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Full Length Article

Identification of Photoperiod Responsive Genes in Wheat Cultivar Jing 841 by Transcriptome Sequencing

Yalan Feng[1,2†], Yongying Zhao[3†], Chao Ma[1,2], Jun Zhang[1,2], Ying Xiong[1,2] and Jun Yin[4,5,6*]
[1]*College of Agriculture, Henan University of Science and Technology, Luoyang 471023, Henan province, P.R. China*
[2]*Dry-Land Agricultural Engineering Technology Research Center in Henan, Luoyang 471023, Henan province, P.R. China*
[3]*Wheat Research Institute, Henan Academy of Agricultural Sciences, Zhengzhou 450002, Henan province, P.R. China*
[4]*National Engineering Research Centre for Wheat, Zhengzhou 450002, Henan province, P.R. China*
[5]*National Key Laboratory of Wheat and Maize Crop Science, Henan Agricultural University, Zhengzhou 450002, Henan province, P.R. China*
[6]*Collaborative Innovation Center of Henan Grain Crops, Zhengzhou 450002, Henan province, P.R. China*
[*]For correspondence: junyindr@163.com; fengyalan2004@163.com
[†]These authors contributed equally to this work

Abstract

The photoperiod pathway is crucial for flowering induction of plants, especially for most of annual plants. Photoperiod response is one of the essential developmental characteristics of wheat, which determines the flowering time of wheat and has an important impact on the ecological distribution, yield and quality of wheat. Therefore, the research on the molecular regulation mechanism of wheat photoperiod response is of great significance for the directional improvement of wheat varieties. The purpose of this research was to identify the photoperiod response genes in wheat Jing 841 by comparing the transcriptome data and the digital gene expression (DGE) profile of photoperiod sensitive wheat cultivar Jing 841 and photoperiod-insensitive wheat cultivar Liaochun 10 under different photoperiod conditions. For each cultivar, seedlings treated with different photoperiods were collected and sequenced by Solexa/Illumina sequencing, and a total of 89,702 unigenes were obtained. Some photoperiodic response genes in Jing 841 were verified through the quantitative reverse transcription polymerase chain reaction (qRT-PCR). According to the functional comment, a total of six categories with 92 DEGs were further identified which were specifically expressing in Jing 841. During a whole light/dark cycle (16 h/8 h and 6 h/18 h light/dark, respectively), three genes showed different expression patterns. The photoperiod response genes specifically expressed in Jing 841 may participate in the photoperiod regulation of wheat and exert an important role. The results provide critical information for the molecular mechanism of photoperiod regulation in wheat, which lays a significant foundation for further research on the development of wheat photoperiod.

Keywords: Wheat; Photoperiod; Solexa/Illumina sequencing; Functional annotation; Expression profiling

Introduction

Flowering time is a key ecologic and agronomic trait for cereal crops, which not only controls the adaptation of cereal crops to environments, but also determines the transition from vegetative growth to reproductive growth phases (Guitton *et al.,* 2018). Photoperiod response is a critical factor in many flowering pathways of plants, which enables the flowering time coincide with seasonal conditions, thus ensuring the reproductive success and crop yield (Ridge *et al.,* 2016). Photoperiod changes with season, and the relative lengths of the light and dark periods alternate every 24 hours (Flis *et al.,* 2016). According to the different photoperiod response, plants could be divided into three categories: long-day (LD), short-day (SD) and day-neutral plant.

As the model plant, there have been extensive studies on the molecular pathways and mechanism of LD-induced floral promotion in *Arabidopsis*. By integrating light signaling and circadian clock output, *Arabidopsis* could perceive and respond to seasonal changes in terms of different photoperiod (Greenup *et al.,* 2009). Clock output of the photoperiod-responsive flowering time is partly regulated *via GIGANTEA* (*GI*), which is mediated by the central clock oscillator comprised of *TIMING OF CAB EXPRESSION 1*(*TOC1*), *LATE ELONGATED HYPOCOTYL* (*LHY*), and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) (Yang *et al.,* 2014). Under LD, GI would activate the expression of *CONSTANS* (*CO*), whose increased CO protein levels might contribute to the activated expression of *FLOWERING LOCUS T* (*FT*) and the formation of "florigen", which moves from leaves to shoot apical meristems (SAM) where it induces floral transition (Valverde, 2011).

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Although the molecular pathways and mechanism of flowering time in response to photoperiod have been well identified in *Arabidopsis*, much less information is known in wheat. Wheat is an important crop whose growth and development is influenced by photoperiod (Wang *et al.,* 2016). As a photoperiodic LD plant, wheat flowers when day length becomes longer than a critical photoperiod (Yang *et al.,* 2014). Most of the naturally variegated wheat varieties with photoperiod sensitivity are related to the mutations in *PHOTOPERIOD 1*(*PPD1*), which is regarded as the crucial photoperiod-regulated gene in wheat (Beales *et al.,* 2007; Wilhelm *et al.,* 2009; Díaz *et al.,* 2012). These three alleles of *PPD1* in wheat are *PPD-A1*, *PPD-B1* and *PPD-D1*, located on the homologous chromosomes 2A, 2B and 2D, respectively (Law *et al.,* 1978; Börner *et al.,* 1993). Bread wheat carrying all three *PPD1* non-functional alleles, flowering later than wild species, which could verify the significant role of *PPD1* in flowering under LD (Shaw *et al.,* 2013). Photoperiod insensitivity in wheat largely depends on the mutations of *PPD-D1* and *PPD-A1*, since both of their promoter region with a larger deletion (Beales *et al.,* 2007; Wilhelm *et al.,* 2009; Nishida *et al.,* 2013), besides, the increased gene copy number of *PPD-B1*, which increases gene expression, also results in photoperiod insensitivity (Díaz *et al.,* 2012).

Wheat *VRN3* is the homolog of *Arabidopsis FT*, and it is the down-stream target of *PPD1* (Turner *et al.,* 2005), whose protein acts as a signaling molecule, transmitting the LD signal from the leaves to the SAM (Corbesier *et al.,* 2007). Based on global expression profiles, *FT* is the main target of *CO* in leaves, besides, *FT* is the major output of *CO* at the shoot apex (Schmid *et al.,* 2003; Wigge *et al.,* 2005). Additionally, the FT could interact with FD and FD PARALOGUE, which are the bZIP transcription factors, then activates the floral integrator genes *APETALA1* (*AP1*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*(*SOC1*), inducing an expressed cascade of down-stream genes, initiating flowering ultimately (Abe *et al.,* 2005).

Although the molecular network of photoperiod response in model plant *Arabidopsis* has been well characterized, the molecular pathway underpinning the photoperiodic flowering is known litter in cereal crops. The study on photoperiod response of cereal crops is still in its infancy. Thus, understanding the molecular regulation mechanism of photoperiod flowering in wheat is of great importance since it will promote the ecological breeding and adaptation of wheat, improve the varieties utilization efficiency, and even optimize the cultivation techniques. In the present study, we tried to identify the genes in response to photoperiod by comparing the transcriptome and DEG profiles between a photoperiod-sensitive wheat cultivar Jing 841 and a photoperiod-insensitive wheat cultivar Liaochun 10, which both underwent an artificial vernalization treatment to eliminate the effect of temperature on development. Furthermore, the expression patterns of three photoperiod responsive genes were investigated during the different photoperiod treatment to analyze their roles during this process.

Materials and Methods

Plant Materials and Growth Conditions

Liaochun 10, a photoperiod-insensitive wheat cultivar, widely grown in northeast China, and Jing 841, a photoperiod-sensitive wheat cultivar, mainly grown in northern China. Seeds of each cultivar were fully soaked and seeded into pots containing sterilized vermiculite after germination, and then were incubated at 4ºC for 30 days without light for vernalization treatment, in order to eliminate the influence of temperature on development. They were transferred into different growth chambers under controlled condition: temperature was 20ºC, the photosynthetically active radiation was 200 μmol·m^{-2}·s^{-1}, and 6 h/18 h and 16 h/8 h light/dark cycle, respectively. After three weeks, the seedlings were sampled at the three leaves stage with consistent growth. For each wheat cultivar, seedlings at the beginning of the light and dark were sampled in order to construct the cDNA libraries (6 h/16 h). Simultaneously, during the light/dark treatment cycle, the seedlings were sampled every 3 h (0 h, 3 h, 6 h, 9 h, 12 h, 15 h, 18 h, 21 h, and 24 h), then immediately frozen in liquid nitrogen and stored in a -80ºC ultra-low temperature freezer for subsequent RNA extraction and qRT-PCR.

Solexa/Illumina Sequencing, Data Assembly and Functional Annotation

The methods of total RNA extraction from leaves and cDNA reverse transcription can refer to the description of Ma *et al.* (2019). Four cDNA libraries were constructed by sampling seedlings at the beginning of the light and dark, respectively. Then, each cDNA library was sequenced, assembled and functionally annotated according to the high-throughput sequencing process of Beijing Genomics Institute (BGI).

Identification of Differentially Expressed Genes (DEGs)

Gene expression level was calculated by using the Cufflinks software (Mortazavi *et al.,* 2008). For each wheat cultivar, these genes differently expressed at the beginning of the light and dark were identified as DEGs according to certain criterion, *i.e*., false discovery rate (FDR) ≤ 0.001 and |log2 (fold change)| >1 (Audic and Claverie, 1997). Using the Benjamin–Hochberg (BH) method (Benjamini and Hochberg, 1995) to adjust the raw *P* value, as a result, the FDR value could be obtained.

Data Processing and Bioinformatics Analysis

The data from Illumina sequencing were coped with software developed by the BGI. Classification of gene

function was performed by Gene Ontology (GO), which is an international standardized system. Detailed analysis can refer to the method of Feng *et al.* (2016).

qRT-PCR Validation

12 unigenes were selected to confirm the expression level through qRT-PCR. The reaction system and procedures can refer to the description of Feng *et al.* (2015). The fold variation of selected gene was calculated by $2^{-\Delta\Delta}$Ct method (Livak and Schmittgen, 2001). Taking the β-actin as an internal control.

Expression Pattern Analysis of three Specific Photoperiod-related Genes in Jing841

Finally, three unigenes (CL4196.Contig1, CL14771.Contig1, and CL2039.Contig2) with significant expression differences were chosen to analyse their expression pattern during a whole light/dark cycle. Their expression levels were detected through qRT-PCR at different time.

Results

Transcriptome Sequencing, Assembly and Functional Annotation

Totally, 75,531,652 raw reads and 6,249,814,560 (6.24 Gb) nucleotides were obtained, with Q20 percentage (1% error rate) and GC percentage of 97.28 and 51.93%, respectively. 196,415 contigs, with an average length of 258 nt, were assembled from the raw short reads. Finally, 89,702 unigenes were obtained with an average length of 550 nt (Table 1).

Identification of DEG in the two Cultivars and Specific DEGs in Jing 841

In total, 2,496 significantly different unigenes were obtained in Jing 841 samples between the short light (J-SL) and long light (J-LL), including 622 up-regulated unigenes and 1,873 down-regulated unigenes (Fig. 1). Meanwhile, 2,436 significantly changed unigenes were identified after comparing short light (LC-SL) and long light (LC-LL) Liaochun 10 samples, including 1,599 up-regulated unigenes and 837 down-regulated unigenes (Fig. 1). After the further comparison, there were 265 common up-regulated genes (Fig. 2, C1) as well as 441 common down-regulated genes between the two cultivars (Fig. 2, C2).

The results of transcriptome and DGE profiles showed that the GEGs involved in photoperiod response in the photoperiod-sensitive variety Jing 841 were relative more than that in photoperiod-insensitive variety Liaochun 10. The up-regulated and down-regulated genes specifically expressed in Jing 841 were significantly lower or higher than that in Liaochun 10. Due to the insensitivity of Liaochun 10 to photoperiod, the length of light has little effect on its developmental process. Therefore, the genes specifically expressed in Jing 841 might be the main genes involved in photoperiod response, which were further classified by functional annotation and expression level screening. Then, a total of six categories with 92 DEGs were further identified which were specifically expressing in Jing 841 according to the functional comment, including 17 photoperiod and photosynthesis related genes, 13 genes involving in metabolism, 3 genes related to flower development, 1 photomorphogenesis gene, 37 genes encoding ribosomal protein, and 24 transcription factors (Table 2). All of them directly or indirectly take part in the photoperiodic response.

qRT-PCR Validation and Rhythm Expression Analysis

Among the 92 DEGs in Jing 841, which were divided into six categories, twelve genes were screened for qRT-PCR validation, including two photoperiod related genes, two photosynthesis related genes, two genes involving in metabolism, one gene related to flower development, one photomorphogenesis gene, two genes encoding ribosomal protein, and two transcription factors. Five of them were upregulated, while the rest were downregulated. These qRT-PCR results were consistent with the sequencing data (Fig. 3 and Table 2).

The rhythmical expression patterns of the three genes (CL4196.Contig1, CL14771. Contig1, and CL2039.Contig2) were further analysed during a whole light/dark cycle (16 h/8 h and 6 h/18 h light/dark, respectively). The results represented three diverse expression patterns (Fig. 4). In the initial stage of light period, the expression levels of the three genes were lower, but with the extension of illumination time the expression showed an increasing trend, and then gradually decreased to the initial level during dark period. The first one, CL4196. Contig1, displayed a rising trend during the light time, and a declined trend during the dark. The expression peaks of CL4196.Contig1 both appeared during the light period at 6h (J-LL/J-SL) (Fig. 4, A1, A2). The expression patterns of CL14771.Contig1 were similar with those of CL4196.Contig1, but the expression peaks appeared during the dark period at 21 h and 12 h (J-LL/J-SL) (Fig. 4, B1, B2). The CL2039.Contig2 represented a fluctuating change with two peaks during the whole light/dark cycle (Fig. 4, C1, C2). The peaks of CL2039. Contig2 in long-light treatment appeared during the light period at 6h and 12h, respectively, and the expression of the second peak was almost twice that of the first one (Fig. 4, C1). However, the expression peaks of CL2039.Contig2 in short-light treatment appeared at 6 h and 12 h during the dark phase, respectively, and the expression levels were similar (Fig. 4, C2).

Discussion

Wheat is a typical long-day plant and the photoperiod is a critical factor regulating its development and flowering time

(Wang and Engel, 1998). Long light could promote the growth and development process of wheat. In addition, the photoperiodic response of different wheat varieties are closely related to their vernalization characteristics (González *et al.,* 2002). For winter varieties, who are short light sensitive before the vernalization requirements are met, that is, short light promotes development. After the vernalization is completed, they are characterized as long light sensitivity (Distelfeld *et al.,* 2009). Therefore, in this study, each variety was vernalized before photoperiod treatment to cater for the vernalization requirements and eliminate the effect of temperature on photoperiod response genes.

The early light induced proteins (ELIPs) are the part of the pigment-binding light-harvesting complexes family (Tzvetkova-Chevolleau *et al.,* 2007). The *ELIP* gene was first discovered in etiolated pea seedlings, which transiently expressed during the seedlings greening and disappeared before the chloroplast development was completed (Meyer and Kloppstech, 1984; Kolanus *et al.,* 1987). Thus, it was speculated that, in the late stage of photosystem assembly during plastid development, ELIP might be replaced by light-harvesting chlorophyll a/b binding protein (LHC) (Grimm *et al.,* 1989; Adamska, 2001; Tzvetkova-Chevolleau *et al.,* 2007). Increasing evidences indicated that the rising expression of *ELIP* might in response to a number of stresses, including cold (Montané *et al.,* 1997), desiccation (Harari-Steinberg *et al.,* 2001), and senescence (Bhalerao *et al.,* 2003). It was reported that ELIPs played the photoprotective effect especially under high light stress. Transgenic *Arabidopsis* overexpressing *BrELIP* (*ELIP* from *Brassica rapa*) showed enhanced tolerance to the high light stress (Lee *et al.,* 2006). However, the *ELIP* mRNA level only increased transiently under these conditions, and the expression returned to normal after these stresses disappeared (Bruno and Wetzel, 2004). These former researches indicated that the expression changes of *ELIP* were closely related to a variety of abiotic stresses, but this does not accord with the results obtained in this study. The expression of CL4196.Contig1(encoding ELIP) was higher in J-LL compared with the J-SL (Fig. 4, A1, A2), although the growth conditions were normal and suitable, and there was no abiotic stress. Hence, the difference in expression might be largely due to the length of illumination. Combined with the above results, in addition to responding to various abiotic stresses, *ELIP* is also very likely to participate in the photoperiodic response of wheat, thereby promoting the growth and development process.

Photosystem II (PSII) is a giant multi-subunit thylakoid membrane protein complex which could catalyze water splitting as well as oxygen evolution and transfer electrons to plastoquinone (PQ). This is also the first step in photosynthesis, which would convert the light energy into chemical energy and produces oxygen. Therefore, photosynthetic hydrolysis is one of the most essential biochemical reactions on the planet (Barber, 2005, 2006). It is clear that the PSII population has the potential to affect

Table 1: Statistics of RNA-seq data

	Total number	Total length (nt)	Median length (nt)
Raw reads	75,531,652		
Clean reads	69,442,384		
Contig	196,415	50,618,453	258
Unigene	89,702	49,304,768	550

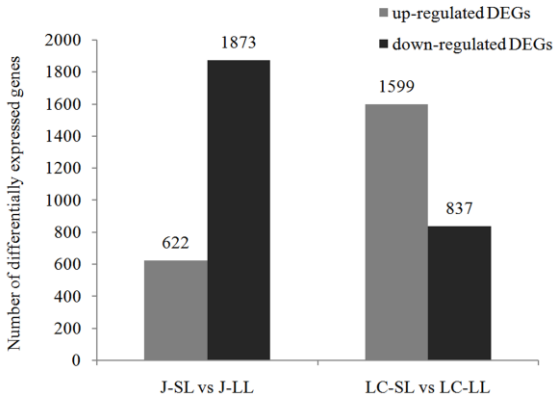

Fig. 1: Number of DEGs in J-SL *vs*. J-LL comparison and LC-SL *vs*. LC-LL comparison. J-SL, Jing 841 in short light; J-LL, Jing 841 in long light; LC-SL, Liaochun 10 in short light; LC-LL, Liaochun 10 in long light.

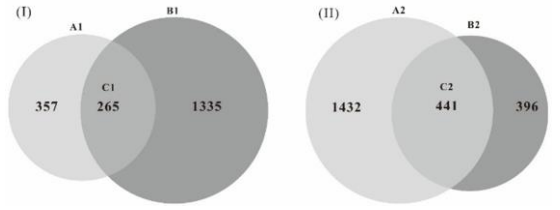

Fig. 2: Venn diagrams showing the number of DEGs exposed to different photoperiod. (**I**) number of up-regulated genes in A1 (J-SL versus J-LL) and B1 (LC-SL versus LC-LL); (**II**) number of down-regulated genes in A2 (J-SL versus J-LL) and B2 (LC-SL versus LC-LL). C1, the shared genes of A1 and B1; C2, the shared genes of A2 and B2. J-SL, Jing 841 in short light; J-LL, Jing 841 in long light; LC-SL, Liaochun 10 in short light; LC-LL, Liaochun 10 in long light

leaf photosynthesis. Previous studies have shown that there were associations between leaf photosynthetic rate grain yield progress, and any increase in wheat grain yield required enhanced photosynthetic efficiency (Parry *et al.,* 2011). Yin *et al.* (2015) reported that a certain extent light intensity and photoperiod would put a significant influence on duckweed growth. Prolonging the photoperiod (24/0 h light/dark), even though under the normal light intensity (20–200 μmol·m^{-2}·s^{-1}), could lead to a remarkable increase of biomass. Although the photoperiod was extended, at the light density of 400 μmol·m^{-2}·s^{-1}, the yield was still lowered (Yin *et al.,* 2015). In this research, some unigenes involved in PSII specifically expressed in photoperiod-sensitive Jing 841, and most of the expression levels were higher in short-light treatment.

Table 2: Some genes specifically expressed in Jing 841

Functional description	Gene	log2 Ratio(J-LL/J-SL)	Remark
Photoperiod and photosynthesis related genes (17)	Unigene11066	-1.08425	photoperiod；U2AF small subunit
	CL4196.Contig1[a,b]	4.450252	early light-inducible protein
	Unigene28920	1.761586	early light-inducible protein
	CL2829.Contig3	1.293389	plastocyanin
	Unigene78	1.263764	Ferredoxin
	Unigene28148	1.066237	Ferredoxin
	CL5193.Contig2	-1.56429	light-induced protein 1-like
	CL893.Contig7[a]	-3.21653	high light protein
	Unigene23764	-1.14253	phytochrome
	CL13963.Contig1[a]	1.473804	photosystem II
	CL13963.Contig2	1.125219	photosystem II
	CL9007.Contig2	1.045603	light harvesting
	CL14771.Contig1[a,b]	-12.7379	Photosystem II 10 kDa polypeptide
	CL6652.Contig2	-1.7856	Photosystem II
	CL1875.Contig6	-1.60703	photosystem I
	CL11051.Contig2	-1.19656	Photosystem II
	Unigene13053	-1.97954	light-harvesting chlorophyll a/b binding protein
Metabolism related (13)	Unigene47967[a]	10.09408	cytochrome P450
	CL12437.Contig4	3.483628	cytochrome P450
	CL9331.Contig1	1.023034	cytochrome P450
	Unigene52104	-9.3837	cytochrome P450
	Unigene14288	-8.2384	cytochrome P450
	Unigene5027[a]	-3.74193	cytochrome P450
	CL9331.Contig3	-2.43588	cytochrome P450
	CL7089.Contig1	-2.11808	cytochrome P450
	Unigene46027	-1.46022	cytochrome P450
	Unigene14270	-1.14301	Cytochrome c
Flower development (3)	CL12024.Contig1	-1.89465	phyllome development
	CL5796.Contig1[a]	-2.10117	flower development;histidine kinase2
	Unigene6344	-1.38167	flower development
Photomorphogenesis gene (1)	CL807.Contig2[a]	-1.07787	photomorphogenesis
Ribosomal protein (37)	Unigene19589[a]	1.250613	ribosomal protein L12
	CL12670.Contig1[a]	-3.291	ribosomal protein S15
	Unigene21054	-2.57393	ribosomal protein S9
	CL1482.Contig1	-2.44748	ribosomal protein S15a
	CL11062.Contig1	-2.03252	ribosomal Pr 117
	Unigene23595	-1.90202	acidic ribosomal protein P2
	CL4779.Contig1	-1.87488	ribosomal protein L6
	CL5472.Contig1	-1.79866	ribosomal protein L7
	CL1482.Contig2	-1.7719	ribosomal protein S15a
	CL12670.Contig2	-1.76193	ribosomal protein S15
	CL12798.Contig1	-1.65099	ribosomal protein L36
	CL6327.Contig2	-1.64489	ribosomal protein L11
	CL478.Contig1	-1.63585	ribosomal protein
	CL5472.Contig2	-1.61111	ribosomal protein L7
	CL273.Contig1	-1.60984	ribosomal protein S6
	Unigene9070	-1.54452	ribosomal protein L18A
	Unigene7234	-1.54414	ribosomal protein L37
	Unigene2089	-1.5097	ribosomal subunit 8E protein
	Unigene21154	-1.47885	ribosomal protein S3
	CL126.Contig2	-1.45198	ribosomal protein L3-A2-II
	Unigene26397	-1.4058	ribosomal protein L24
	CL11319.Contig1	-1.40237	ribosomal protein S26
	Unigene15591	-1.3947	ribosomal protein L21
	CL634.Contig3	-1.38033	ribosomal protein S7
	Unigene796	-1.36532	ribosomal protein L21
	Unigene12763	-1.35796	ribosomal protein L17-2
	CL11062.Contig2	-1.34028	ribosomal Pr 117
	CL9501.Contig3	-1.27966	ribosomal L14 protein
	Unigene18312	-1.24473	ribosomal protein L31
	Unigene23641	-1.17476	ribosomal protein S12
	Unigene12785	-1.14053	ribosomal protein P1
	CL11319.Contig2	-1.12924	ribosomal protein S26
	Unigene6011	-1.11905	ribosomal protein L39

Table 2: Continued

Table 2: Continued

	Unigene22410	-1.09948	acidic ribosomal protein P40
	Unigene15837	-1.08546	ribosomal protein L2
	Unigene21675	-1.02817	ribosomal protein S7
Transcription factors (24)	Unigene11576	8.696968	WRKY transcription factor 3
	CL5172.Contig2 [a]	4.416086	WRKY45 transcription factor
	Unigene18167	2.551104	bZIP protein
	CL13425.Contig1	1.605473	nucleic acid binding transcription factor
	Unigene47538	1.384559	ethylene-responsive factor-like transcription factor
	Unigene23772	1.257711	MYB transcription factor-like
	Unigene10168	1.195632	nucleic acid binding transcription factor
	CL1656.Contig2	-9.12928	WRKY transcription factor 23
	CL653.Contig1	-8.12928	WRKY19 transcription factor
	CL2039.Contig2 [a,b]	-8.12928	VRN-A1
	Unigene24871	-3.21653	WRKY27 transcription factor
	CL5747.Contig4	-2.96963	transcription factor X1
	CL4351.Contig2	-1.78835	nucleic acid binding transcription factor
	Unigene25192	-1.68684	nucleic acid binding transcription factor
	Unigene26461	-1.63373	nucleic acid binding transcription factor
	Unigene25444	-1.60322	NAC-domain transcription factor
	Unigene16592	-1.54024	nucleic acid binding transcription factor
	Unigene12869	-1.49207	homeodomain transcription factor
	CL6896.Contig1	-1.38558	transcription factor AP2D8
	CL8312.Contig1	-1.34987	nucleic acid binding transcription factor
	Unigene6336	-1.34987	nucleic acid binding transcription factor
	CL5727.Contig2	-1.30904	nucleic acid binding transcription factor
	Unigene22009	-1.13132	bZip type transcription factor
	Unigene9999	-1.05795	nucleic acid binding transcription factor

[a] genes validated by qRT-PCR
[b] genes used to analyse the expression pattern under different photoperiod

Taking the CL14771. Contig1(encoding Photosystem II 10 kDa polypeptide) as an example, the expression peaks both appeared in the dark phase regardless of different photoperiod treatments, and the expression level in short light conditions was noticeably higher than that in long light. It is speculated that in order to maintain normal growth, plants need to activate the high expression of PSII to enhance the dark reaction and compensate for the lack of organic substance synthesis caused by insufficient photosynthesis time under short-light conditions.

Ribosome proteins are the major components of ribosomal structures and important components of protein synthesis machinery in plants (Zhang *et al.,* 2016). They have different functions in plant growth and development, like participating in DNA repair (Wool, 1996), cell differentiation (Akanuma *et al.,* 2012), developmental control (Horiguchi *et al.,* 2011) and cold tolerance (Zhang *et al.,* 2016). Furthermore, the expression of ribosomal protein-related genes in plants could respond to different photoperiods. Some genes related to protein synthesis and degradation in chrysanthemum, such as 60S ribosomal protein L38, especially expressed under short-day conditions (Ren *et al.,* 2013). Silencing of the gene encoding 40S ribosomal protein S4 in soybean resulted in stunted plants and extreme flowering time delay. The gene was up-regulated under short-day conditions, indicating that it is involved in the photoperiod flowering pathway (Sha *et al.,* 2014). In the present study, among the six categories of photoperiod-responsive genes specifically expressed in Jing 841, ribosomal protein-related genes had the largest number, and most of them highly expressed under short-light, which was consistent with previous studies. The results indicated that these ribosomal proteins highly possibly involved in the photoperiod regulation and development in wheat. It's worth noting that up-regulation or down-regulation of ribosomal protein expression would be an important clue to regulation of flowering and photoperiod responses, however, current researches known little about the molecular function and regulatory mechanisms for each ribosomal protein, so further research is needed in the future.

Plant transcription factors (TFs) regulate transcription initiation through interaction of specific sequences with DNA binding domains at one or more stages of transcription, which is essential for plant development and environmental response (Lehti-Shiu *et al.,* 2017). Because TFs are involved in the transcription of all functional genes, changes of their expression would greatly affect the expression of downstream genes, which in turn would further influence morphological characteristics and developmental processes (Soltis *et al.,* 2002; Benfey, 2012). The WRKY family is widely distributed in plants (Rushton *et al.,* 2010), which has been shown to be involved in a great range of physiological as well as biochemical processes and play key roles, such as seed development, leaf senescence, plant growth, and stress response (drought, salt, heat, cold) (Chen *et al.,* 2012; Banerjee and Roychoudhury, 2015). The rice plants overexpressing the *OsWRKY11* showed enhanced tolerance to heat and

drought (Wu *et al.,* 2009).

Transgenic *Arabidopsis* plants overexpressing the *GmWRKY34* represented better tolerance to salt stress (Zhou *et al.,* 2015). In addition, the *Arabidopsis* mutants *wrky2* and *wrky34* cause male sterility, defects of pollen development and germination growth (Lei *et al.,* 2017). Under osmotic/salt stress conditions, *Arabidopsis* plants overexpressing *WRKY46* could promote the development of lateral root (Ding *et al.,* 2015). Among the known functional WRKY TFs, most are negative regulators, and only a few are positive regulators (Kim *et al.,* 2008; Xing *et al.,* 2008). In *Arabidopsis*, *AtWRKY38* and *AtWRKY62* encode two structurally similar WRKY TFs which negatively regulate the resistance of pathogen *Pseudomonas syringae*. The disease resistance was improved in the plants with single mutant of *Atwrky38* or *Atwrky62*, as well as the double mutants *Atwrky38*/*Atwrky62*, while the overexpression of *AtWRKY38* or *AtWRKY62* would reduce the disease resistance (Kim *et al.,* 2008). In transgenic plants overexpressing *AtWRKY48*, their susceptibility is enhanced, whereas in *Atwrky48* mutant, the resistance to *Pseudomonas syringae* is enhanced (Xing *et al.,* 2008). These results illustrated that WRKY48 has a negative regulatory impact on the resistance of *Pseudomonas syringae*. Although the function of some WRKYs has been confirmed, most WRKYs, especially those in non-model plants like wheat, are far from being functionally elucidated. Those WRKYs that are specifically expressed in response to photoperiod in Jing 841, some are up-regulated expression in short light, while others are opposite, suggesting that they may participate in photoperiod response through different pathways, however, their specific functions still need in-depth research. Another TF worth concerning is *VRN-A1* (encoding MADS-box TF), which is a known wheat vernalization gene that plays a central role in the flowering pathways of wheat (Danyluk *et al.,* 2003; Trevaskis *et al.,* 2003; Yan *et al.,* 2003). *VRN1* could promote the transcription of *VRN3* in wheat, and its deletion resulted in the down-regulation of *VRN3* (Shimada *et al.,* 2009). The wheat *VRN3* (*TaFT*) is homologous to *FT* gene in *Arabidopsis*, which is known to encode florigen that could move from the phloem to SAM and promote flowering (Corbesier *et al.,* 2007). In wheat, barley and other temperate cereals, *FT* could integrate the signals from the photoperiod pathway *via* interacting with photoperiod pathway-associated genes *PPD1* and *CO* (Turner *et al.,* 2005) as well as vernalization pathway-associated gene *VRN2* (Yan *et al.,* 2006). According to these previous results, *TaFT* was considered to be the intermediate step during the inhibition of *VRN2* by *VRN1* in the flowering model. Up-regulated expression of *FT* requires LD (Turck *et al.,* 2008). When those photoperiod-sensitive wheat or barley cultivars growing under SD (Dubcovsky *et al.,* 2006; Hemming *et al.,* 2008), or when those plants are in the dark phase of the long photoperiod, the transcription levels of *VRN2* and *FT* are simultaneously down-regulated (Shimada *et al.,* 2009). Here, the expression level of *VRN-A1* in J-SL was higher than that in J-LL, however, the decreased expression of *FT* in short light suggesting that there might be other downstream genes regulated by *VRN-A1* under short light, but this still needs further research to confirm.

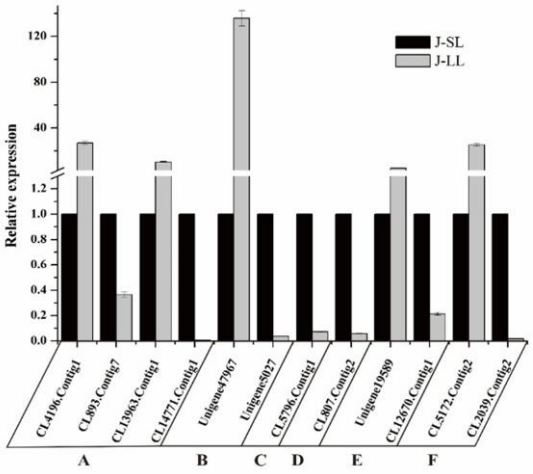

Fig. 3: Relative expression of 12 selected DEGs in Jing 841 at the beginning of the dark (6 h/16 h) by qRT-PCR. **A**, photoperiod and photosynthesis related genes; **B**, metabolism related genes; **C**, flower development-related genes; **D**, photomorphogenesis gene; **E**, ribosomal protein related genes; **F**, transcription factors. J-SL, Jing 841 in short light; J-LL, Jing 841 in long light

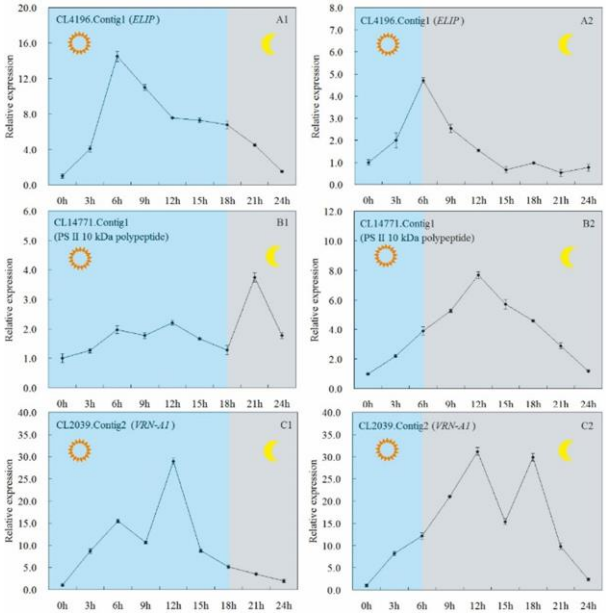

Fig. 4: Expression of 3 selected DEGs in the Jing 841 during a whole light/dark cycle (16 h/8 h and 6 h/18 h light/dark, respectively) by qRT-PCR. **A1**, **A2**: CL4196.Contig1 (photoperiod and photosynthesis related gene, *ELIP*); **B1**, **B2**: CL14771.Contig1 (photoperiod and photosynthesis related gene, PS II 10 kDa polypeptide); **C1**, **C2**: CL2039.Contig2 (transcription factor, VRN-A1).

Conclusion

Through high-throughput sequencing, a photoperiod transcriptome library of wheat was constructed, obtaining a total of 6.24 Gb transcriptome data and 89,702 unigene sequences. By analyzing the DGE profiles of photoperiod-sensitive variety Jing 841 and photoperiod-insensitive variety Liaochun 10 under different photoperiod conditions, the number of DEGs in Jing 841 and Liaochun 10 reached 2,496 and 2,346, respectively. According to the functional annotation, six categories with 92 DEGs were further identified which were specifically expressed in Jing 841, including 17 photoperiod and photosynthesis related genes, 13 genes involving in metabolism, 3 genes related to flower development, 1 photomorphogenesis gene, 37 genes encoding ribosomal protein, and 24 transcription factors. The results provide important information for the molecular mechanism of wheat photoperiod regulation and lay an essential foundation for further research about photoperiod development in wheat.

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