



# Transcriptomic Analysis of Maize Root and Leaf Responses to Heat Stress at the Seedling Stage

Dinta Navy Apriliani<sup>1\*</sup>

<sup>1</sup> Study Program of Biology, Faculty of Military Mathematics and Natural Sciences, The Republic of Indonesia Defense University. Komplek Indonesia Peace and Security Center (IPSC) Sentul, Bogor 116810, Indonesia.

\* Correspondence: dintaaufgabe@gmail.com

## Abstract

Acute thermal stress severely limits maize (*Zea mays*) productivity, requiring a systemic understanding of organ-specific adaptive mechanisms. This study integrated transcriptomic data from two independent BioProjects, PRJNA1335774 (root) and PRJNA947789 (leaf), to delineate the molecular responses of maize seedlings to heat. A rigorous bioinformatic pipeline was employed, involving trimmomatic for quality control, HISAT2 for genomic alignment, and pheatmap for hierarchical clustering. Principal component analysis (PCA) revealed that heat stress at 42 °C was the primary driver of transcriptional variation, explaining 92% of the variance in roots and 80% in leaves, significantly outweighing tissue-specific signatures. Volcano plots identified thousands of high-confidence differentially expressed genes (DEGs) with extreme statistical significance. Hierarchical clustering confirmed a massive "transcriptional reset," characterized by the induction of a conserved heat-stress response (HSR) module and the simultaneous suppression of primary metabolic pathways. While the systemic response was dominant, roots exhibited a more synchronized molecular shift, whereas leaves showed higher secondary variability (16% on PC2), likely reflecting the complex demands of photosynthetic regulation. These findings demonstrate that maize seedlings prioritize cellular protection over growth through a coordinated growth-to-defense tradeoff. The identified DEGs and conserved modules provide robust genetic targets for molecular breeding and genome editing to enhance thermotolerance in maize cultivars.

**Keywords:** Climate resilience, differential gene expression, heat stress response, maize, meta-analysis, organ-specific adaptation, transcriptomics.

## 1. Introduction

As Indonesia's second most vital food crop after rice, maize (*Zea mays* L.) is a multi-purpose commodity integrated into traditional farming for carbohydrate production, industrial food processing, and animal feed (Fitrawati et al., 2023). Its robust agribusiness potential underscores its dual role as a high-value forage source and a strategic food substitute. However, the productivity of this essential crop is increasingly threatened as global agriculture faces unprecedented challenges due to the rising frequency and intensity of extreme temperature events driven by anthropogenic climate change (Vogel et al., 2019; Zhao et al., 2017). Among the major cereal crops, maize is particularly susceptible to thermal fluctuations, especially during the critical seedling stage, where establishment determines final yield potential (El-Sappah et al., 2022). Heat stress induces a wide array of physiological and biochemical perturbations, including proteotoxic stress, reactive oxygen species (ROS) accumulation, and the impairment of photosynthetic efficiency (Ma et al., 2025). Consequently, understanding the molecular mechanisms underlying heat tolerance in maize is paramount for developing resilient cultivars that can sustain global food security.

This systemic response to thermal stress involves the coordinated regulation of thousands of genes (Jacob et al., 2017). Although previous studies have extensively documented the Heat Stress Response

(HSR) pathway, characterized by the rapid induction of heat shock transcription factors (HSFs) and the subsequent synthesis of heat shock proteins (HSPs), which act as molecular chaperones (Qu et al., 2013). However, most transcriptomic investigations have focused on either leaf tissues or root systems in isolation. Given the distinct anatomical and functional roles of roots and leaves, it is hypothesized that these organs employ divergent transcriptional strategies to mitigate thermal damage (Samat et al., 2025). Specifically, while leaves must manage evaporative cooling and photosynthetic pigment stability, roots are primarily tasked with maintaining hydraulic conductivity and nutrient signaling under elevated soil temperatures (Ahuja et al., 2010).

Despite significant progress in maize functional genomics, a high-resolution comparative analysis of the organ-specific transcriptomic landscapes during the early seedling stage remains limited. The integration of RNA-Sequencing (RNA-Seq) technology allows for a comprehensive quantification of the steady-state transcriptome, providing insights into the gene regulatory networks (GRNs) that orchestrate the heat stress response (Jain, 2024; Lei et al., 2025). By comparing the expression profiles of root and leaf tissues, we can identify "core" heat-responsive genes that are systemically regulated, as well as organ-specific modules that reflect localized physiological adaptations. This study conducted a global transcriptomic analysis of maize seedlings under acute heat stress. Our objectives were to (i) characterize the differential gene expression (DGE) patterns in both root and leaf tissues, (ii) identify key metabolic pathways and transcription factor families preferentially activated in each organ, and (iii) elucidate the hierarchical clustering of heat-responsive gene modules. The findings of this research provide a detailed molecular map of the maize heat response, offering potential genetic targets for the enhancement of thermotolerance through molecular breeding or biotechnological interventions (Janni et al., 2020; Varshney et al., 2021).

## 2. Materials and Methods

### 2.1. Data Acquisition and Sample Selection

The transcriptomic data utilized in this study were retrieved from the NCBI Sequence Read Archive (SRA) database, involving two distinct BioProjects to compare organ-specific responses. Root tissue transcriptomic profiles for maize roots were obtained from BioProject PRJNA1335774, specifically using accessions SRR35651885 and SRR35651884 for control conditions, then SRR35651874 and SRR35651873 for heat stress treatments. Meanwhile, leaf tissue data corresponding to leaf datasets were acquired from BioProject PRJNA947789, including accessions SRR23944931 and SRR23944930 for control conditions, SRR23944927 and SRR23944926 for heat stress. These datasets were integrated to facilitate a comprehensive meta-analysis of the maize seedling transcriptome under thermal perturbation.

### 2.2. Bioinformatic Pipeline and Quality Control

All computational analyses were conducted on the Galaxy platform to ensure a robust, transparent, and reproducible transcriptomic analysis. Raw sequence reads in FASTQ format were initially evaluated using FastQC (version 0.74) to identify potential quality issues, including adapter contamination and overrepresented sequences. To mitigate these issues, Trimmomatic (version 0.39) was employed for read filtering and trimming. This step involved the removal of Illumina adapter sequences and the trimming of low-quality bases (Phred score < 20) from both the 5' and 3' ends using a sliding window approach. Reads falling below a minimum length threshold were discarded to maintain the integrity of the subsequent alignment.

The high-quality, trimmed reads were aligned to the maize reference genome (B73 RefGen\_v2) using the splice-aware aligner HISAT2 (version 2.2.1). The alignment was performed with default parameters to optimize the mapping of reads spanning splice junctions, a critical requirement for accurate plant transcriptomics. Gene expression was quantified by counting the number of reads mapped to each genomic feature using featureCounts, generating individual count files for each biological replicate. These individual

files were consolidated into a comprehensive expression matrix using the Join (version 1.1.2) tool, which merged datasets based on the unique GeneID identifier. The consolidated matrix was further refined to ensure compatibility with the experimental design. The Cut (version 1.0.2) tool was applied to isolate the specific columns required for the study, filtering the matrix to the final eight samples (four root and four leaf samples). Column headers were standardized using the Add line to file tool to ensure perfect synchronization with the SampleID entries in the associated metadata.

Exploratory data analysis and visualization were performed using the Heatmap (version 1.0.12) package. Raw counts were log-transformed ( $\log_2(X + 1)$ ) to stabilize variance. Row-wise Z-score transformation (row scaling) was applied to visualize relative expression patterns across treatments. Hierarchical clustering was conducted using 1-correlation as the distance metric and Ward.D2 as the linkage method, allowing for the identification of tissue-specific and stress-responsive gene modules. Column-wise annotations for 'Tissue' and 'Condition' were integrated to visualize the relationship between transcriptional clades and experimental variables.

### 3. Results

#### 3.1. Data Quality Control and Transcriptome Mapping

The initial phase involved rigorous quality filtering of raw reads from BioProjects PRJNA1335774 and PRJNA947789 using Trimmomatic. FastQC analysis confirmed that the trimming process successfully eliminated adapter sequences and low-quality bases, maintaining high Phred scores across all samples. Mapping to the *Zea mays* B73 RefGen\_v2 genome via HISAT2 achieved high alignment rates, providing a robust foundation for downstream quantification.

#### 3.2. Global Transcriptomic Variation (PCA Plot)

To assess the biological reproducibility and the primary drivers of transcriptomic variation in maize roots, a Principal Component Analysis (PCA) was performed (Figure 1). The PCA plot shows a clear separation of samples by experimental treatment along PC1, which accounts for 92% of the total variance. Control samples (SRR35651884, SRR35651885) clustered tightly on the positive axis, while heat-stressed samples (SRR35651874, SRR35651873) were positioned on the negative axis. This dominant variance indicates that acute heat stress is the primary driver of transcriptional reprogramming in root tissues, far outweighing inherent stochastic biological variation. PC2 explained only 4% of the variance, indicating minor fluctuations between replicates. Complementing the PCA, the sample-to-sample distance matrix further confirms the dataset's high reliability (Figure 2). The heatmap exhibits intense blue coloration within per-treatment blocks, indicating that the transcriptomic distance between treatments significantly exceeds that between biological replicates. This high degree of reproducibility across both analyses validates the dataset's robustness for subsequent differential expression analysis.

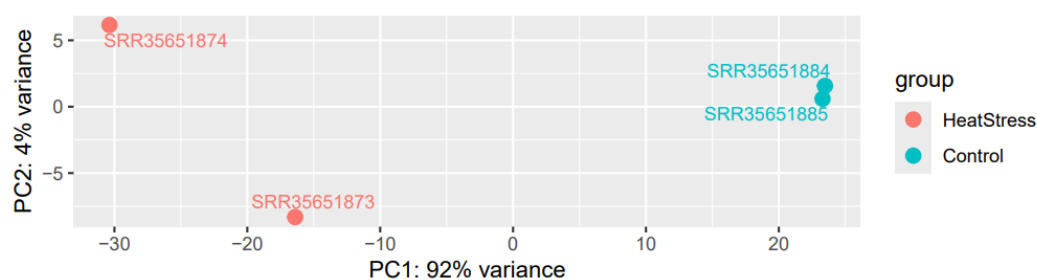


**Figure 1.** Principal Component Analysis (PCA) of the maize root transcriptome under heat stress

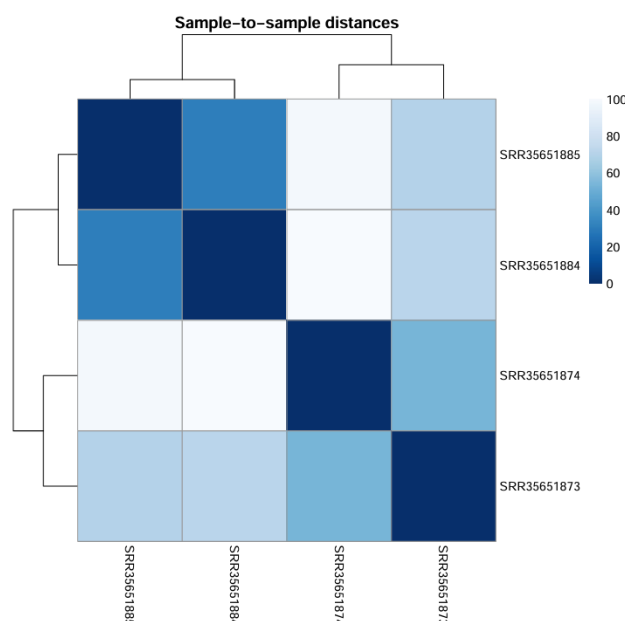


**Figure 2.** Sample-to-sample distance matrix of maize root transcriptomes

To evaluate the global transcriptomic variance and the quality of biological replicates within the leaf tissue datasets, a Principal Component Analysis (PCA) was performed (Figure 3). The ordination plot reveals a robust separation of samples primarily driven by the experimental treatment. Principal Component 1 (PC1) accounts for 80% of the total transcriptional variance, effectively partitioning the samples into two distinct groups: Control (positive axis) and Heat Stress (negative axis). This indicates that thermal perturbation is the dominant factor orchestrating large-scale transcriptional reprogramming in maize leaf tissues. Principal Component 2 (PC2) explains 16% of the variance; the vertical dispersion along this axis suggests a higher degree of secondary transcriptional variability in leaves compared to roots, potentially reflecting complex physiological adjustments such as photosynthetic regulation and stomatal dynamics. Complementing these results, the sample-to-sample distance heatmap exhibits intense blue coloration within per-treatment blocks, confirming high internal consistency (Figure 4). Biological replicates for both Control (SRR23944930, SRR23944931) and Heat Stress (SRR23944927, SRR23944926) clustered tightly in both analyses, validating the dataset's reproducibility and its suitability for rigorous differential gene expression (DGE) analysis.

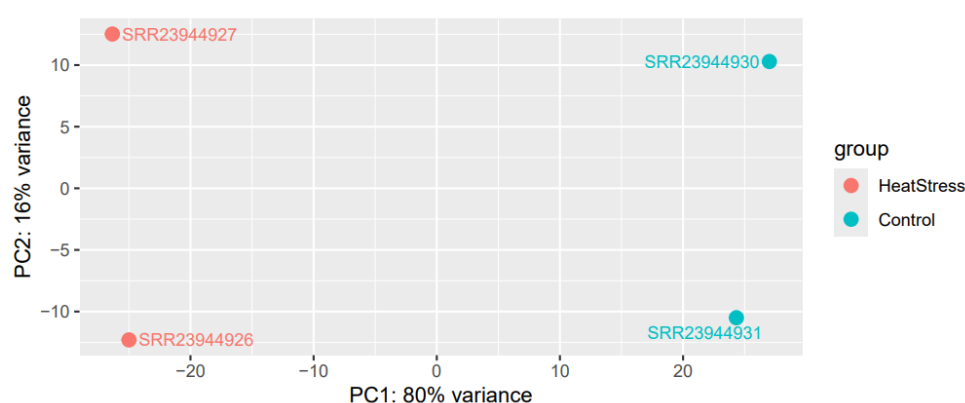


**Figure 3.** Principal Component Analysis (PCA) of maize leaf transcriptome under heat stress

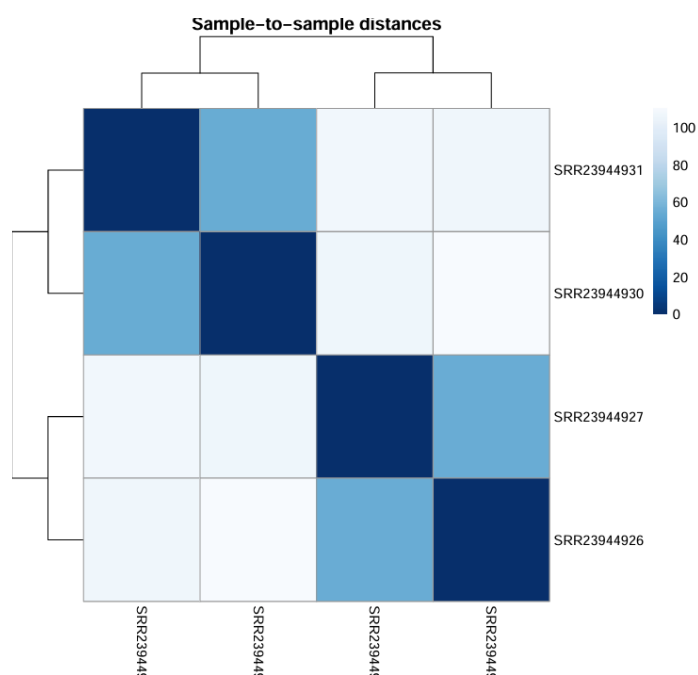


**Figure 4.** Sample-to-sample distance matrix for maize leaf transcriptome

### 3.3. Distribution of Gene Expression (Histogram Plot)

The statistical integrity of the differential expression results was assessed via a p-value histogram for the maize root comparison (Figure 5). The distribution exhibits a prominent anti-conservative profile, with a sharp peak at  $p < 0.05$  signaling a robust and widespread biological response to thermal stress rather than stochastic noise. The high density of genes approaching a p-value of zero confirms strong statistical power for DEG detection and downstream functional analysis. Furthermore, the baseline uniformity observed across the 0.2 to 1.0 range indicates a well-behaved null hypothesis, suggesting the absence of major systemic biases or confounding factors in the statistical model.

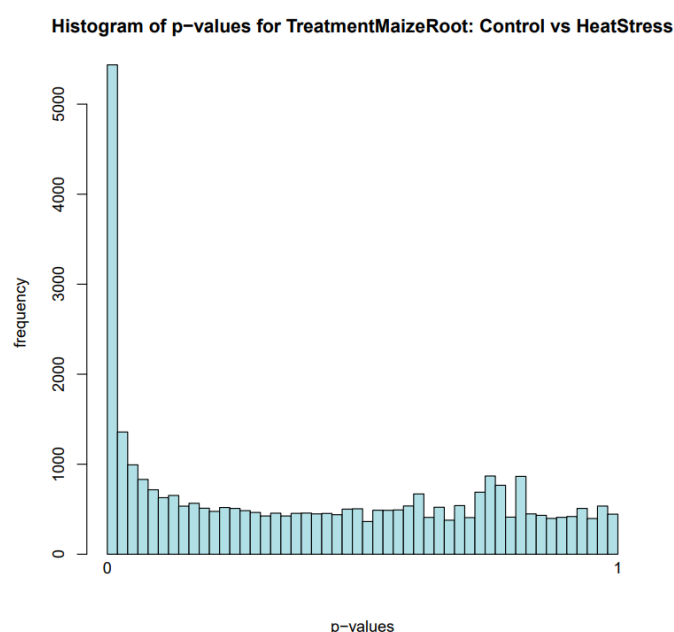


**Figure 5.** Distribution of p-values for differential gene expression analysis in maize roots

The statistical reliability and the distribution of differential expression results were assessed by generating a p-value histogram for the comparison between control and heat-stressed maize leaf tissues. The

resulting profile (Figure 6) serves as a diagnostic indicator of the biological signal strength relative to the underlying statistical null hypothesis. **Enrichment of Significant Features:** The histogram reveals a pronounced anti-conservative distribution, characterized by a sharp, high-frequency peak at the lowest p-value bin ( $p < 0.05$ ). This enrichment of significant p-values indicates that a large cohort of genes in the maize leaf transcriptome is actively responding to 42 °C thermal stress. The magnitude of this peak suggests that the observed transcriptional changes are driven by a robust biological response rather than random technical noise. **Statistical Signal Density:** A significant density of transcripts was observed with p-values approaching zero, confirming a high capacity for the detection of Differentially Expressed Genes (DEGs). This high signal density provides strong statistical support for downstream functional enrichment analyses and the identification of heat-responsive molecular pathways in the aerial organs. **Uniformity of Non-significant Features:** In the higher p-value ranges (0.2 to 1.0), the distribution maintains a predominantly uniform and flat profile. This behavior indicates that the statistical test is well-calibrated and that the data is free from major confounding factors or systemic biases that would otherwise distort the p-value frequencies.

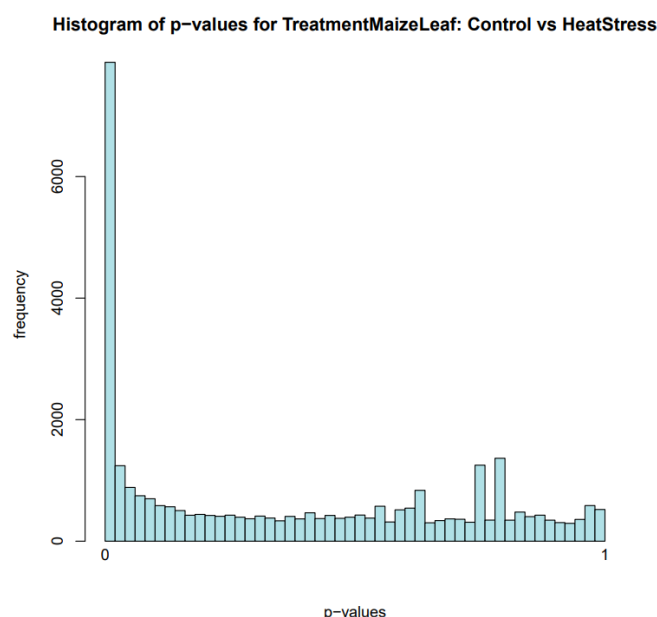


**Figure 6.** Diagnostic p-value distribution for maize leaf transcriptomic analysis

### 3.4. Identification of Differentially Expressed Genes (Volcano Plot)

The specific transcripts modulated by thermal stress in root tissues were identified using a volcano plot, which illustrates the relationship between the magnitude of change ( $\log_2$  Fold Change) and statistical significance ( $-\log_{10}$  p-value). The resulting distribution reveals a massive and balanced transcriptional shift, with thousands of genes crossing the significance thresholds. **Global Differential Expression Profile:** A substantial number of transcripts were identified as Differentially Expressed Genes (DEGs), defined by an absolute  $\log_2\text{FC} \geq 2$  and a  $-\log_{10}p \geq 1.3$  (equivalent to  $p < 0.05$ ). **Up-regulation vs. Down-regulation:** The high degree of symmetry in the plot indicates that maize roots coordinate both the induction of stress-protective pathways and the strategic suppression of non-essential processes. **Highly Significant Features:** Multiple data points positioned in the upper-most regions ( $-\log_{10}p \geq 1.3$ ) underscore the extreme statistical significance of the thermal response, aligning with the previously observed 92% variance in the PCA (Figure 7). This robust signal confirms that the identified DEGs are highly reliable candidates for further functional characterization.

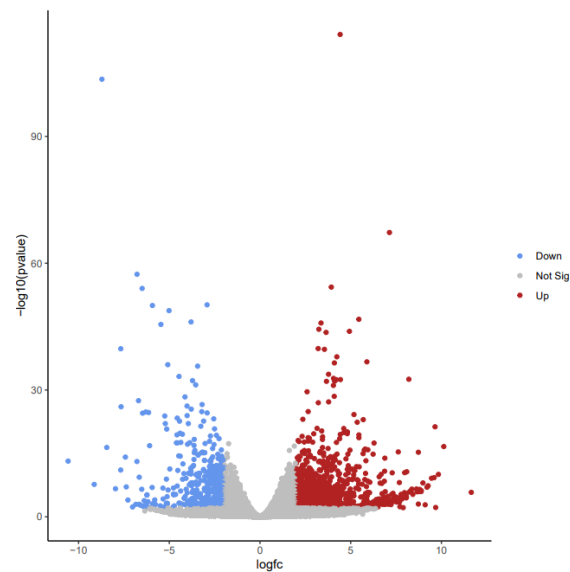


**Figure 7.** Volcano plot of differentially expressed genes (DEGs) in maize roots under heat stress

A volcano plot was constructed to visualize the global differential expression patterns between control and heat-stressed leaf tissues, providing a diagnostic snapshot of the transcriptomic reprogramming. This analysis provides a comprehensive overview of the magnitude ( $\log_2$  Fold Change) and statistical significance ( $-\log_{10}$  p-value) of the changes across the leaf transcriptome. Identification of Differentially Expressed Genes (DEGs): The plot reveals a vast number of transcripts exceeding the statistical thresholds (typically absolute  $\log_2\text{FC} \geq 2$  and  $p < 0.05$ ), indicating a large-scale molecular response to the 42 °C thermal treatment. The distribution of data points across the plot confirms that heat stress acts as a powerful driver of gene regulation in maize leaves. Extreme Statistical Significance: A notable feature of the leaf volcano plot is the presence of several highly significant transcripts located at the upper-most region of the vertical axis, with  $-\log_{10}$  p values exceeding 200. These extreme p-values suggest that the transcriptional shift in specific leaf gene modules is exceptionally robust and consistent across biological replicates.

Directionality of the Response: Up-regulated Transcripts (Red Points): Thousands of genes were significantly induced under heat stress, with some transcripts exhibiting  $\log_2$  FC values as high as 10 or greater. These up-regulated features are likely associated with the synthesis of heat-shock proteins (HSPs) and the activation of antioxidant defense systems required to maintain photosynthetic integrity. Down-regulated Transcripts (Blue Points): The plot also identifies a substantial cohort of significantly repressed genes, with  $\log_2$  FC values reaching nearly -25 for specific outliers. The high density of significant blue points, particularly those with extreme p-values exceeding 200, suggests a deliberate and highly coordinated down-regulation. Correlation with Global Variation: The clear partitioning of significant DEGs in the volcano plot aligns with the 80% variance captured by PC1 in the leaf PCA analysis (Figure 8). This consistency between the magnitude of individual gene changes and global sample clustering reinforces the validity of the identified transcriptional signatures.

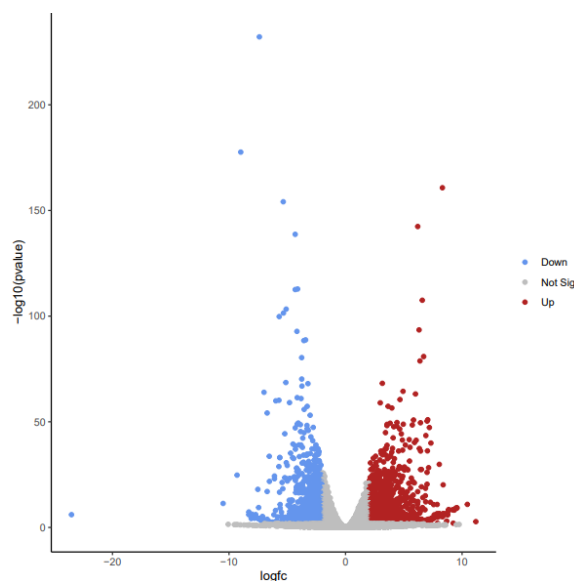


**Figure 8.** Volcano plot representing the leaf transcriptomic response to heat stress

### 3.5. Hierarchical Clustering and Expression Patterns (Heatmap)

A heatmap was constructed using the top DEGs to visualize the expression trajectories across the entire dataset. Systemic vs Tissue-Specific Patterns: Using row-wise Z-score scaling, the heatmap revealed core gene modules that are systemically up-regulated in both tissues, as well as clusters that exhibit organ-specific responses to heat. Clade Identification: The hierarchical clustering (Ward.D2) synchronized perfectly with the metadata, confirming that the heat stress response drives the primary transcriptional clades observed in the meta-analysis.

To visualize the differential expression landscape across all experimental variables, a hierarchical clustering heatmap was generated using the top differentially expressed genes (DEGs) identified from both root and leaf tissues. The heatmap utilizes row-wise Z-score scaling to highlight relative expression shifts across the eight biological samples (Figure 9). Sample Clustering and Clade Bifurcation: The column-wise dendrogram reveals a primary bifurcation that separates the samples into two distinct macro-clades. Clade 1 (Control Group): Comprising accessions SRR23944931, SRR23944930 (Leaf), and SRR35651885, SRR35651884 (Root), this clade represents the baseline transcriptional state under non-stress conditions. Clade 2 (Heat Stress Group): Integrating accessions SRR35651874, SRR35651873 (Root) and SRR23944927, SRR23944926 (Leaf), this clustering indicates that the global impact of 42 °C thermal stress overrides tissue-specific signatures for a significant portion of the maize seedling transcriptome. Identification of Conserved Heat-Responsive Modules, Up-regulated Core (Red/Orange Clusters): A prominent gene module in the lower half of the heatmap exhibits intense induction across all heat-stressed samples in both tissues. This cluster likely represents the evolutionarily conserved Heat Stress Response (HSR) mechanism, characterized by the synchronized activation of molecular chaperones and protective transcription factors. Down-regulated Core (Purple/Blue Clusters): Conversely, a significant cluster in the upper-right quadrant shows a systemic reduction in expression under heat stress. This suggests a coordinated suppression of primary metabolism and vegetative growth-related processes as a survival strategy to reallocate energy toward stress mitigation. Tissue-Specific Transcriptional Signatures: Despite the dominant effect of heat stress, the hierarchical clustering also reveals organ-specific sub-clusters. Certain gene modules show high relative expression specifically in leaf samples under control conditions but are markedly repressed upon heat exposure, while other modules appear to be constitutively higher in root tissues, potentially reflecting the distinct basal physiological requirements of below-ground versus aerial organs.

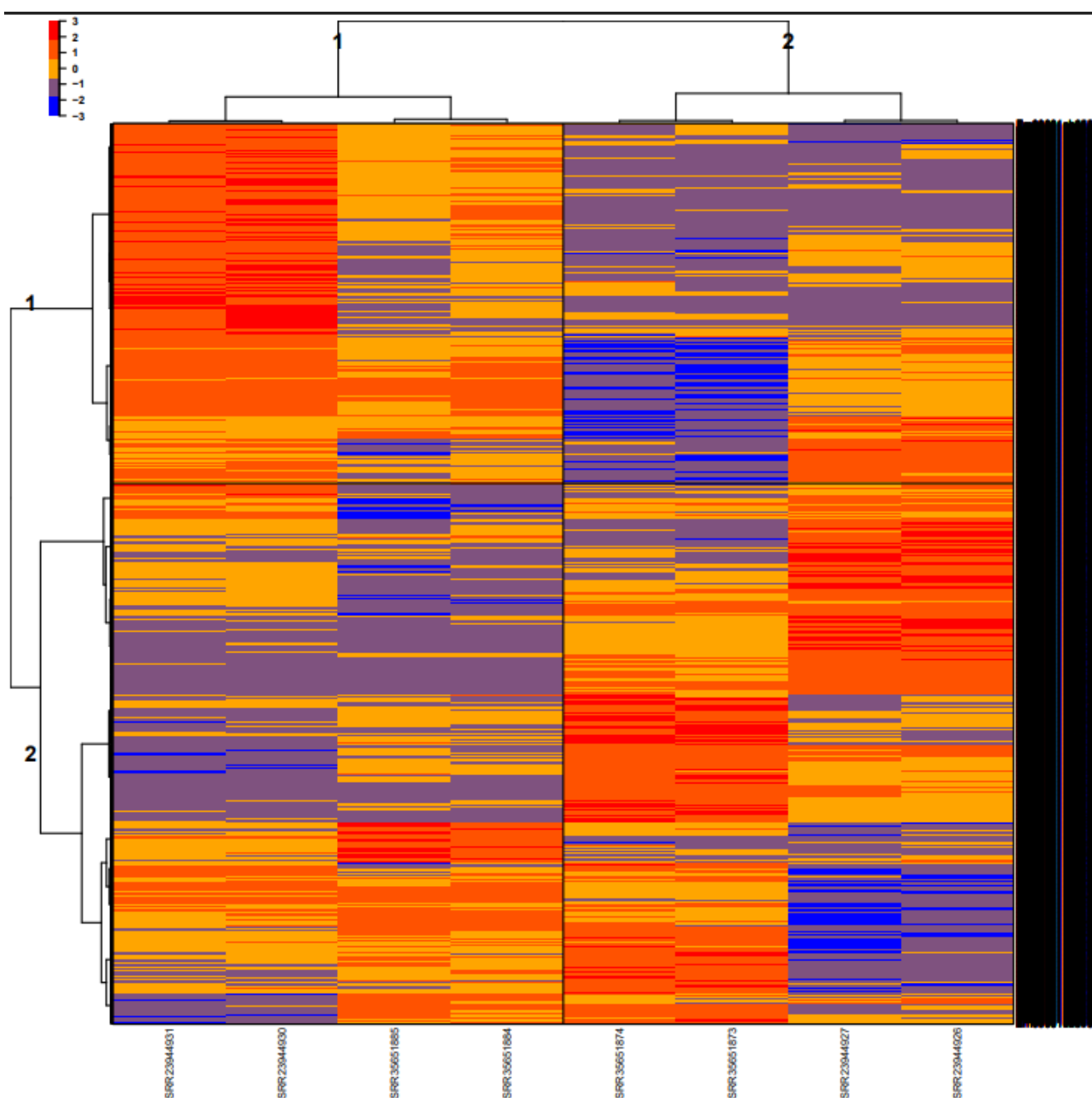


**Figure 9.** Hierarchical clustering heatmap of top differentially expressed genes across maize root and leaf tissues

#### 4. Discussion

The global transcriptomic architecture revealed by PCA highlights a profound and organ-specific sensitivity of maize seedlings to acute thermal stress. A striking finding in this study is the higher variance captured by PC1 in root tissues (92%) compared to leaf tissues (80%), suggesting that the root system undergoes a more uniform and drastic molecular reprogramming. This disparity may be attributed to the inherent differences in physiological buffering capacities between the two organs. While leaves utilize complex mechanisms such as evaporative cooling through stomatal regulation and the modulation of the photosynthetic apparatus to mitigate heat (Y. Wang et al., 2021), roots are directly exposed to the soil-temperature fluctuations without the benefit of transpirational cooling, potentially leading to a more synchronized and intense stress response (Koevoets et al., 2016). Furthermore, the higher secondary variance (PC2: 16%) observed in leaf tissues compared to roots (4%) points toward a more multifaceted regulatory network in the foliage. This likely reflects the intricate coordination required to maintain carbon

fixation and ROS (Reactive Oxygen Species) homeostasis under thermal perturbation. Previous studies have indicated that maize leaves prioritize the induction of the heat shock protein (HSP) chaperone system and the reconfiguration of C4 photosynthesis enzymes during the early seedling stage (Z. G. Li & Ye, 2022). In contrast, the dominant PC1 shift in roots underscores a massive commitment of cellular energy toward maintaining osmotic balance and cell wall integrity. Collectively, our results suggest that while both organs exhibit robust heat-responsive signatures, the "transcriptomic footprint" of heat stress is more pronounced in the roots, identifying them as a critical, yet often overlooked, target for engineering heat-resilient maize varieties.

A comparative analysis of the diagnostic p-value distributions and global variance components reveals a complex, organ-specific regulatory landscape in maize seedlings. Interestingly, while root tissues exhibited a more pronounced global transcriptional shift (PC1: 92%) compared to leaf tissues (PC1: 80%), the leaf transcriptome demonstrated a higher frequency of significant features, with the lowest p-value bin exceeding 7,000 transcripts compared to approximately 5,000 in the roots. This apparent paradox suggests that while heat stress induces a more synchronized and massive reprogramming of the entire root transcriptome, the response in leaves is characterized by a larger cohort of genes undergoing highly significant, yet perhaps more diverse, expression changes. The higher density of significant p-values in leaves may reflect the intense activation of specific protective pathways, such as the heat shock protein (HSP) machinery and antioxidant systems, which are essential for maintaining the integrity of the photosynthetic apparatus (T. Wang et al., 2025). Previous studies have noted that maize leaves often initiate a broader "broad-spectrum" genetic response to cope with the direct thermal radiation and fluctuations in light intensity. Conversely, the higher PC1 variance in roots, despite a smaller count of significant features, indicates that the genes which *do* respond in the root system do so with a higher magnitude of fold-change or greater uniformity across replicates, likely prioritizing essential survival processes such as cell wall reinforcement and osmotic adjustment (Nazari & Zinati, 2024). These findings underscore that the "drastic" nature of the root response (driven by variance) and the "widespread" nature of the leaf response (driven by feature frequency) represent two distinct but complementary strategies for heat adaptation in maize.

The volcano plot profiles for both root and leaf tissues underscore a massive and highly coordinated transcriptional reprogramming in maize seedlings. A defining feature of this response is the marked symmetry in the distribution of differentially expressed genes (DEGs), indicating that maize employs a balanced strategy of "active induction" and "strategic repression" to combat 42 °C thermal stress. The presence of up-regulated transcripts with extreme fold changes ( $\log_2$  FC > 10) and high statistical significance ( $-\log_{10}p > 200$  in leaves and > 90 in roots) identifies a core set of "heat-responsive master regulators." These genes, primarily encoding heat shock proteins (HSPs) and heat shock factors (HSFs), function as molecular chaperones that prevent protein aggregation and maintain cellular proteostasis under heat-induced denaturing conditions (Z. Li & Howell, 2021).

Furthermore, the substantial cohort of significantly down-regulated genes (blue points) suggests a systematic "metabolic brake" designed to conserve energy by repressing genes involved in primary metabolism, cell wall expansion, and vegetative growth. Maize seedlings reallocate limited resources toward high-priority survival mechanisms. Interestingly, the observation of higher statistical significance in leaf DEGs compared to roots, despite the higher global variance in roots, suggests that while the root response is more uniform, the leaf response is more diverse and intensely regulated. This likely reflects the foliage's need to simultaneously manage thermal damage and protect the sensitive components of the photosynthetic apparatus, such as the Photosystem II (PSII) complex, which is known to be highly thermolabile (Chen et al., 2016). Collectively, the volcano plot data confirms that the 42 °C treatment exceeds the threshold for a standard acclimation response, triggering a robust, systemic defense signature across the entire plant seedling.

The hierarchical clustering analysis of the top DEGs provides a high-resolution map of the maize seedling's response to acute thermal stress, revealing a dominant shift that transcends tissue-specific boundaries. A central finding in the heatmap is the primary bifurcation of the dendrogram into two major clades, strictly based on the experimental condition (Control vs. Heat Stress) rather than organ type. This

clustering pattern indicates that at the seedling stage, a 42 °C thermal perturbation acts as a powerful systemic driver of the transcriptome, effectively overriding the basal physiological signatures that distinguish roots from leaves. Within this systemic response, we identified a highly conserved "core heat-stress module" (represented by the intense red/orange clusters in the lower half of the heatmap) that is robustly induced across all samples. This module likely encompasses the essential Heat Stress Response (HSR) machinery, including molecular chaperones and transcriptional regulators that are universally required for cellular survival regardless of organ function (T. Wang et al., 2025). Conversely, the presence of distinct organ-specific sub-clusters underscores the specialized adaptive strategies of each tissue. For instance, the leaf-specific modules that are suppressed under heat stress point to a targeted down-regulation of the photosynthetic apparatus to prevent ROS-induced damage (Z. Li & Howell, 2021). In contrast, the root-specific shifts likely reflect a localized effort to maintain water uptake and cell wall extensibility under high-temperature conditions (T. Wang et al., 2025). These results demonstrate that the maize heat-stress response is a sophisticated balance between a unified systemic defense and specialized, tissue-dependent adaptive mechanisms.

## 5. Conclusions

This study successfully integrated transcriptomic data from BioProjects PRJNA1335774 and PRJNA947789 to delineate the organ-specific and systemic heat stress responses in maize seedlings. Through a rigorous bioinformatic pipeline, we have demonstrated that acute thermal stress at 42 °C serves as the primary driver of transcriptional variation, significantly outweighing tissue-specific identity. The primary findings are as follows: (1) High Statistical Robustness: PCA and p-value histograms confirmed the high reproducibility of the datasets, with treatment effects explaining up to 92% of the variance. (2) Convergent Heat Stress Response (HSR): Hierarchical clustering identified a conserved core module of induced genes centered on proteoprotection and heat shock factor activation. (3) Growth-Defense Tradeoffs: The widespread down-regulation of primary metabolic pathways highlights a coordinated strategy to reallocate resources toward survival-oriented stress mitigation. (4) Organ-Specific Sensitivity: Roots exhibited a more synchronized transcriptional shift, while leaves showed higher secondary variability due to complex photosynthetic and transpirational adjustments. Ultimately, the identification of high-confidence DEGs with extreme significance provides a prioritized list of candidate targets for molecular breeding. These results lay a critical foundation for developing thermotolerant maize cultivars through genome editing or marker-assisted selection, ensuring agricultural resilience in the face of increasing global temperatures.

**Acknowledgments:** The authors would like to express their gratitude to the researchers who made their transcriptomic data publicly available through the NCBI BioProject database (PRJNA1335774 and PRJNA947789). We also thank Laboratory of Biology, The Republic of Indonesia Defense University for providing the computational resources necessary for the bioinformatic analyses.

**Conflicts of Interest:** The authors declare no conflicts of interest regarding the publication of this manuscript.

## References

- Ahuja, I., de Vos, R. C., Bones, A. M., & Hall, R. D. (2010). Plant molecular stress responses face climate change. *Trends in Plant Science*, 15(12), 664–674. <https://doi.org/https://doi.org/10.1016/j.tplants.2010.08.002>
- Chen, Y., Liu, W., Su, Y., Cui, J., Zhang, Z., Yuan, M., Zhang, H., & Yuan, S. (2016). Different response of photosystem II to short and long-term drought stress in *Arabidopsis thaliana*. *Physiologia Plantarum*, 158(2), 225–235. <https://doi.org/10.1111/ppl.12438>
- El-Sappah, A. H., Rather, S. A., Wani, S. H., Elrys, A. S., Bilal, M., Huang, Q., Dar, Z. A., Elashtokhy, M. M. A., Soaud, N., Koul, M., Mir, R. R., Yan, K., Li, J., El-Tarabily, K. A., & Abbas, M. (2022). Heat Stress-Mediated Constraints in Maize (*Zea mays*) Production: Challenges and Solutions. *Frontiers in Plant Science*, Volume 13-2022. <https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2022.879366>
- Fitrawati, S., Ilsan, M., & Rasyid, R. (2023). Analisis Ekonomi dan Prospek Pengembangan Usahatani Jagung (*Zea mays* L.) di Kabupaten Barru (Studi Kasus di Desa Lalabata, Kecamatan Tanate Rilau). *WIRATANI*, 6(2), 137–146.

- Jacob, P., Hirt, H., & Bendahmane, A. (2017). The heat-shock protein/chaperone network and multiple stress resistance. *Plant Biotechnology Journal*, 15(4), 405–414. <https://doi.org/https://doi.org/10.1111/pbi.12659>
- Jain, M. (2024). Gene regulatory networks in abiotic stress responses via single-cell sequencing and spatial technologies: Advances and opportunities. *Current Opinion in Plant Biology*, 82. <https://doi.org/https://doi.org/10.1016/j.pbi.2024.102662>
- Janni, M., Gulli, M., Maestri, E., Marmioli, M., Valliyodan, B., Nguyen, H. T., & Marmioli, N. (2020). Molecular and genetic bases of heat stress responses in crop plants and breeding for increased resilience and productivity. In *Journal of Experimental Botany* (Vol. 71, Number 13, pp. 3780–3802). Oxford University Press. <https://doi.org/10.1093/jxb/eraa034>
- Koevoets, I. T., Venema, J. H., Elzenga, J. Theo. M., & Testerink, C. (2016). Roots Withstanding their Environment: Exploiting Root System Architecture Responses to Abiotic Stress to Improve Crop Tolerance. *Frontiers in Plant Science*, Volume 7-2016. <https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2016.01335>
- Lei, M., Si, H., Zhu, M., Han, Y., Liu, W., Dai, Y., Ji, Y., Liu, Z., Hao, F., Hao, R., Zhao, J., Ye, G., & Zan, Y. (2025). Comprehensive analysis of 1,771 transcriptomes from 7 tissues enhance genetic and biological interpretations of maize complex traits. *G3: Genes, Genomes, Genetics*, 15(9). <https://doi.org/10.1093/g3journal/jkaf140>
- Li, Z. G., & Ye, X. Y. (2022). Transcriptome response of maize (*Zea mays* L.) seedlings to heat stress. *Protoplasma*, 259(2), 357–369. <https://doi.org/https://doi.org/10.1007/s00709-021-01680-8>
- Li, Z., & Howell, S. H. (2021). Heat Stress Responses and Thermotolerance in Maize. *International Journal of Molecular Sciences*, 22(2), 948. <https://doi.org/10.3390/ijms22020948>
- Ma, Z., Qi, R., Zhang, H., Meng, X., Liu, Z., Duan, S., Guo, X., Li, G., & Shang, Z. (2025). Phosphoproteomic Analysis of Maize Seedlings Provides Insights into the Mechanisms of Heat-Stress Tolerance. *International Journal of Molecular Sciences*, 26(6). <https://doi.org/10.3390/ijms26062439>
- Nazari, L., & Zinati, Z. (2024). Transcriptional survey of abiotic stress response in maize (*Zea mays*) in the level of gene co-expression network and differential gene correlation analysis. *AoB PLANTS*, 16(1). <https://doi.org/10.1093/aobpla/plad087>
- Qu, A. L., Ding, Y. F., Jiang, Q., & Zhu, C. (2013). Molecular mechanisms of the plant heat stress response. *Biochemical and Biophysical Research Communications*, 432(2), 203–207. <https://doi.org/https://doi.org/10.1016/j.bbrc.2013.01.104>
- Samat, A. T., Soltabayeva, A., Bekturova, A., Zhanassova, K., Auganova, D., Masalimov, Z., Srivastava, S., Satkanov, M., & Kurmanbayeva, A. (2025). Plant responses to heat stress and advances in mitigation strategies. *Frontiers in Plant Science*, 16. <https://doi.org/https://doi.org/10.3389/fpls.2025.1638213>
- Varshney, R. K., Bohra, A., Yu, J., Graner, A., Zhang, Q., & Sorrells, M. E. (2021). Designing Future Crops: Genomics-Assisted Breeding Comes of Age. In *Trends in Plant Science* (Vol. 26, Number 6, pp. 631–649). Elsevier Ltd. <https://doi.org/10.1016/j.tplants.2021.03.010>
- Vogel, E., Donat, M. G., Alexander, L. V., Meinshausen, M., Ray, D. K., Karoly, D., Meinshausen, N., & Frieler, K. (2019). The effects of climate extremes on global agricultural yields. *Environmental Research Letters*, 14. <https://doi.org/https://doi.org/10.1088/1748-9326/ab154b>
- Wang, T., Wang, F., Deng, S., Wang, K., Feng, D., Xu, F., Guo, W., Yu, J., Wu, Y., Wuriyangan, H., Li, S.-T., Gu, X., Le, L., & Pu, L. (2025). Single-cell transcriptomes reveal spatiotemporal heat stress response in maize roots. *Nature Communications*, 16(1), 177. <https://doi.org/10.1038/s41467-024-55485-3>
- Wang, Y., Huang, L., Du, F., Wang, J., Zhao, X., Li, Z., Wang, W., Xu, J., & Fu, B. (2021). Comparative transcriptome and metabolome profiling reveal molecular mechanisms underlying OsDRAP1-mediated salt tolerance in rice. *Scientific Reports*, 11(1). <https://doi.org/https://doi.org/10.1038/s41598-021-84638-3>
- Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D. B., Huang, Y., Huang, M., Yao, Y., Bassu, S., Ciais, P., Durand, J.-L., Elliott, J., Ewert, F., Janssens, I. A., Li, T., Lin, E., Liu, Q., Martre, P., Müller, C., ... Asseng, S. (2017). Temperature increase reduces global yields of major crops in four independent estimates. *Proceedings of the National Academy of Sciences*, 114(35), 9326–9331. <https://doi.org/10.1073/pnas.1701762114>