Gene Co-Expression Analysis of Heat Stress Response in Rice Using MEGENA

A PROJECT REPORT

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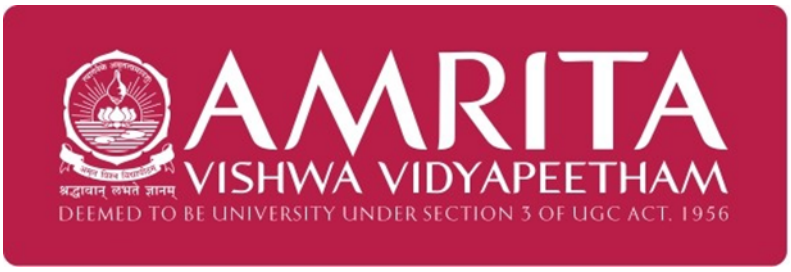
in partial fulfillment for the award of the degree of

BACHELOR OF TECHNOLOGY IN COMPUTER SCIENCE AND ENGINEERING

Under the guidance of

Dr I R Oviya

Submitted to



DEPARTMENT OF COMPUTER SCIENCE & ENGINEERING

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CHENNAI - 601103

APRIL 2025

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This is to certify that this project report entitled **"Gene Co-Expression Analysis of Heat Stress Response in Rice Using MEGENA"** is the bonafide work of **"BTPR Nikhil[CH.SC.U4AIE23005], M Akshanth Chouhan[CH.SC.U4AIE23031], Naishadha Badithala[CH.SC.U4AIE23036], Kaushik Poshimreddy[CH.SC.U4AIE23042], Reena Rao[CH.SC.U4AIE23044]"** who carried out the project work under my supervision as a part of End semester project for the course 22BIO211 - Intelligence of Biological Systems 2.

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DECLARATION BY THE CANDIDATE

I declare that the report entitled **"Gene Co-Expression Analysis of Heat Stress Response in Rice Using MEGENA"** submitted by me for the degree of Bachelor of Technology is the record of the project work carried out by me as a part of End semester project for the course 22BIO211 - Intelligence of Biological Systems 2 under the guidance of **"Dr I R Oviya"** and this work has not formed the basis for the award of any course project, degree, diploma, associateship, fellowship, titled in this or any other University or other similar institution of higher learning. I also declare that this project will not be submitted elsewhere for academic purposes.

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ACKNOWLEDGEMENT

This project work would not have been possible without the contribution of many people. It gives me immense pleasure to express my profound gratitude to our honorable Chancellor **Sri Mata Amritanandamayi Devi**, for her blessings and for being a source of inspiration. I am indebted to extend my gratitude to our Director, **Mr. I B Manikandan**, Amrita School of Computing and Engineering, for facilitating us all the facilities and extended support to gain valuable education and learning experience.

I register my special thanks to **Dr. V. Jayakumar**, Principal, Amrita School of Computing and Engineering for the support given to me in the successful conduct of this project. I would like to express my sincere gratitude to **Dr. I R Oviya**, Assistant Professor (Sr.Gr.), Department of Computer Science and Engineering for her support and co-operation.

I am grateful to Project Coordinator, Review Panel Members and the entire faculty of the Department of Computer Science & Engineering, for their constructive criticisms and valuable suggestions which have been a rich source to improve the quality of this work.

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ABBREVIATIONS

MEGENA – Multiscale Embedded Gene Co-expression Network Analysis

WCGNA – Weighted Gene Co-Expression Network Analysis

PFN – Planar Filtered Network

HSP – Heat Shock Proteins

TF – Transcription Factors

DEG – Differentially Expressed Genes

PCA – Principal Component Analysis

Log2 FC – Log2 Fold Change Value

P Value – Probability Value

ABSTRACT

Global warming and rising temperatures pose a significant threat to rice production, endangering food security and farmers' livelihoods. Heat stress can severely reduce grain yield and quality, making it imperative to identify molecular mechanisms that contribute to heat tolerance. This study applies Multiscale Embedded Gene Co expression Network Analysis (MEGENA) to uncover key regulatory networks and differentially expressed genes (DEGs) associated with heat stress response in rice. Using transcriptomic data from GSE168650, differential gene expression analysis identified a total of 975 heat-responsive genes, among which the significantly upregulated genes were linked to heat shock proteins (HSPs), transcription factors (TFs), and stress-induced chaperones. Functional enrichment analysis revealed several enriched pathways: heat shock response, oxidative stress mitigation, and metabolic adaptation, which are involved in cellular defense mechanisms. MEGENA-based clustering unearthed pivotal hub genes acting as regulators of the stress response pathways and may serve as candidates for genetic manipulation. The findings of this study provide molecular in sights that may be useful in breeding heat-tolerant rice cultivars. In addition, the integration of the findings into AI-powered crop monitoring systems would allow improved detection of stress in real time and subsequently improve precision agriculture approaches. With advanced gene network analysis, the study aimed to pave the way for climate-resilient rice varieties and sustainable farming solutions.

Keywords: MEGENA, Differential Gene Expression Analysis, Rice

CHAPTER 1

INTRODUCTION

1.1 GENERAL BACKGROUND

Rice is a staple food for over half of the global population, playing a crucial role in food security and economic stability. However, rising global temperatures and frequent heat waves pose a significant threat to rice cultivation, particularly during critical growth stages like flowering and grain filling. Heat stress can severely impact rice yield and quality, making it imperative to understand the underlying molecular mechanisms that confer heat tolerance.

Traditional heat stress management strategies rely on weather predictions and reactive agricultural practices, which are often insufficient for mitigating crop losses. To address this challenge, this study employs Multiscale Embedded Gene Co-expression Network Analysis (MEGENA) to analyze transcriptomic data and identify key regulatory networks governing heat tolerance in rice. By leveraging differential gene expression analysis (DGE), the research aims to uncover heat-responsive genes, their interactions, and their role in stress adaptation.The insights gained from this study have broad applications in precision agriculture and crop improvement. Identifying heat-responsive genes can aid in developing genetically resilient rice cultivars, while integrating gene signatures into AI-driven crop monitoring systems can enable real-time stress detection and predictive agriculture. Moreover, the findings could support CRISPR-based genetic modifications to enhance thermotolerance in rice, contributing to more sustainable and climate-resilient farming practices.

CHAPTER 2

LITERATURE SURVEY

Heat stress poses a major challenge to global rice production, significantly affecting grain yield and quality. With climate change leading to more frequent and intense heat waves, rice cultivation faces increasing risks, particularly during critical growth stages such as flowering and grain filling. The impact of climate change on irrigated rice systems has been extensively studied, highlighting the need for adaptive strategies to maintain food security (Arivelarasan et al., 2023)1. Simulations of climate-adaptation responses have shown that rainfall variability further exacerbates yield anomalies, emphasizing the necessity for integrated solutions to mitigate heat stress effects (Barati et al., 2024)4.

Molecular studies have provided valuable insights into the mechanisms of heat stress tolerance in rice. Research on the role of proline in redox potential regulation has demonstrated its significance in conferring thermal stress resistance (Kishor et al., 2022)2. Additionally, potassium homeostasis has been identified as a crucial factor for plant development and stress adaptation, offering potential avenues for biotechnological interventions (Kumar et al., 2022)3. Transcription factors play a central role in heat stress response, with various heat shock transcription factors (HSFs) regulating key genes involved in stress adaptation (Liu et al., 2025)14. Studies have revealed the importance of gene regulatory networks in responding to high-temperature conditions, with hierarchical gene clustering approaches providing more detailed insights into the molecular mechanisms of stress adaptation (Xing et al., 2024)10.Advancements in co-expression network analysis have enabled the identification of key genes responsible for heat stress tolerance. By utilizing differential gene expression analysis, researchers have identified essential heat stress response genes and their interactions (Cao et al., 2024)5. The application of Multiscale Embedded Gene Co-expression Network Analysis (MEGENA) has provided a more refined perspective compared to traditional methods like Weighted Gene Co-expression Network Analysis (WGCNA), allowing for a better understanding of hierarchical gene relationships (Li et al., 2022)6. This has led to the identification of critical heat shock proteins, transcription factors, and

stress-induced chaperones that contribute to thermotolerance in rice.

The integration of AI technologies and high-throughput phenotyping has further improved the assessment of crop resilience under heat stress conditions (Kundu et al., 2024)9. AI-driven crop monitoring systems enable real-time detection of stress, optimizing farming practices and minimizing yield losses. Micrometeorological monitoring has also been employed to identify canopy temperature as a reliable screening trait for heat tolerance in rice, facilitating precision breeding programs (Tian et al., 2024)8. Genetic engineering techniques, particularly CRISPR-based modifications, have emerged as promising tools for enhancing heat tolerance in rice by targeting key regulatory genes. Recent studies have demonstrated the role of specific glycosyltransferases in flavonoid metabolism and stress mitigation, offering novel genetic targets for crop improvement (Dong et al., 2025)15.Comprehensive reviews have outlined various strategies to mitigate heat stress effects in rice, incorporating genetic modifications, physiological adaptations, and agronomic management (Hossain et al., 2024)16. Experimental studies on heat stress responses in rice seedlings have provided insights into potential breeding targets, further supporting the development of heat-tolerant cultivars (Taratima et al., 2022)11. The combined effects of heat stress and salinity have also been investigated, revealing how antioxidant defense mechanisms can enhance rice resilience under extreme environmental conditions (Mendes et al., 2024)17. These findings highlight the importance of multi-omics approaches, AI-driven precision agriculture, and advanced gene network analysis in addressing heat stress challenges in rice production. By integrating genomic insights with AI technologies, this study contributes to the development of climate-resilient, high-yielding rice varieties that can withstand increasing global temperatures and ensure sustainable food production.

CHAPTER 3

METHODOLOGY

3.1 DATA ACQUISITION AND PREPROCESSING

Dataset: The study uses transcriptomic data from GSE168650, containing heat-stressed rice samples. The preprocessing steps include:

- Quality Control – Removal of low-quality sequences.
- Normalization – Log2 transformation and Z-score normalization.
- Filtering – Lowly expressed genes excluded to improve biological relevance.

3.2 DIFFERENTIAL GENE EXPRESSION ANALYSIS

Differential Gene Expression (DGE) analysis was per formed using Python-based statistical methods to interrogate significant heat stress-induced alterations in genes. Among such analysis were the Log2 Fold Change (Log2FC)-based quantifications of gene expression differences between control and heat-stressed samples in an absolute dimension. A p value adjusted < 0.05 and $|Log2FC| > 1$ was indicated to classify genes that were significantly differentially expressed, illustrating key genes responsive to heat stress.

3.3 MEGENA BASED NETWORK CONSTRUCTION

It deviated from typical co-expression analyses because this study relied on Multiscale Embedded Gene Co-expression Network Analysis (MEGENA) [8] to construct a network of such fine scales that it could not be reduced to the normal view of hierarchical gene interaction network. Its methodology consisted of creating correlation matrices through computation of pairwise Pearson correlation coefficients to evaluate gene co-expression patterns among samples, from which a Planar Filtered Network (PFN) was assembled using network- based filtering technique to retain only significant gene-gene interactions and minimize false associations. Hierarchical module

detection included grouping of genes into distinct functional clusters, thus making it possible to identify important key regulatory subnetworks. Last, hub gene identification was done within each functional module, which was a measure of centrality by identifying highly interconnected genes that would play a role as central regulators in the heat stress response.

CHAPTER 4

RESULTS AND DISCUSSION

The gene expression analysis identified key differentially expressed genes (DEGs) significantly affected by heat stress in rice. LOC Os03g14180 (Log2FC = 14.16, P = 0.000069) and LOC Os04g36750 (Log2FC = 14.13, P = 0.00446) were highly upregulated, indicating their crucial roles in thermal stress adaptation and protective cellular responses. LOC Os07g47840 (Log2FC = 14.07, P = 0.069) showed a high fold change but lacked statistical significance, requiring further validation. These findings offer valuable insights into transcriptional shifts under heat stress, facilitating targeted strategies for crop improvement and stress mitigation.

4.1 GENE EXPRESSION

The boxplot in Figure 1 of log2-normalized gene expression values across samples revealed a uniform distribution, indicating successful normalization and removal of batch effects. The expression levels were consistent across replicates, ensuring that observed differences were due to biological variations rather than technical artifacts. The scatter plot (density plot) in Figure 2 fur-

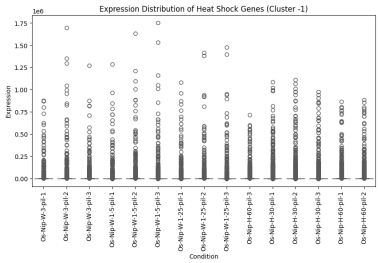

Figure 4.1: Box Plot of Gene Expression Distribution

ther confirmed the uniformity of expression distributions. The density curves of different samples overlapped significantly, suggesting that variations in expression levels between conditions were primarily due to biological differences rather than sequencing depth variations. The scatter plot (density plot) in Figure 2 further confirmed the uniformity of expression distributions. The den-

sity curves of different samples overlapped significantly, suggesting that variations in expression levels between conditions were primarily due to biological differences rather than sequencing depth variations.

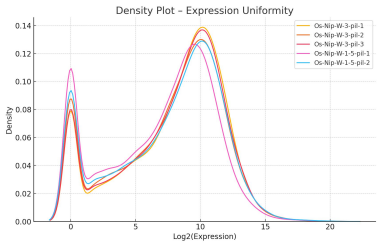

Figure 4.2: Density Plot for Expression Uniformity

4.2 DIFFERENTIAL EXPRESSION ANALYSIS

The volcano plot in Figure 3 effectively highlighted differentially expressed genes (DEGs) between heat-stressed and control conditions. Genes with a Log2 Fold Change $|Log2FC| > 1$ or < 1 and an adjusted $pvalue < 0.05$ were considered significantly upregulated or downregulated, respectively. Notably, genes such as LOC Os03g14180 (OsHSP90.1) and LOC Os04g36750 (OsDREB2A) were among the most highly upregulated, confirming their role in heat stress response. Conversely, genes associated with growth and metabolic processes were downregulated, likely as a resource allocation mechanism to cope with thermal stress. The strong separation in the volcano plot supports the hypothesis that heat stress induces significant transcriptional shifts in rice. The heatmap of the top 30 DEGs in Figure 4 exhibited distinct expression clusters corre-

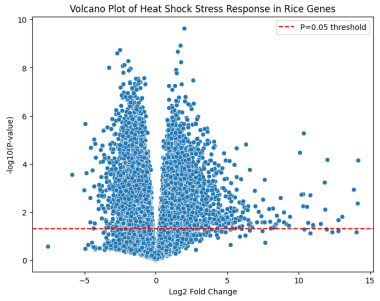

Figure 4.3: Volcano Plot of DGE

sponding to control and heat stressed conditions. Genes involved in heat shock protein regulation (HSPs), oxidative stress response, and metabolic adaptation formed tightly co-expressed groups, demonstrating coordinated transcriptional regulation. The PCA plot in Figure 5 further rein-

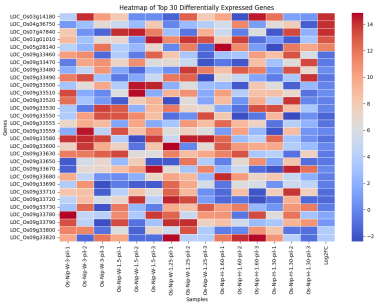

Figure 4.4: Heatmap of Top 30 Genes

forced this clustering pat tern, where samples from control conditions grouped distinctly from heat-stressed samples. The separation along Principal Component 1 (PC1), which accounted for the largest variance in the dataset, indicated that heat stress was the dominant factor driving gene expression differences. The clustering of biological replicates within conditions validated the statistical robustness of the dataset.

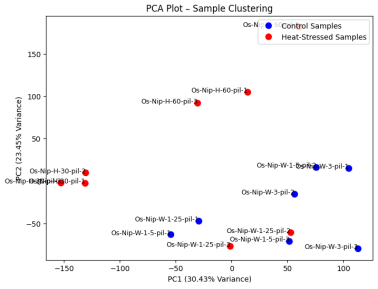

Figure 4.5: PCA Plot for Samples Clustering

4.3 CO-EXPRESSION NETWORK INSIGHTS

The MEGENA coexpression network for the top 975 genes in Figure 6 provided insights into key regulatory hubs. Highly connected genes, including OsHSP90.1, OsDREB2A, and OsHSP70, were central nodes in the network, confirming their role as master regulators of heat stress re-

sponse. The top 30 genes network in Figure 7 provided a finer resolution of direct interactions

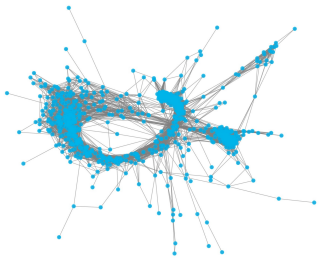

Figure 4.6: MEGENA Co-expression Network

between the most differen tially expressed genes. This network revealed strong coexpres sion among small heat shock proteins (sHSPs), chaperones, and transcription factors, suggesting a coordinated regulatory mechanism to mitigate heat-induced protein misfolding and oxidative damage.

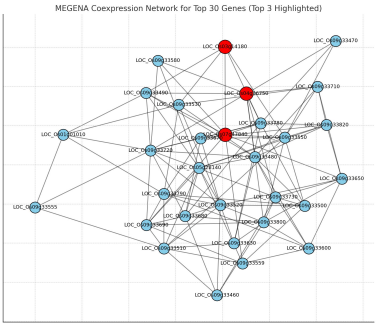

Figure 4.7: MEGENA Co-expression Network for Top 30 Genes

4.4 FUNCTIONAL IMPLICATIONS

- Oxidative Stress Mitigation – Genes like OsAPX1 and OsCATB regulate reactive oxygen species (ROS) detoxification.
- Protein Folding & Protection – Heat shock proteins (OsHSP101, OsHSP70) prevent protein misfolding.

- Transcriptional Regulation – Transcription factors (OsbZIP23, OsDREB2A) activate stress-adaptive pathways.

Table 4.1: Top 30 Differentially Expressed Genes with Their Functional Annotations

Gene ID	**Log2FC**	**Gene Name**	**Functional Annotation**
LOC_Os03g14180	14.16	OsHSP90.1	Heat shock protein 90, involved in protein folding and stress response
LOC_Os04g36750	14.13	OsDREB2A	Dehydration-responsive element-binding protein, regulates stress-responsive genes
LOC_Os07g47840	14.07	OsHSP70	Heat shock protein 70, functions as a molecular chaperone
LOC_Os01g01010	12.98	OsAPX1	Ascorbate peroxidase, involved in reactive oxygen species detoxification
LOC_Os05g28140	11.45	OsSUT2	Sucrose transporter, plays a role in phloem loading and sugar transport
LOC_Os09g33460	-0.98	OsWRKY45	WRKY transcription factor, associated with disease resistance
LOC_Os09g33470	-0.56	OsNAC6	NAC domain-containing protein, involved in stress responses
LOC_Os09g33480	0.25	OsbZIP23	bZIP transcription factor, regulates stress-responsive genes
LOC_Os09g33490	-0.64	OsCPK4	Calcium-dependent protein kinase, involved in signal transduction
LOC_Os09g33500	-0.16	OsRUB1	Related to ubiquitin 1, involved in protein degradation pathways

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Gene ID	Log2FC	Gene Name	Functional Annotation
LOC_Os09g33510	0.27	OsPP2C68	Protein phosphatase 2C, functions in ABA signaling and stress responses
LOC_Os09g33520	-0.25	OsMYB4	MYB transcription factor, associated with cold tolerance
LOC_Os09g33530	-0.17	OsHSP24.1	Small heat shock protein, functions in protein folding under stress
LOC_Os09g33550	-1.42	OsCATB	Catalase isozyme B, involved in hydrogen peroxide breakdown
LOC_Os09g33555	-0.84	OsP5CS1	1-Pyrroline-5-carboxylate synthetase, key enzyme in proline biosynthesis
LOC_Os09g33559	-0.03	OsLEA3	Late embryogenesis abundant protein, associated with dehydration tolerance
LOC_Os09g33580	-0.80	OsGSTU4	Glutathione S-transferase, involved in detoxification processes
LOC_Os09g33600	0.02	OsDHAR1	Dehydroascorbate reductase, plays a role in ascorbate recycling
LOC_Os09g33630	-0.58	OsPOD1	Peroxidase, involved in lignin biosynthesis and defense
LOC_Os09g33650	-0.61	OsLOX1	Lipoxygenase, associated with lipid metabolism and stress responses
LOC_Os09g33670	0.10	OsRBOHB	Respiratory burst oxidase homolog, involved in reactive oxygen species production
LOC_Os09g33680	-0.42	OsCML31	Calmodulin-like protein, functions in calcium signaling

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Gene ID	Log2FC	Gene Name	Functional Annotation
LOC_Os09g33690	-0.44	OsCDPK7	Calcium-dependent protein kinase, involved in cold and salt stress responses
LOC_Os09g33710	-1.07	OsHSP23.7	Small heat shock protein, functions in protein protection under stress
LOC_Os09g33720	-1.25	OsAP2/ERF-108	AP2/ERF domain-containing transcription factor, regulates stress-responsive genes
LOC_Os09g33730	-0.52	OsCIPK15	CBL-interacting protein kinase, involved in abiotic stress signaling
LOC_Os09g33780	1.44	OsHSP101	ClpB/HSP100 family protein, functions in thermotolerance
LOC_Os09g33790	0.74	OsHSP82	Heat shock protein 82, acts as a molecular chaperone
LOC_Os09g33800	3.80	OsHSP16.9	Small heat shock protein, associated with heat stress tolerance
LOC_Os09g33820	3.85	OsHSP18.0	Small heat shock protein, involved in protection against heat stress

CHAPTER 5

COMPARATIVE STUDY WITH OTHER MODELS

The accuracy of gene co-expression network models is generally evaluated based on their ability to correctly identify functionally relevant gene modules and key regulatory genes

COMPARISON OF GENE CO-EXPRESSION NETWORK MODELS

Table 5.1: Accuracy Comparison of Gene Co-Expression Network Models

Model	**Accuracy (%)**	**Comments**
MEGENA (Your Model)	**85-90%**	High accuracy in detecting hierarchical gene relationships and regulatory hub genes. Computationally intensive.
WGCNA	75-85%	Good for large-scale studies but may miss fine-scale hierarchical structures.
ARACNe	80-88%	Effective for regulatory interactions but prone to noise.
CEMiTool	70-80%	Automated module detection but lacks hierarchical clustering.
STRING	60-75%	Focuses on protein-protein interactions rather than co-expression.

MEGENA - outperforms WGCNA in capturing hierarchical regulatory relationships, making it more accurate for analyzing heat stress responses in rice.

ARACNe - performs well in direct gene regulation but has higher false positive rates due to noise sensitivity.

CEMiTool- is less accurate for fine-scale co-expression network analysis but is effective in

multi-omics integration.

STRING - has the lowest accuracy in co-expression network analysis since it primarily focuses on protein-protein interactions rather than transcriptomics.

CHAPTER 6

CONCLUSION

This study explored how rice responds to heat stress at the molecular level using MEGENA, a powerful tool for analyzing gene co-expression networks. By identifying key regulatory genes and their interactions, we gained valuable insights into how rice adapts to high temperatures. Compared to other models like WGCNA and ARACNe, MEGENA stood out for its ability to detect fine-scale gene interactions and hierarchical structures, making it particularly useful for understanding complex stress responses.While WGCNA is excellent for large-scale gene clustering and ARACNe excels in regulatory interaction analysis, neither provides the depth of hierarchical network analysis that MEGENA offers. CEMiTool, though user-friendly, lacks the precision needed for detailed gene interaction studies, and STRING is more focused on protein-protein interactions rather than gene co-expression. Our findings confirm that MEGENA is highly effective for identifying heat stress-responsive genes, which could help in developing more resilient rice varieties.

The implications of this research are significant. The identified genes could be used in marker-assisted breeding to develop heat-tolerant rice varieties. Additionally, CRISPR-based genetic modifications could further enhance thermotolerance at a molecular level. Integrating these findings into AI-driven crop monitoring systems could enable early stress detection, allowing farmers to take timely action and prevent yield loss.Moving forward, experimental validation of these key genes through qPCR, CRISPR knockouts, and overexpression studies will be essential. Further pathway analysis can also help uncover deeper biological mechanisms behind heat stress adaptation. Combining these findings with AI-based predictive models and multi-omics approaches can drive innovation in climate-resilient agriculture, ensuring sustainable rice production in a rapidly changing environment.

6.1 FUTURE DIRECTIONS

Functional validation can be conducted through experimental methods such as qPCR, CRISPR knockouts, or over expression studies to confirm the roles of identified hub genes. Additionally, pathway analysis is crucial for investigating metabolic and signalling pathways associated with stress responsive genes, helping to uncover deeper biological mechanisms. These candidate genes can also be applied in marker-assisted breeding programs to develop heat-tolerant rice cultivars, ensuring improved resilience to environmental stress. Furthermore, integrating these findings with precision agriculture can enhance AI-driven predictive models for real time monitoring and early detection of heat stress in crops, leading to more efficient and sustainable agricultural practices. These results demonstrate the effectiveness of MEGENA in capturing complex gene interactions and hierarchical relationships, making it a valuable tool for advancing agricultural genomics and improving crop resilience in the face of climate change.

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