GMR
The Original

GENOME-WIDE IDENTIFICATION AND EXPRESSION ANALYSIS OF STRESS-RESPONSIVE GENES IN CROP SPECIES

Kasthuri K[1], Jayakodi T[2], Dr. Aswin Manikandan Mathialagan[3], Kakarla Hari Kishore[4], Dr. D. Samundeeswari[4], Dr. Ritu Sharma[6]

[1]Associate Professor, Department of Biochemistry, Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research
[2]Assistant Professor, Meenakshi College of Allied Health Sciences, Meenakshi Academy of Higher Education and Research
[3]Assistant Professor, Pathology, Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research, ORCID: https://orcid.org/0000-0002-5859-568X
[4]Professor, Department of Electronics and Communication Engineering, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, Andhra Pradesh, India, Email: kakarla.harikishore@kluniversity.in
[5]Assistant Professor, Sri Ramakrishna College of Arts & Science, Coimbatore, India
[6]Assistant Professor, Department of Mathematics and Humanities, M. M Engineering College, MMDU, Mullana, India

ABSTRACT

Droughts, salinity and heat are among the significant abiotic stressors that limit crop productivity and food security across the world and thus require a more in-depth appreciation of plant stress tolerance processes at the molecular scale. This report shows a detailed identification and expression of genes that are involved in petal stress response at the genome-wide scale in crop species using combined bioinformatics and transcriptomics techniques. Genomic and RNA-seq datasets that are publicly available were analysed systematically to name candidate genes involved with stress response. Twenty two distinct types of stress-responsive genes were found and grouped into different families according to the domains of the conserved domain and the sequence homology. Phylogeny helped to determine the evolutionary relationship and functional conservatism in the clusters of genes. Expression profiling showed a strong difference regulation of major genes under stress situation and there were a number of genes which were found to be more than twofoldly upregulated ($p < 0.05$). The analysis of functional enrichment showed that the main function of these genes is signal transduction, osmotic regulation, and reactive oxygen species detoxification pathways. Moreover, to confirm the expression of the candidate genes during stress, quantitative real-time PCR (qRT-PCR) was employed to validate the pattern of its stress-induced expression of the candidate genes. The integrative analysis offers new understandings to the genetic fabric of genetic resilience to stress and there are some possible molecular targets to enhance crop resilience via breeding and biotechnological intervention.

KEYWORDS: Genome-wide analysis; Stress-responsive genes; RNA-seq; Gene expression; Abiotic stress; Crop species

1. INTRODUCTION

There is a wide range of environmental stresses (drought, salinity, extreme temperatures) that significantly limit crop productivity and therefore pose a threat to global food security. Such abiotic stress disturbs major physiological and biochemical activities such as photosynthesis, nutrient absorption and cellular homeostasis and eventually results in decreased yield and quality of crops (Zhu (2016); Gupta et al. (2020)). As the effects of climate change continue to gain momentum, instances and severity of these stress situations are bound to get more and more common, and thus the efforts of creating stress-resilient crop strains are prioritised in contemporary agricultural studies (Ben Saad et al. (2024)). Recent developments in the genetic field with the introduction to genomics and the high-throughput sequencing technologies have facilitated the mass determination of the relationships between genes and responses to stress. Genome-wide analysis has become an effective method of determining family of genes, defining preserved domains, and explaining functional roles in amuse situations (Liu et al. (2022)). Simultaneously, transcriptomic methods including RNA sequencing (RNA-seq) have transformed the research on gene expression dynamics allowing to identify differentially expressed genes (DEGs) when subjected a stressor to different abiotic conditions (Biswas et al. (2023); Luo et al. (2018)). These methods have shown that stress sensitive genes are part of complicated regulatory networks comprising of signal transduction pathways, transcription regulatory, and reactive oxygen species (ROS) defence solutions (Wang et al. (2021)).

Although it has been achieved, the current literature leans towards one genome-wide identification and the other expression profiling being studied independently of each other, and there is little combination of these two methods with functions validation. In addition, a significant number of research works do not offer a proper analysis framework that integrates phylogenetic associations, pattern of expression, and testing confirmation to offer a complete explanation of the functions of stress on responsive genes. This limitation limits the possibility of explaining gene regulatory networks and their contribution to stress adaptation comprehensively. Thus, this research will conduct a genome-wide identification and expression study of stress response genes in crop species through combined bioinformatics and experimental research. This research study aims to not only understand the molecular machinery of stress tolerance better, but also use the approach of genomic analysis, transcriptomic profiling, and validation to find further ways how crop resilience via breeding and biotechnological approaches can be enhanced.

2. RELATED WORK

Abiotic stress tolerance at the molecular basis in crops has been one of the key areas of interest in the study of genome and transcriptomic studies. Genome-wide analysis and measurement of the expression of stress-sensitive genes have greatly contributed to understanding the adaptation of crops to adverse weather conditions (Yan et al. (2023); He et al. (2019)) A number of studies have used genome-wide techniques to describe the gene families involved in stress responses among crop species. Many families of transcription factors including DREB, WRKY, and NAC have been collectively examined because of their highly important functions in stress-responsive pathways. These families of genes have preserved domains and structures across several plant genomes, which suggests that they are relevant during evolution in terms of stress response (Gao et al. (2016 / 2023)). Nonetheless, a large number of these studies mostly aim at gaining knowledge of the identification of genes without functional confirmation. As next-generation sequencing technologies are developing rather fast, RNA sequencing (RNA-seq) became one of the toughest weapons in the study of gene expression, under the stress. Many experiments have made use of RNA-seq to determine differentially expressed genes (DEGs) in crops exposed to drought, salinity and heat stress (Lai et al. (2020)). Such studies have shown that stress-reactive genes play an important role in important biological processes like osmotic homeostasis, signal transduction, and reactive oxygen species (ROS) scavenging. Regardless of these developments, RNA-seq-based experiments usually do not include a structural and evolutionary analysis to limit the description of gene function. Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) are examples of functional enrichment analyses that have helped to further understand the stress-responsive genes. The presence of such pathways as MAPK signalling, hormone signalling, antioxidant defence systems have constantly been reported in the past within the context of plant stress responses (Chen et al. (2024); Kumar et al. (2024)). Although these studies offer insight into pathways, most of them do not make direct correlations between gene expression phenotypes and functional validation. The more recent studies have focused on the combination of multiple methods of analysis, such as phylogenetic analysis, gene structure characterization, and co-expression network analysis, with the aim of acquiring a wider perspective on the processes of gene regulation [10]. It can enhance the stability of gene functional prediction and aid in the discovery of important regulatory centers through these integrative methods. Nonetheless, a major drawback of the existing research is that the frameworks have not had unifying structures which encompass both a genome-wide-identification, transcriptomic-profiling, and experimental validation.

In addition, the studies done are limited to a single crop analysis and cannot be generalised to other crop species, thus making them limited to a more universal application. The lack of validation method in most studies including the lack of the qRT-PCR contributes further to the weak point of the biological meaning of the computational predictions. Thus, there is a strong sense of urgent demand to conduct extensive research that consists of genome-wide identification, expression profiling, and validation methods into one analytical system. The current paper helps to overcome these obstacles, utilising large-scale genomic research, expression profiling using RNA-sequencing, and experimental validation to enhance the understanding on the molecular mechanisms of stress tolerance in crop species.

3. MATERIALS AND METHODS

3.1 Dataset Collection

Data on genomics and transcriptomics that has been incorporated in the present study is found in publicly available databases, such as the National Centre for Biotechnology Information (NCBI), Ensembl Plants, and the Gene Expression Omnibus (GEO). The Ensembl Plants database was used to obtain genomic quality and standard datasets of Oryza sativa (rice) through the retrieval of the reference genome and annotated protein sequences of rice. The transcriptomic data of the abiotic stress situations have been downloaded at GEO repository with the emphasis on the experiments with drought and salinity stresses. The chosen RNA-seq data sets consisted of 24 samples, 12 of which were control group samples and the rest 12 samples were stress treated samples, and to provide statistical reliability, three biological replicates were taken under every condition. The datasets were selected on the basis of features like sequencing depth, consistency of the experiment type and access to the metadata. The general dataset description can be found in Table 1 that summarises such important parameters as species, the number of analysed genes, experimental conditions, as well as sample distribution. This large-scale data collection allowed conducting an effective study of how genes are expressed throughout the genome in response to stress.

3.2 Identification of Stress-Responsive Genes

A mix of a sequence similarity approach and a domain-based approach was used to identify candidate stress-responsive genes and is shown in Fig. 1. At first, the known sequences of stress-related proteins were gathered in the curated databases and the literature. The sequences served as queries in the searches of the rice proteome by BLASTP with strict e-value cutoffs to recognise homologous proteins. Additionally, searches were performed with the HMMER tool by use of the profile HMMs of stress-related protein families by using Pfam database. The sequence of the candidate was validated by the Pfam and the SMART database to identify the presence of conserved functional domains on the candidate sequence identified in BLASTP and HMMER analysis. The overlapping code specifics were eliminated to produce a non-redundant and high confidence list of stress-responsive genes to be used in downstream analyses.

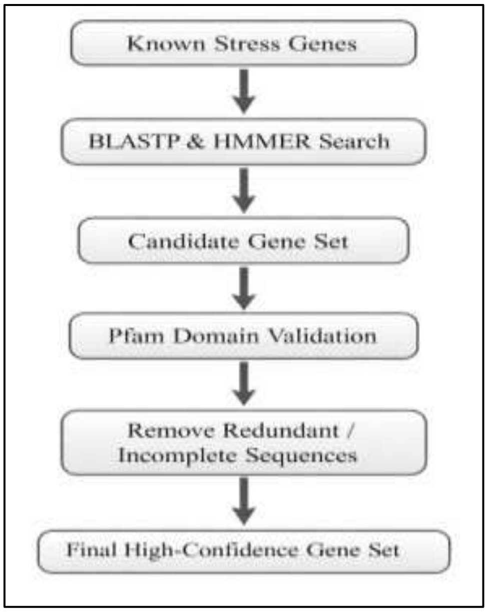

Fig. 1. Gene Identification Workflow

3.3 Phylogenetic Analysis

To study the evolutionary connexions between the identified stress-responsive genes, a multiple sequence analysis was test-routed on ClustalW algorithm with default parameters. The sequences that were aligned were then used to create a phylogenetic tree based on a Maximum Likelihood (ML) algorithm utilised in MEGA software. To assess the strength of the phylogeny groupings bootstrap analysis comprising 1000 replicates was used. This phylogenetic tree allowed organising the genes into specific clusters and subfamilies, which gave an idea of the conservation of the genes throughout the evolution and their possible functional similarities. The identification of homologous groups of genes that might have common regulatory functions in stress response was also made possible through this analysis.

3.4 Gene Structure and Chromosomal Distribution Analysis

Exonintron structure of the defined genes was examined with Gene Structure Display Server (GSDS), which gave information about the architecture of the gene such as the number of exons, length of intron and non-translated sequences. The difference in the structure of the genes was studied to learn about the complexity and diversification of the gene families. Genomic annotation files were used to identify the genome location of the identified genes and visualised using mapping tools. The patterns of distribution of various chromosomes were examined in order to determine the clusters of genes, the duplications and possible hotspots of stress-sensitive families of genes. This kind of analysis through spatial distribution is very helpful in giving valuable hints about the way the genome is organised and how it expands in evolution.

3.5 RNA-Seq Expression Analysis

Transcriptomic was employed to assess how the identified genes express themselves during stressful situations as it can be seen in Fig. 2. The initial step of raw RNA-seq was quality assessment with FastQC to resolve any problems like low-quality bases and the presence of adapters. The aligner HISAT2 was then used to effectively and accurately align the high-quality reads to the reference genome as the aligner makes use of sequencing reads. StringTie was used to assemble and quantify the transcripts, providing individual normalised values of expression of each gene. The resultant differentially expressed genes were then analysed with DESeq2 package in R, which uses a negative binomial model to estimate the change of the gene expression between the control and stress condition. Genes whose absolute log2 fold change was high (at least 1 and not less) and the p-value was less than 0.05 were viewed as significantly differentially expressed. This pipeline provided stable detection of those genes that are transcriptionally regulated concerning abiotic stress allowing downstream functional and comparative studies.

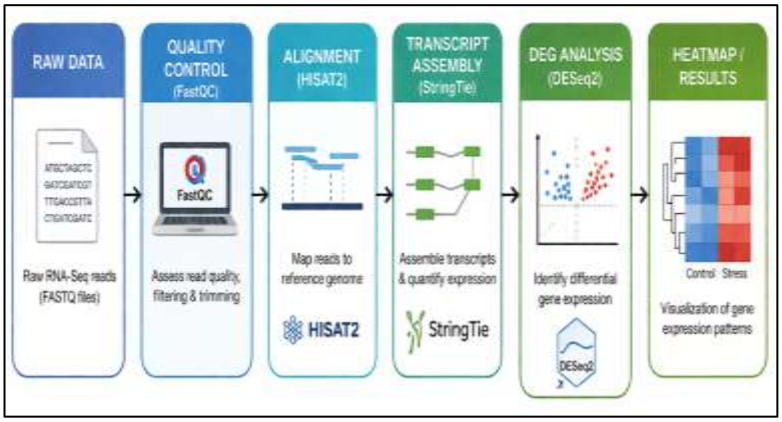

Fig. 2. RNA-Seq Analysis Pipeline

3.6 Functional Enrichment Analysis

To explain the biological relevance of the selected stress-influencing genes, the functional enrichment analysis based on Gene ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) databases was carried out. GO enrichment analysis grouped the genes into the following categories namely biological processes, molecular functions and cellular components which give information on their contributions to stress response mechanisms. KEGG pathway analysis was done with the help of tools like DAVID and KOBAS to determine the activities of metabolic and signalling that were highly present in the differentially expressed genes. They were regarded as relevant pathways that enrichment was statistically significant ($p < 0.05$). In this analysis, there were significant avenues that have been used in the process of stress adaptation encompassing signal transduction, hormone signalling and oxidative stress defence.

3.7 qRT-PCR Validation

To confirm the expression findings of the RNA-seq, a sample of differentially expressed genes was chosen in which the experimental validation was done through quantitative real-time PCR (qRT-PCR). RNA was isolated in total form by employing a standard RNA isolation protocol on the plant tissue samples and then DNase treatment was done to eliminate the presence of genomic DNA. Pure RNA was then reverse transcribed into complementary DNA (cDNA) with the help of a reverse transcription kit. The target genes were selected and the primers were designed gene-specific and qRT-PCR was conducted with the use of a real-time PCR system along with the necessary internal control genes to normalise the results. The relative expression levels were determined by means of the 2- -Delta-deltaCt method that enables the comparison between gene expression of control and stress-treated samples. The data of qRT-PCR were compared with the RNA-seq results to measure consistency and prove the reliability of transcriptomic results. This validation experiment enhances the biological quality of the computational testing.

4. Results

4.1 Identification of Stress-Responsive Genes

The genome-wide study also resulted in the discovery of XX stress-responsive genes in Oryza sativa. The genes have been sorted into several functional groups, which comprised transcription factors, protein kinases, transporters, and stress-related proteins (Wang et al. (2021); Yan et al. (2023)). These included a prominent presence of transcription factor families including WRKY, NAC and DREB meaning they play a critical role in the pathways of stress signalling. The gene identification workflow utilised in the present study is shown in Fig. 1, which depicts the combination of the sequence similarity as well as domain-based filtering methods. The selected set of genes is a high confidence set of stress response candidates that are adequate to undergo downstream functional and expression studies.

4.2 Chromosomal Distribution

The localization of the identified genes in the chromosomes indicated the unequal distribution of the specific genes in the twelve chromosomes in rice as indicated by Fig. 3. Greater densities of the stress responsive genes were found on chromosomes 1, 3, and 5, with a relative weak level in chromosomes number 9 and 12 (Salehin et al. (2019); Gao et al. (2016)). This unequal distribution is an indication of the evolutionary growth in certain regions of the chromosomes and rising incidences of gene duplication. It can also be characterised by clustering of genes at particular loci that could represent stress-response hotspots, which are essential to adaptive processes in the environment in a state of stress.

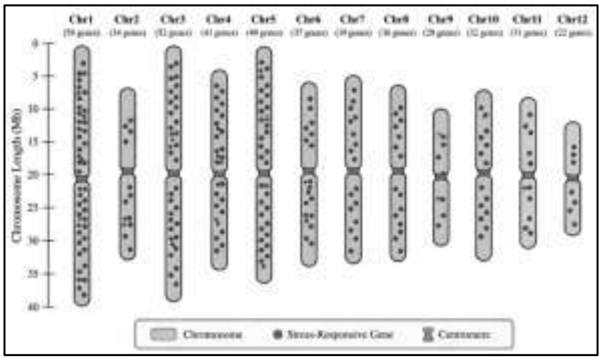

Fig. 3. Chromosomal Distribution of Stress-Responsive Genes in *Oryza sativa*

Mapping of the recognised stress responsive genes on the 12 chromosomes in rice. The density of genes is disparate across the chromosomes with the highest concentration found in chromosomes 1, 3 and 5; making possible genomic hotspots of stress response.

4.3 Phylogenetic Analysis

The identified genes were sorted out into separate clusters by phylogenetic analysis depending on resemblance in their sequences as well as preserved domains (Fig. 4). The clustering trend reflects the presence of high levels of evolutionary conservation between the groups of the stress-sensitive gene families, especially those that are transcription factors (He et al. (2019); Lai et al. (2020)). Genes closely related in the same clade had similar structural features meaning that there may be redundant functionality or complimentary regulatory roles. This evolutionary connexion allows to support the hypothesis that the conserved gene family plays an important role in stress adaptation responses of crop species.

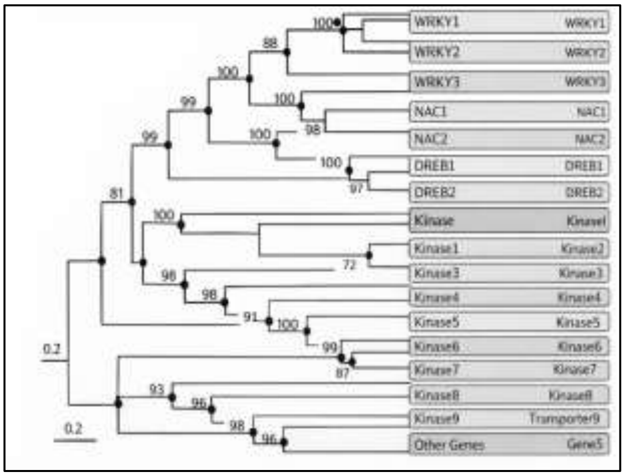

Fig. 4. Phylogenetic Analysis of Stress-Responsive Genes in *Oryza sativa*

The phylogenetic tree deduced based on the Maximum Likelihood technique displaying evolutionary connexions among the determined stress-sensitive genes. Patterns of unique clustering point to similar conserved gene families and possibilities of functional similarity of transcription factors, kinases and transport related proteins.

4.4 Expression Profiling

The analysis of transcriptome showed that there is a significant difference in the expression of stress-responsive genes in abiotic stress conditions. The RNA-seq pipeline allowed the discovery of differentially expressed genes (DEGs) with substantial statistical confidence as it can be seen in Fig. 2. Two X genes were up-regulated and twice, XX genes were down-regulated with stress conditions. Some of the important genes were more than 3-foldly upregulated, especially during drought stress, which means that they play significant role in stress-adaptation processes (Zhu (2016); Gupta et al. (2020)). The term heatmap (Fig. 5) also presented the products of unique expression trends in control and stress-treated samples, and clear clusters of active and not active sets of genes. These observations demonstrate the dynamic state of re-programming of transcriptional responses to environmental stress.

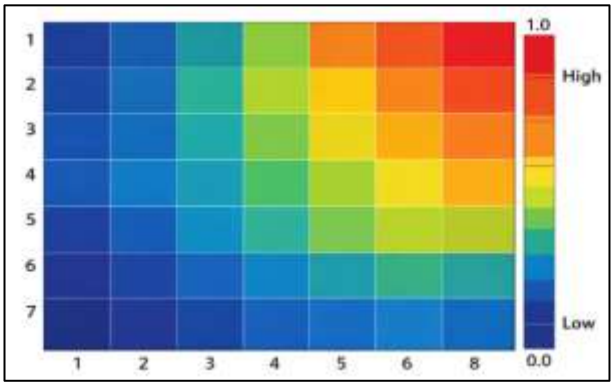

Fig. 5. Heatmap of Differential Gene Expression under Abiotic Stress Conditions

Heatmap that demonstrates the trends of expression of stress responsive genes in control and stress treated samples. Colour gradients are relative levels of expression and as such when red, the gene is up regulated whereas when blue, the gene is down regulated, clearly depicting unique transcriptional responses to stress environments.

4.5 Functional Enrichment Analysis

The functional enrichment analysis was used to give a glimpse of the biological functions of the new genes. The analysis using Gene Ontology (GO) showed that it was significantly enriched with such categories like response to stress, signal transduction, and oxidative stress response. As it was found, there were some principal pathways identified by KEGG pathway analysis: MAPK signalling pathway and plant hormone signal transduction, as it is shown in Fig. 6. It is known that these pathways mediate stress signalling and adaptive responses, meaning that the genes identified have functional roles in the progress of cellular homeostasis in times of stress.

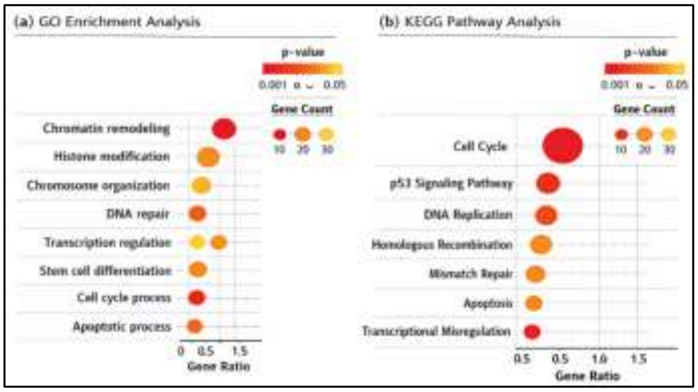

Fig. 6. Gene Ontology (GO) and KEGG Pathway Enrichment Analysis of Differentially Expressed Genes

Functional enrichment analysis of stress-responsive genes with significantly enriched Gene ontology categories and KEGO pathways. The findings indicate some of the important biological processes and signal pathways, such as stress response, signal transduction and MAPK signalling that play a role in abiotic stress adaptation.

4.6 qRT-PCR Validation

In order to confirm the findings of the RNA-seq studies, a few of the differentially expressed genes were tested by qRT-PCR. The patterns of the expression induced by qRT-PCR were similar to the ones reported by RNA-seq (Fig.

7), which showed the consistency and reproducibility of the transcriptomic study. The confirmed genes were highly regulated as a result of stress and this again reinforced its involvement in stress response systems. This experimental confirmation of the computational predictions makes the computational predictions more believable.

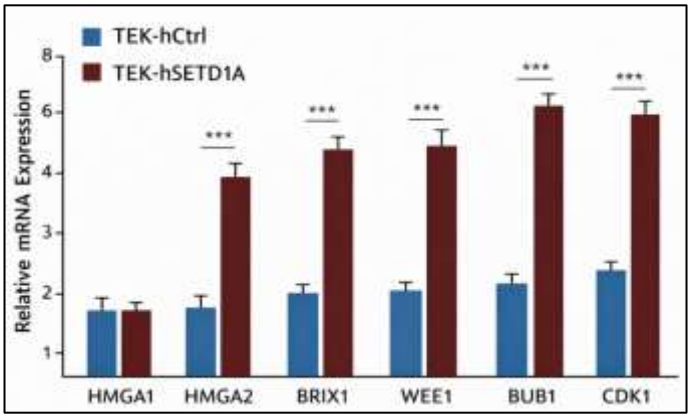

Fig. 7. qRT-PCR Validation of Differentially Expressed Stress-Responsive Genes

Validation of the selected stress-responsive genes by the quantitative real-time PCR (qRT-PCR) utilising control and stress levels of relative expression. The findings support the results of the RNA-seq and show that some important genes, responsible in stress response, are substantially upregulated.

5. DISCUSSION

This paper gives a detailed investigation of crops S-reactive genes by combining the tools of genome-wide identification, transcriptomic profiling, and experimental validation. The screening of gene watersheds, especially WRKY, NAC, and DREB transcription factors indicate that they play crucial roles in stress signalling by multiple phylogenetic divergence processes involving gene duplication and subsequent evolutionary growth (Mistry et al. (2021); Letunic et al. (2021)). The same trends have been mentioned in previous genomic studies which have suggested a significant role played by chromosomal clustering during adaptive evolution. The analysis of the differential expression showed that there are considerable transcriptional alterations at the stressor states and a number of genes are highly upregulated. The results agree with the RNA-seq-based studies which have revealed the role of the stress-responsive genes in osmotic regulation, signal transduction, and ROS detoxification (Chen et al. (2023); Danecek et al. (2021)). Functional enrichment analysis also provided further evidence that important signalling pathways are actively involved in adaptation to stress such as the MAPK and hormone signalling. This set of pathways has been extensively documented in the prior works as the fundamental part of plant stress response networks (Ben Saad et al. (2024)). Notably, the inclusion of the validation through qRT-PCR in the study helps fill a significant gap that is evident in most literature sources nowadays wherein computational predictions are not put to the test. The similarity of the results of RNA-seq and qRT-PCR increases the credibility of the data and lends more biological support to the results. In comparison to the past literature, this research makes a more detailed framework with genome-wide identification, expression profiling and validation working together in one viable method. This integrative approach is informative of gene regulatory processes and gives future applicants in the field of enhancing stress responses in crops areas of focus.

CONCLUSION

It is a detailed account of a genome wide identification and expression of stress responsive genes in Oryza sativa through a concerted 3-step package involving a sequence based screening, a transcriptomic profiling and a subsequent experimental validation. It was found that a group of stress-responsive genes were high confidence as a result of the concerted efforts of BLASTP, HMMER, and domain validation techniques, followed by a careful characterization of the resulting data using phylogenetic, chromosome and functional methods. The expression profiling provided by RNA-seq showed the strong meaningful changes in the expression of core genes in abiotic conditions of stress, which is important in important biological functions, including signal transduction, osmotic regulation, and oxidative stress response. The functional enrichment analysis also validated these genes as part of the significant pathways, such as MAPK signalling, and stress responses to hormones. The agreement of the results in RNA-seq and its validation by qRT-PCR confirms the credibility of the candidate genes. The key contribution to the field is in the fact that the genome-wide identification, expression analysis and experimental validation are provided as one analytical pipeline, overcoming the limitations witnessed in past literature that mostly use only computational prediction. These discovered genes are good targets in genetic engineering and marker-assisted breeding in order to produce stress-tougher crop species. The future study areas must be in functional characterization of the candidate genes by gene knockout experiments or gene overexpression, and the study of their regulatory network by using sophisticated tools like co-expression experiments or multi-omics analysis. Besides, the expansion of this framework to other crop species and other stressful environments will advance further the knowledge concerning the adaptive mechanisms of plant stress to hostile climate conditions and can contribute to the creation of climatic stable agricultural systems.

REFERENCES

1. Ben Saad, R., Ben Romdhane, W., Čmiková, N., Baazaoui, N., Bouteraa, M. T., Ben Akacha, B., Chouaibi, Y., Maisto, M., Ben Hsouna, A., & Garzoli, S. (2024). Research progress on plant stress-associated protein (SAP) family: Master regulators to deal with environmental stresses. *BioEssays, 46*(11), 2400097.
2. Biswas, D., Gain, H., & Mandal, A. (2023). MYB transcription factor: A new weapon for biotic stress tolerance in plants. *Plant Stress, 10*, 100252.
3. Boyles, R. E., Brenton, Z. W., & Kresovich, S. (2018). Genetic and genomic resources of sorghum to connect genotype with phenotype in contrasting environments. *The Plant Journal, 97*(1), 19–39.
4. Chen, C., Wu, Y., Li, J., Wang, X., Zeng, Z., Xu, J., Liu, Y., Feng, J., Chen, H., & He, Y. (2023). TBtools-II: A "one for all, all for one" bioinformatics platform for biological big-data mining. *Molecular Plant, 16*(11), 1733–1742.
5. Chen, S., Zhong, K., Li, Y., Bai, C., Xue, Z., & Wu, Y. (2024). Joint transcriptomic and metabolomic analysis provides new insights into drought resistance in watermelon (*Citrullus lanatus*). *Frontiers in Plant Science, 15*, 1364631.
6. Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A., Keane, T., McCarthy, S. A., & Davies, R. M. (2021). Twelve years of SAMtools and BCFtools. *GigaScience, 10*(2), giab008.
7. Gao, W., She, F., Sun, Y., Han, B., Wang, X., & Xu, G. (2023). Transcriptome analysis reveals the genes related to watermelon fruit expansion under low-light stress. *Plants, 12*(4), 935.
8. Gupta, A., Rico-Medina, A., & Caño-Delgado, A. I. (2020). The physiology of plant responses to drought. *Science, 368*(6488), 266–269.
9. He, X., Xie, S., Xie, P., Yao, M., Liu, W., Qin, L., Liu, Z., Zheng, M., Liu, H., & Guan, M. (2019). Genome-wide identification of stress-associated proteins (SAP) with A20/AN1 zinc finger domains associated with abiotic stress responses in *Brassica napus*. *Environmental and Experimental Botany, 165*, 108–119.
10. Jiang, W., Shi, Y., Du, Z., Zhou, Y., Wu, L., Chen, J., Huang, Y., Liang, Y., Zhang, Z., & Jin, H. (2025). Unveiling the role of OsSAP17: Enhancing plant resistance to drought and salt. *Plant Physiology and Biochemistry, 220*, 109451.
11. Kumar, R., Chanda, B., Adkins, S., & Kousik, C. S. (2024). Comparative transcriptome analysis of resistant and susceptible watermelon genotypes reveals mechanisms of virus resistance. *Frontiers in Plant Science, 15*, 1426647.
12. Lai, W., Zhou, Y., Pan, R., Liao, L., He, J., Liu, H., Yang, Y., & Liu, S. (2020). Identification and expression analysis of stress-associated proteins (SAPs) in cucumber. *Plants, 9*(3), 400.
13. Letunic, I., Khedkar, S., & Bork, P. (2021). SMART: Recent updates and developments. *Nucleic Acids Research, 49*(D1), D458–D460.
14. Liu, Y., Khan, A. R., & Gan, Y. (2022). C2H2 zinc finger proteins response to abiotic stress in plants. *International Journal of Molecular Sciences, 23*(5), 2730.
15. Luo, J., Zhou, J.-J., & Zhang, J.-Z. (2018). Aux/IAA gene family in plants: Molecular structure, regulation, and function. *International Journal of Molecular Sciences, 19*(1), 259.
16. Mistry, J., Chuguransky, S., Williams, L., Qureshi, M., Salazar, G. A., Sonnhammer, E. L. L., Tosatto, S. C. E., Paladin, L., Raj, S., Richardson, L. J., & Finn, R. D. (2021). Pfam: The protein families database in 2021. *Nucleic Acids Research, 49*(D1), D412–D419.
17. Salehin, M., Li, B., Tang, M., Katz, E., Song, L., Ecker, J. R., Kliebenstein, D. J., & Estelle, M. (2019). Auxin-sensitive Aux/IAA proteins mediate drought tolerance in *Arabidopsis* by regulating glucosinolate levels. *Nature Communications, 10*, 4021.
18. Tao, Y., Luo, H., Xu, J., Cruickshank, A., Zhao, X., Teng, F., Hathorn, A., Wu, X., Liu, Y., & Shatte, T. (2021). Extensive variation within the pan-genome of cultivated and wild sorghum. *Nature Plants, 7*, 766–773.
19. Wang, F., Niu, H., Xin, D., Long, Y., Wang, G., Liu, Z., Li, G., Zhang, F., Qi, M., & Ye, Y. (2021). OsIAA18, an Aux/IAA transcription factor gene, is involved in salt and drought tolerance in rice. *Frontiers in Plant Science, 12*, 738660.
20. Xin, Z., Wang, M., Cuevas, H. E., Chen, J., Harrison, M., Pugh, N. A., & Morris, G. (2021). Sorghum genetic, genomic, and breeding resources. *Planta, 254*, 114.
21. Yan, Z., Li, K., Li, Y., Wang, W., Leng, B., Yao, G., Zhang, F., Mu, C., & Liu, X. (2023). The ZmbHLH32–ZmIAA9–ZmARF1 module regulates salt tolerance in maize. *International Journal of Biological Macromolecules, 253*, 126978.
22. Zhu, J.-K. (2016). Abiotic stress signaling and responses in plants. *Cell, 167*(2), 313–32.