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Decoding the Transcriptomic Response of Cucumber (*Cucumis sativus*) Roots to Waterlogging

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Abstract

Waterlogging induces growth and developmental changes in cucumbers during early plant development. In this study, the transcriptional response of cucumber (*Cucumis sativus*) roots to waterlogging stress was investigated using RNA-Seq. Analysis of RNA-Seq data (PRJNA678740) from twelve samples using the Galaxy platform (https://usegalaxy.org) showed a clear separation between control and waterlogging treatments in principal component analysis (PCA). Our analysis revealed 15 genes with significantly altered expression levels, exceeding a 256-fold increase under stress conditions. Key identified genes were the defensin gene *Ec-AMP-D2*, several expansin-like genes involved in cell wall modification, the signaling-related ankyrin repeat-containing protein ITN1, and early nodulin-93. The dramatic increase in the expression of expansin-like genes highlighted their pivotal role in cell wall adaptation. Their marked upregulation suggests a possible involvement in modifying cell wall properties as an adaptive response to the waterlogging conditions of cucumber roots. Also, significant increase in expression was observed in genes involved in cell wall remodeling (xyloglucan endotrans-glucosylase / hydrolase 26 protein), stress signaling (ERF098 transcription factor), oxidative stress response (peroxidase 5), and metabolic adaptation (*FHIT* gene). The results of our study provide a comprehensive understanding of the genetic pathways and molecular mechanisms involved in the response of cucumbers to waterlogging.

Keywords

Abiotic stress*, Cucumis sativus,* Differential expression analysis, RNA-Seq

Introduction

The physiological effects of waterlogging on cucumber plants are significant. Under waterlogging stress, cucumber plants exhibit inhibited growth, reduced leaf count, decreased leaf area, fresh mass, and dry mass. This stress also leads to a decline in net photosynthesis, which adversely affects the accumulation of biomass. However, the plant's capacity to produce adventitious roots in waterlogged conditions aids in gas diffusion and enhances its survival in low oxygen concentrations. A study has found potential genes associated with long-term tolerance to waterlogging. These genes are involved in processes such as improved glycolysis, the growth of adventitious roots, and the metabolism of amino acids (Kęska et al., 2021a). A further investigation examined the function of long non-coding RNAs (lncRNAs) in the response to waterlogging stress. It identified particular lncRNAs that play a role in developing tolerance to low oxygen levels in cucumber plants (Kęska et al., 2021b). Furthermore, studies have demonstrated that the *CsARN6.1* gene, which encodes a protein containing an AAA ATPase domain, plays a pivotal role in the development of adventitious roots induced by waterlogging (Xu et al., 2023). Finally, microRNA sequencing revealed differentially expressed miRNAs involved in waterlogging-triggered adventitious root primordia formation, with target genes related to cell redox homeostasis, cytoskeleton, photosynthesis, and cell growth (Jiang et al., 2023). The objective of this study was to identify and analyze the differentially expressed genes (DEGs) in response to stress conditions in both sensitive and tolerant varieties of cucumber. The focus was on comparing the gene expression profiles between the stress and control samples. By conducting RNA-Seq analysis, this research aimed to compile a comprehensive list of DEGs that exhibit altered expression levels under stress conditions compared to their respective controls in both sensitive and tolerant varieties.

Materials and methods

Sequencing files of twelve cucumber root samples (*Cucumis sativus*) were obtained from the SRA (Sequence Read Archive) platform with accession number PRJNA678740, sequenced using the NEBNext UltraTM RNA Library Kit (Illumina). Among these samples, six were under control conditions, while six were subjected to waterlogging stress, comprising three samples from a sensitive variety and three from a tolerant variety. The Galaxy platform (https://usegalaxy.org) was utilized for RNA-Seq data analysis. Initially, quality control checks for the raw sequencing data were conducted using the FastQC tool. The results indicated satisfactory data quality, eliminating the need for preprocessing or trimming of the raw expression data. Annotation files and the reference genome of cucumber were downloaded from the Ensembl Plants database (https://plants.ensembl.org/info/data/ftp/index.html). The reads were aligned to the cucumber reference genome using the HISAT2 tool. Subsequently, quantification of gene expression was performed using the FeatureCounts tool with the cucumber genome annotation file. Raw counts were normalized in DESeq2 by estimating size factors, and differential expression testing was based on the DESeq2 output. For visualization and variance stabilization, transformed counts were generated using the variance-stabilizing transformation (VST). VST was used only for Principal Component Analysis (PCA) and did not affect the reported log2 fold changes or statistical tests. PCA was used to understand how the stress-treated cucumber samples are distinct from the control samples in the experiment. Genes exhibiting statistical significance with P-adj ≤ 0.0001 and log2 fold change ≥ 8 were considered as key differentially expressed genes. These stringent cut-offs were chosen to prioritize robust candidates for downstream validation.

Results and discussion

Principal component analysis (PCA) of normalized gene expression data in cucumber roots revealed a distinct separation between control (C) and waterlogging stress-treated (S) samples across the first and second components, explaining 92% of the variance (Figure 1). This separation underscores the profound impact of waterlogging stress on cucumber root gene expression profiles.

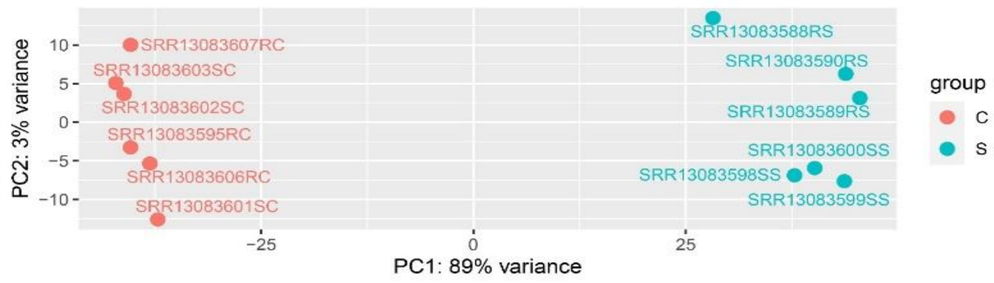

Figure 1- The PCA plot exhibits a distinct segregation between the control sample (C) and stress-treated sample (S) along the first and second principal components, which collectively explain 92% of the overall variation.

Using a threshold of adjusted p-value ≤ 0.0001, we identified 3713 differentially expressed genes, of which 2165 were upregulated and 1548 downregulated. Notably, 15 differentially expressed genes exhibited a strong response, with expression levels increasing more than 256-fold under waterlogging with regard to P-adj ≤ 0.0001 and log2 fold change ≥ 8 (Table 1). The defensin gene *Ec-AMP-D2*, known for its role in plant defense mechanisms, suggests a potentially enhanced protective response in cucumber roots subjected to waterlogging stress. A considerable proportion of the 15 genes were classified as expansin-like suggesting a possible involvement in modifying cell wall properties as an adaptive root response to the waterlogging. Expansins are proteins known for their involvement in the process of cell wall loosening during both growth- and stress-related reactions. The notable upregulation of *ITN1* in response to waterlogging suggests its potential involvement in cucumber's cellular adaptation mechanisms to cope with environmental stress. Ankyrin repeats facilitate interactions between proteins and are involved in various functions such as signalling, cell-cycle control, and stress responses (Zhao *et al*., 2020). The significant increase of early nodulin-93, typically associated with plant responses to symbiotic interactions, such as those with nitrogen-fixing bacteria in legumes, suggests a broader role in response to abiotic stress, which may contribute to the cellular adjustments necessary for survival under such conditions. Xyloglucan endotransglucosylase/hydrolases (*XTHs*) are enzymes involved in cell wall remodeling (Han *et al*., 2023) through the modification of xyloglucan polymers. The significant upregulation of this gene may reflect an active reorganization of cell wall architecture in response to waterlogging, crucial for maintaining cell integrity and function under stress conditions. The increased expression of stigma-specific STIG1-like protein 1 gene under waterlogging stress conditions may indicate a novel function in adaptation to abiotic stress in cucumber. The peroxidase 5 (*Prx5*), belonging to the peroxiredoxin family, is highly conserved in most organisms, scavenges ROS and protects cells from oxidative damage. The *Prx5* upregulation highlights its involvement in detoxifying reactive oxygen species and maintaining cellular homeostasis under waterlogging conditions. The significant expression of *ERF098* in response to waterlogging stress may suggest its involvement in modulating ethylene signaling pathways, which are crucial for plant adaptation and survival under adverse environmental conditions. Ethylene-responsive factors (*ERFs*) are known to play a crucial role in regulating plant responses to abiotic and biotic stresses. Bifunctional bis(5'-adenosyl)-triphosphatase/adenylyl sulfatase *FHIT*, belonging to the *FHIT* (Fragile Histidine Triad) family, plays a role in the process of purine metabolism. The increased expression of this gene in cucumber plants, when exposed to waterlogging stress, suggests a potential adaptive metabolic response. This response may help regulate energy and nucleotide balance in plants during periods of environmental stress. These findings highlight a complex response in cucumbers to waterlogging, involving genes related to defense, cell structure, stress signaling, and metabolism.

Gene Ontology and KEGG pathway enrichment analysis revealed distinct functional categories for the upregulated and downregulated DEGs. Among the upregulated genes, biological process (BF) were significantly enriched for *response to heat* (Count = 22, FDR = 3/42E-06), molecular function (MF) terms were significantly enriched for *RNA binding* (Count = 169, FDR = 7/9E-17), *zinc ion binding* (Count = 100, FDR = 0/000021), and *unfolded protein binding* (Count = 28, FDR = 0/000081), while cellular component (CC) enrichment was most prominent for *intracellular membrane-bounded organelle* (Count = 58, FDR = 6/07E-13). KEGG pathway analysis indicated enrichment for *Protein processing in endoplasmic reticulum* (P-value = 4.12E-11, FDR = 5.35E-09), highlighting the importance of protein processing under waterlogging stress. For downregulated genes, biological process (BP) were significantly enriched for *microtubule-based movement* (Count = 23, FDR = 6/65E-09) , *intracellular protein transport* (Count = 39, FDR = 1/05E-07), and *nucleosome assembly* (Count = 17, FDR = 4/89E-06), molecular function (MF) terms were significantly enriched for *microtubule binding* (Count = 44, FDR = 9/66E-17), *protein heterodimerization activity* (Count = 28, FDR = 6/81E-10), while cellular component (CC) enrichment was most prominent for *nucleosome* (Count = 27, FDR = 6/07E-13), *proteasome core complex (*Count = 12, FDR = 2/18E-07). KEGG pathway analysis indicated significant downregulation for both the Proteasome (P-value = 2.15E-13, FDR = 2.51E-11) and Citrate cycle (TCA cycle) (P-value = 1.19E-06, FDR = 6.98E-05) pathways, underscoring the importance of these metabolic and degradation processes under waterlogging stress. These results suggest that waterlogging stress triggers a survival-oriented cellular reprogramming, shifting resources from routine growth and metabolism (e.g., TCA cycle, cytoskeleton function) towards essential stress protection mechanisms (e.g., protein homeostasis, RNA binding). This indicates a fundamental trade-off between maintenance and defense under anaerobic stress. This RNA-Seq study identified 15 highly upregulated genes in cucumber roots under waterlogging stress, revealing a complex molecular response involving defense, cell wall modification, and signaling for adaptation.

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