BMC Plant Biology

RESEARCH

Open Access

Comparative transcriptome analysis of oat varieties with different flowering performances under a short-day photoperiod

Man Zhang[1†], Yuan Jiang[2†], Haixiao Dong[2], Xiaohui Shan[2], Hao Chen[2], Xueying Li[1], Chunlong Wang[1], Sangpingcuo Ba[1], Junwei Li[1], Laichun Guo[1*], Yaping Yuan[2*] and Changzhong Ren[1*]

Abstract

Background Insensitivity to day length is an essential trait for oat cultivation and its geographical spread to a wide range of latitudes. Daylength-insensitive oat cultivars can flower normally from low to high latitudes and may be especially well suited for a double-cropping system. However, few studies have investigated the regulatory mechanisms involved in flowering in photoperiod-insensitive oats.

Results In this study, we compared the developmental stages of shoot apical meristems (SAMs) and transcriptome profiles between the photoperiod-insensitive oat cultivar VAO-8 and the photoperiod-sensitive oat cultivar Baiyan 2 at four time points under a short-day photoperiod. The development of Baiyan 2 was affected by short-day length, and SAMs persisted in the vegetative stage. VAO-8 responded less to photoperiod and matured normally under a short-day photoperiod. Comparative transcriptome data of the two cultivars revealed a total of 824, 1,189, 646 and 1,145 differentially expressed genes (DEGs) between VAO-8 and Baiyan 2 at four time points. Functional enrichment analysis revealed that metabolic processes related to chlorophyll biosynthesis, photosynthesis, carbohydrate metabolism and the secondary metabolism were significantly enhanced in VAO-8 compared with those in Baiyan 2. The upregulated genes involved in these processes may contribute to the flowering of VAO-8 under a short-day photoperiod. Furthermore, the differential expression of 93 transcription factor genes involved in multiple flowering pathways was observed, and these genes may play important roles in the regulation of VAO-8 flowering.

Conclusions The results provide a comprehensive understanding of the photoperiod-insensitive molecular mechanism of oats at the transcriptional level under a short-day photoperiod, which lay a foundation for breeding photoperiod-insensitive oat cultivars.

Keywords Oat, Photoperiod insensitivity, Short-day photoperiod, Flowering, Transcriptome analysis

†Man Zhang and Yuan Jiang contributed equally to this work.

*Correspondence:
Laichun Guo
guolaichun@126.com
Yaping Yuan
yuanyp@jlu.edu.cn
Changzhong Ren
13500830080@163.com
[1]Key Laboratory of Biotechnology of Jilin Province, Baicheng Academy of Agricultural Science, Baicheng 137000, China
[2]Jilin Engineering Research Center for Crop Biotechnology Breeding, College of Plant Science, Jilin University, Changchun 130000, China

Introduction

Oat (*Avena sativa* L.) is one of the most important coarse cereals and ranks seventh in cereal production worldwide. The success of oat production is determined by proper flowering time in a suitable environment [1]. Flowering time is known to be an important trait for geographical adaptation and crop yield [2]. This trait is regulated by multiple environmental factors, particularly day length (photoperiod). Daylength is one of the major factors influencing the flowering time. Based on their daylength requirements for flowering, plants can be classified into three major types: long-day (LD) plants, short-day (SD) plants and day-neutral (DN) plants [3–4]. The LD and SD plants tend to flower under a specific photoperiod duration, whereas the DN plants or photoperiod-insensitive plants are less affected by photoperiod and can trigger flowering irrespective of the photoperiod [5].

Many studies have indicated that diverse compounds, such as carbohydrates and photosynthetic pigments, play essential roles in flowering regulation. Previous reports have demonstrated that carbohydrates, including sucrose, starch, glucose and trehalose, can be used as energy sources during the plant life cycle. Moreover, they serve as important signalling molecules that regulate of flowering time in many plant species [6]. The level of trehalose-6-phosphate (T6P) is positively regulated by T6P synthase (TPS) and negatively regulated by T6P phosphatase (TPP) [7]. Overexpression of the TPP gene from *Jatropha curcas* resulted in late flowering in *Arabidopsis* and increased soluble sugar accumulation [8]. Moreover, sugar content also modulates the timing of flowering. The expression levels of the sucrose cleavage enzyme-encoding genes sucrose synthase 1 (SUS1) and SUS4 were upregulated in plants that overexpressed INDETERMINATE DOMAIN 8 (AtIDD8) from Arabidopsis, which displayed an early-flowering phenotype, whereas the expression levels of these two genes were suppressed in *Atidd8* mutants [9].

In addition to carbohydrates, chlorophyll may also play a crucial role in the plant flowering time network. Recent studies on several SD plant species, including sweet potato [10], tomato [11], rice [12] and *Medicago truncatula* [13], have shown that alterations in chlorophyll metabolism affect flowering time. For example, in *Medicago truncatula* plants, overexpression of the AP2/ERF transcription factor (TF) *MtRAV3* resulted in a high level of chlorophyll and led to a late phenotype [13]. Photoreceptors also affect chlorophyll accumulation and flowering time. In tomato, *cry2/cry1* double mutants lead to early flowering and decreased chlorophyll levels [11].

The genetic mechanism of photoperiod insensitivity in plants has been studied in several plant species. Several major determinants of the photoperiod response have been identified that confer normal flowering phenotypes to crops under both LD and SD conditions [14]. In barley and wheat, the major regulator PPD1 harbours multiple allelic variations, leading to increased expression levels of several genes, such as *Flowering Locus T* (*FT*), and achieving a photoperiod-insensitive phenotype [15]. Many genes in maize, including *ZmCOL3*, *ZmMADS69*, *ZmCCT9*, *ZmCCT10*, *ZmRAP2.7* and *ZmZCN8*, play important roles in maize adaptation to temperate regions [16]. A major flowering time regulatory gene, GmPRR3b, which belongs to the CCT gene family, was selected during soybean domestication and facilitated the geographic expansion of soybean [17].

Like LD plant species such as wheat, oat is a classical long-day crop, and its flowering is mainly controlled by day length. Under long days (daylengths exceeding 12 h), the flowering time of oat is hastened. In contrast, when the daylength is shorter than a certain threshold, many cultivars exhibit a stronger response to photoperiod and do not proceed to the reproductive stage or produce seeds [18]. Since 1972, Burrows and Sampson reported that several oat cultivars were less sensitive to photoperiod and identified a single, dominate gene *Dil*, for daylength insensitivity [19, 20]. Several technologies were been subsequently applied to identify genes responsible for flowering time in oats. Fifteen major QTLs associated with flowering time were identified by using different oat populations via the restriction fragment length polymorphism (RFLP) and single-nucleotide polymorphism (SNP) methods [21–26]. Recent studies have shown that several genes, including *PHYTOCHROME B* (*PHYB*), *TIMING OF CAB EXPRESSION 1* (*TOC1*), *PSEUDO-RESPONSE REGULATOR5* (*PRR5*), *PRR37* and *GIGANTEA* (*GI*) in the photoperiod pathway, as well as *ARABIDOPSIS RESPONSE REGULATOR 4* (*ARR4*) and *Cytokinin Response 1* (*CRE1*) in the cytokine signalling pathway, are differentially expressed between the oat photoperiod-sensitive cultivar and the cultivar less sensitive to photoperiod under 12 h light/12 h dark conditions [27]. However, previous studies have been limited to two varieties during the transition to a reproductive state, with less emphasis on the differences in photoperiod sensitivity among different varieties at various plant developmental stages under shorter photoperiods. To develop oat varieties with improved photoperiod insensitivity, knowledge of the underlying molecular mechanism that regulates the different flowering performances between photoperiod-insensitive oat varieties and photoperiod-sensitive oat varieties under short-daylength conditions is needed. This research area needs to be further explored.

In our study, we used the photoperiod-insensitive oat variety VAO-8 and the photoperiod-sensitive oat variety Baiyan 2 as materials and observed the developmental stages of the two oat cultivars under a short-day photoperiod. To reveal the different response mechanisms

for flowering under a short-day photoperiod, we analysed the transcriptomic differences between VAO-8 and Baiyan 2 by using RNA-sequencing technology. Our results suggested that, compared with Baiyan 2, VAO-8 could enhance several metabolic, energetic and photosynthesis-related processes under a short-day photoperiod. These processes could lead VAO-8 to flower under a short-day photoperiod. Moreover, 93 differentially expressed TFs involved in the hormone pathway and the photoperiod pathway, which may play important roles in flowering regulation under a short-day photoperiod conditions, were identified between VAO-8 and Baiyan 2. Our results provide theoretical guidance for the breeding of daylength-insensitive oat cultivars and accelerating the widespread adaptation of oats.

Materials and methods

Plant materials and growth conditions

The photoperiod-insensitive oat cultivar VAO-8 used in this study was obtained from Agriculture and Agri-Food Canada (Ottawa, Canada), and the photoperiod-sensitive oat cultivar Baiyan 2 was obtained from Baicheng Academy of Agricultural Science, (Jilin, China). These two cultivars were chosen as plant materials because of their different flowering performances under short-day photoperiod conditions, with VAO-8 being photoperiod insensitive [28, 29]. For the experiments in this study, seeds of VAO-8 and Baiyan 2 were sown in plastic pots (13 cm diameter, 15 cm high) containing field soil. Each pot contained five seeds. All plants were grown in an artificial chamber under short-day photoperiod conditions (10 h light/14 h dark) with a light intensity of 8000 lx. The temperature was maintained at 24℃ during the day and 20℃ at night. Fifty days after growth under a short-day photoperiod, the fourth fully unfolded true leaves of the two cultivars were harvested: this time point was defined as stage one (S1). At 60 d, 70 d and 85 d under a short-day photoperiod, the fourth fully unfolded true leaves of VAO-8 and Baiyan 2 were collected and defined as stage two (S2), stage three (S3), and stage four (S4), respectively. Three biological replicates of the two oat cultivars were taken at four time points, and each replicate consisted of three individual plants. After sampling, the leaves were immediately placed in liquid nitrogen and stored at -80 °C for transcriptome sequencing.

Observation of the developmental stage of the shoot apical meristem

To examine the phenotypic changes in the SAM during development in response to a short-day photoperiod, we dissected the SAMs of the two cultivars and regularly observed the SAM phenotype when the plants were at least at the four-leaf stage. To determine of the developmental stage of the SAMs of VAO-8 and Baiyan 2 cultivars, the leaves surrounding the SAMs were removed manually, and the SAMs were quickly placed in a formaldehyde-acetic acid-ethanol fixative (FAA) solution. After fixation for at least 24 h, the samples were subjected to dehydration via an ascending ethanol series. For SEM, the dried samples were sputter-coated with gold and observed using field emission scanning electron microscopy (FESEM) (SIGMA 300, ZEISS, Germany).

Chlorophyll measurement

The fourth leaves of VAO-8 and Baiyan 2 plants were collected at the four stages. The samples were subsequently immersed in 3 mL of leach liquor and incubated for 24 h at 25℃ in the dark. The liquor was composed of acetone and absolute ethanol, and the volume ratio of acetone: ethanol was 2:1. After 24 h, the absorbance (OD) of each sample at 663 nm and 645 nm was measured by a UV−vis spectrophotometer (WFZ UV-2800AH, UNICO, Shanghai, China). The concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), and chlorophylls (Chls) were calculated with the following formulas using the methods of Arnon [30]:

$$\mathrm{Chl\ a\ (mg/g)} = (12.72 \times \mathrm{OD_{663}} - 2.69 \times \mathrm{OD_{645}}) \times \mathrm{V} \times \mathrm{N/W} \quad (1)$$

$$\mathrm{Chl\ b\ (mg/g)} = (22.88 \times \mathrm{OD_{645}} - 4.68 \times \mathrm{OD_{663}}) \times \mathrm{V} \times \mathrm{N/W} \quad (2)$$

$$\mathrm{Chls\ (mg/g)} = \mathrm{Chl\ a} + \mathrm{Chl\ b} \quad (3)$$

V represents the volume of the leach liquor, N represents the number of dilutions, and W represents the fresh weight.

RNA extraction, library construction and illumina sequencing

Total RNA from oat materials was extracted using an Ultrapure RNA Kit from Beijing ComWin Biotech Co., Ltd. (China), following the manufacturer's protocols. The quality and integrity of total RNA were checked by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Massachusetts, US) and an Agient2100/LabChip GX analyser (Agilent Technologies, California, USA). Then, high-quality RNA was subsequently used for construction of the transcriptome library, and sequenced using the Illumina NoveSeq6000 platform by Biomarker Technologies Co., Ltd. (Beijing, China), PE = 150. The raw RNA data were uploaded to NCBI with the accession number PRJNA1019673.

Differential gene expression analysis and functional enrichment

After the low-quality reads and the reads containing the adaptors were removed, the clean data were obtained. The clean reads from each sample were subsequently mapped to the OT3098 v2 hexaploid oat genome using HISAT2 software [31]. The expression level of each gene was assessed by the mapped results and fragments per kilobase of transcript per million mapped reads (FPKM) value. Based on the above results, the differentially expressed genes between the two cultivars were identified using DESeq2 software [32]. The key indicators of differentially expressed gene selection were set to a false discovery rate (FDR) ≤ 0.05 and a|$\log_2$fold-change (FC)|≥1. The Venn diagrams and heatmaps were plotted by the bioinformatics online platform (https://www.bioinformatics.com.cn/) and TBtools v1.132 software, respectively. The DEGs were subjected to functional annotation using GO, KEGG pathway enrichment and MapMan analyses. GO enrichment and KEGG pathway enrichment analyses were performed on the BMKCloud platform (www.biocloud.net). The significantly enriched GO terms and KEGG terms were set at a corrected p value ≤ 0.05. The bubble plot was drawn using the ggplot2 package in R software. In addition, the DEGs were further analysed and visualized using MapMan functional annotation software (version 3.6.0) [33].

For the plant TFs, we submitted the nucleotide sequences of the DEGs to the online iTAK 1.6 software (http://itak.feilab.net/cgi-bin/itak/index.cgi) and then clas sified the obtained transcription factors (TFs) into different gene families [34].

qRT–PCR analysis

Total RNA was extracted from the samples using the Ultrapure RNA Kit (CWbiotech, Beijing, China) and then reverse transcribed to synthesize cDNA using the UEIris II RT-PCR System for First-Strand cDNA Synthesis with dsDNase (US Everbright). To confirm the RNA-seq results, qRT-PCR was conducted to validate the expression levels of 10 candidate genes on a PCRmax Eco 48 machine (PCRMax, Staffordshire, UK). qRT-PCR was performed using Bimake SYBR Green qPCR Master Mix (Bimake Biotechnology Co., Ltd., Houston, Texas, USA), following the manufacturer's protocol. The $2^{-\Delta\Delta Ct}$ method was applied to calculate the relative expression of the genes [35]. Three biological replicates were performed for each gene. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (AK251456) was used as the internal control to normalize the tested genes [36]. The primer pairs used for the qRT-PCR analysis are listed in Table S1.

Results

The morphology of the SAM in VAO-8 and Baiyan 2

Based on the morphological observations of the shoot apical meristems, the two oat cultivars, VAO-8 and Baiyan 2, were in the vegetative growth stage after 50 d under short-day photoperiod conditions (Fig. 1a and e). After 60 d, the SAM of VAO-8 slowly transitioned to the elongation stage (Fig. 1b). In this stage, the apical dome was enlarged, suggesting that the plants had transitioned to a reproductive state. The SAMs of VAO-8 subsequently progressed to the floret differentiation stage, in which the floret primordium (FP) formed after 70 d of

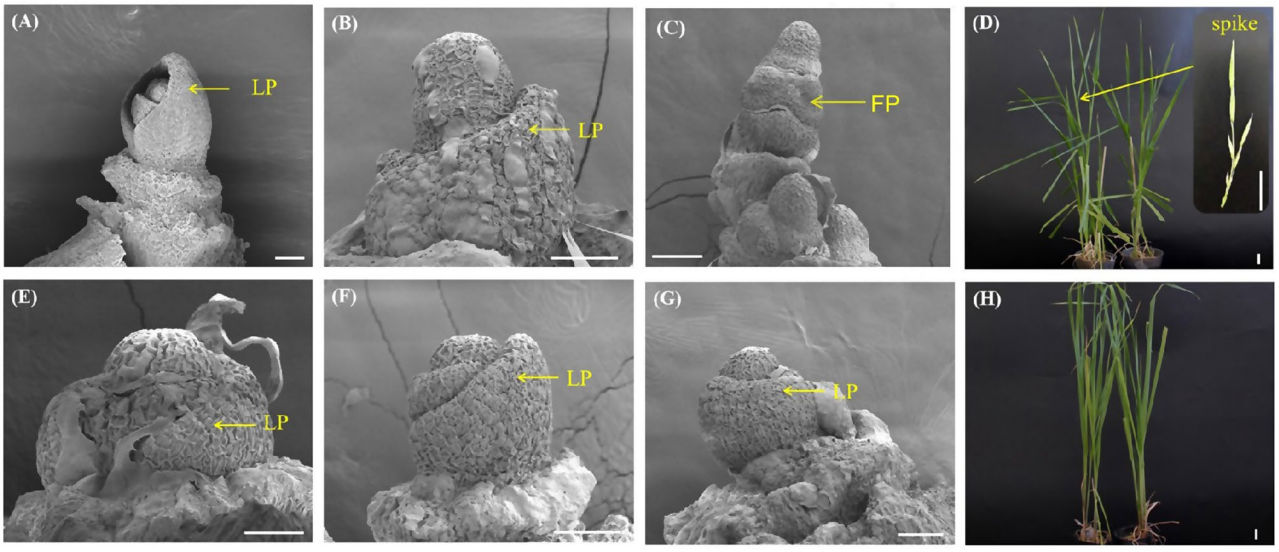

Fig. 1 SAM phenotypic characterization of VAO-8 and Baiyan 2. (**A**–**D**) Developmental stages of VAO-8 under short-day photoperiods for 50 d (**A**), 60 d (**B**), 70 d (**C**), and 85 d (**D**). (**E**–**H**) The SAMs of Baiyan 2 were still in the vegetative stage under short-day conditions at 50 d (**E**), 60 d (**F**), 70 d (**G**) and 85 d (**H**). LP, leaf primordia; FP, floret primordium. Scale bars = 50 μm (**A**–**C**, **E**–**G**) and 2 cm (**D**, **H**)

short-day photoperiod treatment (Fig. 1c). When grown for 85 d under short-day photoperiod conditions, VAO-8 was in the booting stage (Fig. 1d), indicating that VAO-8 can normally head under a short-day photoperiod. In contrast, the SAMs of Baiyan 2 persisted in the vegetative stage (Fig. 1f, g and h, Fig. S1), indicating that the oat cultivar Baiyan 2 is extremely sensitive to photoperiod. These phenotypic results showed that the cultivar VAO-8 is insensitive to photoperiod and can flower normally under a short-day photoperiod.

Overview of the transcriptome analysis

To explore the differences in flowering performance between VAO-8 and Baiyan 2 under a short-day photoperiod, fourth-leaf samples of the two cultivars at S1, S2, S3 and S4 were subjected to RNA-Seq. After data filtering, each sample generated 5.76 Gb of clean data, and the Q30 values for all 24 RNA-seq libraries were greater than 95.33%. The filtered clean data were subsequently mapped to the reference genome (Table S2). The percentage of unique mapped sequences was greater than 85.43% in each sample, indicating that the tested sequences were consistent with the oat reference genome. The Pearson's correlation coefficients between the three biological replicates for each time point of VAO-8 and Baiyan 2 were greater than 0.82, indicating that the repeatability of the three replicates was good (Table S3).

Based on a false discovery rate (FDR) ≤ 0.05 and log$_2$Fold change ≥ 1, all DEGs in the four comparison groups were determined (Table S4). There were 824, 1,189, 646 and 1,145 DEGs in the V8-S1_vs_B2-S1, V8-S2_vs_B2-S2, V8-S3_vs_B2-S3 and V8-S4_vs_B2-S4 comparisons, respectively. Among them, 381, 724, 331 and 663 DEGs were upregulated in the four groups, respectively. There were 443, 465, 315 and 482 downregulated DEGs in the four comparison groups, respectively (Fig. 2a). The Venn diagram results revealed that 45 upregulated DEGs were shared among the four comparison groups (Fig. 2b), and 77 downregulated DEGs were coexpressed in all four groups (Fig. 2c).

To corroborate the accuracy of the RNA-seq data, a qRT−PCR assay was performed on 10 selected DEGs, including three TFs, one gene involved in starch and sucrose metabolism, four genes associated with the EMP−TCA pathway and two genes involved in the chlorophyll biosynthetic process. As shown in Fig. S2a-j, the relative expression levels of these genes as determined via qRT−PCR were consistent with the RNA-seq results, verifying the accuracy of the expression level changes obtained from the RNA-seq data. In addition, the correlation coefficient value was 0.928, indicating a positive correlation between the RNA-seq data and the qRT−PCR results (Fig. S2k).

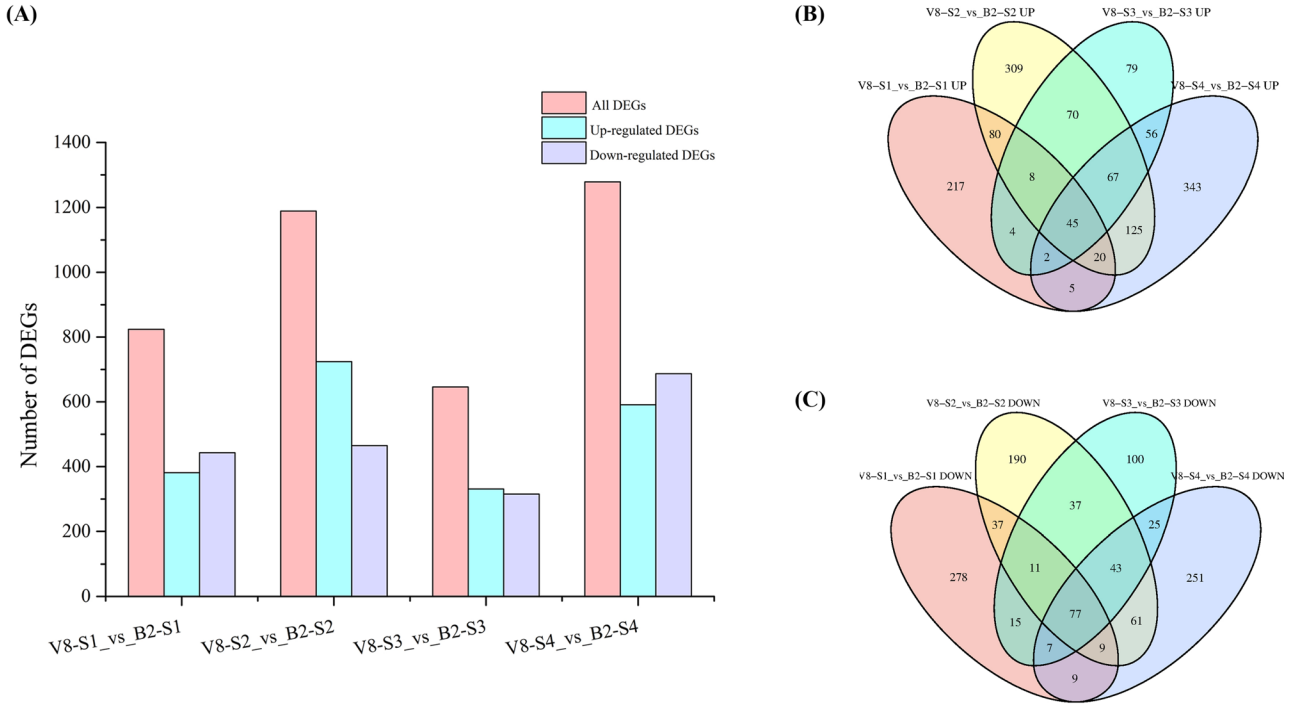

Fig. 2 Statistical analysis of DEGs in the four comparison groups. (**A**) Histogram illustrating significantly upregulated DEGs, downregulated DEGs and all DEGs for the four comparison groups. (**B**) Venn diagrams showing unique and shared upregulated DEGs among the four comparison groups. (**C**) Venn diagrams showing unique and shared downregulated DEGs among the four comparison groups

Different response mechanisms for flowering between VAO-8 and Baiyan 2

To analyse the different response mechanisms for flowering under a short-day photoperiod between Baiyan 2 and VAO-8, we performed gene ontology term enrichment analysis on the differentially expressed genes (DEGs) (Tables S5 and S6). For stage one (S1), only one biological process (BP), 'defense response', was enriched among the upregulated DEGs, and no GO terms were enriched among the downregulated DEGs, indicating that Baiyan 2 did not significantly differ from VAO-8 in terms of flowering during the early vegetative growth stage (Fig. 3a and b). For stage two (S2), two BP terms, 'tricarboxylic acid cycle' and 'glutamine transport', were enriched in the upregulated DEGs, indicating that the energetic metabolism and amino acid metabolism processes were enhanced in VAO-8 during the early flowering stage, which could lead to flowering as compared with Baiyan 2 (Fig. 3a). Moreover, eight BP terms were enriched among the downregulated DEGs, and these could be classified into three groups (Fig. 3b). The first group is related to biosynthetic and metabolic processes, including the

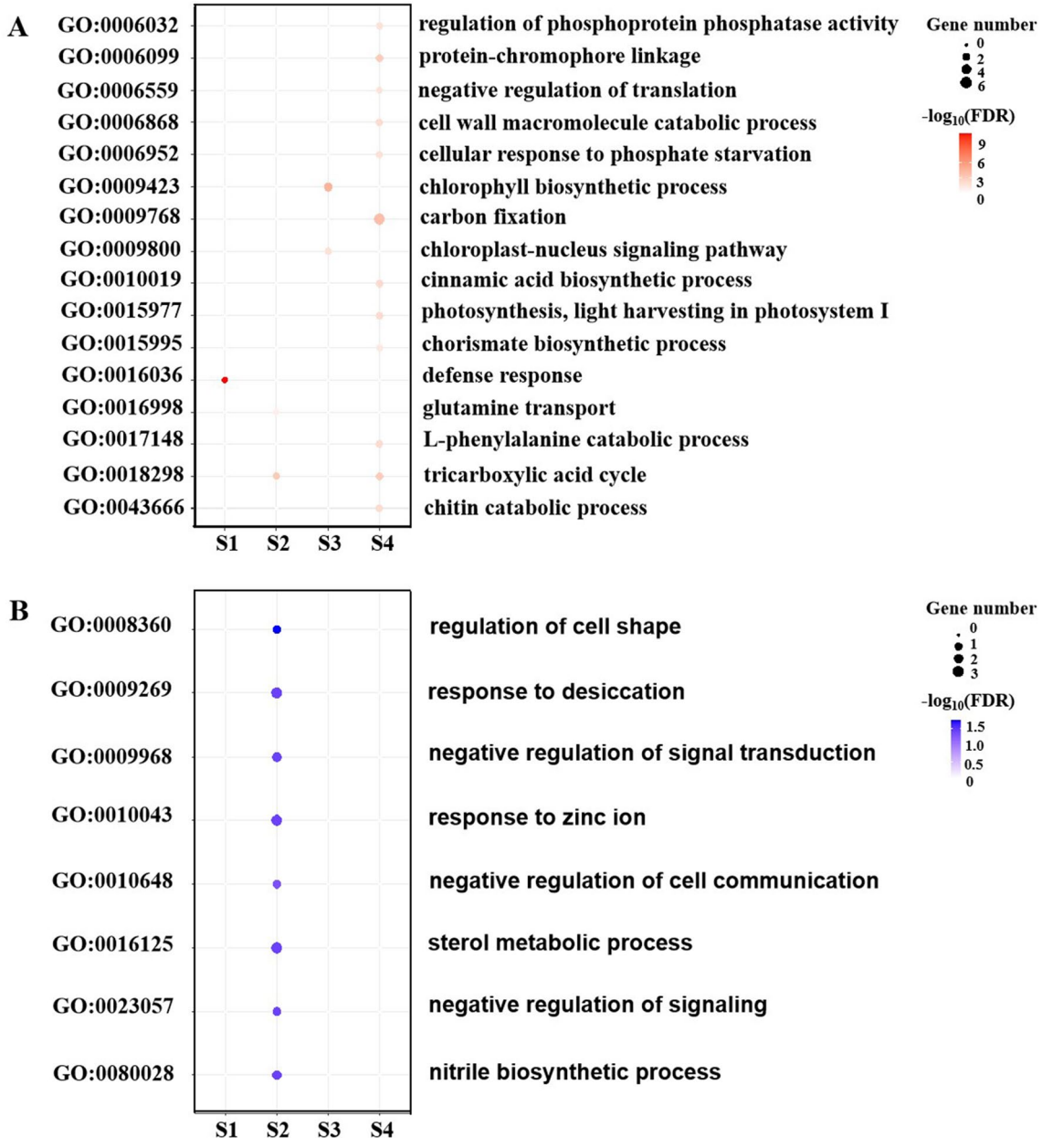

Fig. 3 GO enrichment analysis of the four comparison groups. (**A**-**B**) Biological process (BP) terms enriched with upregulated (**A**) and downregulated (**B**) DEGs between VAO-8 and Baiyan 2 at S1, S2, S3 and S4. The x-axis and y-axis represent the different stages and the BP term names, respectively. Significant BP terms with FDR ≤ 0.05. The size of the bubble dot indicates the gene ratio

'nitrile biosynthetic process' and 'sterol metabolic process'. The second group is related to signal transduction, such as the 'negative regulation of signal transduction'. The third group is related to responses to stimuli, including the 'response to desiccation' and 'response to zinc ions'. Two chlorophyll-related processes, 'chlorophyll biosynthetic process' and 'chloroplast-nucleus signalling pathway', were enriched among the upregulated DEGs in stage three (S3), whereas no significant BP terms were enriched among the downregulated DEGs. For stage four (S4), 12 BP terms were enriched among the upregulated DEGs and were also classified into three groups (Fig. 3a and b). The first group of BP terms was related to substance biosynthesis and energetic metabolism processes, including 'chitin catabolic process', 'chorismate biosynthetic process', 'cinnamic acid biosynthetic process', 'L-phenylalanine catabolic process', 'carbon fixation' and 'tricarboxylic acid cycle'. The second group of BP terms was related to photosynthetic activities such as 'photosynthesis, light harvesting in photosystem I' and 'protein–chromophore linkage'. The third group of BP terms was related to protein modification, including the 'regulation of phosphoprotein phosphatase activity'. The downregulated DEGs were not enriched under S4. The glycometabolism-related processes were shared by both stages, indicating that VAO-8 could regulate saccharide metabolic processes from the early stage of flowering. In addition, we also found that VAO-8 did not significantly differ from Baiyan 2 during the early stage, but VAO-8 enhanced more processes than Baiyan 2 from S2 to S4, which resulted in flowering.

To further investigate the flowering mechanism of VAO-8 under a short-day photoperiod, we compared the gene expression patterns involved in essential pathways between VAO-8 and Baiyan 2. There were 17 BP terms enriched among the upregulated DEGs from S1 to S4, and the 'tricarboxylic acid cycle' was shared by S2 and S3. These terms can be classified into four groups. The first group comprises biosynthesis- and metabolism-related processes, including the 'chlorophyll biosynthetic process' and 'cinnamic acid biosynthetic process'. The BP term 'chlorophyll biosynthetic process' included five DEGs. One, five and two DEGs presented greater expression in VAO-8 than in Baiyan 2 in S2, S3 and S4, respectively, whereas none of the DEGs presented significant changes in S1 (Fig. 4a). Next, we measured the contents of chlorophyll pigments in the fourth-leaf samples of VAO-8 and Baiyan 2 at the fourth stage (Fig. S3). The levels of Chl a were significantly greater in VAO-8 than in Baiyan 2 at S3 and S4, whereas the levels of Chl b were significantly greater in S4 (Fig. S3a, S3b). The Chls content was significantly greater in VAO-8 than in Baiyan 2 at S3 and S4 (Fig. S3c). These results showed that VAO-8 had more chlorophyll pigments than Baiyan 2 under short-day photoperiods.The other BP term, 'cinnamic acid biosynthetic process', also included five DEGs encoding the ammonia-lyase. One and five DEGs presented increased expression levels in VAO-8 compared with those of Baiyan 2 in S2 and S4, respectively (Fig. 4b). All of the above results indicated that VAO-8 could increase secondary metabolite and amino acid biosynthesis processes for flowering under a short-day photoperiod.

The second set of BP terms were photosynthesis-related processes, including 'carbon fixation' and 'photosynthesis, light harvesting in photosystem I'. The BP term 'carbon fixation' included nine DEGs that encode mainly phosphoenolpyruvate carboxylase. Four, one and nine upregulated DEGs were more abundant in VAO-8 than in Baiyan 2 under a short-day photoperiod (Fig. 4c). Moreover, VAO-8 activated a total of five genes associated with the BP term 'photosynthesis, light harvesting in photosystem I' (Fig. 4d). This enhanced ability may allow VAO-8 to capture and transmit more light energy and promote the flowering of oat under a short-day photoperiod. The third set of BP terms is related to protein modification, whereas the fourth set of BP terms is related to stimulus responses. In addition, VAO-8 significantly differed in the tricarboxylic acid cycle pathway compared with Baiyan 2, which could enhance the shift and delivery of energy to facilitate flowering under a short-day photoperiod. S2 and S4, which included three and seven DEGs in oats, respectively, were both enriched in this term (Fig. 4e). Among these genes, one citrate synthase (CS) gene, one cytosolic isocitrate dehydrogenase (ICDH) gene and two succinate dehydrogenase (SDH) genes were identified and presented higher expression levels in VAO-8 than in Baiyan 2.

Taken together, all these results suggested that VAO-8 could increase substance biosynthesis, photosynthesis and chlorophyll-related processes, which could lead to flowering under a short-day photoperiod compared with Baiyan 2.

Pathway enrichment based on KEGG

To further identify the different photoperiod responses between VAO-8 and Baiyan 2 under short-day photoperiod conditions, KEGG pathway enrichment analysis of the four comparison groups was performed. Consistent with the GO enrichment analysis results, there were no pathways that were differentially enriched in S1 between VAO-8 and Baiyan 2. For the upregulated DEGs in VAO-8 compared with those in Baiyan 2 at S2, the most enriched pathways were isoflavonoid biosynthesis, plant–pathogen interaction and starch and sucrose metabolism. Moreover, the upregulated DEGs in VAO-8 compared with Baiyan 2 at S3 were enriched in the plant-pathogen interaction and photosynthesis-antenna proteins pathways. In addition, the upregulated DEGs in VAO-8

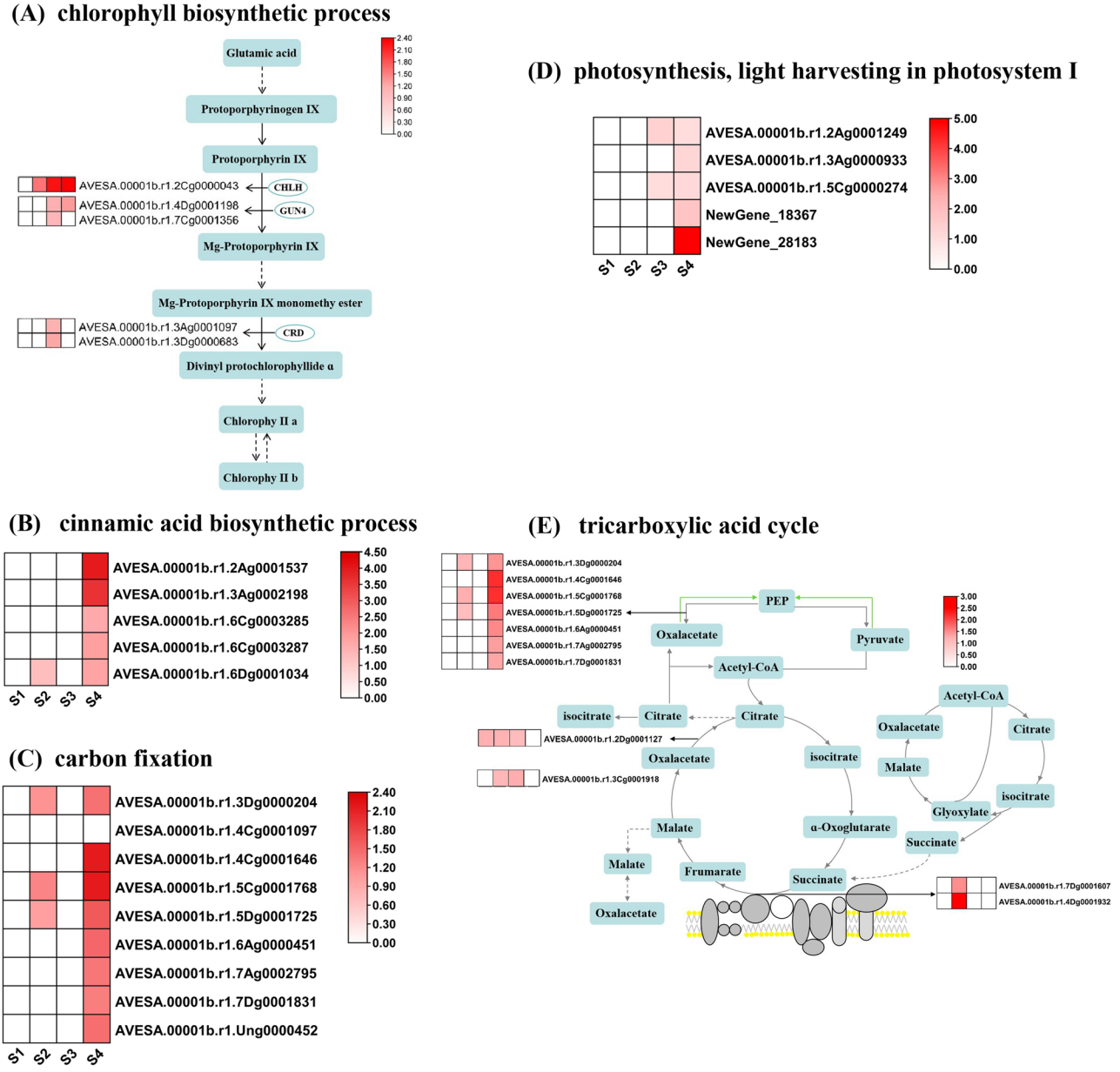

Fig. 4 Expression levels of genes involved in the 'chlorophyll biosynthetic process' (**A**), the 'cinnamic acid biosynthetic process' (**B**), 'carbon fixation' (**C**), 'photosynthesis, light harvesting in photosystem I' (**D**), and the 'tricarboxylic acid cycle' (**E**) between VAO-8 and Baiyan 2 at different stages. Scale bars display the $\log_2$-fold changes

compared with Baiyan 2 at S4 were enriched in five pathways, including photosynthesis-antenna proteins, phenylalanine, tyrosine and tryptophan biosynthesis, carbon fixation in photosynthetic organisms, plant-pathogen interaction and biosynthesis of amino acids. Among these pathways, the photosynthesis-antenna protein pathway was shared by S2 and S3. In addition, only one pathway, benzoxazinoid biosynthesis, was enriched among the downregulated DEGs in VAO-8 at S4. Among these enriched pathways, the photosynthesis-antenna protein pathway and the starch and sucrose metabolism pathway were activated in VAO-8 compared with Baiyan 2. The former pathway was significantly enriched in S2 and S4, and it contained 1, 2, 4 and 7 upregulated DEGs in S1, S2, S3 and S4, respectively (Fig. S4a and table S5). The latter pathway was significantly enriched in S2, and there were three, twenty-one, six and four DEGs were upregulated in S1, S2, S3 and S4, respectively (Fig. S4b and table S5). These results suggested that the enrichment pathways related to energy metabolism and photosynthesis processes were markedly altered between VAO-8 and Baiyan 2. Compared with those in Baiyan 2, the increased photosynthetic activities and energetic metabolism processes

in VAO-8 may lead to flowering under short-day photoperiod conditions.

Metabolism process analysis by MapMan

The metabolic processes of the four comparative groups of DEGs were examined using the MapMan tool. As shown in Fig. 5 and Fig. S5, the metabolic processes and the number of DEGs involved in these processes did not change as much between VAO-8 and Baiyan 2 at the vegetative stage. In contrast, more metabolic processes and DEGs involved in metabolic processes were activated and increased between VAO-8 and Baiyan 2 at the latter stages, especially at S4. In addition, the DEGs involved in photosynthesis, cell wall synthesis, glycolysis, the citrate cycle (TCA cycle) and secondary metabolic processes at three developmental stages (S2, S3 and S4) were mainly upregulated in VAO-8. Moreover, several additional DEGs related to lipid, amino acid and nucleotide metabolism were also affected by the short-day photoperiod (Fig. 5 and Fig. S5).

To further understand the differences in the photoperiod response of VAO-8 and Baiyan 2 under a short-day photoperiod, the MapMan program was used to analyse changes in the transcription of glycolysis process-related DEGs. Our results revealed that significant changes in several genes involved in glycolysis in VAO-8 compared with those in Baiyan 2 at S2, S3 and S4 under a short-day photoperiod. In glycolysis, we identified several genes that were differentially expressed between the two varieties, including one gene encoding UGPase, two genes encoding aldehyde 3-phosphate dehydrogenase, one gene encoding aldolase, three genes encoding phosphoglycerate mutase, one gene encoding enolase, seven genes encoding phosphoenolpyruvate carboxylase (PEPC), and one gene encoding phosphoenolpyruvate carboxylase kinase (PPCK) (Fig. 6a and table S5). More genes were upregulated in VAO-8 than in Baiyan 2 at S2, S3, and especially at S4, whereas only one gene was upregulated at S1. Compared with Baiyan 2, VAO-8 was more active in the metabolism of the Embden–Meyerhof–Parnas pathway (EMP) pathway under short-day conditions. These processes may provide a large amount of energy support for the flowering induction of VAO-8 under a short-day photoperiod.

In addition, we also noted changes in the expression levels of some genes involved in the biosynthetic pathways of isoprenoid synthesis in secondary metabolism. One dimethylallyl diphosphate gene (DMAPP) involved in the MVA pathway and one gene encoding 1-deoxyxylulose-5-phosphate synthase (DXS) involved in the MEP pathway were upregulated in VAO-8 compared with Baiyan 2 at S2, S3, and S4; these two genes were unchanged in S1 (Fig. 6b). Overall, these results revealed that several DEGs related to material and energy metabolism, particularly those involved in glucose catabolism and isoprenoid metabolism, were altered in VAO-8 compared with Baiyan 2, indicating that these metabolic processes may play essential roles in flowering regulation in VAO-8 under a short-day photoperiod.

Identification of differentially expressed transcription factors

On the basis of our transcriptome data, we identified 37, 26 and 52 differentially expressed TFs in VAO-8 compared with Baiyan 2 at S2, S3 and S4, respectively. Apart from eight TFs showing the same expression trend and similar fold changes in stage one and the other three stages between VAO-8 and Baiyan 2, the remaining 93 TFs were categorized into 30 families, of which the GARP, NAC, WRKY, TRAF, AP2/ERF, bHLH, MYB, HSF, Trihelix, C2C2, CCT and MADS-box TFs were the top families (Fig. 7a). Moreover, one ARR-B belonging to the GARP family involved in hormone signalling pathways was also identified and found to be downregulated in VAO-8 compared with Baiyan 2 (Fig. 7a). In addition, our heatmaps revealed that most WRKY TFs (8 genes out of 9) (Fig. 7b) and NAC TFs (5 genes out of 9) (Fig. 7c) were upregulated, whereas most bHLH TFs (5 genes out of 7) were downregulated in VAO-8 compared with Baiyan 2 (Fig. 7d). Taken together, our results indicated that the flowering regulation of VAO-8 under a short-day photoperiod was positively or negatively regulated by several flowering pathways and several transcription factors.

Discussion

Flowering is an important agronomic trait that significantly affects the yield and quality of many plants. Previous studies have revealed that flowering is precisely regulated by genetic and environmental factors. Among the environmental factors, day length is one of the critical signals that affects the flowering behaviour and growth habits in many plant species. The response to photoperiod sensitivity limits the geographical range of many crops such as maize, soybean, wheat and sorghum [37, 38]. Reducing photoperiod sensitivity is important for global crop cultivation for crops to ensure yield [39]. Therefore, several crop varieties that are insensitive to photoperiod have been selected by plant breeders for several decades, and international crop improvement programs have successfully exploited daylength insensitivity to broaden the range of adaptation of wheat and rice cultivars. Moreover, over the past decade, intensive studies have been conducted to elucidate the molecular mechanisms of flowering time in many crops under unsuitable photoperiod conditions. At present, a diverse set of signalling pathways are associated with the flowering process, including the photoperiod, vernalization, autonomous, gibberellin, age, and thermosensory

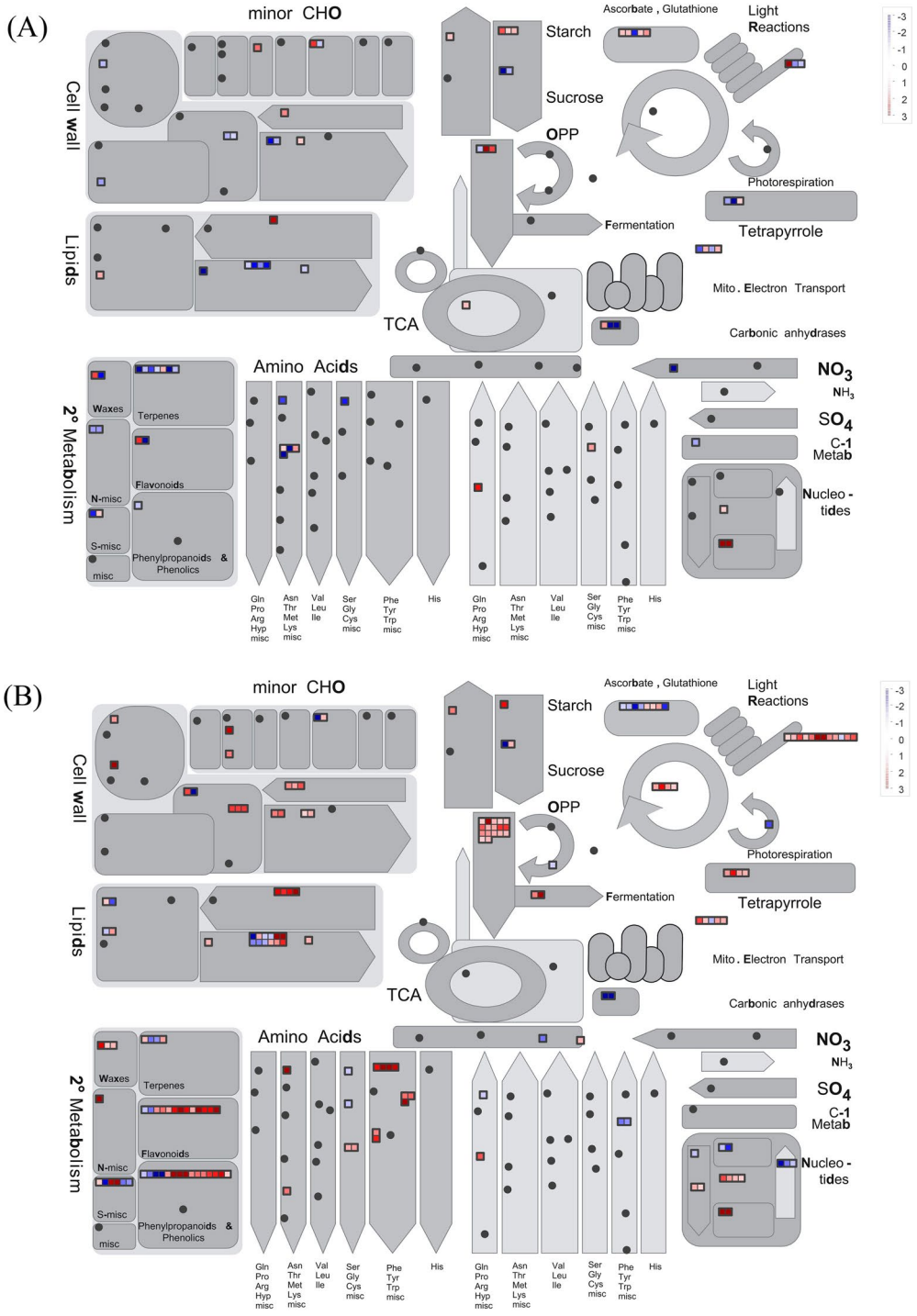

Fig. 5 Overview of the metabolic pathways of genes differentially expressed between VAO-8 and Baiyan 2 visualized by MapMan. (**A**) and (**B**) MapMan analysis of the DEGs between VAO-8 and Baiyan 2 at S1 and S4, respectively. The red lattice indicates the upregulated genes, and the blue lattice indicates the downregulated genes. The colour scale presents the $\log_2$-fold changes

pathways, the sugar pathway, the stress pathway, and the hormonal signalling pathway [40, 41]. Moreover, studies have revealed that several key flowering genes involved in multiple flowering pathways play important roles in flowering regulation.

In our study, we found that the oat cultivar VAO-8 was photoperiod-insensitive, whereas Baiyan 2 was photoperiod-sensitive according to our phenotypic data. Next, we attempted to gain a first comprehensive understanding of the different molecular mechanisms

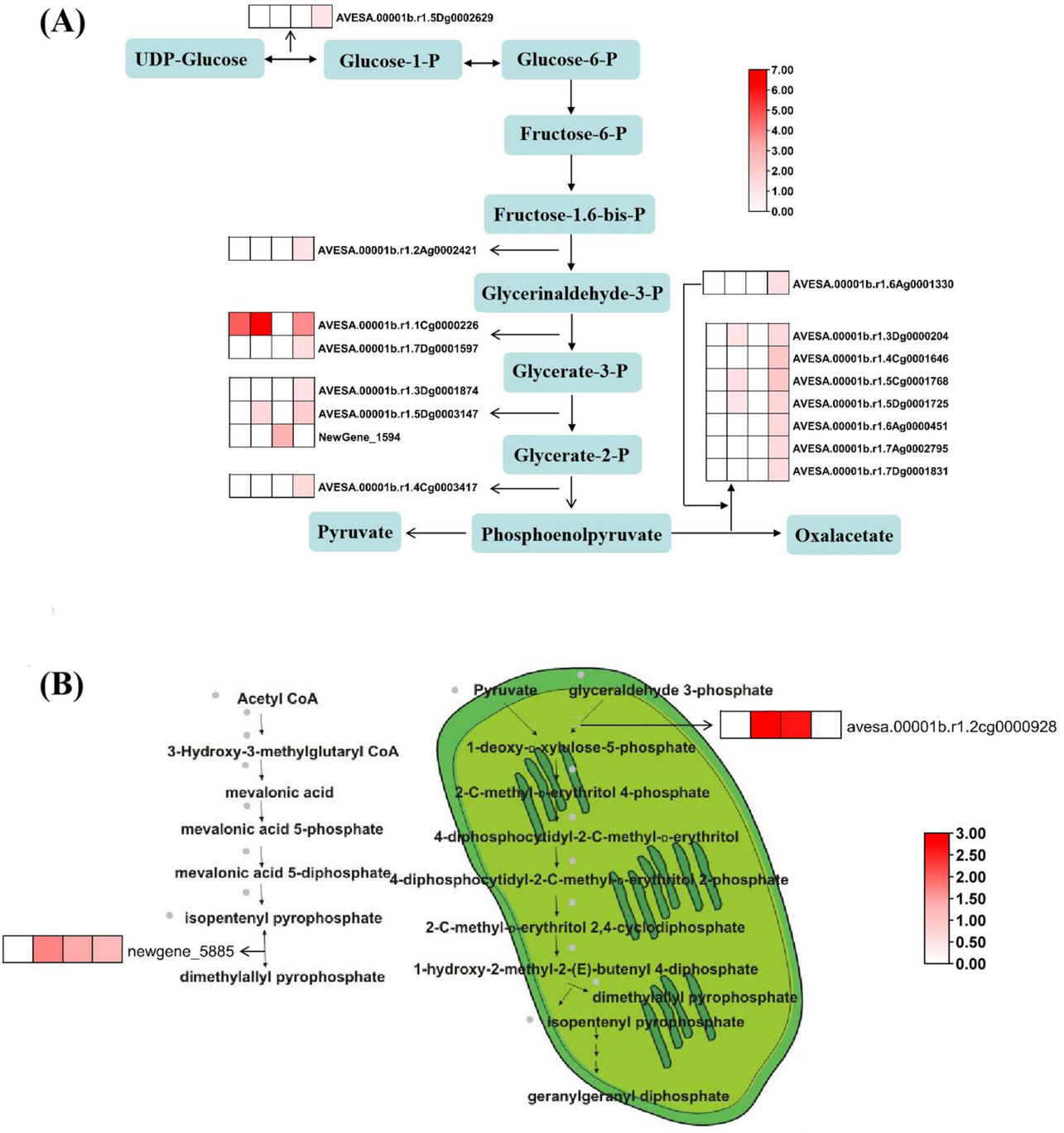

Fig. 6 Changes in the expression of several DEGs associated with the glycolysis process (**A**) and isoprenoid biosynthetic process (**B**) at four stages. From the left, the squares represent the DEGs in S1, S2, S3 and S4 between VAO-8 and Baiyan 2. Scale bars display the log2-fold change

of flowering time between VAO-8 and Baiyan 2 under short-day photoperiod conditions. Based on the results of the present study and previous reports, a model of the flowering regulation network in VAO-8 compared with Baiyan 2 under a short-day photoperiod was proposed and is summarized in Fig. 8. In this model, we consider that the transition from vegetative growth to reproductive growth is regulated by several signalling pathways. Under a short-day photoperiod, the upregulated expression of genes involved in the methylerythritol 4-phosphate (MEP) pathway and the tetrapyrrole biosynthetic pathway (TBP) contributed to the content of chlorophyll pigments in VAO-8 compared with that of Baiyan 2. Chlorophyll content is closely related to photosynthetic efficiency [42]. In the present study, the different contents of chlorophyll pigments between VAO-8 and Baiyan 2

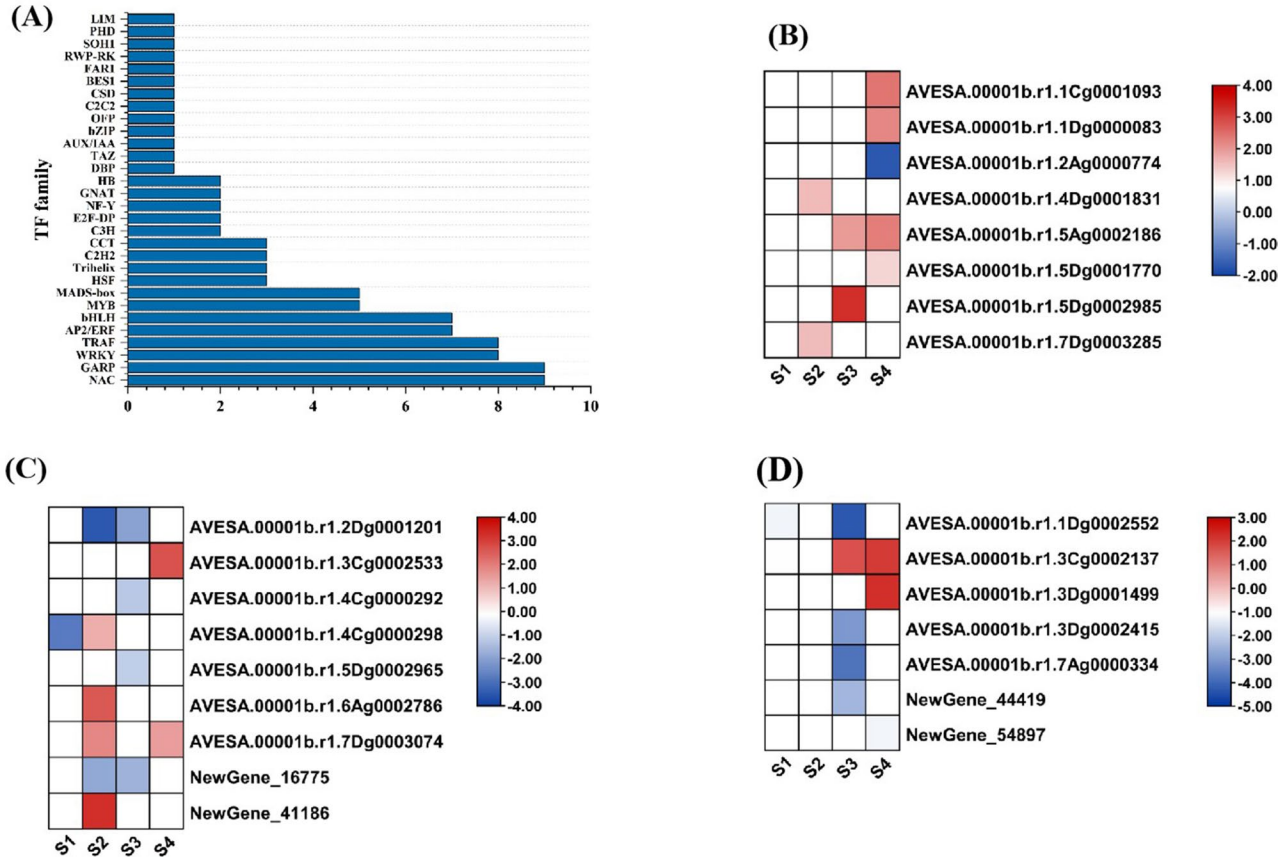

Fig. 7 Statistics of 29 transcription factor (TF) families (**A**) and a heatmap showing the expression patterns of WRKY TFs (**B**), NAC TFs (**C**) and bHLH TFs (**D**) between the VAO-8 and Baiyan 2 groups. Scale bars display log2-fold changes. S1, S2, S3 and S4 represent stage one, stage two, stage three and stage four, respectively. Red blocks indicate increased expression levels, and blue blocks indicate decreased expression levels in VAO-8 compared with Baiyan 2 under short-day photoperiod conditions

led to alterations in photosynthesis. Next, the increased photosynthetic capacity leads to increased carbohydrate and energy metabolism processes, which contribute to flowering in VAO-8. Additionally, several differentially expressed transcription factors were identified between VAO-8 and Baiyan 2. These flowering pathways may act in combination to allow VAO-8 to enter the reproductive stage under a short-day photoperiod.

Enhanced chlorophyll biosynthesis and photosynthetic capacity contribute to flowering in VAO-8 plants

The photoperiod pathway affects flowering not only through interactions with internal and external flowering pathways but also via crosstalk with metabolic pathways [43]. In recent years, increasing evidence has shown that signals from secondary metabolites involved in photosynthesis, such as chlorophylls, affect flowering time when plant species are exposed to long-day or short-day conditions [44].

In plants, there are two distinct biochemical pathways that are responsible for chlorophyll biosynthesis: the MEP pathway and the TBP pathway. The MEP pathway in the cytoplasm produces the isoprenoid phytol tail of the chlorophyll derived from GGPP [43, 45, 46]. Based on the MapMan analysis, one gene encoding DXS in the MEP pathway was identified and upregulated in VAO-8 compared with Baiyan 2. Moreover, one CHLH gene, two CRD genes and two GUN4 genes involved in the TBP biosynthetic process were upregulated in VAO-8 compared with Baiyan 2, suggesting that VAO-8 can accumulate more chlorophyll pigment than Baiyan 2, conferring VAO-8 with the ability to flower normally under a short-day photoperiod. Consistent with the upregulated gene expression patterns of the chlorophyll biosynthesis process, the contents of Chl a and Chl b were synchronously upregulated in VAO-8 compared with those in Baiyan 2 at S3 and S4, implying that chlorophyll pigments may act as a flowering signal, triggering the transition from vegetative to reproductive states. Similar results have been reported for other plant species [43]. In addition, chlorophyll levels can influence the sugar biosynthesis process; however, more research is needed to verify the crosstalk between the chlorophyll pathway and the sugar pathway [47].

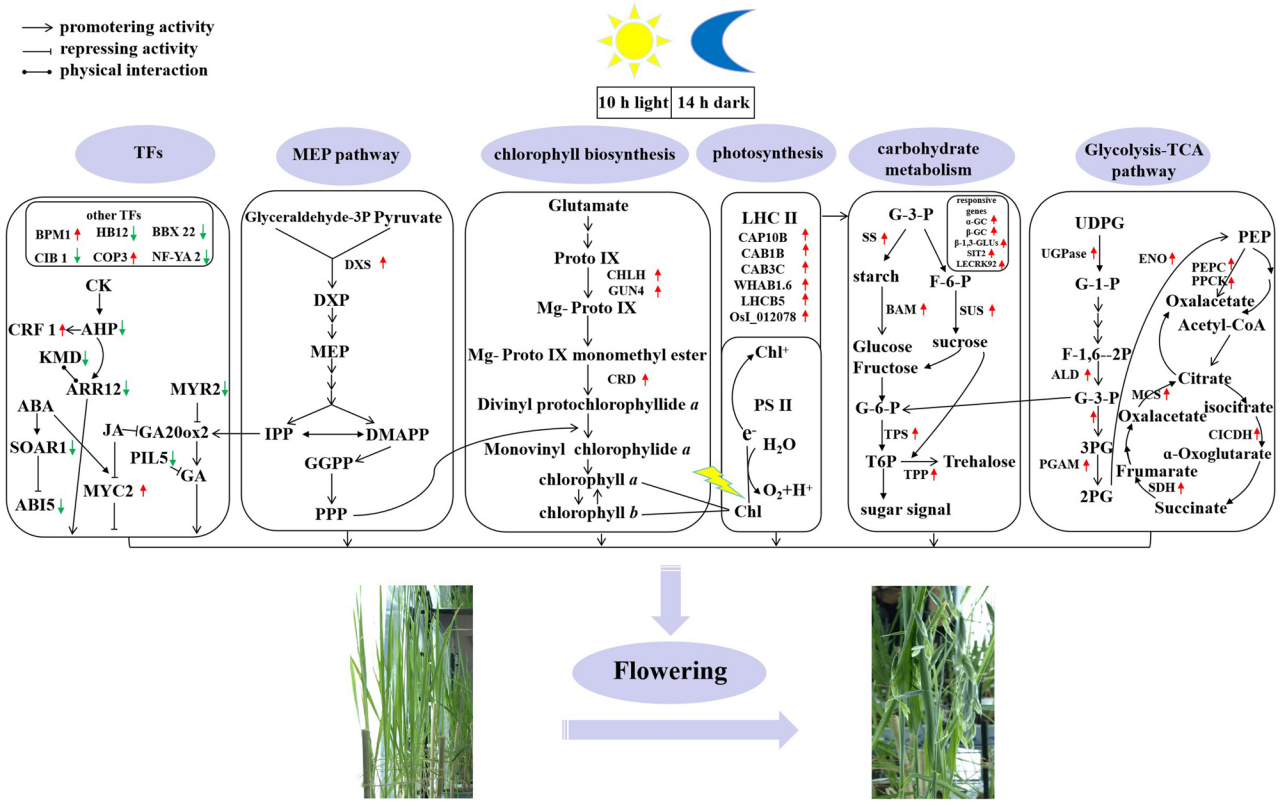

Fig. 8 A proposed working model of different photoperiod-sensitive oat cultivars in response to short-day photoperiod conditions. Red arrows represent upregulated genes, and green arrows indicate downregulated genes. The full names of all genes noted in this model are listed in Table S7

Previous studies have shown that chlorophyll pigments and photosynthesis are positively correlated. The increased chlorophyll levels allow the absorption of more light energy to generate more carbon sources [48]. Moreover, previous studies have reported that the photosynthesis process is related to plant flowering time [49, 50]. High irradiance-mediated photosynthesis promotes flowering in *Anagallis arvensis* [51]. In *Arabidopsis*, photosynthesis in leaves exposed to high irradiance under red-light LDs accelerates flowering via the FT-dependent pathway, supporting the connection between photosynthesis and flowering [52]. In the present study, the KEGG pathway 'photosynthesis-antenna proteins' was significantly enriched among the upregulated DEGs in VAO-8. Seven photosystem II chlorophyll a/b-binding protein genes involved in this pathway were all upregulated in VAO-8 compared with Baiyan 2. In plants, photosystem II (PSII) is a large protein complex that is located on the thylakoid membrane of chloroplasts and consists of the PSII core complex and light-harvesting complex II (LHC II) [53]. LHC II, which is responsible for capturing light during photosynthesis and maintaining the stability of the PSII electron transport chain, participates in plant photosynthesis and regulates plant growth and development [54–56]. Silencing *AtLhcb1* in *Arabidopsis thaliana* hindered the formation of LHC II trimers and thus affected photosynthesis and plant height [57]. Moreover, the overexpression of OsNF-YB2, which belongs to the NF-Y gene family, can promote flowering induction and increase the photosynthesis rate, suggesting that there is a relationship between the floral transition and the photosynthetic process [53]. In our study, the upregulation of seven chlorophyll a/b-binding protein genes may have contributed to the process of electron transfer from photosystem II to photosystem I, resulting in a significant increase in overall photosynthetic activity. The increased photosynthetic activity in VAO-8 may be related to the flowering of VAO-8 under a short-day photoperiod. However, whether enhanced chlorophyll biosynthesis and photosynthetic processes are beneficial for the flowering of VAO-8 under a short-day photoperiod has yet to be determined, and this finding should be verified in future studies.

Altered carbohydrate metabolism is an important factor involved in flowering time

In photosynthetic plants, carbohydrate substances including glucose, sucrose and starch, modulate many developmental processes, including reproduction and flowering time [58–60]. According to the GO enrichment

analysis, the BP terms 'tricarboxylic acid cycle' and 'carbon fixation' were significantly enriched, and the DEGs involved in these processes were upregulated in VAO-8, indicating that more energy was provided in VAO-8 than in Baiyan 2 by carbohydrate metabolism processes and contributed to adverse environmental conditions. Similarly, GO enrichment analysis revealed that the DEGs in the V8-S3_vs_V8-S1 and V8-S4_vs_V8-S1 groups were significantly enriched in the 'carbon fixation' (GO:001597) biological process (Fig. S6), indicating that this process also plays an important role during the flowering induction period of VAO-8 (Fig. S6). However, as we focused primarily on interspecies differences, we did not perform GO analysis on the DEGs among S1, S2, S3, and S4 of VAO-8. In addition, the 'starch and sucrose metabolism' pathway was significantly enriched among the upregulated DEGs in S2 according to KEGG enrichment pathway analysis. Among the DEGs related to the starch process, two starch synthase 1 (SS1) genes, which are responsible for the starch synthesis process, were upregulated in VAO-8 compared with Baiyan 2. In *Arabidopsis thaliana*, the expression of SS1 is induced and regulated by photosynthetic activities to affect flowering, demonstrating the intimate connection between photosynthesis and flowering via sugar metabolism [52]. Moreover, several genes (i.e., BAM9, GC, β-1,3-GLUs, SIT2, and LECRK92), which participate in the sugar degradation process, were also upregulated in VAO-8 compared with Baiyan 2, indicating that the process may produce more soluble sugars in VAO-8. These sugars have been reported to be involved in energy and material metabolism, plant growth and development, and plant responses to biotic and abiotic stresses [61–63]. Through transcriptome analysis, intensive molecular and genetic studies have shown that energy metabolism pathways such as glycolysis, which plays an important role in glucose metabolism, are significantly altered during the flowering process in many plants [64]. The results of the present study revealed showed that all DEGs involved in the glycolysis pathway, including UGPase, GAP-DH, PGM, ENO, PPCK, PEPC, IDH, and SDH, were upregulated as indicated by MapMan analysis, which may contribute to the flowering process in VAO-8.

Additionally, sugar not only acts as an energy source but also functions as a signal that affects flowering time. Trehalose-6-phosphate (T6P), a key sugar-signalling molecule, plays important roles in regulating carbohydrate use for growth and in regulating flowering time [65, 66]. In *Arabidopsis*, increased T6P levels significantly promote flowering time through both *FT* and the repression of miR156 [67]. In plants, T6P levels are regulated by TPS and TPP and are induced by sucrose [7]. Previous studies have reported that *AtTPS1* is important for vegetative development and the transition to flowering [67, 68]. Moreover, increased levels of *MdTPS1* and *MdTPS2* trigger early flowering in apple [69]. In our study, one TPS gene and one TPP gene were significantly expressed in VAO-8 compared with Baiyan 2, indicating that the sucrose metabolism-mediating T6P/TPS gene plays an essential role in VAO-8 under a noninductive photoperiod. Taken together, these data suggest that carbohydrate metabolism plays an important role in the different flowering performances of these two oat varieties under a short-day photoperiod.

Transcription factors involved in multiple pathways affect flowering time in VAO-8

All aspects of plant growth and development are controlled by a complex network of transcription factors. Transcription factors are associated with a variety of biological processes, including the regulation of plant flowering, light morphogenesis, and abiotic stress tolerance [70]. Therefore, we explored changes in the expression of transcription factors between VAO-8 and Baiyan 2 under short-day photoperiod conditions.

In our study, several TFs were identified, and most of the TFs were enriched in the WRKY, GARP, NAC, bHLH and MYB families (Fig. 7a). The expression levels of eight genes from the WRKY family increased in VAO-8, with two genes at stage two, two at stage three, and four at stage four. In contrast, only one WRKY transcription factor was highly expressed in Baiyan 2 at stage four, suggesting the importance of the WRKY family in response to a short-day photoperiod and its positive effect on flowering time regulation in VAO-8. Moreover, the differentially expressed type-B ARRs belonging to the GARP gene family were also identified in this study. Previous studies revealed that type-B ARR proteins can physically interact with the KELCH REPEAT F-BOX gene in *Arabidopsis* and lead to the degradation of type-B ARR proteins, resulting in negative regulation of cytokinin responses [71]. In addition, the ABA-responsive transcription factor ABA-insensitive 5 (ABI5), which belongs to the bZIP gene family, was downregulated in VAO-8 compared with Baiyan 2. In the ABA signalling pathway, genetic evidence indicates that SUPPRESSOR OF THE ABAR OVEREXPRESSOR 1 (SOAR1), a critical and negative regulator of ABA signalling, may function upstream of the ABA-responsive transcription factor ABA-insensitive 5 (ABI5). Changes in SOAR1 expression alter the expression levels of many ABA-responsive genes, including ABI5 [72, 73].

In addition to the GARP and bZIP TFs, HB, MYB and bHLH TFs play critical roles in regulating flowering time, mainly through hormone pathway genes. Moreover, the WRKY, NF-Y, NAC, MYB, bHLH, AP2/ERF, GRAS, MADS-box and CCT genes are also involved in photoperiod-induced flowering [74]. These findings suggest that

various transcription factors specifically and cooperatively regulate the flowering time control of VAO-8 plants under a short-day photoperiod via multiple flowering pathways. Whether these candidate TF family genes are related to the flowering of VAO-8 under short-day photoperiods requires further functional characterization.

Conclusions

In this study, we compared the photoperiod-insensitive oat cultivar VAO-8 and the photoperiod-sensitive oat cultivar Baiyan 2 at four different developmental stages under a short-day photoperiod through phenotypic identification and transcriptomic analysis. VAO-8 could head normally under a short-day photoperiod and exhibited photoperiod insensitivity. Transcriptome analysis revealed that the flowering of VAO-8 under short-day photoperiods may be related to the upregulated expression of several genes involved in chlorophyll biosynthesis, photosynthesis, carbohydrate metabolism, the secondary metabolism processes, and transcription factors. These candidate genes, which will be functionally characterized in future studies, provide in-depth insights into the molecular mechanism of photoperiod insensitivity in oats and lay the foundation for further breeding of photoperiod-insensitive oat cultivars in the future.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06648-x.

Supplementary Material 1: Fig. S1. Phenotypic characterization of Baiyan 2 under short-day conditions for 85 d. LP, leaf primordia. Scale bars = 50 μm

Supplementary Material 2: Fig. S2. Validation of the expression patterns of 10 DEGs between VAO-8 and Baiyan 2 using qRT–PCR. (A-J) Transcript levels of 10 DEGs analysed by qRT–PCR and RNA-seq. The data represent the means ± SD of three biological replicates. The bar graphs and line graphs present the qRT–PCR results and the RNA-seq results, respectively. The scales on the left and right axes are the relative expression levels and the FPKM values of these genes, respectively. V8 represents the oat cultivar VAO-8, and B2 represents the oat cultivar Baiyan 2. The primer information for these genes is listed in Table S1. (K) Correlation of the expression analysis by RNA-seq (X-axis) and qRT–PCR (Y-axis). R^2 represents the correlation between RNA-seq and qRT–PCR data

Supplementary Material 3: Fig. S3. The photosynthetic pigment contents of the fourth leaf were assayed in VAO-8 and Baiyan 2 plants via the spectrophotometric method. The data represent the means ± SD of triplicate experiments. *$p < 0.05$, **$p < 0.01$. The significant difference between VAO-8 and Baiyan 2 was calculated via two-tailed Student's t test

Supplementary Material 4: Fig. S4. Heatmap of seven DEGs in the photosynthesis-antenna protein pathway (A) and 21 DEGs in the starch and sucrose metabolism pathways (B) based on the values of the log2-fold changes. S1, S2, S3 and S4 represent the stages for VAO-8 and Baiyan 2

Supplementary Material 5: Fig. S5. Overview of the metabolic pathways of the genes differentially expressed between VAO-8 and Baiyan 2 using the MapMan tool. (A) and (B) show the MapMan analysis of the DEGs between VAO-8 and Baiyan 2 at S2 and S3, respectively. The red lattice indicates the upregulated genes, and the blue lattice indicates the downregulated genes. Scale bars display log2-fold changes

Supplementary Material 6: Fig. S6. GO enrichment analysis was performed for the three comparisons, including V8-S2_vs_V8-S1, V8-S3_vs_V8-S1 and V8-S4_vs_V8-S1 groups. The x-axis represents the different comparison groups, and the y-axis represents Biological Process (BP) terms. Significant BP terms were identified with a false discovery rate (FDR) ≤ 0.05. The size of the bubble dot indicates the number of genes, and the color scale indicates the values of $-\log_{10}$(FDR)

Supplementary Material 7: Table S1 Sequences of primers used for qRT–PCR. Table S2 Summary of sequencing data for 24 samples. Table S3 Pearson's correlation coefficients among the three replicates for each time point of VAO-8 and Baiyan 2. Table S4 The up- and downregulated DEGs identified among the four comparison groups. Table S5 Description of the differentially expressed genes in specific GO terms, KEGG pathways and metabolic processes. Table S6 Biological process enrichment analysis of the differentially expressed genes between VAO-8 and Baiyan 2 at different stages. Table S7 The full names of all genes noted in the flowering model.

Author contributions

CR, YY and LG designed the research. MZ, YJ, HD, XS, and HC performed the experiments. MZ, YJ and HD analysed the RNA-Seq data. MZ and YJ wrote the manuscript. CR, YY, LG, MZ, YJ, HD, XS, XL, CW, SB and JL revised the manuscript. All the authors contributed to the article and approved the submitted version.

Funding

This work was supported by the China Agriculture Research System of the Ministry of Finance and Ministry of Agriculture and Rural Affairs (CARS07 to C.R.), the Jilin Development and Reform Commission Project, Research and Application of Key Technologies in Oat Molecular Breeding (2021FGWCXNLJSSZ04), the Baicheng Science and Technology Development Program (202211) and Creation of new forage germplasm and breeding and promotion of new varieties (2024NSZC03).

Data availability

The datasets presented in this study can be found in online repositories. The names of the repositories and accession number(s) can be found below: NCBI accession number PRJNA1019673.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 14 October 2024 / Accepted: 29 April 2025
Published online: 10 May 2025

References

1. Kamal N, Tsardakas Renhuldt N, Bentzer J, Gundlach H, Haberer G, Juhász A, et al. The mosaic oat genome gives insights into a uniquely healthy cereal crop. Nature. 2022;606:113–9.
2. Matar S, Kumar A, Holtgräwe D, Weisshaar B. Melzer S.The transition to flowering in winter rapeseed during vernalization. Plant Cell Environ. 2021;44:506–18.
3. Garner WW, Allard HA. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Mon Weather Rev. 1920;48:415.
4. Jeong S, Clark SE. Photoperiod regulates flower meristem development in *Arabidopsis thaliana*. Genetics. 2005;169:907–15.
5. Wang S, Guo T, Shen Y, Wang Z, Kang J, Zhang J, et al. Overexpression of MtRAV3 enhances osmotic and salt tolerance and inhibits growth of Medicago truncatula. Plant Physiol Biochem. 2021;163:154–65.

6. Wingler A. Transitioning to the next phase: the role of sugar signaling throughout the plant life cycle. Plant Physiol. 2018;176:1075–84.
7. Kolbe A, Tiessen A, Schluepmann H, Paul M, Ulrich S, Geigenberger P. Trehalose 6-phosphate regulates starch synthesis via posttranslational redox activation of ADP-glucose pyrophosphorylase. Proc Natl Acad Sci. 2005;102:11118–23.
8. Zhao ML, Ni J, Chen MS, Xu ZF. Ectopic expression of Jatropha curcas TREHALOSE-6-PHOSPHATE PHOSPHATASE J causes late flowering and heterostylous phenotypes in arabidopsis but not in Jatropha. Int J Mol Sci. 2019;20:2165.
9. Seo PJ, Ryu J, Kang SK, Park CM. Modulation of sugar metabolism by an INDETERMINATE DOMAIN transcription factor contributes to photoperiodic flowering in Arabidopsis. Plant J. 2011;65:418–29.
10. Jiang J, Hu J, Tan R, Han Y, Li Z. Expression of IbVPE1 from sweet potato in Arabidopsis affects leaf development, flowering time and chlorophyll catabolism. BMC Plant Biol. 2019;19:184.
11. Fantini E, Sulli M, Zhang L, Aprea G, Jimenez-Gomez JM, Bendahmane A, et al. Pivotal roles of Cryptochromes 1a and 2 in tomato development and physiology. Plant Physiol. 2019;179:732–48.
12. Peng YL, Zou T, Li LM, Tang SW, Li Q, Zhang J, et al. Map-based cloning and functional analysis of ye1 in rice, which is involved in light-dependent chlorophyll biogenesis and photoperiodic flowering pathway. Int J Mol Sci. 2019;20:758.
13. Wang X, Zhou P, Huang R, Zhang J, Ouyang X. A daylength recognition model of photoperiodic flowering. Front. Plant Sci. 2021;12:778515.
14. Wang F, Li S, Kong F, Lin X, Lu S. Altered regulation of flowering expands growth ranges and maximizes yields in major crops. Front Plant Sci. 2023;14:1094411.
15. Wu Y, Liu J, Hu G, Xue H, Xu H, Zhao C, et al. Functional analysis of the green revolution gene Photoperiod-1 and its selection trends during bread wheat breeding. Front. Plant Sci. 2021;12:745411.
16. Jin M, Liu X, Jia W, Liu H, Li W, Peng Y, et al. ZmCOL3, a CCT gene represses flowering in maize by interfering with the circadian clock and activating expression of ZmCCT. J Integr Plant Biol. 2018;60:465–80.
17. Li C, Li YH, Li Y, Lu H, Hong H, Tian Y, et al. A Domestication-Associated gene GmPRR3b regulates the circadian clock and flowering time in soybean. Mol Plant. 2020;13:745–59.
18. Trevaskis B, Harris FAJ, Bovill WD, Rattey AR, Khoo KHP, Boden SA, et al. Advancing Understanding of oat phenology for crop adaptation. Front Plant Sci. 2022;13:955623.
19. Burrows VD. Breeding oats for food and feed: Conventional and new techniques and materials. In: Webster FH, editor. Oats: Chemistry and Technology. St. Paul: American Association of Cereal Chemists; 1986.
20. Sampson DR, Burrows VD. Influence of photoperiod, short day vernalization, and cold vernalization on days to heading in Avena species and cultivars. Can J Plant Sci. 1972;52:471–82.
21. Holland JB, Moser HS, O'Donoughue LS, Lee M. QTLs and epistasis associated with vernalization responses in oat. Crop Sci. 1997;37:1306–16.
22. Holland JB, Portyanko VA, Hoffman DL, Lee M. Genomic regions controlling vernalization and photoperiod responses in oat. Theor Appl Genet. 2002;105:113–26.
23. Klos KE, Huang YF, Bekele WA, Obert DE, Babiker E, Beattie AD, et al. Population genomics related to adaptation in elite oat germplasm. Plant Genome. 2016;9:1–12.
24. Locatelli AB, Federizzi LC, Milach SCK, Wight CP, Molnar SJ, Chapados JT, et al. Loci affecting flowering time in oat under shortday conditions. Genome. 2006;49:1528–38.
25. Siripoonwiwat W, O'Donoughue LS, Wesenberg D, Hoffman DL, Barbosa-Neto JF, Sorrells ME. Chromosomal regions associated with quantitative traits in oat. J Ag Genomics. 1996.
26. Zimmer CM, Ubert IP, Pacheco MT, Federizzi LC. Molecular and comparative mapping for heading date and plant height in oat. Euphytica. 2018;214:1–19.
27. An JH, Yu DY, Yang XH, Rong XP, Han B, Yang C, et al. Combined transcriptome sequencing reveals the photoperiod insensitivity mechanism of Oats. Plant Physiol Biochem. 2020;146:133–42.
28. Zhang M, Tian J, Liu H, Guo L, Wang C, Yuan Y. Screening of SSR molecular markers of oat photoperiod insensitivity gene. Mol Plant Breed (in Chinese). 2017;15:974–8.
29. Zhang M, Jiang Y, Dong H, Shan X, Tian J, Sun M, et al. Transcriptomic response for revealing the molecular mechanism of oat flowering under different photoperiods. Front Plant Sci. 2023;14:1279107.
30. Arnon DI. Copper enzymes in isolated chloroplasts.polyphenoloxidase in Beta Vulgaris. Plant Physiol. 1949;24(1):1–15.
31. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. Nat Methods. 2015;12:357–60.
32. Love MI, Huber W, Anders S. Moderated Estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.
33. Thimm O, Bläsing O, Gibon Y, Nagel A, Meyer S, Krüger P, et al. (MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J. 2004;37:914–39.
34. Zheng Y, Jiao C, Sun H, Rosli HG, Pombo MA, Zhang P, et al. iTAK: a program for genome-wide prediction and classification of plant transcription factors, transcriptional regulators, and protein kinases. Mol Plant. 2016;9:1667–70.
35. Kenneth JL, Thomas DS. Analysis of relative gene expression data using Real-Time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 2001;25:402–8.
36. Jarosová J, Kundu JK. Validation of reference genes as internal control for studying viral infections in cereals by quantitative real-time RT-PCR. BMC Plant Biol. 2010;10:146.
37. Deng W, Casao MC, Wang P, Sato K, Hayes PM, Finnegan EJ, et al. Direct links between the vernalization response and other key traits of cereal crops. Nat Commun. 2015;6:5882.
38. Weller JL, Vander Schoor JK, Perez-Wright EC, Hecht V, González AM, Capel C, et al. (Parallel origins of photoperiod adaptation following dual domestications of common bean. J Exp Bot. 2019;70(4):1209–19.
39. Sedivy EJ, Wu F, Hanzawa Y. Soybean domestication: the origin, genetic architecture and molecular bases. New Phytol. 2017;214:539–53.
40. Kong DY, Chen SJ, Zhou LG, Gao H, Luo LJ, Liu ZC. Research progress of photoperiod regulated genes on flowering time in rice. Hereditas. 2016;38:532–42.
41. Liu L, Zhang Y, Yu H. Florigen trafficking integrates photoperiod and temperature signals in Arabidopsis. J Integr Plant Biol. 2020;62:1385–98.
42. Xie S, Nie L, Zheng Y, Wang J, Zhao M, Zhu S, et al. Comparative proteomic analysis reveals that chlorophyll metabolism contributes to leaf color changes in Wucai (Brassica Campestris L.) responding to cold acclimation. J Proteome Res. 2019;18:2478–92.
43. Gawarecka K, Ahn JH. Isoprenoid-Derived metabolites and sugars in the regulation of flowering time: does day length matter?? Front Plant Sci. 2021;12:765995.
44. Cho LH, Yoon J, An G. The control of flowering time by environmental factors. Plant J. 2017;90:708–19.
45. Dong C, Wang Z, Qin L, Zhang C, Cao L, Li H, et al. Overexpression of Geranyl diphosphate synthase 1 (NnGGPPS1) from Nelumbo nucifera enhances carotenoid and chlorophyll content and biomass. Gene. 2023;881:147645.
46. Swiezewska E, Danikiewicz W. Polyisoprenoids: structure, biosynthesis and function. Prog Lipid Res. 2005;44:235–58.
47. Yang L, Xu M, Koo Y, He J, Poethig RS. Sugar promotes vegetative phase change in *Arabidopsis thaliana* by repressing the expression of MIR156A and MIR156C. Elife. 2013;2:e00260.
48. Stirbet A, Lazár D, Guo Y, Govindjee G. Photosynthesis: basics, history and modelling. Ann Bot. 2020;126:511–37.
49. Cho LH, Pasriga R, Yoon J, Jeon JS, An G. Roles of sugars in controlling flowering time. J Plant Biol. 2018;61:121–30.
50. Peng M, Gan F, Lin X, Yang R, Li S, Li W, et al. Overexpression of OsNF-YB4 leads to flowering early, improving photosynthesis and better grain yield in hybrid rice. Plant Sci. 2023;331:111661.
51. Bernier G, Havelange A, Houssa C, Petitjean A, Lejeune P. Physiological signals that induce flowering. Plant Cell. 1993;5:1147–55.
52. King RW, Hisamatsu T, Goldschmidt EE, Blundell C. The nature of floral signals in arabidopsis. I. Photosynthesis and a far-red photoresponse independently regulate flowering by increasing expression of FLOWERING LOCUS T (FT). J Exp Bot. 2008;59:3811–20.
53. Huang W, Chen Q, Zhu Y, Hu F, Zhang L, Ma Z, et al. Arabidopsis thylakoid formation 1 is a critical regulator for dynamics of PSII-LHCII complexes in leaf senescence and excess light. Mol Plant. 2013;6:1673–91.
54. Allen JF, Bennett J, Steinback KE, Arntzen CJ. Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. Nature. 1981;291:25–9.
55. Nilsson A, Stys D, Drakenberg T, Spangfort MD, Forsén S, Allen JF. Phosphorylation controls the three-dimensional structure of plant light harvesting complex II. J Biol Chem. 1997;272:18350–7.
56. Standfuss J, Kühlbrandt W. The three isoforms of the lightharvesting complex II: spectroscopic features, trimer formation, and functional roles. J Biol Chem. 2004;279:36884–91.
57. Pietrzykowska M, Suorsa M, Semchonok DA, Tikkanen M, Boekema EJ, Aro EM, et al. The light-harvesting chlorophyll A/b binding proteins Lhcb1 and

Lhcb2 play complementary roles during state transitions in Arabidopsis. Plant Cell. 2014;26:3646–60.
58. Belhassine F, Pallas B, Pierru-Bluy S, Martinez S, Fumey D, Costes E. A genotype-specific architectural and physiological profile is involved in the flowering regularity of Apple trees. Tree Physiol. 2022;42:2306–18.
59. Li L, Liu KH, Sheen J. Dynamic nutrient signaling networks in plants. Annu Rev Cell Dev Biol. 2021;37:341–67.
60. Ren S, Hu M, Wu Q, Wang L, Gu H, Chen Z, et al. Flowering time and physiological reaction of dendrobium nobile Lindl in response to TDZ application. Horticulturae. 2023;9:129.
61. Bezrutczyk M, Yang J, Eom JS, Prior M, Sosso D, Hartwig T, et al. Sugar flux and signaling in plant-microbe interactions. Plant J. 2018;93:675–85.
62. Wei XY, Nguyen STT, Collings DA, McCurdy DW. Sucrose regulates wall ingrowth deposition in phloem parenchyma transfer cells in Arabidopsis via affecting phloem loading activity. J Exp Bot. 2020;71(16):4690–702.
63. Yamada K, Osakabe Y. Sugar compartmentation as an environmental stress adaptation strategy in plants. Semin Cell Dev Biol. 2018;83:106–14.
64. Abolghasemi R, Haghighi M, Etemadi N, Wang S, Soorni A. Transcriptome architecture reveals genetic networks of bolting regulation in spinach. BMC Plant Biol. 2021;21:179.
65. Broeckx T, Hulsmans S, Rolland F. The plant energy sensor: evolutionary conservation and divergence of SnRK1 structure, regulation, and function. J Exp Bot. 2016;67:6215–52.
66. Figueroa CM, Lunn JE. A Tale of two sugars: Trehalose 6-Phosphate and sucrose. Plant Physiol. 2016;172:7–27.
67. Wahl V, Ponnu J, Schlereth A, Arrivault S, Langenecker T, Franke A, et al. Regulation of flowering by trehalose-6-phosphate signaling in Arabidopsis thaliana. Science. 2013;339:704–7.
68. Redondo-Gómez S, Mateos-Naranjo E, Moreno FJ. Physiological characterization of photosynthesis, Chloroplast ultrastructure, and nutrient content in bracts and rosette leaves from Glaucium flavum. Photosynthetica. 2010;48(4):488–93.
69. Du L, Qi S, Ma J, Xing L, Fan S, Zhang S, et al. Identification of TPS family members in Apple (Malus X domestica Borkh.) and the effect of sucrose sprays on TPS expression and floral induction. Plant Physiol Biochem. 2017;120:10–23.
70. Baillo EH, Kimotho RN, Zhang Z, Xu P. Transcription factors associated with abiotic and biotic stress tolerance and their potential for crops improvement. Genes (Basel). 2019;10:771.
71. Kim HJ, Chiang YH, Kieber JJ, Schaller GE. SCF(KMD) controls cytokinin signaling by regulating the degradation of type-B response regulators. Proc Natl Acad Sci. 2013;110:10028–33.
72. Bi C, Ma Y, Jiang SC, Mei C, Wang XF, Zhang DP. Arabidopsis translation initiation factors eIFiso4G1/2 link repression of mRNA cap-binding complex eIFiso4F assembly with RNA-binding protein SOAR1-mediated ABA signaling. New Phytol. 2019;223:1388–406.
73. Mei C, Jiang SC, Lu YF, Wu FQ, Yu YT, Liang S, et al. Arabidopsis pentatricopeptide repeat protein SOAR1 plays a critical role in abscisic acid signalling. J Exp Bot. 2014;65:5317–30.
74. Shim Y, Lim C, Seong G, Choi Y, Kang K, Paek NC. The AP2/ERF transcription factor LATE FLOWERING SEMI-DWARF suppresses long-day-dependent repression of flowering. Plant Cell Environ. 2022;45:2446–59.

Publisher's note