

PLANT BIOTECHNOLOGY APPROACHES FOR IMPROVING CROP RESILIENCE TO DROUGHT AND SALINITY: MOLECULAR MECHANISMS AND GENETIC RESPONSES

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ABSTRACT:

Drought and salinity are major abiotic stresses that restrict crop productivity by disrupting water relations, ion balance, cellular metabolism, and gene regulation. This study investigated the molecular and genetic responses associated with these stresses using the publicly available rice transcriptomic dataset GSE21651 from the NCBI Gene Expression Omnibus. The analysis included 16 samples representing control, drought, and salinity conditions and 57,381 probe sets. Genome-wide differential-expression analysis revealed that 23,506 probe sets (40.96%) were significant at adjusted $P < 0.05$, indicating extensive transcriptional reprogramming under stress. Exploratory expression profiling and UMAP analysis further demonstrated distinct global expression patterns among control, drought, and salinity groups. Functional interpretation of significant genes identified major stress-responsive components involved in water transport, osmotic protection, ABA-mediated signalling, antioxidant defence, metabolic adjustment, and transcriptional regulation. Important candidate genes included aquaporins, late embryogenesis abundant proteins, dehydrins, antioxidant enzymes, and WRKY, NAC, MYB, and bZIP transcription factors. The findings demonstrate that drought and salinity tolerance in rice is governed by coordinated molecular networks involving both shared and stress-specific responses. These results highlight the value of transcriptomic analysis for identifying candidate genes that can support marker-assisted breeding, genetic engineering, and genome-editing strategies aimed at developing climate-resilient crops.

KEYWORDS: Drought stress; Salinity stress; Rice transcriptomics; Abiotic stress tolerance; Plant biotechnology

1. INTRODUCTION

The impacts of climate change have increased the duration and intensity of droughts and soil salinization, which are significant impediments to crop productivity and pose a risk to global food security. As a result, the improvement of performance under adverse environmental conditions by targeted modification of stress-responsive traits has become an important strategy for GM, and specific examples of such traits have been identified for both plants and animals.

Strategies for enhancing performance under adverse environmental conditions have therefore become an important GM objective, as have been targeted modification of stress-responsive traits, and specific traits have been identified for both plants and animals. Conventional breeding is complemented by modern plant biotechnology, which allows for the identification, transfer and regulation of stress perception, stress signalling, and stress adaptation genes (Munaweera et al., 2022).

Progress in crop biotechnology has also moved towards molecular informed strategies that can help to speed up the breeding of climate-smart cultivars (Riaz et al., 2025). Drought stress affects plant water relations, photosynthesis, membrane stability, nutrient acquisition and cellular metabolism, and ultimately growth and yield. Plants react by a coordinated physiological and molecular mechanism including stomatal control, osmotic adjustment, antioxidant defence, plant hormone signalling and expression of stress responsive genes (Bashir et al., 2021). These processes are now targeted by biotechnological interventions such as the development of transgenic plants, marker-assisted selection, genomic tools and manipulation of the regulatory genes involved in abiotic stress tolerance (Villalobos-López et al., 2022).

The advent of genome editing technologies has multiplied these possibilities, as precise changes in genes that regulate drought and salinity responses can be made with minimal or no alteration of the plant genome (Shelake et al., 2022). This has led to the development of various strategies for using CRISPR-Cas9 to modify genes associated with drought response, with the aim of creating drought-resistant crops (Rai et al., 2023). Epigenetic regulation, such as DNA methylation, histone modification and stress-related chromatin remodelling, which affect the expression of genes without altering their base sequence, also contribute to plant responses to environmental stress (Sun et al., 2021). Hence, advanced biotechnological approaches are increasingly being used to combine genomic, transcriptomic, epigenetic and genome editing technologies in order to characterize drought-responsive pathways and select candidate genes for crop improvement (Şimşek et al., 2024).

Stress-tolerance alleles and genomic regions can also be introgressed into elite cultivars through molecular breeding, keeping the desirable agronomic traits (Pagnotta, 2025). Excessive exposure to salt causes osmotic stress, ionic toxicity and alters water uptake, as well as causing oxidative damage and metabolic imbalance, thus making salinity an equally complicated stress. With the aid of modern biotechnology, plants can be reprogrammed to achieve enhanced ion homeostasis, osmotic protection, signalling and antioxidant activity in saline environments (Raza et al., 2023). Incorporating plant biotechnology with biological interactions and climate-smart breeding strategies can also result in the creation of resilient crops that can withstand various environmental challenges and continue to yield (Srivastava et al., 2025). Most importantly, drought and salinity often trigger similar physiological and biochemical responses, such as increased production of Reactive Oxygen Species, osmolyte accumulation, protection of membranes, and regulation of stress-responsive genes (Kumar et al., 2023). These molecular responses can be identified by transcriptomic analysis, which allows common and stress-specific regulatory pathways to be identified by evaluating the changes in gene-expression patterns (KhokharVoytas et al., 2023).

The simultaneous effects of drought and salinity at the transcriptome level can thus help identify molecules to target in crop improvement research through biotechnology. In the present study, the molecular and genetic changes associated with drought and salinity stress were characterized by utilization of the publicly available rice transcriptomic. The scope encompassed expression distribution analysis across the world, transcriptomic variability associated with treatments, separation of control, drought and salinity samples as well as identification of stress-responsive genes of biological relevance. The aims were to assess the magnitude of differential expression under the various stress conditions, to identify the key genes involved in water transport, osmotic protection, ABA signalling, oxidative-stress defence and transcriptional regulation, and to assess their potential application in crop biotechnology for increased resilience.

2. MATERIALS AND METHODS

2.1 Dataset Source and Study Design

In the present study, publicly available gene-expression data of the National Centre for Biotechnology Information Gene Expression Omnibus (NCBI, 2020), accession number GSE21651 were used. The data set is comprised of microarray-based transcriptomic profiles of *Oryza sativa* in relation to drought and salinity stress. It was chosen because its experimental conditions were directly related to the study of abiotic stresses associated molecular and genetic mechanisms of crop resistance.

A total of 16 samples were analysed and categorised into three experimental groups (control (c) n = 8; drought (d) n = 4; and salinity (s) n = 4) based on the available sample annotations. Thus, for all sample-level exploratory analyses (expression-distribution plots and dimensionality-reduction analysis), the denominator was 16 samples. There were 57,381 probe sets in the processed expression output. Gene-level statistical interpretation variables were probe-set identifier, adjusted P-value (adj.P.Val), unadjusted P-value (P.Value), F-statistic (F), gene symbol and gene title. The total number of probe sets used (57,381) was kept as the denominator for genome-wide statistical significance calculations.

2.2 Expression Data Assessment and Exploratory Analysis

Prior to differential expression analysis, overall distribution of normalized expression values was evaluated. Box and whisker plots were created to compare the median expression level, interquartile range and the spread of expression measurements in the drought, salinity and control groups at the sample level.

These plots were therefore used descriptively to assess if there were any major distributional abnormalities within the 16 samples. In addition, kernel-density plots were plotted for each sample to explore the distribution of the expression intensities throughout the entire range of expression intensities measured. The density profiles of the controls, drought and salinity groups were compared to determine the overall changes in the transcriptomic distributions associated with the stress.

2.3 Statistical Testing and Differential Gene-Expression Analysis

To assess differences in expression between the different groups (control vs drought vs salinity), the F-statistic provided in the processed differential-expression table was calculated at the probe-set level. The F-statistic was used to test the hypothesis that expression differed among the three experimental conditions as an omnibus test. The groupwise expression model can be represented as:

$$Y_{ij} = \beta_0 + \beta_1 D_j + \beta_2 S_j + \epsilon_{ij}$$

where Y_{ij} represents the expression value of probe set i for sample j , β_0 represents the reference expression level for the control group, D_j represents membership in the drought group, S_j represents membership in the salinity group, and ε_{ij} represents residual variation.

The omnibus null hypothesis was:

$$H_0: \beta_1 = \beta_2 = 0$$

indicating no overall treatment-associated difference in expression. The alternative hypothesis indicated that at least one treatment coefficient differed from zero.

The processed output of differential expression was returned and provided P values after multiple testing, which were used for significance assessment. The adjusted P value of < 0.05 was considered the primary significance value. Other levels of statistical evidence were indicated by adjusted P values of < 0.01 , < 0.001 , < 0.0001 , and < 0.00001 . The percentage of significant probe sets at each threshold was calculated using:

$$\text{Percentage significant} = \frac{\text{Number of significant probe sets}}{57,381} \times 100$$

Descriptive statistics for the F-statistic were computed for the 23,506 probe sets that met adjusted $P < 0.05$. As shown in the Results, the mean F-statistic was around 9.93, with the observed values ranging from around 4.54 to 137.0. A volcano plot was created to visualize the specific salinity-versus-control response with the x-axis of the plot representing $\log_2$ fold change and the y-axis representing $-\log_{10}(P\text{-value})$. The fold change values shown as positive (positive $\log_2$ fold-change) showed higher expression under salinity compared to control, while negative (negative $\log_2$ fold-change) showed lower expression. Statistically significant genes were identified visually from nonsignificant genes.

2.4 Dimensionality Reduction and Identification of Stress-Responsive Candidate Genes

Uniform Manifold Approximation and Projection (UMAP) was used to explore the global transcriptional similarity among samples. All the 16 samples were analysed, which are control, drought, and salinity. Seven nearest neighbors ($n_neighbors = 7$) were used for the UMAP model, as in the final dimensionality reduction plot shown in the study.

Statistically significant and biologically annotated probe sets were then selected to yield candidate stress-responsive genes. Selection was based on the biological relevance to drought and salinity adaptation mechanisms, not on an additional statistical model. Specific functions of interest were water transport, osmotic regulation, abscisic acid signalling, late embryogenesis abundant proteins, dehydrins, metabolic adaptation, reactive oxygen species detoxification, and transcriptional regulation.

2.5 Functional Classification and Missing-Data Handling

Significant probe sets were interpreted by gene symbols and gene-title annotations to assess the molecular functions. Genes with annotations were divided into biologically meaningful groups under the categories of abiotic-stress adaptation. These encompassed aquaporins associated with water transport, LEA proteins and dehydrins associated with cellular protection, ABA-responsive genes associated with stress signalling, antioxidant enzymes associated with reactive oxygen species detoxification, and WRKY, NAC, MYB, and bZIP transcription factors associated with transcription regulation. Inferential statistics for each selected candidate gene were the probe-level F value and adjusted P value from the differential-expression analysis.

3. RESULTS

3.1 Overall Expression Profile and Statistical Significance

The expression data set GSE21651 contained 57,381 probe sets which were analysed in control, drought and salinity conditions. A significant number of genes had statistically significant differences between the experimental groups after multiple testing. In total, 40.96% (23,506 of 57,185) probe sets had an adjusted P-value less than 0.05 (Table 1). Increasingly stringent thresholds reduced the number of significant probes sets to 12,399 at adjusted $P < 0.01$, 3,583 at adjusted $P < 0.001$, 759 at adjusted $P < 0.0001$, and 101 at adjusted $P < 0.00001$. The results show the more extensive transcriptional reprogramming in rice under drought and salinity stress.

Table 1. Distribution of statistically significant probe sets in GSE21651

Adjusted P-value threshold	Significant probe sets (n)	Percentage of total probes (%)
< 0.05	23,506	40.96
< 0.01	12,399	21.61
< 0.001	3,583	6.24
< 0.0001	759	1.32
< 0.00001	101	0.18
Total probe sets	57,381	100.00

Within each sample, the intensity of the expression was also analysed to examine the distribution of expression intensities. The box plot distribution of normalized expression values for the control, drought and salinity samples is shown in Figure 1.

The interquartile ranges and medians of most control and drought samples were similar. Median expression levels were slightly more variable for salinity-treated samples and in a few samples were lower than for the control and drought samples. The large overlap in the distributions, however, suggested that the data set was appropriate to conduct further comparative expression analyses.

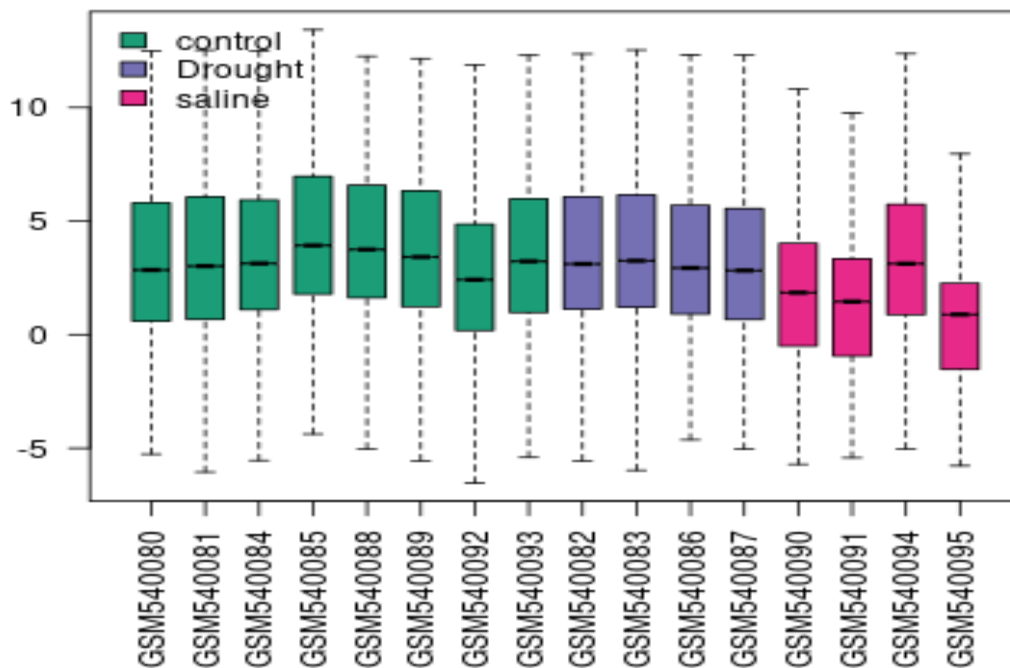


Figure 1. Boxplot distribution of gene-expression intensities across control, drought, and salinity samples in GSE21651

The density distribution confirmed the observations from the boxplot. The expression-density profile for most of the control and drought samples was found to be overlapping with each other, while salinity treated samples had a higher displacement in their density curves, as shown in Figure 2. More specifically, some salinity samples were found to have higher density at lower expression intensities, indicating a more pervasive effect of salt stress on the rice transcriptome.

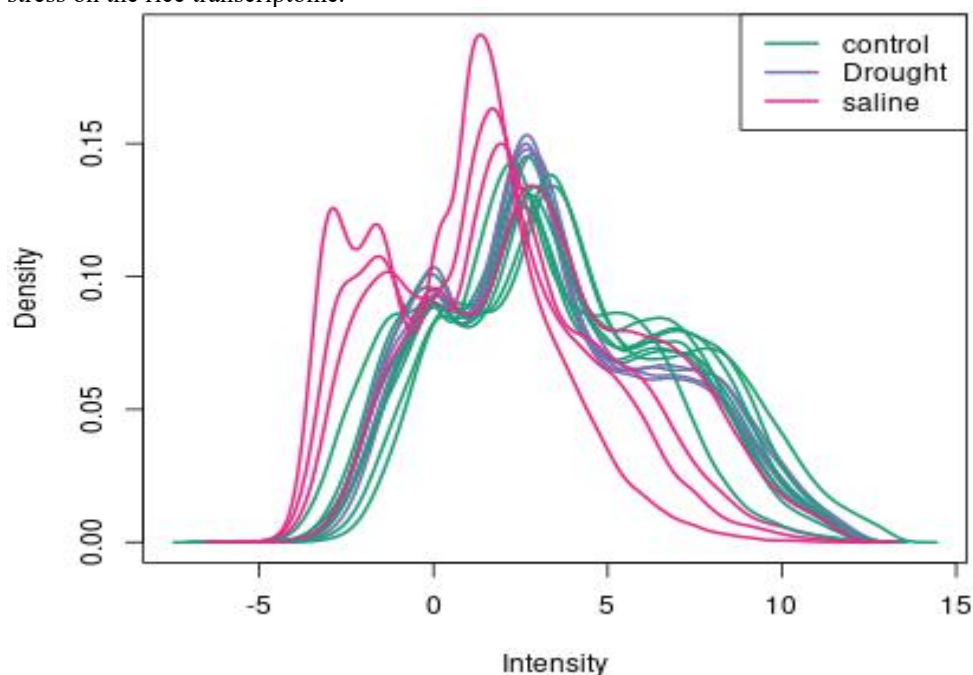


Figure 2. Density distribution of normalized gene-expression intensities among control, drought, and salinity sample

3.2 Global Separation of Drought- and Salinity-Stressed Samples

A Uniform Manifold Approximation and Projection (UMAP) was used to reduce the dimensionality of the experimental samples to analyse the similarities and differences across the globe. The difference between the three experimental conditions is apparent in Figure 3. The drought-treated samples grouped mainly in the lower-right part of the UMAP space while the salinity-treated samples grouped mostly in the upper left part of the UMAP space. The distribution of control samples was wider in the intermediate range.

Clearly, the drought and salinity samples were shifted away from the controls, suggesting two distinct genome-wide transcriptional responses to the two stresses. There was also a greater separation between the drought and salinity groups, indicating that the molecular response to each stress also has common and stress-specific regulatory mechanisms. Intra-group variability may be due to biological or genotype related variability as part of the experimental design.

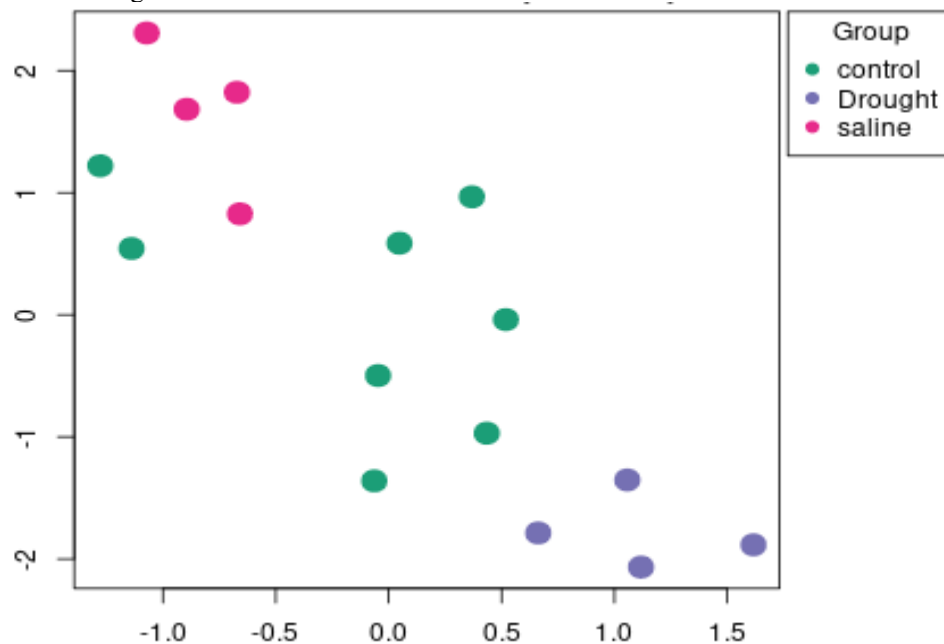


Figure 3. UMAP visualization of global gene-expression profiles in GSE21651

The global F-statistical analysis also showed significant transcriptional differences between the treatments. Of the probe sets significant at adjusted $P < 0.05$, the average F statistic had a mean of ~ 9.93 (range 4.54-137.0). Some of the most statistically significant annotated probe sets in the data set are shown in Table 2. A set of highly responsive genes involved in water transport, Osmo protection, ABA signalling, carbon metabolism and cellular stress response were identified.

Table 2. Selected highly significant annotated genes identified in GSE21651

Probe ID	Gene symbol	Gene annotation	Adjusted P	F-statistic
Os.2250.1.S1_a_at	LOC4339655	Uncharacterized LOC4339655	4.16 $\times 10^{-7}$	72.1
Os.16163.1.S1_at	LOC4328924	Uncharacterized LOC4328924	1.02 $\times 10^{-6}$	62.2
Os.36307.1.S1_at	LOC9266106	Uncharacterized LOC9266106	1.02 $\times 10^{-6}$	62.1
Os.31022.1.S1_at	LOC4339887	Uncharacterized LOC4339887	1.02 $\times 10^{-6}$	61.8
Os.17347.1.S1_at	LOC4328194	Titin homolog	1.70 $\times 10^{-6}$	57.0
Os.47303.1.S1_s_at	LOC4326552	NADP-dependent malic enzyme	2.28 $\times 10^{-6}$	52.7
Os.9824.1.S2_at	LOC107278649/LOC4339090	RETICULATA-related protein/aldose reductase	2.28 $\times 10^{-6}$	52.7
Os.2544.1.S1_s_at	LOC4348981	Probable aquaporin TIP3-1	2.40 $\times 10^{-6}$	52.1
Os.1205.1.S1_at	LOC4326740	LEA protein Lea14-A	2.78 $\times 10^{-6}$	49.0

Os.53526.1.S1_at	LOC4336949	ABA-inducible protein PHV A1	2.90 10 ⁻⁶	×	48.7
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3.3 Differential Gene-Expression Response to Salinity

Salinity treatment resulted in significant differential expression in comparison with control, by pairwise analysis, in several genes. The volcano plot of salinity versus control is displayed in Figure 4. The vast majority of genes with statistically significant fold changes were in the negative $\log_2$ fold change region, suggesting that there were many genes that were repressed when salinity stress was applied, while a smaller number of genes had fold changes in the positive $\log_2$ fold change region and were therefore expressed more under salinity stress. Genes showing changes in $P < 0.05$ and downregulated genes (blue colour) and upregulated genes (red color) are highlighted in the plot.

The high abundance of highly significant genes on the negative side of the volcano plot indicates a large proportion of genes which were suppressed by transcription. The presence of genes with very high expression levels suggests activation of certain adaptive pathways for salt tolerance.

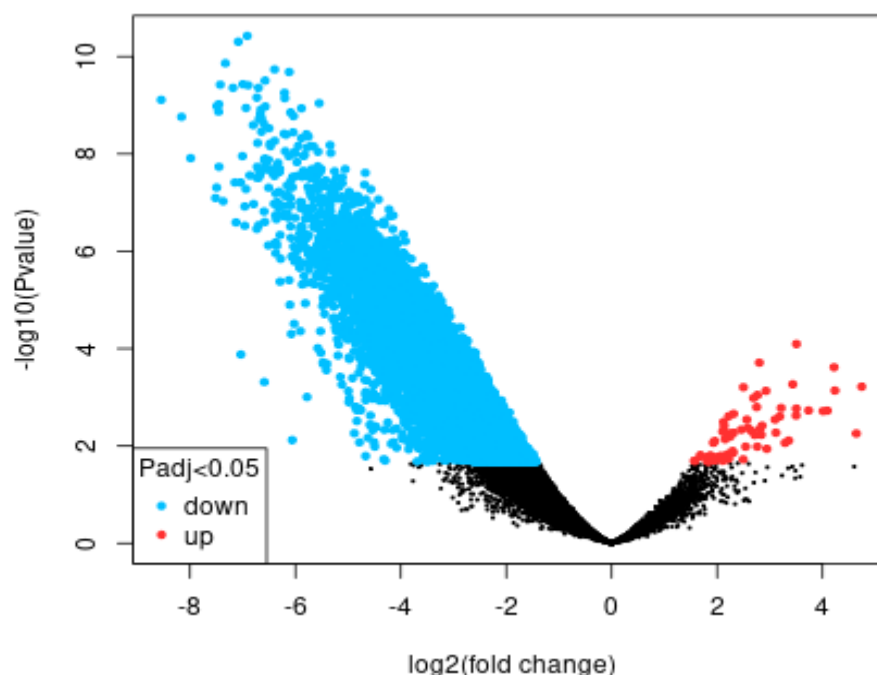


Figure 4. Volcano plot showing differential gene expression between salinity-treated and control samples in GSE21651

The x-axis represents $\log_2$ fold change, and the y-axis represents $-\log_{10}(P\text{-value})$. Blue points indicate significantly downregulated genes, red points indicate significantly upregulated genes, and black points represent genes not meeting the significance criterion.

Since the top-table provided is an overall F-test and not pairwise fold-changes it is important to note that the exact number of genes that are "upregulated" and "downregulated" should be drawn from the differential-expression output for each pairwise comparison of salinity versus control, not from the omnibus table. As a result, the volcano plot is used to primarily understand the direction and overall magnitude of the transcriptional response.

3.4 Molecular and Genetic Components Associated with Drought and Salinity Resilience

The most statistically significant features in the data set included several genes which have been shown to be functionally relevant to abiotic-stress tolerance. Genes associated with water transport and osmotic adjustment were highly represented as can be seen in Table 3. The aquaporin TIP3-1 yielded a probable aquaporin (adjusted P of 2.40×10^{-6}) and an F-statistic of 52.1, whereas aquaporin PIP2-7 also exhibited highly significant differences between treatments.

Aquaporins play a key role in regulating the water flow in and out of cells and tissues and can be targeted towards enhancing plant water-use efficiency. Late embryogenesis abundant proteins and dehydrins were also specifically strongly expressed. LEA14-A showed an adjusted P of 2.78×10^{-6} , while dehydrin Rab25 showed an adjusted P of 6.0×10^{-6} . Dehydration is linked closely to these proteins and their functions of cellular protection. The protein, PHV A1, induced by ABA, was also highly significant indicating the role of ABA-dependent signalling in drought and salt induced stress.

Table 3. Major drought- and salinity-responsive candidate genes associated with osmotic adjustment and stress signalling

Functional category	Gene/protein	Gene symbol	Adjusted P	F-statistic	Potential stress-related role
Water transport	Aquaporin TIP3-1	LOC4348981	2.40×10^{-6}	52.1	Cellular water transport
Water transport	Aquaporin PIP2-7	LOC4347729	8.0×10^{-6}	40.9	Membrane water permeability
Cellular protection	LEA protein Lea14-A	LOC4326740	2.78×10^{-6}	49.0	Protection during dehydration
Cellular protection	LEA protein D-34	LOC4331691	6.0×10^{-6}	42.6	Osmotic/desiccation tolerance
Dehydration response	Dehydrin Rab25	LOC4326935	6.0×10^{-6}	42.8	Cellular stabilization under dehydration
ABA signalling	ABA-inducible protein PHV A1	LOC4336949	2.90×10^{-6}	48.7	ABA-mediated stress signalling
Carbon metabolism	NADP-dependent malic enzyme	LOC4326552	2.28×10^{-6}	52.7	Metabolic adjustment under stress
Osmo protection/metabolism	Aldose reductase-associated probe	LOC4339090	2.28×10^{-6}	52.7	Detoxification/osmotic adaptation

Genes also significantly changed were those related to oxidative-stress management and transcriptional regulation. The results of the analysis revealed the presence of representative antioxidant enzymes and transcription factors, as indicated in Table 4. Peroxidase 1-like (catalase isozyme B) that mitochondrial manganese superoxide dismutase and glutathione S-transferases showed significant treatment-associated responses, suggesting that treatment led to an activation of mechanisms involved in the detoxification of reactive oxygen species. Likewise, there was widespread transcriptional regulation of the drought and salinity stress response as evidenced by the significant responses of WRKY, NAC, MYB and bZIP transcription factors.

Table 4. Selected antioxidant and transcriptional regulatory genes associated with abiotic-stress responses

Functional class	Gene/protein	Gene symbol	Adjusted P	F-statistic
ROS detoxification	Peroxidase 1-like	LOC4340843	1.8×10^{-5}	34.3
ROS detoxification	Catalase isozyme B	LOC4342124	1.04×10^{-4}	23.8
ROS detoxification	Mn-superoxide dismutase	LOC4338417	7.70×10^{-4}	15.4
Detoxification	Glutathione S-transferase GSTF1	LOC4325710	1.25×10^{-4}	22.9
Transcription regulation	WRKY transcription factor 17	LOC4335388	8.1×10^{-5}	25.0
Transcription regulation	NAC domain-containing protein 71	LOC4349963	3.9×10^{-5}	29.5
Transcription regulation	MYB1R1	LOC4327305	1.60×10^{-4}	21.8
Transcription regulation	bZIP transcription factor 17	LOC4344086	9.67×10^{-4}	14.6

Taken together, these results suggest that drought and salinity stress in rice are linked to concerted alterations in water transport, ABA signalling, protective proteins, redox homeostasis, metabolic adjustment and transcriptional regulation. The simultaneous response of the aquaporins, LEA proteins, dehydrins, antioxidant enzymes and major transcription-factor families suggest that these molecular systems could be used as targets in biotechnology to enhance abiotic-stress resilience.

4. DISCUSSION

The GSE21651 dataset was subjected to transcriptomic analysis, which showed that there was a considerable molecular reprogramming in rice plants under drought and salinity stress conditions. In fact, a significant percentage of the probe sets analysed exhibited statistically significant differences, suggesting that abiotic stress responses are not confined to the level of gene expression but are generally large-scale transcriptional changes. This process is similar to the present knowledge that stress tolerance is a co-regulated process involving interdependent physiological, biochemical and regulatory networks which synchronously regulate plant survival in adverse environment conditions (Mohapatra et al., 2024).

The overall expression profile also indicated that salinity induced transcriptional changes over a wider range of samples as compared to drought. These changes might be a consequence of the joint effect of osmotic stress and ionic toxicity, factors which are known to disrupt the cells' water balance, membrane stability, nutrient transport and metabolic homeostasis. The use of biotechnology for screening stress-responsive molecular traits has been gaining importance as a practical tool to develop and identify sensitive and tolerant genotype and future target traits for crop improvement (Nguyễn et al., 2024). The dimensional separation of drought and salinity treated

samples suggested that the two stresses induced different global gene-expression patterns. While each of the two conditions has multiple pathways of stress response, salinity also triggers ion transport, ion exclusion and ionic detoxification pathways which may generate a unique molecular signature.

This discovery is consistent with studies revealing that transcription factors and signalling molecules play a pivotal role in determining salt-responsive genetic networks in crops, alongside other approaches such as next-generation sequencing (Singh et al., 2024). Molecular-level differentiation between the types of stress is also beneficial for identifying genes that are relevant specifically to drought or salinity tolerance or both together (combined stress) (Heikal et al., 2023). A notable observation was the marked and widespread repression of genes with a subset of genes that were highly activated, when comparing the salinity with the control. This unbalanced regulation can be a sign of growth-related and energy-consuming processes being suppressed, and protective/defensive ones being stimulated. Stress-responsive signalling and molecular breeding are likely to be effective approaches towards achieving this balance between survival and productivity in water-limited environments (Wang et al., 2025).

The several genes identified within the analysis which had biological significance were related to water transport, osmosis, cellular protection and hormonal regulation. Water-permeable aquaporins are of special interest as they control the permeability of membranes to water and may affect the hydration status of tissues during drought and salinity stress. Further, the discovery of late embryogenesis abundant proteins, dehydrins, and ABA-inducible proteins further confirms dehydration-protective and hormone-mediated pathways. These gene classes have been known to be significant molecular targets which can be improved using biotechnology techniques for abiotic stress tolerance (Mafakheri & Kordrostami, 2020).

The impact of drought-responsive water transport and cellular protection mechanisms further broadens the application potential of molecular breeding for water-use efficiency and drought tolerance. With the rise of molecular breeding methods in dominant crops, more and more research is focusing on water relations, osmotic balance, and stress-responsive signalling genes, as these traits are closely related to survival under water scarcity (Rasheed et al., 2023). The present results thus confirm the possible role of aquaporins, LEA proteins, dehydrins and ABA-responsive genes as possible targets for crop improvement. The apparent cross-over molecular responses to drought and salinity are also biologically reasonable as both stresses lead to cellular dehydration, osmotic imbalance, oxidative damage and hormonal signalling. Such common responses are responsible for many genes that are active during both types ultimately having similar roles in stress adaptation, regardless of the type of stress triggering event (Waseem et al., 2023).

Candidates for aquaporin activity, protection proteins, and stress-responsive signalling pathways also have been suggested as potential molecular targets for the engineering of abiotic stress tolerance in multiple abiotic stresses (Trono & Pecchioni, 2022). Oxidative-stress defence also clearly became a feature of the response. Large changes in the genes for peroxidase, catalase, manganese superoxide dismutase and glutathione S-transferase indicate active control of detoxification of reactive oxygen species. Under salinity and drought stress, accumulation of high levels of ROS can cause protein, lipid, nucleic acid, and cellular membrane damage (Atta et al., 2023); therefore, efficient antioxidant defence is required. The potential of molecular breeding and biotechnology to increase antioxidant ability could therefore benefit the capacity of crops to withstand chronic stress (Ngongolo & Mmbando, 2024). Among the most important stress-responsiveness genes, there were also transcription factors such as WRKY, NAC, MYB and bZIP family genes. These transcription factors are particularly desirable biotechnology targets as they can control several downstream genes at once and to coordinate intricate adaptation responses. Alteration of such regulatory hubs could be a better way to increase stress tolerance in general than altering a single structural gene (Sharma et al., 2024).

For rice, water transport, osmotic adjustment, antioxidant defence, ABA signalling and transcription-factor activities have been correlated repeatedly with enhanced drought and salinity tolerance (Li et al., 2024). The results are in general agreement that the drought and salinity resistance of rice is linked to the concerted regulation of water transport, cellular protection, antioxidant defence, hormone-responsive signalling, metabolic adjustment, and transcriptional control. The molecular pathways identified offer the basis for future applications of marker assisted selection, genome selection, transgenic and genome editing technologies for creating drought and salinity tolerant crops.

5. CONCLUSION

The analysis of publicly available rice dataset in this study showed that drought and salinity cause large-scale and coordinated transcriptional reprogramming in rice. An appreciable percentage of the probe sets analysed exhibited noticeable differences in response to this treatment, suggesting that abiotic-stress tolerance is not regulated by individual genes but by complex molecular networks. In addition, the separation of drought and salinity treated samples confirmed the activation of common and stress specific genetic responses. Several biologically important candidate genes associated with water transport, osmotic adjustment, cellular protection, ABA mediated signalling, oxidative-stress defence, metabolic regulation and transcriptional control were identified in the analysis. The Aquaporins, LEA proteins, dehydrins, antioxidant enzymes, and the transcription factors WRKY, NAC, MYB, and bZIP were found to be possible parts of drought and salinity-resilient mechanisms. They are genes useful potential targets for future functional validation and crop improvement. In summary the results are in favour of the use of transcriptomics as a useful tool for the identification of molecular

markers and candidate genes associated with abiotic stress tolerance. Combining all these transcriptomic data with marker assisted breeding, genome selection, transgenic technologies, and genome editing will speed up the process of developing drought and salinity resistant crops for future climate scenarios.

REFERENCES

1. Atta, K., Mondal, S., Gorai, S., Singh, A. P., Kumari, A., Ghosh, T., ... & Jespersen, D. (2023). Impacts of salinity stress on crop plants: improving salt tolerance through genetic and molecular dissection. *Frontiers in Plant Science*, 14, 1241736.
2. Bashir, S. S., Hussain, A., Hussain, S. J., Wani, O. A., Zahid Nabi, S., Dar, N. A., ... & Mansoor, S. (2021). Plant drought stress tolerance: Understanding its physiological, biochemical and molecular mechanisms. *Biotechnology & Biotechnological Equipment*, 35(1), 1912-1925.
3. Heikal, Y. M., El-Esawi, M. A., El-Ballat, E. M., & Abdel-Aziz, H. M. (2023). Applications of nanoparticles for mitigating salinity and drought stress in plants: An overview on the physiological, biochemical and molecular genetic aspects. *New Zealand Journal of Crop and Horticultural Science*, 51(3), 297-327.
4. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE21651>
5. KhokharVoytas, A., Shahbaz, M., Maqsood, M. F., Zulfiqar, U., Naz, N., Iqbal, U. Z., ... & AlShaqhaa, M. A. (2023). Genetic modification strategies for enhancing plant resilience to abiotic stresses in the context of climate change. *Functional & integrative genomics*, 23(3), 283.
6. Kumar, R., Sagar, V., Verma, V. C., Kumari, M., Gujjar, R. S., Goswami, S. K., ... & Prasad, P. V. (2023). Drought and salinity stresses induced physio-biochemical changes in sugarcane: an overview of tolerance mechanism and mitigating approaches. *Frontiers in Plant Science*, 14, 1225234.
7. Li, Q., Zhu, P., Yu, X., Xu, J., & Liu, G. (2024). Physiological and molecular mechanisms of rice tolerance to salt and drought stress: advances and future directions. *International journal of molecular sciences*, 25(17), 9404.
8. Mafakheri, M., & Kordrostami, M. (2020). Role of molecular tools and biotechnology in climate-resilient agriculture. In *Plant ecophysiology and adaptation under climate change: mechanisms and perspectives II: mechanisms of adaptation and stress amelioration* (pp. 491-529). Singapore: Springer Singapore.
9. Mohapatra, R., HM, H. K., Naan, T., Chitra, M., Ashwini, R., Rout, A., & Lallawmkimi, M. C. (2024). A review on biotechnological innovations in developing stress-tolerant crops for adverse environmental conditions. *Journal of Scientific Research and Reports*, 30(7), 901-920.
10. Munaweera, T. I. K., Jayawardana, N. U., Rajaratnam, R., & Dissanayake, N. (2022). Modern plant biotechnology as a strategy in addressing climate change and attaining food security. *Agriculture & Food Security*, 11(1), 26.
11. National Center for Biotechnology Information. (2020). *Gene expression analysis of drought and salinity stress response in rice* (GEO Series GSE21651) [Data set]. Gene Expression Omnibus:
12. Ngongolo, K., & Mmbando, G. S. (2024). Harnessing biotechnology and breeding strategies for climate-resilient agriculture: pathways to sustainable global food security. *Discover sustainability*, 5(1), 431.
13. Nguyễn, C. T., Gammatantrawet, N., Susawaengsup, C., Tandee, K., Ramli, A. N. M., Tongkoom, K., ... & Bhuyar, P. (2024). Drought-ready plant resilience: Harnessing nano-biotechnology techniques for swift screening and selection of organic crop varieties. *South African Journal of Botany*, 169, 553-566.
14. Pagnotta, M. A. (2025). Molecular breeding for abiotic stress tolerance in crops: recent developments and future prospectives. *International Journal of Molecular Sciences*, 26(18), 9164.
15. Rai, G. K., Khanday, D. M., Kumar, P., Magotra, I., Choudhary, S. M., Kosser, R., ... & Pandey, S. (2023). Enhancing crop resilience to drought stress through CRISPR-Cas9 genome editing. *Plants*, 12(12), 2306.
16. Rasheed, A., Zhao, L., Raza, A., Mahmood, A., Xing, H., Lv, X., ... & Jie, Y. (2023). Role of molecular breeding tools in enhancing the breeding of drought-resilient cotton genotypes: An updated review. *Water*, 15(7), 1377.
17. Raza, A., Tabassum, J., Fakhar, A. Z., Sharif, R., Chen, H., Zhang, C., ... & Varshney, R. K. (2023). Smart reprogramming of plants against salinity stress using modern biotechnological tools. *Critical reviews in biotechnology*, 43(7), 1035-1062.
18. Riaz, M., Yasmeen, E., Saleem, B., Hameed, M. K., Saeed Almheiri, M. T., Saeed Al Mir, R. O., ... & Gururani, M. A. (2025). Evolution of agricultural biotechnology is the paradigm shift in crop resilience and development: a review. *Frontiers in Plant Science*, 16, 1585826.
19. Sharma, A., Dheer, P., Rautela, I., Thapliyal, P., Thapliyal, P., Bajpai, A. B., & Sharma, M. D. (2024). A review on strategies for crop improvement against drought stress through molecular insights. *3 Biotech*, 14(7), 173.
20. Shelake, R. M., Kadam, U. S., Kumar, R., Pramanik, D., Singh, A. K., & Kim, J. Y. (2022). Engineering drought and salinity tolerance traits in crops through CRISPR-mediated genome editing: Targets, tools, challenges, and perspectives. *Plant Communications*, 3(6).
21. Şimşek, Ö., Isak, M. A., Dönmez, D., Dalda Şekerci, A., İzgü, T., & Kaçar, Y. A. (2024). Advanced biotechnological interventions in mitigating drought stress in plants. *Plants*, 13(5), 717.

22. Singh, A. K., Pal, P., Sahoo, U. K., Sharma, L., Pandey, B., Prakash, A., ... & Imbrea, F. (2024). Enhancing crop resilience: the role of plant genetics, transcription factors, and next-generation sequencing in addressing salt stress. *International Journal of Molecular Sciences*, 25(23), 12537.
23. Srivastava, A. K., Riaz, A., Jiang, J., Li, X., Uzair, M., Mishra, P., ... & Xie, X. (2025). Advancing climate-resilient sorghum: the synergistic role of plant biotechnology and microbial interactions. *Rice*, 18(1), 41.
24. Sun, C., Ali, K., Yan, K., Fiaz, S., Dormatey, R., Bi, Z., & Bai, J. (2021). Exploration of epigenetics for improvement of drought and other stress resistance in crops: A review. *Plants*, 10(6), 1226.
25. Trono, D., & Pecchioni, N. (2022). Candidate genes associated with abiotic stress response in plants as tools to engineer tolerance to drought, salinity and extreme temperatures in wheat: an overview. *Plants*, 11(23), 3358.
26. Villalobos-López, M. A., Arroyo-Becerra, A., Quintero-Jiménez, A., & Iturriaga, G. (2022). Biotechnological advances to improve abiotic stress tolerance in crops. *International Journal of Molecular Sciences*, 23(19), 12053.
27. Wang, M., Zhao, Y., Huang, Y., & Liu, J. (2025). Integrating drought stress signalling and smart breeding for climate-resilient crops: regulatory mechanisms and genetic strategies. *Plants*, 14(24), 3714.
28. Waseem, M., Liu, P., & Aslam, M. M. (2023). Salinity and drought stress in plants: understanding physiological, biochemical and molecular responses. *Frontiers in Plant Science*, 14, 1277859.