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Original article

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

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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 pathway 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

Abbreviations: MSA, Multiple Sequence Alignment; DNA, Deoxyribonucleic Acid; RNA, Ribonucleic Acid; JTT, Jones-Taylor-Thornton; ML, Maximum Likelihood; eFP, Electronic Fluorescence Pictogram; KEGG, Kyoto Encyclopedia of Genes and Genomes; BIC, Bayesian Information Criterion; CDS, Coding Sequenc; UTR, Untranslated Region; GCOS, GeneChip Operating Software; SAM, Shoot Apical Meristem; AOX, Alternative Oxidase; ALDH, Aldehyde Dehydrogenase; ORF, Open Reading Frames; OsNAC, *Oryza sativa* NAC-domain transcription factor; OsDHN, *Oryza sativa* Dehydrin; OsDREB, *Oryza sativa* DRE-binding protein; OsERF, *Oryza sativa* Ethylene-Responsive Factor; OsSTLK, *Oryza sativa* Serine/Threonine Protein Kinase; OsPP2C, *Oryza sativa* Protein Phosphatase 2C; OsSADR, *Oryza sativa* Salt and Drought Resistance protein; OsNIN, *Oryza sativa* NIN-like protein; OsNCA, *Oryza sativa* Non-Coding Acidic protein; SDG, Sustainable Development Goal.

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of these genes. These findings can be employed to develop gene-based molecular markers for breeding rice cultivars with enhanced resistance to abiotic stresses, and contribute to the successful application of computational biology in plant breeding towards sustainable development goal.

1. Introduction

Plants inhabit dynamic environments, subject to continuous fluctuations that can be detrimental to their growth and development (Zhu et al., 2016). Environmental stressors can be broadly classified into biotic and abiotic stress (Debnath et al., 2013). Biotic stress refers to adverse effects resulting from herbivore attacks and pathogen infections, while abiotic stress pertains to unfavourable environmental conditions such as cold, heat, 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 being 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, as crops lacking stress tolerance require excessive inputs like fertilizers, pesticides, and irrigation. Understanding plant stress response necessitates examining stress signals, which facilitate communication between various molecular responses 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, including reduced photosynthesis, stomatal closure, increased reactive oxygen species (ROS) scavenging, stunted leaf growth, and elongated roots (Cohen and Leach, 2019). Whereas, biotic stress factors, such as pathogens, can also induce stomatal closure and decrease photosynthesis. Defensive mechanisms against biotic stress include 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, are crucial for plant immunity against pathogenic factors (Lata et al., 2011). Transcription factor families involved in abiotic stress response include abscisic acid (ABA)-dependent and ABA-independent transcription factors. 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 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 thought to regulate downstream stress-responsive genes by binding directly 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, respectively. Rabbani et al., 2003 used a cDNA microarray technique to profile rice gene expression under various environmental stresses, such as drought, cold, high salinity, and abscisic acid. 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 significant overlap between gene expression and mechanisms of action in response to abiotic and external stress stimuli. Consequently, abiotic stress response genes play a critical role in stress regulation and assisting rice plants in coping with adverse conditions induced by abiotic stress. Throughout their evolution, plants have developed various strategies and processes to deal with stressors such as dryness, heat, cold, and excessive salinity (Debnath et al., 2022).

Plant stress responses are 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 modulation of gene expression and post-translational modification. Gaining a comprehensive understanding of the genes responsible for abiotic stress response in rice plants and their expression patterns in response to abiotic stress can contribute valuable insights for modulating genetic expression. Additionally, studying the roles of different abiotic stress-responsive genes can help in understanding their importance in developing stress-tolerant rice varieties.

Despite the wealth of information on regulating various stress-responsive genes in rice, there remains a significant gap in the literature regarding a comprehensive study that investigates the

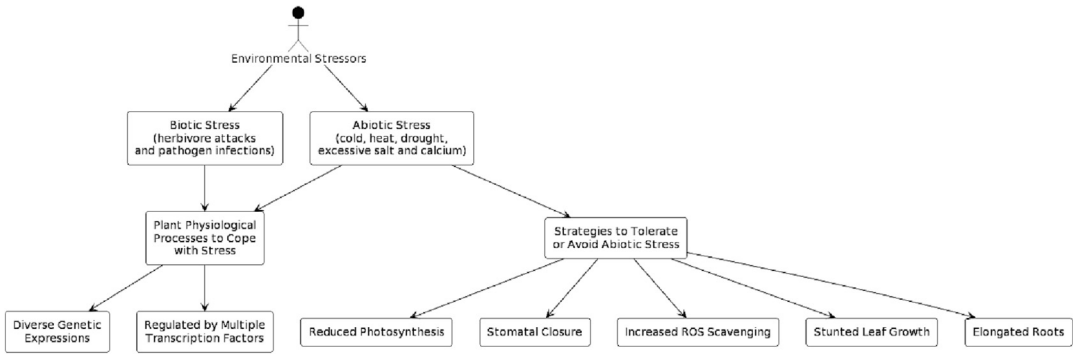

Fig. 1. Strategies employed by plants against environmental stressors (Source: Author's creation with PlantText UML Editor).

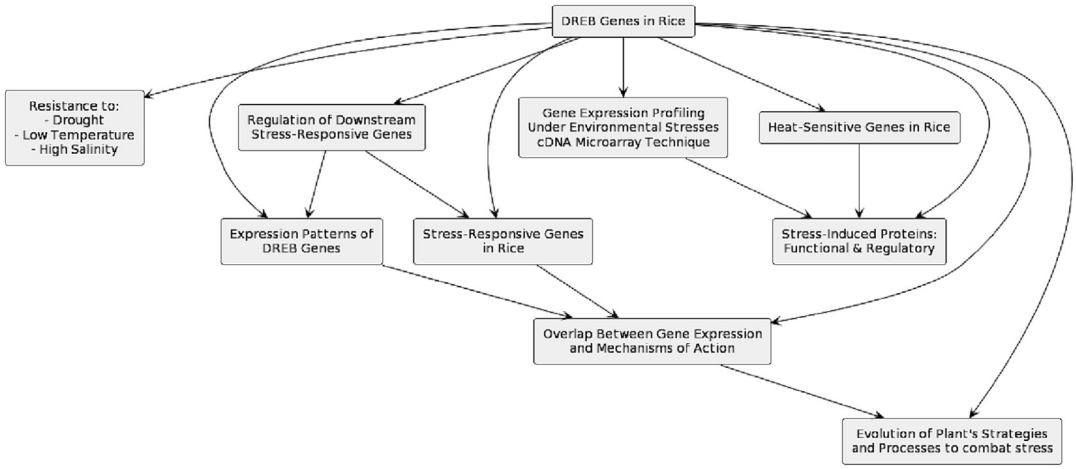

Fig. 2. Functions of DREB gene in rice combating different abiotic stresses (Source: Author's creation with PlantText UML Editor).

expressions of abiotic stress-responsive genes and their interplay within different stress response pathways in rice plants. Numerous primary articles found in electronic databases offer insight into the expression patterns of the aforementioned genes through systematic reviews. However, considering the lack of a comprehensive study on this topic, it is essential to formulate a hypothesis for a comprehensive analysis of the expression patterns of abiotic stress-responsive genes in *O. sativa*. 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 to achieve sustainable development goal.

2. Materials and methods

2.1. Identification of genes

Data mining is essential for the effective screening and analysis of the retrieved 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 used as sources for collecting relevant literature. However, this research focused on publications available in CINAHL, PubMed, and Google Scholar databases due to their public accessibility. To perform 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 in relation to the research question (Supplementary file 1). Initially, title and abstract screening were used to collect publications from online databases. Subsequently, a full-text assessment was conducted to evaluate the relevance of the selected articles.When mining the literature for genes of interest, it is crucial to establish and apply appropriate inclusion and exclusion criteria. Using an evidence-based screening strategy alone may not be sufficient to maintain the relevance of the search results if no control is exercised during the literature mining process. Inclusion of extraneous publications in the search results may occur without proper guidance. To ensure the validity of the gathered literature for this study, the authors applied specific inclusion and exclusion criteria (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 (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. The database is supported by the National BioResource Project (NBRP).The Michigan State University (MSU) IDs of these genes were extracted from Oryzabase, which facilitated locating the genes 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. The genomic sequence data of the genes in FASTA format (.fasta /.fa) were obtained using the G-Browse tool of RAP-DB. To identify the ORF of the genes and reveal the protein-coding sequences (CDS), the NCBI ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/) was employed. 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 the gene sequences using the NCBI BLASTX (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to obtain the translated protein sequences of the genes. This methodology allowed for 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 genes, Multiple Sequence Alignment (MSA) is employed. The translated protein sequences of the genes were subjected to Multiple Sequence Alignment 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, one thousand bootstrap replications were performed during tree construction using the MEGA11 software (Tamura et al., 2021).This methodological approach provides 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 in stress tolerance.

2.4. Protein-Protein interaction and gene co-occurrence study

The investigation of interactions among proteins and small molecules is vital for a deeper comprehension of molecular and cellular functions, including metabolism, signalling, and drug treatments, as they may apply. Gaining insight into the interactions between proteins and other biomolecules is imperative for a thorough understanding of molecular and cellular processes such as metabolic pathways and signalling cascades.

STRING (https://string-db.org/) is an online resource that provides information on functional associations between various biomolecules for different species by integrating known and predicted protein–protein interactions (Szklarczyk et al., 2021). The interactions among the translated proteins from the identified genes were examined using STRING. To enhance the understanding of how these genes interact across different taxa beyond *Oryza*, gene co-occurrence and co-expression were also investigated within the STRING platform which provided greater insights into their interactions across various taxa.

2.5. In silico expression study

The electronic fluorescence pictogram (eFP) program is a widely recognized tool for visualizing transcriptome data and has been employed in various model organisms. To understand 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 facilitates the visual analysis of gene expression by providing illustrative graphics for different tissue types, with distinct colors representing the expression levels of a specific gene in each tissue (Jung et al., 2011).Bioinformatic analysis revealed that six out of the fourteen genes, namely *AOX1a*, *AOX1b*, *ALDH2a*, *ALDH2b*, *OsNAC6* and *OsDHN1*, exhibited the potential to be expressed under all abiotic stresses investigated. Consequently, these six genes were further screened using the Rice eFP Browser to evaluate their expression potential through in silico analysisfor exploring a more exhaustive understanding of the stress-responsive expression patterns of the identified genes in *O. sativa*.

These methodological approaches followed in this study (Fig. 3) collectively allows for a comprehensive examination of the characterization, evolutionary relationship, 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 of rice have been identified through a comprehensive literature mining process. These genes were classified into three significant abiotic stresses that rice plants experience throughout their lifetime, i.e., drought, salt, and low temperature. Only three identified genes are expressed under individual stresses: *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 showed significant overlapping in their mechanisms of action, as reported by Mondini et al. (2015). *OsPP2C1* and *OsSADR1* are expressed under salt and drought stress (Jiang et al., 2011; Park et al., 2018), *OsNIN6* and *OsNCA1A* are expressed in response to salt and low temperatures (Liu et al., 2019; Yao et al., 2009), and *OsDREB1B* is expressed under both drought and cold stress (Figueiredo et al., 2012). Remarkably, six genes (*AOX1a*, *AOX1b*, *ALDH2a*, *ALDH2b*, *OsNAC6* and *OsDHN1*) are found to be expressed under all three categories of abiotic stress, i.e., drought, salt, and low temperature (Feng et al., 2009; Tsuji et al., 2003; Nakashima et al., 2007; Kumar et al., 2014) (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, 9,

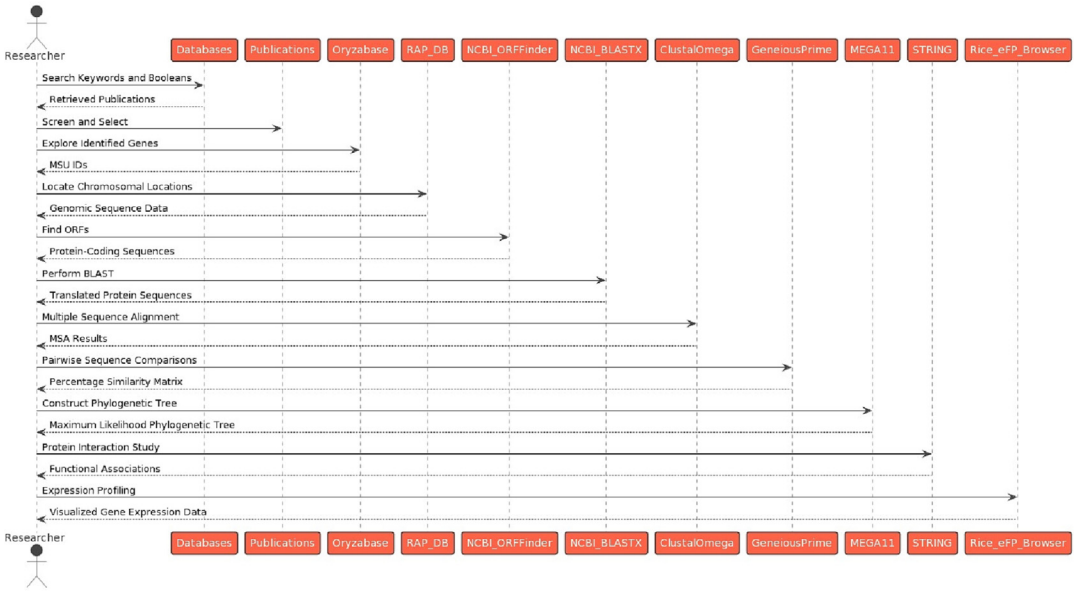

Fig. 3. Methodological approaches followed in this study to identify abiotic stress-responsive genes in *O. sativa*. (Source: Author's creation with PlantText UML Editor).

and 11 each, and only one gene is located on chromosomes 5, 6, and 8 each. 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 indicates an average protein length of 416 aa and a pairwise identity of 9.2% (Supplementary file 4). The MSA revealed that the proteins have an average molecular weight of 45.353 kDa and an average isoelectric point of 6.88. The percentage similarity matrix (Fig. 4) was constructed using the BLOSUM62 matrix to assess the amino acid substitution rates in clusters of the relevant protein translated by these genes. The matrix primarily highlights the evolutionary relationships among the genes. In terms of evolutionary divergence, genes sharing higher percentages of similarities are more closely related, as confirmed by the subsequent phylogenetic analysis.

The phylogenetic analysis, based on the Maximum Likelihood method, resulted in four clusters of genes (Fig. 5). The resulting dataset had 948 positions from the 14 amino acid sequence analyses. In agreement with the similarity matrix constructed, *ALDH2a*, *ALDH2b*, *AOX1a* and *AOX1b* formed cluster 1 in the phylogenetic tree, as they showed the highest values of percentage similarities among them, ranging from 82.7 to 91.9%. Cluster 2 is formed by *OsERF28*, *OsDREB1B* and *OsDREB1G*, corresponding to percentage similarities ranging from 41.6 to 57.9%. *OsNAC6* and *OsDHN1* formed cluster 3, showing a 22.2% similarity. Lastly, cluster 4 is formed by *OsSTLK*, *OsNCA1A* and *OsNIN6*, expressing percentage

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

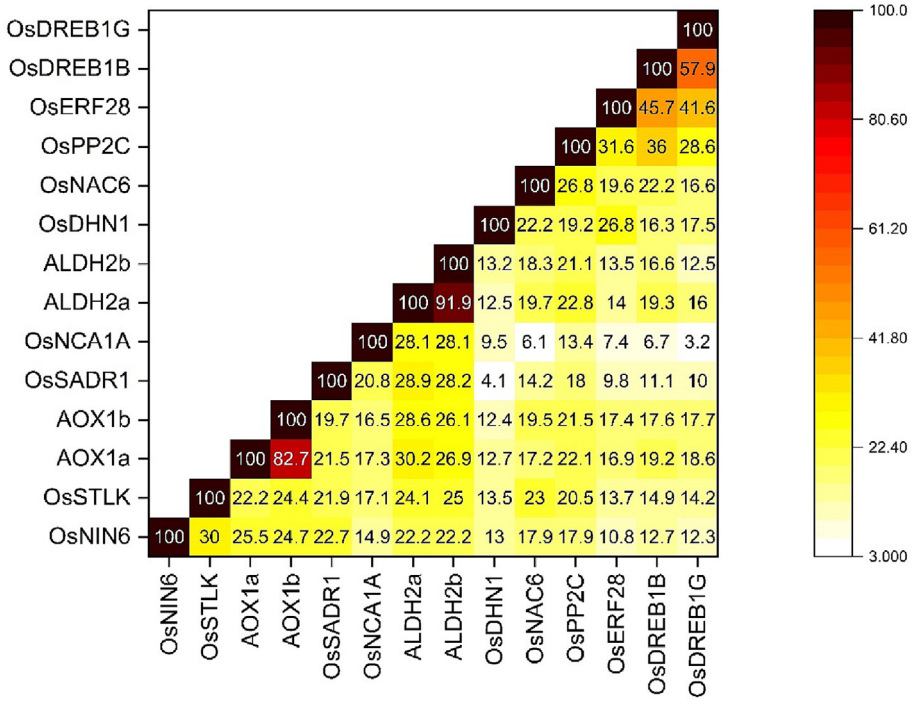

Fig. 4. Percentage similarity matrix of 14 identified abiotic-stress responsive genes in *O. sativa*. The colour scheme indicates the heatmap of similarities among the genes (Source: Author's creation with OriginPro 2022 v.9.9.0.225).

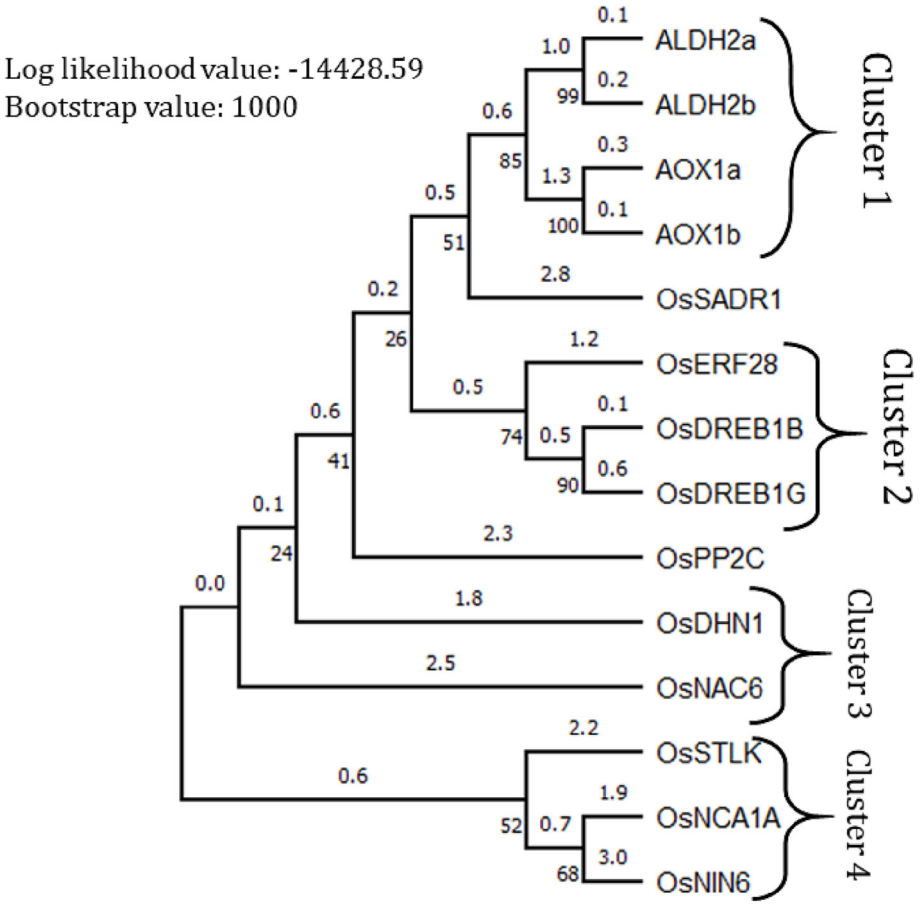

Fig. 5. Phylogenetic tree of 14 identified abiotic-stress responsive genes in *Oryza sativa* made in MEGA11 (20). The Maximum Likelihood (ML) phylogenetic tree is computed with the highest log likelihood value of −14428.59. Above the branches is the branch length and below is the proportion of replicate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

similarities ranging from 14.9 to 30%. *OsSADR1* and *OsPP2C*are not clustered in the phylogenetic tree, although they show a percentage similarity of 18% per the matrix constructed.

3.4. Protein-protein interaction and gene co-occurrence study

The phylogenetic analysis suggests that these genes share homology, potentially evolving from a single ancestral gene. The protein–protein interaction (PPI) network, based on high confidence values as defined by the STRING software, revealed 19 nodes in the network of proteins located in functional subsystems, with an average node degree of 1.68, an average local clustering coefficient of 0.458, and a PPI enrichment p-value of 0.000639 (Fig. 6). In addition to the 14 proteins in the search query (Supplementary file 5), five more proteins (OsJ_04113, OsJ_24269, OS03T0297600-01, OS07T0188800-01, and OsJ_06966) were predicted as functional partners by the network. The STRING software recorded 17 interactions among the query proteins (Supplementary file 6). The k-means clustering identified 3 clusters of interactions at the protein level with a high level of interaction confidence. Cluster 1 groups 6 genes (AOX1a, AOX1b, DHN1, DREB1G, OS11T0175500-02, OsJ_33156), Cluster 2 groups 7 genes (DREB1H, NAC6, OS01T0104100-01, OS03T0297600-01, OS05T0305900-01, OS08T0545500-00, OsJ_027745), and Cluster 3 groups 6 genes (Aldh2a, Aldh2b, OS07T0188800-01, OsJ_04113, OsJ_06966, OsJ_24269). The KEGG pathway analysis identified that the query proteins are involved in 14 potential KEGG pathways (Table 2). The co-occurrence patterns of the 14 identified abiotic-stress responsive genes in rice and 5 predicted functional protein partners are also examined across various taxa (Supplementary file 7). These genes were further subjected to a co-expression study in STRING, which provided an initial understanding of the pattern of expression of these genes under various abiotic stresses (Supplementary file 8).

3.5. In silico expression study

The expression potential of each of the six genes was examined individually using an electronic fluorescent pictogram (eFP) to better understand how these genes may respond under stressful conditions (Fig. 7). The expression potential of each gene was calculated through the GCOS expression signal (Supplementary file 9). *ALDH2a* showed the highest and lowest expression potential during inflorescence P3 and lowest in young leaves *ALDH2b* showed the highest expression potential in young leaves and the lowest in SAM Meanwhile, *AOX1a*showed the highest expression potential in seed S1 and the lowest in SAM.*AOX1b* showed the highest expression potential in seed S1 and the lowest in inflorescence P2. *OsDHN1* showed the highest expression potential in young inflorescence and the lowest in seed S5. *OsNAC6* showed the highest expression potential in the mature leaves and the lowest in SAM.

In summary, the comprehensive analysis of 14 abiotic stress-responsive genes in rice, including their expression patterns, evo-

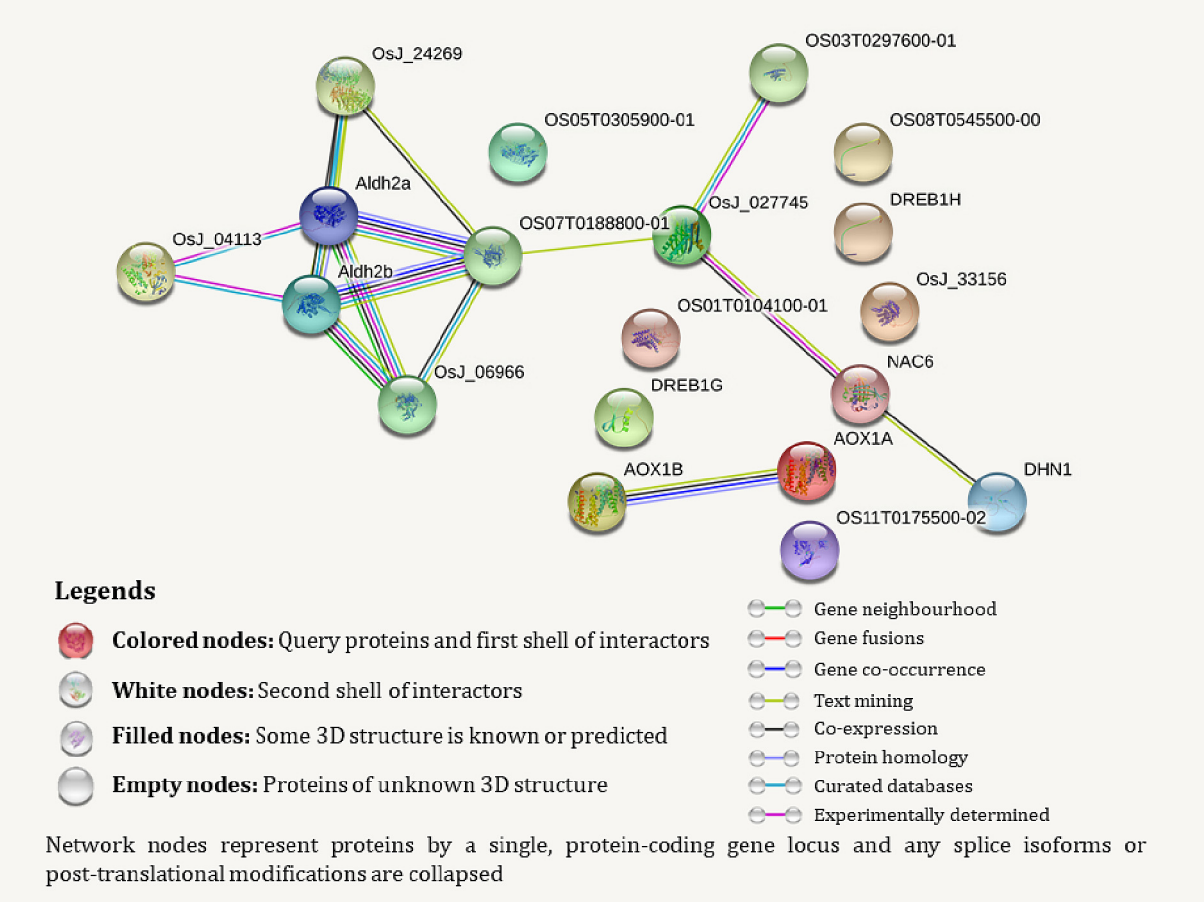

Fig. 6. Protein-protein interaction among 14 identified abiotic-stress responsive genes in *O. Sativa* along with 5 predicted functional protein partners made in STRING.

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

lutionary relationships, protein–protein interactions, and co-expression, provides valuable insights into their potential roles in abiotic stress response. Further experimental validation and functional characterization of these genes will contribute to a better understanding of their roles in stress tolerance and may help develop strategies for improving rice crop resilience.

4. Discussion

The 14 abiotic-stress-responsive genes in *O. sativa* identified for this study are distributed across different chromosomes, suggesting that multiple interconnected cascades regulate the abiotic-stress-response. The presence of more than one gene on a single chromosome further indicates the prominent cross-talk in the response pathways due to the prevalence of abiotic stress (Zarattini et al., 2021). The transcript length, CDS length, and translated amino acid length (Supplementary file 10) of these genes varied significantly, as demonstrated by the analysis carried out in this study. This variation implies individual gene occupancy in 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

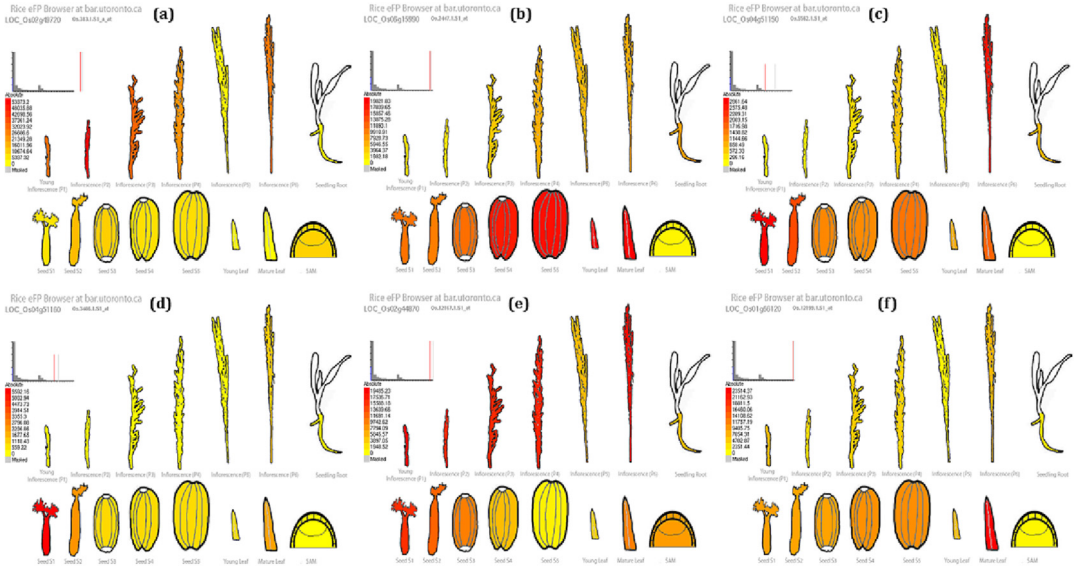

Fig. 7. Expression profiling of 6 identified abiotic-stress responsive genes in *Oryza sativa,* analysis done in Rice eFP browser: (a) *ALDH2a*, (b) *ALDH2b*, (c) *AOX1a*, (d) *AOX1b*, (e) *OsDHN1*, (f) *NAC6.*

revealed a wide range of variation in percentage similarity among the genes of interest, ranging from 3.2 to 91.9%. The broad range of similarity percentages is well supported by the phylogenetic analysis conducted in this study.

The base tree(s) for the heuristic search were automatically generated during the development of the phylogenetic tree using the Neighbor-Join and BioNJ algorithms and then determining 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 abundant information on the evolutionary development of the genes involved in the analysis. The phylogenetic tree was constructed using the Maximum Likelihood (ML) approach, which employs various substitution models to account for multiple changes at the exact sequence location along the evolutionary timeline. The Jones-Taylor-Thornton (JTT) substitution model (Jones et al., 1992) was considered while constructing the phylogenetic tree, as this model best fits the phylogeny by corresponding to the lowest Bayesian Information Criterion (BIC) score calculated. Bootstrapping was performed using 1000 replicates (Felsenstein, 1985; Hillis and Bull, 1993). The clusters in the phylogenetic tree indicate the similar potential ancestry of genes within the same cluster. Although each cluster is somehow interrelated according to the phylogenetic tree, genes within the same cluster showed significant percentage similarity in the pairwise similarity matrix, justifying the phylogenetic tree.

The protein–protein interaction analysis revealed three prominent clusters of interactions. The most robust interactions were found among *Aldh2a*, *Aldh2b*, *OS07T0188800-01* and *OsJ_04113* in one cluster and between *AOX1a* and *AOX1b* in another cluster. Notably, the KEGG pathway analysis identified two genes, *ALDH2a* and *ALDH2b*, to participate in every pathway except propanoate metabolism. The profiles of 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 lifetime. Each gene demonstrated peaks of expression at different stages of plant growth. These findings underscore the significant roles of these genes in the abiotic-stress response in *O. sativa* and suggest intense cross-talk of 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 crop for more than half of the world's population, and its production must keep pace with the 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 help plant breeders and biotechnologists to design targeted breeding strategies, including marker-assisted selection and genetic engineering approaches, for developing stress-tolerant rice cultivars (Sun et al., 2022). The resulting improved rice varieties would enhance agricultural productivity and ensure food security in the face of climate change.

Furthermore, understanding the cross-talk of response mechanisms 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 the cross-talk, thereby enabling the plant to withstand multiple 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. Moreover, the study of gene regulatory networks and the identification of transcription factors involved in the regulation of these stress-responsive genes will provide a comprehensive understanding of the molecular mechanisms underlying abiotic stress responses in rice. Therefore, 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, thus ensuring sustainable rice production and global food security.

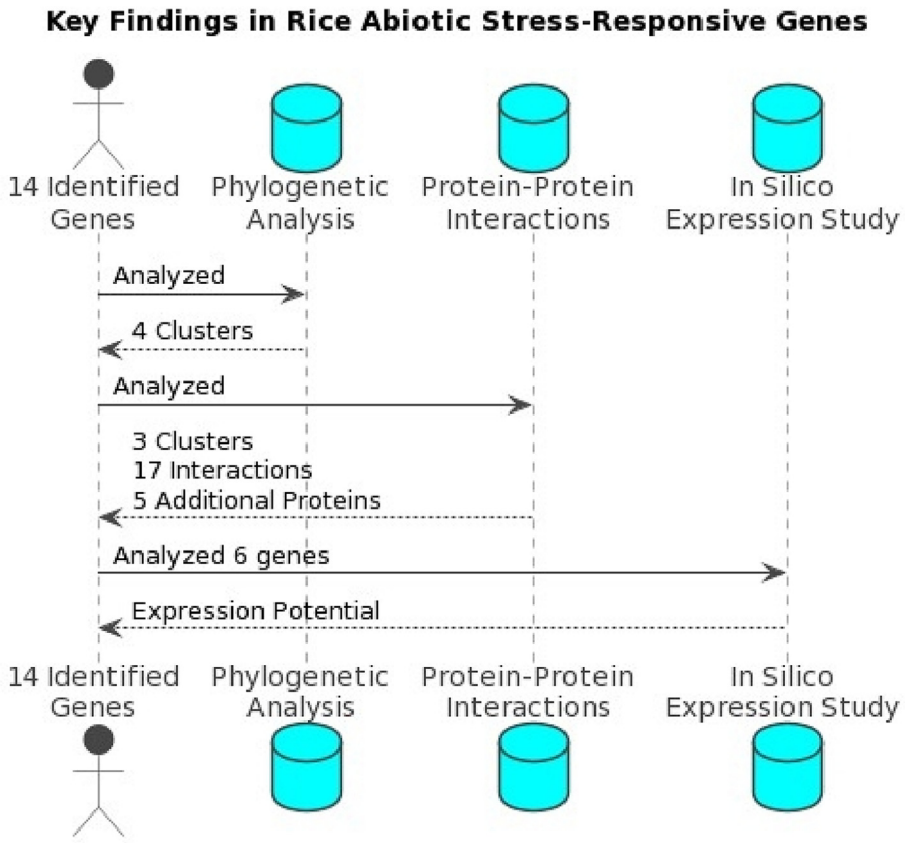

Fig. 8. Key findings of the study conducted on the genes playing significant regulatory roles in abiotic stress responses in *O. Sativa* (Source: Author's creation with PlantText UML Editor).

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 *O. sativa*. The expression profiles of these genes were found to vary across different stages of the rice life cycle, such as young and mature leaves, young inflorescence, and seed development stages. These results indicate that these genes participate in interconnected cascades throughout the plant's life cycle, providing protection against abiotic stresses. The cross-talk between response mechanisms of these abiotic-stress responsive genes was also corroborated by phylogenetic analysis, protein–protein interaction, and gene co-expression studies. Therefore, using these genes as targets for developing gene-based molecular markers could enable the breeding of rice cultivars with enhanced resistance to challenging environmental conditions. Furthermore, these findings have the potential to contribute to the successful application of computational biology in plant breeding by reducing the expense, complexity, and time required for traditional biological studies.

CRediT authorship contribution statement

Sandip Debnath: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Shaik Aisha:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing - original draft, Writing - review & editing. **Ayushman Malakar:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing - original draft, Writing - review & editing. Kahkashan Perveen: Manuscript preparation, reviewing, and editing. **Alanoud Alfagham:** Manuscript preparation, reviewing, and editing. **Mehrun NishaKhanam:** Manuscript preparation, reviewing, and editing. **Biswajit Pramanik:** Manuscript preparation, reviewing, and editing. **Ahmed Mohammed:** Manuscript preparation, reviewing, and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2023.102786.

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