2014) was used to remove adaptors from raw sequencing reads following the following parameter: -q 25 -paired -length 36 -stringency 3 -e 0.1. Clean data were aligned to the Chinese Spring reference sequence from the International Wheat Genome Sequencing Consortium (IWGSC) (version 1.1) using the STAR software (v.2.7.0c; Dobin et al., 2013). RSEM software (v.1.3.3; Li and Dewey, 2011) was used for quantification analysis. Differentially expressed genes were identified using DESeq2 software (Love et al., 2014) with *P* value < 0.05 and $|\log_2$ fold changes| \geq 1, 2, or 3. STEM analysis was performed using the OmicStudio online software (Lyu et al., 2023). The TGT Online website (<http://wheat.cau.edu.cn/TGT/m3/?navbar=GOenrichment>) was used for GO analysis on differentially expressed genes (Chen et al., 2020).

2.4. Chromatin immunoprecipitation (ChIP) assay

ChIP assay was performed according to a previous report (Zhao et al., 2020) with minor modifications. For each ChIP experiment about 0.2 g of tissue was used. Fresh seedlings were cut into small pieces and placed in MC buffer (10 mM sodium phosphate, pH 7, 50 mM NaCl and 0.1 M sucrose) that contained 1% formaldehyde for cross-linking. The samples were vacuum-fixed twice (10 min for each time). Then 0.125 M glycine was added to terminate the crosslinking. After vacuuming for 5 min, the samples were washed with MC buffer and stored at -80 °C for later use. Samples were ground into powder using liquid nitrogen and chromatin was fragmented into 200–600 bp by sonication (Covaris S220) at 200 cycles per burst, peak power for 10 min. Chromatin was immunoprecipitated using anti-H3K27me3 (9733S, CST) or anti-H3K4me3 (ab8580, Abcam). After de-crosslinking and proteinase K treatment, the precipitated DNA was purified by QIAquick® PCR Purification Kit (28106, QIAGEN) for later qPCR analysis.

2.5. Quantitative polymerase chain reaction (qPCR) and ChIP-qPCR

Gene expression analysis was carried out with the 2X SYBR Green Fast qPCR Mix Kit (RK21204, ABclonal Corporation). qPCR reactions included 1 μ L cDNA, 10 μ L 2X Supermix, 0.8 μ L Primer mix, and 8.2 μ L ddH₂O. Amplification was performed on a CFX96 Real-Time System PCR instrument (Bio-Rad) and fluorescence values were collected. The relative expression levels of genes were calculated using the $2^{-\Delta\Delta Ct}$ method with TaActin as the internal reference gene. For ChIP-qPCR assay, input DNA and immunoprecipitated DNA were used simultaneously for qPCR detection, with input DNA serving as control. The primers used for qPCR and ChIP-qPCR are listed in Table S6.

3. Results

3.1. The effect of vernalization on the expression of major vernalization genes in both winter and spring wheat

Spring wheat varieties can be planted in autumn in areas where winter is not too harsh. In this case, plants would experience low

temperature in winter. To study the effect of vernalization on spring wheat varieties, we compared the expression of *VRNs* in four wheat varieties, including two spring varieties (i.e., Chinese Spring, CS and Fielder) and two winter varieties (i.e., Aikang58, AK58 and Kenong199, KN199), that were grown under five vernalization conditions: no vernalization; two- or four-week vernalization; two- or four-week vernalization followed by 7 days at room temperature (Fig. 1A). We found that in both spring and winter wheat varieties the homologs of *VRN1* (*VRN1A*, *VRN1B*, and *VRN1D*) were expressed at higher levels following vernalization treatment. In spring wheat varieties, the expression of all the three *VRN1s* continued to increase after plants were returned to normal temperatures, except in Chinese Spring wheat following the two-week vernalization treatment (Fig. 1B, C and D). In winter wheat varieties, expression of *VRN1s* decreased greatly after plants were returned to normal temperatures, with the largest decrease in expression observed in plants vernalized for four weeks. These results indicate that vernalization induces expression of *VRN1* genes in both spring and winter wheat. However, in winter wheat varieties, *VRN1* expression must be maintained by low temperatures.

In both spring and winter wheat varieties, *VRN1* and *VRN2* expression patterns were negatively correlated. Specifically, in winter wheat, when plants were returned to normal temperatures after two- and four-week vernalization treatments, *VRN1* expression decreased, whereas *VRN2* expression increased (Fig. S1A). Our findings suggest that *VRN1* acts as a major upstream negative regulator of *VRN2* in both winter wheat and spring wheat, which is

consistent with models put forth in previous studies (Xu and Chong, 2018). *VRN3* was also expressed at higher levels following a four-week vernalization treatment (Fig. S1B). However, *VRN3* expression did not decrease in plants returned to normal temperatures following either a two- or four-week vernalization treatment. These results indicate that factors other than *VRN1* and *VRN2* regulate *VRN3* expression.

3.2. Common and also divergent histone modification dynamics on *VRN1* gene in spring and winter wheat regulated by vernalization

Gene transcription is highly associated with histone modification. Furthermore, in barley and wheat vernalization has been shown to induce *VRN1* expression by altering chromatin state (Oliver et al., 2009). To determine whether histone modifications underlie differences in *VRN1* expression between winter and spring wheat, we evaluated dynamic changes of H3K27me3 and H3K4me3 on the *VRN1* gene in Fielder and KN199 wheat varieties grown with no vernalization, a four-week vernalization, and a four-week vernalization followed by a return to normal temperatures (Fig. 2A). We found that H3K4me3 modification levels at the *VRN1* gene increased following vernalization in both spring and winter wheat varieties (Fig. 2B). However, H3K4me3 modification levels decreased in KN199 but not in Fielder wheat following the return to a normal temperature. In Fielder wheat, levels of H3K27me3 modification at the *VRN1A* gene decreased after a four-week vernalization and a four-week vernalization

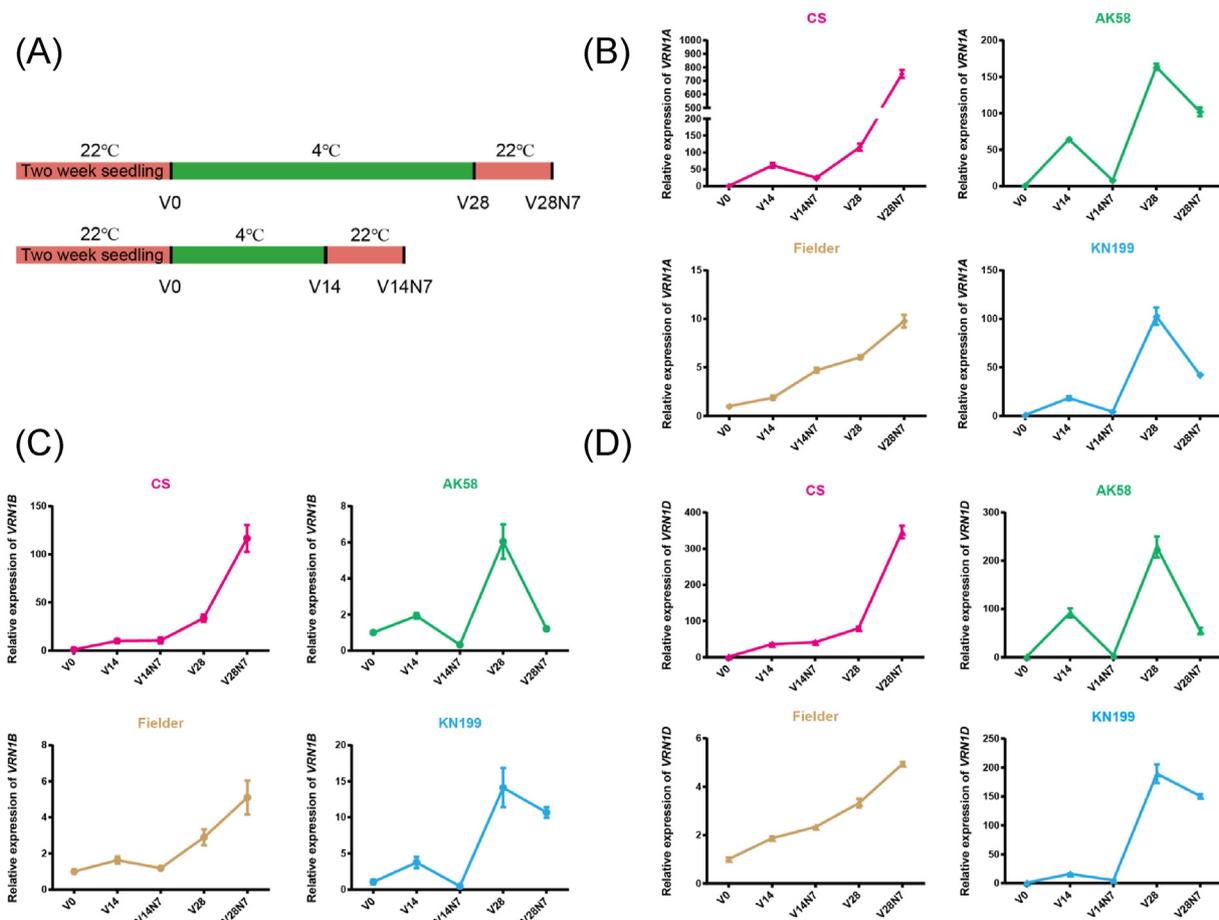


Fig. 1. The effect of vernalization on the expression of *VRN1A* in different wheat varieties. (A) A schematic diagram illustrating five vernalization treatment conditions. The red color represents plant growth at 22 °C, the green color represents plant grown at 4 °C. The expression of *VRN1A* (B), *VRN1B* (C) and *VRN1D* (D) in four wheat varieties under five vernalization conditions are shown. The internal reference is *TaActin*. Results represent data from three biological replicates. Bar = mean ± sd.

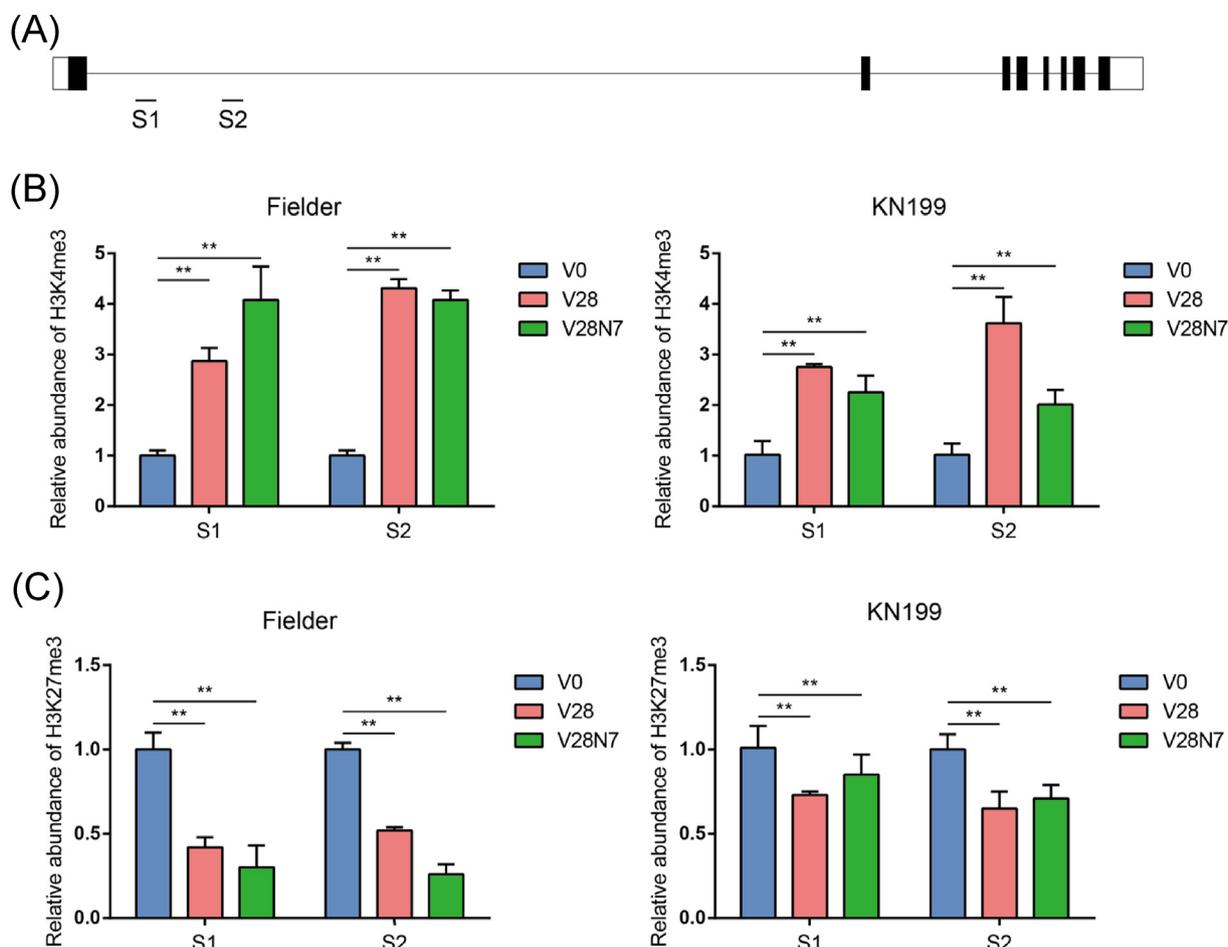


Fig. 2. The effect of vernalization on the levels of H3K27me3 and H3K4me3 modifications at the *VRN1A* locus. (A) Gene structure diagram of *VRN1A*. The black lines represent introns, black rectangles represent exons, and white regions represent non-coding regions. S1 and S2 indicate the positions of primers for ChIP-qPCR detection. Changes in H3K4me3 modification levels (B) and H3K27me3 modification levels (C) of *VRN1A* in Fielder and KN199 varieties grown with no vernalization (V0), a four-week vernalization (V28), and a four-week vernalization followed by a return to room temperature (V28N7). The calculated values represent relative enrichment levels of ChIP-DNA compared to input DNA with three replicates, and was normalized to histone modification levels in plants that were not vernalized (V0). Significance was obtained from the student's *t*-test. ***p* < 0.01.

followed by a return to normal temperatures. After returning plants from vernalization to normal temperatures, levels of H3K27me3 modification at the *VRN1A* gene decreased in KN199, but not as much as in Fielder wheat (Fig. 2C). This indicates that although H3K27me3 and H3K4me3 modifications are both involved in the vernalization response in wheat and have an impact on *VRN1* expression, the catabolism of H3K4me3 and H3K27me3 on *VRN1* induced by vernalization may differ between winter and spring wheat.

3.3. Genome wide gene transcription alteration by vernalization treatment in AK58

We also examined the genome-wide effect of vernalization on wheat gene expression in a winter variety (AK58) grown with no vernalization, a four-week vernalization, and a four-week vernalization followed by 1 week at normal temperatures. For these treatments, RNA sequencing (RNA-seq) generated 106,154,530, 1,203,515,301, and 125,884,336 reads on average, respectively. To assess the quality of the transcriptome data, we confirmed that the expression profiles of six randomly selected genes were consistent with those of the transcriptome (Fig. S2). In addition, PCA analysis clustered replicates from the same treatment together (Fig. 3A), indicating the reproducibility of the data.

To determine which genes respond to vernalization, we identified genes that were differentially expressed (DEGs) between different vernalization treatments. Genes with the highest probability of being regulated by vernalization were those that were differentially expressed between the following two treatments: no vernalization vs. a four-week vernalization; and no vernalization vs. a four-week vernalization followed by a return to normal temperatures. In total, 13,256 and 6776 genes (fold change greater than 2) were differentially expressed between these treatments. Moreover, 5673 and 1719 DEGs with fold changes greater than 4 and 2991 and 671 DEGs with fold changes greater than 8 were also identified in each treatment (Fig. 3B, C and D).

For the complementary comparisons (i.e., a four-week vernalization vs. no vernalization; a four-week vernalization followed by return to normal temperature vs. no vernalization), there were a total of 3560 DEGs (fold change greater than 2) in common (Fig. 3E). Among these genes, 1919 genes were downregulated during the four-week vernalization and 1906 genes were downregulated in plants that were returned to normal temperatures following the vernalization. These DEGs are enriched for genes that play roles in the flavonoid biosynthesis, protein autoprocesing and the positive regulation of anthocyanin metabolism (Fig. 4A and C). Specifically, these DEGs include members of the cytochrome P450 superfamily, chalcone and stilbene synthase gene family, N-terminal

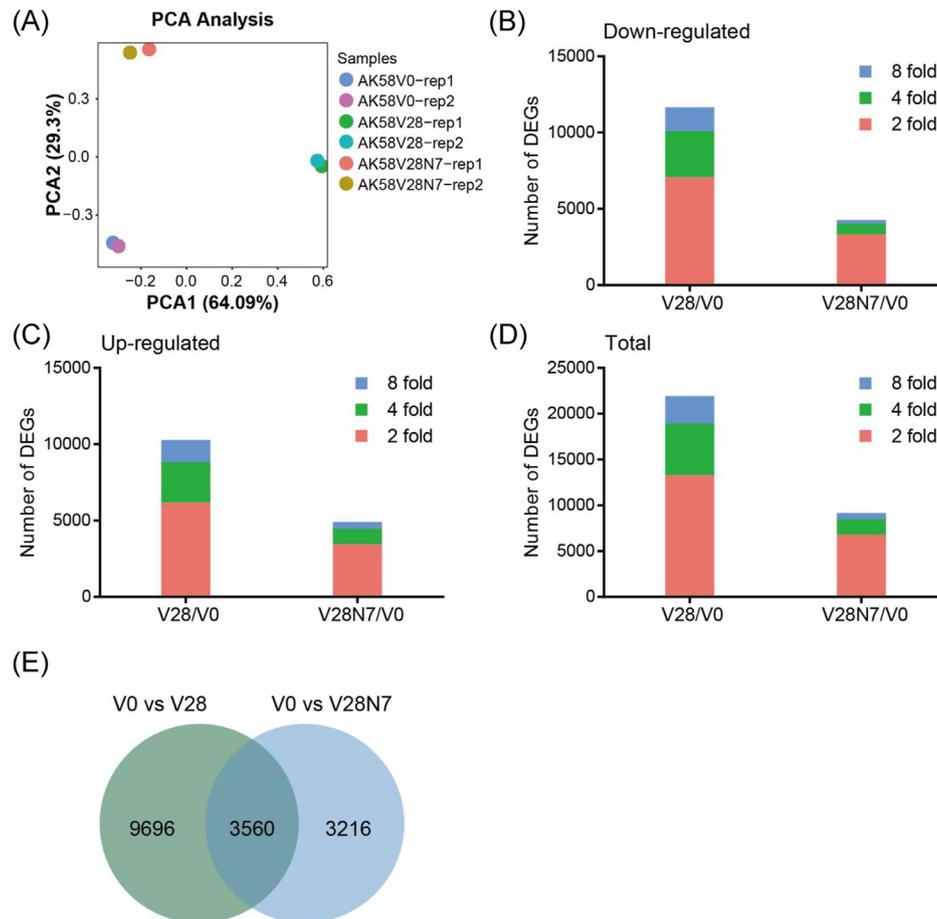


Fig. 3. Variation in gene expression profiles in a winter wheat variety (AK58) under different vernalization treatments. (A) PCA analysis of differentially expressed genes in AK58 grown with no vernalization (V0), a four-week vernalization (V28), and a four-week vernalization followed by a return to normal temperatures (V28N7). Comparisons were performed with AK58 grown after a four-week vernalization (V28) and those returned to room temperature following a four-week vernalization (V28N7). The number of downregulated genes (B), the number of upregulated genes (C) and the total number of differentially expressed genes (D) are shown in comparison with the V0 condition. (E) Differentially expressed genes between V28 and V0 conditions and between V28N7 and V0 conditions are also shown.

nucleophile aminohydrolases (Ntn hydrolases) superfamily and basic-leucine zipper (bZIP) transcription factor family (Table S1). In addition, 1641 genes were upregulated in response to a four-week vernalization treatment and 1654 genes were upregulated in plants that were returned to normal temperatures after vernalization. These genes are mainly enriched for the leucine catabolic process pathway (Fig. 4B and D). These findings suggest that vernalization activates flavonoid biosynthesis, protein autoprocesing, anthocyanin metabolism, and leucine catabolic processes.

3.4. Possible genes that may involve in the dynamic gene expression of *VRN1* during vernalization

VRN1 is a key regulator during vernalization. Our transcriptome data show that *VRN1* expression is strongly induced by vernalization and significantly decreased after being returned to normal temperatures (Figs. 1 and S1A). To identify genes involved in the dynamic expression of *VRN1* following a change in temperature, we used Short Time-series Expression Miner (STEM) analysis of 3560 genes to trace their dynamic expression during the vernalization process. These 3560 genes were divided into 16 clusters (Cluster 0–15; Fig. 5A, Table S2). Expression of genes in Cluster0, Cluster1, Cluster3, Cluster4, Cluster11, and Cluster14 changed significantly across three pseudo-time points. These clusters contain a total of 2531 genes. The expression patterns of genes in Cluster11 and

Cluster14 were similar to that of *VRN1*, whereas genes in Cluster1 showed the opposite pattern. Cluster11 and Cluster14 are enriched for genes that are involved in the negative regulation of red or far-red light signaling pathway and leucine catabolic process.

Cluster1 is enriched for genes in the flavonoid biosynthetic process (Fig. S3; Table S3). Clusters1, 11, and 14 collectively contain 1575 genes, including 110 transcription factors (Table S4). Transcription factor expression patterns differed among plants treated with no vernalization, a four-week vernalization, and a four-week vernalization followed by a return to normal temperatures (Fig. 5B). These transcription factors include MADS-box, MYB, TCP, and WRKY genes. Previous studies that identified DNA elements that bind to these transcription factors (Inukai et al., 2017) allowed us to predict the regulatory relationships among these transcription factors. Accordingly, we speculate that *VRN1* regulates *VRN2* and *FPF*, which is in line with previous reports (Greenup et al., 2010; Deng et al., 2015). In addition, four cold-regulated genes (*TaCORs*), a class of genes believed to respond to low-temperatures (Park et al., 2015), appear to be targets of *VRN1*. Moreover, *VRN1* has an indirect effect on Wheat PHYTOCLOCK 1 (*WPCL1*) mediated by *VRN2*. *WPCL1* is a homologous to *LUX ARRHYTHMO* (*LUX*)/PHYTOCLOCK 1 (*PCL1*) in *Arabidopsis*, which participates in the regulation of the circadian clock and flowering time (Mizuno et al., 2012, 2016). *VRN1*, *VRT2*, *HSFB*, *ERF*, and *ZHD* are all involved in the regulation of *WPCL1*, indicating the link of vernalization and circadian clock. In

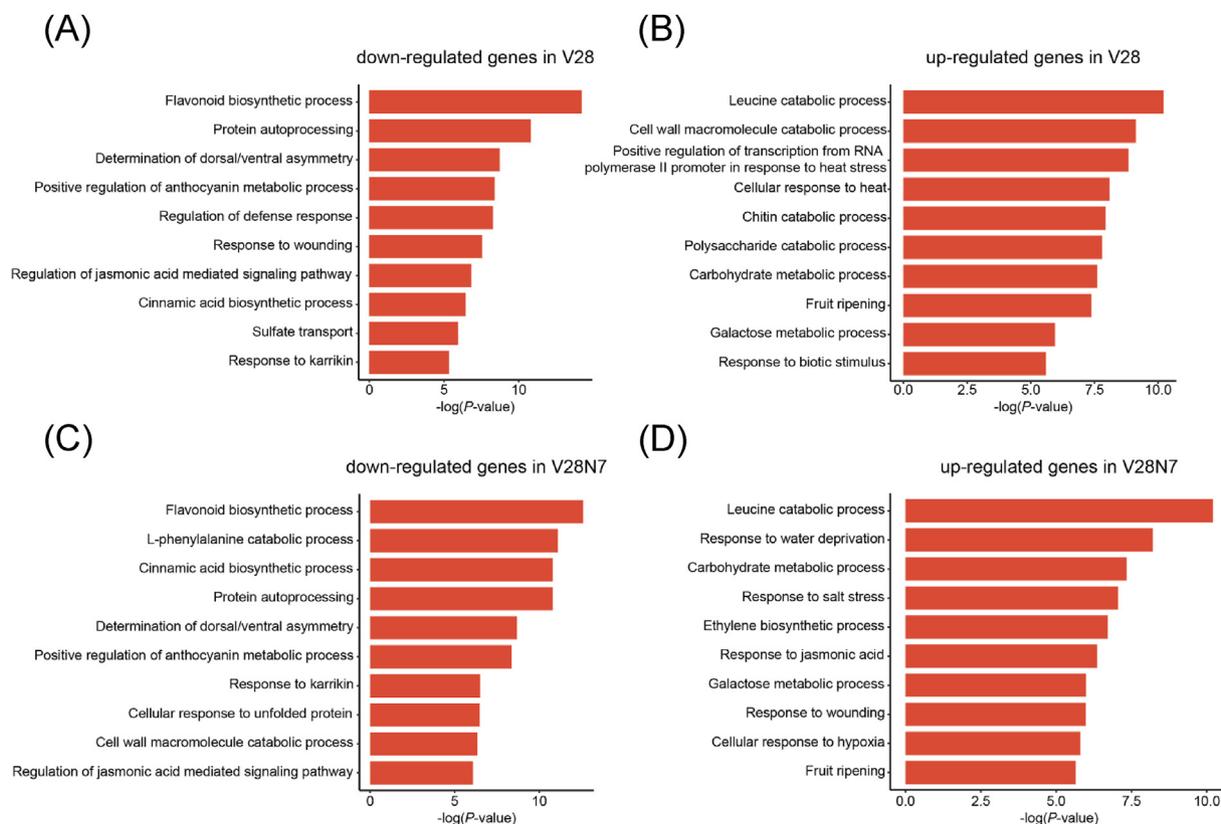


Fig. 4. GO analysis of genes differentially expressed under different vernalization conditions.

addition, *VRN2* appears to be regulated by multiple transcription factors, including TCP, ARF, HSF1 and ABI. *TaFT-5A*, a homolog of *VRN3*, is also regulated by TCP.

To identify the possible regulators that mediate changes of histone modifications on the *VRN1* gene during vernalization, we analyzed the expression of genes encoding histone writers or erasers in wheat (Wang et al., 2021). We found that the expression of some H3K4me3 methyltransferases in wheat was induced by vernalization, whereas the expression of other H3K4me3 demethylases was inhibited by vernalization. The expression of some H3K27me3 methyltransferases was inhibited by vernalization, whereas the expression of some H3K27me3 demethylases was induced by vernalization. However, the expression of H3K4me3 demethylase, JM14 and large proportion of H3K27me3 methyltransferases, including MEA, LHP1 and FIS, was induced also by vernalization (Fig. S4). This indicates that only particular histone writers or erasers are involved in the vernalization-induced transcription of *VRN1A* in wheat. Further investigation on these vernalization-related transcription factors will provide new insights into wheat vernalization.

4. Discussion

4.1. The impact of vernalization time on wheat

Wheat can be cultivated in a wide range of regions and high yield is greatly enhanced by appropriate flowering time (Gororo et al., 2001; Kamran et al., 2014). As a major gene controlling the flowering of wheat, *VRN1* mainly gives wheat its spring or winter characteristics depending on different genotypic alleles (Yan et al., 2003). The combination of *VRN1* genotype alleles and other genes controlling flowering time determines the regions where wheat

can be grown. Winter wheat cannot complete its reproductive transition without vernalization. The expression level of *VRN1* in winter wheat is strongly induced by vernalization and maintained at a high level after vernalization, preparing wheat for reproductive growth.

In this study, the expression levels of *VRN1*, *VRN2*, and *VRN3* were examined in four wheat varieties under five vernalization treatment conditions. It was found that sufficient vernalization time (28 days of vernalization followed by 7 days of recovery at room temperature) could promote the expression of *VRN1*, while insufficient vernalization time (14 days of vernalization followed by 7 days of recovery at room temperature) could not fully induce the expression of *VRN1*. This indicates that both spring and winter wheat varieties respond to vernalization, but the degree of response varies among different wheat varieties. Additional vernalization treatments on more wheat varieties will lay a foundation for determining the time of response to vernalization for different wheat varieties and provide more insight into wheat breeding.

4.2. The effect of epigenetic factors on the expression of vernalization gene *VRN1*

The expression of the vernalization gene *VRN1* is affected by epigenetic factors, such as DNA methylation, non-coding RNA, and histone modifications (Khan et al., 2013; Huan et al., 2018; Xu et al., 2021). Studies have shown that DNA methylation and demethylation are also involved in the vernalization process in plants. CG methylation exists throughout the *VRN1* gene region, while non-CG methylation mainly exists in the first intron of *VRN1*. Low temperature specifically induces an increase in non-CG methylation in the first intron of *VRN1*, and this change in methylation is reset in the

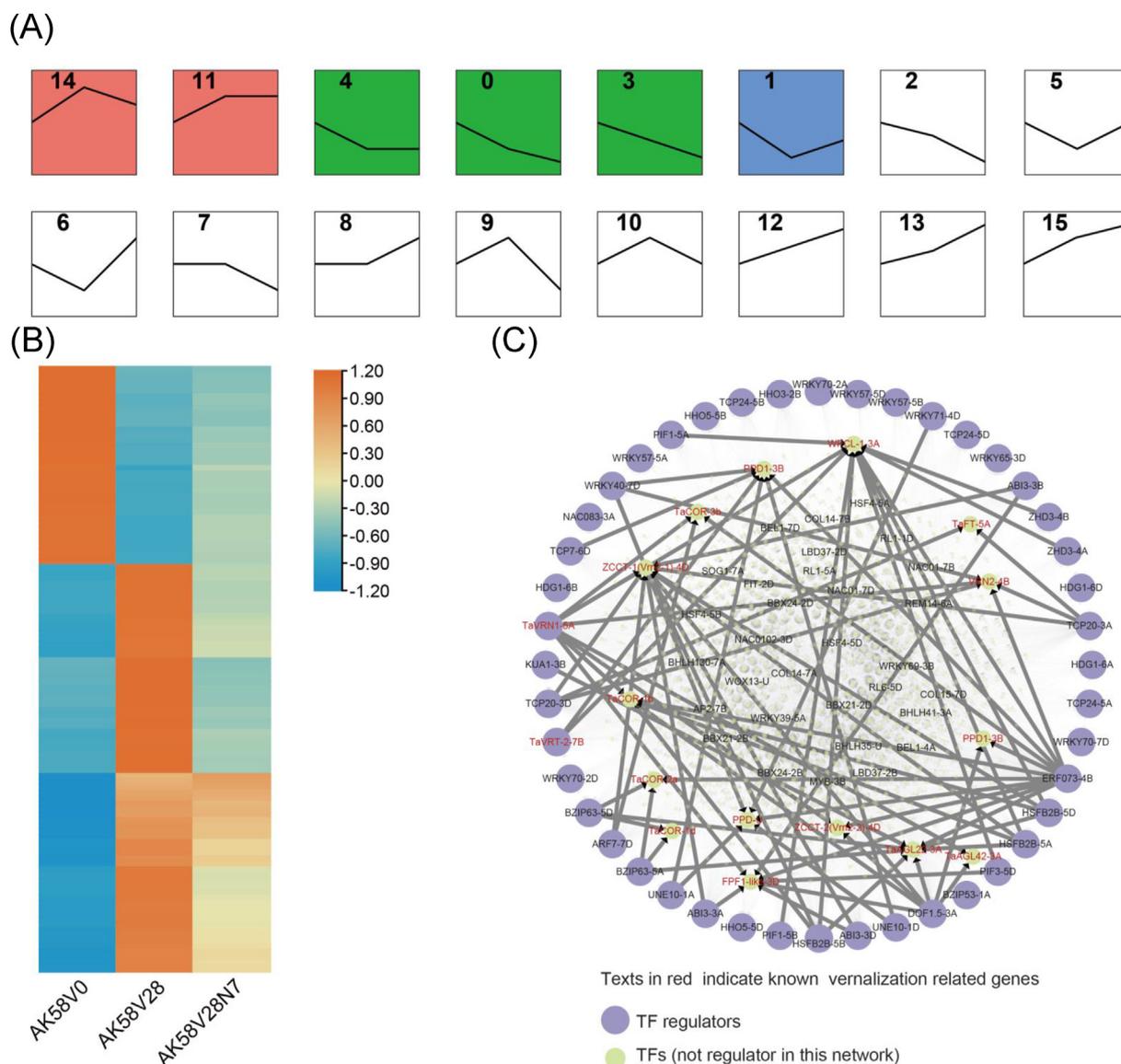


Fig. 5. Identification and functional analysis of vernalization-responsive genes in wheat. (A) STEM analysis of vernalization-responsive genes in wheat. A total of 16 clusters were identified with Cluster 0, Cluster 1, Cluster 3, Cluster 4, Cluster 11, and Cluster 14 showing significant gene expression dynamics. (B) Heatmap of expression dynamics of 110 transcription factors under V0, V28, and V28N7 treatments. (C) Regulatory network based on transcription factors differentially expressed in response to vernalization.

next generation (Khan et al., 2013). In addition to DNA methylation, the expression of *TaVRN1A* is also regulated by non-coding RNA. *VAS* is a non-coding RNA generated from the antisense strand of *TaVRN1A* that only exists in winter wheat. During early vernalization, *VAS* expression is induced, which promotes wheat flowering by positively regulating the expression of *TaVRN1A*. During mid-vernalization, *VAS* helps the complex formed by *TaRF2b* and *TaRF2a* anchor in the *Sp1* site of *TaVRN1A*, activating the expression of *TaVRN1A* (Xu et al., 2021).

Both H3K27me3 and H3K4me3 modifications are involved in the wheat vernalization process. In this study, the H3K27me3 and H3K4me3 modification levels at the *VRN1A* locus in Fielder and KN199 varieties were tested before vernalization (V0) and after vernalization (V28N7). The results showed that in these two varieties, vernalization could reduce the H3K27me3 modification level at the *VRN1A* locus, and it could increase the H3K4me3 modification level at the *VRN1A* locus. Transcription data indicated that specific histone writers or/and erasers are involved in the vernalization-induced transcription of *VRN1A* in wheat (Fig. S4).

This may help us to discover the upstream regulator of vernalization response in wheat. In rice, distinct forms and levels of histone modification vary across the genome and among varieties (Zhao et al., 2020), and may also occur among wheat varieties.

4.3. Regulation of vernalization in cereal crops

The distribution of H3K4me3 and H3K27me3 modifications in the genome before and after vernalization in *Brachypodium distachyon* revealed that H3K27me3 modification is selectively regulated in multiple regions during vernalization and Chromosome 4 may be the main target of this selective regulation, whereas H3K4me3 modification does not have this feature (Huan et al., 2018). During vernalization, the levels of H3K4me3 and H3K27me3 modification on thousands of genes change. Cluster analysis of gene function classifies these genes into several categories, including maintenance of vernalization memory, low temperature response, and flowering and stress response. Vernalization changes the overall distribution of H3K27me3 and

H3K4me3 modifications in *B. distachyon*. However, the effect of vernalization on the overall distribution of H3K27me3 and H3K4me3 modifications in hexaploid wheat are not clear yet and requires further study.

By comparing the transcriptome data of non-vernalized, vernalized and post-vernalized *Brachypodium distachyon*, 674 genes that continuously respond to vernalization were defined as vernalization-memory-related genes (Huan et al., 2013). There were conserved and specific vernalization-memory-related genes found in *B. distachyon* and barley. In this study, transcriptome analysis of wheat seedlings grown under different vernalization conditions identified seven clusters of 2531 genes that continuously respond to vernalization. Many of these genes play roles in leucine catabolism, the biosynthesis of unsaturated fatty acid, monoterpene biosynthesis, and flavonoid biosynthesis; in addition, several of these genes are known to act as negative regulators in the red or far-red light signaling pathway and positive regulators during anthocyanin metabolism. Twelve of these genes overlap with orthologues in *Brachypodium* and barley (Table S5), indicating that vernalization response is both conserved, although with some species specificity, in temperate plants.

Furthermore, we discovered a large number of transcription factors that may be involved in the vernalization response in wheat. We have used data on differentially expressed transcription factors to propose a regulatory network that highlights novel regulatory relationships in the vernalization response, e.g., VRNs regulate *WPCL1*. Our findings improve our understanding of vernalization based on the response to environmental stimulates.

Data availability

The RNA-Seq data generated in this study have been deposited in the NCBI/Sequence Read Archive (SRA) database under accession code PRJNA1019635.

CRediT authorship contribution statement

Yunzhen Li: Investigation, Formal analysis, Data curation, Writing – original draft. **Liujie Jin:** Resources, Validation. **Xinyu Liu:** Resources. **Chao He:** Software, Formal analysis. **Siteng Bi:** Software. **Sulaiman Saeed:** Writing – review & editing. **Wenhao Yan:** Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pld.2024.02.005>.

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