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RESEARCH

Growth and protein response of rice plant with plant growth-promoting rhizobacteria inoculations under salt stress conditions

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Abstract

Soil salinity has been one of the significant barriers to improving rice production and quality. According to reports, *Bacillus* spp. can be utilized to boost plant development in saline soil, although the molecular mechanisms behind the interaction of microbes towards salt stress are not fully known. Variations in rice plant protein expression in response to salt stress and plant growth-promoting rhizobacteria (PGPR) inoculations were investigated using a proteomic method and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Findings revealed that 54 salt-responsive proteins were identified by mass spectrometry analysis (LC–MS/MS) with the *Bacillus* spp. interaction, and the proteins were functionally classified as gene ontology. The initial study showed that all proteins were labeled by mass spectrometry analysis (LC–MS/MS) with *Bacillus* spp. interaction; the proteins were functionally classified into six groups. Approximately 18 identified proteins (up-regulated, 13; down-regulated, 5) were involved in the photosynthetic process. An increase in the expression of eight up-regulated and two down-regulated proteins in protein synthesis known as chaperones, such as the 60 kDa chaperonin, the 70 kDa heat shock protein BIP, and calreticulin, was involved in rice plant stress tolerance. Several proteins involved in protein metabolism and signaling pathways also experienced significant changes in their expression. The results revealed that phytohormones regulated the manifestation of various chaperones and protein abundance and that protein synthesis played a significant role in regulating salt stress. This study also described how chaperones regulate rice salt stress, their different subcellular localizations, and the activity of chaperones.

Keywords LCMS/MS mass spectrometry · PGPR · Proteomics · Rice · Salinity · SDS-PAGE

Introduction

Rice (*Oryza sativa* L.) is a crucial cereal that feeds more than half the world's population. Soil salinity is a major abiotic factor affecting the production of rice worldwide. Growing soil salinity causes crop productivity to decline in many places of the world (Kumar et al. 2020). Salt stress causes ion toxicity, osmotic stress, ion imbalance, and decreased water potential, harshly affecting plant physiology and metabolism. Salinity stress impacts numerous plant development elements and affects seed germination, plant growth and development, spike formation, and reproductive growth, among others (Ke et al. 2020). High salinity can affect stomatal conductance, leaf area, and photosynthetic efficiency (Hassan et al. 2021; Solangi et al. 2021). Conventional reproduction, genomic engineering to develop salt-resistant crops, and chemical treatments are used to address

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the problem of excess salt (Ladeiro 2012). However, these measures are not always feasible; some may even harm soil ecology. Chemical fertilizers and pesticides are widely used in modern agriculture, slowly altering nutrient supply, reducing microbial activity and diversity, and affecting soil health (Ali et al. 2021a, b).

Soil microorganisms provide another method for soil remediation and salt tolerance in plants. Symbiotic bacteria boost plant development and reduce salt stress by supplying minerals (such as N, P, and K) and hormones (such as auxin, cytokinin, and abscisic acid) or by lowering ethylene synthesis (Gao et al. 2022). The beneficial microbes colonize the plant's rhizosphere, stimulate plant growth, and increase N, P, and K content in the stems and storage roots of the paddy (Ali-Tan et al. 2017). Ashik et al. (2023) reported that enhancing rice growth and yield under salinized circumstances was more successful for a prospective bacterial consortium than for a single strain that could withstand salt. Sirajuddin Khan et al. (2016) discovered that inoculating rice plants with *Bacillus pumilus* leads to salt stress easiness. The rhizospheres of plants harbor a variety of microorganisms, some of which can resist salt stress. These halotolerant PGPR help plants withstand saline environments by producing proteins and metabolites. The word "metabolites" refers to low molecular weight chemicals (1000 Da) essential to microbes and plant growth. Previous research has shown that the salt-tolerant *Bacillus tequilensis* and *Bacillus aryabhattai* have functional EPS groups that can help bind and chelate Na^+ in the soil, minimizing plant contact with the ion under saline conditions (Shultana et al. 2021). Bacteria also initiate a gene expression program in response to osmotic stress caused by high NaCl concentrations (Khan et al. 2021a, b), which manifests itself in a number of proteins that are produced in greater amounts in response to the stress (Paul and Nair 2008).

Plant responses to saltiness can be reviewed from many biological and molecular perspectives. Proteomics effectively identifies proteins that respond to salt (Kosova et al. 2011). Numerous research has been conducted to analyze proteomic patterns in various rice tissues, including shoots (Parker et al. 2006), and leaf membranes (Nohzadeh et al. 2007), in response to salt stress. Unlike the genome, the proteome study is a dynamical entity that is influenced not only by physiological state but also by cell type and developmental stage. Proteomics is the most eminent tool for uncovering the dynamic expression of whole proteins towards salt stress with microbial interactions. The introduction of mass spectrometry (MS) and IPG strips has improved proteomic analysis's sensitivity, reliability, and performance. Chickpea salt stress was lessened as a result of the *Bacillus tequilensis* inoculation; additionally, Haroon et al. (2023) showed that the existence of proteins and carbohydrates that bind to sodium ions (Na^+) and give salt tolerance was revealed by Fourier-transformed infrared spectroscopy. Zhao et al. (2022) reported that soluble proteins expressed with the application of plant growth promoting PGPR *Bacillus* sp. Wp-6 under salt stress condition in *Triticum aestivum* plant.

A thorough proteomic study of the leaf sheath proteins of rice grown under high salinity conditions is presented here. The current proteomic study aimed to investigate the molecular incidents and changes produced or affected during the interaction of rice with *Bacillus* spp. because of salt stress. The effectiveness of *Bacillus* spp. strains in stimulating rice plant growth under salt stress conditions was explored.

Materials and methods

Experimental site and soil preparation

The experiment was conducted in a greenhouse with the salt-tolerant rice variety Pokkali. Sterilized, dry, 2-mm sieved soil was weighed, and 3 kg was placed in an undrained plastic pot. Urea, TSP, and MOP fertilizers were applied at 170–80–150 kg ha^{-1}.

Plant material and treatments

Seeds of the selected rice variety were sterilized by soaking in ethanol (95%) for 10 s. After removing the ethanol, the seeds were immersed in 3% NaOCl six more times before rinsing with sterilized dH_2O water. The sterilized seeds were allowed to germinate aseptically in a humidity chamber prepared in Petri dishes coated with filter paper (Whatman No. 1). The seed germination percentage was monitored daily for 7 days. Then, roots were immersed in overnight-grown bacterial cell suspensions of *Bacillus tequilensis* (UPMRB9), *Bacillus aryabhattai* (UPMRE6), and mixed strains (a combination of *Bacillus tequilensis* and *Bacillus aryabhattai*) at approximately 10^8–10^9 CFU/mL and distilled water (as a control) for 1 h. Fresh *Bacillus* spp. bacterial cultures were inoculated into Tryptic Soy Broth (TSB) medium and shaken for 24 h (orbital shaker, model 722). Fourteen days after cell planting, plants received a second microbial inoculation of 5.0 mL (pellets were harvested at 6500 g for 10 min by centrifugation and resuspended in 10 mM $MgSO_4$) in the roots. The soil was salinized 14 days after planting by adding sodium chloride (NaCl) in amounts of 30 mM and 60 mM to each pot for 7 days to achieve electrical conductivity (EC) of 5 dSm^{-1} and 10 dSm^{-1}, respectively, while irrigated tap water served as a control treatment. The effect of the inoculum on the salt-sensitive protein of rice grown in saline soils was studied with four replicates and twelve combinations ($12 \times 4 = 48$). Data was recorded on the root and shoot length on the 7th and 21st days after planting. Each replicate's root and shoot lengths were measured independently with a ruler.

Afterward, all treated plants were harvested after 21 days, as were the untreated controls (Fig. 1). The proximal third of the leaf blade of leaf 4 from each duplicated plant of each treatment was immediately frozen in liquid nitrogen and stored at − 80 °C for ion analysis and protein extraction.

Extraction of protein from the rice leaf sheath

Twenty-one-day-old frozen rice leaf samples weighing 100 mg were pulverized and crushed in a mortar with liquid nitrogen. The plant cells were lysed with 350 µl of buffer RP1 (including reducing agent) and 3.5 µl (0.07%) of ß-mercaptoethanol (ß-ME) with vigorous shaking. The precipitated proteins were filtered using a NucleoSpin® filter to minimize viscosity. The NucleoSpin® filter (violet ring) was placed in a collection tube, the mixture was transferred to the violet ring, and the protein pellet was centrifuged at 11,000 × g for 1 min. RNA binding conditions were adjusted by discarding the NucleoSpin® filter and adding 350 µl of ethanol (70%) to the homogenized lysate and mixing by pipetting up and down (approximately 5 times) or vortexing (2 × 5 s). The NucleoSpin® RNA/protein column (light blue ring) was placed in a collection tube for RNA binding, and the lysate was loaded (per treatment) and centrifuged at 11,000 × g for 30 s. NucleoSpin® RNA/protein was added to the column in a fresh collection tube (2 mL). The column membrane was coated with RNA and DNA, filling the flow-through with protein. An appropriate volume of 35 µl of flow and 35 µl of protein separator was added to a fresh 1.5-mL collection tube. The mixture was mixed vigorously before incubation at room temperature for approximately 10 min and centrifugation at 11,000 × g for 5 min. The supernatant was removed as completely as possible by pipetting or decantation. After washing the protein with 500 µl of 50% ethanol, it was centrifuged at 11,000 × g for 1 min. The protein pellet was dried at 25 °C for 5–10 min with the cap unlocked. A total of 100 µl of PSB-TCEP (protein-solving buffer with reducing agent) was added to prepare the protein sample. To aid in subsequent protein dissolution, the large and visible pellets were digested with a pipette tip and kept alive at 95—98 °C for 3 min to allow complete protein dissolution and denaturation. After the sample cooled to room temperature, it was centrifugated at 11,000 × g for 1 min to pellet the remaining insoluble material. Finally, the supernatant was saved and cooled to 4 °C. BSA was used as a reference and a commercial dye reagent (Bio-Rad Laboratories, Hercules, CA, USA) to quantify the protein concentration of each sample using the Bradford procedure (Bradford 1976).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

To investigate the change between the different MW (molecular weight) of protein molecules, we executed western blot analyses using reducing 12% sodium dodecyl sulfate (SDS)-PAGE with a vertical electrophoresis unit (Bio-Rad, Hercules, CA) to detect total proteins as described by Laemmli (1970) and Sambrook et al. (1989). A 5% stacking gel and a 10% separation gel were prepared for both systems. Water (dH_2O), a 30% acrylamide mixture, 1.5 M Tris (pH = 8.8), 10% SDS, and 10% ammonium persulfate TEMED were combined to prepare the separation gel. The chemicals used to prepare the stacking gel were the same as those used to prepare the separating gel, except for 1.0 M Tris (pH = 6.8). Each sample received an equal amount of protein (14 µl) mixed with 14 µl of gel loading buffer,

Fig. 1 Plant material used for the extraction of protein

heated in 100 °C boiling water for 1 min, and loaded into the gel. A 1 × Tris–glycine buffer was used for electrophoresis at 10 mA for 1 h, followed by 15 mA for 5 h. Coomassie Brilliant Blue (CBB) staining solution (G-250) was used for staining and re-staining as described in Sambrook et al. (1989). For each biological replicate, a high-resolution gel with different run times was carefully chosen for further analysis. Protein band relative abundance was assessed by densitometry (GS-700, Bio-Rad).

Protein identification and protein digestion

Differentiated protein stains on preparative gels were removed. The cut-out protein spots were digested with trypsin using the MassPREP station (Waters). Excised spots were decolorized in 50 µl of 50 mM ammonium bicarbonate and 50 µl of 50% acetonitrile, washed one time in 50 µl of 100 mM ammonium bicarbonate and 50 µl of dehydrated acetonitrile. During 5 h at 37 °C, 6 ng μl^{-1} trypsin was digested in 25 µl of 50 mM ammonium bicarbonate. The digested proteins were separated twice: once with 1% formic acid (30 µl) and once with 1% formic acid (12 µl)/50% acetonitrile (12 µl). The digested proteins were mixed and stored at 4 °C in a PCR plate for subsequent analysis. Two-dimensional liquid chromatography (ESI MS) (Agilent 1100 series 2D nano LCMS/MS) was used for protein identification and sequencing. Tryptic digested protein was column separated, followed by reverse phase separation. The electrospray ionizer ionized peptides in the liquid phase were then split (MS/MS) and identified. For analysis, the data was sent to the MASCOT search engine (Agilent).

Databank exploring with MS/MS spectra

MS/MS spectra were compared with the NCBI nonredundant protein databank. This was done using the MS/MS Ion Search Engine, a computer program that performs protein documentation by matching a protein's MS/MS spectra with a protein or DNA sequence database http://www.matrixscience.com/search_form_select.html. The mouse scoring system was used to determine the relevance of the protein match to the ion score. The ion score was calculated as − 10 LOG10(p), where p is the absolute probability that the observed match is a random event. Thus, a low P value indicates that the match between the identified protein and the MS/MS spectra is not random. Because a substantial specific match raises the ion core, a high score indicates a highly significant match (MASCOT help; http://www.matrixscience.com/help/scoring_help.html). A significant match was defined as a single protein with a score greater than the minimum significance level score ($p < 0.05$). The minimum significance level score was reported in each MASCOT search result constructed based on the definite possibility and length of the series catalog explored.

Statistical analyses

A completely randomized design was used in this experiment. Results were expressed as ± standard deviation (SD) of four replicates ($n = 4$). ANOVA determined means using the least significant difference (LSD) test at $p \leq 0.05$. Statistical analyses were carried out using the bioinformatics tool R programming. The R programming software was used to generate the graphical representations of the hierarchical cluster heat map for protein expression. The protein was identified using the GenBank website. The proteomic approach and a database matching program (MASCOT assistance, http://www.matrixsciences.com) were used to evaluate the protein expression of plant samples.

Results

The effect of Bacillus spp. and different salt levels on the growth of rice plants

The influence of *Bacillus* spp. on rice growth was studied using the bacterial strains UPMRB9 (*Bacillus tequilensis*), UPMRE6 (*Bacillus aryabhattai*), and their consortium (*Bacillus tequilensis* and *Bacillus aryabhattai*).

Under non-stress conditions, *Bacillus* spp. UPMRB9 and UPMRE6 strains increased rice shoot lengths by 3.34% and 4.87%, respectively, and root lengths by 5.37% and 8.61%, correspondingly, compared to the control (Table 1). Treatment with mixed strains (UPMRB9 and UPMRE6) had the most significant positive impacts on rice growth, increasing shoot and root lengths by 6.76% and 16.31%, respectively. These data showed that rice seeds inoculated with mixed strains of *Bacillus* spp. had better growth parameters than single and control strains. Under salt stress, significant root and shoot length changes were observed at salinity stages of 5 dSm^{-1} and 10 dSm^{-1}. Significantly higher root length was observed for the mixed cultivar at salinities of 5 dSm^{-1} (18.27 cm) and 10 dSm^{-1} (16.14 cm). The lowest root length reduction was observed for the bacterial consortium at 5 dSm^{-1} (4.78%) and 10 dSm^{-1} (17.47%). Moreover, the highest root length reduction was measured by isolating UPMRE6 (4.87%, 24.52%) and UPMRB9 (4.79%, 24.28) at 5 dSm^{-1} and 10 dSm^{-1}, respectively.

The effect of Bacillus spp. on the protein concentration of rice under normal and stressed conditions

The amount of total protein recovered differed significantly between normal plant tissue and bacterial-treated tissue at different salinity levels. This indicates that PGPR-treated plants can significantly increase the amount of total protein compared to untreated plants.

Table 1 Effect of different *Bacillus* spp. and salinity levels on rice plant growth

Treatments	Shoot length (cm)		Root length (cm)	
	7 D	21 D	7 D	21 D
Control S0	46.50^{ij}	84.67^{h}	5.67^{ef}	16.36^{e}
UPMRB9 S0	47.86^{fg}	87.5^{cd}	6.34^{cd}	17.24^{d}
UPMRE6 S0	49.77^{bc}	88.8^{b}	7.17^{b}	17.77^{c}
Mixed S0	52.17^{a}	90.4^{a}	8.53^{a}	18.96^{a}
Control S5	46.10^{j}	83.81^{fg}	5.26^{f}	15.57^{g}
UPMRB9 S5	47.50^{gh}	86.30^{d}	6.24^{d}	16.22^{ef}
UPMRE6 S5	48.57^{de}	87.13^{bc}	6.95^{c}	16.48^{d}
Mixed S5	50.23^{b}	89.01^{a}	8.27^{a}	18.27^{b}
Control S10	46.93^{hi}	85.17^{gh}	4.77^{g}	13.15^{j}
UPMRB9 S10	47.67^{g}	83.5^{i}	5.86^{e}	13.86^{i}
UPMRE6 S10	48.43^{ef}	84.67^{h}	6.37^{cd}	14.27^{h}
Mixed S10	49.13^{cd}	86.27^{ef}	6.86^{c}	16.14^{f}
B X S	***	***	***	***

Annotation: *Bacillus* strains, namely UPMRB9 (*Bacillus tequilensis*), UPMRE6 (*Bacillus aryabhattai*), and mixed (*Bacillus tequilensis* + *Bacillus aryabhattai*) suspensions, were used to evaluate growth-promoting activity on rice (Pokkali) by the standard rolling towel method, where treatments with control = no bacterial strains; S0 = no salinity; S5 = NaCl 30 mM, EC 5 dSm^{-1}; S10 = NaCl 60 mM, EC 10 dSm^{-1}. Different letters indicate significant differences, as determined by Fisher's least significant difference test at $p = 0.05$

The Bradford assay confirmed the higher protein concentrations after treatment (Fig. 2). After treatment of rice plants with *Bacillus* mixed strains, the highest protein level of 136.77 µl/ml was obtained under non-stress conditions. This concentration was extensively different compared to the control and single inoculation strains. A related trend was remarked for total protein concentration under NaCl salt stress levels, with the maximum (128.35 µl/ml) reached when inoculated with mixed strains. The lowest amount of protein (108.03 µl/ml, 92.88 µl/ml) was found in non-inoculated and non-stressed leaf tissues (control). Similarly, a significant difference was found between inoculations at both salt levels.

Proteomic profile of SDS-PAGE gel electrophoresis of rice leaves and its changes induced by Bacillus spp. in response to salt stress

To better recognize the molecular systems and changes triggering the interaction between *Bacillus* spp. and rice seedlings (Pokkali cultivar) under salt tension, proteins were isolated from rice leaves and separated by SDS-PAGE western blotting. Figure 2 shows the reduced SDS-PAGE electrophoresis and structural analysis of the recombinant protein. Many targeted salt-sensitive pure protein bands were found on the gels of the control and inoculated plants as a monomer of about 60 kDa (Fig. 3), which was expressed in the leaf based on MW. The protein bands were visible on the gel complexes under control and NaCl salt stress, also produced by inoculation of *Bacillus* spp. isolates.

Comparative proteomics using LC–MS/MS (the label-free quantitative shotgun system) to characterize the proteomic responses of salt-responsive PGPR-treated rice plants

The label-free assessable shotgun proteomic analysis was performed to describe the salt-induced proteomic responses of normal and PGPR-treated experimental rice seedlings that differed in their susceptibility to salt stress. Soluble protein extracted from normal water-soaked seedlings and 7-day-old seedlings soaked with plant growth-promoting rhizobacteria were grown for 21 days under different levels of salt stress (30 mM NaCl, EC-5 dSm^{-1} and 60 mM NaCl, EC-10 dSm^{-1}), and soluble protein was subjected to label-free quantitative shotgun proteomic analysis. The study identifies

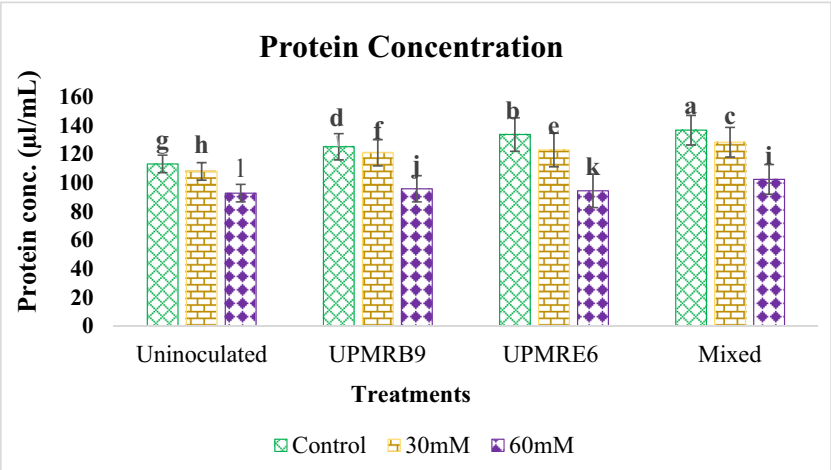

Fig. 2 Effect of *Bacillus* spp. strains on the total protein concentration of rice leaves from 21 DAT (determined by Bradford assay). Plants were treated with Bacillus spp. suspensions and for salinity with 30 mM NaCl and 60 mM NaCl salt, while water served as a control. Several letters denote significant differences, as established by Fisher's least significant difference test at $p = 0.05$

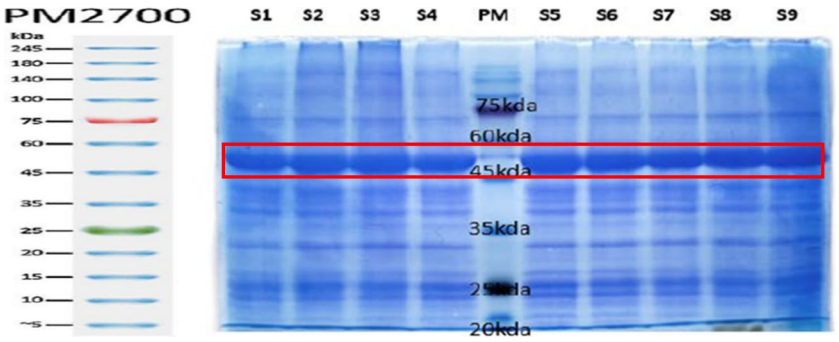

Fig. 3 Structural analysis of the salt-responsive protein 60 kDa chaperonin in the leaf of *Oryza sativa*. Using 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions, the refined chaperonin protein was discovered

numerous non-redundant proteins with high confidence in normal and NaCl salt stress evolved plants (Table 2).

MALDI-Tendam mass spectroscopy (MALDI-TOF MS/MS) and LC–MS/MS chromatogram analysis of control or normal (Fig. 3) and treated plants (Figs. 4 and 5) revealed a total of 54 reproducible proteins that varied considerably in abundance. Mascot analysis of the bulk data revealed 36 (66.67%) up-regulated proteins and 18 (33.33%) down-regulated proteins (Table 2) (Fig. 4). These altered protein levels can be categorized as salt-stress-responsive proteins. The molecular weight range of the defined proteins was found to be higher for the identified rice proteins. Most rice proteins have isoelectric points pI from 4.63 to 9.85 and molecular masses from 15 to 175 kDa (Table 2 identifies rice proteins in normal and stressed rice plants). All quantitative data on the peptides from the soluble fractions (Figs. 5, 6, and 7) were examined to identify the proteins.

A common functional proteomic response in the interaction of Bacillus spp. with rice under salinity stress

The abundant proteins found in response to PGPR under salinity stress fitted to several diverse functional classifications that provide an understanding of the proteome under excessive salinity and useful microbial response mechanisms. Changes in protein content in various functional categories demonstrate the complexity and adaptability of plant responses to varying degrees of salinity and microbial interactions.

Figure 8 shows the gene ontology analysis (GO) of the fifty-four proteins abundantly expressed in response to microbial effects under all normal and stressed environments. This showed that the proteins were abundantly expressed with the application of PGPR, and salinity was mainly associated with the functions of (1) photosynthesis and energy catalyst (Up, 17; Down, 5); (2) antioxidation and detoxification (Up, 4); (3) protein synthesis (Up, 8; Down, 2); (4) transporters, folding, assembly, and degradation (Up, 4; Down, 2); (5) metabolism, transduction, and signaling (Up, 3; Down, 7); and (6) signal transcriptional regulation (Down, 2).

Proteins from the photosynthesis, protein synthesis and metabolism, transduction, and signaling pathway functional categories were the most abundant, while proteins from the signal transcription regulation, antioxidants, and detoxification categories were the least abundant. In response to salt-tolerant bacteria under salt stress, numerous functional categories of transporters, folding, assembly, and degradation were expressed. Most proteins discovered were associated with photosynthesis, stress mechanisms (protein synthesis), and metabolic signaling networks.

After bacterial inoculation at different salt stress levels, significant changes occurred in proteins involved in photosynthesis, primary metabolism, and stress mechanisms. Bacterial inoculation upregulates the expression of proteins involved in photosynthesis, defense, protease inhibition, and protein synthesis. Proteins involved in cell division, defense, protein synthesis, and stress resistance were down-regulated in the manifestation of NaCl. The highest increase in protein upregulation in response to bacterial inoculation at different salt levels was observed for photosynthesis and energy catalyst (77.28%); protein synthesis (80%); antioxidation and detoxification (100%); and transporter, folding, assembly, and degradation (66.67%) (Table 2). In addition, the down-regulated proteins belonged to metabolism, transduction, and signaling pathways (70%); signal transcription regulation (100%); protein degradation (33.34%); and stress-related proteins (20%) (Table 2).

Analysis of cluster for differentially expressed proteins

A total of 54 differentially expressed proteins common to all treatments (normal and stressed levels) were used for hierarchical cluster analysis (Fig. 8) among different treatments (protein with its functional name and treatment level). These 54 proteins were selected based on their higher abundance. The cluster contains 36 up-regulated and 18 down-regulated proteins under non-stressed and stressful situations.

Examples of proteins that were severely decreased underneath salt stress include fructose-1,6-bisphosphatase,

Table 2 Effect of different *Bacillus* spp. and salinity level on rice plant growth

Sl. no	Accession No	Description	Peptides matched	Mascot score	Coverage (%)	pI	Theoretical Mr (kDa)/pI	Up/down	Functional category
Photosynthesis and energy catalyst									
1	Q6ENG6	Ribulose bisphosphate carboxylase large chain	32	325.31	60.5	6.68	52.8	Up	Energy catalyst
2	P0C2Z7	ATP synthase subunit beta, chloroplastic	30	297.56	81.9	5.5	53.9	Up	Photosynthesis
3	Q01859	ATP synthase subunit beta, mitochondrial	21	125.9	59.6	6.37	58.8	Up	Catalyst mitochondria
4	P93431	Ribulose bisphosphate carboxylase/oxygenase activase, chloroplastic	15	78.6	52.1	5.62	51.4	Up	Energy catalyst
5	B8AME2	Catalase isozyme C	14	70.0	45.5	7.42	56.7	Up	Energy catalyst
6	Q6ENH7	ATP synthase subunit alpha, chloroplastic	13	68.7	35.5	6.25	55.6	Up	Photosynthesis
7	Q6AVT2	Glucose-1-phosphate adenylyltransferase large subunit 1, chloroplastic/amyloplastic	15	53.4	40.7	7.37	55.3	Up	Photosynthesis
8	Q69SV0	Probable L-ascorbate peroxidase 8, chloroplastic	10	50.1	35.7	5.53	51.1	Up	Photosynthesis
9	P0C520	ATP synthase subunit alpha, mitochondrial	12	38.9	28.6		55.3	Up	Energy Catalyst mitochondria
10	Q6K669	Leucine aminopeptidase 2, chloroplastic	9	35.0	21.4	8.1	61.7	Up	Energy catalyst
11	Q7XDY9	Rubisco accumulation factor 1, chloroplastic	8	26.9	26.2	5.62	51.5	Up	Energy catalyst
12	P0C433	Photosystem II protein D1	3	11.2	10.4	5.36	38.9	Up	Photosynthesis
13	P12330	Chlorophyll a-b binding protein 1, chloroplastic	2	5.51	17.7	5.26	27.9	Up	Photosynthesis
14	Q10HD0	Chlorophyll a-b binding protein, chloroplastic	2	4.33	17.1	5.91	28.4	Up	Photosynthesis
15	P0C363	Photosystem II CP47 reaction center protein	1	4.02	2.55	6.54	56.1	Up	Photosynthesis
16	Q6YXW6	Sucrose-phosphatase 2	1	2.87	3.30	5.82	47.2	Up	Energy catalyst
17	Q94E75	Probable sucrose-phosphatase 1	1	2.44	2.60	6.06	47.0	Up	Energy catalyst
18	P0C389	Cytochrome f	1	2.33	4.68	8.91	35.4	Down	Photosynthesis
19	O64422	Fructose-1,6-bisphosphatase, chloroplastic	1	1.60	3.20	5.12	43.5	Down	Energy catalyst
20	Q9LDN2	Uridine 5'-monophosphate synthase	1	2.34	5.45	7.21	50.7	Down	Energy catalyst
21	P83646	Oxygen-evolving enhancer protein 3, chloroplastic	1	1.62	6.45	9.85	22.9	Down	Photosynthesis

Table 2 (continued)

Sl. no	Accession No	Description	Peptides matched	Mascot score	Coverage (%)	pI	Theoretical Mr (kDa)/pI	Up/down	Functional category
22	Q69RJ0	Ferredoxin-dependent glutamate synthase, chloroplastic	1	1.54	0.61	6.84	174.9	Down	Photosynthesis
Antioxidation and detoxification									
23	Q42971	Enolase	10	52.0	35.6	5.57	47.9	Up	Antioxidation and detoxification
24	P00761	Trypsin	4	38.7	25.1	7.18	24.3	Up	Antioxidation and detoxification
25	Q0E4K1	Catalase isozyme A	2	4.40	5.08	7.01	56.6	Up	Antioxidation and detoxification
26	Q70G58	Thioredoxin reductase NTRC	1	2.43	3.49	6.49	56.1	Up	Antioxidation and detoxification
Protein synthesis									
27	E0WCX8	60 kDa chaperonin	6	72.1	26.2	7.23	61.5	Up	Protein Synthesis,
28	O64937	Elongation factor 1-alpha	9	34.2	26.1	9.06	49.2	Up	Protein Synthesis
29	P35683	Eukaryotic initiation factor 4A-1	8	27.2	24.1	5.57	47.0	Up	Protein synthesis
30	P15280	Glucose-1-phosphate adenylyltransferase small subunit 2, chloroplastic	6	21.8	18.8	7.03	56.0	Up	Protein synthesis
31	Q5Z627	Elongation factor 1-gamma 3	4	11.1	13.94	6.47	47.36	Up	Protein Synthesis,
32	Q6YW46	Elongation factor 1-gamma 2	4	10.5	11.0	6.73	47.3	Up	Protein Synthesis,
33	Q9SLY8	Calreticulin	2	8.87	7.54	4.63	48.2	Up	Protein Synthesis,
34	A2YL07	Glutamate–cysteine ligase B, chloroplastic	2	5.76	5.24	7.02	56.1	Up	Protein Synthesis,
35	Q9SNN8	1-aminocyclopropane-1-carboxylate synthase 6	1	1.80	3.69	8.78	59.4	Down	Protein synthesis
36	Q75HQ0	Heat shock 70 kDa protein BIP4	1	1.87	1.60	5.22	74.2	Down	Protein Synthesis
Transporter, fold, assembly, and degradation									
37	Q5W676	Hexokinase-5	3	8.68	7.69	6.11	54.6	Up	Transport
38	Q851S8	Adenylosuccinate synthetase 2, chloroplastic	4	8.43	9.40	6.92	52.6	Up	Transport
39	Q8LQ68	Hexokinase-6	3	5.42	10.2	6.34	55.0	Up	Transport
40	Q25A68	Glutamyl-tRNA (Gln) amidotransferase subunit A, chloroplastic/mitochondrial	1	4.19	2.76	6.18	57.21	Up	Fold and assembly
41	Q6K6K7	Ubiquitin-like modifier-activating enzyme	1	1.87	2.85	4.75	45.5	Down	Folding and degradation
42	Q6K6K7	Ubiquitin-like modifier-activating enzyme 5	1	1.84	2.85	4.75	45.5	Down	Folding and degradation

Table 2 (continued)

Sl. no	Accession No	Description	Peptides matched	Mascot score	Coverage (%)	pI	Theoretical Mr (kDa)/pI	Up/down	Functional category
Metabolism, transduction, and signal pathway									
43	Q9LI00	6-phosphogluconate dehydrogenase, decarboxylating 1	5	15.4	15.6	6.18	52.6	Up	Metabolism
44	Q4677	Fructose-bisphosphate aldolase, chloroplastic	2	4.84	10.3	6.8	41.9	Up	Metabolism
45	B8A8C9	Phospholipase A1-II 5	1	2.69	5.37	6.98	50.3	Up	Metabolism
46	Q0JM17	DEAD-box ATP-dependent RNA helicase 56	1	1.64	2.31	5.72	48.6	Down	Signal pathway
47	P14655	Glutamine synthetase, chloroplastic	1	1.58	4.43	6.34	46.6	Down	Metabolism
48	Q10LG8	Tubby-like F-box protein 6	1	1.56	2.23	9.36	44.8	Down	Signal transduction
49	Q2QQS5	Cyclin-T1-4	1	1.49	3.49	7.36	61.3	Down	Signal transduction
50	Q6L5H6	Protein phosphatase 2C 50	1	1.40	3.35	6.11	41.5	Down	ABA signaling pathway
51	Q7XX84	Ethylene receptor 2	1	1.39	3.40	6.38	84.7	Down	Signaling pathway
52	B8AIW3	Very-long-chain aldehyde decarbonylase GL1-2	1	2.40	2.38	9.42	70.9	Down	Signaling pathway
Signal transcriptional regulation									
53	A2ZVI7	Calcium-dependent protein kinase 1	1	2.80	2.89	6.51	58.7	Down	Signal transcriptional
54	Q5D0W8	BTB/POZ domain and ankyrin repeat-containing protein NPR1	1	1.72	1.71	5.67	63.8	Down	Transcriptional regulation

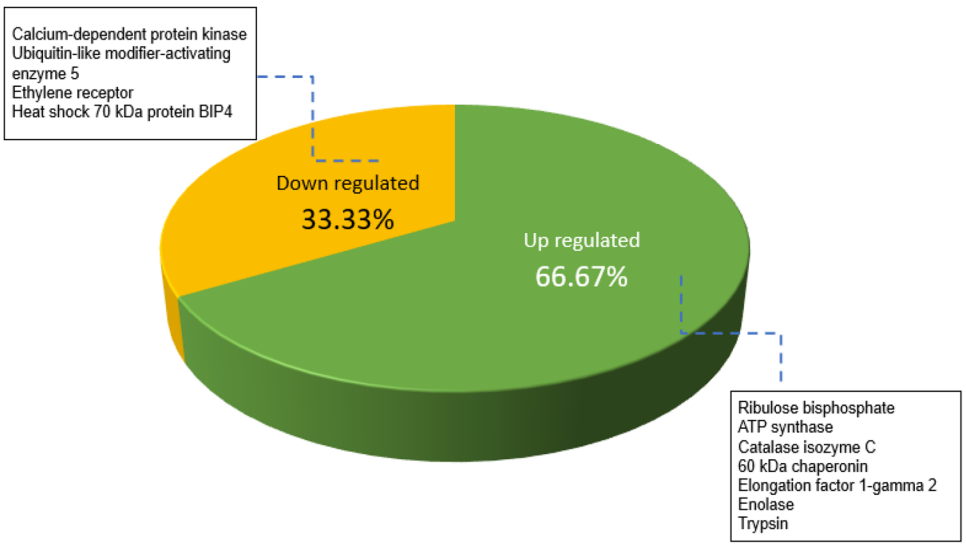

Fig. 4 Representation of some significant up-regulated and down-regulated metabolites at different salinity levels with inoculation of beneficial rhizobacteria. The functional names of the differentially stated proteins represent the up-regulated and down-regulated proteins

chloroplastic (O64422), ferredoxin-dependent glutamate synthase, chloroplastic (Q69RJ0), and cytochrome f (P0C389), which are functionally defined as photosynthetic and energy catalytic proteins. The other down-regulated proteins belonging to stress-induced chaperone proteins are the 70-kDa heat shock protein BIP4 (Q75HQ0),

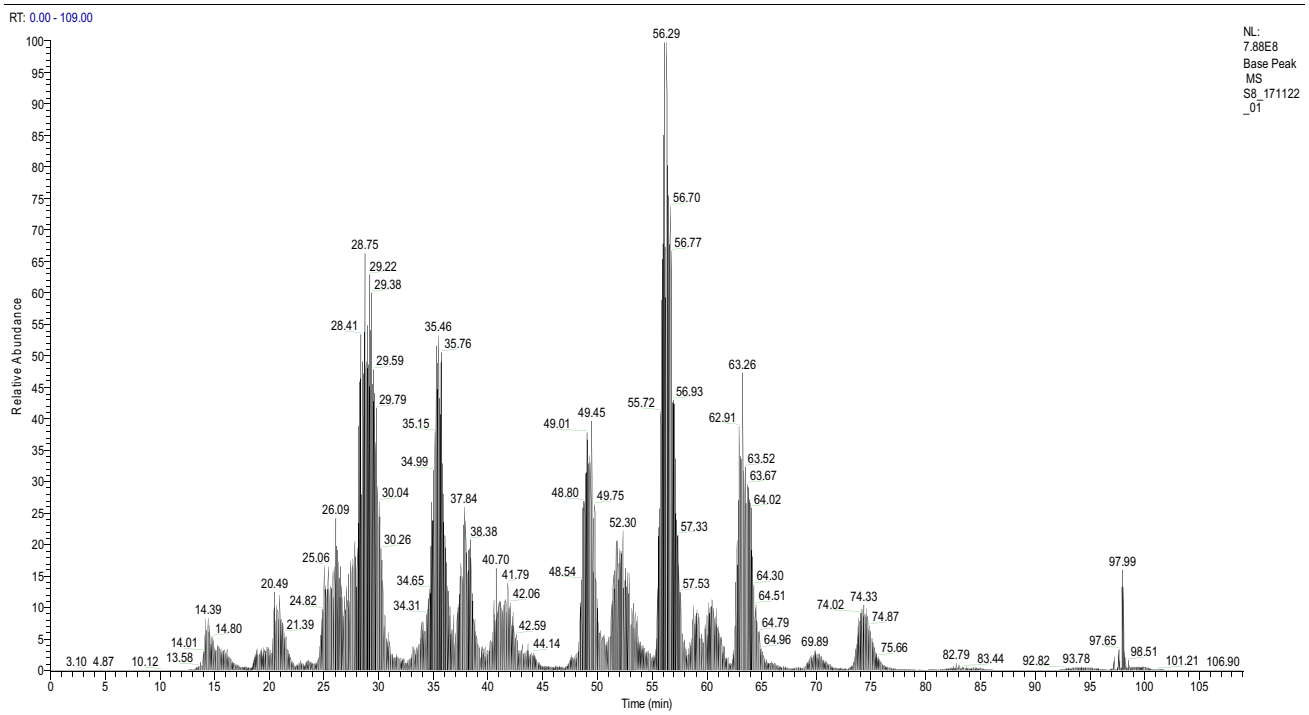

Fig. 5 LC–MS/MS spectra of separated protein under normal conditions in raised seedlings of a salt-tolerant rice cultivar (*Oryza sativa* L., Pokkali)

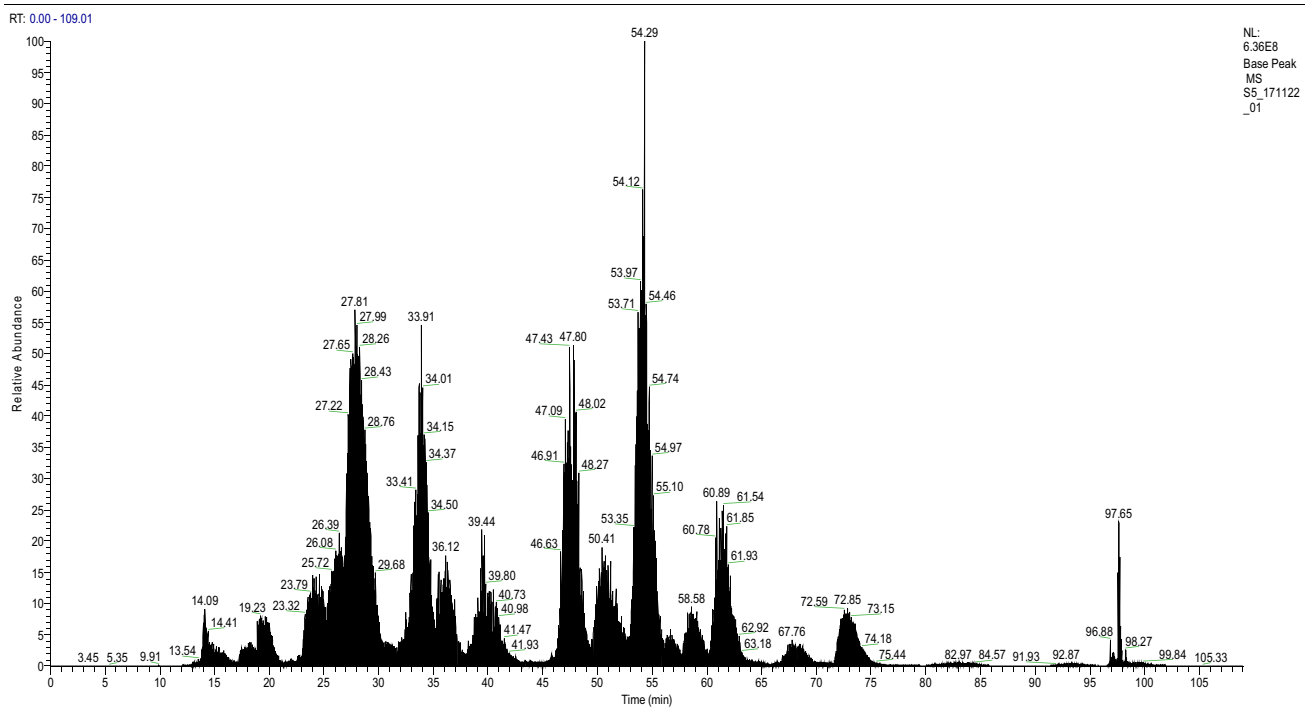

Fig. 6 LC–MS/MS spectra of separated protein under salt-stress conditions (30 mM NaCl) in raised seedlings of a salt-tolerant rice cultivar (*Oryza sativa* L., Pokkali)

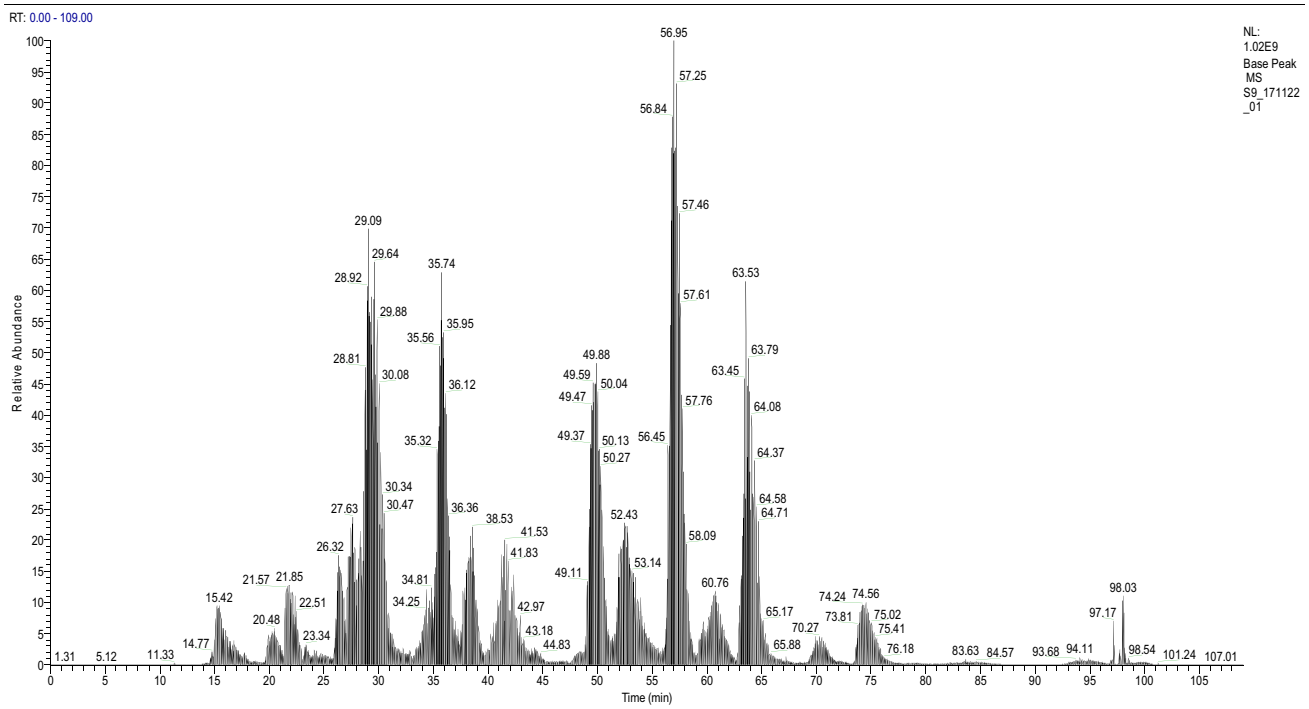

Fig. 7 LC–MS/MS spectra of separated protein under salt-stressed conditions (60 mM NaCl) in raised seedlings of a salt-tolerant rice cultivar (*Oryza sativa* L., Pokkali)

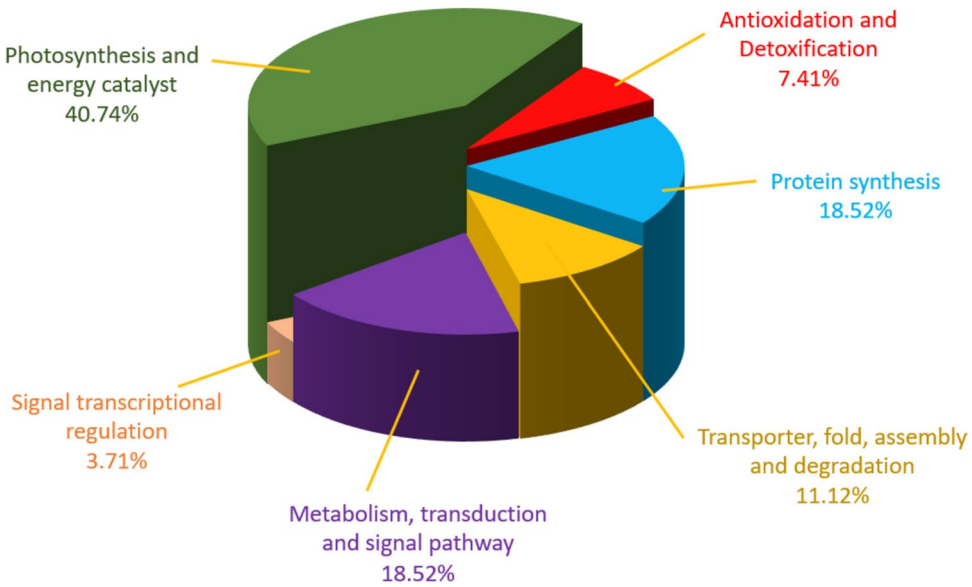

Fig. 8 Functional classification and gene ontology (GO) enrichment analysis of differentially expressed proteins during the interaction between PGPR and rice plants in response to normal and two salt stress levels. The pie chart shows the percentage of different biological functions of common proteins whose abundance changed significantly in response to all treated salt stress levels and PGPR interactions

1-aminocyclopropane-1-carboxylate synthase 6 (Q9SNN8), and the important folding and degradation protein ubiquitin-like modifier-activating enzyme (Q6K6K7). Some proteins of biosynthetic metabolism and signaling pathways were significantly downregulated: glutamine synthetase, chloroplastic (P14655), protein phosphatase 2C 50 (Q6L5H6), and very long chain aldehyde decarbonylase GL1-2 (B8AIW3). Another important function is signal transcriptional regulation described by BTB/POZ domain- and ankyrin repeat-containing proteins NPR1 (Q5D0W8) and calcium-dependent protein kinase 1 (A2ZVI7), which downregulates proteins under salt stress conditions.

Under non-saline stress, bacterial inoculation increased the expression of photosynthetic and energy catalyst proteins such as ATP synthase subunit-β, chloroplastic (P0C2Z7), ribulose bisphosphate carboxylase large chain (RuBisco)

(Q6ENG6), and photosystem II protein D1 (P0C433). Under salt stress of 30 mM and 60 mM NaCl, bacterial inoculation improved the demonstration of the salt-stress-related proteins 60 kDa chaperonin (Q89LB1), calreticulin (Q9SLY8), and glutamate cysteine ligase B, chloroplastic (A2YL07). The up-regulated proteins in the antioxidation and detoxification functional category are enolase (Q42971) and trypsin (P00761). Significant proteins regulating transport, folding, and assembly are hexokinase-6 (Q25A68), 6-Phosphogluconate dehydrogenase, decarboxylating 1 (Q9LI00), fructose-bisphosphate aldolase, and chloroplastic (Q4677), which belong to the functional group biosynthetic metabolism (Fig. 9).

Discussion

Previous studies have shown that inoculation with PGPR can up or down-regulate protein expression towards salinity and other conditions (Gagne et al. 2016). This study investigated the NaCl treatment-induced change in proteome expression in rice plants inoculated with PGPR *Bacillus* spp. It is known that plants exposed to various stresses such as temperature, drought, and salinity develop one or more additional processes that are considered adaptive mechanisms to persist under certain stress conditions. These adaptive traits (changes) can occur at the genetic, molecular, membrane, and cellular levels. The current study showed that plants have some adaptive structures to tackle executed stress by regulating genes for proteins involved in stress, photosynthesis, transcriptional control, metabolism, signaling pathways, and protein synthesis under NaCl stress (Shrivastava and Kumar 2015). This altered gene expression to NaCl stress leads to the up or down-regulation of numerous stress-associated proteins, thereby protecting the plant from stress. Therefore, identifying the distinction expressions could offer comprehension of the plant's response to NaCl stress. In this study, we used the LC–MS/MS chromatography-proteomics method for protein identification because it allows large changes and even deeper proteome exploration. The functional groups assigned to the different categories have been studied in detail in the following sections.

Proteins implicated in photosynthesis and energy catalyst

Photosynthesis is a physiological system that is very sensitive to salt stress. The main consequence of salt stress is a reduction in stomatal opening in leaves, which reduces CO_2 availability and, thus, energy available for plant growth. During rice growth and development, 22 proteins engaged in cellular mechanisms, photosynthesis, and energy metabolism were significantly expressed, up-regulated, and down-regulated. We found that at salt concentrations of 30 mM and 60 mM NaCl, the level of the large subunit of ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO)

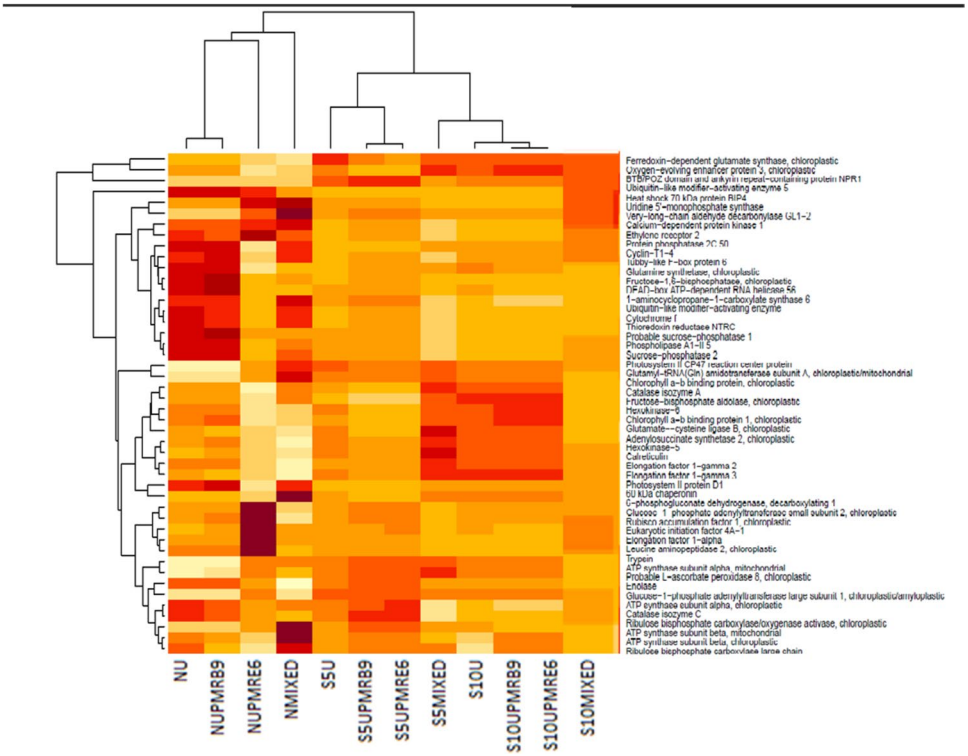

Fig. 9 Heatmap analysis combined with hierarchical cluster analysis of 54 differentially expressed proteins common to the experimental treatments of metabolites in the samples of rice plants under controlled and salt stress conditions with NaCl concentrations at 30 mM (5 dSm^{-1}) and 60 mM (10 dSm^{-1}) using normalized data with mean-centered via R-Program. Treatments were indicated by NU, normal uninoculated; S5, salinity level 5 dSm^{-1}; S10, salinity 10 dSm^{-1}; UPMRB9, *Bacillus tequilensis*; UPMRE6, *Bacillus aryabhattai*; and mixed, UPMRB9 + UPMRE6. Up-regulation or down-regulation indications represent the red and orange colors, respectively

increased significantly. Plant photosynthesis is functioned by a chloroplastic enzyme that uses ATP hydrolysis energy to eradicate inhibitors from RubisCO. According to some studies, it may act in reworking abiotic stress (salt and drought stress). According to Agrios (2005), RuBisCO shows a notable function in photosynthesis and chlorophyll formation. The amount of chaperonin 60 in plant chloroplasts, implied in the assembly of RuBisCO holoenzyme, is generally tuned to RuBisCO (Avni et al. 1989). With increasing NaCl and Na_2SO_4 concentrations, chlorophylls were significantly higher in *Kalidium foliatum* (Pall.) Moq. than in controls, which is widely supplied in the salty soil of the Hetao irrigation area in Inner Mongolia, China (Gong et al. 2018). Shotgun proteomics revealed the upregulation of respective key proteins such as RubisCO subunits, glyceraldehyde-3-phosphate dehydrogenase. The salt stress response proteins, for example, CAT and glutathione S-transferase (antioxidants), and proline-rich precursor protein (osmolyte), increased stress acceptance in soybean by co-injection of *Rhizobium* sp. SL42, *Hydrogenophaga* sp. SL48, and *Bradyrhizobium japonicum* 532C (Ilangumaran et al. 2022). Given the catalytic activity of RuBisCO, enhanced activase action may be necessary to survive lasting salt tension due to a direct decline in stomatal conductance and resulting low CO_2 levels. It is a stroma-localized protein that accounts for up to half of all chloroplast proteins. This leads to the hypothesis that overexpression of RuBisCO may improve photosynthetic activity in treated plants to achieve elevated growth and that this is likely related to plant defense under salt stress conditions. Other enzymes that increased were ATP synthase subunit beta, chloroplastic, cytochrome f, photosystem II protein D1, cytochrome b, photosystem II CP47 reaction center protein, ferredoxin-dependent glutamate synthase, chloroplastic, and others. Electron transfer between photosystem I (PSI) and photosystem II (PSII) is mediated by cytochrome f. As stated by Willey et al. (1984), cyclic electron flow and the PSI and PSII around the photosystem transmit the electrons once more. The chloroplastic subunit (small isoform) of the ATP synthase, subunit beta (β), was upregulated 2.6- and fivefold. The 400 kDa ATP synthase complex comprises an integral membrane CF0 segment and an extrinsic CF1 segment. The CF0 part represents a transmembrane ion channel for proton transport. The level of ATP synthase, which is mainly involved in energy production, increased in response to salt stress and bacterial inoculation. Following NaCl stress in the existence of bacterial inoculation, we discovered a number of differentially upregulated proteins related to energy catalysts and metabolism. Fructose-1,6-bisphosphatase, chloroplastic, cytochrome f, uridine 5′-monophosphate synthase, oxygen-evolving enhancer protein 3, chloroplastic, ferredoxin-dependent glutamate synthase, and chloroplastic were the photosystem and catalyst proteins that were downregulated.

Proteins involved in antioxidation and detoxification

An antioxidant defense system in plants under stress conditions is critical as it delays programmed cell death. When there are insufficient antioxidant enzymes in plants to scavenge excessive ROS, cell organelles cannot function properly, resulting in lipid peroxidation, protein oxidation damage, degradation of DNA molecules and nucleic acids, and inhibition of various enzymes (Dumanovic et al. 2021). By providing comprehensive cellular protection, plant stress tolerance is increased. At the protein level, plant stress tolerance capacity has been observed to increase in case of drought, salt, cold, and ABA in rice leaf sheaths (Zang and Komatsu 2007). The presence of phenolics and plant-protective enzymes increases after PGPR inoculation. Primary components of plant resistance to salt and drought include higher levels of phenolics and other defense enzymes (Sharma and Sharma 2017). Inoculation with *Piriformospora indica* and *Azotobacter chrococcum* lessens salinity in maize and rice by increasing the production of non-enzymatic antioxidants such as carotenoids, trypsin, proline, and polyphenols (Jogawat 2019). In this study, enolase and trypsin levels were elevated in saline soil together with catalase isoenzyme A and thioredoxin reductase. Furthermore, catalase A was found to have a particular expression in rice anthers by inoculation of PGPR in reducing abiotic stress reported by Qian et al. (2018). Consequently, our results suggest that antioxidants may be an interesting new target candidate in protein profiling to advance crop tolerance to abiotic stresses. Corresponding to El-Esawi et al. (2018), using *Serratia liquefaciens* KM4 moderates salt stress in maize by upregulating genes implied in the production of antioxidant enzymes. An important study (Barkla et al. 2009) exhibited that a rise in the amount of the VHA-B subunit was linked with a surge in the amount of aldolase and enolase proteins at the tonoplast but not with changes in the amount of VHA-E or VHA-A in *Arabidopsis thaliana* under salt conditions. In rice plants, relative catalase-A action was determined in the manifestation of various salts and decreased with increasing salt concentration (Nuchanat et al. 2011). Various antioxidant catalase enzymes were more susceptible to the ROS burst caused by stress conditions in rice anthers (Qian et al. 2018). Compared to salt-stressed soybean plants, antioxidant levels of peroxidase (POD) and polyphenol oxidase (PPO) were significantly higher (21–68%) in soybean plants injected with *B. aryabhattai* ALT29 and *A. woluwensis* ALT43 (Khan et al. 2021a).

Proteins involved in protein synthesis and composition

We discovered ten expressed proteins that are up and down-regulated and involved in protein synthesis and composition. A probable 60 kDa chaperonin was

successfully detected, demonstrating that PGPR inoculation dramatically increases the expression of chaperone proteins. A chaperonin of 60 kDa was upregulated in MSP-393 in response to the salt shock at 50 mM NaCl salt level, and chaperonins are involved in refolding denatured proteins (Hartl et al. 1994) and are considered important stress proteins. In another study, the PGPR-reactive protein was located to be a chaperone, a protein that connects specifically to denatured proteins to block their destruction and to aid in the refolding of ATP proteins (Rochester et al. 1986). A well-known study found that using PGPR restored the expression of a 20 kDa chaperonin protein in cucumber roots in a stressful environment (Du et al. 2016). When rice plants were inoculated with *Bacillus* spp. CPN20 (Spot 23), one of the most abundant aldo/keto reductase protein molecules which functions as cell defense and rescue proteins highlighting stress' effect on the post-translational modification mechanism (Zhang et al. 2015), presented parallel results. HSP20-like proteins of the chaperone superfamily affect protein folding and are connected with abiotic stressors and death (Gugger et al. 2017). Another group includes three proteasome subunits involved in protein degradation: alpha-gamma type-1,2 elongation factor and glucose-1-phosphate adenylyl transferase small subunit 2, chloroplastic. The proteasome has been attributed to a specific function in the deterioration of dissolved proteins created by various stressors to avoid blocking plant metabolism (Polge et al. 2009). This study discovered that other defense response proteins are down-regulated, including heat shock protein 70 (HSP70) and a protein similar to 1-aminocyclopropane-1-carboxylate synthase 6. Inoculation with the endophytic bacterium *Bacillus aryabhattai* promotes the growth of *Arabidopsis* and tobacco plants and induces the production of heat shock protein 70 kDa against various stresses (Xu et al. 2022). Based on a BLAST search, the protein synthesis molecule calreticulin (CRT) was identified as an important secondary messenger in rice plant signal transduction pathways. Calreticulin is critical for maintaining proper calcium levels in rice plants, among other functions. Mendlovic and Conconi (2010) discovered that calreticulin acts as a chaperone, aiding other proteins to fold appropriately. Calreticulin (CRT) is a Ca^{+}-binding protein that acts as a molecular chaperone in microbial inoculation but helps reduce several stresses (Joshi et al. 2019).

Proteins associated in transport, folding, assembly, and degradation

Four proteins are upregulated due to salt treatment. These include hexokinase-5, 6 from the hexokinase family, glutamyl-tRNA (Gln) amidotransferase from the eudicot gene family, and adenylosuccinate synthetase from the adenylosuccinate synthetase family, which inserts bonds into folding proteins, transport them, and shows an imperative role in various pathway of purine nucleotide biogenesis and protein degradation (String Consortium 2023). Two down-regulated proteins, ubiquitin-like modifier-activating enzyme 5, which were highly expressed during salinity, were discovered and showed identity with maize and potato (Liu et al. 2019). Hexokinase has been described to have a significant influence on cell digestion and other sugar signaling pathways that rely on phosphorylated hexoses and glycolytic intermediates, and it is the only protein in plants that can phosphorylate Glc (Paulina and Sobeida 2017). Guanine-3′, 5′-bispyrophosphate (ppGpp), a hyperphosphorylated guanine ribonucleotide, is identified, for example, a comprehensive regulator in PGPR and adjusts the roles of numerous mechanisms in procaryotic cell systems under many stress circumstances in plants (Boutte and Crosson 2013). Yuhta et al. (2014) present compelling evidence for modulating the adenylosuccinate synthetase protein in rice chloroplasts to numerous stress environments. Qin et al. (2016) showed that Gln-tRNAGln is required to assemble protein transport in mitochondria, markedly ATP fabrication was reformed in Osgatb root tip-up cells subjected to salt stress. Drought and saltiness increased the appearance of a ubiquitin-like modifying enzyme in soybean, whereas *Arabidopsis* plants overexpressing GmUBC2 were more tolerant to salt and drought conditions than uninoculated plants (Zhou et al. 2010).

Proteins engaged in metabolism, transduction, and signaling pathways

Salt treatment was reported to alter the lipid content of broccoli roots and alfalfa cultivars (Rahman et al. 2015). In our work, 6-phosphogluconate dehydrogenase, involved in lipid binding and molecular transducer activity in protein metabolism, was upregulated in rice encoding the important enzyme. It metabolizes the third step in the PPP oxidative phase reaction and has been investigated for its function in rice defense induced by various abiotic stresses. In the current study, glutamine synthetase and chloroplast proteins were identified as metabolic proteins involved in plant development and halophilic due to the application of a microbial consortium (Surabhi et al. 2021). Under osmotic and salt stressors, the expression of the down-regulated tubby-like protein CsTLP8 increases, involving stress reactions with unclear metabolic pathways (Wang et al. 2018). Ni et al. (2018) found that the downregulation of very long chain (VLC) alkanes, which are important components of cuticle waxes in rice leaves, shows a critical task in the plant reproductive system and alkane production

during plastid differentiation of anther development in rice. Another signaling metabolite studied in plants is cyclin-T1;3, downregulated because of salinity. Down-regulation of cyclin-T1;3 in rice leads to tinier grains, suggesting that cyclin-T has a novel function in cell cycle regulation. The current study detected changes in the expression of several transduction factors, including 6-phosphogluconate dehydrogenase protein, glutamine family synthetase, cyclin, and others, suggesting that a microbial consortium may cause significant variations in plant production and facilitate salt tolerance over the participation of several transduction and signaling pathways in metabolism.

Proteins implicated in the regulation of signal transcription

Calcium-dependent proteins are implied in controlling signal transcription, cellular growth response, plant root formation, and regulatory processes. Activation and downregulation of Ca^{2+}-dependent protein kinase signaling are universal responses to various abiotic stresses that enable cells to respond rapidly to environmental stimuli. Recognition of plant growth microbes leads to an influx of Ca^{+}-dependent proteins that activate various signaling pathways responsible for relaying immunological signals important for tolerance to abiotic stress or disease-causing agents (Bredow and Monaghan 2019). It is thought to function upstream of mitogen-activated protein kinase (MAPK) in ABA signaling, leading to seed sprouting, root growth, H_2O, and oxidative stress adaptation (Chen et al. 2021). The BTB/POZ domain and ankyrin repeat-encompassing protein NPR1 were discovered in plants as transcriptional signaling proteins that perform a role in alleviating abiotic stresses in the model plant rice. This is essential primarily when a plant is exposed to salt tension. Alterations in membrane potential also correlate with the beginning of several other signaling transcriptional regulatory processes, particularly those implicated in pathogens and stress (Yuan et al. 2007). The regulation and signal transcriptional protein family contains Zinc Finger (BTB/POZ) domains (Li et al. 2006). The multiple functional roles of NPR1-like proteins indicate a complex and significant functional family connected to immune reactions and plant development.

According to the results of this study, the up- and down-regulation of several salt-responsive proteins by applying microorganisms seems to serve to repair or prevent more severe damage to the plant's biological, molecular, or cellular activities. In young rice seedlings, photosynthesis, protein synthesis, and protein metabolism responded more strongly to salt stress than osmotic stress. It will be interesting to learn more about the engagement of the above proteins in the rice seedling's response to salt stress.

Conclusion

The reaction of plants to salt tension is a complicated process whose processes are difficult to decipher when only one or a few genes are studied. "Omics" studies have been a valuable tool for studying complex biological phenomena. Proteomic methods have recently provided important insights into plant biology, microbiology, human diseases, and other fields. This study presents a thorough proteomic study of rice leaf sheath proteins under high salinity conditions. Fifty-four different salt strain-responsive proteins were found, each implying various cellular roles, including glucose, nitrogen, energy metabolism, ATP synthase, mRNA and protein synthesis, and antioxidant stability. Ten of these proteins are novel salt stress-responsive proteins, such as 60 kDa chaperonin, 70 kDa heat shock protein BIP4, and calreticulin, to name a few. These findings form the basis for further research into their role using proteomics and other techniques. The proteome of any life form is extremely dynamic and has an infinite number of potential variants. The proteins in this study signify a minor portion of the rice proteome. Many more proteins that respond to salt stress remain to be discovered. Non-cytosolic proteins, for instance, membrane proteins and nuclear proteins, are thought to perform important tasks in osmo-sensing, ion transport, and signal transduction. A more thorough proteome study may help us better realize the mechanism. Future research combining transcriptomics, proteomics, and metabolomics will be of great benefit in elucidating the mechanisms of the salt stress response. All these techniques have great potential in the field of microbiology and will significantly impact our understanding of associated abiotic stressors. If data analysis can keep pace with advances in next-generation sequencing, transcriptomics will lead the way. 2-DE will remain the dominant technique in proteomics for the foreseeable future. However, as gel-free proteomics technology becomes more accessible, its application will increase. The improved resolution of mass spectrometers will benefit the field of metabolomics. There will be fewer and fewer "unknowns" thanks to the new generation of instruments that will make the identification of chemicals much easier. Nevertheless, there is still much untapped potential for each of these platforms, and systems biology research will benefit from the simultaneous assessment of transcripts, proteins, and metabolites from the same sample.

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Author contribution ATKZ: conceptualization, methodology, validation, formal analysis, investigation, writing—original draft, writing—review and editing, visualization, supervision, project administration,

and funding acquisition; SSC: conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft, writing—review and editing, and visualization; AMA: conceptualization, supervision, and funding acquisition; TGH: conceptualization, supervision, and funding acquisition; AHAG: conceptualization and supervision; BMS: resources and data curation; AA: resources and data curation; MER: software; HOR: software, writing—review and editing, and visualization.

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Data availability All data that support the findings of this study were included in this manuscript.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare no competing interests.

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