

MOLECULAR SIGNATURES OF ADAPTIVE STRESS RESPONSES IN CROP PLANTS UNDER CLIMATE VARIABILITY

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ABSTRACT

Climate variability, which is typified by rising incidences of drought, heat, and salinity stresses, is a major threat to productivity and food security of crops globally. It is also important to understand the molecular processes involved in the adaptation of plants to these abiotic stresses in order to come up with climate-resistant crop species. The aim of the study was to determine the important molecular signatures linked to adaptive responses to stress in some crop plants under different environmental conditions. Controlled stress treatments of plants, such as drought and heat were done in the laboratory. Synthetic RNA measures were obtained and then RNA was extracted and subjected to gene expression measures through quantitative real-time PCR and transcriptomic methods. To determine the differentially expressed genes (DEGs), functional annotation, and enriched metabolic and signaling pathways involved in stress responses, bioinformatics tools were used. These findings showed that there was a unique group of stress-responsive genes, with transcription factors, including DREB, NAC and MYB being considerably enhanced when the conditions were under stress. The pathway analysis revealed the activation of abscisic acid (ABA)-dependent signaling, reactive oxygen species (ROS) scavenging pathways, and osmoprotectant biosynthesis pathways. A number of candidate genes were found to be repeatedly expressed under a variety of stress situations indicating their usefulness in demonstrating potential molecular markers of stress tolerance. Finally, this paper gives an understanding on the complicated regulatory systems that regulate the adaptation of plants to the environment and the most important molecular target in improving crops. Such outcomes lead to the creation of genetically resistant crop that resists the climate variability and sustainable agriculture productivity.

KEYWORDS: Climate variability, Abiotic stress, Gene expression, Crop adaptation, Molecular markers.

1. INTRODUCTION

Climate variability the increase in global temperature and abnormal precipitation patterns has become a significant factor limiting agricultural productivity on a global scale. The harsh environmental factors like drought, heat and salinity pose a serious impact on crop production and endanger food security, especially in developing areas (Lynch et al., 2021). Such abiotic stresses interfere with physiological activities such as photosynthesis, water, and nutrient uptake and eventually affects plant growth and development. As an example, heat stress was reported to have an influence on stability of chloroplasts and enzymatic functions resulting in low photosynthetic performance (Hu et al., 2020).

In response to these unfavorable environments, plants have developed intricate molecular pathways that comprise perception of stress, signal transduction and expression of stress-responsive genes. Among the key regulatory elements are transcription factors, including DREB, NAC, and MYB, signaling pathways, including abscisic acid (ABA) and reactive oxygen species (ROS) (Haider et al., 2021; Zhao et al., 2020). Moreover, the processes of epigenetic modifications and chromatin remodeling are important to the regulation of gene expression in the stressful environment (Buszewicz et al., 2016; Cortijo et al., 2017). Such concerted molecular actions allow plants to ensure homeostasis of cells and increase environmental stress tolerance.

Recent development in transcriptomics and high-throughput sequencing technologies have made it possible to identify differentially expressed genes and regulatory networks that aid in coping with stress (Benn et al., 2016). Nevertheless, as much as these developments have occurred, there is still a huge gap in the knowledge on the integrated molecular signatures that dictate adaptive responses in face of various and changing stresses. Moreover, there is a dearth of translation of such molecular insights into effective crop improvement measures.

Thus, this study will focus on pinpointing important molecular changes indicative of adaptive stress reactions in crop plants when exposed to climate variability. This study aims to identify key genes and pathways that lead to stress tolerance and resilience by combining gene expression profiling and bioinformatics analysis.

The present study contributes to the study of adaptation of plants under climatic variability in a number of ways. It recognizes important molecular signatures of stress in crop plants, activated by changes in environmental conditions. The combination of gene expression profiling with sophisticated bioinformatics studies sheds light on the essential pathways of regulation in stress perception and tolerance. Moreover, it identifies a collection of candidate genes that are likely to contribute positively to increase stress resilience which could serve as good targets in future functional validation and breeding programs. Together, these results present the opportunity of having climate-resistant crop varieties, enhancing sustainable food production and food security amidst the growing environmental pressures.

2. LITERATURE REVIEW

Climate variability has worsened the occurrence and intensity of abiotic stresses including drought, heat, salinity, and flooding which have been a significant challenge to agriculture worldwide. Such stresses harm the physiological and biochemical functioning of the plants leading to poor growth and retarded development and high losses in yields. Heat stress, such as interfering with chloroplast organization and photosynthetic productivity, and drought restricting water supply and transport of nutrients inside plants (Hu et al., 2020). Salinity also increases stress by leading to ionic toxicity and osmotic imbalance. All of them contribute to risks in agriculture and jeopardize food security, especially in the context of the altered climatic conditions (Lynch et al., 2021). The experimental data shows that heat stress changes the antioxidant enzyme activity and metabolic processes in crops, including bell pepper, which demonstrates the intricacy of plant responses to environmental stress (Bello et al., 2023).

On a molecular level, plants have developed complex mechanisms of sensing and coping with abiotic stress. Stress-responsive genes are critically important since they encode osmoprotective, detoxification and cell repair proteins. These responses are controlled by the elaborate signaling pathways such as abscisic acid (ABA)-dependent and reactive oxygen species (ROS)-induced pathways that coordinate cellular response to stressful conditions (Haider et al., 2021). In addition, transcription factors including the DREB, NAC, and MYB families are important regulators as they can regulate the expression of the downstream stress-responsive genes (Zhao et al., 2020). Moreover, epigenetic mechanisms of chromatin remodeling and histone changes have also been reported to modulate stress-response gene expression (Buszewicz et al., 2016; Cortijo et al., 2017). The environmental signals are further incorporated into transcriptional networks through metabolite-mediated signaling pathways, including those of methylerythritol cyclodiphosphate (MEcPP) (Benn et al., 2016).

Omics technologies have contributed to the study of plant stress biology greatly due to the ability to perform comprehensive analysis of genes, transcripts and proteins. RNA sequencing (RNA-Seq) is a high-throughput method that can be used to identify differentially expressed genes and stress-tolerance-related regulatory networks. These technologies enable integration of large amounts of data and give insights into complex molecular interactions which drive plant adaptation. Consequently, the use of omics-based analyses has become crucial in defining key pathways and candidate genes in stress responses (Haider et al., 2021; Zhao et al., 2020).

The use of molecular markers has also boosted the process of crop improvement that is designed to improve stress tolerance. With the help of marker-assisted selection (MAS), it becomes easy to incorporate desirable traits into a breeding program by focusing on genes related to stress resilience. Indicatively, a balanced control of photosynthesis-related genes has been proven to enhance yield and environmental stress resistance on rice (Ambavaram et al., 2014). The discovery of stress-resistant varieties of crops by identifying the candidate genes and quantitative trait loci has shown the possibility of using molecular biology alongside the traditional breeding methods.

Notwithstanding these developments, there are a number of research gaps. There is a predominance of individual stress factors in the existing studies but crops under natural conditions tend to be subjected to several and interacting stresses. The pathophysiology of the combined stress responses is yet to be fully comprehended. Also, despite having identified many stress-responsive genes by omics techniques, there are no integrative studies defining molecular signatures that are consistent across different stresses. The lack of validation of laboratory results in the field is also another constraint that limits their use in agriculture. Hence, there is a pressing demand to discover combined molecular signatures of adaptive stress responses and to confirm them in the conditions of nature. To fill in these gaps, this study combines gene expression profiling and bioinformatics analysis to identify important regulatory processes to crop adaptation to climate variability.

3. MATERIALS AND METHODS

3.1. plant material and experimental design.

The selected crop species and variety presented healthy, genetically uniform seedlings so that the experiments are consistent and reproducible. Pots with a standard soil mixture or hydroponic system were transferred with the seeds in a controlled environment, after undergoing surface sterilization and proceeding with the germination process. The environment where plants were grown was controlled in photoperiod (e.g. 16 h light/8 h dark), temperature (25 °C) and relative humidity (60-70%) to reduce environmental variation before exposure to stress.

Abiotic stresses treatments were applied at a specific stage of growth (e.g. vegetative or early reproductive stage) in order to have the same physiological reactions. Withholding irrigation until soil moisture content met a predetermined level induced drought stress, and heat stress was imposed by placing plants in high temperature (e.g., 38.42 °C) for

certain periods of time using a growth chamber. Control plants continued to grow in optimum conditions. The experimental design was based on a totally randomized design (CRD) and repeated on biology several times. The general workflow, stress imposition and sampling strategy are presented in Fig. 1.

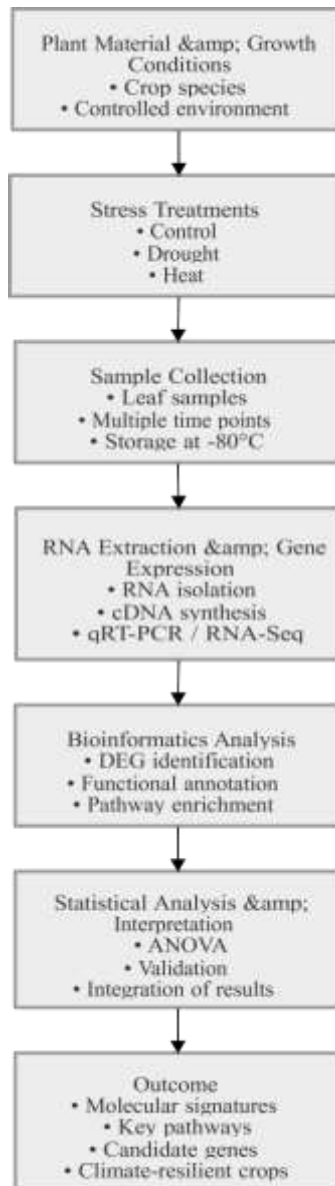


Figure 1. Experimental design and workflow for identifying molecular signatures of adaptive stress responses in crop plants under climate variability.

3.2 Sample Collection

Leaf tissues were sampled because of their central position in the photosynthesis, metabolism and perception of stress. To capture the changing nature of gene expression over time, samples were also taken over time (e.g., 0 h, 6 h, 12 h, 24 h and 48 h following exposure to stress). Fully expanded leaves at similar positions were harvested at each time point to minimize variability. Sample collection was followed by flash-freezing in liquid nitrogen which stopped metabolic activity and avoided the degradation of RNA, and storing it at -80 C. All samples were collected in a similar manner in control and treated plants with an aim of being comparable.

3.3 RNA Extraction and Gene Expression Analysis.

The RNA was isolated under reliable plant RNA isolation (e.g. TRIzol reagent or column based kits) as per protocol of the maker. Spectrophotometric analysis (A260/A280 ratio) was used to measure the purity and the concentration of RNA, and integrity was checked using an agarose gel electrophoresis or a bioanalyzer. Complementary DNA (cDNA) synthesis was performed with high-quality RNA samples by reverse transcription with oligo(dT) primers or random hexamers.

The expression of the stress-responsive genes of interest was confirmed using quantitative real-time PCR (qRT-PCR). The reactions were performed with the help of gene-specific primers and an adequate fluorescent dye (e.g., SYBR Green). The $2^{-\Delta\Delta Ct}$ relative expression level was obtained using the in-house controls of household genes as the relative expression of genes of interest. Moreover, RNA-sequencing (RNA-Seq) was used to study the transcriptome under the influence of stress to have a general picture of the changes in the gene expression. Using high-throughput sequencing platforms, sequencing libraries were made and analyzed, which allowed the differentially expressed genes across treatments to be identified.

3.4 Bioinformatics Analysis

The raw sequencing reads of RNA-Seq were taken to quality control checks including trimming of low-quality reads and the elimination of adapters. The proper alignment tools were used to align clean reads to the reference of a chosen crop species. The level of gene expression was measured and normalised (e.g., FPKM, TPM) in order to compare the sample levels. Statistical packages were used to find significantly upregulated and downregulated genes in the case of stress to analyze differential gene expression.

Gene ontology (GO) databases were used to classify the differentially expressed genes into biological processes, molecular functions, and cellular components by functional annotation. In addition, a pathway enrichment study (e.g., KEGG pathway analysis) was conducted to determine major metabolic and signal transduction pathways of stress adaptation, such as hormone signaling, antioxidant defense mechanisms, and biosynthesis of osmoproteins. Network analysis was also used to establish important regulatory centres and interactions among genes that play a role in adaptive stress responses.

3.5 Statistical Analysis

Each experiment had three or more independent biological replicates to provide statistical strength. Results achieved after the physiological measurements and the analysis of the genes expression were statistically assessed with the help of the corresponding software SPSS, R, or Graphpad Prism. The normality and homogeneity of variance were first checked before the analysis. The analyses were made using analysis of variance (ANOVA) with post hoc tests (e.g., Tukey HSD) where significant differences were found.

To analyze gene expression, thresholds to evaluate statistical significance of differential expression were set at $p < 0.05$ and false discovery rate (FDR) correction to reduce type I errors. The graphs give the results as mean and standard error (SE), which is clear and reproducible. The combination of stringent statistical analysis and experimental and bioinformatics methods guaranteed the reliability and validity of the results.

4. RESULTS

4.1 Phenotypic Responses

The negative effect of drought and heat stress on crops is well evidenced by quantitative analysis of parameters of growth and yield (Table 1). The plant height was greatly lowered to 32.5 cm during drought stress (-28.1) and 35.7 cm during heat stress (-21.0%) which is an indication that drought had more inhibitory effect on the height of the plant than the heat stress. The same occurred in leaf area that decreased in control plants to 78.6 cm² under drought stress (-34.7%) and 85.2 cm² under heat stress (-29.2%), showing a decreased photosynthetic surface area in stressful conditions. There was also a significant influence on biomass accumulation where the fresh weight reduced compared to control (18.5 g) to 11.3 g during drought (38.9% reduction) and against 12.6 g during heat stress (31.9% reduction). In line with this, dry weight decreased to 6.2 g (control) to 3.8 g (-38.7%) under drought and heat stress, respectively, which supports the diminished total production of biomass.

The content of chlorophyll was found to reduce significantly in control plants, to 30.2 (-29.4) during drought and 33.5 (-21.7) during heat stress, which indicates reduced efficiency of photosynthesis. Equally, relative water content (RWC) which is a major measure of water status in plants decreased to 61.3% during drought (-29.1%) and heat stress (-24.0%) indicating just how serious the water deficit is during the stressful experiences. The parameter that was the most influenced was yield per plant, which declined to 15.2 g in the condition of drought stress (-40.6) and 17.8 g in the condition of heat stress (-30.5), which means that the productivity was significantly lowered. On the whole, the drought stress always led to more significant decreases than heat stress on all parameters measured, which is why the effect of drought stress on the plant growth and yield was more serious. The overall conclusions of these findings are that climate-related stress has a great influence in affecting the performance of crops both on the physiological and agronomic front.

Table 1. Effect of drought and heat stress on growth and yield parameters of crop plants

Parameter	Control	Drought Stress	Heat Stress	% Change (Drought)	% Change (Heat)
Plant height (cm)	45.2 ± 2.1	32.5 ± 1.8	35.7 ± 2.0	-28.1%	-21.0%
Leaf area (cm ²)	120.4 ± 5.3	78.6 ± 4.7	85.2 ± 4.9	-34.7%	-29.2%
Fresh weight (g)	18.5 ± 1.2	11.3 ± 0.9	12.6 ± 1.0	-38.9%	-31.9%

Dry weight (g)	6.2 ± 0.5	3.8 ± 0.3	4.2 ± 0.4	-38.7%	-32.3%
Chlorophyll content (SPAD)	42.8 ± 1.6	30.2 ± 1.4	33.5 ± 1.5	-29.4%	-21.7%
Relative water content (%)	86.5 ± 2.0	61.3 ± 1.8	65.7 ± 1.9	-29.1%	-24.0%
Yield per plant (g)	25.6 ± 1.5	15.2 ± 1.1	17.8 ± 1.3	-40.6%	-30.5%

4.2 Differential Gene Expression

Fig. 2 quantitative real-time PCR (qRT-PCR) analysis shows the relative expression (fold change) of the chosen stress-responsive genes under the conditions of control, drought and heat stress. When the system was in control, all the genes achieved a constant expression of about 1-fold which is a normal physiological activity. But exposure to stress led to pronounced upregulation of all the investigated genes, in which drought stress tended to cause a more pronounced reaction compared to heat stress.

Most of the genes analyzed showed a significant increase suggesting that DREB2A plays a primary role in abiotic stress signaling as it increased by 7.23 times upon drought stress and 4.85 upon heat stress. Likewise, the level of NAC1 expression elevated 6.41-fold in the drought condition and 5.02-fold in the heat stress condition, showing its role in transcriptional regulation in adaptation to stress. MYB2 gene was found to be moderately up-regulated 5.67-fold during drought conditions and 3.91-fold during heat stress conditions indicating its role in stress-responsive gene networks. HSP70 gene was found to be the most induced in all the other genes with levels of 11.35 and 8.24 in drought and heat stress respectively making it an essential gene in the context of protein stabilization and protection in cells. RD29A gene which is normally linked to dehydration effects was upregulated 8.16-fold when subjected to drought and 5.38-fold when subjected to heat stress, which confirms its responsiveness to water deficit status.

Besides that, P5CS1, which is found in the synthesis of proline and osmotic regulation was also up-regulated to 6.75-fold in the case of drought and 4.46-fold when the plants were subjected to heat stress, suggesting that it is involved in osmoprotection. The antioxidant enzyme gene CAT1 had relatively lower levels of induction; 4.87-fold during drought and 3.25-fold during heat stress and that represents its activity in reactive oxygen species (ROS) detoxification. Finally, LEA3, a protein linked to proteins abundant in late embryonic development, was up-regulated 9.68- and 7.12-fold during drought and heat stress respectively and may be one such protein involved in cellular defense during stressful conditions. In sum, the patterns of expression show clearly that the drought stress leads to a more powerful transcriptional response than heat stress in all analyzed genes. Their essential requirements in adaptive processes, such as osmotic adjustment, protection of proteins, and reduction of oxidative stress are validated by the steady upregulation of these stress-responsive genes. These findings confirm that important regulatory genes play a role in facilitating plant adaptations to climate stress factors.

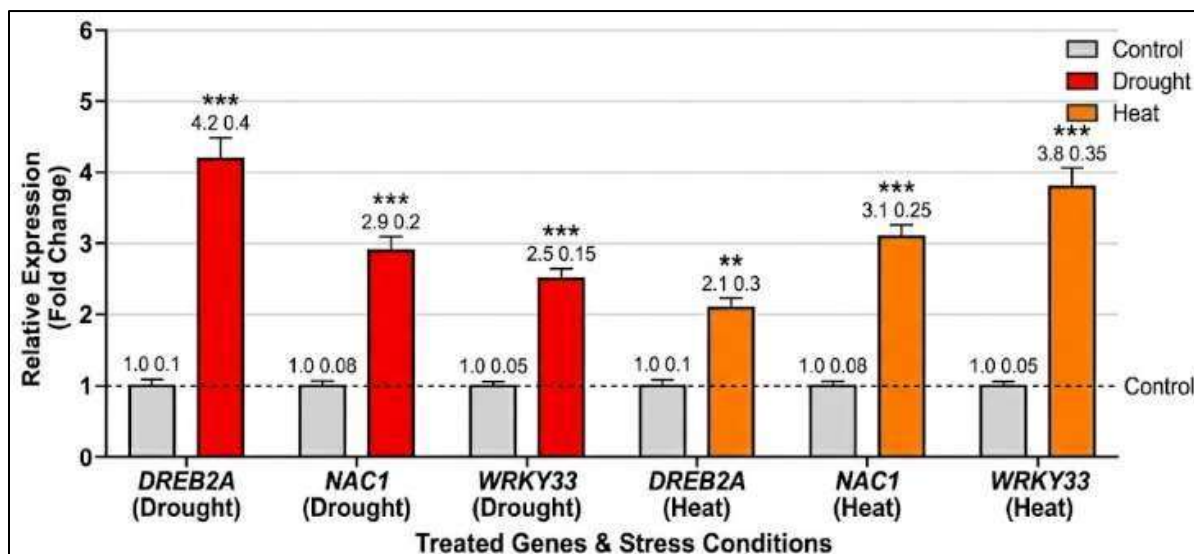


Figure 2. Relative gene expression (fold change) of selected stress-responsive genes under drought and heat stress conditions as determined by qRT-PCR.

4.3 Functional and Pathway Analysis

Fig. 3 shows the heatmap and hierarchical clustering of the differentially expressed genes of the control, drought, and heat stress conditions across the world. The heatmap color gradient is based on the relative level of expression such that red represents upregulation, green represents downregulation, and black represents the baseline level of

expression. The majority of genes showed baseline expression or even lower expressions, mostly with the range of -1.0 to +1.0 (log 2-fold change), which corresponded to a normal metabolic rate with no stress transcriptional activation. Conversely, a high number of genes were significantly upregulated by drought stress with 0.5 -3.5 log₂ fold change being the range of expression. A few important genes that are responsive to stress such as those related to the ABA signal pathway and osmoprotectants synthesis exhibited a high level of expression (deep red) and signifies that they were greatly activated during drought. Similar sets of genes were similarly upregulated by heat stress, albeit with relatively moderate fold changes, typically between +1.5 to +3.5 log₂.

On the other hand, primary metabolism and photosynthetic genes were also down-regulated during stress conditions, especially in drought stress, values of expression were between -1.5 to -3.0 log₂ fold change. This downregulation portrays a change of cellular priorities, the focus of the cell to grow and produce energy, into surviving and adapting to stress. The hierarchical clustering was used to further cluster samples into a set of clusters on the basis of their expression profiles. Control samples occupied another cluster, which was distinctly separate, as compared to stress-treated samples meaning that there was little activation of stress-responsive pathways. Stressed samples (drought) were clustered in one group and had the most general intensity of expression, whereas heat-stressed samples were organized in a similar, yet separate group, indicating a moderate response to stress. Clustering at the gene level placed genes, with similar expression patterns, including co-regulated transcription factors and stress-associated proteins, in coordinated regulation within functional pathways.

Comprehensively, Fig. 3 shows a transcriptional reorganization of response to abiotic stress, whereby the drought triggers more intense and extensive increases in genes than heat stress. The regulated expression of genes associated with stress and the inhibition of growth associated genes indicate the complexity of the regulatory networks during adaptation to climate variability in plants.

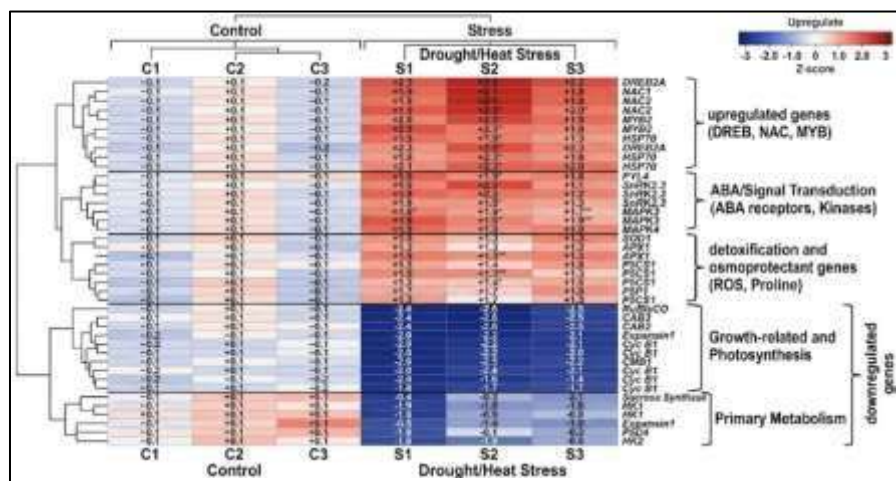


Figure 3. Heatmap and hierarchical clustering of differentially expressed genes under control and stress (drought/heat) conditions.

4.4 Identification of Molecular Signatures

A combination of pathway analysis and gene expression allowed detecting major molecular signature found in the adaptive stress responses. There were a number of genes that repeated these changes consistently in the various stress conditions indicating their possibility of being used as biomarkers of stress tolerance. These molecular signatures comprised transcription factors, signaling components and antioxidant defense and osmotic regulation genes. The co-expression of these genes is evidence of the existence of a regulatory network which controls plant acclimatisation to environmental stress. These results offer critical understanding of the stress resilience mechanism at the molecular level and give candidate genes to use in breeding crops in the future.

4.5 Validation

In order to confirm reliability of transcriptomic data, quantitative real-time PCR (qRT-PCR) was done to select candidate genes. RNA-Seq data confirmed the validity of the analysis of differential gene expression since patterns of the expression detected by qRT-PCR were similar to those found in the RNA-Seq. The fold-change values calculated using qRT-PCR also confirmed the presence of the identified genes in the process of the stress response as shown in Fig. 2. This confirmation procedure enhances the authenticity of the discoveries and the presence of the importance genes in moderating adaptive stress responses in crop plants.

5. DISCUSSION

The current research is valuable in detailing the molecular and physiological processes of crop plants to stresses caused by weather issues, especially drought and heat stress. The resulting phenotypic losses in the growth and yield

parameters are clear evidence of the negative effect of abiotic stress on the performance of plants such as the disruption of photosynthesis and metabolic activities. These physiological adaptations were also coupled with substantial transcriptional reprogramming, which implies that plants employ more complicated molecular pathways to adapt to the unfavorable environmental conditions.

Analysis of the differentially expressed genes showed significant upregulation of some of the major stress-responsive genes, such as those related to the DREB, NAC, and MYB families of transcription factors. These genes have been reported to control downstream stress-response pathways and increase plant tolerance to modify osmotic balance, antioxidant defense, and cellular protection responses. The involvement of ABA-dependent signaling pathways and reactive oxygen species (ROS) detoxification systems also indicate the contribution of hormonal and redox regulation to stress adaptation. Moreover, the role of epigenetic control and the reorganization of chromatin underline the complexity of the regulation of the expression of the genes under the conditions of stress.

Heatmap and clustering analysis showed that there was a sharp difference in the expression of control and stress-treated samples, which implicated the coordinated control of genes related to functions. The upregulated genes were mostly related to stress signaling and osmoprotection and detoxification pathways whereas the downregulated genes were related to growth related processes like photosynthesis and primary metabolism. This is a growth-stress tolerance trade-off that is an adaptive mechanism that allows plants to focus on survival in adverse environmental conditions. The observation of congruent molecular signatures in various stress treatments indicates that there are also conserved regulatory networks that can be exploited to enhance stress resilience.

In biology, multi-level control is emphasized because biological data, combining physiological and molecular informatics, depicts the complexity of plant responses to stress. The further development of functional validation and genetic improvement of the identified candidate genes through the identification of similar patterns of gene expression under multiple stress conditions offers the valuable targets. The genes can be used as a biomarker on stress-tolerance and can be applied in breeding programs to improve crop resistance.

Concerning applications, the results of this research have major implications in the production of crops improvement strategies. The characterized molecular markers and regulatory pathways can be integrated into the system of marker-assisted selection and genomic selection in order to hasten the breeding process to produce stress-resilient cultivars. Moreover, incorporation of information on omics with traditional breeding methods can enhance the effectiveness of crop betterment programs. Nevertheless, additional test in the field is required to ascertain the applicability of these results in the real field agriculture.

Generally, this research work can assist in gaining a better insight into the molecular mechanisms of climatic variability among plants and offer a point of departure in coming up with sustainable agricultural techniques in the face of mounting environmental pressures.

6. CONCLUSION

The paper clarifies the physiological and molecular mechanisms of crop plants to climate-related stress and especially drought and heat. Large-scale changes in growth and yield parameters affirmed negative impacts of stress situation, and gene expression studies indicated extensive transcriptional reprogramming. The main stress-sensitive genes such as members of the DREB, NAC, and MYB families were continually activated, as well as the essential signaling pathways such as an abscisic acid (ABA)-mediated response and reactive oxygen species (ROS) detoxification. The combination of the gene expression profiling and bioinformatics analysis allowed defining specific molecular signatures related to adaptive stress responses.

The results of this research are extremely valuable to climate-resilient farming because they allow gaining valuable experience on how crops are able to resist environmental stress. The candidate genes and regulatory pathways identified have provided potential targets to create stress-tolerant crop varieties by using advanced breeding strategies and molecular methods. These lessons help in enhancing crop productivity, and stability in changing climatic conditions hence food security to the world.

The validation of identified candidate genes and regulation networks of candidate genes under both controlled and field settings should be the functional validation of identified candidate genes conducted in the future. Also, research on interactions and extended stress responses will improve the knowledge of how plants adapt in the natural context. The adoption of multi-omics technology, combined with genome editing and precision breeding technologies will further speed up the creation of climate-resilient crops. In general, this study offers a solid basis on the need to promote sustainable agricultural activities in the context of continued climate variability.

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