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ORIGINAL ARTICLE

Comparative transcriptome profiling of rice colonized with beneficial endophyte, *Piriformospora indica*, under high salinity environment

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Abstract

The salinity stress tolerance in plants has been studied enormously, reflecting its agronomic relevance. Despite the extensive research, limited success has been achieved in relation to the plant tolerance mechanism. The beneficial interaction between *Piriformospora indica* and rice could essentially improve the performance of the plant during salt stress. In this study, the transcriptomic data between *P. indica* treated and untreated rice roots were compared under control and salt stress conditions. Overall, 661 salt-responsive differentially expressed genes (DEGs) were detected with 161 up- and 500 down-regulated genes in all comparison groups. Gene ontology analyses indicated the DEGs were mainly enriched in "auxin-activated signaling pathway", "water channel activity", "integral component of plasma membrane", "stress responses", and "metabolic processes". Kyoto Encyclopedia of Genes and Genomes pathway analysis revealed that the DEGs were primarily related to "Zeatin biosynthesis", "Fatty acid elongation", "Carotenoid biosynthesis", and "Biosynthesis of secondary metabolites". Particularly, genes related to cell wall modifying enzymes (e.g. invertase/pectin methylesterase inhibitor protein and arabinogalactans), phytohormones (e.g. Auxin-responsive Aux/IAA gene family, ent-kaurene synthase, and 12-oxophytodienoate reductase) and receptor-like kinases (e.g. AGC kinase and receptor protein kinase) were induced in *P. indica* colonized rice under salt stress condition. The differential expression of these genes implies that the coordination between hormonal crosstalk, signaling, and cell wall dynamics contributes to the higher growth and tolerance in *P. indica*-inoculated rice. Our results offer a valuable resource for future functional studies on salt-responsive genes that should improve the resilience and adaptation of rice against salt stress.

Keywords Rice · RNA-seq · *Piriformospora indica* · Symbiosis · Salt stress

Introduction

A significant part of the cultivated and irrigated land in the world is affected by high salinity to the extents that pose a severe threat to agronomically important crops [1]. Soil

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salinity has a profound effect on crop productivity due to the toxic Na^+ and Cl^- ions accumulation and nutrient imbalance [2]. The salinity stress impaired the plant growth by reducing the soil osmotic potential that concomitantly causes physiological drought in the plant [3]. The disproportionate amount of Na^+ and Cl^- ions in the cell leads to structural damage of enzymes and other macroelements, disruption to the cell organelles as well as perturbation of plant metabolism and physiological functions such as photosynthesis, cellular respiration, and protein synthesis [2, 4].

Previous research provided information regarding cellular, metabolic, molecular, and physiological changes related to salt stress in various plants [5, 6]. Many genes involved in salt tolerance such as cyclophilin, Na^+/H^+ antiporter (*NHX*), salt overly sensitive (*SOS*), superoxide dismutase (*SOD*), pyrroline-5-carboxylate synthetase, have been identified and characterized in many plant varieties [7–9]. During the past decade, genetic manipulation of crops provided efficient ways to produce the salt-stress tolerant transgenic varieties [10, 11].

The constant demand to develop crops with higher salt tolerance has been an incentive for research. Studies have demonstrated that the plants associated with mycorrhizal fungi exhibit improved growth and yield under salt stress [12, 13]. For instance, the higher chlorophyll contents have been detected in the leaves of mycorrhizal plants, indicating that the symbiotic interaction plays a crucial role in regulating photosynthetic and other metabolic activities [12]. Further, mycorrhizal fungi have also been shown to influence stomatal conductance and transpiration in the host plants [14]. It has also been reported that the mycorrhizal symbiosis can improve the osmotic balance in the host plant by enhancing osmolytes production, such as proline, during salinity stress [15, 16].

P. indica, an axenically cultivable root endophyte of basidiomycota, has manifested growth-promotional activity in a wide range of plant species [17]. *P. indica* has shown enormous potential to ameliorate the plant vigor, nutrient acquisition, and provide tolerance against abiotic and biotic stresses [18]. Plant colonized with *P. indica* has shown higher antioxidants level during salt stress, which minimizes the salinity-induced lipid peroxidation and membrane fatty acid desaturation in leaves [19]. Further, a cyclophilin like A protein in *P. indica* (PiCyPA) has reported being involved in various abiotic stresses such as salinity [8]. Despite the profound research advances in the past years, progress is slow; therefore, it is imperative to develop salt-tolerant plants with higher yield and productivity by employing eco-friendly biological means. The in-depth analysis of stress tolerance mechanism in plant–microbe system will be effective in developing strategies to improve salt tolerance.

Taking advantage of RNA-seq technology, many studies were conducted to identify genes involved in plant development and stress responses [20, 21]. Here, we compared the transcriptome of rice root colonized with and without *P. indica* under control and salt stress condition by using RNA-seq analysis. The treated root samples were sequenced in order to identify the repertoires of salt-responsive differentially expressed genes (DEGs). Functional annotation of DEGs was performed to reveal biological processes and metabolic pathways involved in salt stress responses. The present work provides a further exploration into the molecular events involved in salt tolerance in rice during beneficial interaction.

Materials and methods

Plant, fungus co-cultivation, salt stress treatment and tissue sampling

Seeds of rice (*Oryza sativa*) var. Pusa basmati 1 were germinated aseptically on Murashige and Skoog [22] media for ten days. Rice seedlings were planted to the PNM agar medium [23] and inoculated with *P. indica* spores (~ 1×10^5 spores/ml) after 24 h. For salt stress, rice plants were placed on the PNM medium containing 0 M, 0.05 M, 0.1 M, 0.15 M, and 0.25 M NaCl with and without *P. indica* for 14 days. Rice seedlings were kept in a culture room equipped with 2000 lx fluorescent light, 28 ± 2 °C temperature. *P. indica* colonization in roots was checked by staining root tissues with 0.05% lactophenol blue and visualized under the light microscope at × 20 or × 40 (Additional Supplementary Fig. 1). Total four groups of treated and untreated root samples were collected (15 plants each) on 14 days after *P. indica* inoculation (dai) i.e. control roots (C), only salt-treated roots (Salt), only *P. indica*-treated roots (C + Pi), and salt- and *P. indica*-treated roots (Salt + Pi). Samples were immediately frozen in liquid nitrogen and stored at − 80 °C before the RNA extraction.

Determination of malondialdehyde (MDA) content

Rice leaves were collected after 14 dai with *P. indica* and salt treatment (0.25 M NaCl) to analyze the MDA content. Leaf tissues (0.1 g) were homogenized with 0.5 ml of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 10,000×*g* for 10 min. The resulting supernatant was separated and mixed with 1.5 ml of 20% (w/v) TCA and 0.5% (w/v) thiobarbituric acid (TBA). The mixer was incubated at 95 °C for 25 min. After cooling on the ice, mixer was centrifuged at 10,000×*g* for 10 min. The absorbance of the supernatant was recorded at 532 nm and 600 nm [24].

RNA extraction, library construction and transcriptome sequencing

Total RNA was extracted using the RNeasy plant mini kit (Qiagen) according to the manufacturer's protocol. The RNA quality was assessed using 1% agarose gels and NanoDrop 1000 spectrophotometer (Thermo Scientific, USA). Total RNA was treated with DNaseI to remove DNA traces. The purity and quantity of RNA were analyzed by NanoDrop 1000 and 21,000 Bioanalyzer RNA Nanochip (Agilent Technologies GmbH, Germany). RIN (RNA integrity number) values were > 6 for all samples. In order to generate the cDNA library, total RNA was fragmented and amplified by illumina primer (oligonucleotide sequences© 2006–2010 Illumina Inc) and reverse transcriptase. After quality assessment with Agilent 2100, a total of four cDNA libraries were generated with the Illumina Hiseq 2500 platform following the manufacturer's instructions (Illumina, USA).

Preprocessing, assembly, and data analysis

The paired-end raw reads were generated by Illumina Hiseq 2500 and analyzed by FastQC (v.0.11.3). The adapter sequences and low-quality bases were trimmed by AdaperRemoval-v2 (version 2.2.0) followed by rRNA removal by Sliva database using Bowtie2 (version 1.0.13). The clean reads were aligned to the Rice genome and gene model downloaded from MSU Rice Genome Annotation Project website. The read alignment was performed using the STAR program (version 2.5.3a). The aligned reads were then used for estimating the gene expression using the cufflinks (version 2.2.1). The normalized gene expression level was computed as fragments per kilobase (kb) pair of exon model per million fragments mapped (FPKM). Differential expression analysis between samples was analysed using the cuffdiff program of cufflinks package. Genes with log2 fold change ≥ 2 and/or p-value ≤ 0.05 were used as cutoff criteria for DEGs. The p-values were adjusted using Benjamini and Hotchberg's False Discovery Rate method.

Functional categorization and annotation

The functional annotation of rice genes was performed with blastx search against the rice genome database. The GO-term distribution was generated using in-house scripts based on Uniprot annotations obtained for DEGs. The GO-enrichment analysis was executed using GOseq R package. GO terms with p-value ≤ 0.05 were depicted as significantly enriched. KEGG Orthology-Based Annotation System (KOBAS 3.0) was used to assess the enrichment of DEGs in KEGG pathways (Fisher's exact test, $p < 0.05$) for metabolic pathway prediction. The expressed genes were used as the background set for GO and KEGG enrichment analyses.

Validation of RNA-Seq data by quantitative real-time PCR (qRT-PCR)

The RT-qPCR was performed on the Roche real-time PCR system (Roche, Switzerland) using LightCycler 480 SYBR Green I Master (Roche, Switzerland) according to the manufacturer's instruction. The cycle threshold value (CT) was assessed and differential expression was calculated using the $2^{-\Delta\Delta CT}$ method [25] with the actin gene of rice as an endogenous reference [26]. All data are the means of three biological replicates and three technical replicates ± SD. Gene-specific primers used for qRT-PCR were listed in Supplementary Table S3.

Data availability

All the raw sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra) under the accession number PRJNA535402.

Results

Effect of salt treatment on the growth of rice seedlings in the presence and absence of *P. indica*

Rice seedlings were treated with different salt concentrations of 0 M, 0.05 M, 0.1 M, 0.15 M, and 0.25 M NaCl for 14 days with and without *P. indica* inoculation (Supplementary Fig. S1). Root length, shoot length, and fresh weight of treated and control plants, were measured and compared (Fig. 1). We observed that root and shoot length of rice plants were gradually decreased with increasing salt concentration in comparison to control. However, plants colonized with *P. indica* showed higher root and shoot length as compared to salt-treated plants without *P. indica*. A similar observation was recorded in the case of fresh weight of treated and control rice. The root length and fresh weight of *P. indica*-inoculated rice were shown slight increment at 0.25 M NaCl concentration in comparison to control; however, the shoot length of *P. indica*-inoculated rice was significantly higher as compared to uninoculated plant (Fig. 1). This result suggests that *P. indica* positively supports the plant growth during salt stress to a certain extent, however; the growth rate was slower with increasing salt concentration. Based on the above finding, we decided to explore the molecular events in rice affected by higher salinity stress and the molecular changes occurred in the presence of beneficial endophytic interaction during stress.

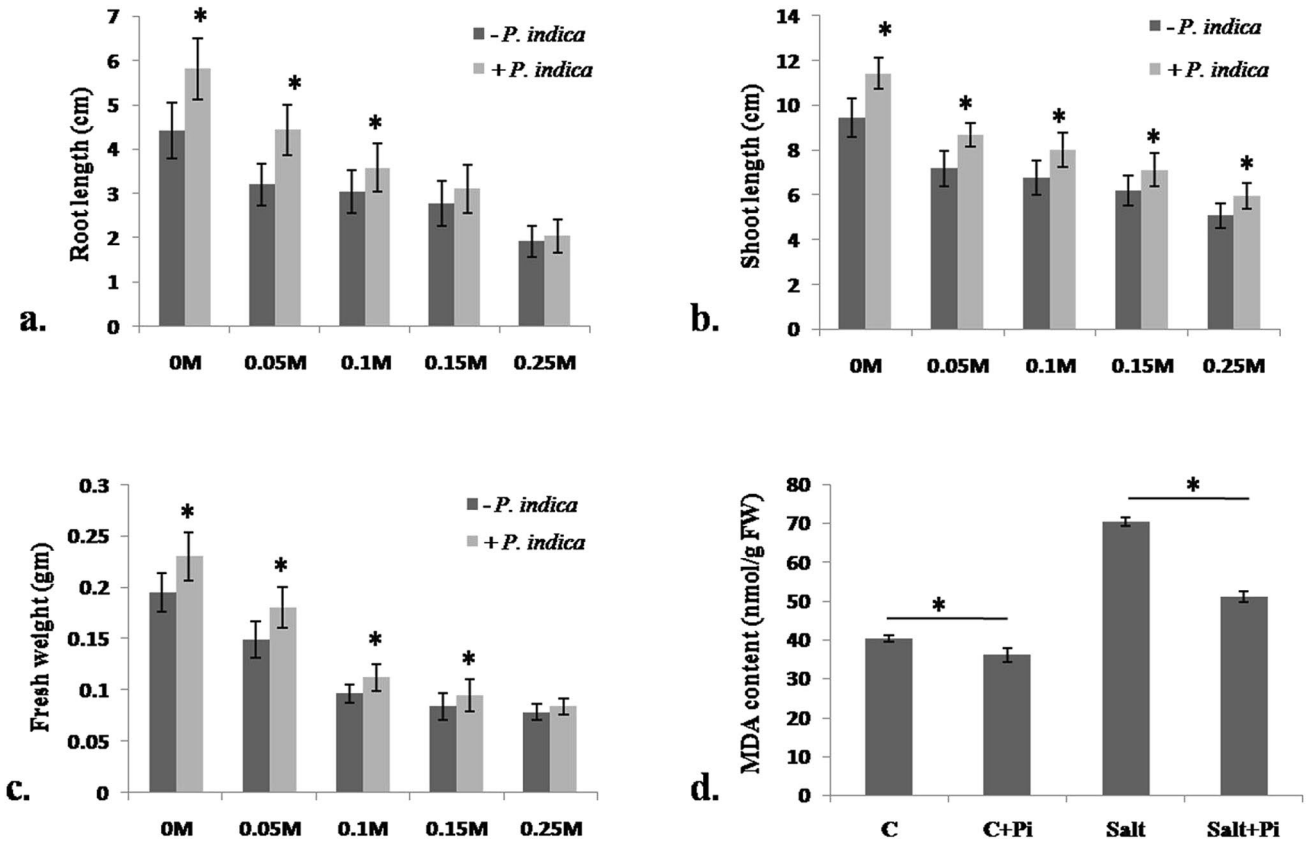

Fig. 1 Measurement of root length (**a**), shoot length (**b**), and fresh weight (**c**) of rice under different concentration of salt (NaCl) in the presence and absence of *P. indica* interaction at 14 days after inoculation (dai). (**d**) Effects of *P. indica* interaction on MDA level under salt stress. The MDA content was measured in leaf samples collected after salt treatment (0.25 M NaCl) for 14 days with or without *P. indica*. Values are the mean ± SD (n = 15). Asterisks indicate statistical significance between *P. indica* treated and untreated plants (p-value < 0.05) based on *t*-test

Effect of *P. indica* colonization on lipid peroxidation in salt-treated rice

Salinity causes oxidative stress and lipid peroxidation in plant tissues which results in a higher level of MDA. Our result showed that the MDA content was increased upon salt treatment (0.25 M NaCl). However, *P. indica* colonized plant showed significantly lowered MDA level in both control and salt-treated plant (Fig. 1d).

RNA sequencing and mapping

We performed a comprehensive RNA-seq profile with rice root under the given high salt stress in the presence and absence of *P. indica* interaction to explore the global gene expression changes in root tissues. High-throughput sequencing of the four root RNA samples generated a total of 237.2 M reads of length 100 bp (paired-end), with over 11 Gbps of raw read data. An average 97.15% of total reads passed ≥ 30 Phred score. After trimming, removal of adapter and low-quality sequence, a total of 72,415,218, 52,503,258, 64,794,380, and 46,234,048 clean reads were obtained from RNA samples of C, C + Pi, Salt, and Salt + Pi respectively. The percentages of aligned reads were 97.28%, 94.86%, 98.27%, and 96.99% respectively. The summary of reads and mapping statistics is given in Tables 1 and 2. The total number of genes, unique genes, and common genes expressed in C, C + Pi, Salt, and Salt + Pi samples are provided in Supplementary Table S1.

Differential expression of rice genes in three comparison groups

A total of 1610 DEGs were identified in all sample combinations. The FPKM values of several DEGs were observed to be < 1; therefore genes with FPKM ≥ 1 at least in one sample were also taken into consideration. Out of 1610 DEGs only 661 genes were considered substantial between all the comparison groups (C vs Pi, C vs salt, C vs Salt + Pi, and Salt vs Salt + Pi), with 161 up- and 500 down-regulated genes (Fig. 2, Online Resource 1), indicating that the number of down-regulated genes

Table 1 Statistics of transcriptome sequencing

Sample name	Number of raw reads (paired-end)	Number of bases (Gb)	GC (%)	% data ≥ Q30	Raw read length (bp)
C	36,253,006	7.25%	52.38	97.2	100 × 2
Pi	26,358,362	5.27%	52.65	97.2	100 × 2
Salt	32,428,506	6.49%	51.69	97.2	100 × 2
Salt + Pi	23,590,104	4.72%	51.60	97.0	100 × 2
	Total reads (paired-end) = 118,629,978	Total no. of bases = 23.73%			

Table 2 Summary of read mapping with reference genome

Sample name	Total read count	Clean reads	QC pass %	Aligned reads %	Unaligned reads %
C	72,506,012	72,415,218	99.87	97.28	2.72
Pi	52,716,724	52,503,258	99.60	94.86	5.14
Salt	64,857,012	64,794,380	99.90	98.27	1.73
Salt + Pi	47,180,208	46,234,048	97.99	96.99	3.01

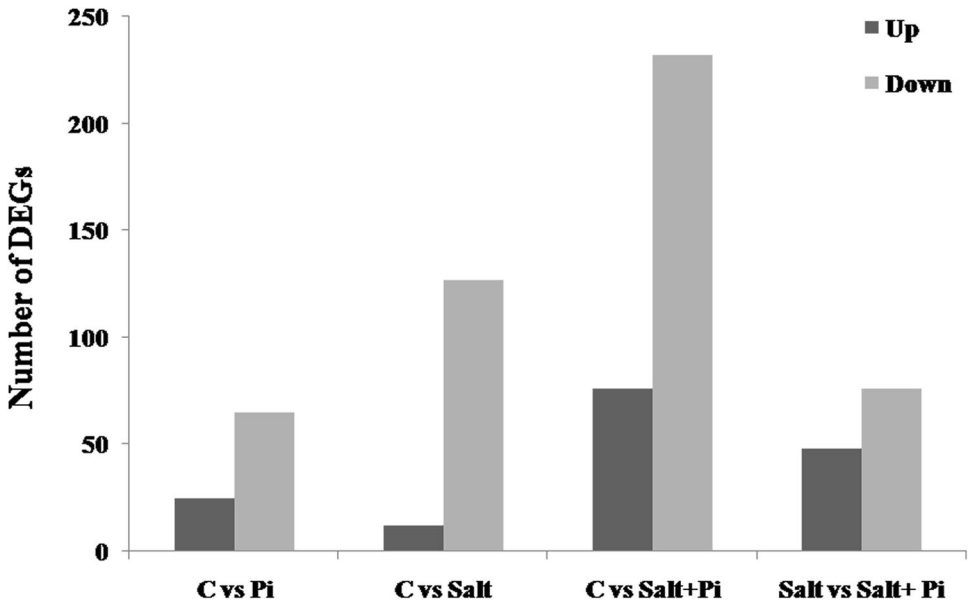

Fig. 2 Total number of up- and down-regulated DEGs of rice inresponse to salt stress and *P. indica* interaction. Total 90 (25 up/65 down), 139 (12 up/127 down), 308 (76 up/232 down), and 124 (48 up/76 down) DEGs were detected in C vs Pi, C vs Salt, C vs Salt + Pi, and Salt vs salt + Pi group combinations, respectively. DEGs were selected based on log2fold change (> two fold) and statistical significance of $p \leq 0.05$

was higher in the samples exposed to salinity. The total number of DEGs varied among the salt and *P. indica*-treated comparison groups. There were fewer DEGs in the C vs Pi group as compared to other treatment groups. The total number of up- and down-regulated DEGs in each group were shown in Figs. 2 and 3. FPKM plots and heatmap for expressed genes of all comparison groups were shown in Supplementary Fig. S2, Fig. S3, and Fig. S4. To explore the potential functions of these genes, all the transcripts were annotated using UniProt databases.

GO and KEGG enrichment analysis

We employed GO enrichment analysis to identify the major gene groups affected by salt stress and beneficial interaction with *P. indica*. We observed 13 up- and 30 down-regulated GO terms that were over-represented (p-value ≤ 0.01) in Salt vs Salt + Pi group. The most enriched up-regulated terms in biological processes (BP), cellular component (CC), and molecular function (MF) categories were ion transmembrane transport (2 DEGs),

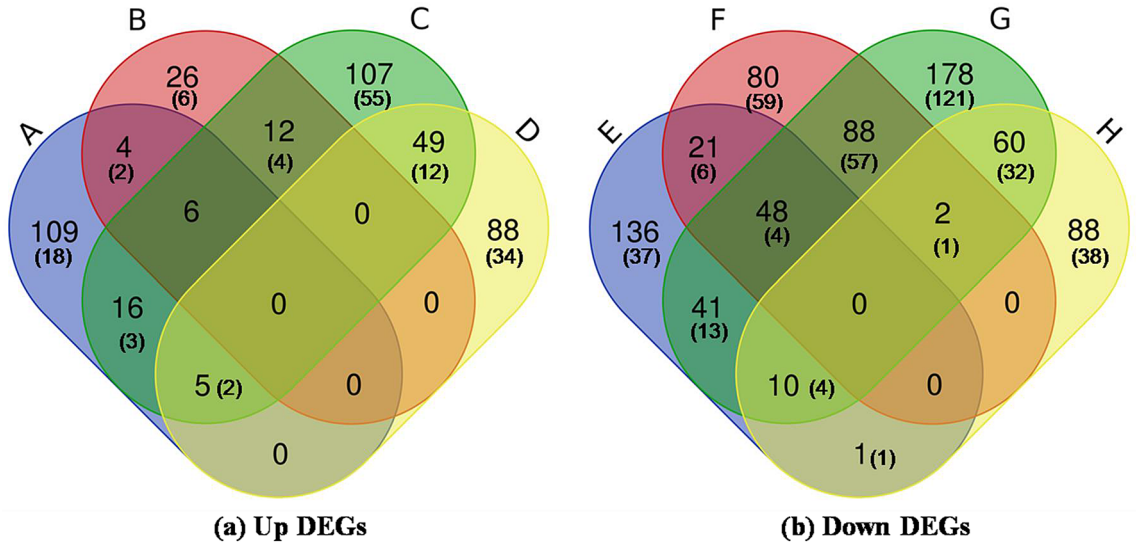

Fig. 3 Venn diagrams show common, salt and *P. indica* specific DEGs in four comparison groups. **a** Up-regulated DEGs and **b** down-regulated DEGs, where A, B, C, and D indicate all DEGs in C vs Pi, C vs Salt, C vs Salt + Pi, and Salt vs salt + Pi groups, respectively. DEGs with log2fold change (> two fold) are shown in brackets

integral component of plasma membrane (3 DEGs), and syn-copalyl diphosphate synthase activity (1 DEG), respectively. Whereas the enriched down-regulated terms in BP, CC, and MF categories were response to stress (30 DEGs), extracellular region (10 DEGs), and catalytic activity (25 DEGs), respectively (Fig. 4a, b). Next, the

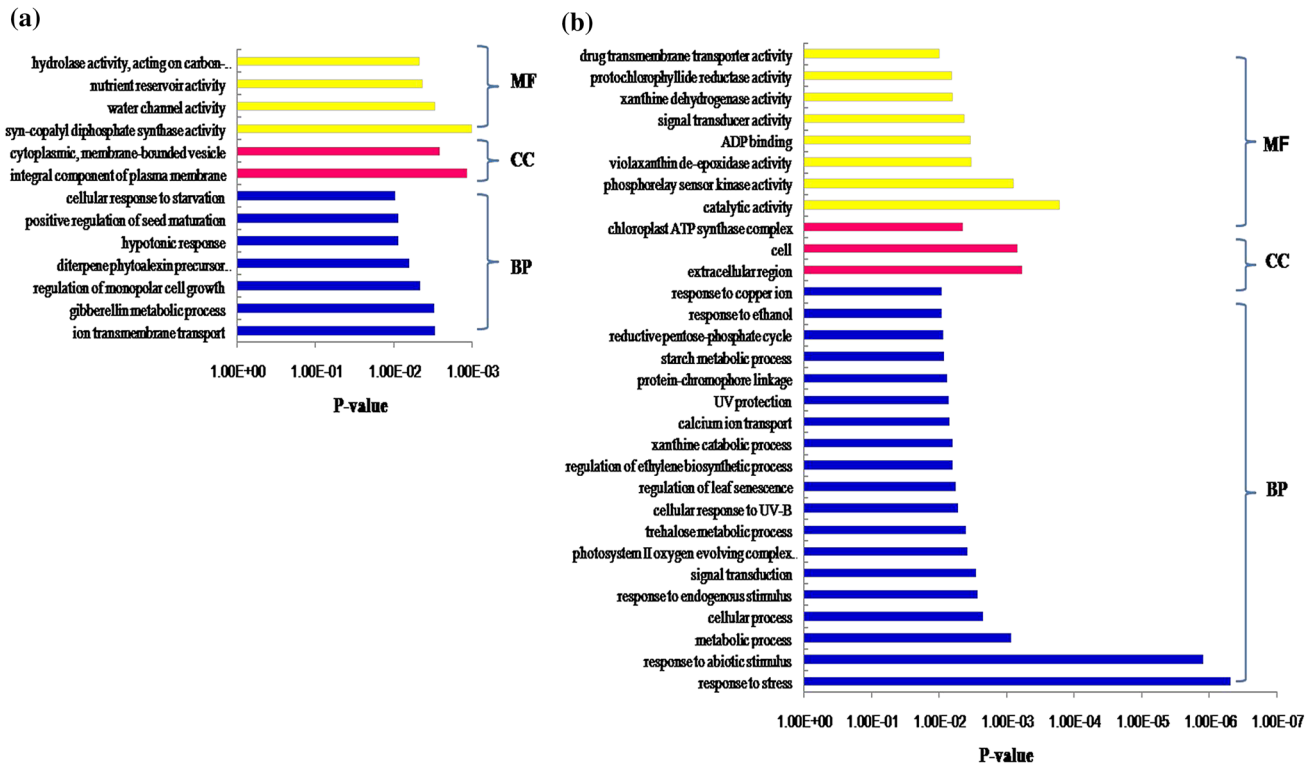

Fig. 4 GO enrichment of DEGs in Salt vs salt + Pi group showing enriched BP, CC and MF categories. **a**, **b** indicate the up and down GO terms, respectively

most enriched GO term in the C vs Pi group were cytoplasmic, membrane-bounded vesicle (17 DEGs) and lipid metabolic process (18 DEGs) in up- and down-regulated term, respectively (Supplementary Fig. S5). In the C vs Salt sample, the most enriched up-regulated and down-regulated GO term were response to stress with 11 and 47 DEGs, respectively (Supplementary Fig. S6). Further, we found most enriched GO terms in the C vs Salt + Pi group with cytoplasmic, membrane-bounded vesicle (27 DEGs) and response to stress (90 DEGs) in the up- and down-regulated GO terms, respectively (Supplementary Fig. S7).

Next, KEGG pathway analysis was performed to investigate the DEGs enriched in various metabolic pathways. In this analysis, 9 DEGs in C vs Pi, 4 DEGs in C vs Salt, 62 DEGs in C vs Salt + Pi group, and 9 DEGs in Salt vs Salt + Pi were classified into 4, 6, 7, and 7 functional categories, respectively. Carotenoid biosynthesis (9-*cis*-epoxycarotenoid dioxygenase 1, *LOC_Os02g47510*), cutin, suberin, and wax biosynthesis (*WAX2*, *LOC_Os02g40784.1* and *HOTHEAD*, *LOC_Os09g19930.1*), and fatty acid biosynthesis pathways (acyl-desaturase, *LOC_Os01g65830.1* and AMP-binding enzyme, *LOC_Os11g35400.1*) were significantly enriched among the up- and down-regulated DEGs in the C vs Pi group. In the C vs Salt group, the most enriched up- and down-regulated pathways were flavonoid biosynthesis (chalcone synthase, *LOC_Os11g32650.1*) and galactose metabolism (glycosyl hydrolases, *LOC_Os04g56930.1* and *LOC_Os04g45290.1*), respectively. Metabolic pathways (alpha-amylase, *LOC_Os09g28420.1*; dehydrogenase, *LOC_Os11g10510.1,* and others) biosynthesis of secondary metabolites (lactate/malate dehydrogenase, *LOC_Os06g01590.1*; 3-ketoacyl-CoA synthase, *LOC_Os02g49920.1,* and others), and glycolysis/gluconeogenesis (dehydrogenase, *LOC_Os11g10510.1*; pyruvate decarboxylase, *LOC_Os11g38910.1,* and others) were the most enriched pathways among the down-regulated DEGs in the C vs Salt + Pi group. However, no significant pathway was detected in up-regulated DEGs in the C vs Salt + Pi group. On the other hand, in the Salt vs Salt + Pi group, pathways related to zeatin biosynthesis (cytokinin dehydrogenase, *LOC_Os10g34230*), carotenoid biosynthesis (9-*cis*-epoxycarotenoid dioxygenase 1, *LOC_Os12g42280*), and diterpenoid biosynthesis (ent-kaurene synthase, *LOC_Os04g09900*) were up-regulated, whereas protein processing in endoplasmic reticulum (hsp20/alpha crystallin family protein, *LOC_Os01g04360* and *LOC_Os03g16020*), and phenylpropanoid biosynthesis (peroxidase, *LOC_Os03g25370* and beta-glucosidase, *LOC_Os09g31430*) were among the down-regulated DEGs (Table 3). The enrichment analysis results for all GO enrichment and KEGG pathways are provided in Online Resource 3.

Identification of DEGs related to transcription factors and transporters

Transcription factors are the regulatory proteins which play an important role in stress responses. In our data, a total of 36 transcripts encoding for transcription factors were identified, among which 7 TFs were up-regulated whereas 29 TFs were found to be down-regulated. A total of 3, 6, 17, and 10 TF genes showed differential expression in the C vs Pi, C vs salt, C vs Salt + Pi, and Salt vs Salt + Pi groups, respectively. Among the 14 families represented in these DEGs, the bHLH (7 DEGs) and ERF family (6 DEGs) TFs were the most abundant followed by G2-like (5 DEGs) family. Besides, NAC (3 DEGs), WRKY- domain (3 DEGs), and MYB (2 DEGs) TFs were also significantly represented among DEGs (Supplementary Table S2).

In order to maintain Na^+ homeostasis in plants, a variety of ion pumps and membrane proteins play a vital role. Here, we observed a total 33 DEGs encoding transporter proteins with 9 up-regulated and 24 down-regulated genes. Gene coding for amino acid transporter, aluminum-activated malate transporter, and auxin efflux carrier are up-regulated in the C vs Pi and C vs Salt + Pi groups. Further, nodulin MtN3 family protein, aquaporin protein, and peptide transporter *PTR2* genes were up-regulated in the Salt vs Salt + Pi group. Surprisingly, gene encoding potassium channels KAT1, high-affinity potassium transporters (OsHKT1, OsHKT2), aquaporin protein, major facilitator superfamily antiporter and inorganic phosphate transporter were among the down-regulated genes (Table 4).

DEGs related to signaling and phytohormones

Signaling cascade mediated by protein kinases (PKs) play a critical role in response to environmental stresses as well as in plant growth. We observed that genes related to receptor protein kinases (RPKs) and AGC kinases were up-regulated in Salt vs Salt + Pi groups. On the other hand, gene encoding wall-associated kinases (OsWAK9), RPK, PK, wall-associated receptor kinase, and AGC kinases were up-regulated in C vs Salt + Pi group. However, other genes coding for receptor-like kinases (RLKs), lectin receptor-like kinases (LecRKs), mitogen-activated protein kinases (MAPKKK), ACG kinases, and other kinases were down-regulated in all groups (Table 4).

The plant hormone signaling pathway is one of the crucial pathways which activate the required physiological changes during adverse conditions to ensure the plant growth and development [27]. Genes related to plant hormones biosynthesis and signaling, were also differentially expressed in response to salt and *P. indica*. We found that genes related to ent-kaurene synthase, 12-oxophytodienoate reductase, cytokinin dehydrogenase, and 9-*cis*-epoxycarotenoid

Table 3 Analysis of KEGG enrichment for DEGs in rice comparison groups

KEGG pathway	Number of genes	Pathway ID	p-value
C vs Pi			
Carotenoid biosynthesis	1	osa00906	0.04637
Diterpenoid biosynthesis	1	osa00904	0.04637
Cutin, suberine and wax biosynthesis	2	osa00073	0.00344
Fatty acid biosynthesis	2	osa00061	0.02096
Limonene and pinene degradation	1	osa00903	0.04572
Fatty acid metabolism	2	osa01212	0.04613
C vs Salt			
Flavonoid biosynthesis	1	osa00941	0.01762
Circadian rhythm—plant	1	osa04712	0.01854
Carotenoid biosynthesis	1	osa00906	0.02403
Galactose metabolism	2	osa00052	0.05066
C vs Salt + Pi			
Fatty acid elongation	3	osa00062	0.00297
Glycolysis/gluconeogenesis	6	osa00010	0.00395
Biosynthesis of secondary metabolites	18	osa01110	0.01011
Starch and sucrose metabolism	6	osa00500	0.01011
Cutin, suberine and wax biosynthesis	2	osa00073	0.01773
Propanoate metabolism	2	osa00640	0.03404
Metabolic pathways	25	osa01100	0.04814
Salt vs Salt + Pi			
Zeatin biosynthesis	1	osa00908	0.02775
Carotenoid biosynthesis	1	osa00906	0.02873
Diterpenoid biosynthesis	1	osa00904	0.03655
Protein processing in endoplasmic reticulum	2	osa04141	0.02648
Phenylpropanoid biosynthesis	2	osa00940	0.02876
Porphyrin and chlorophyll metabolism	1	osa00860	0.04717

dioxygenase were up-regulated in Salt vs Salt + Pi groups. Genes such as *OsIAA26*, *AIR12*, auxin efflux carrier, *OsSAUR50*, jasmonate-induced protein, phytosulfokines precursor were up-regulated in the C vs Salt + Pi group. Further, in the C vs Pi group, gibberellin receptor *GID1L2* and 9-*cis*-epoxycarotenoid dioxygenase were among the up-regulated DEGs. On the contrary, genes encoding OsSAUR10, cytokinin-*O*-glucosyltransferase 2, 1-aminocyclopropane-1-carboxylate oxidase, gibberellin receptor GID1L2, and 12-oxophytodienoate reductase were down-regulated in C vs Salt group (Table 4).

Identification of genes related to antioxidants and other stresses

Salt exposure can cause oxidative stress and induces various anti-oxidative enzymes along with the other stress-responsive genes. We observed that two genes related to peroxidase and glutaredoxin (OsGrx_C8) were up-regulated in C vs Salt + Pi group, whereas one gene related to peroxidase was up-regulated in Salt vs Salt + Pi groups (Table 4).

Among other stress and disease-related genes, SCP-like extracellular protein, chitinase (CHIT4), laccase and pathogenesis-related Bet v I family protein were induced exclusively in the C vs Pi group. Gene encoding OsSub12, legume lectins beta domain-containing protein, and RALFL28 were up-regulated in C vs Salt + Pi group, whereas OsSub12, antifreeze glycoprotein, and thaumatin related genes were up-regulated specifically in Salt vs Salt + Pi groups. However, many stress-related genes encoding salt stress root protein RS1, disease resistance protein, hypersensitive-induced response protein, chitinases (CHIT2, CHIT3), dehydration stress-induced protein, wound-induced protein (WIP3, WIP4, WIP5), and metallothionein were found to be down-regulated in all salt-treated group (Table 4).

Identification of cell wall-related DEGs

Cell wall dynamics play an important role in providing resistance to plants against biotic and abiotic stresses. Evidently, structural alteration in the cell wall during salt stress has been reported in many plants [28, 29]. A significant number of genes associated with cell wall modification

Table 4 Salinity responsive DEGs in four comparison groups of rice:

Gene ID	Fold change (log2)				Description
	C vs Pi	C vs salt	C vs salt + Pi	Salt vs salt + Pi	
Transporter					
LOC_Os11g09020	2.0788				Amino acid transporter, putative, expressed
LOC_Os02g14840	− 2.61381		− 2.29646		Potassium channel KAT1, putative, expressed
LOC_Os02g50799	− 3.36617				Nuclear-pore anchor, putative, expressed
LOC_Os04g44610	− 4.09353		− 4.23169		White-brown complex homolog protein 11, putative, expressed
LOC_Os04g34010		2.43852	2.73904		Aluminum-activated malate transporter, putative, expressed
LOC_Os01g65110		− 3.10861	− 4.93532		POT family protein, expressed
LOC_Os01g42090		− 2.96817		2.66589	Nodulin MtN3 family protein, putative, expressed
LOC_Os02g30910		− 2.96512	− 3.61978		Nodulin MtN3 family protein, putative, expressed
LOC_Os02g43410		− 3.00967	− 4.41244		Transposon protein, putative, unclassified, expressed
LOC_Os04g10690		− 2.1534			Inorganic phosphate transporter, putative, expressed
LOC_Os04g44060		− 2.27294	− 2.07218		Aquaporin protein, putative, expressed
LOC_Os06g48810		− 3.44672	− 4.45281		OsHKT2;1—Na + transporter, expressed
LOC_Os07g26660		− 2.09793	− 2.16435		Aquaporin protein, putative, expressed
LOC_Os11g04020		− 2.45533	− 2.91195		Major facilitator superfamily antiporter, putative, expressed
LOC_Os12g03830		− 2.17869	− 3.56899		Major facilitator superfamily antiporter, putative, expressed
LOC_Os11g04190			2.09004		Auxin efflux carrier component, putative, expressed
LOC_Os09g33830			− 2.74704		Solute carrier family 35 member F1, putative, expressed
LOC_Os01g45990			− 2.72344		q
LOC_Os01g65880			− 2.0225		Nodulin MtN3 family protein, putative, expressed
LOC_Os01g71710			− 2.26153		Amino acid permease family protein, putative, expressed
LOC_Os02g43370			− 2.4058		Transposon protein, putative, unclassified, expressed
LOC_Os02g46680			− 3.17241		Multidrug resistance protein, putative, expressed
LOC_Os03g09970			− 3.055		Sulfate transporter, putative, expressed
LOC_Os03g54000			− 2.41503		Oligopeptide transporter, putative, expressed
LOC_Os04g51820			− 2.07325		OsHKT1;1—Na + transporter, expressed
LOC_Os10g40600			− 2.08961		Peptide transporter PTR2, putative, expressed
LOC_Os11g03240			− 2.65461	− 3.24813	MATE efflux family protein, putative, expressed
LOC_Os08g43800			2.05793		Carrier, putative, expressed
LOC_Os02g13870				2.28334	Aquaporin protein, putative, expressed
LOC_Os03g60850				2.20987	Peptide transporter PTR2, putative, expressed
LOC_Os04g50940				2.15662	Peptide transporter PTR2, putative, expressed
LOC_Os03g09970				− 2.96998	Sulfate transporter, putative, expressed
LOC_Os11g03484				− 2.3096	MATE efflux family protein, putative, expressed
Phytohormones					
LOC_Os11g04190			2.09004		Auxin efflux carrier component, putative, expressed
LOC_Os09g35870			2.13389		OsIAA26—Auxin-responsive Aux/IAA gene family member, expressed
LOC_Os12g12720			2.32694		Jasmonate-induced protein, putative, expressed
LOC_Os08g41290			2.0474		AIR12, putative, expressed
LOC_Os02g30810		− 2.23541	− 2.19263		OsSAUR10—Auxin-responsive SAUR gene family member, expressed
LOC_Os02g36830		− 2.1959	− 2.04333		Cytokinin-O-glucosyltransferase 2, putative, expressed
LOC_Os04g51190			− 3.09181		Growth-regulating factor, putative, expressed
LOC_Os11g08380			− 2.10313		1-aminocyclopropane-1-carboxylate oxidase, putative, expressed
LOC_Os07g44850		− 2.69862			Gibberellin receptor GID1L2, putative, expressed
LOC_Os06g11200		− 2.12112			12-oxophytodienoate reductase, putative, expressed
LOC_Os07g41590	2.08998				Gibberellin receptor GID1L2, putative, expressed
LOC_Os06g42680			3.08149		Phytosulfokines 1 precursor, putative, expressed

Table 4 (continued)

Gene ID	Fold change (log2)				Description
	C vs Pi	C vs salt	C vs salt + Pi	Salt vs salt + Pi	
LOC_Os02g47510	2.06033				9-*cis*-epoxycarotenoid dioxygenase 1, chloroplast precursor, putative, expressed
LOC_Os04g09900				2.44931	Ent-kaurene synthase, chloroplast precursor, putative, expressed
LOC_Os06g11200				2.39574	12-Oxophytodienoate reductase, putative, expressed
LOC_Os10g34230				2.41441	Cytokinin dehydrogenase precursor, putative, expressed
LOC_Os12g42280				2.09456	9-*cis*-epoxycarotenoid dioxygenase 1, chloroplast precursor, putative, expressed
Stress/disease related genes					
LOC_Os01g28450	2.3277				SCP-like extracellular protein, expresse
LOC_Os03g30470	2.27246				CHIT4—Chitinase family protein precursor, expressed
LOC_Os10g20610	2.24038				Laccase-15 precursor, putative, expressed
LOC_Os12g36880	2.42656				Pathogenesis-related Bet v I family protein, putative, expressed
LOC_Os01g38229	− 4.51714				Peptidyl-prolyl isomerase, putative, expressed
LOC_Os01g40560		− 3.93062	− 2.17239		Hypersensitive-induced response protein, putative, expressed
LOC_Os04g41620		− 2.87011			CHIT2—Chitinase family protein precursor, expressed
LOC_Os04g41680		− 3.34787	− 2.7598		CHIT3—Chitinase family protein precursor, expressed
LOC_Os10g21790		− 3.50086	− 2.15527		Dehydration stress-induced protein, putative, expressed
LOC_Os11g37950		− 3.26908	− 3.37545		WIP3—Wound-induced protein precursor, expressed
LOC_Os11g37960		− 2.42526			WIP4—Wound-induced protein precursor, expressed
LOC_Os11g37970		− 3.36757	− 2.06798		WIP5—Wound-induced protein precursor, expressed
LOC_Os01g13210			− 2.13877		Salt stress root protein RS1, putative, expressed
LOC_Os01g25720			− 2.21237		Disease resistance protein RGA4, putative, expressed
LOC_Os01g25740			− 2.29708		Powdery mildew resistance protein PM3F, putative, expressed
LOC_Os02g38386			− 2.55406		NBS-LRR disease resistance protein, putative, expressed
LOC_Os02g46680			− 3.17241		Multidrug resistance protein, putative, expressed
LOC_Os04g48350			− 3.1595		Dehydration-responsive element-binding protein, putative, expressed
LOC_Os04g54240			− 2.60229		Wound induced protein, putative, expressed
LOC_Os06g17950			− 2.51958	− 2.24379	NBS-LRR disease resistance protein, putative, expressed
LOC_Os06g48520			− 2.62551		Disease resistance protein RPM1, putative, expressed
LOC_Os08g32880			− 2.52435		Disease resistance protein RPM1, putative, expressed
LOC_Os09g14490			− 2.16511		TIR-NBS type disease resistance protein, putative, expressed
LOC_Os04g41640			− 5.48263		HEV2—Hevein family protein precursor, expressed
LOC_Os10g04110			− 2.94825		MLA6 protein, putative, expressed
LOC_Os02g10520			2.9991	2.60218	OsSub12—Putative Subtilisin homologue, expressed
LOC_Os04g01950			2.29645		legume lectins beta domain containing protein, putative, expressed
LOC_Os06g29730			2.02086		RALFL28—Rapid ALkalinization Factor RALF family protein precursor, expressed
LOC_Os01g69290				2.68168	Antifreeze glycoprotein, putative, expressed
LOC_Os12g43450				2.95388	Thaumatin family domain containing protein, expressed
LOC_Os01g06870				− 2.00922	Resistance protein SlVe1 precursor, putative, expressed
LOC_Os02g45450				− 2.18529	Dehydration-responsive element-binding protein, putative, expressed
LOC_Os04g48350				− 2.94756	Dehydration-responsive element-binding protein, putative, expressed
LOC_Os06g47800				− 2.33465	Disease resistance protein RGA3, putative, expressed
LOC_Os11g12050				− 2.03769	NBS-LRR type disease resistance protein, putative, expressed
LOC_Os12g38051				− 2.32468	Metallothionein, putative, expressed
LOC_Os12g38290				− 2.58327	Metallothionein, putative, expressed

Table 4 (continued)

Gene ID	Fold change (log2)				Description
	C vs Pi	C vs salt	C vs salt + Pi	Salt vs salt + Pi	
LOC_Os12g38300				− 2.38779	Metallothionein, putative, expressed
Oxidative stress related genes					
LOC_Os03g44170	− 2.76481		− 3.0338		Glutathione *S*-transferase, putative, expressed
LOC_Os07g48040		2.44551		− 2.35065	Peroxidase precursor, putative, expressed
LOC_Os01g18970		− 3.45926	− 3.82462		Peroxidase precursor, putative, expressed
LOC_Os07g01420		− 2.24421	#VALUE!		Peroxidase precursor, putative, expressed
LOC_Os07g31610		− 3.1358			Peroxidase precursor, putative, expressed
LOC_Os02g30850			2.03845		OsGrx_C8—glutaredoxin subgroup III, expressed
LOC_Os12g02060			2.25782		Peroxidase precursor, putative, expressed
LOC_Os01g72140			− 2.002		Glutathione S-transferase, putative, expressed
LOC_Os06g48020			− 3.87125		Peroxidase precursor, putative, expressed
LOC_Os07g01410			− 2.0116		Peroxidase precursor, putative, expressed
LOC_Os07g48010			− 2.17931		Peroxidase precursor, putative, expressed
LOC_Os10g38580			− 2.06434		Glutathione *S*-transferase, putative, expressed
LOC_Os10g38780			− 2.64911		Glutathione *S*-transferase, putative, expressed
LOC_Os10g39160			− 2.9655		Peroxidase precursor, putative, expressed
LOC_Os08g32840			− 2.19559		Bifunctional monodehydroascorbate reductase and carbonic anhydrasenectarin-3 precursor
LOC_Os01g73200				2.47063	Peroxidase precursor, putative, expressed
Protein kinases					
LOC_Os06g38830		2.44252			Receptor-like protein kinase precursor, putative, expressed
LOC_Os03g16260		− 2.82823			Protein kinase, putative, expressed
LOC_Os04g56430		− 2.86479			Cysteine-rich receptor-like protein kinase, putative, expressed
LOC_Os01g26300			2.22788		OsWAK9—OsWAK receptor-like cytoplasmic kinase OsWAK-RLCK, expressed
LOC_Os01g57510			2.03103		Receptor protein kinase, putative, expressed
LOC_Os04g01890			2.35511		Protein kinase, putative, expressed
LOC_Os08g24630			2.65161		Protein kinase domain containing protein, expressed
LOC_Os12g29434			3.08948		Wall-associated receptor kinase 3 precursor, putative, expressed
LOC_Os12g42020			2.1836	2.03758	AGC_PVPK_like_kin82y.20—ACG kinases include homologs to PKA, PKG and PKC, expressed
LOC_Os01g60060			− 3.25285	− 2.31109	Leucine-rich repeat family protein, putative, expressed
LOC_Os02g13510			− 2.33426		Receptor-like protein kinase 5 precursor, putative, expressed
LOC_Os02g53030			− 3.13135	− 2.75594	Mitogen-activated protein kinase kinase kinase 1, putative, expressed
LOC_Os03g56160			− 2.0162		Lectin-like receptor kinase 7, putative, expressed
LOC_Os05g16920			− 2.61052		SHR5-receptor-like kinase, putative, expressed
LOC_Os06g14260			− 2.14477		Lectin-like receptor kinase, putative, expressed
LOC_Os11g01140			− 2.67218	− 2.26715	AGC_PVPK_like_kin82y.18—ACG kinases include homologs to PKA, PKG and PKC, expressed
LOC_Os12g01140			− 2.52297	− 2.15338	AGC_PVPK_like_kin82y.2—ACG kinases include homologs to PKA, PKG and PKC, expressed
LOC_Os04g01980				2.05692	Receptor protein kinase, putative, expressed
LOC_Os02g06150				− 2.63883	Brassinosteroid LRR receptor kinase precursor, putative, expressed
LOC_Os06g35850				− 2.38377	Lectin protein kinase family protein, putative, expressed
LOC_Os08g27810				− 2.76807	OsWAK115—OsWAK receptor-like protein OsWAK-RLP, expressed
LOC_Os10g22890				− 2.73169	Receptor-like kinase, putative, expressed

Table 4 (continued)

Gene ID	Fold change (log2)				Description
	C vs Pi	C vs salt	C vs salt + Pi	Salt vs salt + Pi	
Cell wall related genes					
LOC_Os06g47360	3.36366				Beta-expansin precursor, putative, expressed
LOC_Os09g29710	2.33895				Beta-expansin precursor, putative, expressed
LOC_Os06g21410	2.04315		2.4412		Arabinogalactan peptide 23 precursor, putative, expressed
LOC_Os08g24750		− 2.59974			Xyloglucan fucosyltransferase, putative, expressed
LOC_Os01g71670		− 3.51594			Glycosyl hydrolases family 17, putative, expressed
LOC_Os01g71820		− 2.11408	− 2.45131		Glycosyl hydrolases family 17, putative, expressed
LOC_Os01g71860		− 2.9985			Glycosyl hydrolases family 17, putative, expressed
LOC_Os04g56930		− 2.03331	− 2.37388		Glycosyl hydrolases, putative, expressed
LOC_Os05g15880		− 4.94042	− 2.6345	2.30592	Glycosyl hydrolase, putative, expressed
LOC_Os11g47500		− 2.28966	− 2.76625		Glycosyl hydrolase, putative, expressed
LOC_Os04g57860		− 2.6923			Endoglucanase precursor, putative, expressed
LOC_Os10g31660		− 2.24074			Glycine-rich cell wall structural protein 2 precursor, putative, expressed
LOC_Os10g31330			2.51234		Glycine-rich cell wall structural protein 2 (Glycine-rich protein 1) (GRP-1)
LOC_Os02g16730			− 2.77289		EXPANSIN precursor, putative, expressed
LOC_Os04g46630			− 2.65249	− 2.10962	Expansin precursor, putative, expressed
LOC_Os01g66830			− 2.18769	− 2.13861	pectinacetylesterase domain containing protein, expressed
LOC_Os01g20980			2.41771		Pectinesterase, putative, expressed
LOC_Os04g01570			2.53233	2.0369	Invertase/pectin methylesterase inhibitor family protein, putative, expressed
LOC_Os05g01380			2.30567		Polygalacturonase inhibitor precursor, putative, expressed
LOC_Os04g27980			− 3.57982	− 2.73879	Glycosyl hydrolase, putative, expressed
LOC_Os09g31430			− 2.46894	− 2.8203	Os9bglu30—beta-glucosidase, similar to Os4bglu12 exoglucanase, expressed
LOC_Os10g17650			− 3.18935		Os10bglu34—beta-glucosidase homologue, similar to Os3bglu6, expressed
LOC_Os07g36750			2.33883		CSLF3—cellulose synthase-like family F; beta1,3;1,4 glucan synthase, expressed
LOC_Os01g48540			3.18072		Glyoxal oxidase-related, putative, expressed
LOC_Os12g12290			2.12665		Exostosin family domain containing protein, expressed
LOC_Os08g37630			2.69785	2.46664	Elastin precursor, putative, expressed
LOC_Os12g38100			2.28655		Membrane associated DUF588 domain containing protein, putative, expressed
LOC_Os02g52700				2.75714	Alpha-amylase precursor, putative, expressed
LOC_Os07g41650				2.17969	Pectinesterase, putative, expressed

and metabolism have been observed to be differentially expressed in our study. Two genes encoding beta-expansion and one arabinogalactan peptide were up-regulated in C vs Pi. Genes involved in cell wall modification such as pectinesterase, pectin methylesterase inhibitor, polygalacturonase inhibitor, arabinogalactan peptide 23, glycin-rich cell wall structural protein 2, *CSLF3*, glyoxal oxidase, exostosin family domain-containing protein, elastin precursor, and membrane-associated DUF588 domain-containing protein were up-regulated in the C vs Salt + Pi and Salt vs Salt + Pi groups. On the contrary, several genes related to glycosyl hydrolase, endoglucanase, and beta-glucosidase were down-regulated in the salt-treated groups (Table 4).

Genes involved in secondary metabolism and other processes

We, further, observed that genes related to diterpenoid metabolisms such as terpene synthase and ent-kaurene synthase were overexpressed in the C vs Pi and Salt vs Salt + Pi groups respectively. Several genes related to lipid transfer proteins or lipid transfer protein-like (LTPs or LTPL),

metallothionein, thaumatin, cytochrome P450 and others were responded to salt stress and *P. indica* in this data. We noticed that genes belong to LTPL121 and LTPL101 protease inhibitor/seed storage/LTP family protein were up-regulated in C vs Pi and C vs Salt + Pi groups, respectively. Apart from that, genes related to ubiquitin, Pollen Ole e I allergen and extensin family protein, and thaumatin were induced in the C vs Salt + Pi and Salt vs Salt + Pi groups (Online Resource 2).

RNA-Seq data validation

To validate the gene expression patterns obtained by RNA-Seq, we conducted qRT-PCR on 11 selected salt-responsive DEGs correspond to each condition. The genes were selected from DEGs that were expressed in at least one of the comparison group including both up- and down-regulated genes. The results of qRT-PCR experiment for the selected genes are shown in Fig. 5 and Online Resource 4. We observed that the relative expression patterns of the qRT-PCR results were consistent with the RNA-Seq data.

Discussion

Salinity stress is destructive to the plant growth and affects crop production worldwide [2]. Recent studies have provided significant evidence regarding the mechanism of stress regulation in plants emphasizing morphological, physiological, and molecular responses [30, 31]; however, exploitable results on plant resilience are still relatively limited.

In our study, rice colonized with *P. indica* has shown higher growth in comparison to the plants without *P. indica* during high salt stress (0.25 M NaCl). We also observed that the significant reduction in MDA content in *P. indica* inoculated rice during salt stress suggesting that *P. indica* helps the plant to overcome the harmful effect of salinity induced lipid peroxidation. We, further, employed an RNA-Seq based transcriptomic approach to investigate the gene expression in rice in response to salt stress and beneficial interaction with *P. indica*. In our transcriptome analysis, overall, 235.94 million clean reads were generated from four samples and a total of 1610 DEGs were identified in the four comparison groups. GO enrichment analysis of DEGs revealed that ion membrane transport, response to stress, endogenous stimulus, flavonoid biosynthetic process, secondary metabolic process, auxin-activated signaling pathway, and oxidoreductase activity were activated in response to *P. indica* during salt stress. KEGG analysis revealed the

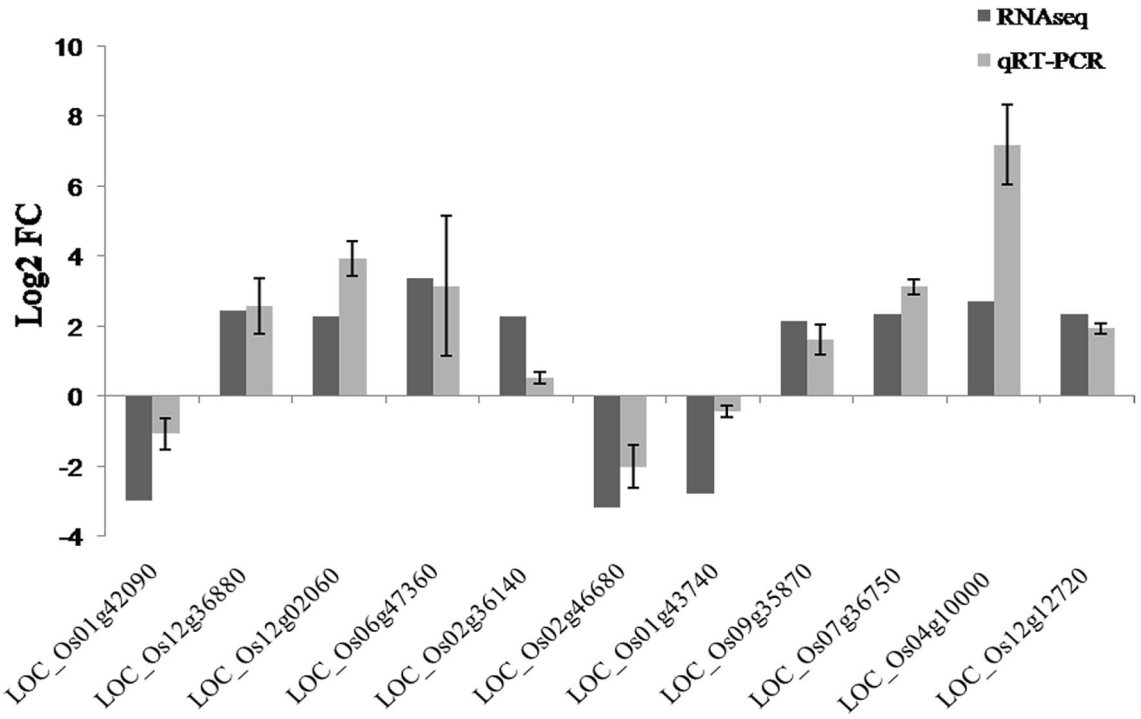

Fig. 5 Validation of RNA-Seq results by using qRT-PCR analysis. We selected 11 genes from all the treated rice groups for real-time PCR and used rice actin2 gene as an endogenous reference. Values are means of three replicates and the bar represents the ± SD

pathways that were mainly involved in zeatin biosynthesis, carotenoid biosynthesis, diterpenoid biosynthesis, flavonoid biosynthesis, fatty acid elongation, biosynthesis of secondary metabolites and cutin, suberine, and wax biosynthesis in response to *P. indica* and salt stress. These observations imply that the coordinated response of gene networks involved in various metabolic pathways related to transport, plant hormone signaling, stress responses, secondary metabolites biosynthesis etc. is crucial for the plant adaptation to environmental stresses as reported previously [32, 33].

Transcription factors control the expression of numerous target genes during stress. Here, in *P. indica* colonized rice, genes related to ERF, bZIP and WRKY TF family are induced during salt stress which are involved in the regulation of many hormones and stress-responsive genes [33]. High salt stress results in an intracellular ionic imbalance due to excess of sodium ion (Na^+). The synchronized action of various ion pumps help in the exclusion of excess Na^+ ions and vacuolar compartmentalization thereby preventing ionic toxicity in the cytosol. In this study, the activation of the transporter genes such as aquaporin, peptide transporter *PTR2*, and nodulin MtN3 family protein in Salt vs Salt + Pi group suggests their role in the maintenance of water flow and plant growth in the presence of beneficial interaction during salt stress [34, 35]. An *ALMT* gene (*LOC_Os04g34010*) is up-regulated in C vs Salt + Pi group which has considered important for plant tolerance to abiotic stress as well as in stomatal opening and GABA signaling [36]. Genes related to OsHKT1;1, OsHKT2;1, potassium channel KAT1, aquaporin protein, major facilitator superfamily antiporter were down-regulated in our data in contrast to previous studies [37, 38]. However, in some reports, genes for OsHKT1 and OsAKT1 were found to be down-regulated after the osmotic shock and salt stress [39, 40].

Protein kinases participate in many plant signal transduction pathways and play a crucial role in response to environmental stresses as well as plant development [41]. Here, the up-regulation of genes related to wall-associated kinases (OsWAK9), AGC kinases, RPKs, and PKs in the C vs Salt + Pi and Salt vs Salt + Pi groups implicating their role in cell wall expansion and activation of stress responses [42]. Gene related to AGC kinases (*LOC_Os12g42020*) or barren inflorescence 2 was reported to be involved in salt tolerance in rice seedlings [43]. Our result suggests that these protein kinases might regulate fungal invasion inside the root cortex by regulating cell wall-cytoskeleton interface, priming defense/immune system of the plant thereby conferring resistance against stresses.

The interplay between plant hormone signal transduction and stress signaling pathways confers plant acclimatization to adverse conditions. Studies revealed that symbiotic interaction with *P. indica* affects the plant hormone regulation and homeostasis and is required for plant growth and immunity [18, 44, 45]. We observed the overexpression of a jasmonate-induced protein (*LOC_Os12g12720*) in the C vs Salt + Pi group which indicates the increase in JA activity. Further, a gene encoding 12-oxophytodienoate reductase which is involved in jasmonic acid (JA) biosynthesis was found to be induced in Salt vs Salt + Pi. An increase in JA has been observed in tomato, rice, and other plants during salt stress and might be responsible for reduced photosynthesis activity [46, 47]. Abscisic acid (ABA) is a phytohormone that contributes to plant survival under hostile conditions [48]. Two genes related to 9-*cis*-epoxycarotenoid dioxygenase 1 involved in ABA biosynthesis were found to be up-regulated in C vs Pi and Salt vs Salt + Pi groups. Two genes encoding gibberellin receptor GID1L2 which are involved in the gibberellins signaling pathway, and gene encoding ent-kaurene synthase (*LOC_Os04g09900*), a key enzyme in gibberellic acid (GA) synthesis, were up-regulated in the C vs Pi and Salt vs Salt + Pi groups, respectively. It was reported that the gibberellin receptor is required for *P. indica* host colonization and GA induced defense and metabolism [18]. Further, genes involved in ethylene biosynthesis and cytokinin signaling (1-aminocyclopropane-1-carboxylate oxidase and cytokinin-O-glucosyltransferase 2) were down-regulated. Also, a cytokinin degrading enzyme, cytokinin dehydrogenase, was up-regulated in response to *P. indica* and salt stress indicating that the ethylene and cytokinin signaling might be compromised in the presence of high salt and endophytic colonization which is essentially required for stress tolerance [18, 27]. Furthermore, several auxin gene family members were induced in C vs Salt + Pi group such as *OsIAA26* (*LOC_Os09g35870*), putative auxin-induced protein (*LOC_Os08g41290*), auxin efflux carrier (*LOC_Os11g04190*) and *OsSAUR50* (*LOC_Os09g37452*). A gene code for *OsIAA6* was reported to be induced in rice under high salt and drought conditions [49]. Similarly, the Arabidopsis *SAUR41* subfamily genes were shown to regulate cell expansion during salinity stress [50] which further supports our results. The differential expression pattern of phytohormones might explain that *P. indica* actively involved in the modulation of hormonal crosstalk in rice enabling plant growth as well as immunity to cope with salt stress.

Further, we observed that many stress and disease-related genes such as laccase, chitinase, and pathogenesis-related Bet v I family proteins were induced in C vs Pi group. Moreover, genes related to OsSub12 (*LOC_Os02g10520*), thaumatin (*LOC_Os12g43450*), legume lectins beta domain-containing protein (*LOC_Os04g01950*), and RALFL28 (*LOC_Os06g29730*) were induced in the C vs Salt + Pi and Salt vs Salt + Pi groups. These genes play essential roles in plant immunity, pathogen recognition, defense, abiotic stress, and flower development which suggests that *P. indica* influences these genes to provide tolerance against salt stress [51–53]. On the other hand, several genes related to WIP,

chitinase, hypersensitive-induced response protein, salt stress root protein RS1, and disease-related proteins were shown down-regulation upon salt treatment insinuating that *P. indica* might be regulating the expression of these genes in order to lower the defense response to prevent further damage by salt stress. Apart from this, genes such as peroxidase and glutaredoxin (*OsGrx*) were among the up-regulated DEGs in the C vs Salt + Pi and Salt vs Salt + Pi groups. This result suggests that these antioxidants help in alleviating the toxic effects of oxidative stress in salt-stressed rice during symbiotic interaction.

Cell wall architecture is a crucial factor in stress-responsive signaling pathways. Cell wall proteins and cell wall-modifying enzymes such as xyloglucan endo-β-transglucosylases/hydrolases, expansins, and pectin acetylesterase are involved in modulating cell wall extensibility and plasticity [54–56]. In this transcriptome profile, we observed that genes encoding pectinesterase (*LOC_Os01g20980*, *LOC_Os07g41650*), pectin methylesterase inhibitor (PMEIs, *LOC_Os04g01570*), polygalacturonase inhibitor (PGIPs, *LOC_Os05g01380*), alpha-amylase (*LOC_Os02g52700*), and arabinogalactan proteins (OsAGP, *LOC_Os06g21410* and *LOC_Os08g37630*) were up-regulated in C vs Pi, C vs Salt + Pi and Salt vs Salt + Pi group. The activities of PGIPs and PMEIs are important to maintain the pectin level for cell wall rigidity during disease and salt stress as evident by earlier studies [28, 57]. Arabinogalactan protein possesses adhesive and water-holding properties, reported to be induced upon salt exposure [58, 59]. Further, the activation of genes related to cellulose synthase-like protein (*LOC_Os07g36750*) glycin-rich cell wall structural protein 2 (*LOC_Os10g31330*), exostosin family domain-containing protein (*LOC_Os12g12290*), and membrane-associated DUF588 domain-containing protein (*LOC_Os12g38100*) indicates their involvement in the regulation of ROS, cell differentiation and development, and cell wall integrity during stress condition [60–62]. In the C vs Pi group, genes related to beta-expansin (*LOC_Os06g47360* and *LOC_Os09g29710*), acanthoscurrin-1 (*LOC_Os07g09620*) and arabinogalactan (*LOC_Os06g21410*) were found to be induced. The *P. indica* colonized rice seems to undergo a major cell wall alteration under high salinity by influencing the expression of cell wall-modifying proteins and thereby maintaining the wall-plasma membrane continuum to provide the necessary protection against stress.

Furthermore, many genes related to cytochrome P450 were up-regulated in *P. indica* treated roots which regulate the plant growth and defense mechanism during salt stress by catalyzing secondary metabolites, regulation of plant hormone metabolism, and other metabolic processes as evident by previous research [45, 63]. Apart from this, many genes related to ubiquitin, LTPLs, and pollen Ole e I allergen and extensin family protein, were also showed altered expression in salt and *P. indica* treated rice. These genes involved in plant development, defense and stress regulation, indicating rice-*P. indica* symbiotic system employed the unique coping mechanism against higher salinity by modulating the functions of these genes [64, 65]. Also, a gene related to lipase (*LOC_Os01g51360*) that involved in lipid peroxidation, was downregulated in Salt vs Salt + Pi group, which further supports our result that *P. indica* can alleviate the process of lipid peroxidation. Due to low transcript expression, many previously studied salt-responsive genes could not be included in our study except one gene related to osmolyte (TPP, *LOC_Os02g44235*).

Based on the above results, we proposed a schematic model of the signaling cascades involving important rice DEGs in response to high salt stress and *P. indica* interaction (Fig. 6). Since most of the genes showed down-regulation, the common protection mechanism of rice against salt stress seems to be controlled in a specific manner at the higher salinity condition. However, *P. indica* colonized rice seem to follow the pathways that rely on maintaining cell wall integrity and plant growth which is consistent with the improved growth performance in the Salt + Pi treated rice.

Conclusion

We conclude that the transcriptome of *P. indica* colonized rice root under salt stress revealed the expression variations among several genes with emphasis on the genes related to cell wall remodeling, phytohormones, and receptor-like kinases, suggesting *P. indica* positively influences the plant growth during salt stress. Functional annotation of salt-responsive DEGs revealed the intricate networks of biological processes and metabolic pathways that might be crucial for growth and stress tolerance in rice. Several DEGs were identified in this study such as RALFL28 (*LOC_Os06g29730*), membrane-associated DUF588 domain-containing protein (*LOC_Os12g38100*), and pollen Ole e I allergen and extensin family protein (*LOC_Os12g28770*) which were not clearly described earlier for their role in symbiotic interaction during stress. Further functional validation of these genes will establish their role in fungal mediated stress response in plants. The results demonstrated here, offer a fresh perspective into the activation and regulation of salt tolerance mechanisms during plant–microbe beneficial interaction and provide a valuable addition to the genetic improvement of plant development and salt tolerance.

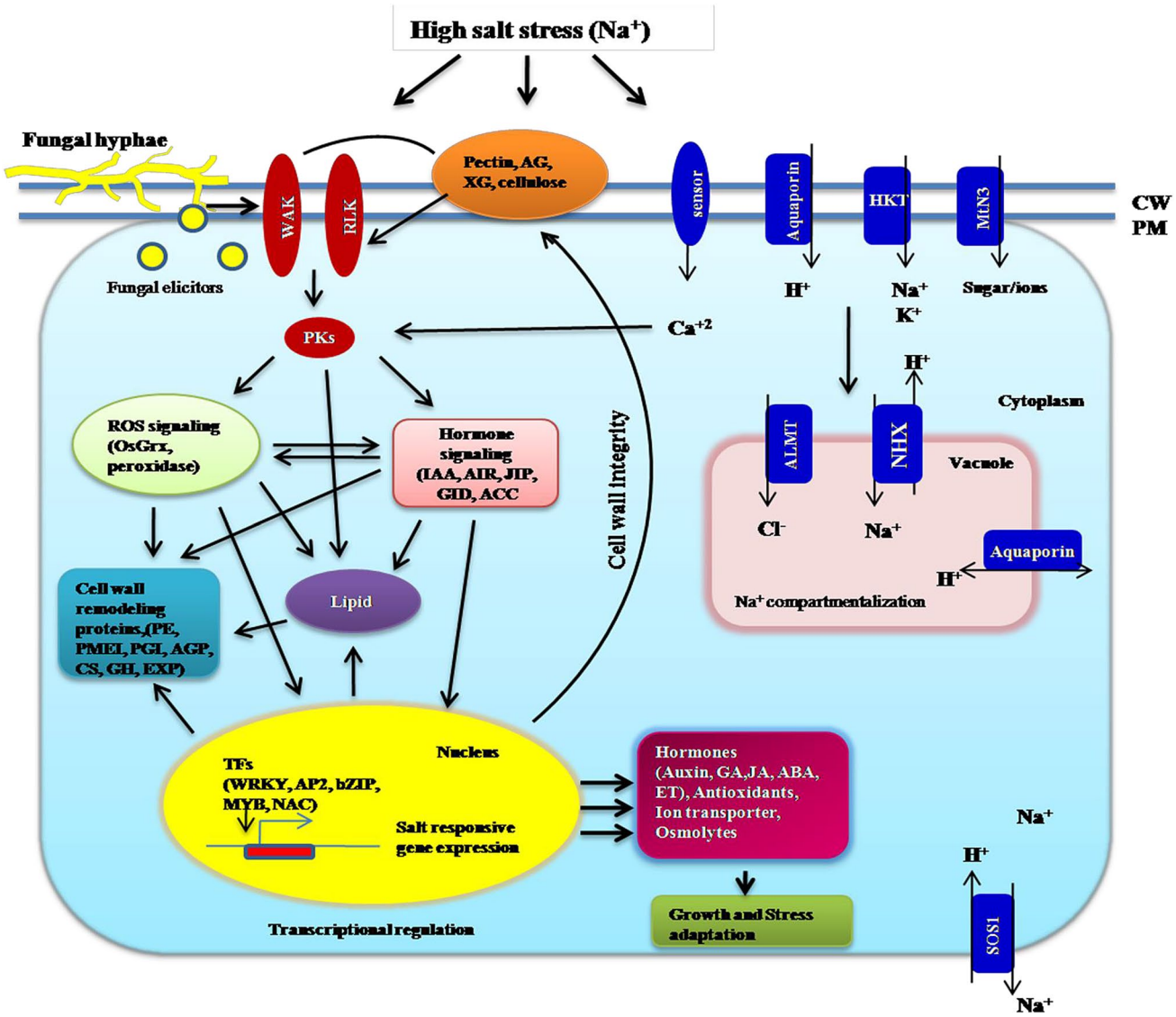

Fig. 6 A schematic representation of cell signaling cascade based on differential gene expression pattern in *P. indica* colonized roots in response to salt stress. (Genes related to SOS1 (Na^+/H^+ antiporter), NHX (Na^+/H^+ exchanger), and Ca^{+2} sensor were not detected in the present study and only included in the model for easier explanation)

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Author contributions N conducted the experiments and wrote the initial drafts of manuscript. RKG performed the data analysis, and helped in the manuscript editing. SK contributed in performing quantitative RT-PCR for data validation. SI and KA contributed in sample collection and RNA extraction. N and MZA planned and designed the research, evaluated the scientific implications of the transcriptome data, and prepared the final maunscript. All author approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights The present study did not involve any Human participants and/or Animals.

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