VALIDATION OF *CIPK7* GENE EXPRESSION UNDER LOW TEMPERATURE CONDITIONS IN DIFFERENT MAIZE INBRED LINES

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INTRODUCTION

For several decades, climate change causes detrimental effect on crop production worldwide. Maize is widely negatively affected by this factor, too. Avoidance of extremely high temperatures during the most sensitive period (flowering and grain filling) could be achieved by earlier sowing. Thus, better tolerance of breeding material to low temperatures in early spring is very important for overcoming this problem. Another benefit of cold tolerance improvement is synchronization of flowering between maize hybrids parental components with different sensitivity to low temperatures.

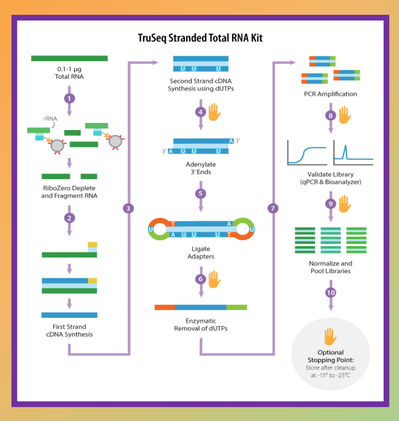
MATERIAL AND METHODS

Eight maize inbred lines belonging to different heterotic groups (four Lancaster and four non-Lancaster inbreds) contrasting for their performance under low temperatures, were chosen for the analysis of genes potentially involved in maize response to cold stress.

Ten plants per genotype were grown until V4 in the greenhouse under optimal conditions. After total RNA extraction from bulked samples, the Next Generation Sequencing of leaf transcriptome was done (MiSeq Illumina,TruSeq Stranded Total RNA Sample Library Prep Kit with Ribo Zero, MiSeq Reagent kit v2, standard flow cell) followed by bioinformatic analysis.

Genes related to stress response, according to literature data, were chosen for further research from differentially expressed genes (DEGs) detected between Lancaster and non-Lancaster maize lines. One of these genes was CBL-interacting kinase 7 (*CIPK7*). Analysis of *CIPK7* expression under optimal (22 ºC) and cold stress (6ºC for 24h) conditions in V4 growth stage was performed. Samples for qPCR expression validation were taken 6 and 24 hours after beginning of stress application and after 48 hours period of recovery. Total RNA extraction (Thermo Scientific GeneJet RNA Purification Kit), DNA removal (Ambion DNA free kit) and cDNA synthesis (Thermo Scientific RevertAid First Strand cDNA Synthesis Kit) followed. The reaction mixture consisted of 6.25µl 5xHOT FIREPol EvaGreen qPCR Mix Plus (ROX) (Solis BioDyne), 0.5µl each primer and 4.25µl H_2O. The qRT-PCR was performed in StepOne Plus (Applied Biosystems). All reactions were performed in triplicates.

NGS



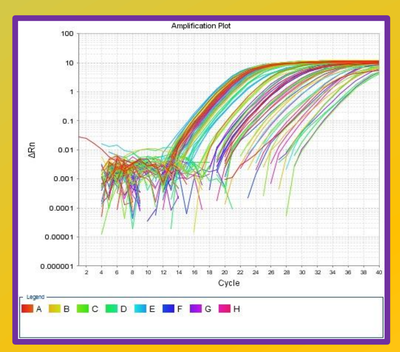
MiSeq

Bioinformatic analysis

- **Sequence quality control**
- **Preprocessing**
- **Assembling** — **processing**
- **Mapping**
- **Mapping quality control** — **postprocessing**
- **Analysis of sequences (SNPs, indels, DGE, etc.)**

Growth chamber

qPCR



RESULTS AND DISCUSSION

Research aimed to identify early response of maize *CIPK7* gene, as well as to follow its expression level upon prolonged exposure to low temperatures and subsequent 48h of recovery period. Results (Figure 1) showed increased *CIPK7* expression level in five out of eight tested inbreds in all analyzed time points. In six genotypes maximal expression level was detected 24h after the exposure to low temperatures, while in remaining two genotypes the level of expression was almost the same after 6h as it was after 24h of exposure to cold stress. Upon plants' recovery, *CIPK7* expression level decreased to the level characteristic for 6h of cold stress (or even lower) for all tested genotypes.

Interestingly, tested non-Lancaster lines, proved to be tolerant to low temperatures in field experiments, showed generally higher rise of *CIPK7* expression level after 24h of cold stress onset than the one displayed in cold sensitive Lancaster lines. Therefore, the difference between levels of expression after 6h and 24h seems to be pronounced to a lesser extent in Lancaster than in non-Lancaster lines. Also, *CIPK7* was expressed to a higher extent after 6h in sensitive Lancaster lines. After 48h period of recovery, clear trend of *CIPK7* level in tolerant versus sensitive inbreds was not observed.

According to the literature data, *CIPK7* is involved in plant abiotic stress response, including cold stress, but to the authors best knowledge this is the first such report for *CIPK7* in maize. Mechanisms of regulation are not completely defined, but current model in *Arabidopsis thaliana* is that Calcineurin-B like proteins (CBLs) interact with CIPKs, creating CBL-CIPK signaling network. This network detects Ca^{2+} based signals which are set off by environmental changes, but more detailed analysis is needed in order to elucidate possible roles of *CIPK7* in response to cold stress in maize.

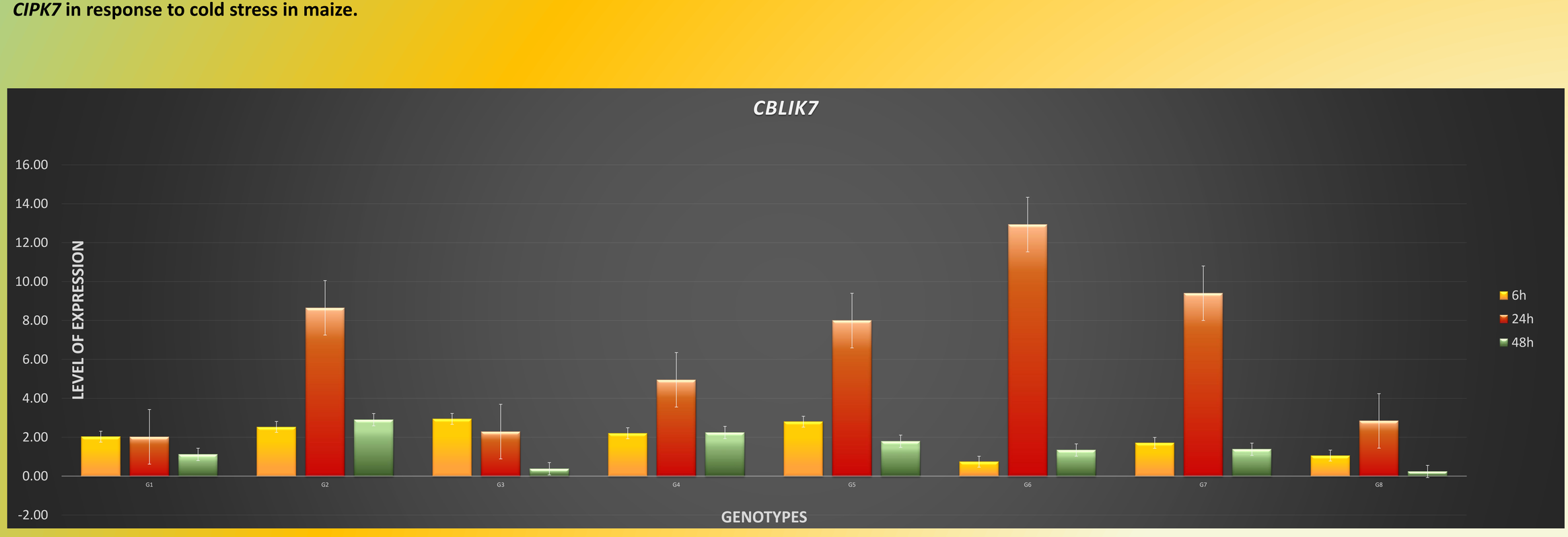


Figure 1. *CIPK7* expression in eight maize inbreds under low temperature conditions (G1-G4 – Lancaster, G5-G8 – non-Lancaster inbreds)

FUTURE PLANS:

- **Testing other DEG in these genotypes**
- **Further characterization of validated DEG**
- **Gene network modeling for validated DGE in cold stress**
- **Phylogenetic analysis of DGE in higher plants**