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Original article

Identification of pathways and genes with differential expression in sugarcane after cold stress, through RNA-seq analysis

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Extended abstract

Introduction

In order to identify cold tolerant cultivars in sugarcane, in December 2015 and one week after the occurrence of -1.2 °C, by morphological study of 454 sugarcane cultivars, the reaction of these cultivars and their tolerance to cold were investigated.

Materials and methods

In the first stage of the experiment (morphology), 54 cultivars were introduced as resistant and tolerant cultivars and 400 cultivars were introduced as cold and very sensitive cultivars. In 2016, to evaluate cultivars based on biochemical indices, out of 54 resistant cultivars, 5 cultivars, and out of 400 susceptible cultivars, 5 cultivars were selected and the amount of free amino acids, Proline and Malondialdehyde, in them before and after the onset of cold, it was measured. Among the selected cultivars of the previous stage, BR00-01 cultivar was selected as the most tolerant cultivar, and TUC66-107 cultivar as the most sensitive cultivar to cold, to apply cold stress in the cold storage and continue the project in the molecular stage. After planting them in plastic pots, leaf sampling was performed in the refrigeration under stress and to extract TotalRNA. After RNA extraction, the quantity and quality of RNA samples were measured. Using the Illumina Hiseq250 platform in the RNAseq technique, after confirming the quantity and quality of the extracted RNAs, the TruSeq cDNA library was constructed by the Chinese company Novogene and sequencing for RNAseq was done as a pair-end with a length of 150 nucleotides. After sequencing, the data were downloaded in compressed files in Fastq format from the Novogene website. The quality of the raw reads was checked using FastQC software. Trimmomatic software was used in the trimming step. After aligning the reads to the sugarcane reference genome, quantified reads were normalized, differential expression analysis was performed, and genes with differential expression were analyzed and pathways were mapped. After analyzing the differential expression of genes and obtaining the values, GO enrichment analysis was performed to classify the genes based on the placement of their products. Next, the path analysis was performed using the kobas database. Then, in order to enrich the pathways, significant pathways were identified with Corrected P-Value <0.05 for genes with increased and decreased expression.

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Results and discussion

he results of differential expression of genes showed that out of a total of 62285 expressed genes, 12674 genes have differential expression in different comparisons between treatments, of which 6939 genes decreased expression and 5735 genes increased expression. After enriching the pathways, genes that could be 95% probable in 18 treatment comparisons to attribute their change in expression to cold stress were identified, and marker genes were identified in these comparisons. According to the results of the metabolic study, increasing the concentration of free amino acids, Proline and Malondialdehyde increased the plant's tolerance to cold. In this study, due to cold stress, various biosynthetic and metabolic pathways were activated under the influence of the activity of genes controlling these pathways to produce their products. These pathways include the biosynthesis of the amino acids arginine and Proline, the biosynthesis of Phenylpropanoid, the riboflavin metabolism, and the MAPK messenger pathway, in which the genes involved increased expression during cold stress. Meanwhile, the PR1 gene was detected in the MAPK messenger pathway, which showed an increase in expression with decreasing temperature Increased expression of genes in MAPK, Phenylpropanoid, sulfur, Glycerophospholipid, arachidonic acid, lipid ether, riboflavin and Proline signaling pathways indicates that the products of some genes controlling these pathways play a role in increasing cold tolerance of sugarcane.

Conclusions

The results of morphological, metabolic and molecular studies showed that there is a correlation between these indices and their values in resistant cultivar. When screening sugarcane clones for cold, the best clones can be selected by measuring the amount of secondary metabolites. In this study, genes affecting cell physiology pathways were identified and their role in increasing sugarcane tolerance to cold was elucidated. Using these genes, you can use them in a sugarcane breeding program to screen for cold-resistant clones. In the sugarcane breeding program, these genes can be used as markers in the screening of clones.

***Keywords*:** Cold stress, Differential expression of genes, Malondialdehyde, Proline, Sugarcane

Table 1. Scoring the studied traits in morphological study stage

Morphological trait measured	Descriptive expression indicating the status of adjective	Quantitative score
Amount of damage to terminal meristem	No damage, mild, moderate, severe	0-1-2-3
Percentage of canopy greenery	Completely green, Half green, Low greenness, Complete drying	0-1-2-3
Damage to buds	No damage, mild, moderate, severe	0-1-2-3
Rate of Pith development	No development, low Developed, Medium development, High developed	0-1-2-3

Table 2. Comparison table of mean cold tolerance (C.T) in sugarcane cultivars

	Cold tolerance (C.T.)	
Group	Mean	cultivars
A	10.09	BJ97-19
A	10.84	CP50-28
A	10.76	C88-356
A	9.70	L61-67
A	9.32	BR00-01

Table 3. analysis of variance for cold tolerant trait (C.T) in sugarcane cultivars

S.O.V	df	MS (C.T)
Rep.	2	0.52
Treatment	53	17.87**
Error	106	0.16

R^2=0.98, CV=5.13, **= Significant at 1% Level, ns= non-Significant

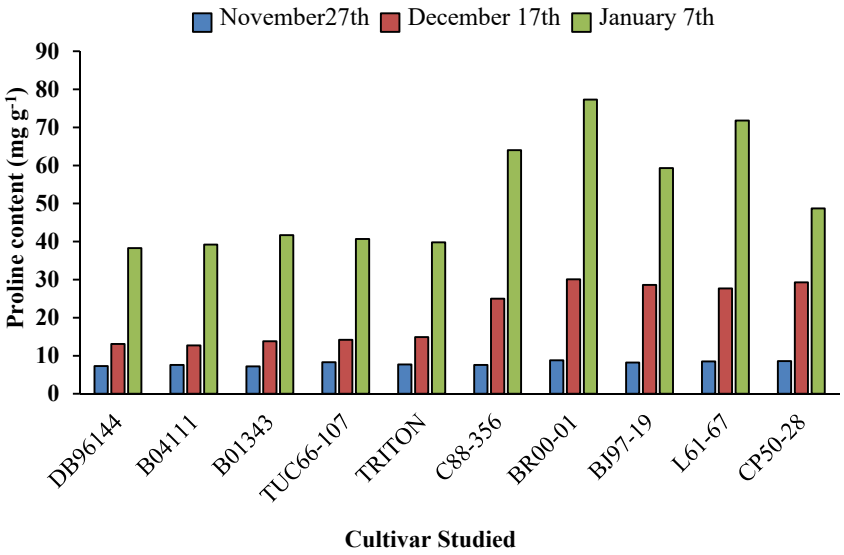

Fig. 1. Proline content, on three different dates, in studied cultivars

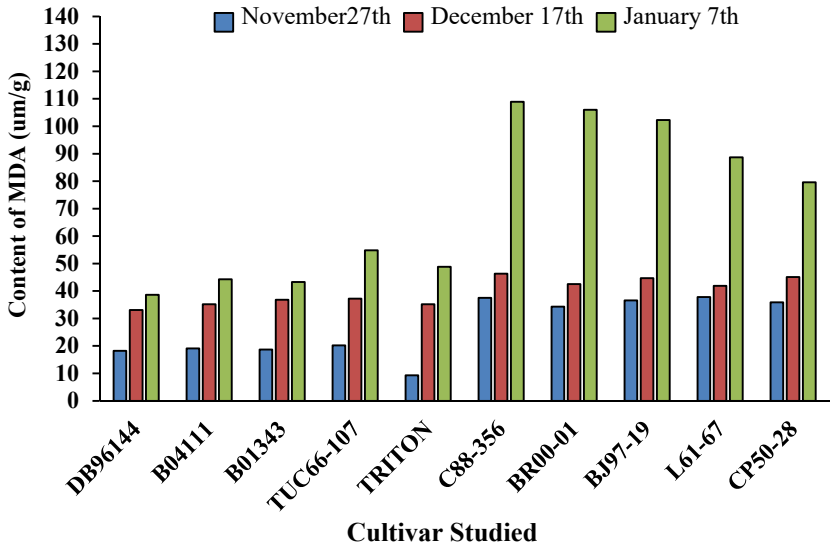

Fig. 2. Malondialdehyde content (MDA), in three different histories, in studied cultivars

Table 5. Summary of quantitative and qualitative review of submitted samples

NO	Sample name	Sample ID	$OD_{260/OD280}$	$OD_{260/OD230}$	RNA RIN	sample QC Result
1	19452	FKRN190315691-1A	2.79	0.8	8.1	PASS
2	19069	FKRN190315693-1A	1.75	0.64	8.2	PASS
3	19018	FKRN190315694-1A	1.7	1	8.4	PASS
4	19866	FKRN190315695-1A	2.33	0.96	8.1	PASS
5	19507	FKRN190315696-1A	2.6	0.57	7.7	PASS
6	19881	FKRN190315697-1A	5	1.11	7.9	PASS

Table 6. Summary of gene statistics obtained from differential expression analysis with q <0.05

Row	Comparison	UP Rigulate	Down Rigulate	Total
1	H_0 vs H_6	1435	1497	2932
2	H_0 vs H_{12}	1412	1245	2657
3	H_6 vs H_{12}	31	21	52
4	H_0 vs M_0	281	229	510
5	H_6 vs M_6	457	655	1112
6	H_{12} vs M_{12}	269	304	573
7	M_0 vs M_6	863	1809	2672
8	M_0 vs M_{12}	901	1140	2041
9	M_6 vs M_{12}	86	39	125
	Total	5735	6939	12674

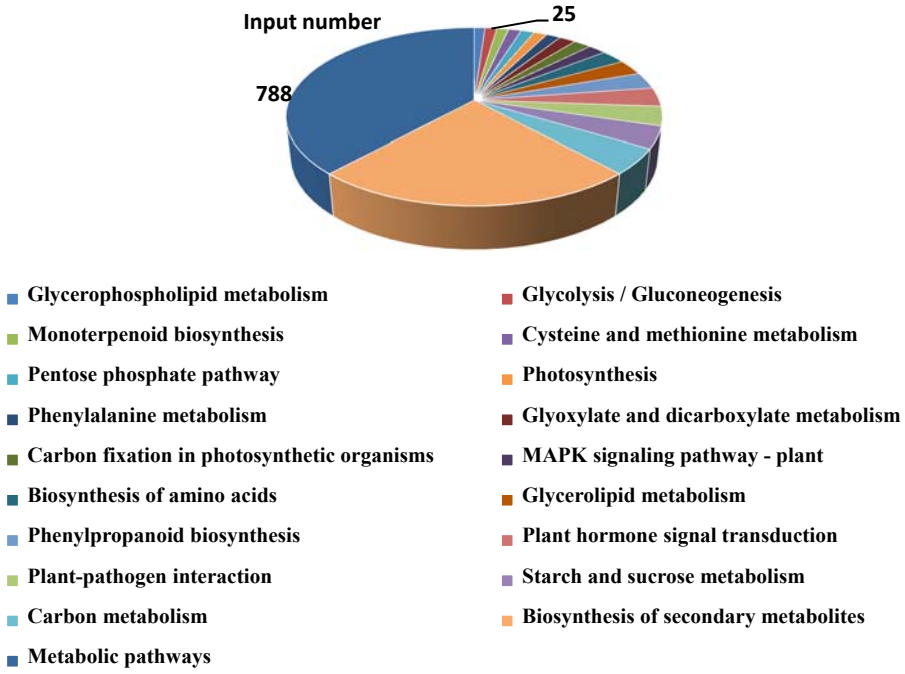

Fig 3. Functional classification of genes with differential expression in different treatment comparisons based on KEGG classification

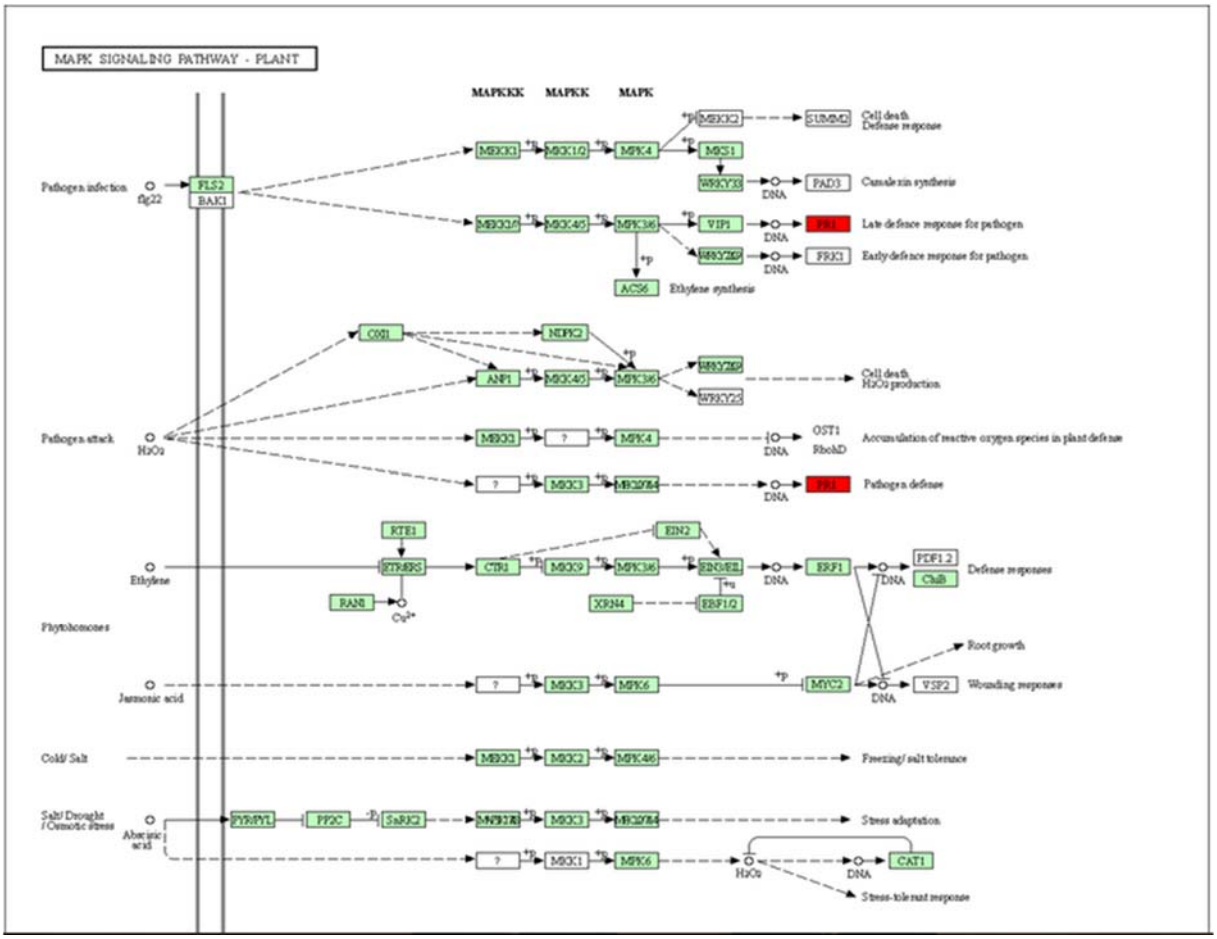

Fig. 4. PR1 gene (In red), effective in MAPK messenger pathway: This gene had increased expression in sensetive cultivar after 12 hours of cold stress.

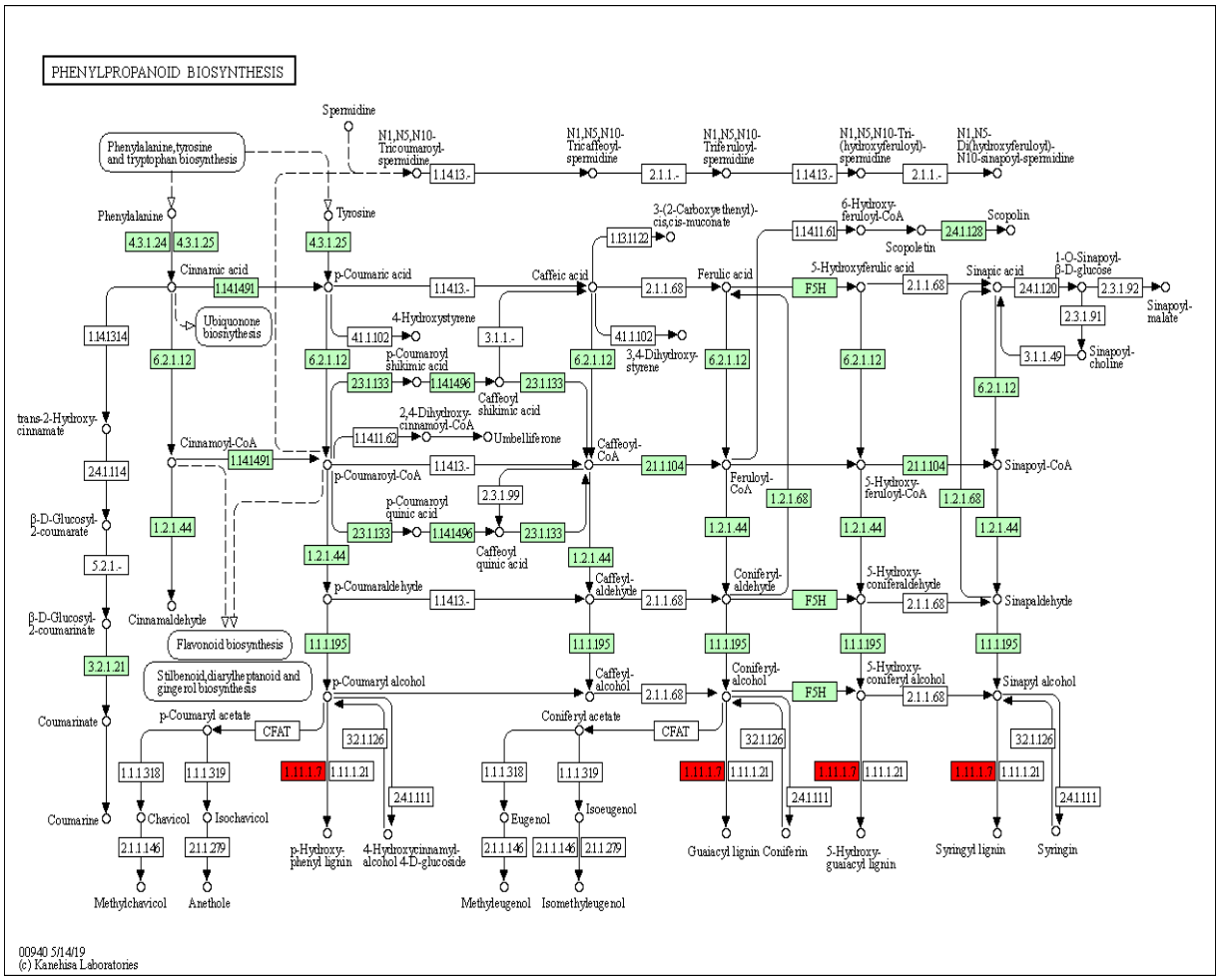

Fig. 5. Genes affecting the biosynthesis pathway of phenylpropanoid (In red): These genes showed increased expression in resistant cultivar after 6 hours of cold stress.