Transcriptomic analysis of flowering-time genes in wild and cultivated chickpea

Gretsova M.[1], Surkova S.[1], Kanapin A.[1], Samsonova A.[1], Scherbakov A.[2], Logacheva M.[3], Nuzhdin S.[1,4] and Samsonova M.[1]

[1] Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia
[2] All-Russia research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia
[3] Skolkovo Institute of Science and Technology, Moscow, Russia
***[4]* University of Southern California, Los Angeles, USA**

Objectives

The cultivated chickpea (*Cicer arietinum* L.) is an important grain legume and a good source of plant-based protein.
During domestication, chickpea was transformed from an autumn-sown crop to a spring-sown crop. Wild species, including *C. reticulatum* and *C. echinospermum,* possess a strong vernalization requirement.
Transition from vegetative to reproductive state plays a key role for the performance of crop species. In this study, we acquired high-throughput transcriptomic sequencing data to analyze changes of gene expression during flowering transition in cultivated and wild genotypes.

Materials and methods

The dataset

144 sequenced chickpea samples.
Tissue types: leaves before and after flowering initiation and the flowering buds at the initial stages of their formation (FB1 and FB2).
Genotypes: wild *Cicer*: 1) *C. reticulatum*, 2) *C. echinospermum;*
Cultivated chickpea: 1) elite early flowering cultivar ICCV96029 ("mutant") containing mutation in the photoperiod / circadian clock gene *ELF3,* 2) other *C. arietinum genotypes.*
Conditions: long day with and without vernalization.
Gene networks involved in flowering-time control are well studied in *Arabidopsis* and summarized in the FLOR-ID database (Bouche et al., 2016).
We identified 274 *C. arietinum* coding sequences highly homologous to *A. thaliana* flowering time genes.

Results

1. An overview of the flowering time-related gene expression data

We have built a heatmap of the expression levels of 274 estimated flowering time genes (columns) in 144 sequenced chickpea samples (rows). The normalized expression profiles were clustered according to genotype, tissue type and condition by the complete linkage method, and the Euclidean metric was taken as a measure of distance (Fig. 1).
The most clear difference in gene expression is detected between the sample clusters corresponding to a tissue type.

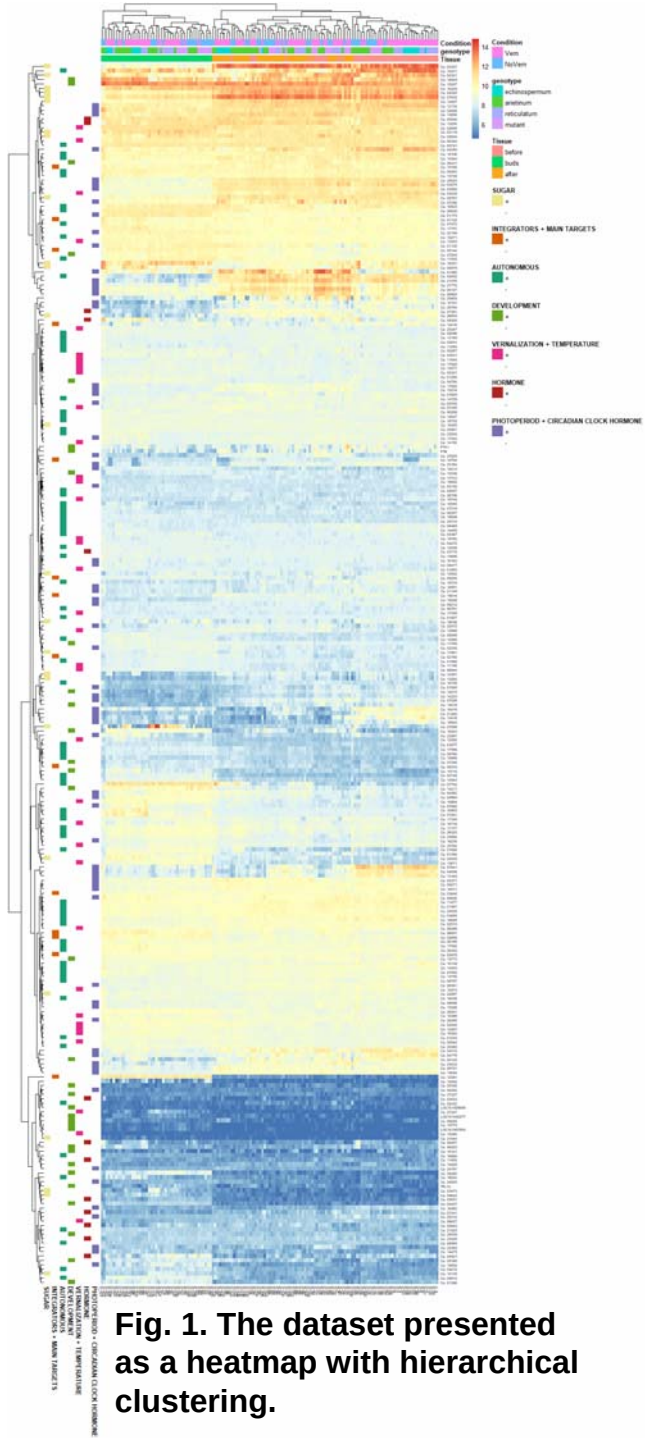

Fig. 1. The dataset presented as a heatmap with hierarchical clustering.

2. Tissue-specific expression of the flowering time genes

The maximum difference is observed between leaves at any stage and the buds. We detected 66 differentially expressed genes (DEGs) between the leaves before and after flowering initiation, 128 DEGs between the leaves after flowering initiation and buds, and 147 DEGs between leaves before flowering initiation and buds. This supports the existence of different transcriptional programs in the vegetative and flower chickpea tissues (Singh et al, 2013).

CA_ID	name	arietinum NoVern	arietinum Vern	mutant NoVern	mutant Vern	reticulatum Vern	echinospermum Vern
Ca_01175	HAP5C,NF-YC9	buds	buds	buds	buds	buds	buds
Ca_01365	LHY,LHY1	before	before	-	before	-	buds
Ca_01386	LFY,LFY3	buds	buds	buds	buds	buds	buds
Ca_02427	FTIP1	buds	buds	buds	buds	buds	buds
Ca_02452	ATGLK1,GLK1,GPRI1	before	before	before	before	before	before
Ca_03275	ATCOL5,BBX6,COL5	before	before	before	before	before	before
Ca_03357	ADG1,APS1	before	before	before	before	before	before
Ca_03475	ATSUS4,SUS4	buds	buds	-	buds	buds	buds
Ca_22383	CDF2	buds	-	buds	before	buds	buds
Ca_26853	ATTPS1,TPS1	before	before	before	before	before	before

Table 1. An example of differential gene expression between tissue types.

Our results showed that each individual DEG is up or down-regulated in the specific tissue, and the sign of regulation does not change with respect to genotype and condition. The regulation sign changed only in two genes from photoperiod/circadian clock pathway: *LHY* and *CDF2*. Thus, for these genes the differences in gene expression between tissues significantly depend on a genotype and condition.

3. Differential gene expression between chickpea genotypes

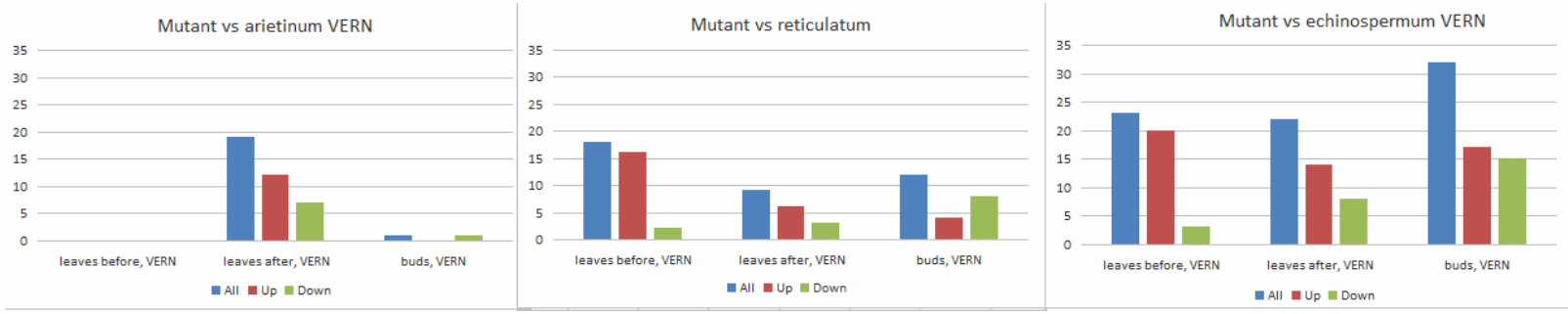

Fig. 2. Bar plots showing total number of DEGs between mutant and *C. arietinum*, *C. reticulatum* and *C. echinospermum* (blue) in each tissue type. Upregulated genes are shown in red and downregulated in green.

We compared gene expression between mutant and the other *C. arietinum* genotypes with a special emphasis on the photoperiod/circadian clock genes. Presumably, the mutation in the chickpea homologue *of Arabidopsis ELF3* gene does not lead to the severe alterations of the circadian clock gene function in the mutant genotype, nevertheless somehow providing early induction of the *FLOWERING LOCUS T (FT)* genes (Ridge et al., 2017).

The number of DEGs between mutant and *C. arietinum* depends on a tissue type. The differential expression is mostly evident in the leaves after flowering initiation (Fig. 2). In this tissue type, we detect six DEGs from the photoperiod/circadian clock pathway: *CPK6; LHY; CDF2; MYR2; ELF4; APL (WDY).* Interestingly, all these genes are upregulated in mutant.

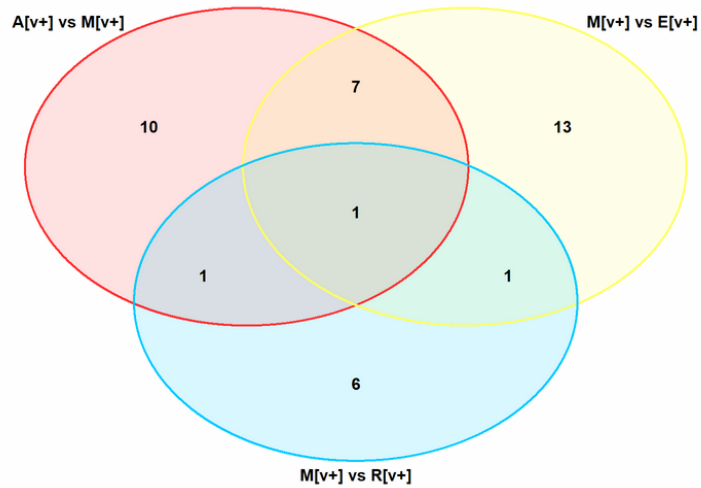

Fig. 3. Venn diagram showing the number of DEGs which are common among the comparisons between mutant and *C. arietinum*, mutant and *C. echinospermum*, mutant and *C. reticulatum* in the leaves after flowering initiation with vernalization.

With respect to differential expression between cultivated and wild genotypes, the maximum number of DEGs is detected between the mutant and *C. echinospermum* (Fig. 2). This is expected because mutant is an elite cultivar and *C. echinospermum* is a wild genotype which is evolutionary more distant from the cultivated chickpea than *C. reticulatum*. We analyzed differential expression for each specific tissue type and revealed genes which are common among different comparisons (Fig. 3). For example, when we compared mutant with *C. reticulatum* and *C. echinospermum* in the leaves after flowering initiation, common DEGs were *CPK6* from the photoperiod/circadian clock pathway and *FTa1.* Differential expression of *CPK6* gene was also common for the comparisons of *C. arietinum* with wild genotypes in this tissue type.

4. Vernalization response in cultivated chickpea

The existence of a vernalization response in cultivated chickpea has been widely discussed and is somewhat controversial. As expected, the number of genes differentially expressed between two conditions (with and without vernalization) in the cultivated chickpea is very small. We detect differential expression of the *FTa3* gene between conditions in all tissues both in *C. arietinum* and mutant. In all cases, *FTa3* expression is upregulated with vernalization.

References

1. Bouché F, Lobet G, Tocquin P, Périlleux C. FLOR-ID: an interactive database of flowering-time gene networks in Arabidopsis thaliana. Nucleic Acids Res. 2016 Jan 4;44(D1):D1167-71.
2. Ridge S, Deokar A, Lee R, Daba K, Macknight RC, Weller JL, Tar, an B (2017) The chickpea Early Flowering 1 (Efl1) locus is an ortholog of Arabidopsis ELF3. Plant Physiology 175, 802–815.
3. Singh VK, Garg R, Jain M. (2013). A global view of transcriptome dynamics during flower development in chickpea by deep sequencing. Plant Biotechnol J. 11(6):691-701. doi: 10.1111/pbi.12059.

Acknowledgements

The research was funded by the Ministry of Science and Higher Education of the Russian Federation as part of World-class Research Center program: Advanced Digital Technologies (contract No. 075-15-2020-934 dated 17.11.2020).