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Responses of rice plant to multiple abiotic stresses revealed by transcriptome meta-analysis and identification of novel genetic factors

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Plant responses to abiotic stresses have a complex polygenic nature including main and epistatic genetic factors. Several tolerant rice varieties were subjected to drought, salt and cold stresses and their transcriptomic responses were evaluated using affymetrix probe set. Meta-analysis of standardized microarray data was conducted to identify specific and common genes responding to multiple abiotic stresses. 375 and 298 genes were up- and downregulated under drought stress, 281 and 313 genes were up- and downregulated under salt stress, and 1,273 and 2,996 genes were up- and downregulated under cold stress. In addition to many specific genes for each stress condition, common genes were identified for response to drought and salt (n=91), drought and cold (n=121), and salt and cold (n=108), while 14 genes were common for response to all 3 stresses. 12 out of 14 genes were downregulated under the 3 stresses; however, only 2 upregulated genes (including an auxin-responsive protein and a LRR protein) were common among the 3 stresses. One of the common downregulated genes is a non-ABC transporter belonging to proton-dependent oligopeptide transport (POT) family protein which is novel and has a vital role in uptake of nutrients, particularly nitrate, and in recognizing plant defense compounds and hormones. In addition, two other non-ABC transporters (*OsAAP7C* and *OsGT1*) were identified which were downregulated under drought, salinity and cold stresses. This finding can explain why and how the uptake of necessary nutrients for growth and development of plants decreases under these oxidative stresses. Another novel downregulated gene under the 3 stresses is a TraB-related protein with vital role for normal mitochondrial function. These results open new insights into genetic engineering and molecular breeding of plants for tolerance to abiotic stresses.

Keywords Cold, Drought, Microarray, Proton-dependent oligopeptide transport, Salt, TraB

Abiotic stressors are thought to be serious agricultural challenges because they significantly reduce crop growth and productivity. Since plants are sessile and must endure harsh environmental conditions, they have evolved various responses to adapt[1,2]. Plants possess sophisticated sensory systems that detect subtle changes in growing conditions and trigger signal transduction cascades, leading to the activation of stress-responsive genes and subsequent physiological and biochemical changes[3–5]. Understanding how plants respond to these challenges and the underlying mechanisms of stress tolerance can contribute to increasing global food supply.

Over half of the world's population depends on rice (*Oryza sativa* L.) as a staple crop, yet it is susceptible to several abiotic stresses, including salt, drought, and cold[6]. With the projected increase in the frequency, intensity, and duration of these stresses due to climate change, rice productivity and global food security are at risk[7]. Many resources reported considerable damage of abiotic stresses to rice yield. For instance, severe drought stress in the flowering stage can cause more than 70% decrease in yield[8].

Abiotic stresses induce the production of reactive oxygen species (ROS) in plants, resulting in oxidative damage and reduced growth and yield. These ROS, including superoxide, OH^- radicals, H_2O_2, and singlet oxygen, accumulate in cells due to various stress factors, disrupting cellular homeostasis[9]. However, plants maintain a

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baseline ROS level essential for regulating vital cellular processes and metabolic pathways. To counteract ROS accumulation, plants have developed intricate antioxidative defense mechanisms, including enzymatic processes involving superoxide dismutase, catalase, peroxidase, and glutathione reductase, which help prevent or repair oxidative damage caused by stress[10,11].

Abiotic stress significantly impacts plant growth and productivity. Salt stress, for instance, not only affects the absorption of essential nutrients but also interacts with various metabolic processes in plants. Moreover, temperature fluctuations during critical growth stages can profoundly influence crop yield, especially in rice[12–16]. On the other hand, the genetic regulation of stress responses offers potential strategies for enhancing plant resilience. On the other hand, the genetic regulation of stress responses offers potential strategies for enhancing plant resilience. The core components of strigolactone (SL) biosynthesis and signaling, MAX1–MAX4, are crucial for enhancing freezing tolerance in plants. Additionally, C-REPEAT BINDING FACTOR/DEHYDRATION RESPONSE ELEMENT BINDING FACTOR 1 (CBF/DREB1) transcription factors play essential roles in cold acclimation, with the F-box protein MAX2 facilitating the degradation of WRKY41 to enhance freezing tolerance[17,18]. Moreover, the involvement of genes like DEAR4 in stress responses is evident, as overexpression of DEAR4 leads to reduced seed germination rates under ABA and salt stress, along with decreased drought tolerance[19]. Likewise, in lettuce, RD29A and RD29B respond differently to various stresses, with RD29A being more responsive to drought and cold stresses, while RD29B is highly responsive to salt stress[20,21]. Furthermore, carotenoid cleavage oxygenases (CCOs), including NCEDs (9-Cis-epoxycarotenoid dioxygenases), CCDs (Carotenoid cleavage dioxygenases), and CCD-like genes, are vital for rice's response to stress[22]. Transcriptome analysis highlights the upregulation of OsNCED6 and OsNCED10 during abiotic stress, suggesting their potential for stress-resistant breeding[23]. Understanding the intricate genetic mechanisms underlying stress responses, such as those involving CBF/DREB1 transcription factors, WRKY41, DEAR4, RD29A, RD29B, and CCOs, provides insights into developing stress-resistant crop varieties. This knowledge can significantly contribute to improving agricultural productivity in the face of challenging environmental conditions.

Rice is susceptible to various abiotic and biotic stresses, often occurring simultaneously within a single cropping season, significantly impacting rice growth and yield. To address this, rice mega varieties have been introduced, which exhibit tolerance to multiple stresses[24–26]. Yadav et al.[25] developed the rice breeding line IR 91,648-B-1-B-3-1 using a funnel mating design to incorporate targeted QTLs and genes. Jamaloddin et al.[26] evaluated gene-pyramided rice lines TH-625-159 and TH-625-491, both showing resistance to blast and bacterial blight (BB) across multiple locations. These lines have the potential to become mega varieties for different agro-climatic zones and valuable resources in pre-breeding rice research.

Meta-analysis, a statistical technique combining information from various genomic studies, offers a comprehensive understanding of the problem being studied[27]. It helps identify candidate genes showing differential expression across studies[28] and elucidates the molecular mechanisms and crosstalk between individual and combined abiotic stresses by comparing transcriptome data[29]. Meta-analyses are particularly suitable for such comparisons, offering greater power to discover genes with consistent responses that may be overlooked in individual studies or vary among them. For instance, a meta-analysis on drought stress response in cereal crops identified 69 conserved drought tolerant-related (CDT) genes, with 20 of these genes being potential novel candidates for drought tolerance[30]. Meta-analysis of transcriptome studies reveals key regulatory hub genes, such as NSP2, DRE1D, ERF61, CDF1, and TLP3 for drought and TLP1, TLP, ERF109, ELF4, and ATHB7 for salt stress, which are associated with significant differential expression and offer potential candidate genes for improving *Gossypium hirsutum* genotype selection against drought and salt stress conditions. In this study, we explored the expression pattern of rice genes in response to drought, salt, and cold stresses using a large-scale meta-analysis based on publicly accessible microarray data. This study aims to open new insights into genetic engineering and molecular breeding of plants for tolerance to abiotic stresses.

Material and method

Microarray data

The Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) was used to download microarray datasets from the Affymetrix platform Rice Genome Array, which were obtained from rice (*Oryza sativa*) seedlings grown under normal conditions and subjected to different stresses including drought, salinity and cold. Totally, ten GEO datasets were used in the study (Table 1). The expression data sets were processed separately using different methods depending on the platform used[31]. The raw data was preprocessed with quantile normalization and Robust Multi-Array Average background correction, and then probes with low intensity and non-informative data were removed based on the program's standard settings. The probes were also transformed to their corresponding genes.

Differential gene expression and meta-analysis

Gene expression analysis was conducted for three treatment conditions versus normal condition. R specific pakages including edgeR and Limma were used for detection of differentially expressed genes (DEGs). A linear model was fitted to the data and a simple empirical Bayes model was used to adjust the standard errors. For each contrast in every gene, moderated t-statistic and log-odds of differential expression were calculated. Genes with a q-value cut-off of ≤ 0.05 and $-1 \geq \log2$ fold change ≥ 1 were identified as differentially expressed genes (DEG) in each of the 3 stresses.

RNA-seq profiling of DEGs under three stresses

To validate the meta-analysis of microarray data, three independent RNA-seq data sets (including GSE8081: drought, PRJEB4671: salt, PRJNA506503: cold) were retrieved from GEO/SRA data bases and subjected to DEG

GEO ID	Sample count (treatment: control)	Platform	Tissue	Type of stress
GSE41647	6:3	Affymetrix Rice Genome Array	Young seedlings	Drought
GSE21651	2:2	Affymetrix Rice Genome Array	Young seedlings	Drought
GSE24048	6:6	Affymetrix Rice Genome Array	Seedlings	Drought
GSE26280	9:9	Affymetrix Rice Genome Array	Seedlings	Drought
GSE25176	4:4	Affymetrix Rice Genome Array	Seedlings	Drought
GSE41650	12:6	Affymetrix Rice Genome Array	Young seedlings	Salinity
GSE21651	2:2	Affymetrix Rice Genome Array	Young seedlings	Salinity
GSE16108	4:4	Affymetrix Rice Genome Array	Seedlings	Salinity
GSE37940	17:3	Affymetrix Rice Genome Array	Seedlings	Cold
GSE38023	12:3	Affymetrix Rice Genome Array	Seedlings	Cold

Table 1. Characteristics of the individual studies included in study.

Condition	Downregulated	Upregulated	Unchanged
Drought	298	375	56,585
Salt	313	281	56,664
Cold	2996	1273	52,989

Table 2. The number of down- and upregulated genes (DEGs) under three stresses in rice microarray data.

analysis using edgeR and Limma packages in R. The gene expressions with $|\log2 \text{ fold change}| \geq 1$ and FDR < 0.05 were considered significant.

Validation of candidate genes by qRT-PCR

In order to validate the results of identifying candidate genes by meta-analysis, the genes were subjected to real-time PCR under drought stress in rice cultivar Neda. This cultivar is a high-yielding breeding line with relatively low water demand that is cultivated in Northern Iran. The seeds of this cultivar were obtained from Rice Research Institute of Iran. All the plant experiments/protocols were performed with relevant institutional, national, and international guidelines and legislation. Relevant permissions were obtained for plant sample collection. The plants were raised up to 5–6 leaf stage in hydroponic conditions with 4 replicates and the drought stress was treated for 6 h via imposing the roots to air and then leaf samples were collected under both normal and stress conditions. In addition, another experiment was conducted for examining salinity response, so that seedlings (at 5–6 leaf stage) were imposed to salinity stress (150 mM NaCl) for 24 h and then the leaf samples (in 3 replicates) were collected. Furthermore, in another experiment, the cold response of rice was examined. For this, the seedlings (at 5–6 leaf stage) were subjected to low temperature (4 °C) for 24 h and then leaf samples (in 3 replicates) were collected for RNA extraction. RNA was extracted from samples using QIAGENE extraction kit. The cDNA was synthesized using a commercial kit (ParsTous cDNA synthesis kit, Iran). Primer pairs for 2 and 6 up- and downregulated DEGs were designed using Primer 3.0 online tool (https://www.primer3plus.com/index.html) (Supplementary Table S1). The *OsActin* gene was used as housekeeping reference gene. Real-time qRT-PCR was conducted on cDNA samples and the statistical analysis of Ct values of selected genes was done using REST software[32]. The relative expression in stressful condition vs. normal condition was calculated using $2^{-\Delta\Delta Ct}$ method[33].

Network analysis

Protein-protein Interaction (PPI) network was drawn by Cytoscape software ver. 3.9.1 and information derived from the website STRING (https://string-db.org). The minimum required interaction score was set to 0.40 for more confidence.

Results

Differential gene expression analysis

Meta-analysis was used to find out the most probable genes responsible for tolerance of rice to drought, salinity and cold stresses (Table 1). The analysis was conducted on more than 57,000 probes set, and 2-sided clustering showed adequate resolution between samples and genes under different stress conditions, especially under drought stress (Supplementary Fig. S1A–C). Meta-analysis of standardized microarray data was conducted to identify specific and common genes responding to multiple abiotic stresses. In the DEG analysis it was obtained 3,607 downregulated and 1,929 upregulated genes under all conditions (Table 2). Drought stress resulted in up- and downregulation of 375 and 298 genes, respectively. 281 and 313 genes were up- and downregulated under salt stress, and 1,273 and 2,996 genes were up- and downregulated under cold stress) Table 2; Supplementary Table S2. The identified DEGs showed a discrete relative expression pattern under 3 stresses as revealed by Volcano plot analysis (Fig. 1A–C). Ten top specific DEGs for each stress condition were depicted in Table 3. As seen, 7 genes (including *OsEnS-22*, *OsPP2C51*, *OsCAF1A*, *BZIP12*, *OsRLCK243* and two *DUF581*) were

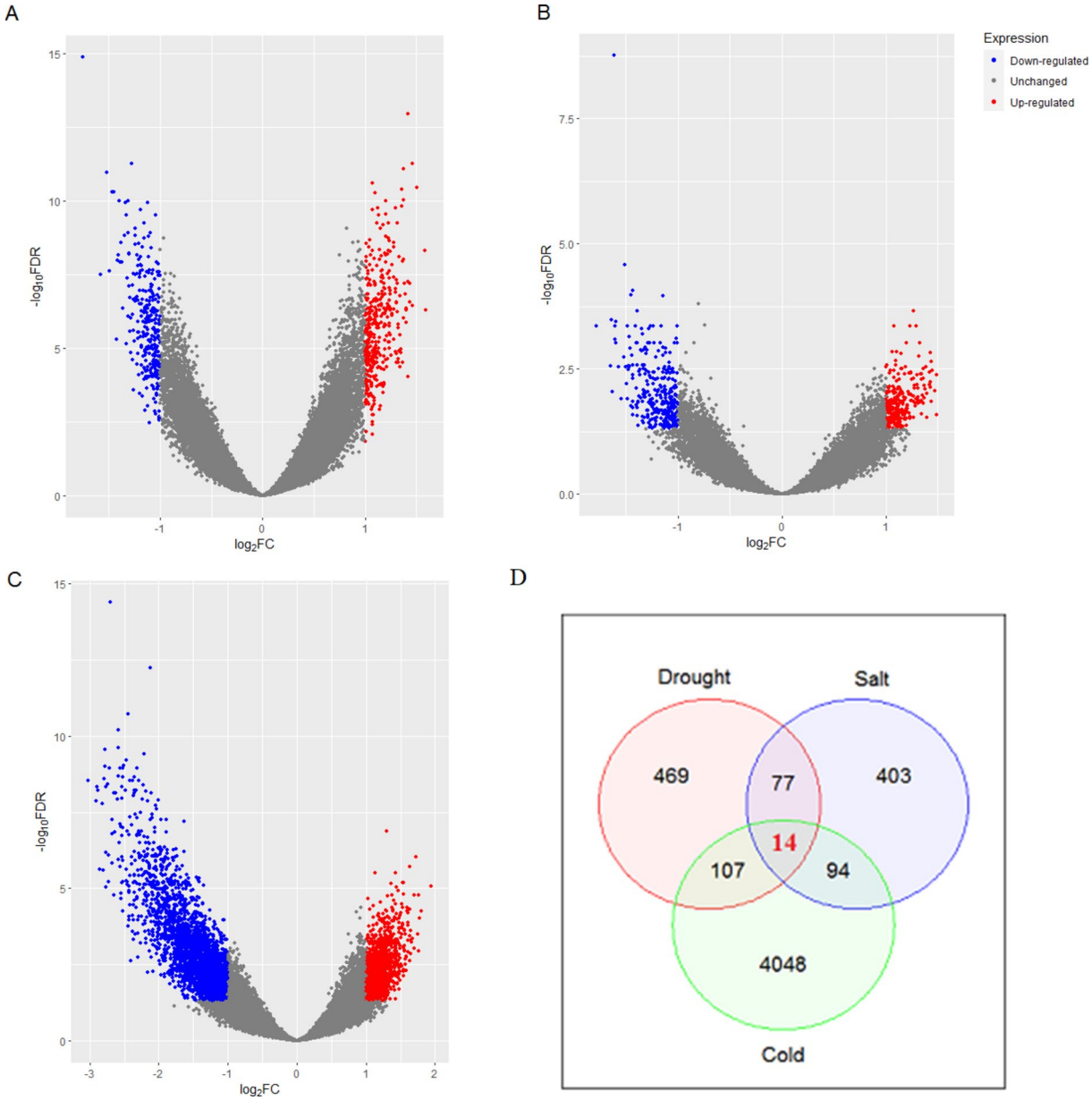

Fig. 1. Volcano plots showing differential expression under 3 stresses. (**A**) drought, (**B**) Salinity, (**C**) cold. (**D**) Venn diagram showing the number of unique and common DEGs between 3 stresses.

upregulated in drought stress; 3 genes (*OsRCCR1*, *OsLTPd1* and *OsLTPd6*) were upregulated in salt stress, and 3 genes (*CDKC*, *OSNPB_030848600* and *OsCML22*) were upregulated in cold stress.

Common genes were identified for response to drought and salt (91), drought and cold (121), and salt and cold (108), while 14 genes were common for response to all 3 stresses (Fig. 1D). Heatmap analysis indicated the discriminated expression pattern of all DEGs under the studied stress conditions (Fig. 2A). The meta-analysis revealed that 12 out of 14 common genes were downregulated, and only 2 genes were upregulated (Fig. 2B). Notably, two genes *Os01g0741900* (*IAA6*) and *Os03g0221800* (a *LRR-like*) were upregulated, and three non-ABC transporters including *Os10g0111700*, *Os02g0102200* and *Os06g0125500* (*POT*, *OsAAP7C* and *OsGT1*, respectively) were downregulated under all three stresses.

RNA-seq profiling of common DEGs under three stresses

To further validate the DEG identification obtained by meta-analysis on microarray data, the RNA-seq profiling of the 14 common DEGs was conducted. In overall a good consistency existed between the two data sets, particularly a high consistency detected between DEGs under drought stress (Table 4). The correlation between results of two assays was 0.919 (drought), 0.843 (salt) and 0.873 (cold). Among 14 common DEGs, *IAA6* (*Os01g0741900*) which is an auxin-responsive protein[34] was significantly upregulated under severe drought, salt and cold stresses. Additionally, a LRR protein coding gene (*Os03g0221800*), was significantly upregulated

Stress	Gene ID	Gene symbol	Description	LogFC
Drought	*Os01g0332800*	*OsSCP3*	Putative serine carboxypeptidase homologue	− 1.75
	Os01g0950900	*OsEnS-22*	Early-responsive to dehydration protein-related	1.42
	Os01g0801901	*OsbZIP07*	bZIP transcription factor domain containing protein	− 1.28
	Os05g0572700	*OsPP2C51*	Protein phosphatase 2 C	1.38
	Os08g0159500	*OsLSD1*	Zinc finger domain containing protein	− 1.52
	Os08g0440300	*OsCAF1A*	CAF1 family ribonuclease containing protein	1.03
	Os06g0223700	*DUF581*	DUF581 domain containing protein	1.51
	Os01g0867300	*BZIP12*	bZIP transcription factor domain containing protein	1.36
	Os07g0695300	*OsRLCK243*	Protein kinase APK1B	1.10
	Os11g0659200	*DUF581*	DUF581 domain containing protein	1.38
Salt	*Os12g0260500*	*Os12g0260500*	Sex determination protein tasselseed-2	− 1.61
	Os05g0213900	*Os05g0213900*	Clumping factor A precursor	− 1.43
	Os10g0389200	*OsRCCR1*	Red chlorophyll catabolite reductase	1.43
	Os01g0801901	*OsbZIP07*	bZIP transcription factor domain containing protein	− 1.15
	Os08g0174500	*HD5*	Histone-like transcription factor	− 1.39
	Os04g0415800	*OsLTPd6*	Protease inhibitor/seed storage/LTP family protein	127
	Os10g0111700	*Os10g0111700*	Peptide transporter PTR2	− 1.64
	Os01g0914100	*OsLTPd1*	Protease inhibitor/seed storage/LTP family protein	1.19
	Os06g0228200	*LSI6*	Aquaporin protein	− 1.16
	Os02g0112100	*NRT2.1*	Transporter, major facilitator family	− 1.26
Cold	*Os02g0525100*	*Os02g0525100*	Expressed protein	− 2.69
	Os06g0269200	*Os06g0269200*	Integral membrane protein	− 2.12
	Os05g0389700	*CDKC*	Cyclin-dependent kinase C-1	2.34
	Os10g0496900	*FGL*	Oxidoreductase, short chain Dehydrogenase/reductase family	− 2.58
	Os03g0848600	*OSNPB_030848600*	Nitrate-induced NOI protein	2.63
	Os09g0451500	*PDIL2*	Protein disulfide isomerase PDIL2-3	− 2.58
	Os02g0732900	*OsISC41*	APO protein 2	− 2.78
	Os04g0492800	*OsCML22*	Calmodulin-related calcium sensor protein	2.13
	Os06g0132400	*ChlM*	Magnesium-protoporphyrin O-methyltransferase	− 2.21
	Os12g0569500	*Os12g0569500*	Osmotin	− 2.78

Table 3. Ten top specific annotated DEGs for each stress condition in rice seedlings identified using microarray data analysis. Significant relative expression values (in view of LogFC) are presented in last column.

only under drought and cold stresses. However, eight genes including *Os03g0221700* (serine/threonine protein kinase), *Os01g0619900* (*OsTCL2*), *Os10g0111700* (POT family protein), *Os04g0387900* (AT.I.24-6 protein), *Os05g0481100*, *Os04g0531750*, *Os06g0125500* (*OsGT1*) and *Os08g0545700* (TraB protein-related) are among the downregulated genes under all 3 stresses. A non-ABC transporter (*OsAAP7C*) and *Os12g0438400* (hypothetical protein) were downregulated under drought and cold stresses. Two other genes (*Os08g0174500* and *Os12g0583500*) showed downregulation under both drought and salt stresses.

Validation of meta-analysis results by qRT-PCR

The expression of selected genes of up- and downregulated classes that were identified in meta-analysis, were examined using real-time qRT-PCR, and the obtained results are depicted in Fig. 3. As seen in the figure, both direction and magnitude of expression of the studied genes are comparable to meta-analysis results. Two upregulated DEGs (*IAA6* and *LRR-like*) showed 4.4 and 2.9 fold changes under drought stress, and 3.1 and 1.9 fold changes under salinity stress, and 1.6 and 2.3 fold changes under cold stress, respectively. Six downregulated DEGs showed − 2.8 to − 4.3 fold changes under drought stress. Similarly, the DEGs showed − 3.9 to − 7.0, and − 2.0 to − 4.6 fold changes, respectively, under salinity and cold stresses. With these results, real-time qRT-PCR confirmed the DEGs identified by meta-analysis.

Protein–protein interaction (PPI) network of meta DEGs

The predicted protein-protein interaction network of *Os10g0111700* (POT protein, which is a non-ABC crucial peptide/nutrient transporter) and *Os08g0545700* (TraB protein, which plays a crucial role in autophagic degradation and is vital for the recycling process) was generated using the STRING database (Fig. 4). It is predicted that *Os10g0111700* interacts with manganese-dependent ADP-ribose/CDP-alcohol diphosphatase (*OsJ_25650*) and calcineurin-like phosphoesterase (*OsJ_34100*). On the other hand, the protein of the downregulated gene *OS08g0545700* shows more diverse interactions. The most confident interaction is between *Os08g0545700* and molybdenum cofactor sulfurase 3 (*Os08g0545000*). Additionally, inositol-1-monophosphatase, transcription

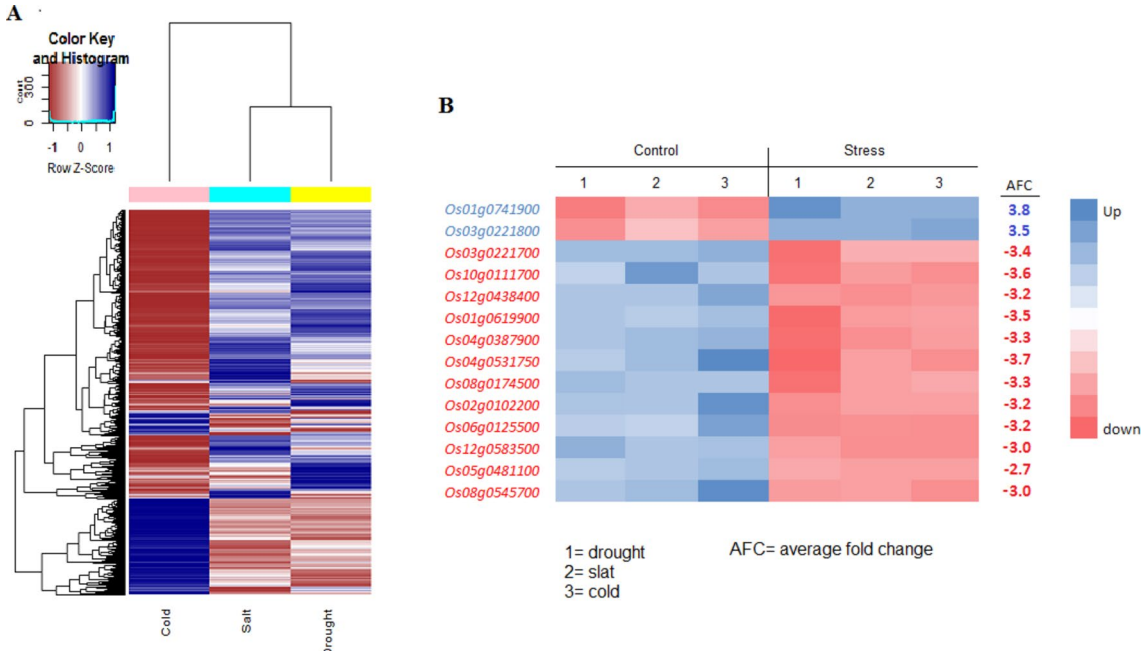

Fig. 2. (**A**) Heat map of all identified DEGs in the study under 3 stresses. (**B**) A heat map showing expression pattern of 14 common DEGs between 3 stresses. *AFC*: average fold change under stress.

	RAP_ID	Name	Description	Drought (GSE8081)	Salt (PRJEB4671)	Cold (PRJNA506503)
				Severe stress	150 mM	4 °C
Upregulated DEGs	*Os01g0741900*	*IAA6*	Auxin-responsive protein, drought tolerance, control of tiller outgrowth	**2.37**	**1.61**	**1.57**
	Os03g0221800	*LRR-like*	Similar to leucine rich repeat family protein	**1.39**	0.92	**1.45**
Downregulated DEGs	*Os03g0221700*	*Os03g0221700*	Serine/threonine protein kinase-related domain containing protein	*−2.43*	*−1.73*	*−2.79*
	Os10g0111700	*POT*	Similar to POT family protein, expressed	*−3.03*	*−1.49*	*−1.53*
	Os12g0438400	*Os12g0438400*	Hypothetical conserved gene	*−2.62*	0.17	*−1.17*
	Os01g0619900	*OsTCL2*	Similar to DNA binding	*−4.38*	*−1.36*	*−1.59*
	Os04g0387900	*Os04g0387900*	Similar to AT.I.24-6 protein	*−3.63*	*−1.9*	*−1.57*
	Os04g0531750	*Os04g0531750*	Similar to OSIGBa0125M19.13 protein	*−3.14*	*−1.09*	*−1.11*
	Os08g0174500	*HD5*	Putative HAP3 subunit of the CCAAT box-binding transcription factor, flowering time, short-day promotion, long-day repression	*−2.74*	*−1.42*	−0.16
	Os02g0102200	*OsAAP7C*	Similar to transmembrane amino acid transporter protein	*−4.45*	−0.23	*−1.3*
	Os06g0125500	*OsGT1*	Similar to oligopeptide transporter 9	*−1.99*	*−1.38*	*−1.69*
	Os12g0583500	*Os12g0583500*	BTB domain containing protein	*−2.88*	*−1.34*	−0.64
	Os05g0481100	*Os05g0481100*	Similar to cDNA clone: J013034D11	*−3.25*	*−1.57*	*−1.28*
	Os08g0545700	*TraB-rlated*	Similar to TraB protein-related	*−1.95*	*−1.13*	*−1.32*

Table 4. RNA-seq profiling of DEGs under three stresses in rice supported by public results, retrieved from GEO/SRA databases. Significant up/dowregulated DEGs in view of log2 fold change are presented in bold and italics colors, respectively.

initiation factor IIA subunit 2, and cytochrome P450 703A2 are predicted to interact with *Os08g0545700*. However, the database could not find any connection between these two downregulated genes (Fig. 4).

Discussion

As shown in this microarray-based meta-analysis, *IAA6* and an LRR protein were upregulated under three abiotic stresses including drought, salinity and cold. Aux/IAA genes are known to play a crucial role in the tolerance of plants to abiotic stresses[35]. Studies have reported differential expression of many Aux/IAA genes under stress conditions such as salinity and cold in soybean[36]. A transcriptomic profiling study conducted on rice grown under abiotic stress showed that several OsIAA proteins, including OsIAA9 and OsIAA20 (XP_015641983.1), were significantly upregulated under salt and drought stresses[37]. Recent research has revealed that auxin-sensitive Aux/IAA proteins such as IAA5, IAA6, and IAA19 regulated aliphatic GLS levels in Arabidopsis plants

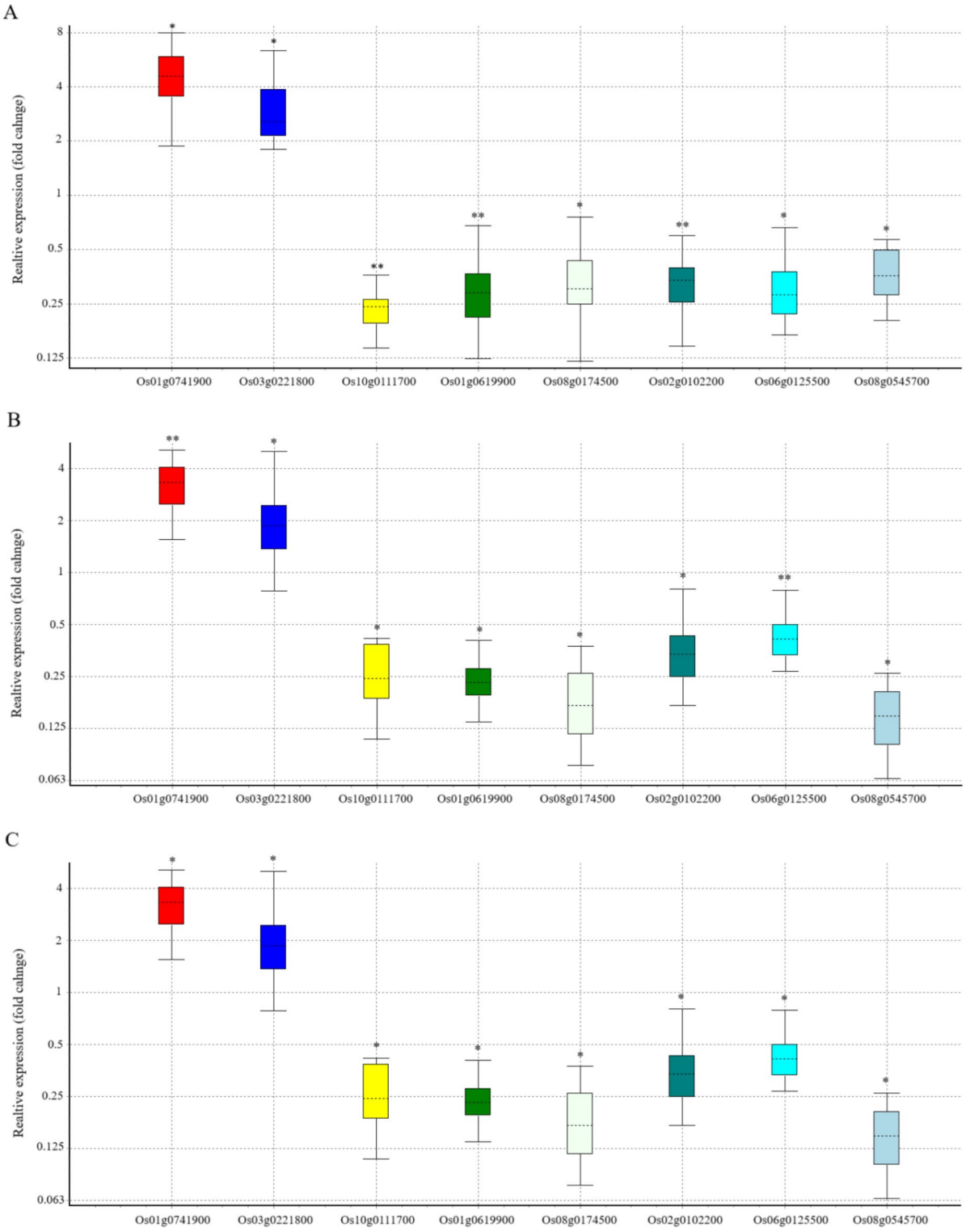

Fig. 3. Relative expression results of 2 upregulated and 6 downregulated DEGs under abiotic stresses in rice seedlings. A: under drought stress; B: under salinity stress. C: under cold stress. * and ** represent the significance of relative expression at $p \leq 0.05$ and $p \leq 0.01$ levels, respectively.

exposed to drought conditions, and loss of IAA5/6/19 resulted in decreased drought tolerance[38]. Additionally, these IAAs were found to be involved in stomatal regulation and drought stress response. The OsIAA20 gene was also found to display increased expression levels under drought stress, salt stress, and ABA treatment[39]. A study found that drought stress induces the rice gene *OsIAA6*, which plays a key role in the response to drought stress and the regulation of tiller growth. Overexpression of *OsIAA6* in transgenic rice plants improves drought

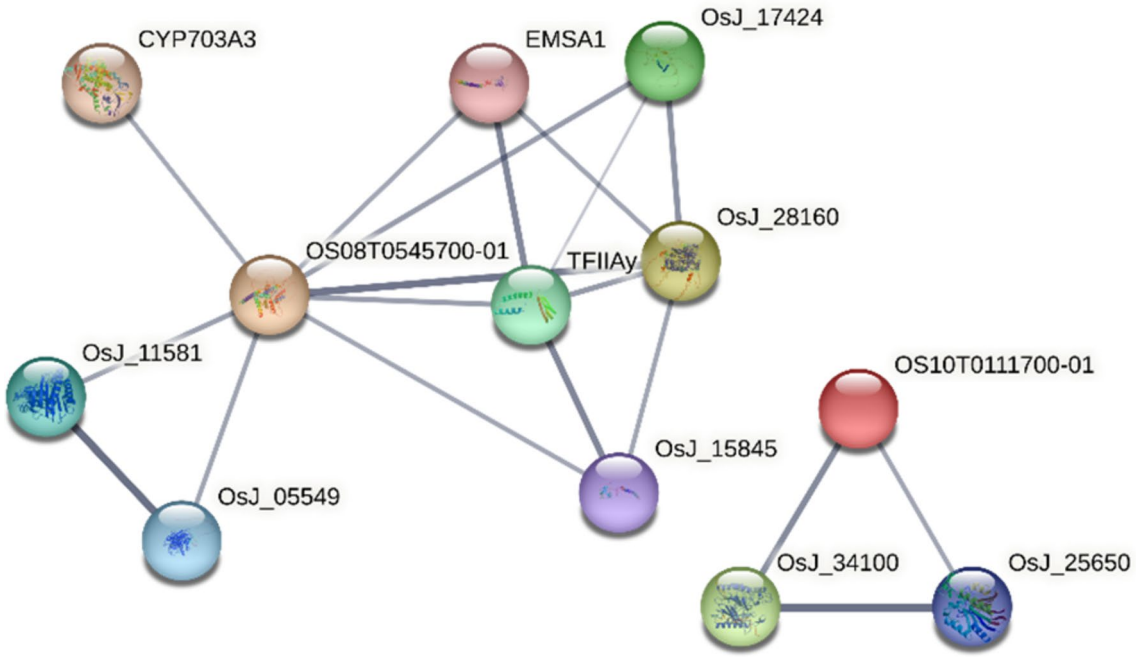

Fig. 4. Predicted protein-protein interaction network of *Os10g0111700* (POT protein) and *Os08g0545700* (TraB protein). The first network contains 8 nodes, and the second contains 3 nodes. Line thickness indicates the strength of data support.

tolerance by regulating auxin biosynthesis genes. The gene is specifically expressed in the axillary meristem of the basal stem, which gives rise to tillers. A knock-down mutation of *OsIAA6* resulted in abnormal tiller outgrowth, suggesting that *OsIAA6* is involved in the control of tiller growth through the regulation of *OsPIN1* and *OsTB1*[40]. Many studies have demonstrated the importance of auxin biosynthesis genes in improving drought tolerance. Overexpression of *OsIAA6* and *OsABF1/57* in rice resulted in drought tolerance[40,41]. In tobacco, overexpression of iiaH and iiaM genes, involved in auxin biosynthesis, led to improved heat tolerance and water retention capacity. These findings suggest that auxin biosynthesis plays a crucial role in enhancing plant drought tolerance[42].

LRR (leucine-rich repeat) proteins play a crucial role in the response of plants to abiotic stress conditions. These proteins are involved in various signaling pathways that regulate plant growth, development, and stress responses. LRR proteins act as receptors or co-receptors, perceiving external signals and transmitting them to downstream signaling components[43]. During abiotic stress, such as drought, salinity, extreme temperatures, or oxidative stress, LRR proteins are known to participate in stress perception and signal transduction. They can activate specific defense pathways, regulate gene expression, and modulate physiological and biochemical responses to help plants adapt and survive under adverse conditions[44]. The specific mechanisms through which LRR proteins function in abiotic stress responses can vary depending on the plant species and the nature of the stress. Some LRR proteins are involved in the perception of stress signals and the activation of defense-related genes, while others participate in stress tolerance by regulating ion homeostasis, osmotic adjustment, or antioxidant defense systems[45,46].

Our meta-analysis on microarray data showed that *Os10g0111700* (POT family protein), which is a non-ABC crucial peptide/nutrient transporter, was downregulated under three abiotic stresses. Furthermore, two additional non-ABC transports (viz. *OsGT1* and *OsAAP7C*) that are involved in amino acid/oligopeptide transport, were downregulated under 3 stresses that their expression was validated under drought/salt/cold by RNAseq analysis (Table 4). The import of nutrients from outside the cell is crucial for the survival of all living organisms. While some nutrients can cross the cellular membrane by passive diffusion, others require specific transport proteins for facilitated transport. These transport processes can be powered by either primary or secondary energy sources. The uptake of nitrogen from the surroundings through the peptide transport is a significant pathway for cells[47]. Disruption of the absorption and transfer of nutrients, especially nitrogen (which is crucial for synthesis of amino acids and peptides) into cells and tissues will reduce cell growth and proliferation. The downregulation of the abovementioned genes under abiotic stresses can explain why and how the leaves of stressed plants turn yellow. The POTs, also known as PTR2s uses the electrochemical proton gradient for the uptake of their substrate. The proton-dependent oligopeptide transporters from different kingdoms of life can generally be classified into two groups: the PEPT-like POTs and the PHTs. The PEPT1-like POTs are the most abundant and can be found in bacteria, fungi, protists, and plants. The POT family is included in the major facilitator superfamily (MFS) in the Transporter Classification Database[48]. The PTR system in *S. cerevisiae* is composed of three genes that are mutually dependent. Ptr2, the most well-known member of the PTR family, is encoded by *PTR2* and is an integral membrane transporter. Isoforms of Ptr2 have also been found

in *Candida albicans* and *A. thaliana*[49,50]. The ability to transport other nitrogen sources, for example, by nitrate permease *AtCHL1* in *(A) thaliana*, has been acquired by members of the POT family in plants[51]. The NPF family, a vital group of NRTs in plants, is not only regulated by nitrate but also by other nutrients, playing roles in various signaling pathways. In *Brassica napus*, *BnaNPFs* respond to nitrogen (N), phosphorus (P), potassium (K) stresses, and NH_4^+ toxicity in both leaves and roots, suggesting their involvement in nutrient sensing and crosstalk. Moreover, *BnaA05.NPF1;1* emerges as a key regulator under diverse nutrient conditions, including NH_4^+ toxicity, highlighting its role in *(B) napus* resistance[52]. In rice, maize, sorghum, peanut, soybean, and Arabidopsis, PTR genes (part of the NPF family) are involved in N uptake and utilization, especially under low N conditions. They are distributed across various genomic regions and may interact to regulate N metabolism, particularly through hub genes like protein kinases[53]. In cotton, *GhNPF* genes show differential expression under abiotic stresses, with *GhNPF8* exhibiting varied responses to cold, heat, salt, and drought stresses, suggesting their involvement in stress response mechanisms[3]. This research provides insights into the role of NPF genes in nutrient sensing, crosstalk and stress responses across different plant species[54].

Nitrogen is essential for plant growth and productivity, and rice growth relies heavily on the activity of PTR/NRT1 transporters[55]. Fan et al.[56] suggest that upregulating the expression of *OsPTR6* could enhance rice growth by increasing the expression of ammonium transporters and the activity of glutamine synthetase (GSA). When the *OsPTR6* gene was overexpressed in the Nipponbare rice cultivar, it led to increased rice growth via increased ammonium transporter expression and GSA activity[56]. The rice nitrate transporter 1/peptide transporter family 8.1 (*OsNPF8.1*) is an important peptide transporter that plays a significant role in the rebalancing of plant growth and tolerance to abiotic stresses like N deficiency, salt, drought, and ABA by facilitating stress-induced organic N transportation. Its activity helps in redistributing organic N during stress conditions, resulting in changes in water potential and proline levels that aid in stress tolerance[57].

The meta-analysis of multiple experiments examining plant transcriptional responses to drought, salinity and cold stresses revealed that *Os10g0111700*, a member of the POT family of proteins, was consistently downregulated across all conditions. This finding strongly suggests that the suppression of this transporter protein is a key response of rice to oxidative stress. The recently solved crystal structure of *NRT1.1* indicates that *Thr101* is located on 3rd transmembrane motif (TM3) and faces a hydrophobic pocket created by residues on TM2 and TM4, which make up the extracellular and intracellular gates, respectively. It is suggested that phosphorylation of this residue would interfere with the helix packing and allow *NRT1.1* to cycle faster, potentially lowering the energy barrier for the return step in the transport cycle[58]. This disruption may also trigger a transition from dimer to monomer in the membrane, which could subsequently impact the regulation of the nitrate signaling pathway that activates the high-affinity nitrate uptake system[59,60].

The interactions between the endoplasmic reticulum (ER) and mitochondria are crucial for mitochondrial division and the exchange of signals and substrates between these compartments. While animal proteins involved in this process have been extensively studied, only a few plant proteins have been identified as regulators of ER-mitochondrial interactions[61–63]. Li et al.[64] identified the evolutionarily conserved *TraB* family proteins as important regulators of two pathways: ER-mitochondrial interactions and mitophagy, the degradation of damaged mitochondria. They indicated that TRB1 has a critical role in maintaining mitochondrial homeostasis, and its dysfunction leads to an accumulation of damaged mitochondria and impaired mitochondrial function. *TraB* is a DNA translocase similar to *FtsK* and is accountable for the transfer of conjugative plasmids in Streptomyces. Unlike other conjugative systems that rely on a type IV secretion system, the transfer of the plasmid as double-stranded DNA in Streptomyces is only dependent on the TraB protein[65]. Researchers have shown that TraB-family proteins, which act as mitophagy receptors, have been identified in plants. *TRB1* and *TRB2* play a crucial role in mitophagy by interacting with ATG8 to mark damaged mitochondria for autophagic degradation and are crucial for the recycling process. Unlike animal and yeast cells, plant cells have chloroplasts that can produce ATP, so a mild disturbance in mitochondrial function, as seen in the *trb1trb2* double mutant, may not cause a significant growth defect under normal circumstances[65]. It is reported that, the accumulation of *TRAB-1* transcript and protein was observed to be higher in the tolerant rice cultivar Nonabokra when subjected to cadmium and salt stresses[66,67]. Our analysis suggests that the downregulation of TraB family proteins during periods of stress is a significant consequence that causes severe damage to rice plants. However, further investigations, such as conducting experiments where these proteins are overexpressed in stressed plants, are necessary to validate this hypothesis.

The prediction of the protein-protein interaction network of *Os10g0111700* (coding POT protein) (Fig. 4) revealed a connection between the protein and manganese-dependent ADP-ribose/CDP-alcohol diphosphatase (*OsJ_25650*). Manganese-dependent ADP-ribose/CDP-alcohol diphosphatase (mADPR/CDP-alcohol pyrophosphatase) is an enzyme found in plants that is involved in the metabolism of the signaling molecule ADP-ribose (ADPR). This enzyme can hydrolyze both ADPR and CDP-alcohols, which are important intermediates in nucleotide metabolism. It has been suggested that mADPR/CDP-alcohol diphosphatase may play a role in regulating plant growth and development, as well as in the response of plants to biotic and abiotic stresses[68]. Additionally, there is another interaction reported between the POT protein and calcineurin-like phosphoesterase (*OsJ_34100*), which is a family of enzymes found in plants involved in various cellular processes, including the regulation of ion channels and the response to abiotic stresses. Specifically, CAs play a role in regulating the levels of calcium ions (Ca^{2+}) in plant cells, which are essential for signal transduction in response to various environmental stresses. CAs achieve this by dephosphorylating certain proteins involved in the transport of calcium ions across membranes in plant cells. Overall, CAs help to maintain calcium homeostasis in plant cells, which is crucial for normal plant growth and development, as well as for the response to various abiotic stresses[69,70]. On the other hand, the protein encoded by the downregulated gene *Os08g0545700* (*IAA6*) shows a confident interaction with molybdenum cofactor sulfurase 3 (*Os08g0545000*). It is suggested that MCSU3 plays a critical role in maintaining plant growth and development under normal conditions by

regulating the activity of enzymes involved in sulfur and molybdenum metabolism. The study also proposed that MCSU3 may be involved in stress response pathways in plants, but further research is needed to confirm this[71].

Conclusion

The results of transcriptome meta-analysis of rice under multiple stresses indicate the single stress-specific and common responses. Two and twelve genes were up- and downregulated in all stress conditions. Non-ABC transporters including *POT*, *OsAAP7C* and *OsGT1* were downregulated, a phenomenon that is resulted to inability of the plant to uptake and transfer nitrate and amino acids under stressful conditions, and hence, is resulted to decrease of plant growth which is obvious from turning the green tissues to yellow color. On this basis, preventing the reduction of the expression of these genes under stress conditions, especially through the control of their upstream transcription factor genes, can help reduce the adverse effects of abiotic stress in reducing the absorption and transfer of nitrogen or amino acids.

Data availability

Data is provided within the manuscript or supplementary information files or available from the corresponding author upon reasonable request.

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Author contributions
A.A. collected the transcriptome material and conducted the analyses. M.S. conducted qRT-PCR and contributed in analyses. A.S.T. prepared the MS draft and contributed in analyses. All authors read and approved the final MS.

Declarations

Competing interests
The authors declare no competing interests.

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