

Detection and investigation of genes with circadian expression pattern in common wheat

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MOTIVATION AND AIM

Plant circadian rhythms coordinate such physiological processes as growth, transition to flowering, photosynthesis, response to stress, metabolism and phytohormone signal transduction under the daily and seasonal changes in environmental conditions. Such concurrence has a positive effect on plant growth, development and their adaptability. Genes regulated by circadian rhythms include genes for CO_2 assimilation, starch accumulation/degradation in leaves, and storing nutrients (lipids and fatty acids) in seeds. Thus, a large part of the plant transcriptome is controlled by circadian rhythms, which play an important role in the daily regulation of plant physiological processes.

Transcriptome analyses of the circadian mechanisms and its influence on various metabolic processes were performed for such plants as Arabidopsis, Chinese cabbage and soybeans. The genes of the central oscillator are conservative and their interactions are very similar in different plant species, nevertheless, there are species-specific differences. In addition, in most plant species, the genes of metabolic pathways affected by circadian rhythms are investigated very poorly.

Common wheat is an allopolyploid (genome AABBDD), so the presence of homoeologous genes in the genome can greatly affect the functioning and regulation of circadian rhythms. Literature analysis and the search in the databases did not find studies exploring the daily expression of the wheat transcriptome, information about the daily expression of the genes and their interaction is practically absent.



MATERIALS & METHODS

- Material: Chinese Spring (CS)
- Growth conditions: climatic chamber, short day (9 hours)
- **Growth stage:** GS13 (3 leaves unfolded), 21 days after germination
- **Features:** leaf apices were harvested in four time points during the 24-hour period (0, 3, 9, 16 hours since the light was turned on)



- 12 libraries were produced using TruSeq Stranded mRNA LT Sample Prep Kit (Illumina); Sequencing was performed using a 75-bp single-end reads on a Illumina NextSeq 550
- Number of sequences was about 35.292.368 (from 25.176.261 to 45.139.550) reads for CS and 32.552.993 (from 21771050 to 55147200) reads for CS-5Bdic
- Average read quality (Quality Score) was 34.5

- After preprocessing and quality control using the FASTX-toolkit utility the obtained reads were mapped onto the wheat reference transcriptome using Bowtie software.
- Kallisto v0.42.3 software on default parameters was used to quantifying abundances of transcripts and the trimmed mean of M-values (TMM)normalized fragments per kb of Transcripts Per Million (TPM) reads mapped for each transcript.
- To identify rhythmically expressed genes, we applied the JTK-CYCLE circadian transcript analysis software.

RESULTS

- 18 748 genes with circadian expression pattern (p-value < 0,05)
- 2704 genes with circadian expression pattern (BH q-value < 0,05)
- 7 expression modules



RESULTS

Gene enrichment analysis revealed metabolic pathways with diurnal expression:

- Cellular amino acid biosynthesis
- Photosynthesis
- Light reaction
- Generation of metabolites and energy
- Response to abiotic stimulus
- Nitrogen compound metabolism
- Multicellular organismal movement
- Carbohydrate metabolism

small molect metabolic prod	ule oxio pess	dation-reduction process	small molecule biosynthetic process	organic substance biosynthetic process	photosynthesis	metabolism	response to abiotic stimu response to abiotic stimul	us 1,6-k	sphate olic ss fructose fructose oisphosphate etabolism
cellular biosynthetic process	organonitroger compound biosynthetic process	n aromatic compound biosynthetic process F	lipid aromati amino ac process biosynthe process	c sulfur compound biosynthetic process cellular nitrogen			response to temperature stimulus response to high light intensity	elular ponse mulus process to mulus	te process fuctore 6-phosphate metabolic process
cellular amino acid biosynthetic process	cellular ar organic acid metabolic process	cellular compound biosynthetic n norcess	tthesise metabolic process trapyrrole hetabolic process process	addie compound metabolic process rcle starch etic metabolic s process		generation of precursor metabolites and energy	regulation of oregulation of biological quality	carbohydrate derivative mgtakoljsm	biosynthetic biosynthesis process
ether metabolic process	compound metabolic process organic cyclic	dicarboxylic acid biosynthetic process aspartate family amino are	ne-containing acid apo compound metabolic process amine metabolic biosynthetic no process amine metabolic bio biosynthetic	carotenoid netabolic process sucrose osynthetic tamino acid process arimo acid family	biological_process		homeostasis metabolic process m	compound etabolic proces	s
glycerol ether metabolic process	biosynthetic process fructose metabolic	acid metabolic process pyruvate metabolic of bit	response process isoprenoid is metabolic bi process metabolic bi process proces	process metabolic process soprenoid carbohydrate osynthetic catabolic process process licarboxytic regulation of isoprenoid metabolic		cellular process	complex assembly chloroplast organization	nitrogen	rhythmic process
	process	nic substance	primary metabolic	cellular aromatic compound metabolic	photosynthesis photosynthesis, light reaction	one-carbon urea		ompound etabolism	biological regulation sulfur
cellular metabolic p	rocess cell meta	ular metabolism abolic process	process phosphorus metabolic proce	heterocycle metabolic process	light reaction	transmembrane transport one-carbon compound transport transport fluid transport fluid transport	er metabolism res	linkage sponse to stimulus	rotein folding

REVIGO Gene Ontology treemap

g:Profiler + REVIGO



SUMMARY

- System analysis of the daily wheat transcriptome let us to identify genes expressed by the circadian type, and metabolic pathways under their control.
- It was shown, that significant part of the transcriptome is under the control of daily rhythms, and the expression of these genes can vary greatly depending on time.
- We detected seven prominent expression patterns, characterized by peaks in different time points, and described genes underlying these patterns.
- We analyzed enrichment of gene ontology terms of different patterns and described major metabolic pathways in every group.

The study was funded by RFBR, project number 20-316-80003