Understanding the cross-talk of major abiotic-stress-responsive genes in rice: A computational biology approach

Paladi Srinivasulu [1]

Dr. Anita Balwada [2]

Phd Research Scholar, Department of Botany, Sunrise University, Alwar, Rajasthan.[1]

Assistant Professor, Department of Botany, Sunrise University, Alwar, Rajasthan.[2]

Abstract

This study aims to identify key abiotic-stress-responsive genes in Oryza sativa and investigate their expression profiles, protein–protein interactions, and co-expression patterns to better understand the molecular mechanisms underlying stress response in rice. The ultimate goal is to employ these findings in the development of gene-based molecular markers to breed rice cultivars with enhanced resistance to challenging environmental conditions. A total of 14 abiotic-stress-responsive genes in O. sativa were identified through literature mining. These genes were analyzed for their chromosomal distribution, transcript length, CDS length, and translated amino acid length. Pairwise similarity matrix, phylogenetic analysis, and protein–protein interaction networks were employed to understand the evolutionary relationships and functional interactions among these genes. Expression profiles of six key genes (AOX1a, AOX1b, ALDH2a, ALDH2b, OsNAC6, OsDHN1) were investigated using the electronic fluorescent pictogram (eFP) program. KEGG pathway analysis and co-expression studies were also conducted to further understand the roles of these genes in abiotic stress response pathways. The 14 abiotic-stress-responsive genes were distributed across different chromosomes of O. sativa, suggesting the presence of interconnected cascades regulating abiotic-stress-response. Phylogenetic analysis revealed four clusters of genes, indicating their potential shared ancestry. Protein-protein interaction analysis identified three prominent clusters of interactions, with the strongest interactions occurring among Aldh2a, Aldh2b, OS07T0188800-01, and OsJ_04113 in one cluster, and between AOX1a and AOX1b in another cluster. Expression profiles of the six key genes varied across different stages of the rice life cycle. KEGG path way analysis showed that ALDH2a and ALDH2b participated in almost all pathways except propanoate metabolism. The study demonstrated that the six key genes play significant regulatory roles in abiotic stress responses in O. sativa. The expression profiles, phylogenetic analysis, protein–protein interactions, and gene co-expression studies revealed interconnected cascades and cross-talk in response mechanisms.

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1. Introduction

Plants exist in highly dynamic environments, continuously subjected to fluctuations that can hinder their growth and development (Zhu et al., 2016). Environmental stressors are broadly classified into biotic and abiotic stress (Debnath et al., 2013). Biotic stress refers to adverse effects caused by herbivore attacks and pathogen infections, while abiotic stress involves unfavorable environmental conditions such as extreme temperatures, drought, and excessive salt and calcium concentrations. Plants possess inherent physiological processes to cope with these stresses, relying on diverse genetic expressions regulated by multiple transcription factors (Atkinson et al., 2012).

The ability to withstand stress varies among plant species, with different genes responsible for stress tolerance against specific stressors (Seth et al., 2020). Rabadanova et al. (2018) emphasize the importance of enhancing plant stress tolerance for increased productivity and environmental sustainability. Crops that lack stress tolerance often require excessive inputs like fertilizers, pesticides, and irrigation, making stress tolerance vital for sustainable agricultural practices. Understanding plant stress responses necessitates examining the signaling mechanisms that

facilitate communication between various molecular pathways related to stress resistance. This study will specifically focus on the abiotic stress response of rice (*Oryza sativa*).

Plants employ various strategies (Fig. 1) to tolerate or avoid abiotic stresses, such as reducing photosynthesis, closing stomata, increasing reactive oxygen species (ROS) scavenging, stunting leaf growth, and elongating roots (Cohen and Leach, 2019). While abiotic stresses induce these changes, biotic stress factors, such as pathogens, can also lead to stomatal closure and decreased photosynthesis. Defensive mechanisms against biotic stress include the secretion of phytotoxins (e.g., ROS, phytoalexins, and secondary metabolites) and localized cell death.

Phytohormones, such as salicylic acid (SA), jasmonic acid (JA), and ethylene, play crucial roles in plant immunity against pathogenic factors (Lata et al., 2011). Additionally, transcription factor families involved in abiotic stress responses include both abscisic acid (ABA)-dependent and ABA-independent pathways. Kim et al. (2020) identified stress-responsive genes in *O. sativa* using expressed sequence tags (ESTs) generated from drought-stressed seedlings, revealing distinct gene families responsible for the abiotic stress response.

According to Zhao et al. (2010), dehydration-responsive element-binding (DREB) genes confer resistance to drought, low temperature, and high salinity stress in rice. Most DREB genes are believed to regulate downstream stress-responsive genes by directly binding to drought-responsive elements (DRE) and cis-elements (GCC box) (Fig. 2). Studies by Zhang et al. (2013) and Ranawake et al. (2012) investigated the expression patterns of DREB genes and identified numerous stress-responsive genes in rice. Similarly, Rabbani et al. (2003) utilized a cDNA microarray technique to profile rice gene expression under various environmental stresses, such as drought, cold, high salinity, and abscisic acid.

In a more recent study, Sevanthi et al. (2021) identified six highly heat-sensitive genes in rice, while Riccio-Rengifo et al. (2021) categorized stress-induced proteins into functional and regulatory proteins. Mondini et al. (2015) suggested that there is a significant overlap between gene expression and the mechanisms of action in response to both abiotic and external stress stimuli. As a result, abiotic stress response genes play a critical role in stress regulation, aiding rice plants in coping with adverse conditions induced by abiotic stress.

Throughout their evolution, plants have developed various strategies and processes to manage stressors such as drought, heat, cold, and excessive salinity (Debnath et al., 2022). The ability of plants to respond to stress is crucial not only for their economic value but also for maintaining environmental homeostasis. If rice plants lose their stress resistance capacity, they will require more water and fertilizers, which is neither cost-effective nor environmentally sustainable, given the immense agricultural significance of rice (Seth et al., 2020).

Shi and Chan (2014) proposed enhancing plant stress responses through the modulation of gene expression and post-translational modification. A comprehensive understanding of the genes responsible for abiotic stress response in rice plants, along with their expression patterns, can provide valuable insights for genetic modulation. Moreover, studying the roles of different abiotic stress-responsive genes can enhance our understanding of their importance in developing stress-tolerant rice varieties.

Despite the substantial amount of research on regulating stress-responsive genes in rice, a significant gap remains in the literature concerning a comprehensive study that investigates the

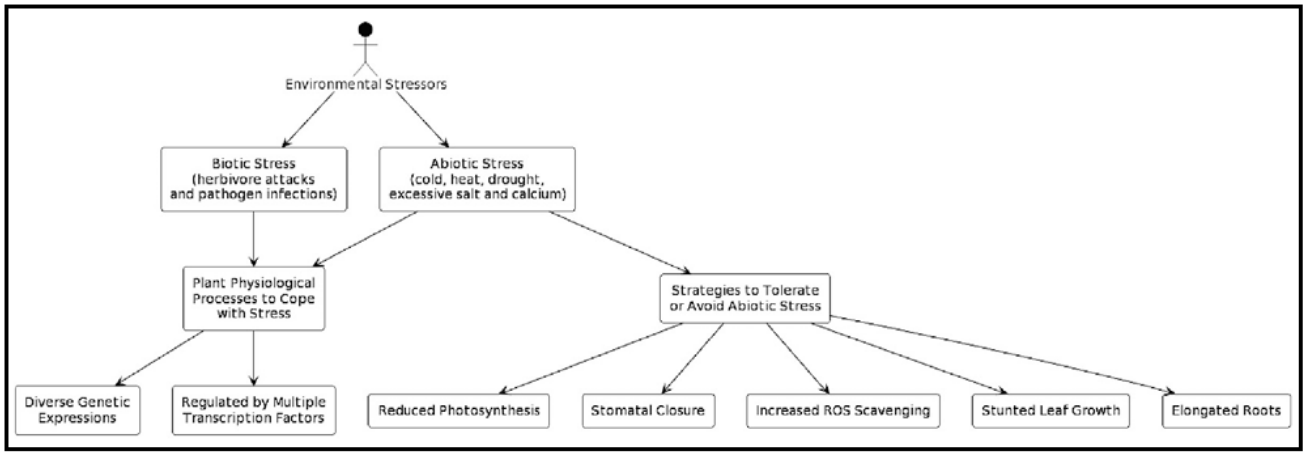

Fig. 1. Strategies employed by plants against environmental stressors (Source: Author's creation with PlantText UML Editor).

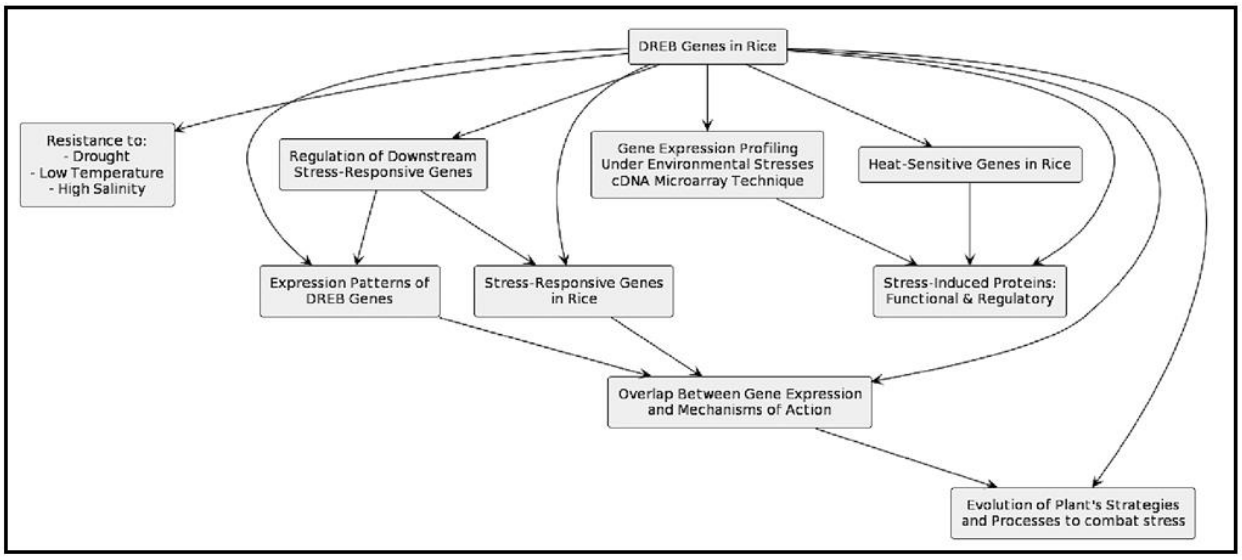

Fig. 2. Functions of DREB gene in rice combating different abiotic stresses (Source: Author's creation with PlantText UML Editor).

expressions of abiotic stress-responsive genes and their interplay with different stress response pathways in rice plants are crucial to understanding how rice (*Oryza sativa*) copes with environmental challenges. Numerous primary articles available in electronic databases provide insights into the expression patterns of these genes through systematic reviews. However, there is a significant lack of comprehensive studies on this topic. To address this gap, formulating a hypothesis for a thorough analysis of the expression patterns of abiotic stress-responsive genes in *O. sativa* is essential. This will enable researchers to identify key factors and mechanisms that can be harnessed to improve stress tolerance in rice plants, ultimately enhancing productivity and minimizing negative environmental impacts, contributing to sustainable development goals.

2. Materials and Methods

2.1 Identification of Genes

Data mining plays a vital role in the effective screening and analysis of relevant information. In this study, a systematic approach to literature mining was employed to identify abiotic stress-sensitive genes in *O. sativa* by searching public databases. Various electronic databases, such as PubMed, CINAHL, EBSCO (HOST), Web of Science, and Google Scholar, were utilized. However, this research focused primarily on publicly accessible publications available in CINAHL, PubMed, and Google Scholar databases.

To conduct a comprehensive search, several keywords and Boolean operators were used, including “rice plant,” “abiotic stress,” “stress-responsive genes,” and “abiotic stress-responsive genes.” A two-step screening process was applied to ensure the relevance of the literature retrieved. Initially, titles and abstracts were screened to collect publications from the online databases. Then, a full-text assessment was conducted to evaluate the relevance of the selected articles.

When mining literature for genes of interest, it is crucial to establish and apply appropriate inclusion and exclusion criteria. Relying solely on an evidence-based screening strategy may not be sufficient to maintain the relevance of search results if no control is applied during the literature mining process. Including extraneous publications in the search results can occur without proper guidance. To ensure the validity of the literature gathered for this study, specific inclusion and exclusion criteria were applied (see Supplementary File 2). By adhering to these criteria, the

research team was able to focus on pertinent publications, thereby enhancing the reliability and accuracy of the data analysis.

2.2 Exploration of Identified Genes

To systematically explore the identified genes, the Oryzabase database (https://shigen.nig.ac.jp/rice/oryzabase/) was utilized. Oryzabase is a comprehensive rice research database established in 2000 by a group of rice researchers in Japan. The initial aim of the database was to consolidate information ranging from traditional rice genetics to modern genomics, supported by the National BioResource Project (NBRP).

The Michigan State University (MSU) IDs of these genes were extracted from Oryzabase, facilitating their location in The Rice Annotation Project Database (RAP-DB) (https://rapdb.dna.affrc.go.jp/). This allowed for the extraction of specific chromosomal locations of the genes. Genomic sequence data in FASTA format (.fasta/.fa) were obtained using the G-Browse tool of RAP-DB. To identify the open reading frames (ORFs) of the genes and reveal protein-coding sequences (CDS), the NCBI ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/) was used.

By analyzing the data acquired from RAP-DB and NCBI ORF Finder, the lengths of the 5' untranslated region (UTR), CDS, and 3' UTR for the genes were determined. Finally, a Basic Local Alignment Search Tool (BLAST) analysis was conducted for all gene sequences using NCBI BLASTX (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to obtain the translated protein sequences. This methodology enabled a detailed characterization of the identified abiotic stress-sensitive genes in *O. sativa*.

2.3 Multiple Sequence Alignment and Phylogenetic Analyses

To investigate the evolutionary relationships and identify similar patterns across the genes, Multiple Sequence Alignment (MSA) was performed. The translated protein sequences of the genes were subjected to MSA using the Clustal Omega 1.2.2 program (Sievers et al., 2014). Pairwise sequence comparisons were carried out by calculating the percentage similarity matrix using the BLOSUM62 matrix in the Geneious Prime 2022.2.2 software. Phylogenetic analysis was conducted by constructing a Maximum Likelihood (ML) phylogenetic tree based on the Jones-Taylor-Thornton (JTT) substitution model (Jones et al., 1992). To assess the reliability and accuracy of the generated phylogenetic tree branches, 1,000 bootstrap replications were performed during tree construction using the MEGA11 software (Tamura et al., 2021). This methodological approach provided a comprehensive understanding of the evolutionary relationships among the identified abiotic stress-sensitive genes in *O. sativa*. Facilitating further analysis of their biological functions and potential roles instress tolerance.

2.4 Protein-Protein Interaction and Gene Co-Occurrence Study

Investigating interactions between proteins and small molecules is crucial for a deeper understanding of molecular and cellular functions, including metabolism, signaling, and drug responses. Understanding protein-protein and biomolecule interactions is vital for comprehending processes such as metabolic pathways and signaling cascades.

To examine these interactions, the STRING database (https://string-db.org/) was utilized. STRING is an online resource that provides information on functional associations between biomolecules for various species by integrating known and predicted protein-protein interactions (Szklarczyk et al., 2021). The interactions among the translated proteins from the identified genes were analyzed using STRING. Additionally, gene co-occurrence and co-expression studies were performed within the STRING platform to gain further insights into how these genes interact across various taxa beyond *Oryza*.

2.5 In Silico Expression Study

The electronic fluorescence pictogram (eFP) program is widely recognized for visualizing transcriptome data in various model organisms. To assess the expression levels of the six identified genes under stress conditions, their expression profiles were analyzed using the Rice eFP Browser (https://bar.utoronto.ca/transcriptomics/efp_rice/cgi-bin/efpWeb.cgi?dataSource=rice_leaf_gradient), developed at the University of Toronto. This tool provides graphical representations of gene expression across different tissue types, with distinct colors illustrating the expression levels in each tissue (Jung et al., 2011).

Bioinformatic analysis revealed that six out of the fourteen identified genes, namely *AOX1a*, *AOX1b*, *ALDH2a*, *ALDH2b*, *OsNAC6*, and *OsDHN1*, exhibited potential expression under all the abiotic stresses investigated. Consequently, these six genes were further screened using the Rice eFP Browser to evaluate their expression potential through in silico analysis, providing a more comprehensive understanding of the stress-responsive expression patterns of the identified genes in *O. sativa*.

The methodologies employed in this study (Fig. 3) collectively offer a thorough examination of the characterization, evolutionary relationships, functional associations, potential roles, and expression patterns of the identified abiotic stress-sensitive genes in *O. sativa*.

3. Results

3.1 Identification of Genes

A total of 14 abiotic stress-responsive genes in rice were identified through a comprehensive literature mining process. These genes were classified according to the three major abiotic stresses rice plants encounter during their lifecycle: drought, salt, and low temperature. Only three identified genes are expressed under individual stress conditions: *OsDREB1G* under cold stress response (Moon et al., 2019), *OsERF28* under drought stress response (Mawlong et al., 2014), and *OsSTLK* under salt stress response (Lin et al., 2020). The other 11 genes exhibited significant overlap in their mechanisms of action, as reported by Mondini et al. (2015).

Several genes showed expression under multiple stress conditions. *OsPP2C1* and *OsSADR1* were expressed under both salt and drought stress (Jiang et al., 2011; Park et al., 2018), while *OsNIN6* and *OsNCA1A* were expressed in response to salt and low temperatures (Liu et al., 2019; Yao et al., 2009). *OsDREB1B* was expressed under both drought and cold stress (Figueiredo et al., 2012). Remarkably, six genes (*AOX1a*, *AOX1b*, *ALDH2a*, *ALDH2b*, *OsNAC6*, and *OsDHN1*) were found to be expressed under all three categories of abiotic stress: drought, salt, and low temperature (Feng et al., 2009; Tsuji et al., 2003; Nakashima et al., 2007; Kumar et al., 2014) (see Supplementary File 3).

3.2 Exploration of Identified Genes

The specific chromosomal locations of the identified genes were determined, revealing that the genes are distributed across multiple chromosomes of *O. sativa*. The top three genes are located on chromosome 2, followed by two genes on chromosomes 1, 4, and 9.

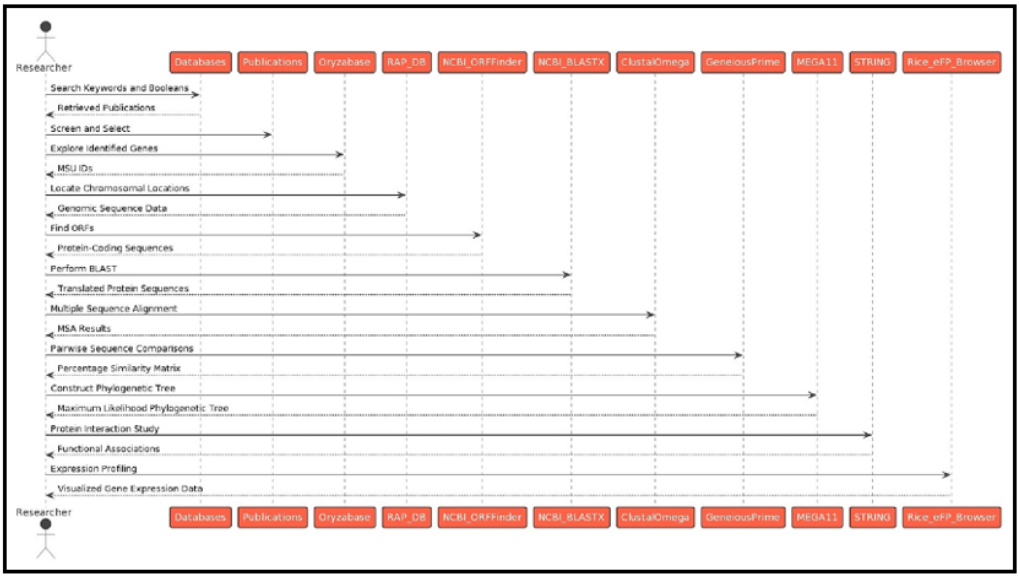

Fig. 3. Methodological approaches followed in this study to identify abiotic stress-responsive genes in *O. sativa*. (Source: Author's creation with PlantText UML Editor).

and 11 each, while only one gene is located on chromosomes 5, 6, and 8, respectively. The transcript length, 5' UTR length, CDS length, 3' UTR length, and translated protein length for each gene are listed in Table 1.

3.3 Multiple Sequence Alignment and Phylogenetic Analyses

The multiple sequence alignment (MSA) of the translated proteins revealed an average protein length of 416 amino acids (aa) and a pairwise identity of 9.2% (Supplementary File 4). The MSA indicated that the proteins have an average molecular weight of 45.353 kDa and an average isoelectric point of 6.88. To assess the amino acid substitution rates in clusters of the relevant proteins, a percentage similarity matrix was constructed using the BLOSUM62 matrix (Fig. 4). This matrix highlights the evolutionary relationships among the genes, where higher percentages of similarity indicate closer evolutionary relationships.

The phylogenetic analysis, based on the Maximum Likelihood (ML) method, resulted in four distinct clusters of genes (Fig. 5). The dataset contained 948 positions derived from the 14 amino acid sequence analyses. Consistent with the similarity matrix, *ALDH2a*, *ALDH2b*, *AOX1a*, and *AOX1b* formed Cluster 1 in the phylogenetic tree, showing the highest percentage similarities among them, ranging from 82.7% to 91.9%. Cluster 2 consisted of *OsERF28*, *OsDREB1B*, and *OsDREB1G*, with percentage similarities ranging from 41.6% to 57.9%. *OsNAC6* and *OsDHN1* formed Cluster 3, exhibiting a 22.2% similarity. Lastly, Cluster 4 was composed of *OsSTLK*, *OsNCA1A*, and *OsNIN6*, with percentage similarities noted.

Table 1
List of 14 identified abiotic-stress responsive genes in *O. sativa* with their MSU IDs, Exhaustive chromosomal location, transcript length, 5′ UTR length, CDS length, 3′ UTR length and translated protein length.

Sl. No.	Gene Name	MSU ID	Chromosome Number	Exhaustive Position	Transcript length	5′ UTR length	CDS length	3′ UTR length	Protein length
1	OsPP2C1	LOC_Os09g15670.1	9	chr09:9567471.0.9568877	1407 bp	80 bp	1077 bp	250 bp	358 aa
2	OsDREB1G	LOC_Os02g45450.1	2	chr02:27652935.0.27654206	1272 bp	45 bp	675 bp	552 bp	224 aa
3	OsERF28	LOC_Os08g43210.1	8	chr08:27320678.0.27325475	981 bp		981 bp		326 aa
4	OsSTLK	LOC_Os05g24010.1	5	chr05:13834116.0.13839997	3312 bp	194 bp	2832 bp	286 bp	943 aa
5	OsSADR1	LOC_Os11g07450.1	11	chr11:3749274.0.3755192	1680 bp	60 bp	1437 bp	183 bp	478 aa
6	OsDREB1B	LOC_Os09g35010.1	9	chr09:20395279.0.20396175	897 bp	15 bp	657 bp	225 bp	218 aa
7	NCA1A	LOC_Os01g01420.1	1	chr01:209771.0.214173	1642 bp	165 bp	1092 bp	385 bp	363 aa
8	OsNIN6	LOC_Os11g07440.1	11	chr11:3739630.0.3743522	2320 bp	325 bp	1647 bp	348 bp	548 aa
9	AOX1a	LOC_Os04g51150.1	4	chr04:30287197.0.30289860	2712 bp	413 bp	999 bp	107 bp	332 aa
10	AOX1b	LOC_Os04g51160.1	4	chr04:30291463.0.30293040	1580 bp	207 bp	1008 bp	146 bp	335 aa
11	ALDH2a	LOC_Os02g49720.1	2	chr02:30392547.0.30396729	4110 bp	964 bp	1752 bp	107 bp	553 aa
12	ALDH2b	LOC_Os06g15990.1	6	chr06:9091026.0.9096474	5664 bp	226 bp	1650 bp	479 bp	549 aa
13	OsNAC6	LOC_Os01g66120.1	1	chr01:38398996.0.38401481	3017 bp	984 bp	744 bp	161 bp	303 aa
14	OsDHN1	LOC_Os02g44870.1	2	chr02:27165514.0.27166898	1465 bp	379 bp	873 bp	119 bp	300 aa

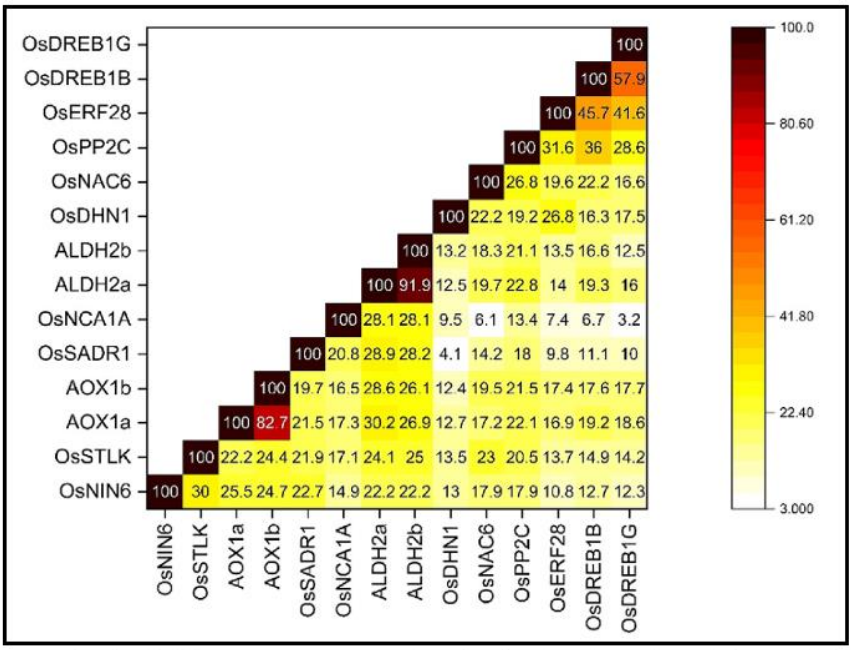

Fig. 4. Percentage similarity matrix of 14 identified abiotic-stress responsive genes in *O. sativa*. The colour scheme indicates the heatmap of similarities among the genes (Source: Author's creation with OriginPro 2022 v.9.9.0.225).

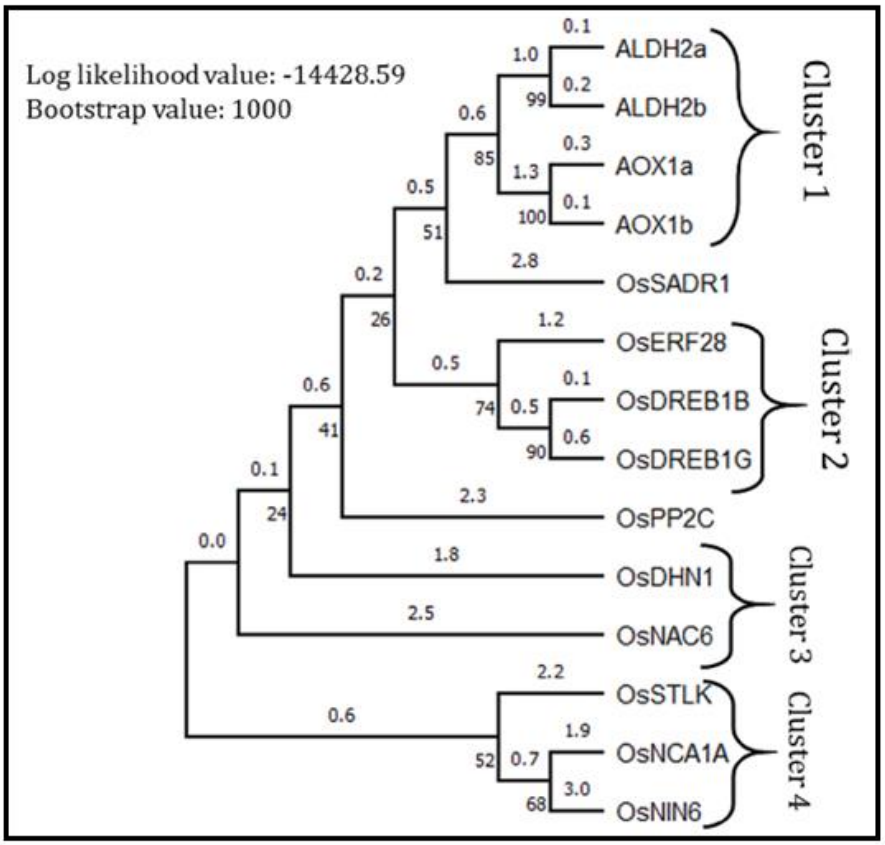

Fig. 5. Phylogenetic tree of 14 identified abiotic-stress responsive genes in *Oryza sativa* made in MEGA11 (20). The Maximum Likelihood (ML) phylogenetic tree is computed with the highest log likelihood value of −14428.59. Above the branches is the branch length and below is the proportion of replicate trees in which the related taxa grouped together in the bootstrap test with 1000 repetitions.

The gene similarities ranged from 14.9% to 30%. Notably, *OsSADR1* and *OsPP2C* were not clustered in the phylogenetic tree, although they showed a percentage similarity of 18% based on the similarity matrix.

3.4 Protein-Protein Interaction and Gene Co-Occurrence Study

The phylogenetic analysis suggests that these genes share homology, indicating potential evolution from a common ancestral gene. The protein-protein interaction (PPI) network, analyzed using the STRING database with high-confidence values, revealed 19 nodes in the protein network associated with functional subsystems. The network had an average node degree of 1.68, a local clustering coefficient of 0.458, and a PPI enrichment p-value of 0.000639 (Fig. 6).

In addition to the 14 query proteins (see Supplementary File 5), five additional proteins (*OsJ_04113*, *OsJ_24269*, *OS03T0297600-01*, *OS07T0188800-01*, and *OsJ_06966*) were predicted to be functional partners by the network. STRING recorded 17 interactions among the query proteins (see Supplementary File 6).

K-means clustering identified three main clusters of protein interactions: Cluster 1: Included 6 genes (*AOX1a*, *AOX1b*, *DHN1*, *DREB1G*, *OS11T0175500-02*, *OsJ_33156*). Cluster 2: Consisted of 7 genes (*DREB1H*, *NAC6*, *OS01T0104100-01*, *OS03T0297600-01*, *OS05T0305900-01*, *OS08T0545500-00*, *OsJ_027745*). Cluster 3: Comprised 6 genes (*ALDH2a*, *ALDH2b*, *OS07T0188800-01*, *OsJ_04113*, *OsJ_06966*, *OsJ_24269*). KEGG pathway analysis identified the involvement of these proteins in 14 potential KEGG pathways (Table 2). The co-occurrence patterns of the 14 identified abiotic stress-responsive genes, along with their five predicted functional protein

partners, were also analyzed across various taxa (see Supplementary File 7). A co-expression study conducted through STRING further provided insights into the expression patterns of these genes under different abiotic stresses (see Supplementary File 8).

3.5 In Silico Expression Study

The expression potential of the six key genes was analyzed individually using an electronic fluorescent pictogram (eFP) to gain insights into how these genes may respond under stress conditions (Fig. 7). The expression potential for each gene was calculated using the GCOS expression signal (see Supplementary File 9). *ALDH2a* had the highest expression in inflorescence P3 and the lowest in young leaves. *ALDH2b* showed the highest expression in young leaves and the lowest in the shoot apical meristem (SAM). *AOX1a* exhibited the highest expression in seed S1 and the lowest in SAM. *AOX1b* had the highest expression in seed S1 and the lowest in inflorescence P2. *OsDHN1* showed the highest expression in young inflorescence and the lowest in seed S5. *OsNAC6* exhibited the highest expression in mature leaves and the lowest in SAM.

In Summary the comprehensive analysis of 14 abiotic stress-responsive genes in rice, including their expression patterns, evolutionary relationships, protein–protein interactions, and co-expression provides valuable insights into the potential roles of the identified genes in the abiotic stress response of rice. Further experimental validation and functional characterization of these genes will contribute to a better understanding of their roles in stress tolerance and may help in developing strategies for enhancing rice crop resilience.

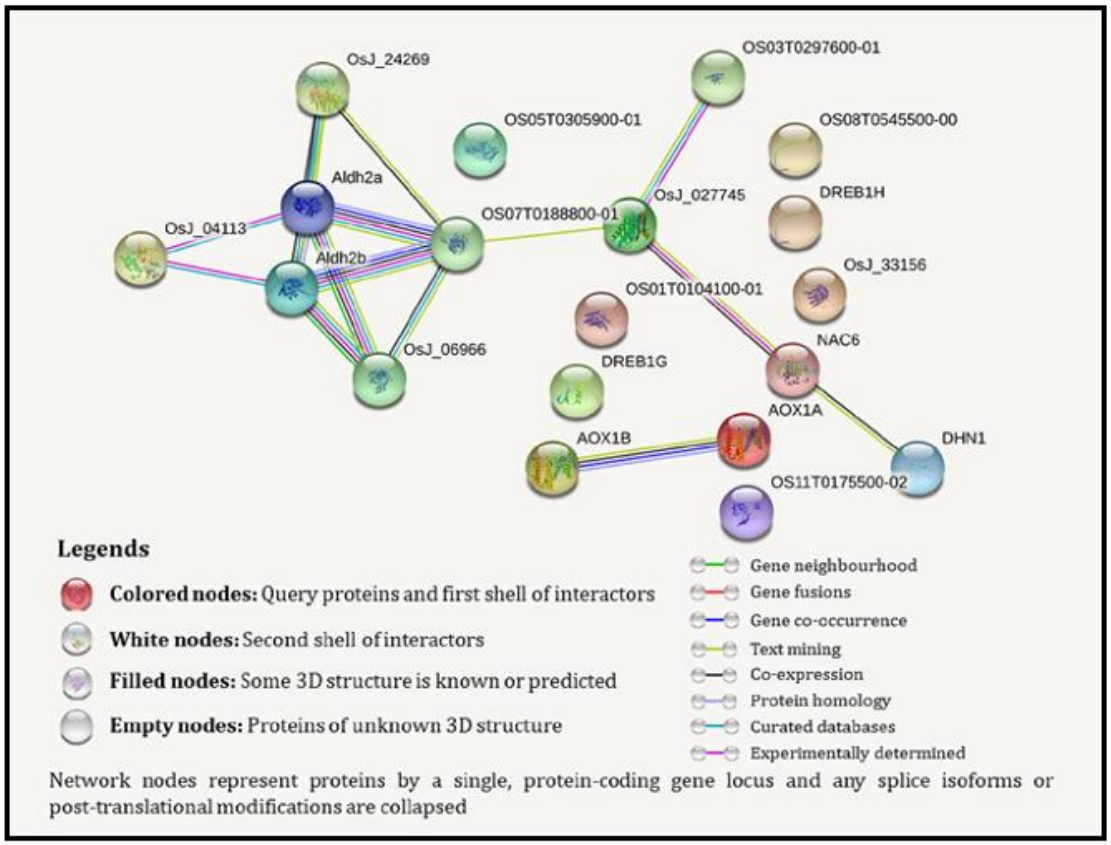

Fig. 6. Protein-protein interaction among 14 identified abiotic-stress responsive genes in *O. Sativa* along with 5 predicted functional protein partners made in STRING.

Table 2
KEGG pathway analysis for the query proteins.

KEGG Pathways	Observed gene count	Background gene count	Strength	False discovery rate	Matching proteins in network
beta-Alanine metabolism	4	55	2.14	3.97E-06	Aldh2a, Aldh2b, OS07T0188800-01, OsJ_24269
Fatty acid degradation	3	60	1.97	0.00026	OsJ_04113, Aldh2a, Aldh2b
Valine, leucine and isoleucine degradation	3	65	1.94	0.00026	Aldh2a, Aldh2b, OS07T0188800-01
Pantothenate and CoA biosynthesis	3	52	2.03	0.00026	Aldh2a, Aldh2b, OsJ_24269
Limonene and pinene degradation	2	9	2.62	0.00046	Aldh2a, Aldh2b
Pyruvate metabolism	3	110	1.71	0.00075	OsJ_06966, Aldh2a, Aldh2b
Histidine metabolism	2	24	2.19	0.0019	Aldh2a, Aldh2b
Glycolysis / Gluconeogenesis	3	179	1.5	0.0023	OsJ_06966, Aldh2a, Aldh2b
Propanoate metabolism	2	42	1.95	0.0043	OsJ_06966, OS07T0188800-01
Ascorbate and aldarate metabolism	2	65	1.76	0.009	Aldh2a, Aldh2b
Arginine and proline metabolism	2	80	1.67	0.0122	Aldh2a, Aldh2b
Lysine degradation	2	88	1.63	0.0135	Aldh2a, Aldh2b
Tryptophan metabolism	2	104	1.56	0.0172	Aldh2a, Aldh2b
Glycerolipid metabolism	2	110	1.53	0.0178	Aldh2a, Aldh2b

4. Discussion

The 14 abiotic stress-responsive genes identified in *Oryza sativa* are distributed across different chromosomes, suggesting that multiple interconnected pathways regulate the plant's abiotic stress response. The occurrence of more than one gene on a single chromosome further highlights the cross-talk between response pathways due to the complex nature of abiotic stress (Zarattini et al., 2021).

The transcript length, CDS length, and translated amino acid length (Supplementary File 10) of these genes showed significant variation, as revealed by the analysis conducted in this study. This variation suggests that individual genes play distinct roles within the abiotic stress response pathways.

A pairwise similarity matrix is a fundamental but effective tool for understanding the co-expression of various genes. The matrix revealed a wide range of variation in percentage similarity among the genes of interest, ranging from 3.2% to 91.9%. This broad range of similarity is well-supported by the phylogenetic analysis conducted in this study.

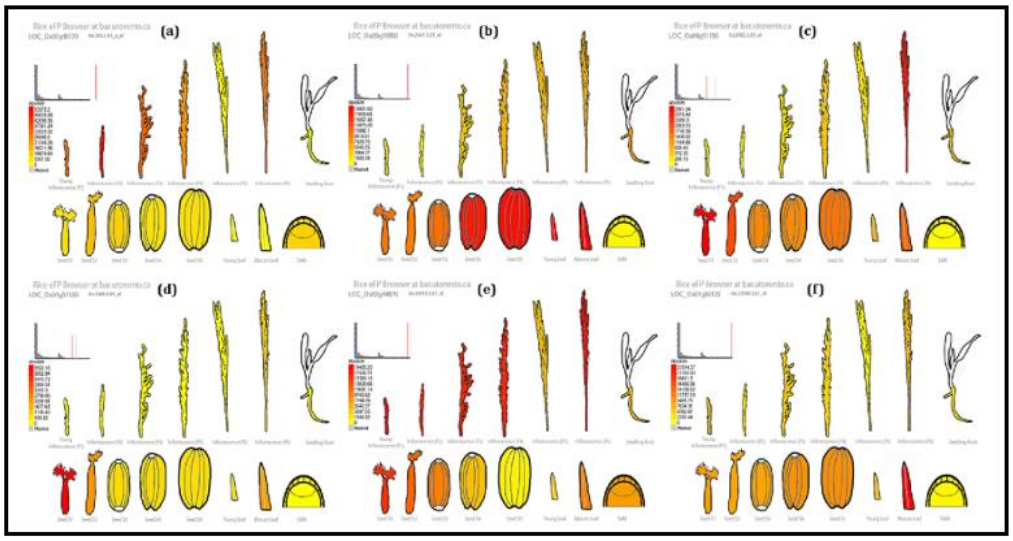

Fig. 7. Expression profiling of 6 identified abiotic-stress responsive genes in *Oryza sativa*, analysis done in Rice eFP browser: (a) *ALDH2a*, (b) *ALDH2b*, (c) *AOX1a*, (d) *AOX1b*, (e) *OsDHN1*, (f) *NAC6*.

The base trees for the heuristic search were automatically generated during the development of the phylogenetic tree using the Neighbor-Join and BioNJ algorithms, which determined the topology with the highest log-likelihood value. The branch lengths of the phylogenetic trees were inferred by computing the number of substitutions per site,

providing significant insights into the evolutionary development of the genes analyzed. The phylogenetic tree was constructed using the Maximum Likelihood (ML) approach, which employs various substitution models to account for multiple changes at specific sequence locations along the evolutionary timeline. The Jones-Taylor-Thornton (JTT) substitution model (Jones et al., 1992) was selected for constructing the phylogenetic tree, as this model best fit the phylogeny with the lowest Bayesian Information Criterion (BIC) score. Bootstrapping was performed with 1,000 replicates (Felsenstein, 1985; Hillis and Bull, 1993). The clusters in the phylogenetic tree indicate potential ancestral relationships between genes within the same cluster. Although all clusters were interrelated according to the phylogenetic tree, genes within the same clusters exhibited significant percentage similarity in the pairwise similarity matrix, which supports the phylogenetic relationships.

The protein-protein interaction (PPI) analysis revealed three prominent clusters of interactions. The most robust interactions were found between *ALDH2a*, *ALDH2b*, *OS07T0188800-01*, and *OsJ_04113* in one cluster, and between *AOX1a* and *AOX1b* in another cluster. Notably, the KEGG pathway analysis identified that two genes, *ALDH2a* and *ALDH2b*, participated in every pathway except propanoate metabolism. The profiles of the potential expression levels of the analyzed six genes indicated that resistance or tolerance mechanisms under various abiotic stress stimuli gradually develop throughout the plant's lifecycle. Each gene demonstrated peaks of expression at different stages of plant growth, underscoring their significant roles in the abiotic stress response in *O. sativa* and suggesting intense cross-talk between response mechanisms.

From an agricultural perspective, understanding the abiotic stress-responsive genes in *O. sativa* is crucial for improving crop resilience and maintaining yield stability under adverse environmental conditions (Paul et al., 2023). Rice is a staple food for more than half of the world's population, and its production must keep pace with growing demand. However, the increasing frequency and severity of abiotic stress factors, such as drought, salinity, and cold, due to climate change, pose significant challenges to rice production worldwide. The findings of this study on the 14 abiotic stress-responsive genes, their interactions, and their potential expression levels under various stress conditions provide valuable insights for developing rice varieties with enhanced tolerance to multiple abiotic stresses (Fig. 8).

Identifying and characterizing these genes and their roles in abiotic stress response pathways can assist plant breeders and biotechnologists in designing targeted breeding strategies, such as marker-assisted selection and genetic engineering, for developing stress-tolerant rice cultivars (Sun et al., 2022). These improved rice varieties would boost agricultural productivity and ensure food security in the face of climate change.

Furthermore, understanding the cross-talk among these abiotic stress-responsive genes can contribute to the development of rice cultivars with broad-spectrum stress tolerance (Husaini, 2022). This can be achieved by manipulating multiple genes or regulatory elements involved in cross-talk, enabling the plant to withstand various stress factors simultaneously. Future research should focus on validating the roles of these identified genes through functional genomics approaches, such as gene knockout or over-expression studies, in rice plants grown under controlled stress conditions. Additionally, studying gene regulatory networks and identifying transcription factors that regulate these stress-responsive genes will provide a comprehensive understanding of the molecular mechanisms underlying abiotic stress responses in rice.

Thus, the insights gained from this study on the abiotic stress-responsive genes in *O. sativa* have significant implications for agriculture, especially in the context of climate change. By harnessing the potential of these genes and understanding their interactions and cross-talk, researchers and plant breeders can develop new rice varieties with enhanced tolerance to multiple abiotic stresses, thereby ensuring sustainable rice production and global food security.

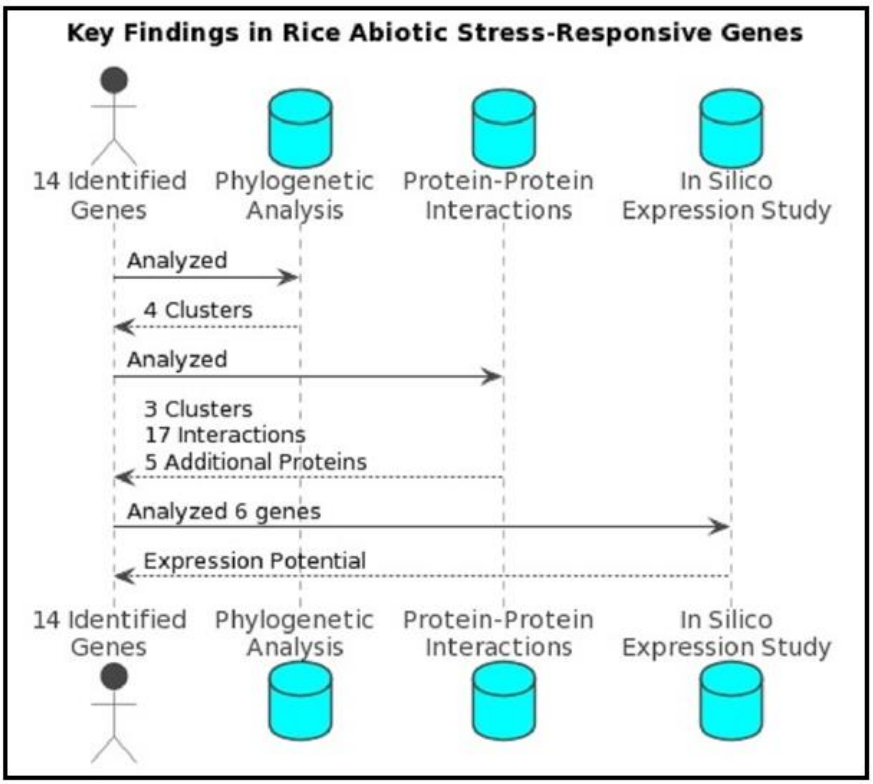

Fig. 8. Key findings of the study conducted on the genes playing significant regulatory roles in abiotic stress responses in *O. Sativa* (Source: Author's creation with PlantText UML Editor).

5. Conclusion

The findings of the present study suggest that six key genes (*AOX1a*, *AOX1b*, *ALDH2a*, *ALDH2b*, *OsNAC6*, *OsDHN1*) play significant regulatory roles in abiotic stress responses in *Oryza sativa*. The expression profiles of these genes were found to vary across different stages of the rice life cycle, including young and mature leaves, young inflorescence, and seed development stages. These results indicate that these genes are involved in interconnected cascades throughout the plant's lifecycle, providing protection against abiotic stresses.

The cross-talk between the response mechanisms of these abiotic stress-responsive genes was also supported by phylogenetic analysis, protein-protein interaction, and gene co-expression studies. Therefore, targeting these genes for the development of gene-based molecular markers could facilitate the breeding of rice cultivars with enhanced resistance to challenging environmental conditions.

Additionally, these findings have the potential to contribute to the successful application of computational biology in plant breeding by reducing the cost, complexity, and time associated with traditional biological studies. This could pave the way for developing stress-tolerant rice varieties, ultimately improving crop resilience and productivity in the face of climate change.

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