Journal of Animal & Plant Sciences, 34(3): 2024, Page: 756-767
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2024.3.0762

EXPRESSION ANALYSIS OF COLD-RESPONSIVE GENES AND MECHANISMS INVOLVED DURING COLD STRESS IN SUGARCANE CELLULAR ORGANELLES

S. U. Rehman[1,*], K. Muhammad[2], K. Osmonaliev[1] M. Abdullah[1], A. Nazira[3] and M. U. Nurlanbek[1].

[1]Faculty of Medicine Ala-Too International University, 720048, Bishkek, Kyrgyzstan

[2]Department of Biotechnology and Genetic Engineering, Hazara University Mansehra, Khyber Pakhtunkhwa, Pakistan
[3]Department of Engineering and Informatics Ala-Too International University, 720048, Bishkek, Kyrgyzstan

*Corresponding Author,s email: shafeeur.rehman@alatoo.edu.kg

ABSTRACT

Sugarcane is a major cash crops cultivated in tropical and subtropical regions around the globe. This crop is mainly considered cold sensitive, although some cultivars showed resistance to cold stress. The crops respond to cold stress in diverse mechanisms and among these mechanisms, genes expression is one of the significant biological pathways. Our goal was to study the involvement of gene expression in cell organelles and cognate targets in the tolerance to low temperatures in sugarcane. The results reported here indicated that 2,324 genes were found upregulated in cultivar GT08-1108 organelles in a total of 5,649 upregulated genes, while 1,252 genes were found downregulated in a total of 3,289 downregulated genes. Furthermore, the cultivar ROC22 gene expression analysis showed that a total of 2,223 genes were found upregulated in 5,558 total upregulated genes, while 1,449 genes were found downregulated in a total of 3,252 downregulated genes. The gene expression analysis in both cultivars showed that during cold stress, the gene expression in the vacuole was found significantly downregulated. Hence, from these findings, we concluded that during cold stress, the most affected organelle is the vacuole. Collectively, our findings suggested that cold stress seriously affected cellular organelles in sugarcane. Hence, more research is needed to identify more cold tolerant cultivars using transcriptomics approaches in sugarcane, which could be significant for better production and quality of sugarcane.

Keywords: Sugarcane; Cellular organelles; Gene expression; Sequence Read Archive; Cold stress.

Published first online March 27, 2024 Published final May 31, 2024

INTRODUCTION

Sugarcane is one of the important cash crop cultivated among the tropical and subtropical region of the globe. The quality and production of the crop is seriously affected by various biotic and abiotic factors, among these cold stress is one of the significant factor affect the quality and yield of sugarcane worldwide (Lopez-carbonell *et al.,* 1994). The response of plant to cold stimuli showed diverse mechanisms and pathways, among these cold responsive gene expression is the key pathways. The effect of cold stress lead disturbance in cell function and activity. Inside the cell the most affected organelles during cold stress is chloroplast, mitochondria and other organelles (Lopez-carbonell *et al.,* 1994; Kratsch and Wise, 2000; Li *et al.*, 2023; Li *et al.,* 2023). However, recent research showed that some polar and alpine plants resisted cold stress on organelles level (Li *et al.,* 2023).

The cloroplast, which are bound by a double membrane and contain stroma and a thylakoid system, are present in the cytoplasm of plants (Giełwanowska, 2003; Giełwanowska *et al.,* 2005; Giełwanowska and Szczuka, 2005; Chen *et al.,* 2023). In chloroplast, a vast area is free, filled with stroma and long protrusion stromules (Holzinger *et al.,* 2007a, b; Shaw and Gray, 2011). Antarctic lichen algae mitochondria cells with similar chloroplast were observed (Giełwanowska and Olech, 2012).

A flowering plant native to the Antarctic region has a dense stroma and a well-organized system of internal membranes, which are the significant characteristics of mitochondria of mesophyll cells in these plants (Giełwanowska *et al.,* 2005). Mesophyll cells of polar and alpine vascular plants have a dense matrix in mitochondria, chloroplast, and large peroxisome (Lütz and Engel, 2007). Crystalline protein structures have been found in the peroxisome matrix of polar Poaceae crops. (Giełwanowska *et al.,* 2005). Most peroxisomes are spherical, although elongated and other shapes are also reported (Muench and Mullen, 2003). In flowering plants, mesophyll cells usually possess spherical nuclei, although they can also have different shapes. It has

become possible to examine unusual properties of cell nuclei, such as their capacity to form grooves and invaginations, using a variety of visualization techniques (Singh *et al.,* 1998; Collings *et al.,* 2000).

The plant body responds to environmental stresses in different ways, among them gene expression, physiological changes, and stop of transpiration. Gene expression is a complex mechanism involving gene expression that encodes proteins to give strength to the plant (Rehman *et al.,* 2022). The effect of cold on crops could reduce yield and affect quality. *Saccharum* hybrid is an economically significant crop, providing sugar and a rich source of biofuel (Rehman *et al.,* 2022). The crop sugarcane is affected by the cold, which reduces yield quality (Rehman *et al.,* 2019; Rehman *et al.,* 2020). However, the response of sugarcane crops to cold depends on the magnitude of stress, time, temperature and gap of sugarcane harvesting (Rehman *et al.,* 2020). Studying physiological and molecular changes in cells and their organelles is very important. Hence, new technologies have made studying plant response at the molecular level easier. The Next generation sequencing, omics and computational biology make it easier to understand the mechanism (Dharshini *et al.,* 2016; Shiyun *et al.,* 2018). The expression analysis of genes responsive to cold and the mechanisms involved in cold stress in sugarcane cellular organelles has not been specifically studied. As a result, we described the processes of cold-sensitive gene expression in sugarcane cellular organelles under cold stress in this study.

MATERIALS AND METHODS

Study Samples: In the current research, two sugarcane cultivars, ROC22 and GT08-1108, were selected. The RNA sequence (57.41) data of two selected sugarcane cultivars, ROC22 and GT08-1108, were downloaded from the Sequence Read Archives (SRA) NCBI database. In the previous study, Shiyun *et al*. (2018) experimented and mapped reads with reference transcriptome assembly. In the current analysis, the reads were mapped with *Saccharum spontaneum* genome derived from sugarcane variety AP85-441 (Zhang *et al.,* 2018) (Figure 1).

Experimental design: In the current study, the downloaded data of RNA sequence were further analyzed using different bioinformatics pipelines. The Raw RNA sequence data were analyzed using FASTQC and Trimmomatic software to remove low quality reads and adapters from the Transcriptomics data (Bolger *et al.,* 2014). In this study, the reads were mapped with the reference genome and aligned using STAR and HTseq software (Zhang *et al.,* 2018). The number of reads from each library aligned in each gene was used as the measurement of gene expression levels.

Data normalization and analysis of differential expression: Furthermore, the examination of differential expression analysis of Transcriptomics data was carried out using software edge R (Robinson *et al.,* 2010). Data was filtered for normalization, and genes were counted 1 per million for at least two excluded samples. Trimmed mean (TMM) software was used for normalization, equalization and minimizing high gene expression (Robinson and Oshlack, 2010). For differential expression analysis of genes in both control and stress treated samples, Fisher extract software was used (Robinson and Smyth, 2008). At the same time, conditional maximum likelihood (CML) was used for sequence analysis. For the significance analysis of the differential expression of genes, the False Discovery Rate (FDR) and P value were calculated.

Gene enrichment analysis: After normalization and differential expression analysis of cold responsive genes in cell organelles, the genes expression was further investigated using Agrigo gene enrichment analysis (Tian *et al.,* 2017). Enrichment and gene ontology of expressed genes were categorized into three categories: 1) Biological processes, 2) Molecular function and 3) Cellular components.

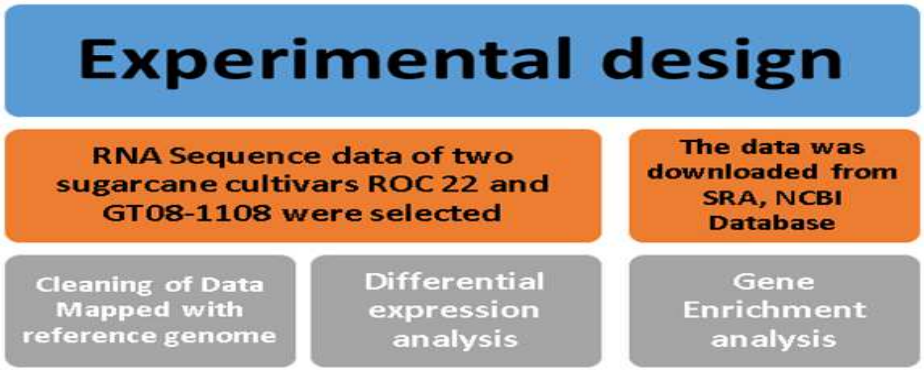

Figure 1: Schematic view of experimental design and data analysis of cold-responsive gene expression in cell organelles in sugarcane.

RESULTS

Differentially expressed genes associated with cold stress in cellular organelles of GT08-1108 and ROC 22: In cultivar GT08-1108, 5,649 genes were upregulated during cold stress (Figure 2 and Table 1). Among these 647 genes were differentially upregulated in the cell wall, 405 in mitochondria, 1096 in the chloroplast, 347 in ribosomes, 996 in the nucleus, 733 in the plastid, 10 in the vacuole, 115 in the Golgi apparatus and 2324 in the Cytoplasm (Figure 2 and Table 1). While 3289 genes were found downregulated during cold stress in cultivar GT08-1108 (Figure 2). Among these, 71 genes were differentially downregulated in the cell wall, 297 in mitochondria, 620 in chloroplast, 28 in ribosomes, 306 in the nucleus, 619 in plastid, 163 in vacuole, 91 in Golgi apparatus and 1252 in the cytoplasm.

In cultivar ROC22, 5649 genes were found upregulated during cold stress. Among these, 554 genes were differentially upregulated in the cell wall, 408 in mitochondria, 602 in the chloroplast, 288 in ribosomes, 1063 in the nucleus, 1587 in the plastid, 133 in the vacuole, 175 in the Golgiapparatus and 2223 in Cytoplasm (Figure 3 and Table 2). In ROC22, 3289 genes were found downregulated during cold stress. (Table 2). Among these, 179 genes were differentially downregulated in the cell wall, 297 in mitochondria, 637 in chloroplast, 62 in ribosomes, 289 in the nucleus, 1239 in plastic, 26 in vacuole, 69 in Golgi apparatus and 1270 in the cytoplasm.

Table 1: Differentially expressed cold-responsive genes associated with cold stress in cellular organelles of sugarcane cultivar GT08-1108.

Serial no	Cell organelle	Upregulated genes	Total upregulated genes	Downregulated genes	Total downregulated genes
1	Cell wall	647	5649	71	3289
2	Mitochondria	405	5649	297	3289
3	Chloroplast	1096	5649	620	3289
4	Ribosome	347	5649	28	3289
5	Nucleus	996	5649	306	3289
6	Plastid	733	5649	619	3289
7	Vacuole	10	5649	163	3289
8	Golgi apparatus	115	5649	91	3289
9	Cytoplasm	2324	5649	1252	3289

Table 2: Cold-responsive differentially expressed genes during cold stress in cellular organelles sugarcane of cultivar ROC22.

Serial no	Cell organelle	Upregulated genes	Total upregulated genes	Downregulated genes	Total downregulated genes
1	Cell wall	554	5649	179	3289
2	Mitochondria	408	5649	297	3289
3	Chloroplast	602	5649	637	3289
4	Ribosome	288	5649	62	3289
5	Nucleus	1063	5649	289	3289
6	Plastid	1587	5649	1239	3289
7	Vacuole	133	5649	26	3289
8	Golgi apparatus	175	5649	69	3289
9	Cytoplasm	2223	5649	1270	3289

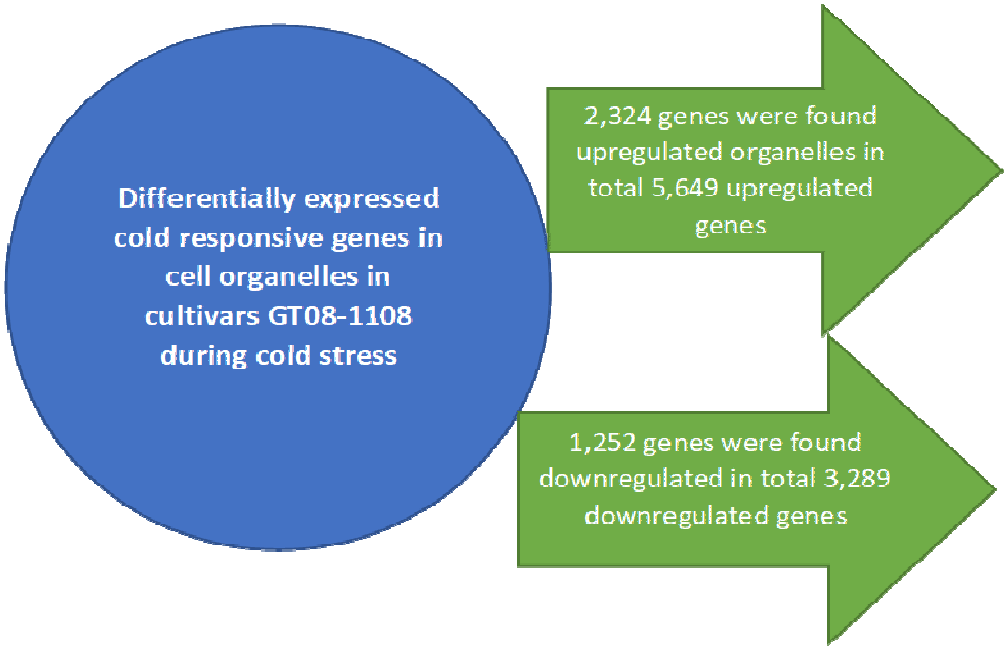

Figure 2: Differentially expressed cold-responsive genes associated with cold stress in cellular organelles of sugarcane cultivars GT08-1108.

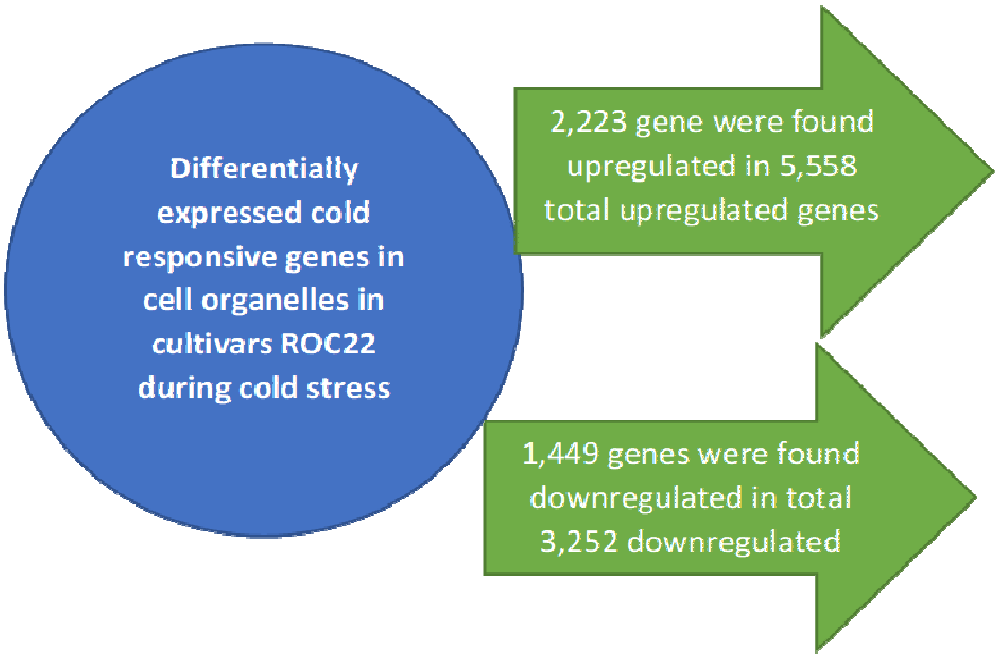

Figure 3: Differentially expressed cold-responsive genes associated with cold stress in cellular organelles of sugarcane cultivars ROC22

Gene ontology and enrichment study of Upregulated and downregulated genes in cellular organelles of GT08-1108 and ROC 22: Agrigo gene enrichment analysis was employed for gene ontology and enrichment research. Gene ontology and enrichment analysis were divided into cellular components, biological processes, and molecular functions.

i. ***Cell wall:*** In our study, the cold-responsive gene in the cell wall showed that in cultivar GT08-1108, 647 upregulated genes out of 5,649 were found in the cell wall. At the same time, the expressed genes were enriched in cell wall modification by enhancing metabolic processes of macromolecules and other essential organic compounds. While, 71 genes were

found downregulated in cell wall in cultivars GT08-1108. These genes were enriched in cell wall organization (Figure 4 and Figure 5).

In cultivar ROC22, 554 genes were found to be upregulated. At the same time, the expressed genes were enriched in cell wall modification by enhancing metabolic processes of macromolecules and other important organic compounds. Out of the 3289 downregulated genes, 179 genes were downregulated in the cell wall in cultivar ROC22. These genes were enriched in cell wall organization (Figure 6 and Figure 7).

ii. ***Mitochondria:*** In mitochondria, 405 out of 5649 upregulated genes were recorded in cultivar GT08-1108. The gene enrichment analysis showed that these genes were significantly enriched in mitochondrial respiratory complexes assembly, mRNA modification, transport, protein modification, ribosome modification and management of mitochondrial outer and inner membranes. In comparison, 297 genes were found downregulated in mitochondria respectively, in 3289, total downregulated genes in cultivar GT08-1108. These genes were enriched in mitochondrial metabolic pathways and membrane organization (Figure 4 and Figure 5).

In our study, the effect of cold stress on mitochondria revealed that 408 out of 5649 upregulated genes were elevated in cultivar ROC22. The gene enrichment analysis showed that these genes were significantly enriched in mitochondrial respiratory complexes assembly, mRNA modification, transport, protein modification, ribosome modification and management of mitochondrial outer membrane and inner membrane. Out of the 3289 downregulated genes in cultivar ROC22, 297 genes were found downregulated in mitochondria. These genes were enriched in mitochondrial metabolic pathways and membrane organization (Figure 6 and Figure 7).

iii. ***Chloroplast:*** Chloroplasts are double-membrane organelles found in plant cells that are responsible for photosynthesis. In this study, the gene expression analysis of cold responsive genes in chloroplast showed that 1096 genes were significantly expressed upregulated in total of 5,649 upregulated genes in cultivar GT08-1108. These genes were enriched in managing chloroplasts, mostly stroma, thylakoid, membrane, and RNA processing. In cultivar GT08-1108, out of 3,289 downregulated genes, 620 genes were found downregulated in the chloroplast. These genes were enriched in chloroplast organization (Figure 4 and Figure 5). In cultivar ROC 22, 642 genes were found upregulated in chloroplast in 5,649 total upregulated genes. Meanwhile, in cultivar ROC 22, 637 downregulated genes were found in the chloroplast out of 3,289 downregulated genes. These expressed genes were enriched in chloroplast organization (Figure 6 and Figure 7).

Nucleus and Ribosome: The nucleus is the heart of the cell, contains heredity materials and controls all cell activity. In our study, in cultivar GT08-1108, 1096 genes were differentially expressed upregulated in a total of 5,649 upregulated genes in nucleus. while in cultivar GT08-1108, 347 genes were expressed upregulated in ribosome out of 5649 total upregulated genes. At the same time, 28 genes in the ribosome and 306 genes the nucleus in a total of 3289 downregulated genes in cultivar GT08-1108. These genes were enriched in the nucleus and ribosome organization (Figure 4 and Figure 5).

In cultivar, ROC22, 288 genes in the ribosome and 1063 genes in the nucleus were found significantly expressed upregulated in a total of 5649 upregulated genes. In comparison, there were, 62 downregulated genes in the ribosome, and 289 downregulated genes in the nucleus, were recorded in total 3289 total downregulated genes. These expressed genes were enriched in ribosome orgonisation and nucleus orgonisation (Figure 6 and Figure 7).

iv. ***Plastid, Vacuole, Golgi apparatus and cytoplasm:*** The gene expression in plastids during cold stress showed that 733 genes were upregulated in a total of 5649 upregulated genes in cultivar GT08-1108. These expressed genes were enriched in the organization of the plastid and enhancement of transcription from the plastid promoter. Furthermore, 10 genes were upregulated in vacuole, 115 genes were significantly upregulated in the Golgi apparatus, 2,324 were shown to be highly increased in the cytoplasm among 5,649 total upregulated genes in cultivar GT08-1108. Moreover, in plastid 619 genes were found downregulated, while in 163 genes were downregulated in Golgi complex, 91 genes were downregulated in vacuole and 1252 genes were found downregulated in cytoplasm in total 3289 downregulated genes in cultivar GT08-1108. (Figure 4 and Figure 5).

In cultivar ROC22, among 5649 total upregulated genes, 133 were expressed upregulated in vacuoles, 175 genes were significantly increased in the Golgi apparatus, 2223 genes had a significant upregulation in the cytoplasm. For the cultivar ROC22, there were 1239 downregulated genes in the plastid, 26 in the Golgi complex, 69 in the vacuole, and 1270 downregulated genes in the cytoplasm, for a total of 3289 downregulated genes (Figure 6 and Figure 7).

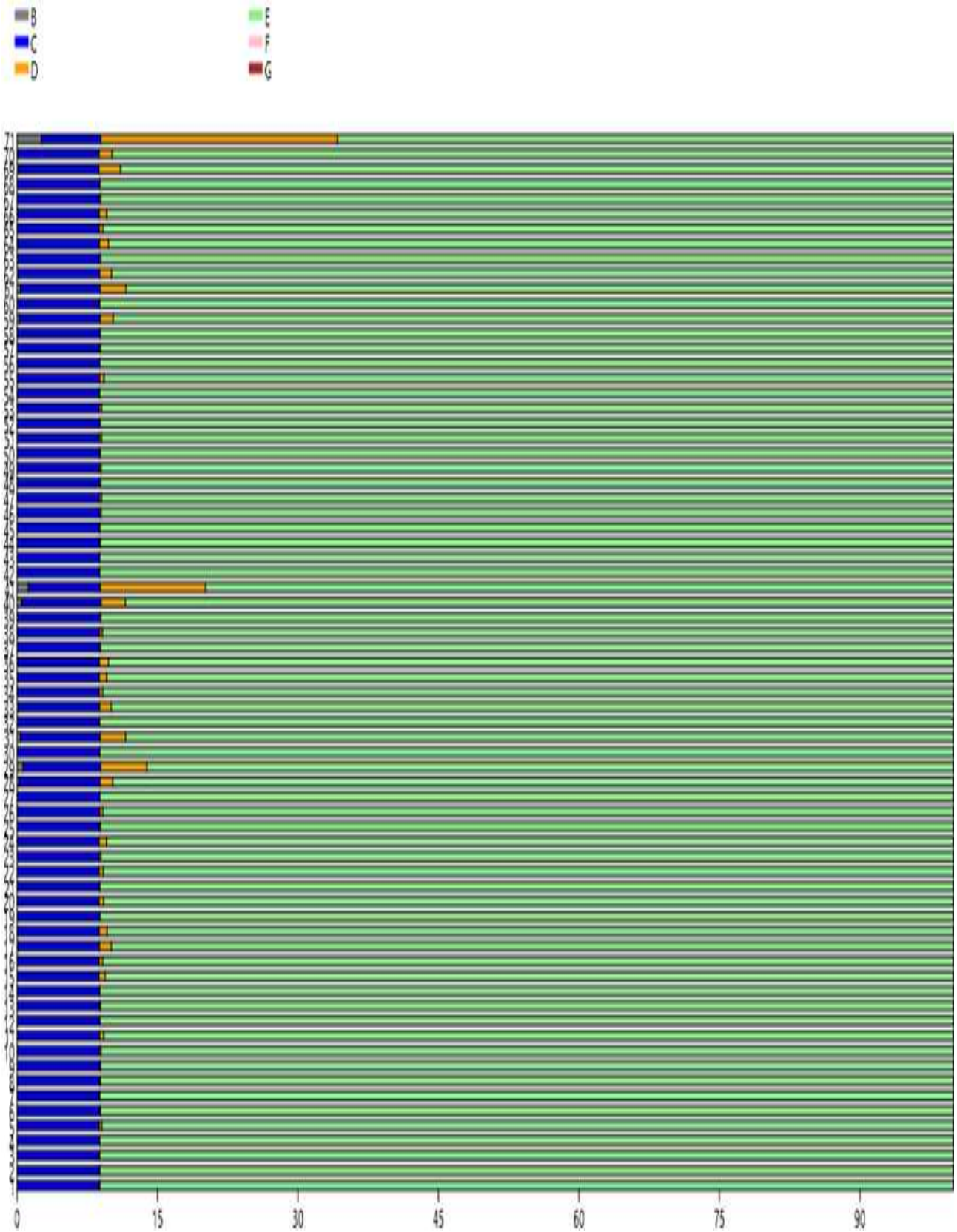

Figure 4: Stacked Chart showed the Gene ontology and enrichment study of Differentially expressed Upregulated cold responsive genes in cellular organelles of sugarcane cultivar GT08-1108.

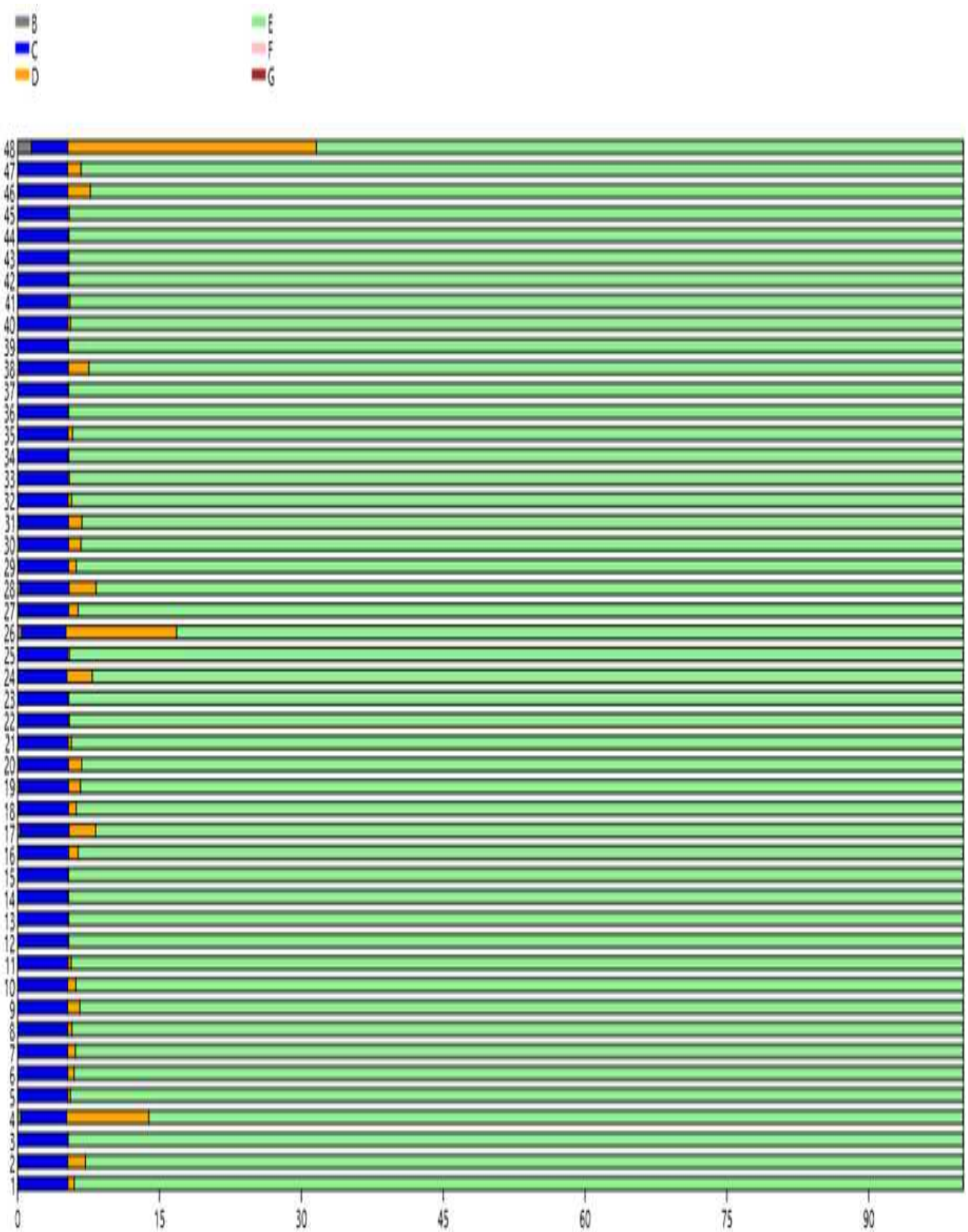

Figure 5: Stacked Chart showed the Gene ontology and enrichment study of Differentially expressed downregulated cold responsive genes in cellular organelles of sugarcane cultivar GT08-1108

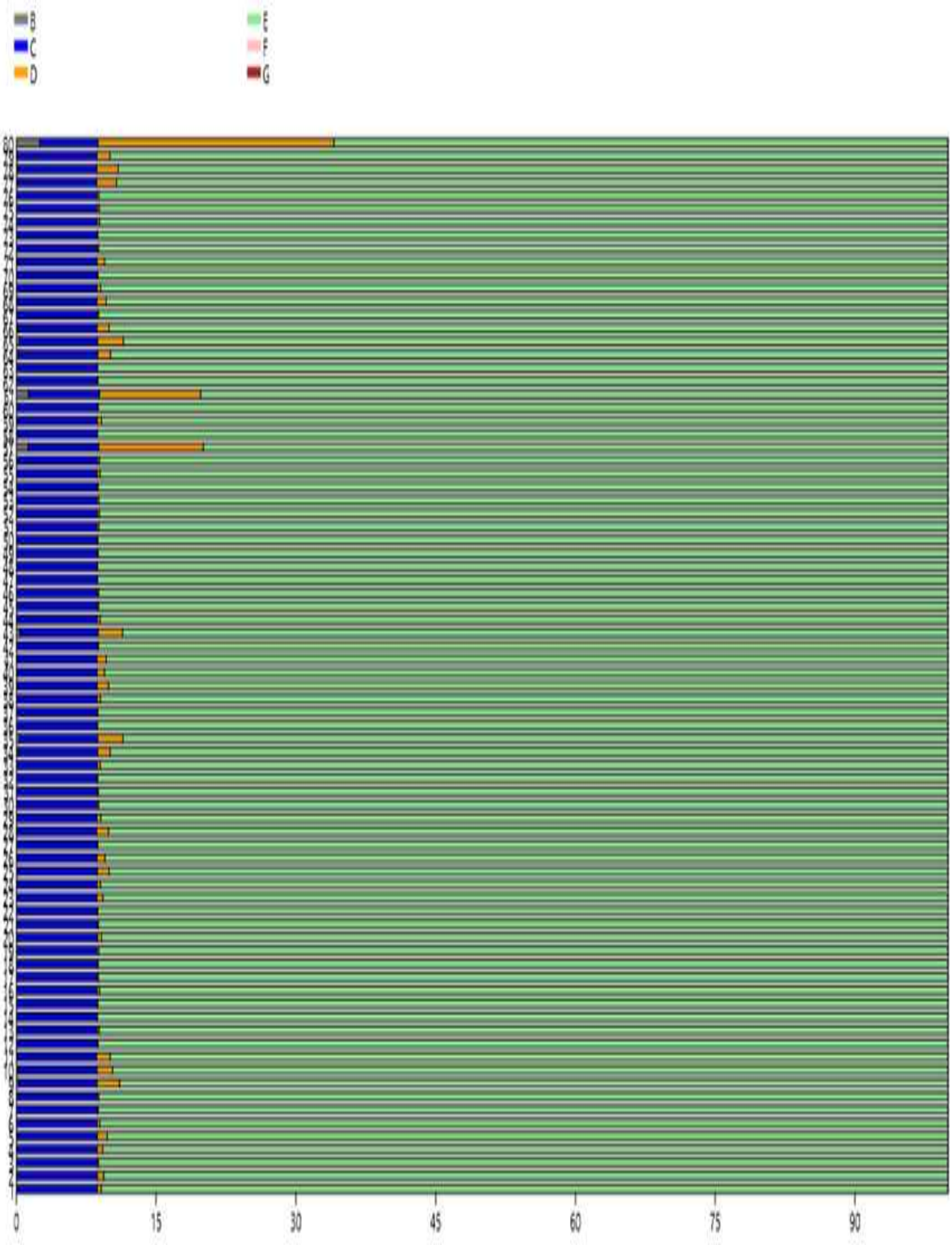

Figure 6: Stacked Chart showed the Gene ontology and enrichment study of Differentially expressed upregulated cold responsive genes in cellular organelles of sugarcane cultivar ROC22.

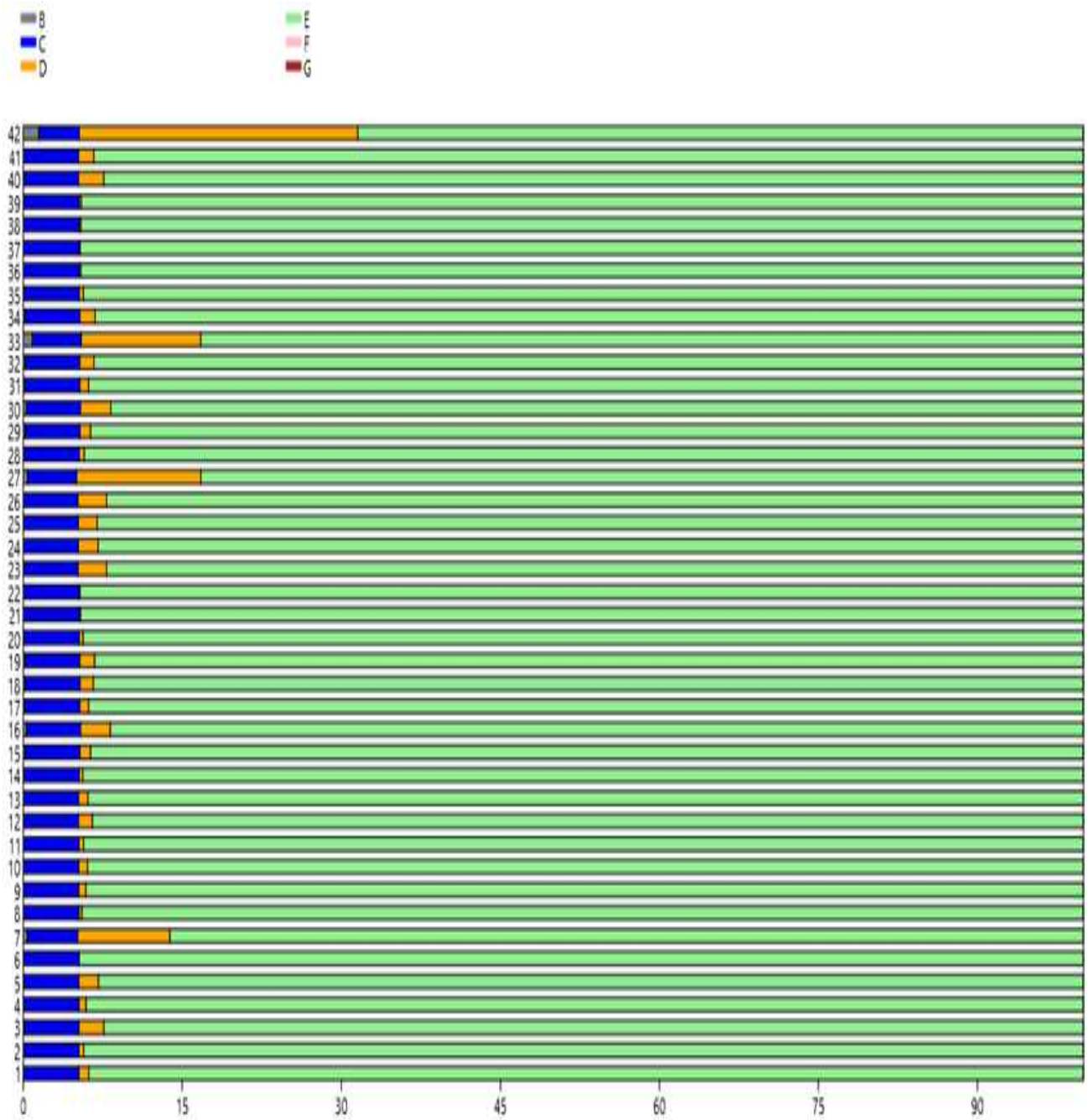

Figure 7: Stacked Chart showed the Gene ontology and enrichment study of Differentially expressed downregulated cold responsive genes in cellular organelles of sugarcane cultivar ROC22

DISCUSSION

Sugarcane is one of the important and major crops cultivated around the globe and a good source of biofuel and sugar (Rehman *et al.,* 2020; Rehman *et al.,* 2022; Afghan *et al.,* 2023). Climate change and global warming have affected crop productivity and quality worldwide. Among these factors, cold stress is one of the major ones that reduces the production and quality of sugarcane worldwide (Rehman *et al.,* 2022; Härter *et al.,* 2023). The plant's response to these stresses in a diverse way among these gene expressions is one of the important biological pathways. The expression of these genes provides different mechanisms for plants to survive in cold stress (Rehman *et al.,* 2022; Huang *et al.,* 2023). In plant cell, the chloroplast, Mitochondria, vacuole and other organelle are the significant structures responsible for photosynthesis, turgidity, energy production and

protein synthesis, and these organelles are seriously affected during cold stress (Lütz *et al.,* 2012).

This study was conducted in sugarcane for the first time to understand the mechanism of gene expression in cellular organelles. We evaluated the gene expression analysis of cold-responsive genes in sugarcane (ROC22 and GT08-1108) cellular organelles during cold stress. In cultivar GT08-1108, 5649 genes were found to be differentially expressed and upregulated during cold stress, while 3,289 were found downregulated. During cold stress the most affected organelle is chloroplast (Holzinger *et al.,* 2007a, b). Still, in our study, the expression of genes in chloroplast was found to be higher (Giełwanowska and Olech, 2012). In the chloroplast, 1096 genes were found to be upregulated, while 620 genes were found to be downregulated (Giełwanowska, 2003; Giełwanowska *et al.,* 2005; Giełwanowska and Szczuka, 2005; Holzinger *et al.,* 2007a, b; Shaw and Gray, 2011). In plastid, 733 genes were upregulated, and 619 were found to be downregulated. In ROC22, 5649 genes were found differentially expressed and upregulated during cold stress, while 3,289 were found to be downregulated. In ROC22, the gene expression in chloroplast was downregulated, 602 genes were upregulated, while 637 genes were downregulated, while in the plastid, 1587 genes were upregulated, and 1239 were downregulated. In GT08-1108, the gene expression in the vacuole was found downregulated, 10 genes were upregulated while 163 genes were found downregulated. In ROC22, 133 genes were upregulated, and 26 genes were downregulated (Kratsch and Wise, 2000; Zeng *et al.,* 2023).

Furthermore, the gene expressions in other cellular organelles were upregulated; in mitochondria, 405 genes were upregulated, 297 genes were downregulated in GT08-1108, 408 upregulated genes and 297 downregulated genes were found in ROC22. In the cytoplasm, GT08-1108 had 2324 upregulated genes and 1252 downregulated genes, whereas ROC22 contained 2223 upregulated and 1270 downregulated genes (Giełwanowska and Szczuka, 2005).

In response to low temperatures, plants undergo various cellular, molecular, physiological, and biochemical changes to cope with stress, including gene expression (Zhu *et al.,* 2007). In our study, the activity of cold-responsive genes in the cell wall, ribosome, golgi apparatus and nucleus were significantly upregulated. In response to low temperatures, plants undergo various cellular, molecular, physiological, and biochemical changes to cope with stress, including gene expression (Zhu *et al.,* 2007). In our study, the activity of cold-responsive genes in the cell wall, Ribosome, Golgi apparatus and nucleus were significantly upregulated. The expressed genes were enriched in cell wall orgonisation, mitochondrial orgonisation and enhancement of metabolic activities, nucleus orgonisation and expression of cold tolerance genes, synthesis of cold tolerant protein, and chloroplast organization.

Summary of the experiment: In this study, we utilized the RNA sequence data of two sugarcane cultivars ROC22 and GT08-1108. The sequence data was retrieved from SRA NCBI database. Insilco analysis was carried out to investigate the differential expression analysis of cold-responsive genes in sugarcane cell organelles. This study was the first study conducted to investigate the cold-responsive gene expression in sugarcane cell organelles during cold stress. The results of this study showed that gene expression was found to be highly upregulated in cellular organelles like chloroplasts and mitochondria and within the cytoplasm. Still, we observed that the gene expression in the vacuole was found downregulated. Hence, it concluded that the most affected organelle in this study is the vacuole, and this effect can lead the cell to lose turgidity and could affect the growth and production of the sugarcane crop. The current study provided some significant insights for future sugarcane breeding programs.

Data availability: All the data is available in the manuscript.

Acknowledgment

The authors are very thankful to the faculty of medicine, at Ala-too International University for supporting this study.

Conflict of interest: The authors declare that they have no conflict of interest.

REFERENCES

Afghan, S., M. E. Khan, W. R. Arshad, K. B. Malik, and A. Nikpay (2023). Economic Importance and Yield Potential of Sugarcane in Pakistan. In Sugarcane-Its Products and Sustainability. IntechOpen. https://doi.org/10.5772/intechopen.105517.

Bolger, A. M., M. Lohse and B. Usadel (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics, 30(15): 2114-2120. https://doi.org/10.1093/bioinformatics/btu170.

Chen, X. L., X. L. Zheng, T. Xu, J. P. Zou, W. D. Jin, G. H. Wang, P. Yang and J. Zhou (2023). Role of selective autophagy receptors in tomato response to cold stress. J Exp Bot,213:105426. https://doi.org/10.1016/j.envexpbot.2023.105426.

Collings, D. A., C. N. Carter, J. C. Rink, A. C. Scott, S. E. Wayatt and A. N. Stromgren (2000). Plant nuclei can contain extensive grooves and

invagination. The Plant Cell,12: 2425–2439. https://doi.org/10.1105/tpc.12.12.2425.

Dharshini, S., M. Chakravarthi, V. M. Manoj, M. Naveenarani, R. Kumar, M. Meena, B. Ram and C. Appunu (2016). De novo sequencing and transcriptome analysis of a low temperature tolerant Saccharum spontaneum clone IND 00-1037. . Biotechnol,231:280–294. https://doi.org/10.1016/j.jbiotec.2016.05.036.

Gielwanowska, I (2003). Deschampsia antarctica responses to abiotic stress factors. Acta Physiol Plan,25(3):61–62.

Gielwanowska, I and M. Olech (2012). New ultrastructural and physiological features of the thallus in Antarctic lichens. Acta Biol Cracov Bot,54:1–13. https://doi.org/10.2478/v10182-012-0004-0 .

Giełwanowska, I and E. Szczuka (2005). New ultrastructural features of organelles in leaf cells of Deschampsia antarctica Desv. Polar Biol,28:951–955. https://doi.org/10.1007/s00300-005-0024-2.

Gielwanowska, I., A. Bochenek, E. Gojlo, R. Gorecki, W. Kellmann, M. Pastorczyk and E. Szczuka (2011). Biology of reproduction of Colobanthus quitensis (Kunth) Bartl. Pol Polar Res,32:139–155. https://doi.org/10.1007/s00300-005-0024-2.

Gielwanowska, I., E. Szczuka, J. Bednara and R. J. Gorecki (2005). Anatomic features and ultrastructure of Deschampsia antarctica (Poaceae) leaves from different growing habitats. Ann Bot, 96:1109–1119. https://doi.org/10.1093/aob/mci262.

Holzinger, A., G. O. Wasteneys and C. Lütz (2007). Investigating cytoskeletal function in chloroplast protrusion formation in the arctic-alpine plant Oxyria digyna. Plant Biol,9(03):400-410. https://doi.org/10.1055/s-2006-924727.

Holzinger, A., O. Buchner, C. Lütz and M. R. Hanson (2007). Temperature-sensitive formation of chloroplast protrusions and stromules in mesophyll cells of Arabidopsis thaliana. Protoplasma,230:23-30. https://doi.org/10.1007/s00709-006-0222-y.

Härter, A., W. R. Antunes, A. A. Azeredo, C. D. Silva and R. A. D.Oliveira (2023). Early screening for cold tolerance in sugarcane breeding. Pesquisa Agropecuária Brasileira,58:02711. https://doi.org/10.1590/S1678-3921.pab2023.v58.02711.

Huang, X., Y. Liang, R. ZhanB. Zhang, X. Song, J. Liu, M. Lu, Z. Qin, D. Li, S. Li and Y. Li. (2023). Genome-Wide Identification of the PP2C Gene Family and Analyses with Their Expression Profiling in Response to Cold Stress in Wild Sugarcane. Plants,12(13):2418. https://doi.org/10.3390/plants12132418.

Kratsch, H. A and R. R. Wise (2000). The ultrastructure of chilling stress. Plant Cell Environ,3(4):337-350. https://doi.org/10.1046/j.1365-3040.2000.00560.x.

Li, X., Q. Liu, R. Wu, J. Bing, L. Zheng, B. Sumbur, Y. Zhou and F. Gao (2023). Proteomic Analysis of the Cold Stress Response of Ammopiptanthus mongolicus reveals the role of AmCHIA in Its Cold Tolerance. Horticulturae,9(10):1114. https://doi.org/10.3390/horticulturae9101114.

Lopez-Carbonell, M., L. Alegre and H. Van Onckelen (1994). Changes in cell ultrastructure and endogenous abscisic acid and indole-3-actic acid concentrations in Fatsia japonica leaves under polyethylene glycol-induced water stress. J Plant Growth Regul,15:165-174. https://doi.org/10.1007/BF00024106.

Lutz, C and L. Engel (2007). Changes in chloroplast ultrastructure in some high−alpine plants: adap−tation to metabolic demands and climate? Protoplasma, 231:183–192. https://doi.org/10.1007/s00709-007-0249-8.

Muench, D. G and R. T. Mullen (2003). Peroxisome dynamics in plant cells: a role for cytoskeleton. Plant Science,164: 307–315. https://doi.org/10.1016/S0168-9452(02)00426-0.

Rehman, S. U., K. Muhammad, Y. Que, A. U. Rehman, E. Novaes and S. Khan (2019). Presence of seventeen genes potentially involved in cold tolerance in sugarcane and *Saccharum spontaneum* genotypes. Int J Biosci,14(1):346-355. http://dx.doi.org/10.12692/ijb/14.1.346-355.

Rehman, S. U., Y. Que, S. Khan, E. Novaes and K. Muhammad (2020). Molecular study of CBF and WCOR14 genes in selected sugarcane cultivars and its wild relative *Saccharum spontaneum* L. J Anim Plant Sci,30(4): 913-922. http://dx.doi.org/10.36899/JAPS.2020.4.0107

Rehman, S. U., K. Muhammad, A. C. M. Porto, Q. Youxiong and E. Novaes (2022). A view of transcriptome during cold stress in sugarcane using *Saccharum spontaneum* genome. Not Bot Horti Agrobo,50(3):12765-12765. https://doi.org/10.15835/nbha50312765.

Robinson, M. D. and A. Oshlack (2010). A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol,11(3):1-9. https://doi.org/10.1186/gb-2010-11-3-r25.

Robinson, M. D and G. K. Smyth (2008). Small-sample estimation of negative binomial dispersion, with applications to SAGE data.

Biostatistics,9(2):321-332. https://doi.org/10.1093/biostatistics/kxm030.

Robinson, M. D., D. J. Mccarthy and G. K. Smyth (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics,26(1):139-140. https://doi.org/10.1093/bioinformatics/btp616.

Shaw, D.J and J. C. Gray (2011). Visualization of stromules in transgenic wheat expressing a plastid−targeted yellow fluorescent protein. *Planta.* 233: 961–970. https://doi.org/10.1007/s00425-011-1351-x.

Shiyun, T., Y. Litao and L. Yangrui (2018). Compartive Analysis of Transcriptomes of different sugarcane varieties under low temperature stress. Biotechnol Bull,34(12):116-124. https://doi.org/10.13560/j.cnki.biotech.bull.1985.2018-0522.

Singh, S., M. D. Lazzaro and B. Walles (1998). The nuclear reticulum in placental cells of Lilium regale is a part of endomembrane system. Protoplasma,2003:144–152. https://doi.org/10.1007/BF01279471.

Tian, T., Y. Liu, H. Yan, Q. You, X. Yi, Z. Du, W. Xu and Z. Su (2017). Agrigo v2.0: a GO analysis toolkit for the agricultural community, 2017 update. Nucleic Acids Res,45(W1):W122-W129. https://doi.org/10.1093/nar/gkx382.

Zhang, J., X. Zhang, H. Tang, Q. Zhang, X. Hua, X. Ma, F. Zhu, T. Jones, X Zhu, J Bowers, C.M. Wai, C. Zheng, Y. Shi S. Chen, X. Xu, J. Yue, D. R. Nelson, L. Huang, Z. Li, H. Xu, D. Zhou, Y. Wang, W. Hu, J. Lin, Y. Deng, N. Pandey, M. Mancini, D. Zerpa, J. K. Nguyen, L. Wang, L. Yu, Y. Xin, L. Ge, J. Arro, J. O. Han, S. Chakrabarty, M. Pushko, W. Zhang, Y. Ma, P. Ma, M. Lv, F. Chen, G. Zheng, J. Xu, Z. Yang, F. Deng, X. Chen, Z. Liao, X. Zhang, Z. Lin, H. Lin, H. Yan, Z. Kuang, W. Zhong, P. Liang, G. Wang, Y. Yuan, J. Shi, J. Hou, J. Lin, J. Jin, P. Cao, Q. Shen, Q. Jiang, P. Zhou, Y. Ma, X. Zhang, R. Xu, J. Liu, Y. Zhou, H. Jia, Q. Ma, R. Qi, Z. Zhang, J. Fang, H. Fang, J. Song, M. Wang, G. Dong, G. Wang, Z. Chen, T. Ma, H. Liu, S. R. Dhungana, S. E. Huss, X. Yang, A. Sharma., J. H. Trujillo, M. C. Martinez, M. Hudson, J. J. Riascos, M. Schuler, Li-Qing Chen, D. M. Braun, L. Li, Q. Yu, J. Wang, K. Wang, M. C. Schatz, D. Heckerman, M. A. V. Sluys, G. M. Souza, P. H. Moore, D. Sankoff, R. VanBuren, A. H. Paterson, C. Nagai and R. Ming (2018). Allele-defined genome of the autopolyploid sugarcane *Saccharum spontaneum* L. Nat Genet,50(11):1565-1573. https://doi.org/10.1038/s41588-018-0237-2.

Zhu, J., C. H. Dong and J. K. Zhu (2007). Interplay between cold-responsive gene regulation, metabolism and RNA processing during plant cold acclimation. Curr Opin Plant Biol,10:290–295. https://doi.org/10.1016/j.pbi.2007.04.010.

Zeng, L., X. Zhou, X. Fu, Y. Hu, D. Gu, X. Hou, F. Dong, and Z. Yang (2023). Effect of the biosynthesis of the volatile compound phenylacetaldehyde on chloroplast modifications in tea (Camellia sinensis) plants. Horticulture Research,10(3): 003. https://doi.org/10.1093/hr/uhad003.