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Transcriptome Analysis for Abiotic Stresses in Rice (*Oryza sativa* L.)

Ashutosh Kumar and Prasanta K. Dash

Abstract

Rice, a model monocot system, belongs to the family Poaceae and genus *Oryza*. Rice is the second largest produced cereal and staple food crop fulfilling the demand of half the world's population. Though rice demand is growing exponentially, its production is severely affected by variable environmental changes. The various abiotic factors drastically reduce the rice plant growth and yield by affecting its different growth stages. To fulfill the growing demand of rice, it is imperative to understand its molecular responses during stresses and to develop new varieties to overcome the stresses. Earlier, the microarray experiments have been used for the identification of coexpressive gene networks during various conditions in crop plants. Though the microarray experiments provided very useful information, the unviability of genome-wide information did not provide complete information about the regulatory gene networks involved in the stress response. The advancement of molecular techniques provided breakthrough to understanding the complex regulatory gene networks and their signaling pathways during stresses. The high-throughput RNA sequencing data have opened the floodgate of transcriptome data in rice. Here we have summarized some of the transcriptome data for abiotic molecular responses in rice, which further help to understand their complex regulatory mechanism.

Keywords: abiotic stresses, cold stress, drought, micronutrients, rice, RNA-Seq, salt stress, submergence, trace element stress, transcriptome

1. Introduction

Rice is the most important staple food crop across the globe and is a model monocot system [1]. It is the second largest produced cereal fulfilling the demand of half world's population. Rice belongs to family Poaceae and genus *Oryza*. Two species *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice) out of 23 species have been cultivated worldwide [2]. The *O. sativa* is native to tropical and subtropical southern and southeastern Asia, while *O. glaberrima* is grown only in South Africa. A third species, *O. rufipogon*, has also been grown in South Asian, Chinese, New Guinean, Australian, and American farms. In Asia, *O. sativa* is separated into three subspecies according to its geographical environment: indica, japonica, and javanica. The variety indica refers to the tropical and subtropical varieties grown throughout South and Southeast Asia and Southern China. The variety japonica is grown in temperate areas of Japan, China, and Korea, while javanica varieties are

grown alongside of indica in Indonesia (<http://agropedia.iitk.ac.in/?q=content/botanical-classification-rice>).

Rice is an annual plant, even though in tropical areas, it is cultivated perennially. It is self-pollinated (wind pollination) tropical C3 grass that evolved in a semi-aquatic, low-radiation habitat having aerenchymatic tissues [3]. Rice is cultivated in more than 100 countries, with a total harvested area till 2017 is of approximately 165 million hectares, and produced ~700 million tons (503.9 million tons of milled rice) (<http://www.fao.org/3/I9243EN/i9243en.pdf>). About 91% of the rice in the world is grown in Asia (nearly 640 million tons) where 60% of the world's population lives. Rice is also cultivated in Sub-Saharan Africa and Latin Americas, and evenly poised in the Eastern and Western Asia. China and India, which account for more than one-third of global population, supply over half of the world's rice. The China produces ~30% of total world rice production followed by India (21%), Indonesia (9%), and Bangladesh (6%). On the other hand, rest of Asia, Americas, and Africa produce 37, 5, and 3%, respectively, of the total world rice production [4]. However, demand of the rice is still growing day by day, as the world population is mounting exponentially. To fulfill the demand of growing population, yield needs to be increased by the application of agricultural as well as biotechnological approaches.

Rice production is severely affected by changing environment including extreme variability in temperature and rainfall pattern along with other factors [5]. The abiotic stresses including drought, high salinity, high or low temperatures, flooding, high light, ozone, low nutrient availability, mineral deficiency, heavy metals, pollutants, wind and mechanical injury, drastically reduce the rice plant growth and yield by affecting it during different growth stages [6]. However, rice has very antagonistic character about tolerances and susceptibilities to abiotic stresses, as compared to other crops. It is very well known that rice paddy grows in standing water containing soil and can tolerate submergence at levels that would kill other crops. However, it is moderately tolerant to salinity and soil acidity but highly susceptible to drought and cold. Drought influences all physiological processes involved in plant growth and development [5]. Drought at vegetative stage can moderately reduce yield, but entire yield is lost if it occurs during pollen meiosis or fertilization [7]. The high salt concentration disrupts the ability of roots for efficient water uptake, leading to perturbation of crucial metabolic reactions inside the cell restricting plant growth and yield potential [8]. Low temperature reduces germination, causes poor establishment, delays phenological development, and increases spikelet sterility [9], and other physiological and metabolite changes causing low yield [10]. Furthermore, rice can tolerate partial submergence as paddy rice or deepwater rice because it is very well adapted to waterlogged conditions as it has well-developed aerenchyma that facilitates oxygen diffusion and prevents anoxia in roots [11–13]. However, it was damaged when submerged partially or completely for a relatively longer period [14] due to the shortage of oxygen during submergence. The response of plants to low oxygen stress comprises complex biochemical and genetic programs that include the differential expressions of a large number of genes. Importantly, abiotic stress conditions not only harm the crop but also influence the manifestation and extent the pathogen infection, attack of insects, and growth of weeds [6]. Though rice has superior response to abiotic stresses, development of their improved tolerant germplasm is indispensable [11]. Besides abiotic stress, the deficiency of micronutrients also affects the crop production.

The crop plants are very sensitive and respond to environmental stimuli through signal perception. The plant responds accordingly for a specific environmental stimulus instigating specific physiochemical changes. These physiochemical changes or adaptations are administered by complex molecular regulatory mechanism of involving various sensors regulated by transcriptional factors/regulators. Various studies have been carried out for understanding the regulatory mechanism of plants during stress

conditions. Earlier, *CIPK* genes (*OsCIPK01–OsCIPK30*) in the rice genome were studied for their transcriptional responses to various abiotic stresses [15]. The results showed that 20 *OsCIPK* genes were differentially induced by at least one of the stresses, including drought, salinity, cold, polyethylene glycol, and abscisic acid treatment. Most of the genes induced by drought or salt stress were also induced by abscisic acid treatment but not by cold. A few *CIPK* genes containing none of the reported stress-responsive *cis*-elements in their promoter regions were also induced by multiple stresses [15]. The proteins possessing A20/AN1 zinc-finger, named *SAP* gene family in rice and *Arabidopsis*, were inducible by one or the other abiotic stresses indicating that the *OsSAP* gene family is an important component of stress response in rice [16]. In addition, the role of *SAP* gene family in abiotic stress conditions was established by expression profiling under abiotic stress conditions. Seven Expansin A (*ExpA*) mRNAs were accumulated in leaves of deepwater rice, and their abundance was upregulated by submergence [17]. Similarly, the drought response in rice incites a signaling cascade through osmolyte synthesis that involves perception and translation of drought signal [18, 19].

Earlier, microarray experiments have been used for expression analysis of multiple genes during various conditions in different tissues for crop plants. The microarray experiments helped to identify the coexpressive genes during a stress condition [20–23]. Though the microarray experiments provided very useful information, the unviability of genome-wide information about the transcripts did not provide the complete information about the regulatory gene networks involved in the stress response. Nowadays, the availability of high-throughput techniques, achieved through advancement of molecular techniques, provided breakthrough in the understanding of complex regulatory gene networks and their signaling pathways involved in stress responses [24]. The techniques are comprised of whole genome transcriptome analyses, small RNA sequencing analysis (RNA-Seq), proteomic analyses, epigenetic sequencing analysis, and metabolomic analyses [25]. These high-throughput techniques use sequence-based approaches instead of hybridization-based approaches (like microarray), which require known genomic sequences, rather able to determine the transcript sequences directly from new genomes, able to map and quantify them [26, 27]. The RNA-Seq has superiority among these techniques due to its in-depth coverage of genome, global expression of transcripts, and also providing detailed information about alternative splicing and allele-specific expressions [27]. The inception of RNA-Seq technique has reformed the perception of complex and dynamic nature of the genomes, further helps to comprehensively elucidate the complex regulatory gene networks pertaining to different physiological and developmental stages of plants [28]. Currently, the various transcriptome analyses of rice genome, accomplished through RNA-Seq, during various abiotic stresses have generated enormous data. Further, these data have been able to decipher the complex regulatory gene networks in rice during various abiotic stresses which helped to understand the adaptive physiological measures taken by rice at cellular level and ascertain the development of tolerant rice varieties. Here, we are describing some of the different transcriptome studies carried out to understand the molecular responses in rice genome during various abiotic stresses.

2. Transcriptome data for submergence/flooding

Flooding is considered as a major threat to the rice crops, as irregular flash floods are very common in the Southeast Asia (major rice producing region), severely affecting the rice productivity [29]. Rice produces high yields, when it is grown in water-logged rice paddies. It can tolerate partial submergence as paddy rice or deepwater rice. However, it is damaged when submerged for a relatively longer

period [14] due to the slow diffusion of oxygen in water fails to match the demands of respiration [30] resulting in anaerobic metabolism and energy crisis [12]. Also, in deepwater rice, energy generation through fermentative metabolism, aerenchyma development in parenchymal tissues that improves access to O₂, activation of ethylene promoted gibberellic acid (GA)-mediated internode elongation cause foliage to shoot up above the water surface for gas exchange and restricting growth and conserving available energy until floodwater recedes [12, 13]. Similarly, flood-tolerant rice varieties have developed the capacity to generate ATP without the presence of oxygen and/or to develop specific morphologies that improve the entrance of oxygen [31]. Moreover, the phytohormonal regulation revealed that gibberellin (GA) has negative effects on submergence tolerance, whereas paclobutrazol (PB), chemical inhibitor of GA, acted contrary to GA [32]. The transcriptome analysis between GA- and PB-treated samples and control identified 3936 differentially expressed genes largely associated with the stress response, phytohormone biosynthesis and signaling, photosynthesis, and nutrient metabolism. It was observed that the PB improved the rice survival during submergence through sustaining the photosynthesis capacity and by dropping nutrient metabolism [32].

Despite knowledge of adaptive mechanisms and regulation at the gene and protein level, our understanding of the mechanisms behind plant responses to submergence is still limited. Even in flood-intolerant species, such as *Arabidopsis thaliana*, many genes are triggered in response to flooding stress [33, 34]. The response of plants to low oxygen stress comprises complex biochemical and genetic programs that include the differential expressions of a large number of genes (**Table 1**). Gene expression is altered under low oxygen stress, and the existence of *anaerobic response elements* (AREs) along with their binding factors has already been reported [35]. Eventually, a *SUB1* locus and three ethylene response factors (ERFs) were identified within the locus in tolerant rice varieties (e.g., FR13A), whereas *SUB1* is a major determinant of tolerance [36]. Introduction of the *SUB1A* gene into submergence-intolerant rice variety significantly increased its flooding tolerance, thus demonstrating the importance of the *SUB1* locus for flooding tolerance [36]. Two different types of molecular mechanisms are adapted by rice ecotypes to survive under stress, *SUB1A*-mediated “quiescence strategy” [37, 38] and “escape strategy” induced by *SNORKEL1/2* [13]. The submergence response in rice consists of the differential expression of genes related to gibberellin biosynthesis, trehalose biosynthesis, anaerobic fermentation, cell wall modification, and transcription factors that include ethylene-responsive factor genes [39]. Though the regulatory mechanism in rice during submergence response has been comprehensively studied, the genome-wide gene expression as well as allelic variation among the cultivars for specific quantitative traits remained elusive. One of the studies was conducted in six rice genotypes to estimate the coleoptile elongation rates during submergence [39]. The result postulated that the coleoptile elongation was augmented by transcriptional regulation. Further, the reason for the variation in anaerobic germination was due to the allelic variation caused by the small-to-large deletions in the coding region of susceptible varieties [39].

Recently, a study on *SUB1A-1* genotypes is carried to understand the molecular mechanism pertaining to the physiological function upon desubmergence through transcriptomic analysis [29]. The results enumerated around 1400 genes that were differentially expressed to recover from the stress to preserve the plastid integrity, and the genes regulating the cell division, chromatin structure, and signaling associated with starch catabolism [29]. They also found that the rice plants recover shoot transcriptome significantly to the control state and return to homeostasis during the 24-h recovery period. It also regulated the GA-responsive starch metabolism

Abiotic stress condition	Gene/s responsible for tolerance	Downstream key gene/s	Physiological functions
Submergence	<i>SUB1A</i>	<i>ERFs</i> regulating genes of GA-responsive starch metabolism, anaerobic fermentation, cell wall modification, JA-mediated internode elongation, and biotic responsive	Quiescence strategy to stop all physiological functions
	<i>SNORKEL1/2</i>		Escape strategy to supersede water level
Drought	<i>DREBs</i> (<i>DREB1A-D/CBF1-4</i> and <i>DREB2</i>)	ABA-responsive genes, <i>LEA</i> , <i>NAC</i> , <i>DBP</i> , α -linolenic acid metabolic pathway genes, osmolyte biosynthesis genes, phospholipid metabolism genes; water channel protein, sugar and proline transporters, and detoxification enzyme-encoding genes; and signaling molecule-encoding genes	Stomatal closure, repression of cell growth, photosynthesis and activation of respiration and production of phytohormone ABA
Salt	<i>SOS1</i> , <i>NHX</i> , <i>HKT2</i> , <i>CAX1</i> , <i>AKT1</i> , <i>KCO1</i> , <i>TPC1</i> , <i>CLC1</i> , <i>NRT1</i> , <i>CDPK7</i> , <i>MAPK5</i> , <i>CaMBP</i> , <i>GST</i> , <i>LEA</i> , <i>V-ATPase</i> , <i>OSAP1</i> , and <i>HBP1B</i>	Genes related to antioxidants, transcription factors, signaling, ion and metabolic homeostasis and transporters	Imbalance in ion homeostasis of cells at plasma membrane and sequestration of vacuolar ion, and stomatal closure which causes higher leaf temperature and reserve shoot elongation
Cold	<i>CBF1</i> , <i>DREB1A</i> , and <i>DREB1B</i>	ABA-responsive genes, <i>ABF</i> , <i>NAC</i> , <i>NACRS</i> containing genes, <i>ERF922</i> , <i>WRKY25</i> , and <i>WRKY74</i> , gene related to signal transduction, phytohormones, antioxidant system and biotic stress	Altered chlorophyll content and fluorescence causing reduction in photosynthesis, increases content of ROS and malondialdehyde causing oxidative damage to cells
Cadmium (Cd)		Cd-responsive transporters, ROS-scavenging enzymes, chelators, and metal transporter-encoding genes and many drought stress-related genes	Fatal damage to rice seedlings during their development
Phosphorus (P)		RNA transport and mRNA monitoring path genes	Important for energy transfer, signal transduction, photosynthesis, and respiration
Manganese (Mn)		TFs, transporters, transferase protein genes, catalytic protein encoding genes, <i>WRKY</i> , and potassium transporter-related genes, <i>Aux/IAA</i> family, and sodium transporter-related genes	Important for catalyzing the water-splitting reaction of oxygen-evolving complex in photosystem II (PSII), acts as cofactor that activates different enzymes, such as Mn-superoxide dismutase and others, to protect against oxidative stresses
Alkaline stress	Alkali-responsive genes	Alkaline resistant genes, TFs related to hormone signal transduction and secondary metabolite biosynthesis pathways	

Table 1.
Regulatory role of different abiotic stress-responsive genes based on RNA-Seq analysis.

indirectly through *SUB1A* and downstream regulatory network to resume the photosynthesis [29]. Similar studies have also been carried between two contrasting deepwater growth rice cultivars [40]. The RNA-Seq analysis was conducted from different tissues, shoot base region, including basal nodes, internodes, and shoot apices of seedlings at two developmental stages. The study elucidated the possible role of jasmonic acid-mediated internode elongation and expression of biotic stress-related genes during submergence response [40].

3. Transcriptome data for drought stress

One of the major abiotic stresses that severely affect the rice production is drought stress. Drought stress causes a series of physiological and biochemical changes which included stomatal closure, repression of cell growth, photosynthesis, and activation of respiration along with production of the phytohormone abscisic acid (ABA) [41]. In response to the drought stress, ABA triggers stomatal closure and induces expression of stress-related genes (**Table 1**) [41]. However, some of drought-related genes were not expressed by the external ABA treatment. Therefore, the drought response is either of ABA-independent or of ABA-dependent or both inducible gene regulatory system networks [42]. These regulatory networks are the amalgamation of interaction between transcription factors and their respective promoter *cis*-elements. It was observed that the promoters of ABA-dependent genes have ABA-responsive element (*ABRE*) and, dehydration- and cold-responsive element (*C-repeat/DRE*) [42]. The transcription factors, which specifically bind to *ABRE* are known as DREBs, trigger the expression of ABA-responsive genes [43], which further encode AP2 domain-containing transcription factors regulating the stress-related genes in an ABA-independent manner [44]. The *DREB* gene family has two groups *DREB1/CBF* and *DREB2*, whereas *DREB1/CBF* consists of *DREB1A* (CBF3), *DREB1B* (CBF1), *DREB1C* (CBF2), and *DREB1D* (CBF4). However, five *DREB* homologs were identified in rice, *OsDREB1A*, *OsDREB1B*, *OsDREB1C*, *OsDREB1D*, and *OsDREB2A* [45, 46]. These gene-encoded proteins are classified into two: the first group belongs to the functional proteins included chaperones, late embryogenesis abundant (LEA) proteins, osmotin, anti-freeze proteins, mRNA-binding proteins, enzymes for osmolyte biosynthesis, water channel proteins, sugar and proline transporters, and detoxification enzymes; the second group is of regulatory proteins (signal transduction and stress-responsive) including various transcription factors, protein kinases, protein phosphatases, enzymes involved in phospholipid metabolism, and other signaling molecules such as calmodulin-binding protein [22, 41]. Interestingly, it was found that many of these proteins, especially *DREBs*, are also involved in transcriptional regulation of stress-response mechanism during cold and salt stresses [46, 47].

The rice is the only crop which is grown in the waterlogged fields and it has very low water-use efficiency [48]. Therefore, it is imperative to decipher the molecular regulatory mechanism to increase the water usage efficiency of rice or the drought tolerance. Nowadays, the drought stress is continuously affecting the rice productivity due to the harsh environmental condition. The transcriptome studies proved to be the boom for researchers due to its global genomes depth and all at once allele mining among different rice genotypes. Earlier, a transcriptome analysis between drought-tolerant and drought-sensitive cultivars was carried out for the identification of novel genetic regulatory mechanisms [48]. This study suggested that the upregulation of genes related to carbon fixation, glycolysis/gluconeogenesis, and flavonoid biosynthesis, whereas the downregulation of genes associated with starch and sucrose metabolism during drought. Further, they also found the upregulation

of genes associated with α -linolenic acid metabolic pathway in tolerant genotype during the stress which supported the previous findings. Consecutively, the analysis of consensus *cis*-motif among the coexpressed drought-induced genes led to the identification of novel *cis*-motifs [48]. Similar comparative studies have been carried out between tolerant and susceptible rice cultivars and in other crops to understand the regulatory mechanisms during drought [49–51]. Their result suggested that 801 transcripts differentially expressed in tolerant cultivar including the TFs NAC and DBP, and thioredoxin involved in phenylpropanoid metabolism [49].

To sustain the drought condition, the roots have a very important role. To understand the molecular regulation in rice seedling roots (4-weeks old) during drought condition, comparative RNA-Seq analysis has been carried out between wet and dry soil conditions [52]. This analysis suggested that 68% of identified genes were novel, and also found that the one of the enzymes RING box E3 ligases from ubiquitin-proteasome pathway was induced by drought. Interestingly, it was found that the *OsPhyB* represses the activity of ascorbate peroxidase and catalase-mediated reactive oxygen species (ROS) processing machinery required for drought tolerance of roots in soil condition, contrary to the previous results [52].

4. Transcriptome data for salt stress

Some of the abiotic stresses are complementary to each other such as the drought and salt, drought and cold stresses, etc., affecting the rice productivity. It is evident that excessive loss of water from the soil evaporation due to drought causes salt accumulation in soil. The salinity is defined as deposition of sodium chloride from natural accumulation or irrigation in soil. It causes imbalance in ion homeostasis of cells regulated by ion influx and efflux at the plasma membrane and sequestration of vacuolar ion [8]. The salt stress affects stomatal closure causing increased leaf temperature and reserved shoot elongation [53]. Studies on the salinity tolerant in rice have shown the regulation of genes related to antioxidants, transcription factors, signaling, ion and metabolic homeostasis, and transporters (**Table 1**) [54]. The identified important class of genes regulated during a salt stress in rice are *OsSOS1*, *OsNHX1* (Na^+/H^+ antiporters), *OsHKT2;1* (Na^+/K^+ symporter), *OsCAX1* (H^+/Ca^+ antiporter), *OsAKT1* (K^+ inward-rectifying channel), *OsKCO1* (K^+ outward-rectifying channel), *OsTPC1* (Ca^{2+} permeable channel), *OsCLC1* (Cl^- channel), *OsNRT1;2* (nitrate transporter), *OsCDPK7*, *OsMAPK5*, *CaMBP* (*calmodulin motif binding protein*), *GST* (*glutathione-S-transferase II*), *LEA* (*late embryogenesis abundant protein*), *V-ATPase* (*vacuolar ATP synthase 16KD proteolipid subunit*), *OSAP1* (zinc finger protein), and *HBP1B* (histone binding protein, TF) [55–63]. The salt stress response mechanism is moreover of complex physiological process pertaining to metabolic and morphological changes, which is comprehensively studied, but in rice, the molecular regulatory mechanism to salt tolerance is elusive [64]. Some of the transcriptome analyses have been completed in conjugation with the drought stress to understand the salt tolerance in rice [46, 49, 59]. Earlier, a comparative study has been carried out between salt tolerant and susceptible rice cultivars to understand the regulatory mechanisms [49]. The result suggested higher expression of bHLH and C_2H_2 TF family members, which might be regulating the genes associated with wax and terpenoid metabolism pathways [49]. Similarly, to understand the salinity stress, a comparative leaf transcriptome analysis at three time points on rice seedlings has been completed [65]. They identified 1375 novel genes, whereas 286 differentially expressed genes exclusively found in tolerant cultivar. They validated two genes: disease resistance response protein 206 and *TIFY10A* to understand the molecular response to salinity stress [65].

5. Transcriptome data for cold stress

The cold stress is defined according to the temperature affecting the plant growth and development which ranges 0–15°C (chilling stress) and <0°C (freezing stress) [66]. The tropical origin of rice makes it more susceptible to cold, critically affecting reproductive stages and grain quality leading to yield reductions [67]. The cold stress affects chlorophyll content and fluorescence causing reduction in photosynthesis, increases content of reactive oxygen species (ROS) and malondialdehyde (MDA) causing oxidative damage to cells in rice [68]. The molecular regulation of cold stress is identified in conjugation of drought stress (**Table 1**) [45]. Many stress-inducible genes are regulated via ABA-independent pathway, characteristically having a *cis* element responsible for dehydration (*DRE*) as well as low-temperature-induced expression. The low-temperature-inducible genes possess C-repeat (*CRT*) and low-temperature-responsive element (*LTRE*). The *DRE*-binding proteins encoding genes *CBF1*, *DREB1A*, and *DREB1B* were induced by cold stress [46]. During cold stress, ABA also accumulates and initiates the ABA signaling cascade, which regulates the ABA-responsive genes through *ABRE* and the *ABRE*-binding bZIP transcription factor ABF [69]. The *OsNAC* gene transduces the ABA signal through an *ABRE* in its promoter and regulates the expression of *NACRS*-containing genes to control cold tolerance in rice [67]. Further, to understand comprehensively the regulation of genes during cold stress, a transcriptome study is carried out between weedy and cultivated rice [70]. The analysis suggested that some typical cold stress-related genes were of basic helix-loop-helix (bHLH) gene and leucine-rich repeat (LRR) domain genes, and several genes associated with phytohormones like abscisic acid (ABA), gibberellic acid (GA), auxin, and ethylene [70]. Similarly, the wild rice, *O. longistaminata*, tolerates nonfreezing cold temperatures, is used for the identification of molecular mechanisms in response to low temperature in its shoots and rhizomes at seedling and reproductive stages using transcriptome analysis [71]. They found photosynthesis pathway-related genes were prevalent in shoots, whereas metabolic pathways and the programmed cell death process-related genes were expressed only in rhizomes. Further, they found that the TFs *CBF/DREB1*, *AP2/EREBPs*, *MYBs*, and *WRKYs* were synergistically expressed in shoots, whereas *OsERF922*, *OsNAC9*, *OsWRKY25*, *OsWRKY74*, and eight antioxidant enzymes encoding genes were expressed in rhizomes during cold stress. The *cis*-regulatory element analysis suggested the enrichment of ICE1-binding site, GATA element, and W-box in both tissues. And the highly expressed genes in shoots were associated with photosynthesis, whereas signal transduction-related genes were highly expressed in rhizomes [71].

Furthermore, a transcriptome analysis is performed in germination phase for contrasting cultivars of rice in cold stress [72], suggesting the higher expression of gene related to signal transduction, phytohormones, antioxidant system, and biotic stress during germination in cold stress [72].

6. Transcriptome data for trace element stress

The rice is the staple food fulfilling the dietary needs of a large population around the world. Besides dietary energy and proteins, it also contains trace elements (Li, B, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Ba, Pb, and Bi) in low amounts [73]. Some of these trace elements Se, Mo, Cr, Mn, Fe, Co, Cu, Zn are micronutrients that help in proper functioning of human biological systems, while nonessential heavy elements such as Pb, As, Cd, Hg are referred as toxins for consumption [73, 74]. However, the trace elements in rice are invariably increasing

either due to the use of agrochemicals or irrigation with contaminated water. The deficiency or accumulation of these trace elements in soil hampers plant growth and development. On the other hand, their biofortification helps to add nutrition supplement. Henceforth, the detailed study about the effects of these trace elements on the rice is indispensable. There are many reports about trace element stresses on rice achieved through transcriptome studies (**Table 1**).

The higher concentration of heavy metal cadmium (Cd) severely hampers the rice growth. Therefore, to understand the molecular mechanism during Cd stress, transcriptome analysis has been completed by exposing rice to higher concentrations of Cd [75]. They found constitutively expressed genes were less affected by low Cd concentrations, whereas high Cd concentration causes fatal damage to rice seedlings during their development. They also found some novel Cd-responsive transporters encoding genes [75]. Previously, they found the upregulation of many genes related to ROS-scavenging enzymes, chelators, and metal transporters during Cd exposure along with upregulation of many drought stress-related genes [76].

Phosphorus (P) is an essential trace element required for proper plant growth and development where it plays an important role in energy transfer, signal transduction, photosynthesis, and respiration [77]. A comparative transcriptome study has been carried out in leaf and root tissues during phosphorus stress to elucidate their molecular mechanisms [78]. The transcriptome analysis suggested that many differentially expressed TFs and functional genes were uniquely involved in multiple regulatory pathways (including RNA transport and mRNA monitoring path) during phosphorus deficiency tolerance [78].

Manganese (Mn) is an essential trace element which plays an important role in catalyzing the water-splitting reaction of oxygen-evolving complex in photosystem II (PSII). It also acts as a cofactor that activates different enzymes, such as Mn-superoxide dismutase and others, to protect against oxidative stresses in plants [79]. However, higher Mn affects the physiological and biochemical pathways associated with plant growth and development. Therefore, to decipher the molecular mechanisms in leaves of Mn-sensitive rice exposed to high Mn stress, transcriptome analysis has been done [79]. The analysis suggested that a large number of TFs, transporters, transferase proteins, catalytic proteins encoding genes were differentially expressed having a major role in primary and secondary metabolisms. Further, it was found that the *WRKY* family and potassium transporter-related genes were significantly upregulated, whereas *Aux/IAA* family and sodium transporter-related genes were strongly downregulated [79].

7. Transcriptome data for other stresses

Besides common abiotic stresses, some other stresses are also studied with the help of transcriptome analysis. A transcriptome study has been carried out for alkaline stress caused by alkaline NaHCO_3 and Na_2CO_3 [80]. The study reported the identification of 926 differentially expressed important alkali-responsive genes including 28 alkaline-resistant genes and 74 transcription factor genes. These genes were related to hormone signal transduction and secondary metabolite biosynthesis pathways [80].

The RNA-Seq or transcriptome analysis has tremendous potential to divulge the complex molecular machinery of plant regulatory response during stress conditions. However, this large number of transcriptome data of abiotic stresses in rice has contributed significantly to rice researchers. It helped to understand complete molecular mechanism pertaining to their physiological and biochemical changes. Such data mining could be a high impact methodical source for identification of candidate gene through integration of functional genomics approach. This will also

help to establish the hierarchical relationships between specific signaling components and downstream effector genes to cope up the stress conditions.

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