### **Abstract**

Sugarcane is a tropical and subtropical plant that produces 70% of the total world's sugar. So its impact on the global economy is transparent and undeniable. The main problem with most sugarcane cultivars is their extreme sensitivity to cold, especially during the harvest season, which reduces the yield, sugar content and quality of their syrup. Despite several management strategies to improve cold tolerance in sugarcane cultivars, so far there has been no comprehensive report on the identification of genes involved in freezing tolerance and the relationships between them in sugarcane. For this purpose, transcriptome changes of sensitive (Saccharum officinarum var CP69-1062) and tolerant (Saccharum spontaneum) cultivars of sugarcane were investigated before and after cold stress. To compare the transcripts, first total RNA was extracted separately from leaf samples of both sensitive and tolerant cultivars before (25 °C) and after cold stress (0 °C and -4 °C). The obtained RNA, was sequenced with the Illumina platform by Novogene company in China, after quantitative and qualitative confirmation. Then, using bioinformatics software including Linux packages and CLC Genomics Workbench software, comparative analysis of gene expression was performed and genes with different expression (DEGs) were identified. By studying 347,840,362 reads generated, 16,088 genes showed a significant difference in expression at the 1% probability level. Finally, Gene ontology analysis was performed for 413 DEGs. The results of molecular analysis showed that at 0 °C, the amount and intensity of biological activities of cold-sensitive cultivar is increased. By reducing the temperature to -4 °C, the catabolic activity of the cold-sensitive cultivar is drastically reduced. Reconstruction of the expression network showed that the LOS1 gene is a key gene in the biological process of sugarcane. Its expression profile showed that the level of expression in all six samples tested is more than six thousand transcripts. It seems that SVR3 gene plays a key role in the cold stress tolerance in tolerant cultivars. SVR3 gene expression profiling showed that with increasing cold intensity, its expression in sensitive cultivar decreases. At 0 °C the difference in SVR3 gene expression between the susceptible and tolerant cultivars is more than 5-fold, and at -4 °C this difference is more than 13-fold. Considering the role of SVR3 gene in cell translation process, it can be considered as a factor of superiority of tolerant cultivar against cold stress.

**Key Words**: Illumina, Gene Expression, RNA-Sequencing, Cold stress, Sugarcane



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# Transcriptome analysis of Saccharum officinarum under freezing stress by RNA-Seq

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