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Transcriptomic Analysis of Leaf Tissues in *Capsicum annuum L.* Subjected to Drought Stress

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Abstract

Drought stress presents a significant risk to the viability of *Capsicum annuum L.*, compelling the plant to execute a very intricate molecular response. This study seeks to delineate the transcriptome profile of chilli pepper leaf tissues subjected to severe water scarcity in order to uncover critical genetic factors that facilitate adaptation. Gene expression profiles were comparatively assessed between control and treatment groups using an RNA-Seq method and differential analysis with DESeq2 software. Quantitative data analysis demonstrated significant increase of transcriptional regulation in the plastid translational apparatus. The loci encoding chloroplast ribosomal RNA components, specifically C329_r008 (log2 FC 9.38), demonstrated the most significant up-regulation. This condition signifies the hyperactivation of de novo chloroplast repair mechanisms to mitigate photosystem breakdown resulting from the accumulation of Reactive Oxygen Species (ROS). Moreover, an unexpected defensive aspect was identified through the heightened responsiveness of secondary genetic elements. Non-coding RNAs (e.g., LOC107852800) and several pseudogenes had a pronounced response, hypothesised to act as molecular sponges that mitigate post-transcriptional suppression. This genomic mapping effectively discovered clusters of orphan genes whose roles remain uncharacterized, yet have a high level of relevance (P-adj < 0.05) for cellular defence. This reorganisation of molecular architecture demonstrates that chilli pepper adaptation is not exclusively governed by traditional protein-coding genes. The discovery of these non-coding regulatory elements and dark loci profoundly alters the paradigm of genetic selection, offering new and vital biomarker resources for the development of future breeding programmes for chilli pepper cultivars resistant to climate anomalies.

Keywords: *Capsicum annuum*; drought stress; non-coding RNA; uncharacterized genes; RNA-Seq.

1.Introduction

Chilli peppers (*Capsicum annuum L.)* are a significant commodity within Indonesia's agricultural framework. The escalating severity of environmental challenges due to climate change, such as drought, presents a significant barrier to their production, especially on poor terrain. Chilli peppers, being a high-value commercial crop, operate both as a flavour enhancer and as a raw material for the pharmaceutical and functional food sectors. In agriculture, several abiotic pressures result in substantial and progressively extensive production reductions attributable to the effects of global climate change (Stefanelli *et al.*, 2010). Chilli peppers are rich in useful elements such as carbohydrates, minerals, proteins, amino acids, antioxidants, phytochemicals, and vitamins (Olatunji & Afolayan, 2018). In recent years, the demand for healthy, nutrient-dense, environmentally sustainable, and palatable chilli peppers has surged; consequently, production has been steadily escalating in various global places. Global production of dried chilli peppers rose

from 1.4 million tonnes in 1980 to 4.6 million tonnes in 2017 (FAO, 2019). Drought is the primary abiotic element influencing plant growth and development (Ferrara *et al.*, 2011).

Plants have evolved intricate defence systems at the organelle level to guarantee their survival in conditions of water scarcity. Leaves, being the hub of metabolic and photosynthetic processes, function as the principal sensors for identifying water scarcity signals and promptly activate adaptive responses by modulating stomatal conductance. Plants subjected to water scarcity exhibit alterations at the cellular and molecular levels, characterised by diminished growth rates, lower leaf area, and an elevated root-to-shoot ratio (Nio Song & Banyo, 2011). Water scarcity directly impedes photosynthesis by restricting the diffusion of CO_2 into drought-affected leaves due to diminished conductivity, especially in the stomatal and mesophyll tissues (Ntanasi *et al.*, 2025). Numerous transcription factor genes associated with plant stress responses display both unique and overlapping expression profiles, underscoring the complexity, specificity, and interconnectivity of plant gene regulatory networks (Bray, 2004).The transcriptome refers to the complete collection of RNA molecules transcribed from a single tissue or cell at a distinct functional or developmental phase, encompassing both mRNA (messenger RNA) and ncRNA (non-coding RNA). Consequently, comprehension of transcriptomics enhances studies pertaining to the functioning of cells, organs, and organisms (Tyagi *et al.*, 2022).

Despite extensive documentation of genes that encode functional proteins, our comprehension of non-protein regulatory factors in relation to abiotic stress is still somewhat constrained. Most prior transcriptome investigations have generally neglected sites regarded as genomic "junk" elements, including pseudogenes and non-coding RNAs (ncRNAs). Recent high-throughput transcriptome investigations have demonstrated that eukaryotic genomes transcribe as much as 90% of genomic DNA. Merely 1–2% of these transcripts encode proteins, whereas the predominant portion is produced as non-coding RNA (ncRNA) (Kaikkonen *et al.*, 2011). Recent scientific research indicates that ribosomal units, such as rrn23, and numerous non-coding regulatory elements are essential in influencing the expression of target genes. Concentrating on the characterisation of these unexplored elements is a strategic approach to elucidating the regulatory framework of the chilli pepper genome.

This study seeks to investigate the functional capabilities of previously uncharacterized genes in response to drought stress in chilli pepper plants. Bioinformatics analysis was performed utilising a secondary transcriptome dataset from NCBI (BioProject: PRJNA668245) to discover Differentially Expressed Genes (DEGs) in leaf tissue. This study primarily analyses the up-regulation patterns of the rrn23 gene, ncRNA, and pseudogenes to offer new insights into plant energy conservation methods at the transcriptional level. This study's results are anticipated to provide a robust scientific basis for the future generation of drought-tolerant chilli pepper variants via genome editing technology.

2. Materials and Methods

This research employed a secondary transcriptome dataset sourced from the National Centre for Biotechnology Information (NCBI) database, accession number BioProject PRJNA668245. This dataset comprises RNA-Seq expression profiles from the leaf tissues of chilli pepper (*Capsicum annuum L.*) subjected to control and drought stress conditions. All raw data in Sequence Read Archive (SRA) format were processed utilising the Galaxy platform to guarantee reproducible analytical standards. The initial steps were quality control of sequences by FastQC, thereafter matching the reads to the C. annuum reference genome (UCD10Xv1.1) utilising the HISAT2 algorithm. Transcript abundance quantification was conducted to provide a raw read count matrix for subsequent statistical analysis. Differentially expressed genes (DEGs) were found with the DESeq2 programme. The assessed statistical metrics were normalised mean expres-

sion (Base mean), expression change (log2 Fold Change), standard error (StdErr), statistical test values (Wald-Stats), significant level (P-Value), and adjusted significance value (P-adj). The significance level was established at P-adj < 0.05. The ten genes with the highest log2 Fold Change values (Top 10 DEGs) were identified to elucidate the plant's principal molecular response to water scarcity.

The functional annotation of the candidate genes was performed using a homology-based approach using the Phytozome v14 genomic portal. BLASTP analysis was specifically applied for protein-coding genes versus curated protein databases, while BLASTN was utilised for non-coding elements. The protein domain design was verified using the SMART (Simple Modular design Research Tool) software to identify certain functional characteristics, such as transcription factors and catalytic domains. All visual representations of the differential expression analysis results were generated using the R programming language, version 4.3.3, in the RStudio environment. Transcriptomic data were subjected to logarithmic transformation to stabilise variance prior to hierarchical clustering. Heatmaps were generated using the pheatmap software, with the colour scale determined by Z-scores to illustrate relative expression profiles across samples. The definitive results, including a list of genes and all statistical variables, were exported in .csv format for the preparation of scholarly manuscripts.

3. Results

An extensive analysis of RNA-Seq profiles utilising DESeq2 software revealed substantial changes in gene expression in the leaf tissues of Capsicum annuum due to acute water scarcity. The quantitative data indicated a distinct profile marked by substantial up-regulation of most critical genes. This transcriptional activation pattern functions as an initial biological signal indicating that plants actively respond to stress by augmenting particular biological activities for molecular defence and cellular flexibility.

Table 1 presents the ten genes demonstrating the most pronounced transcriptional changes, referred to as the Top 10 Differentially Expressed Genes (DEGs). This table encapsulates the normalised mean expression (Base mean), the expression alteration (log2 Fold Change) to denote the extent of up-regulation, and the adjusted significant value (P-adj < 0.05) using the Benjamini-Hochberg false discovery rate (FDR) approach. All 10 potential genes displayed positive log2 Fold Change values, signifying robust transcriptional activation in response to the drought therapy.

Table 1. Catalogue of principal gene candidates and their significance scores pertaining to drought stress response in chilli plants.

Gene ID	Gen Function	Base Mean	log2 Fold Change	P-adj
C329_r002	Encodes 23S ribosomal RNA, a structural element of the major subunit of the plastid ribosome (rrn23)	149.2009	6.0747	0.017
C329_r007	Encodes 23S ribosomal RNA, a structural element of the major subunit of the plastid ribosome (rrn23)	359.017	7.162	0.0208
C329_r008	Encodes 16S ribosomal RNA (rrn16), a crucial element of the small subunit of the chloroplast ribosome involved in the start of protein synthesis	304.219	9.386	0.0002
LOC107852325	Encodes an uncharacterized protein; demonstrates heightened sensitivity to drought stress	35.861	7.779	0.016
LOC107852397	Encodes an uncharacterized protein; demonstrates heightened sensitivity to drought stress	34.94	5.2116	0.009

Gene ID	Gen Function	Base Mean	log2 Fold Change	P-adj
LOC107852464	Encodes an uncharacterized protein; demonstrates heightened sensitivity to drought stress	655.08	5.312	0.003
LOC107852465	Encodes an uncharacterized protein; demonstrates heightened sensitivity to drought stress	895.848	5.041	0.003
LOC107852800	An uncharacterized non-coding RNA (ncRNA) believed to be involved in the transcriptional control of the stress response.	1621.306	5.039	0.0036
LOC107852871	A pseudogene with an uncharacterized function, thought to be involved in the regulation of gene expression during stress responses.	1866.29	5.025	0.0092
LOC107853112	A pseudogene with an uncharacterized function, thought to be involved in the regulation of gene expression during stress responses.	1031.24	4.452	0.0049

A significant activation was noted in genes related to the plastid translational machinery among the principal differentially expressed genes (DEGs). Further analysis of the NCBI Gene database revealed the C329_r002 and C329_r007 loci as the rrn23 gene, which encodes the 23S ribosomal RNA, crucial for peptidyl transferase activity in organelle protein synthesis. The rrn23 gene exhibited a significant increase in expression (log2 FC 6.07; P-adj = 0.017, and log2 FC 7.16; P-adj = 0.0208, respectively). The greatest substantial increase was observed in the C329_r008 locus (log2 FC 9.38; P-adj = 0.0002), which encodes the 16S ribosomal RNA, an essential structural element of the small subunit of the chloroplast ribosome. This simultaneous increase suggests that the plant markedly enhances its chloroplast protein synthesis capacity, perhaps as a restorative mechanism to replace proteins compromised by oxidative stress.

In addition to the ribosomal components, numerous genes producing uncharacterized proteins exhibited a significant response to water constraint. The loci comprise LOC107852325 (log2 FC 7.77; P-adj = 0.016), LOC107852397 (log2 FC 5.21; P-adj = 0.009), LOC107852464 (log2 FC 5.31; P-adj = 0.003), and LOC107852465 (log2 FC 5.04; P-adj = 0.003). Despite the absence of exact functional annotations for these proteins in the NCBI database, their substantial increase in expression indicates a vital, yet unexamined, functional involvement in the plant's molecular adaptation and survival strategies under extreme drought conditions.

Moreover, the research underscored the active involvement of secondary genetic factors. The non-coding RNA (ncRNA) locus, LOC107852800, exhibited a significant elevation in expression (log2 FC 5.03; P-adj = 0.0036). Although its molecular mapping is incomplete, this significant up-regulation clearly suggests its function as a regulatory factor influencing target genes in the physiological defence system. Furthermore, two pseudogenes, LOC107852871 (log2 FC 5.02; P-adj = 0.0092) and LOC107853112 (log2 FC 4.45; P-adj = 0.0049), demonstrated notable transcriptional activity. This overexpression indicates their likely role as non-coding regulators that coordinate the cellular response to mitigate drought stress.

A hierarchical clustering technique was employed to visually assess the consistency and distribution of these gene expression patterns, shown as a heatmap (Figure 1). This visualisation features columns that reflect separate biological replicates for both control (Control_1, 2, 3) and drought stress conditions (Drought_1, 2, 3), with rows indicating individual top differentially expressed genes. The colour gradient represents normalised relative expression levels (Z-scores), with deep red indicating high transcript

abundance (up-regulation) and deep blue indicating low baseline expression (down-regulation). The dendrogram lines depict the statistical proximity of expression patterns among the genes on the left axis and the samples on the top axis.

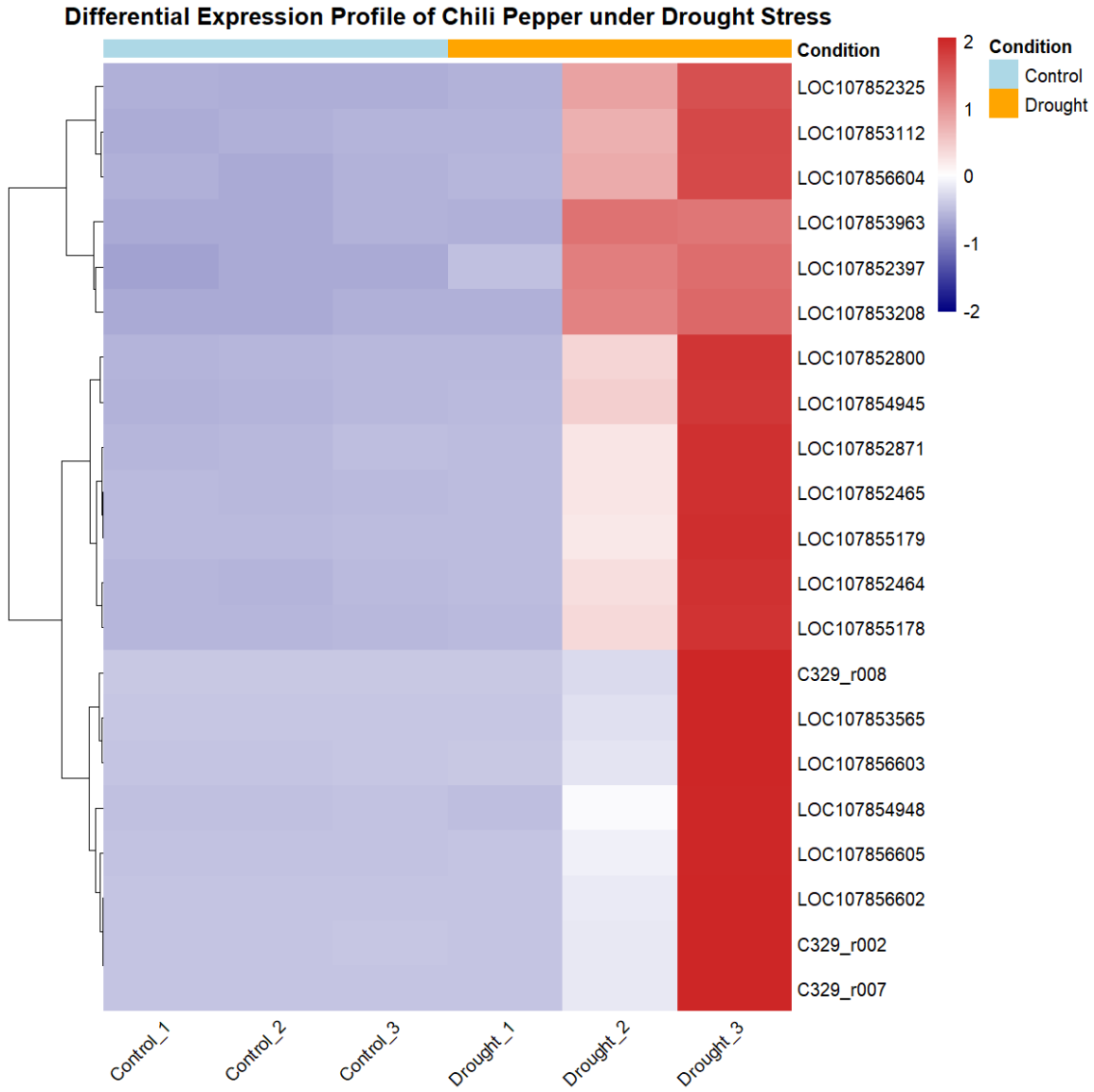

Figure 1. Hierarchical clustering and heatmap of expression profiles for the ten most differentially expressed genes (Top 10 DEGs) in C. annuum leaves subjected to drought stress. Columns denote biological replicates for the control (Control_1, Control_2, Control_3) and drought stress (Drought_1, Drought_2, Drought_3). Rows denote distinct genes. The colour gradient denotes levels of expression.

The heatmap analysis demonstrated a significant and consistent difference in expression profiles between the treatments. The sample dendrogram at the top demonstrates full and perfect cluster segregation among the treatment groups, consolidating the three biological replicates for each condition into the same clade. This confirms a significant level of data repeatability, indicating that the transcriptional changes are a purposeful reaction to the treatment rather than incidental technological fluctuation. The control group is constantly represented by blue hues, indicating modest basal expression, while the drought-stressed group displays prominent red hues, signifying substantial transcript accumulation. Additionally, the gene dendrogram on the left axis illustrates robust co-expression patterns, indicating that the loci encoding chloroplast ribosomal components (C329 group) are tightly grouped with non-coding RNA elements and uncharacter-

ized proteins. This alignment signifies that these genes function within a coordinated signalling network utilised by the plant for cellular defence and chloroplast repair under water constraint.

4. Discussion

Transcriptomic investigation of chilli pepper plants (*Capsicum annuum*) under drought stress conditions demonstrated highly coordinated molecular adaption processes. This study's principal findings reveal that chilli plants demonstrate a significant reaction marked by the pronounced overexpression of genes associated with the plastid translational machinery, specifically the C329_r008, C329_r007, and C329_r002 loci. These loci encode the 16S and 23S rRNA components, which are essential for ribosome biosynthesis and organelle structural function. Both 16S and 23S rRNA are essential for ribosome biogenesis (Sergiev *et al.*, 2018). In water-scarce situations, the restriction of carbon absorption caused by stomatal closure frequently induces the generation of reactive oxygen species (ROS). Malondialdehyde (MDA), the final result of lipid peroxidation due to reactive oxygen species (ROS), can function as a drought indicator to evaluate plasma membrane damage and a plant's capacity to endure drought stress. (Rosawanti *et al.*, 2015; Zhang *et al.*, 2021). Chilli plants enhance the formation of new ribosomes to expedite de novo protein synthesis, crucial for replacing photosystem protein complexes that deteriorate under stress conditions.

This approach underscores the significant impact of secondary genetic elements alongside genes that encode functional proteins. The identification of non-coding RNA (LOC107852800) and pseudogenes (LOC107852871, LOC107853112) that are markedly up-regulated corresponds with these findings Baruah *et al.* (2021) It effectively identified thousands of lncRNAs that respond to diverse abiotic stressors in *Capsicum annuum*. Non-coding RNAs (ncRNAs) serve as regulatory entities that modulate gene expression via precise interactions with DNA, RNA, and proteins. Long non-coding RNAs (lncRNAs), generally exceeding 500 nucleotides in length, participate in cellular mechanisms that govern several biological processes by modulating gene expression at various levels: transcriptional, post-transcriptional, and post-translational (Mattick *et al.*, 2023). Conversely, microRNAs (miRNAs), often measuring 20–25 nucleotides, predominantly operate in post-transcriptional gene silencing by attaching to messenger RNA (mRNA) to obstruct its translation or facilitate its destruction (Pooresmaeil *et al.*, 2025).

This study effectively conducted transcriptome mapping of a series of uncharacterized proteins with substantial significance (P-adj < 0.05) and considerable fold alterations. The elevated transcriptional activity at locations such as LOC107852464, LOC107852397, LOC107852465, and LOC107852325 suggests that the *Capsicum annuum* genome contains several uncharacterized genes with undefined roles that are crucial for survival in harsh settings. Prior comparative studies examining the transcriptome profiles of drought-resistant and drought-sensitive chilli pepper cultivars have experimentally revealed a range of stress-responsive genes. The mapping indicates that most targets are associated with protein-coding genes lacking explicit functional annotations, however their presence is significantly correlated with drought tolerance. This study offers preliminary molecular insights into drought tolerance in *Capsicum annuum* and produces significant transcriptome data concerning its response to water scarcity. While these results offer significant direction for future investigations into drought tolerance mechanisms, the essential intrinsic processes governing the drought resistance of chilli pepper plants remain to be thoroughly clarified. The finding of this gene cluster presents substantial opportunity for additional functional genomic study and may serve as potential biomarker candidates in future drought-tolerant chilli pepper breeding programmes.

5. Conclusions

The adaptive response of *Capsicum annuum* to drought stress is mediated by a molecular network that transcends traditional functional gene boundaries. This study establishes that the survival of chilli plants is significantly dependent on emergency chloroplast repair mechanisms. The hyperactivation of plastid ribosomal genes, especially the C329 locus, is vigorously initiated to expedite de novo protein synthesis for the restoration of the photosynthetic apparatus damaged by oxidative stress. Moreover, the existing defence system depends on ancillary genetic elements. Significant increases in non-coding RNA elements,

pseudogenes, and orphan gene clusters demonstrate the presence of a hitherto neglected hierarchical stress signalling mechanism. This assemblage of uncharacterized locations may no longer be regarded as mere transcriptional leftovers. Their identification fundamentally alters the framework for recognising biomarker candidates, supplying crucial primary genetic material for the accurate development of future chilli pepper types resistant to climatic irregularities.

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