PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.9049

eISSN 2348-1900

RESEARCH ARTICLE

Comparison of the protein system in early response to salinity stress of three rice varieties (*Oryza sativa* L.) with contrast phenotypes

Cuong Duong Quoc[1,2,3], Anh Bui Lan[1,2], Thia Le Hong[4], Gia-Buu Tran[5], Tien T Dang[6], Thinh Nguyen Hung[7], Tuan Nguyen Huu Ngoc[7], Ha Nguyen Cong[8], Ngoc Nguyen Thi Le[8] & Nam Trinh Ngoc[3*]

[1]Faculty of Biology and Biotechnology, University of Science, Ho Chi Minh City 700 000, Vietnam
[2]Vietnam National University, Ho Chi Minh City 700 000, Vietnam
[3]Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City 700 000, Vietnam
[4]Institute for Environmental Science, Engineering and Management, Industrial University of Ho Chi Minh City 700 000, Vietnam
[5]Research Group in Pharmaceutical and Biomedical Sciences, Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City 700 000, Vietnam
[6]Institute of Advanced Technology, Vietnam Academy of Science and Technology, Ho Chi Minh City 700 000, Vietnam
[7]Faculty of Basic Medical Science, Pham Ngoc Thach University of Medicine, Ho Chi Minh City 700 000, Vietnam
[8]Institute of Food and Biotechnology, Can Tho University, Can Tho City 900 000, Vietnam

*Correspondence email - trinhngocnam@iuh.edu.vn

Received: 23 April 2025; Accepted: 04 August 2025; Available online: Version 1.0: 03 December 2025

Cite this article: Cuong DQ, Anh BL, Thia LH, Gia-Buu T, Tien TD, Thinh NH, Tuan NHN, Ha NC, Ngoc NTL, Nam TN. Comparison of the protein system in early response to salinity stress of three rice varieties (*Oryza sativa* L.) with contrast phenotypes. Plant Science Today (Early Access). https://doi.org/10.14719/pst.9049

Abstract

Rice (*Oryza sativa* L.), a staple food for over 40 % of the global population, faces significant yield losses due to salinity stress, particularly at the germination stage. This study compared early proteomic responses to NaCl stress in three Vietnamese rice varieties with contrasting tolerance: OM 9577 (salt-tolerant), OC 10 (moderately tolerant) and Dai Thom 8 (salt-sensitive). Germinating seeds were subjected to NaCl treatment and proteins were extracted for analysis by Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) and Two-Dimensional gel Electrophoresis (2-DE) combined with LC-MS/MS. In total, 160 peptide fragments corresponding to 45 differentially abundant proteins were identified, spanning 13 functional categories, including metabolism, stress response, transcriptional regulation, signal transduction, cellular transportation, cell growth, cell division and protein modification. 2-DE profiles confirmed characteristic proteins in the tolerant and moderately tolerant varieties: OC 10 exhibited three proteins associated with cellular transportation, stress response, signal transduction and transcription, while OM 9577 exhibited eight proteins related to nucleotide metabolism, transport, signal transduction, stress response, cell growth, cell division and transcriptional regulation. These proteins likely underline enhanced salt tolerance and are proposed as candidate biomarkers for molecular screening and the improvement of salt-tolerant rice via conventional breeding and gene transfer.

Keywords: 2-DE; LC-MS/MS; *Oryza sativa* L.; proteomic; stress NaCl

Introduction

Cereal crops play a crucial role in human society; they serve as essential staples for global populations and hold significant economic value as the most traded agricultural commodities worldwide (1). Among cereal crops, rice (*Oryza sativa* L.) is recognized as one of the least salt-tolerant species due to its classification as a glycophyte. Unfavourable environmental conditions, including drought and salinity, significantly contribute to the decline in agricultural productivity on a global scale (2). Salt stress affects more than 900 million hectares of agricultural land (about 20 %) under cultivation globally (3), reduces agricultural land productivity by 30 % and is expected to decrease by more than 50 % by 2050 (4,5).

Salt stress is the accumulation of increased salt levels in the soil beyond the level required by plants, causing growth inhibition, inhibition of photosynthesis, reduction of water potential, cell dehydration, ionic stress, leaf fall, membrane and protein instability, oxidative stress, leading to cell death and plant death (6). In rice, salt tolerance changes during different growth stages of the rice plant (7). Salt tolerance occurs at the seedling stage, then becomes very sensitive during the seedling stage (leaf age 2-3), then becomes tolerant during the growth stage, followed by the pollination and fertilization period and finally shows salt tolerance response during the grain maturity period. The germination stage of rice is one of the most sensitive stages to salt stress. Therefore, for rice, the screening process at the morphological level, based on the physiological and agronomic characteristics of roots, leaves, shoots, etc., can be performed at the seedling stage, which is the

most sensitive stage of rice (3). In addition, studying the biochemical and molecular biological mechanisms of rice during the seedling stage contributes to providing a scientific basis for improving resistance throughout the plant's life cycle (8). Therefore, many researchers must divide their studies into different growth stages to comprehensively understand the salt tolerance mechanisms in rice (7,9).

In Vietnam, rice is the main food crop of agriculture and is grown in all large and small delta regions across the country (9). Of these, the Mekong Delta and the Red River Delta have the highest rice productivity rates of 51 % and 15 %, respectively (7). However, the Mekong Delta, a region with 1.7 million hectares of agricultural land, has 45 % of its area affected by saltwater intrusion (7). The area of re-salinized land accounts for about 46 %, with some regions experiencing salinity intrusion up to 50 km inland (Ben Tre, Tien Giang, Vinh Long, Hau Giang) (9). Recently, several scientists in Vietnam have enhanced rice varieties to withstand salinity stress through traditional breeding techniques and/or molecular marker selection, including marker-assisted selection (10). For instance, several salt-tolerant rice varieties, such as OM 9577, have been developed to withstand salinity levels of approximately 4–6 ‰. Additionally, the rice variety OC 10 is a local rice variety from Ben Tre province in Vietnam, recognized for its potential in salt tolerance, as it can endure salinity levels of 3–4 ‰. Previously, scientists concentrated on developing high-yield varieties, including the rice variety Dai Thom 8. However, its limited tolerance to salt can negatively impact production yields amid extreme climate variations and the expansion of saline soil areas (7,9,11).

Plants significantly increased protein content in response to salt stress at appropriate concentrations, such as energy and carbohydrate metabolism proteins, defense systems, osmolyte biosynthesis enzymes, ROS free radical scavenging enzymes, cell membrane protection proteins and proteins involved in the photosynthetic pathway (4,12,13). Proteome analysis is important and effective for studying plant adaptation under salinity and is widely used to understand the molecular mechanisms of plant response to salt stress (4,14). Specifically, the proteomic approach (non-gel based) by LC-MS/MS allows the assessment of the entire protein on a large scale with efficient reproducibility (15,16). In addition, the 2-DE method is the first technique to separate protein mixtures, combined with polypeptide sequencing by liquid chromatography coupled to mass spectrometry to allow accurate identification of expressed proteins to be analysed (15). For example, A previous study identified over 500 protein spots utilizing 2-DE. They successfully identified 44 proteins through matrix-assisted laser desorption/ionization-time of flight mass spectrometry, which corresponds to 18 distinct functions (13). In addition, another study employed 2-DE to assess protein expression and utilized nano-liquid chromatography coupled with tandem mass spectrometry to identify the names and functions of proteins expressed in rice roots in response to salinity stress (17). A previous study utilized nano-liquid chromatography coupled with tandem mass spectrometry analysis across various rice varieties to elucidate the mechanisms underlying the salinity stress response (16). The studies underscore the efficacy of proteomic analysis in examining plant adaptation in response to polygenic salinity stress (12,13,16,18).

At present, the rice varieties OM 9577, OC 10 and Dai Thom 8 are extensively cultivated and provide significant yields for the population in Ben Tre province, Vietnam. Nonetheless, the southern regions, particularly Ben Tre, are experiencing significant salinity issues, resulting in a decline in both rice productivity and quality. Consequently, a recent study conducted a study examining the physiological and biochemical characteristics of these three rice varieties to elucidate the mechanisms underlying the salt tolerance of each variety (7). Furthermore, the research conducted by the authors highlights the significance of sugar compounds, enzymes and amino acids in safeguarding cells from salinity stress (9). Although these studies examined all morphological, physiological, biochemical and genetic characteristics in roots of three rice varieties (OM 9577, OC 10 and Dai Thom 8) in response to salt stress, in particular, the correlation between physiological, biochemical and genetic responses in each rice variety with NaCl stress concentration was shown. However, proteomic analysis of roots of three rice varieties (OM 9577, OC 10 and Dai Thom 8) at the germination stage has not been studied yet. Despite advances in breeding salt-tolerant rice, the molecular mechanisms-especially proteomic differences-underlying the stress responses in OM 9577, OC 10 and Dai Thom 8 remain unclear. This study aims to analyse the protein expression profiles in 6-day-old roots of these varieties under salt stress using LC -MS/MS, complemented by 2-DE and sequencing of selected protein spots, to identify key contributors to salinity tolerance.

Materials and Methods

Plant materials

The research materials were three rice varieties with different responses to NaCl stress: OM 9577 (salt-tolerant), OC 10 (moderately tolerant) and Dai Thom 8 (salt-sensitive). All rice varieties are provided by Loc Troi Company and the Center for High-Tech Agriculture Application of the Department of Agriculture and Rural Development of Ben Tre province.

Sterilization and root collection process

Six-day-old rice seedlings were prepared following a previously described procedure (7). Rice seeds were disinfected with 5 % NaOCl for 15 min and washed three times with distilled water. A total of 20 seeds were then placed in petri dishes containing water and incubated in the dark for 6 days. The 6-day-old seedlings with root lengths of 2-2.5 cm were subsequently treated with 50 mM NaCl (Dai Thom 8) or 100 mM NaCl (OC 10 and OM 9577) solutions for 72 hr. After treatment, root tips were collected and stored at -80 °C for use in further experiments, such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), 2-DE and LC-MS/MS analysis.

SDS-PAGE

Rice roots were ground with liquid nitrogen and 200 µL phosphate buffer saline, pH 7.4, were added. The mixture was subjected to ultrasound (15 min) and centrifuged at 13000 rpm for 30 min (4 °C). The supernatant was collected and protein concentration was determined using the Lowry method (19). SDS-PAGE was performed with a total of 100 µg protein on a polyacrylamide gel (with 4.5 % stacking gel and 12 % separating gel); the voltage was set constantly at 100 V for 3 hr. The gel was then washed three times with water and stained with Coomassie Blue (Thermo, USA) to visualize the protein profile (20).

Determination of proteins in total lysates using LC-MS/MS

Total proteins were extracted from rice roots by PierceTM Plant total protein kit (Thermo, USA) and purified using a 2-D clean-up kit (Cytiva, USA); the protein was subsequently stored in -80 ˚C before using for total protein analysis by LC-MS/MS and 2-DE combined with protein sequencing. A total of 10 mg of protein was solubilized in the solution containing NH_4HCO_3 and β-mercaptoethanol. Following reduction, alkylation and precipitation, the protein underwent cleavage with trypsin and endoproteinase Glu-C enzymes (Sigma-Aldrich, USA) to yield peptide fragments. The precipitated protein was dissolved in 200 µL of ammonium bicarbonate buffer at a concentration of 100 mM, with a pH of 8. The mixture was analyzed using LC-MS/MS. The LC-MS/MS system utilized was a Triple TOF 6600 mass spectrometer (AB Sciex) paired with an Xbridge C18 column (3.5 µm 2.1 x 150 mm). The gradient cycle was set as following conditions: the solvent system for buffer A consisted of 0.1 % formic acid in miliQ-water, while buffer B comprised 0.1 % formic acid in acetonitrile, with a flow rate of 0.3 mL/min (21). The software utilized for reading and processing mass spectrometry/mass spectrometry results was ProteinPilot 5.0 software.

Two-dimensional gel electrophoresis

A total of 200 mg of protein was dissolved in 300 µL DeStreakTM Rehydration buffer (Cytiva, USA) and 6 µL IPG buffer with pH 4 - 7 (Cytiva, USA) containing 0.1 mg dithiothreitol (Thermo, USA). The protein mixture and ReadyStrip IPG strip (11 cm, pH 4–7) were put in the cytiva strip holder. A layer of drystrip cover fluid was applied on the top to cover the surface. The first electrophoresis was conducted in the ettan IPGphor 3 system (GE HealthCare, USA) according to the IEF (Isoelectric Focusing) program: 50 V for 1 hr, 250 V for 1 hr and 30 min, 500 V for 1 hr, 1000 V for 1 hr, 5000 V for 4 hr, 6000 V for 7 hr and 500 V for 5 hr (18).

The Immobilized pH Gradient (IPG) strip equilibration phase was performed using sodium dodecyl sulfate (SDS) solution containing dithiothreitol (Thermo, USA) and iodoacetamide (Thermo, USA): IPG strip was incubated in 2 mL of SDS buffer [75 mM Tris-HCl (pH 8.8), 6 M urea, 29.3 % (v/v) glycerol, 2 % (w/v) SDS and bromophenol blue] and 0.02 g dithiothreitol for 15 min on a shaker (80 rpm). Next, the IPG strip was incubated in 2 mL of SDS buffer [75 mM Tris-HCl (pH 8.8), 6 M urea, 29.3 % (v/v) glycerol, 2 % (w/v) SDS and bromophenol blue] containing 0.05 g iodoacetamide for 15 min on a shaker (80 rpm). After equilibration, the IPG strips were electrophoresed on 12.5 % polyacrylamide gels with voltage cycling of 80 V for 2 hr, 100 V for 2 hr and 130 V for 3 hr. The gel is then stained with Coomassie Blue (Thermo, USA) to visualize the protein spots (13,18) and the protein spots were extracted and sequencing using LC-MS/MS.

Identification of protein spots extracted from 2-DE by LC-MS/MS

The protein spots separated by 2-DE were cut, chopped and stored in an Eppendorf. The samples were washed with distilled water (3 times) and incubated for 20 min with 100 µL NH_4HCO_3 (100 mmol/L in 50 % acetonitrile) (3 times). The gel cubes were reduced with 100 µL of dithiothreitol (10 mmol/L) (Thermo, USA) for 1 hr (room temperature and dark) and the gel was alkylated with 100 µL of iodoacetamide (25 mmol/L) (Thermo, USA) for 1 hr (room temperature and dark). Finally, the samples were dried with 500 µL of acetonitrile (Sigma-Aldrich, USA).

Protein spots were digested with 100 µg/µL trypsin (Sigma-Aldrich, USA) and 100 µg/µL endoproteinase Glu-C enzymes (Sigma-Aldrich, USA) at 37 ℃ for 16 hr to obtain peptide fragments. The peptide fragment was extracted from the gel using 50 mM NH_4HCO_3 solution with 50 % acetonitrile and 5 % trifluoroacetic acid (TFA) (Sigma-Aldrich, USA) (22). The mixture of peptide fragments was vacuum-dried and then dissolved in 0.1 % formic acid solution (Sigma-Aldrich, USA). The peptide fragments were sequenced via LC -MS/MS analysis as described above (21).

Software

The experiments were repeated 3 times. 2-DE images were analysed using SameSpots 5.1.012 software. Histograms were drawn using Excel 2016. The software used to read and process mass spectrometry/mass spectrometry results was ProteinPilot 5.0 software. The names and functions of some proteins were determined based on the UniProt database (https://www.uniprot.org) and the NCBI database. Determine pI and kDa of proteins using Expasy application - Compute pI/Mw tool.

Results

Visualization of protein profile of three varieties using SDS-PAGE

The SDS-PAGE was performed to primarily separate and visualize the difference in the protein profile of roots during salinity stress of three varieties according to protein molecular weights. The results in Fig. 1 showed that majority of proteins in this study had molecular weights ranging from 14.4 kDa to 97 kDa. Exposure to salinity stress changed several protein bands of rice roots' expressions. Proteins were divided into three groups based on their molecular weights.

Group 1 included some proteins with molecular weights from 50 to 97 kDa and 4 protein bands. Band 3 (66 kDa) had strong expression. However, when comparing the protein bands in group 1, the three rice varieties did not show much difference. Group 2, with molecular weight from 25 to 50 kDa, had a few bands. Among them, band 5 (35 kDa) expression of rice roots exposed to salinity stress was stronger than that of the control. Interestingly, the expression levels of these bands in group 2 of OC 10 were the highest among the three rice varieties during salinity stress. Group 3 contained 4 protein bands with molecular weights from 14.4 to 25 kDa. All 4 protein bands were upregulated during salinity stress as compared to the control. Note that there was a significant difference in protein band expression between the three varieties. For example, the rice variety Dai Thom 8 tended to increase the expression of band 8 and band 9, while the rice varieties OM 9577 and OC 10 focused on increasing band 6 and band 7 expression. From there, it was shown that three rice varieties had different protein profiles according to molecular weights when responding to NaCl stress than the control. However, to in-depth investigate the difference in protein expression of three rice varieties in response to NaCl stress, we employed protein sequencing of total lysates obtained from the rice roots via LC-MS/MS analysis.

Protein sequencing of total proteins of three varieties via LC-MS/MS

A total of 160 peptide fragments was obtained from the total proteins extracted from the roots of three rice varieties in response to NaCl stress (Table 1–3). A comparative analysis of peptide fragments extracted from the roots of three rice varieties (OC 10, OM 9577 and Dai Thom 8) under NaCl stress revealed 160 peptides generated via

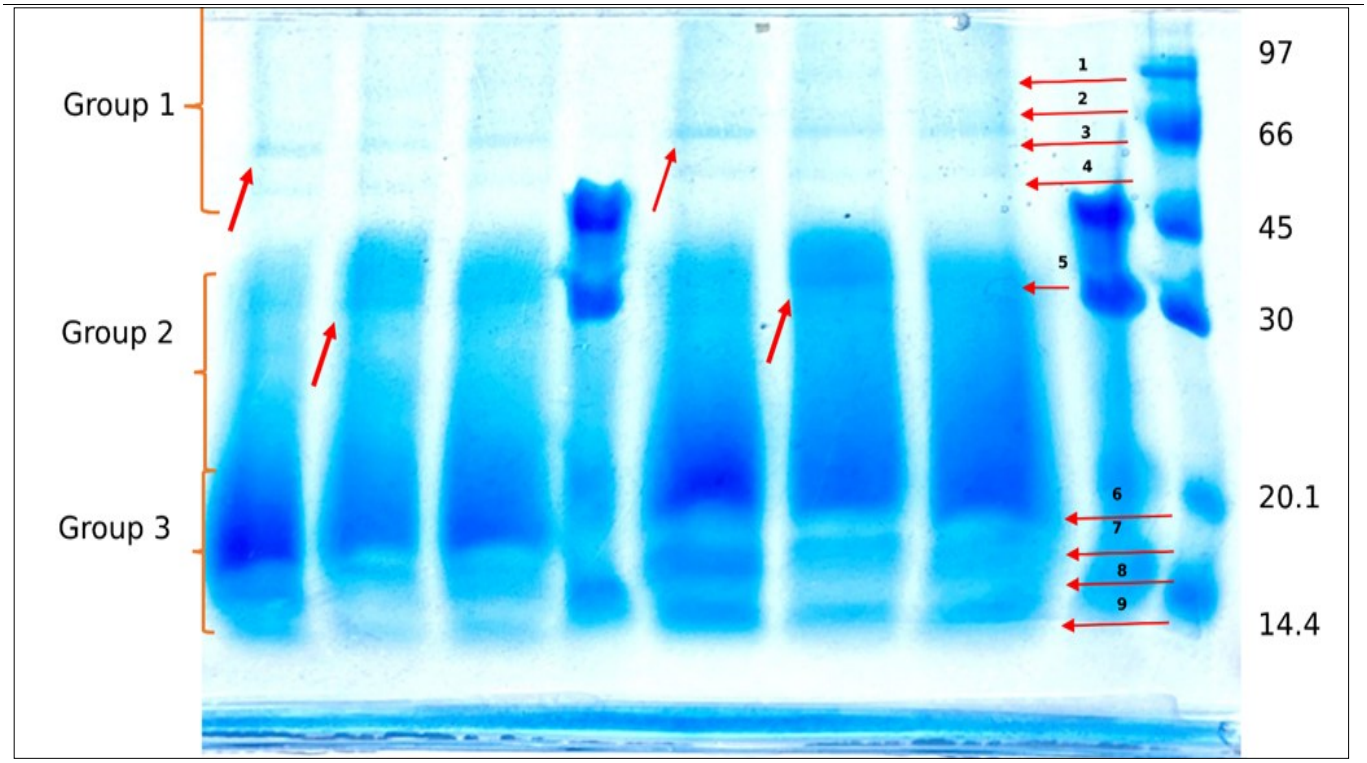

Fig. 1. Coomassie Blue stained gel of SDS-PAGE of proteins extracted from roots of three rice varieties in response to NaCl stress (50 mM and 100 mM) and control (0 mM NaCl).

Table 1. Selected proteins corresponding to peptide fragments expressed in the roots of rice variety OM 9577 under NaCl stress

Salt-tolerant (OM 9577)			
Peptide sequences are cut by trypsin and endoproteinase Glu-C		**Functions of proteins**	**Protein names contain peptide sequences**
IVLTIIR DLSQADFGRLE AEFGPSQPFK	SGKVPDPESTDNAEFK LVGVSEETTTGVK LYQMQETGALLFPAINVNDSVTK HSLPDGLMR	Amino acid metabolic Metabolic process Nucleic acid metabolic	Adenosylhomocysteinase (A0A0P0Y1Y5) Adenosylhomocysteinase (Q0ISV7)
SGDELTSLK SDLVNNLGTIAR GIVDSEDLPLNISR	STNRTKIAE YAVGQLKEFE DTSGEQLGR	Signal transduction Stress response	Heat shock protein 81-3 (Q07078) Heat shock protein 82 (Q5QLP0)
GFEVIDAIK GLLHSDQE DGVNLLGGPTWSVALGR DMTALSGAHTIG	SGGDANLAPFDVQTPDAFDNAYYQNLVSQR GLLHSDQELFNGGSQDGLVR QYSTNPSQFSSDFVSAMVK MGNLLPSSGTATEVR DMTALSGAHTIGR	Stress response	Peroxidase (B9FTP0)
	FQELGLEK SLSALQGALR QQGLNITPRI	Carbohydrate metabolic Metabolic process Protein metabolic process Cell growth	Sucrose synthase (P31924)
	LNVQVTDVK FISTVQQR MELVDAAFPLLK	Metabolic process Nucleic acid metabolic Protein metabolic process	Malate dehydrogenase (Q7XDC8)
TGSIVDVPAGK	NENVGIVVFGSDTAIKE	Transport	ATP synthase subunit alpha (P0C522)
TLADYNIQK TITLEVESSDTIDNVK	VESSDTIDNVKAK IQDKEGIPPDQQR TLADYNIQKE	Protein modification	Os02g0161900 (A0A0P0VF30)
EITALAPSSMK	SYELPDGQVITIGAER	Cell division Cell growth	Actin-2 (A3C6D7)
SSMDAFSQILK	QFNGLVDVYR	Transport	ADP/ATP translocase (A0A8J8Y950)
VQQLLQDFFNGK IIANDQGNR TAGGVMTVLIPR	ITITNDKGR DNNLLGKFE NYAYNMR	Stress response	Heat shock protein cognate 70 (A3AP50)

Table 2. Selected proteins corresponding to peptide fragments expressed in the roots of rice variety OC 10 under NaCl stress

Moderately tolerant (OC 10)			
Peptide sequences are cut by trypsin and endoproteinase Glu-C		**Functions of proteins**	**Protein names contain peptide sequences**
TAAAPIER GNTANVIR GAGANIL	TGSIVDVPAGK GSIVDVPAGK NVGIVVFGSDTAIKE	Transport	ADP/ATP translocase (A0A8J8XVC4) ATP synthase subunit alpha (P0C522)
KDDLQNTIVK VAEKYNVEAMPTFLFIKDGAE		Transport Stress response	Thioredoxin H1 (Q0D840)
SGMASAVR TCPNLATIVR DGVNLLGGPTWSVALGR TASQSAANSNLPG	GLLHSDQE LFNGGSQDGLVRQYSTNPSQFSSDFVSAMVK SAGPNANSAR PGSSLATLISMFGNQGLSAR	Stress response	Peroxidase (B9FTP0)
DITGNPR IIANDQGNR AKRLIGRR EIAEAFLSTTIK GIDFYATITR	TAGGVMTVLIPR DNNLLGKFE ITITNDKGR IERMVQEAEK NYAYNMR	Stress response	Heat shock cognate 70 kDa protein (Q10NA1)
SDLVNNLGTIAR DTSGEQLGR	YAVGQLKEFE IYYITGESKK	Stress response	Heat shock protein (Q0J0U8)
TLADYNIQKE	IQDKEGIPPDQQR VESSDTIDNVKAK	Protein modification	Ubiquitin-ribosomal protein eL40z (P0CH34)
SYELPDGQVITIGAER EITALAPSSMK		Cell division Cell growth	Actin-2 (A3C6D7)
MELVDAAFPLLK VLVVANPANTNALILKEFAPSIPEK FISTVQQR		Metabolic process Nucleic acid metabolic Protein metabolic process	Malate dehydrogenase (Q7XDC8)
AEFGPSQPFK DLSQADFGRLE SGKVPDPESTDNAEFK LVGVSEETTTGVK	HSLPDGLMRATDVMIAGK IDPICALQALME GLQVLTLEDVVSE IDMLGLETYPGVKR TGALLFPAINVNDSVTK	Amino acid metabolic Metabolic process	Adenosylhomocysteinase (A0A0P0Y1Y5)
PKKLNHEPNNED	EMGVSVGIKHK SSSSPSGHLL	Transduction	Nicalin 3 (B9FYM6)
TATNESD	LLDNSAE	Transcription	Auxin-responsive protein IAA10 (Q0DWF2)
LDPQYLLHDR	CLIAFYSAGYPLEK DGSYIYE	Metabolic process	Inositol hexakisphosphate and diphosphoinositol-pentakisphosphate kinase (A0A0P0V8W2)
YSPISQPYLSK	IKQKLE	Stress response	Peroxidase (Q0DCY)
VVQTVKQPR	LATTLERIE	Signal transduction Photosynthesis	Phototropin-2 (Q9ST27)
TSYYQNLLAGR	SVVLAGGTPYR	Stress response	Peroxidase (A3ADK4)
SHTAAEIRWGGAK	ILAQTTLDAE	Transcription	Phytochrome A (Q10DU0)
EVSGFHINPLNGKE	LMINDILDTVD	Metabolic process	Sucrose synthase (B9F4P4)
SSPSLKDPVMDTR	LFLILE	Cell growth	Os07g0603300 protein (A0A0P0X8U0)
ISQSSVQTTGSE	VPPFIGLIR	Transcription	BAH domain (B9F2Y3)
AAAGSEKPEGSE	SLTSMYNSK	Metabolic process	DNA helicase (A0A0P0WJI0)

Table 3. Selected proteins corresponding to peptide fragments expressed in the roots of rice variety Dai Thom 8 under NaCl stress

Salt-sensitive (Dai Thom 8)			
Peptide sequences are cut by trypsin and endoproteinase Glu-C		**Functions of proteins**	**Protein names contain one or more peptide sequences**
TGSIVDVPAGK	NVGIVVFGSDTAIKE	Transport	ATP synthase subunit alpha (P0C522)
SDLVNNLGTIAR AVENSPFLEK SGDELTSLK EDEEEKKD	IAENKEDYNK STNRTKIAE YAVGQLKEFE GLKLDESEDE DTSGEQLGR	Signal transduction Nucleic acid metabolic	Heat shock protein (Q0J0U8)
SGKVPDPESTDNAEFK LVGVSEETTTGVKRL TGALLFPAINVNDSVTK HSLPDGLMR		Nucleic acid metabolic Amino acid metabolic Metabolic process	Adenosylhomocysteinase (Q0ISV7)
EITALAPSSMK SYELPDGQVITIGAER DAPRAVFPSIVGRPR		Cell division Cell growth	Actin-2 (A3C6D7)
LVGVSEETTTGVK SGKVPDPESTDNAEFK	HSLPDGLMR TGALLFPAINVNDSVTK	Metabolic process Amino acid metabolic	Adenosylhomocysteinase (A0A0P0Y1Y5)
VESSDTIDNVK TLADYNIQKE	IQDKEGIPPDQQR QQRLIFAGKQLE	Protein modification	Polyubiquitin 3 (Q58G87)
LFNGGSQDGLVR QYSTNPSQFSSDFVSAMVK	GLLHSDQE GFEVIDAIKTQVE	Stress response	Peroxidase (B9FTP0)
LVDAAFPLLK NVSIYKSQASALE GIVATTDVVE		Metabolic process Nucleic acid metabolic Protein metabolic process	Malate dehydrogenase (Q7XDC8)
TAGGVMTVLIPR SDNQPGVLIQVYE DNNLLGKFE	IIANDQGNR FTDTERLIGD GIFEVKATAGDTHLGGE	Stress response	Heat shock protein cognate 70 (A3AP50)
LDFEPFNASFPRPSLSK	FFEKCQE LYRYICDTK	Carbohydrate metabolic	Sucrose synthase (P31924)
FEIIIFGD	LMLKYNGGKAHAE	Metabolic process	Inositol hexakisphosphate and diphosphoinositol-pentakisphosphate kinase (A0A0P0V8W2)
VSGFHINPLNGKE	LQTALLLAE	Metabolic process	Sucrose synthase (B9F4P4)

trypsin and endoproteinase Glu-C digestion. The rice variety OC 10 exhibited the highest peptide diversity (68 fragments), followed by OM 9577 and Dai Thom 8 (each > 45 fragments). These peptides corresponded to 45 proteins linked to 13 functional categories, including amino acid synthesis, nucleic acid synthesis, carbohydrate synthesis, signal transduction, stress response, metabolic process, transport, protein modification, cell division, protein metabolic process, cell growth, photosynthesis and transcription were identified (Table 4). Interestingly, there were some differences in the number of proteins and their functions among three cultivars in the early phase of salinity stress response (Table 4). The number of proteins in OC 10 was the highest (21 proteins), followed by OM 9577 (12 proteins) and Dai Thom 8 (12 proteins). OC 10's profile included 21 proteins such as adenosine diphosphate/adenosine triphosphate (ADP/ATP) translocase, thioredoxin H1 and phytochrome A, with enrichment in transcription and photosynthesis-related pathways. In contrast, OM 9577 and Dai Thom 8 each had 12 proteins, lacking transcriptional and photosynthetic components. All cultivars shared stress-responsive proteins like peroxidases (e.g., B9FTP0, Q0DCY2) and heat shock proteins, alongside metabolic enzymes such as malate dehydrogenase and sucrose synthase. The rice variety OC 10 uniquely lacked carbohydrate metabolic proteins, while OM 9577 and Dai Thom 8 showed no proteins associated with photosynthesis or transcription (Table 4). The protein profiles of all three rice cultivars commonly shared many functional proteins related to amino acid metabolic, nucleic acid metabolic, signal transduction, stress response, cell transport system and metabolic process.

Table 4. Functions of proteins in total lysates of three rice varieties during salinity stress

No.	Function of proteins	100 mM NaCl		50 mM NaCl
		OM 9577	OC 10	Dai Thom 8
1	Amino acid metabolic	1	1	2
2	Nucleic acid metabolic	2	1	2
3	Signal transduction	1	2	1
4	Stress response	3	6	3
5	Carbohydrate metabolic	1	0	1
6	Metabolic process	3	5	5
7	Transport	2	3	1
8	Protein modification	1	1	1
9	Cell division	1	1	1
10	Protein metabolic process	2	1	1
11	Cell growth	2	2	1
12	Photosynthesis	0	1	0
13	Transcription	0	3	0

The number of proteins in the rice variety OC 10 related to transcription, photosynthesis, stress response, metabolic process and transport was higher than those of the rice varieties OM 9577 and Dai Thom 8, while the number of proteins related to cell growth and protein metabolic process of the rice variety OM 9577 was higher those of the rice varieties OC 10 and Dai Thom 8. These differences suggest cultivar-specific adaptations, with OC 10 prioritizing transcriptional regulation and redox balance, whereas OM 9577 and Dai Thom 8 emphasize protein metabolism and stress signalling during early NaCl stress.

Identification of protein spots by 2-DE

As shown in Table 5, more than 100 protein spots were detected using SameSpots 5.1.012 software (Supplementary Fig. 1-3). In response to NaCl stress, the proteins in the roots of all three rice varieties had a common feature of being concentrated into three groups. Group 1 had a pI from 4-5 and a molecular weight below 25 kDa; group 2 had a pI from 4.5-5.5, a molecular weight from 26-66 kDa; and group 3 had a pI from 5.5-6.5 and a molecular weight from 30 to 97 kDa.

There were differences in protein spots numbers between rice varieties in each group. In detail, within group 1, the rice variety OC 10 had the most protein spots (about 21). Next the rice variety OM 9577 had 8 protein spots and the least of all was the rice variety Dai Thom 8 (6 protein spots). In group 2, the number of protein spots of OM 9577 was the largest number (21 protein spots), followed by those of Dai Thom 8 (10 protein spots) and OC 10 (8 protein spots). In group 3, the rice variety OM 9577 possessed the most abundance in protein spots (more than 35), followed by the rice variety Dai Thom 8 (more than 34 protein spots) and the rice variety OC 10 with 6 protein spots (Fig. 2-4).

Table 5. The protein spots on 2-DE gel of 3 rice varieties as exposing to NaCl stress were determined using SameSpots 5.1.012 software

Dai Thom 8						OC 10						OM 9577								
Spot	pI	kDa	**Spot**	pI	kDa	**Spot**	pI	kDa	**Spot**	pI	kDa	**Spot**	pI	kDa	**Spot**	pI	kDa	**Spot**	pI	kDa
53	6.22	59	**282**	6.38	19	**7**	4.58	65	**111**	4.35	18	**29**	6.10	94	**69**	6.25	46	**109**	5.12	33
58	6.12	57	**295**	4.43	18	**9**	6.04	63	**114**	4.72	17	**32**	6.10	85	**70**	6.42	45	**110**	4.91	32
61	6.26	56	**297**	4.29	18	**18**	5.74	58	**115**	5.27	17	**35**	5.02	80	**71**	6.71	44	**111**	4.95	32
62	6.15	54	**302**	4.26	18	**20**	6.02	58	**116**	5.41	17	**37**	4.46	78	**72**	5.70	44	**112**	4.93	32
63	6.39	54	**303**	5.38	18	**21**	6.48	57	**119**	5.53	17	**39**	5.81	77	**73**	5.86	43	**114**	5.53	32
66	5.96	52				**29**	6.11	46	**120**	4.35	17	**40**	6.78	77	**75**	6.02	43	**117**	5.42	29
81	6.41	45				**30**	6.22	45	**121**	4.37	17	**41**	5.31	76	**77**	4.94	42	**119**	5.23	27
82	6.12	45				**33**	4.68	44	**122**	4.40	17	**42**	6.02	76	**78**	4.46	41	**120**	4.73	26
83	6.37	45				**34**	5.56	43	**123**	4.45	17	**44**	4.80	75	**79**	4.98	41	**121**	4.78	26
103	6.34	41				**35**	5.05	43	**126**	4.26	17	**45**	5.62	74	**80**	5.40	40	**123**	6.01	25
104	6.65	41				**38**	5.74	41	**127**	4.28	17	**46**	6.46	74	**82**	6.44	40	**124**	4.65	24
117	6.45	39				**41**	4.93	40	**130**	4.98	17	**48**	6.71	71	**84**	5.80	40	**127**	4.64	22
121	5.28	39				**42**	5.64	40	**138**	6.75	16	**50**	5.41	66	**85**	5.14	39	**131**	6.01	20
131	6.25	38				**44**	6.51	39	**139**	5.72	16	**51**	6.25	66	**86**	5.02	39	**133**	5.50	20
133	5.89	37				**48**	6.25	37	**141**	4.91	16	**52**	6.34	64	**87**	5.76	39	**136**	5.62	20
136	6.62	37				**50**	4.94	33	**144**	5.30	16	**53**	5.32	64	**88**	6.55	39	**140**	5.33	19
146	6.58	35				**52**	4.90	32	**145**	4.73	16	**55**	5.68	58	**89**	6.71	39	**142**	4.47	18
151	6.22	35				**53**	4.83	32	**154**	4.93	15	**56**	5.91	57	**91**	6.54	38	**145**	5.95	18
152	5.19	35				**54**	4.87	32	**163**	4.81	15	**57**	5.31	56	**92**	4.74	38	**146**	5.36	18
157	5.08	35				**57**	4.73	24				**58**	6.47	56	**93**	5.52	37	**149**	5.93	18
160	4.97	34				**58**	4.68	23				**59**	6.34	56	**94**	6.40	37	**151**	4.77	18
164	5.36	33				**93**	5.04	18				**60**	5.30	56	**96**	5.52	36	**152**	4.63	17
177	5.53	31				**97**	6.73	18				**61**	5.40	56	**97**	6.08	36	**153**	5.77	17
183	4.88	30				**101**	6.50	18				**62**	5.61	51	**98**	5.94	36	**154**	5.72	17
203	5.37	29				**102**	6.63	18				**63**	6.71	49	**99**	5.81	36	**155**	4.95	17
206	6.48	28				**105**	6.49	18				**64**	6.46	48	**101**	5.38	35	**158**	5.75	17
247	5.27	23				**108**	4.48	18				**65**	5.59	47	**103**	5.33	34	**160**	5.57	17
272	6.61	20				**109**	5.09	18				**66**	4.73	47	**104**	5.69	34			
												67	4.75	46	**105**	5.68	34			
												68	4.77	46	**106**	5.98	34			

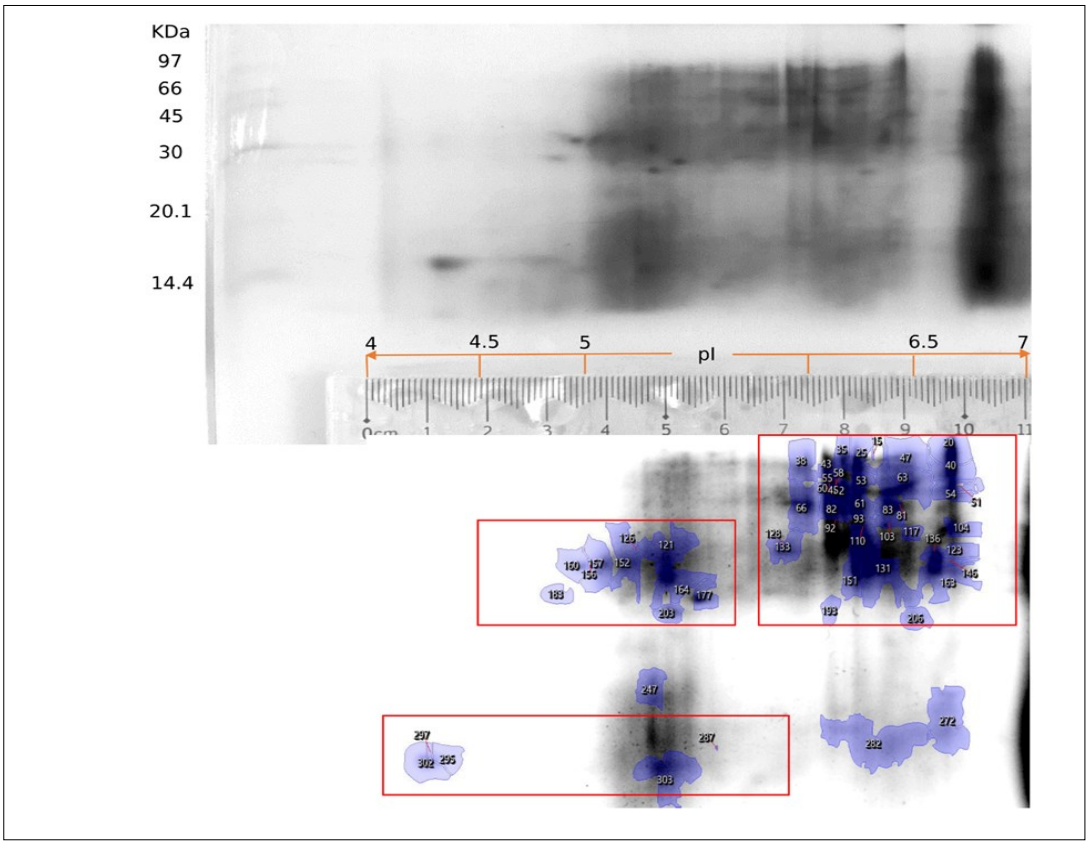

Fig. 2. Protein spots in the roots of the rice variety Dai Thom 8 displayed on a 2-DE gel.

pI 4-7; molecular weight 14.4-97 kDa; strip length 11 cm; 12.5 % polyacrylamide gel

The 2D gel image on the top was taken from ImageQuant LAS 500. The 2-DE gel image was analyzed by SameSpots 5.1.012 software to determine protein spots

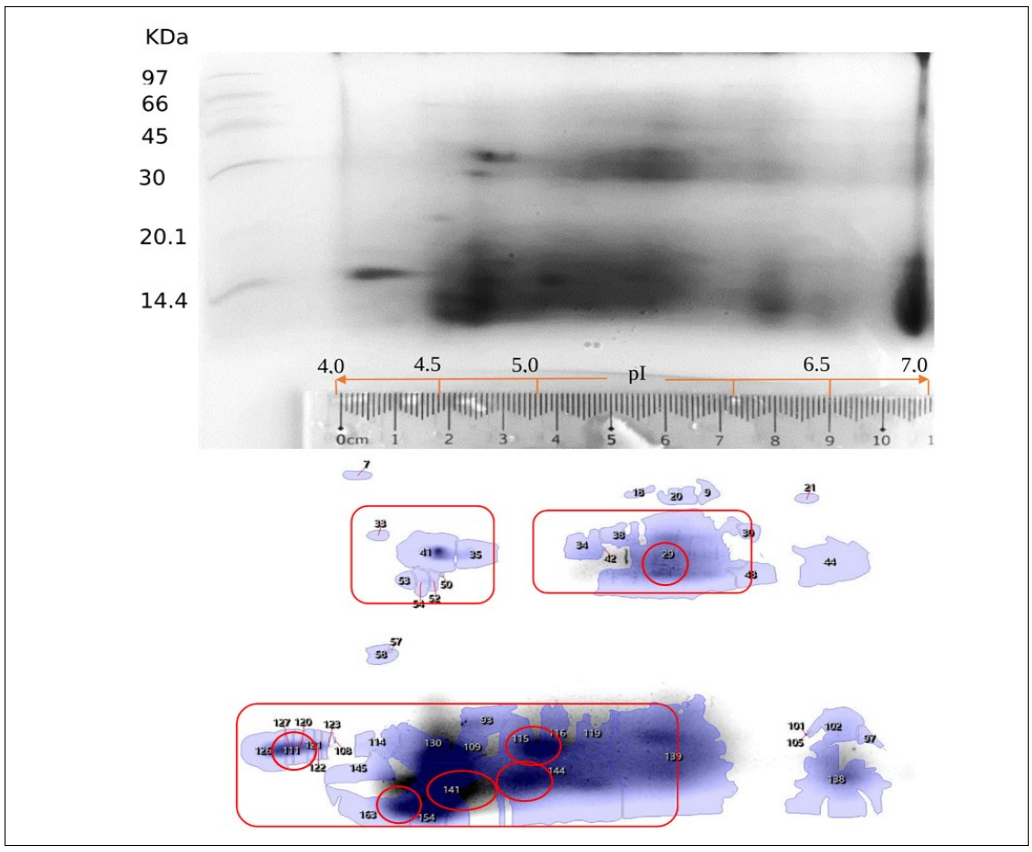

Fig. 3. Protein spots in the roots of the rice variety OC 10 displayed on a 2-DE gel.

pI 4-7; molecular weight 14.4 to 97 kDa; strip length 11 cm; 12.5 % polyacrylamide gel

The 2D gel image on the top was taken from ImageQuant LAS 500. The 2-DE gel image was analyzed by SameSpots 5.1.012 software to determine protein spots

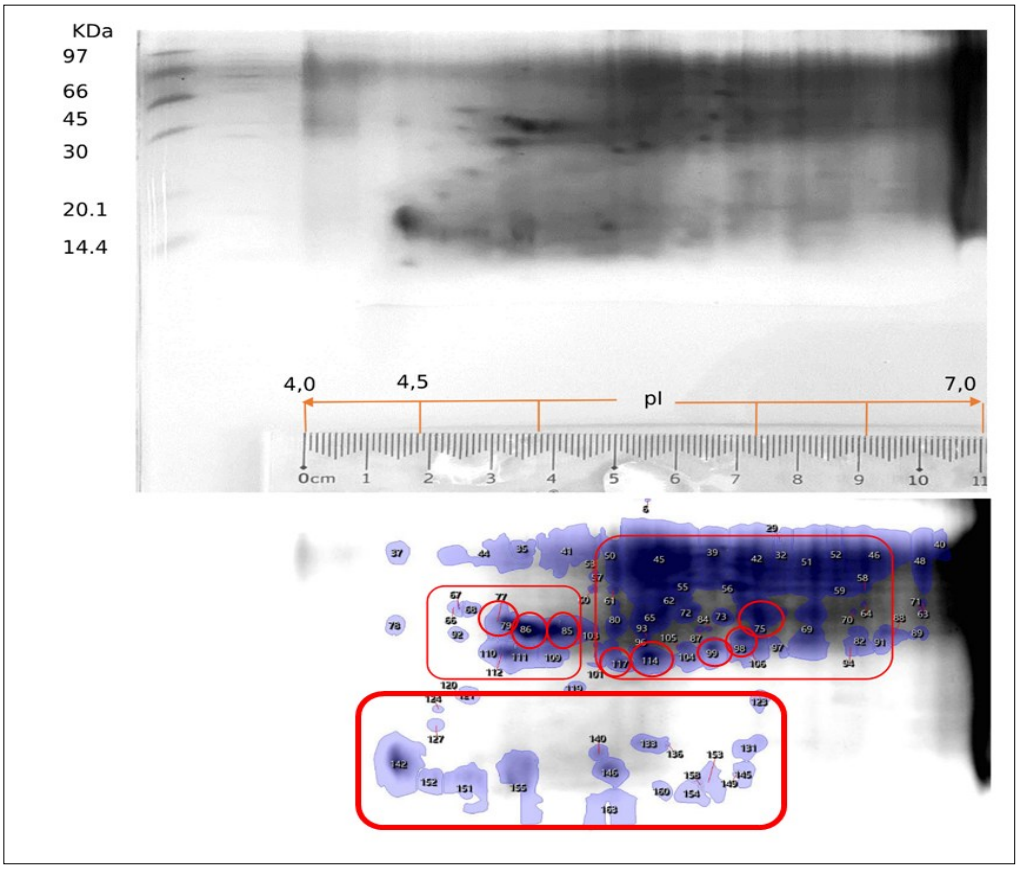

Fig. 4. Protein spots in the roots of the rice variety OM 9577 displayed on 2-DE gel.

pI 4-7; molecular weight 14.4-97 kDa; strip length 11 cm; 12.5 % polyacrylamide gel

The 2D gel image on the top was taken from ImageQuant LAS 500. The 2-DE gel image was analyzed by SameSpots 5.1.012 software to determine protein spot

To further investigate the name and functions of some putative protein spots on 2-DE, we sequentially cleaved proteins with trypsin and endoproteinase Glu-C. We extracted the peptide fragments for sequencing via LC-MS/MS analysis and comparison with protein sequences from the National Center for Biotechnology Information (NCBI) (pBLAST) and Uniprot databases. The putative protein spots in Fig. 2–4 were marked with red circles and were further analysed using LC/MS-MS.

Protein identification via LC-MS/MS of protein spots extracted from 2-DE

Proteins from the rice varieties OM 9577 and OC 10, exposed to 100 mM NaCl, were functionally characterized using p-BLAST on NCBI (www.ncbi.mlm.nih.gov/BLAST/) and the UniProt database (https://www.uniprot.org). In the rice variety OC 10, six proteins were identified: Forkhead-Associated (FHA) domain-containing protein, hypothetical protein EE612_036920, thioredoxin H-type, Os02g0829700, uncharacterized protein (Gi Number: BAD31129.1) and Os02g0778100 protein (Table 6). Among these, the FHA domain-containing protein and thioredoxin H-type are associated with rice transcription regulation and stress response mechanisms. In contrast, the hypothetical protein EE612_036920 is linked to the MAPK signaling pathway. The remaining three proteins (Os02g0829700, Os02g0778100 and BAD31129.1) remain uncharacterized, with no confirmed functional annotations. Besides that, 3 proteins such as Os02g0829700 protein, Os02g0778100 protein and uncharacterized protein (Gi Number: BAD31129.1), are unknown functions. In contrast, the rice variety OM 9577 exhibited eight proteins under the same saline conditions: MYB18 protein, peroxidase, adenylosuccinate lyase, protein-serine/threonine phosphatase, lactoylglutathione lyase, cyclin-T1-3, HAT (histone acetyltransferase) family dimerization domain-containing protein and soluble inorganic pyrophosphatase (Table 7). These proteins span diverse functional categories, including cell division and growth (cyclin-T1-3), signal transduction (protein-serine/threonine phosphatase), transcription regulation (MYB18), cellular transport, stress response (peroxidase) and metabolic processes (adenylosuccinate lyase, lactoylglutathione lyase). The functional diversity highlights the complex molecular adaptations of OM 9577 to salinity stress compared to OC 10.

Discussion

Salinity stress is one of the abiotic stresses that has the most influential effect on agriculture worldwide. Salinity stress first affects photosynthesis and cell growth (14). Specifically, it reduces the growth of shoots and roots (7). According to a previous study, almost all previous studies focus on analysing the proteomic response to the salinity stress of leaves. Still, roots are the first organs to respond to salinity stress, signal transduction and ion balance (23). In addition, the protein concentrations in roots change more rapidly in response to salinity stress than those in leaves and stems (24). This suggests that roots act as a partial barrier to NaCl transport to other parts of the plants. In this study, we performed proteomic analysis to identify the protein profile and expression of rice roots with different levels of salt tolerance exposed to salinity stress. While salinity stress impacts multiple physiological processes, roots are particularly critical in early signal perception and protein-level responses,

Table 6. Protein identification of protein spots of the rice variety OC 10 extracted from 2-DE

Spot No.	Protein	Gi number	Molecular function	pI	Mr (×10³)	Role	Organism
			Moderately tolerant (OC 10)				
29	FHA domain-containing protein	EAZ06542.1	Developmental protein DNA-binding Protein phosphate inhibitor Repressor RNA-binding	6.17	45	Stress response Transcription	*Oryza sativa* L.
111	Hypothetical protein EE612_036920	KAB8104199.1	MAPK signaling pathway - plant	4.37	19	Aallergen V5/Tpx-1 related family protein	*Oryza sativa* L.
115	Thioredoxin H-type	AAB51522.1	Protein-disulfide reductase activity	5.17	13	Transport Stress_response	*Oryza sativa* L.
141	Os02g0829700	BAS81731.1	Unknown protein	5.12	13	Predicted protein	*Oryza sativa* L.
144	Uncharacterized protein	BAD31129.1		5.32	14	Predicted protein	*Oryza sativa* L.
163	Os02g0778100 protein	BAS81185.1		5.0	16	Predicted protein	*Oryza sativa* L.

Table 7. Protein identification of protein spots of the rice variety OM 9577 extracted from 2-DE

Spot No.	Protein	Gi number	Molecular function	pI	Mr (×10³)	Role	Organism
			Salt-**tolerant (OM 9577**)				
75	MYB18 protein	CAD44612.1	DNA binding	5.9	47	Cell division Cell growth Signal transduction Transcription Transport Stress response	*Oryza sativa* L.
79	Peroxidase	BAF25353.1	Heme binding Lactoperoxidase activity Metal ion binding	5.06	38	Stress response	*Oryza sativa* L.
85	Adenylosuccinate lyase	ABF95601.1	N6-(1,2-dicarboxyethyl)AMP AMP-lyase (fumarate-forming) activity	5.3	35	Metabolic process	*Oryza sativa* L.
86	Protein-serine/ threonine phosphatase	EEE54792.1	Calmodulin-dependent protein phosphatase activity. Histone H2AXS140 phosphatase activity. MAP kinase serine/ threonine phosphatase activity. Metal ion binding. Myosin phosphatase activity. RNA polymerase II CTD heptapeptide repeat S2 phosphatase activity. RNA polymerase II CTD heptapeptide repeat S5 phosphatase activity. RNA polymerase II CTD heptapeptide repeat S7 phosphatase activity. RNA polymerase II CTD heptapeptide repeat T4 phosphatase activity. RNA polymerase II CTD heptapeptide repeat Y1 phosphatase activity	5.0	34	Cell growth Transcription Stress response	*Oryza sativa* L.
98	Lactoylglutathione lyase	BAT04179.1	Actoylglutathione lyase Metal ion binding	5.9	32	Stress response	*Oryza sativa* L.
99	Cyclin-T1-3	ABA91550.2	Cyclin-dependent protein serine/threonine kinase activator activity	5.8	30	Transcription Cell division	*Oryza sativa* L.
114	HAT family dimerisation domain containing protein	ABA99411.1	Protein dimerization activity	5.6	38	Transcription	*Oryza sativa* L.
117	Soluble inorganic pyrophosphatase	EAY87213.1	Inorganic diphosphate phosphatase activity Magnesium ion binding	5.56	24	Metabolic process	*Oryza sativa* L.

warranting a focused proteomic analysis.

The protein profile of roots was separated on SDS-PAGE and visualized by Coomassie Blue staining. Gel electrophoresis can also be used to identify the changes in protein expression and the diversity of proteins in roots involved in the NaCl stress response in previous studies (25). As shown in Fig. 1, protein expression of these rice varieties in bands 6, 7, 8 and 9, ranging from 14.4–20.1 kDa, had upregulated as compared to those of control (0 mM NaCl). Next, band 5 in the rice varieties OM 9577 and OC 10 increased its expression as compared to those of control as well as that of Dai Thom 8 as exposed to salinity stress (50 mM NaCl). On the contrary, the expression of band 5 of the rice variety Dai Thom 8 was unchanged during the salinity stress. Finally, expression of band 1, 3 and 4 increased on all three varieties during salinity stress as compared to control (0 mM NaCl).

Furthermore, the number of protein bands visualized on gel electrophoresis of OM 9577 and OC 10 were higher than those of Dai Thom 8. Protein expression of protein bands from 14.4–20.1 KDa and 45–97 KDa of OM 9577 and OC 10 were increased as compared to those of control, while the protein bands from 20.1–30 KDa were decreased. According to a previous study, differences in the number of proteins in rice roots implied the general and genotype-specific changes during the salinity stress. A comparison of proteomic changes in varieties with different salt tolerances can reveal both general and genotype-dependent responses (14). From there, more precise conclusions can be drawn about the biological roles of proteins expressed during exposure to salinity stress (14). Proteomic analysis of total lysates extracted from roots of three varieties with different salinity stress tolerances, as exposed salinity stress, revealed a significant difference in the number of proteins and their functions (Table 4).

Our study was based on previous proteomic investigations (26-29), among which one identified a total of 25364 peptides corresponding to 5.589 proteins in QSM2. There were 30 overlapping proteins among the three hexaploid triticales genera in response to salt stress (26). In addition, another study identified proteins on the plasma membrane (PM) from *Oryza sativa* L. using LC-MS/MS. The results identified 438 proteins on the basis of two or more peptide fragments, 367 proteins were identified on the basis of single peptides and protein functions were determined based on similarity to proteins with known functions or the presence of functional domains (27). The results of this study revealed that 160 peptide fragments were extracted from the roots of three rice varieties during salinity stress, which correspond to 45 proteins. These proteins were related to 13 functions, of which 5 functions, including (stress response, metabolic process, cell growth, transcription and transport) ranked as the highest number of proteins (Fig. 5). Note that the numbers of proteins related to rice transport, protein metabolic process and cell growth in OM 9577 were higher than in Dai Thom 8. Interestingly, carbohydrate metabolism-related proteins were absent in OC 10, while OM 9577 and Dai Thom 8 lacked proteins linked to photosynthesis and transcription, possibly reflecting stress-induced reallocation of resources. The difference in the number of proteins and their functions may be linked to superior growth and salt tolerance of OC 10 during salinity stress. Salinity stress alters many gene expressions, which leads to changes in protein profile expression, especially in the protein system associated with stress response in the plant (14). The protein identification via LC-MS/MS is a useful tool in the investigation of salinity stress response in rice, not only in roots but also in other parts of rice. The functions of proteins in this study were consistent with the findings of another study, in which the authors identified 54 proteins in rice leaves using LC-MS/MS and about 18 proteins related to salt stress tolerance, such as chaperonin 60 kDa, heat shock protein BIP 70 kDa and calreticulin. Some proteins involved in protein metabolism and signalling pathways had also significantly changed in the previous study (28). Moreover, another investigation identified 64 proteins in rice grains by LC-MS/MS and most of them were low molecular weight (10-25 kDa) and pI ranged from 5–7 and from 7–9. Among them, there were 13 proteins associated with biological processes, 5 proteins related to cellular components and 17 proteins associated with molecular functions (29).

The difference in protein expression is one important factor related to the salt-tolerant abilities of rice varieties. A previous study investigated protein expression and its functions among crops during salinity stress via 2-DE and LC-MS/MS analysis to build the basis for a better understanding of biochemical pathways in stress response as well as for the selection of biomarkers of stress tolerance (14). This study is in line with the previous study that conducted a proteomics analysis of roots from three rice varieties with different tolerances.

The results of 2-DE showed that the number of proteins of the rice variety Dai Thom 8 was the lowest value (with 28 protein spots), while those of OC 10 (47 protein spots) and OM 9577 varieties (85 protein spots) were higher. The rice variety OC 10 had 6 putative protein spots expressed differently from those on OM 9577 and Dai Thom 8 varieties (Fig. 3, 4). The rice variety OC 10 has a protein with antioxidant function (thioredoxin H-type), a protein with cell growth function (FHA domain-containing protein) and a protein related to the MAPK signalling pathway (hypothetical protein EE612_036920). The others were unknown function proteins, including Os02g0829700 protein, Os02g0778100 protein and uncharacterized protein (Gi Number: BAD31129.1). In the rice variety OM 9577, 8 putative proteins were selected and sequenced. Among them, there is 1 protein with cell division function (cyclin-T1-3), there are 3 proteins with transcription function (MYB18 protein, protein-serine/threonine phosphatase and HAT family dimerization domain-containing protein), there are 2 proteins with stress response function (peroxidase and lactoylglutathione lyase), there are 2 proteins with metabolic process function (adenylosuccinate lyase and soluble inorganic pyrophosphatase). This finding indicates that the salt tolerance of the rice variety OM 9577 is higher than that of OC 10 and Dai Thom 8 and may be related to these pathways.

The results of protein expression studies on 2-DE gels are consistent with previous studies (13,16). According to one of these studies there were 514 proteins expressed in the salt-tolerant rice variety (Vytilla-4) and 770 proteins expressed in the sensitive rice variety (Jhelum) in response to salt stress. The differentially expressed proteins were found to be associated with major metabolic pathways, including photosynthesis, energy metabolism, amino acid metabolism, nitrogen assimilation and stress and signalling pathways (16). The other study used proteomic analysis to understand the mechanism of rice variety 527 (mature stage) exposed to NaCl stress (0 mM and 300 mM NaCl). The results showed that there were more than 500 protein spots, 44 of which were different from the control. Using matrix-assisted laser desorption/ionization-time of flight mass spectrometry, 44 proteins were identified, representing 18 different protein classes and classified

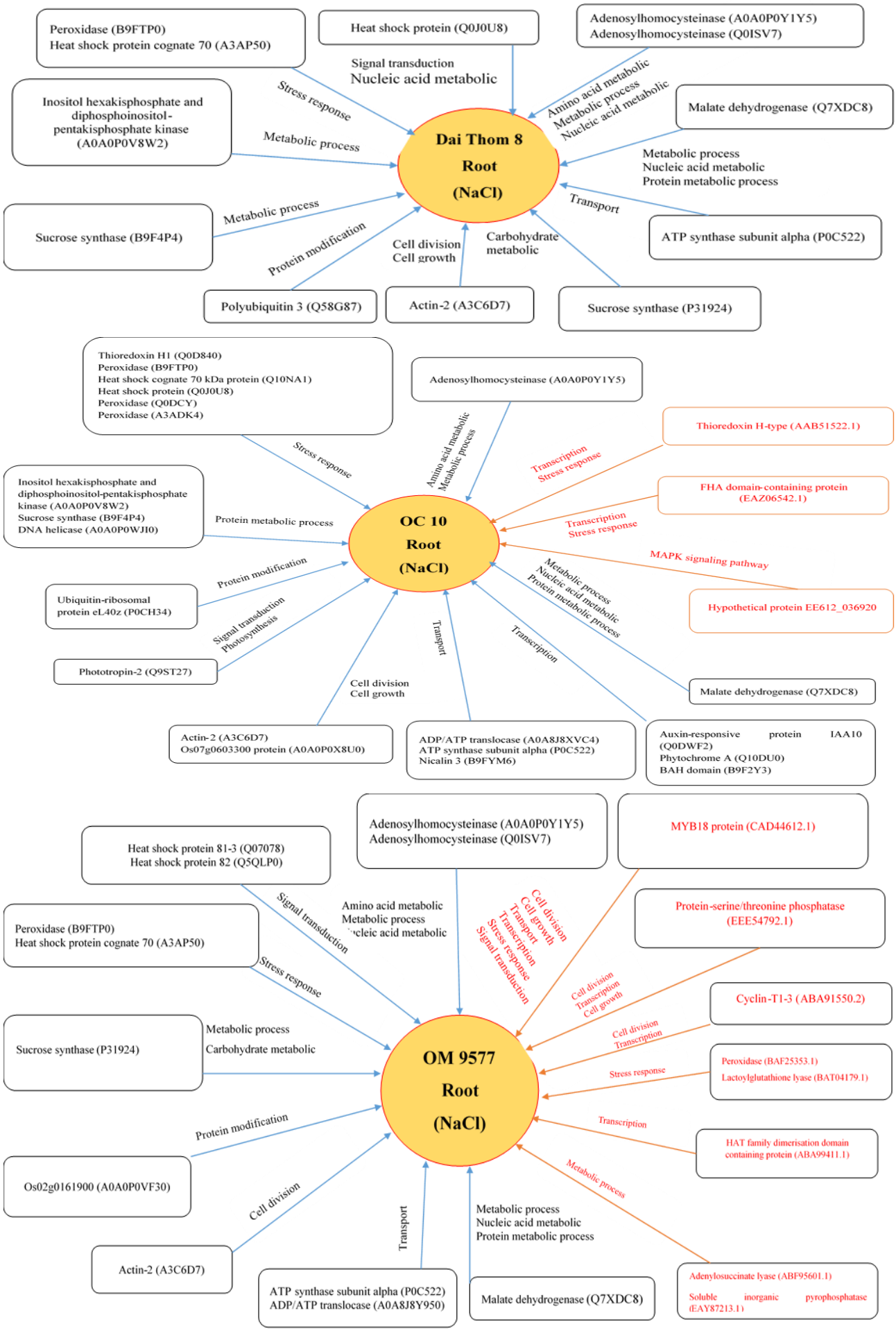

Fig. 5. Protein expression in roots of Dai Thom 8, OC 10 and OM 9577 rice varieties in response to NaCl stress.

Proteins obtained by LC-MS/MS are shown in black label, whereas those identified by 2-DE combined with LC-MS/MS are shown in red labels

into functions such as photosynthesis-related proteins, translation, signal transduction, cell wall-related proteins, energy metabolism, transcription factor, reactive oxygen species scavenging and defense (13).

Salt-tolerant rice varieties when responding to NaCl stress-expressed protein peroxidase, thioredoxin, adenylosuccinate synthetase and aldehyde dehydrogenase (16). Protein expression in the rice variety OC 10 was similar to previous study, which was the expression of antioxidant protein (Trx) (13). However, the difference in the current study is that the rice variety OM 9577 has proteins involved in transcription (MYB18 protein and HAT family dimerization domain-containing protein), cellular defence and antioxidant systems (soluble inorganic pyrophosphatase), cell division and cell growth (cyclin-T1;3, phosphoprotein phosphatase (serine/threonine) and OC 10 had a cell growth protein (FHA domain-containing protein) that was not found in these studies. This shows that each rice variety will have a characteristic protein expression mechanism when responding to NaCl stress. In which, MYB18 protein, HAT family dimerization domain-containing protein, soluble inorganic pyrophosphatase, cyclin-T1;3, phosphoprotein phosphatase (serine/threonine) are the characteristic proteins of the rice variety OM 9577 when responding to NaCl stress. FHA domain-containing proteins are the characteristic proteins of the rice variety OC 10 when responding to NaCl stress.

The FHA domain-containing protein is a protein that specifically recognizes phosphothreonine epitopes of other proteins. In the *Arabidopsis* genome, there are 18 genes encoding proteins containing the FHA domain (KAPP, FHA2, FHA3, PS1, DDL, NBS1, TDP1 and ABA1). Of these, kinase-associated protein phosphatase (KAPP) helps regulate plant growth, stress adaptation and hormone metabolism (30). Abscisic acid 1 (ABA1) is predicted as a zeaxanthin cyclooxygenase, responsible for the abscisic acid synthesis (31). The mutations in DAWDLE (DDL), an FHA domain-containing protein found in the nucleus, result in slow growth and reduce fertility in *Arabidopsis* plants. AtFHA2 regulates the proliferation and differentiation of flower-developing cells (31). These results suggest that FHA domain-containing protein is involved in the regulation of the plant growth and development pathways (30). Therefore, the FHA domain-containing protein helped the rice variety OC 10 increase root and leaf length and develop some lateral roots when exposed to NaCl stress (7,9,11). In addition, OC 10 has the protein thioredoxin (Trxs), which is one of the cellular antioxidants and cellular uses thiol-disulfide processes to control the redox balance of target proteins (32). Trxf, Trxm, Trxx and Trxy, found in chloroplasts, have a regulation function in the activities of enzymes belonging to pentose phosphate and C_4 pathways. Trxf and Trxm also play an important role in plant germination and reproduction. Trxo found in mitochondria is related to physiological pathways in mitochondria. Trxh is mainly found in the cytoplasm and is related to heat shock tolerance mechanisms in plants via chaperone (33). Furthermore, H-type Trx also plays a role in sensing and responding to cellular oxidative stress (34). Moreover, a previous study indicate that the mitogen-activated protein kinase (MAPK) signaling pathway may primarily regulate plant response against salinity stress via controlling salt-associated gene expression, alleviating hypertonic stress and regulating redox balance (35). Therefore, the presence of hypothetical protein EE612_036920, which is related to the mitogen-activated protein kinase (MAPK) signalling pathway, may contribute to the salinity stress response mechanism of the rice variety OC 10.

MYB18 protein, HAT family dimerization domain-containing protein, soluble inorganic pyrophosphatase, cyclin-T1;3, phosphoprotein phosphatase (serine/threonine) are the characteristic proteins of the rice variety OM 9577 when responding to NaCl stress. The first is MYB protein, which plays important roles in cell and organ morphogenesis, regulation of secondary metabolism, meristems, cell cycle of plants (36). A 2024 study reported that the gene expression R2R3-MYB gene showed a noticeable increase after 3 days of being treated with NaCl 0.8 % (37). According to a study in 2025, overexpression of *OsMYB2* and *OsMYB6* enhances salt tolerance in rice via accumulation of proline content, activating antioxidant systems such as catalase (CAT) and superoxide (SOD) and decreasing malondialdehyde (MDA) content. MYB transcription factors also regulate the *OsHKT1;1* gene expression to balance K^+ content during salinity stress in rice (38).

The second is cyclin-T1;3, also known as cyclin 1 in some studies, plays an important role in cell division and is involved in the control of the G2 phase (39). Cyclin T1;3 regulates the elongation phase of RNA polymerase II via the formation of the positive transcription elongation factor b (P-TEFb) complex with cyclin-dependent kinase 9 (CDK9) and may influence cell proliferation. The phosphorylation of RNA polymerase II-associated proteins implies the role of cyclin-T1;3 in cell division regulation via GLABRA3.1-mediated (GL3.1) pathway (39). This result is consistent with the study of Qi et al. (2012), which states that the function of cyclin-T1;3 is also associated with cell cycle regulation. Therefore, the decrease of cyclin-T1;3 in rice results in shorter rice grains (39).

The third is histone acetyl transferase (HAT), which is a group of enzymes that catalyze the addition of acetyl group in proteins (histones) and helps regulate mRNA transcription. HATs and histone deacetylase coordinate (HDAC) to adjust the acetylation level of histones, which in turn helps cell growth and development, as well as adaptation to environmental stresses (40,41). According to a previous study, some abiotic stresses, such as salinity, cold or heat shock stresses, can alter HAT and HDAC activities (42). Another study concluded that the expressions of *OsHAT* and *OsHDAC* genes are changed to respond to environmental stress and stimulation of phytohormones, which suggests the important role of these genes in rice tolerance to environmental stresses (41).

Next is the catalytic inorganic pyrophosphatase, which hydrolyzes PPi (inorganic pyrophosphate) into two orthophosphates (2Pi) in the presence of divalent metal cations (43). Pyrophosphate is extensively produced as a by-product of 200 intracellular metabolic reactions (DNA, RNA, protein and saccharide synthesis). In plant cells, PPi plays an intracellular energy-providing role (H^+-PPase, UDP-glucose pyrophosphorylase, fructose-6 phosphate 1-phosphotransferase (PFP) and pyruvate phosphate dikinase (PPDK)). Therefore, the regulation of PPi is essential for maintaining cellular activity (44). Instead of pyrophosphate hydrolysis, the main function of the enzymes is to pump ions to create proton or sodium gradients under low-energy conditions (45). Therefore, soluble inorganic pyrophosphatases may associated with stress responses. In a previous study conducted in *Arabidopsis* sp., PPP is a key component of several signaling pathways involved in the regulation of abiotic stress responses and plant development (46).

Finally, phosphoprotein phosphatase (serine/threonine), which in eukaryotic cells includes PPP (serine/threonine-specific phosphoprotein phosphatases), protein-mitochondrial

phosphatases (PPM), protein tyrosine (Tyr) phosphatases (PTP) and aspartate (Asp)-dependent phosphatases. Of these, protein phosphatases (PPPs) and protein-mitochondrial phosphatases (PPMs) are serine (Ser)/threonine (Thr)-specific phosphatases and Tyr-specific phosphatases (PTPs). On the contrary, dual-specificity phosphatases (DsPTPs/DSPs) are Ser, Thr and Tyr-specific proteins (47). PPPs play a role in metabolic regulation, regulation of the cell cycle and stress signaling (48).

According to a recent study, the rice variety OM 9577 can grow and develop more effectively than the rice varieties OC 10 and Dai Thom 8 when responding to NaCl stress (7). Because the rice variety OM 9577 has some proteins expressed to cell division and cell growth (cyclin-T1;3, adenylosuccinate lyase and phosphoprotein phosphatase (serine/threonine)).

Differentially expressed proteomes among rice cultivars with contrasting salt tolerances are important findings to explore potential mechanisms by which rice roots respond to salt stress (16). The research team proposed to validate the protein data at the transcript expression level related to the proteins expressed in the study (MYB18, HAT family dimerization domain-containing protein, soluble inorganic pyrophosphatase, cyclin-T1;3, phosphoprotein phosphatase (serine/threonine) and FHA domain-containing protein) by qPCR technique. Proteins play a role in transcription, cell division, antioxidant enzymes, etc. This is the scientific basis that has helped rice plants adapt to salt stress. Therefore, this is a very important direction that our research group will carry out in the future. This aligns with the direction highlighted in a previous study, which reported that validating protein data using qPCR provides deeper insight into gene expression and transcriptional regulation (16).

Environmental conditions are increasingly harsh and we cannot control or change the impacts of the environment; we must adapt to them. Therefore, the problem for breeders is to apply scientific and technological advances in breeding to be able to crossbreed and select new rice lines/varieties that are resistant to climate change conditions, in which resistance to salinity is very necessary. Therefore, our study applied modern analytical techniques such as 2-DE and LC-MS/MS to identify proteins with specific expression in salt-tolerant rice varieties in response to NaCl stress. These are MYB18 protein, HAT family dimerization domain-containing protein, soluble inorganic pyrophosphatase, cyclin-T1;3, phosphoprotein phosphatase (serine/threonine) and FHA domain-containing protein. These findings provide potential biochemical indices to be used as biochemical markers for selecting salt-tolerant rice varieties or for practical application to enhance salt tolerance of rice varieties through hybridization or gene transfer.

Conclusion

Total protein analysis by LC-MS/MS identified 12 proteins in Dai Thom 8, 12 proteins in OM 9577 and 21 proteins in OC 10. The proteins have functions in amino acid synthesis, nucleic acid synthesis, carbohydrate synthesis, signal transduction, stress response, metabolism, transport, protein modification, cell division, protein metabolism, cell growth, photosynthesis, transcription and lipid metabolism of rice. Identification of protein spots from 2-DE combined with LC-MS/MS showed that the rice variety OC 10 has a characteristic FHA domain-containing protein, thioredoxin H-type, hypothetical protein EE612_036920. The rice variety OM 9577 has MYB18 protein, HAT family dimerization domain-containing protein, peroxidase, lactoylglutathione lyase/glyoxalase, soluble inorganic pyrophosphatase, cyclin-T1;3, phosphoprotein phosphatase (serine/threonine), adenylosuccinate lyase. These characterized proteins related to several functions such as cellular transportation, nucleotide metabolism, stress response, signal transduction and transcription. The presence of these characterized proteins validates the total protein identification results and plays a role in enhancing and maintaining salt tolerance in the rice varieties OM 9577 and OC 10. These findings also provide potential biochemical and molecular markers for screening salt-tolerant rice varieties and improving the salt tolerance phenotype of commercially available rice varieties through breeding and hybridization methods.

Acknowledgements

The authors would like to express their sincere gratitude to colleagues in the Plant Tissue Culture Laboratory of the Biotechnology Center of Ho Chi Minh City, the Faculty of Basic Medical Science, Pham Ngoc Thach University of Medicine, the Institute of Applied Material Science, Vietnam Academy of Science and Technology, Ho Chi Minh city, Vietnam and the Institute of Food and Biotechnology, Can Tho University, Can Tho City, Vietnam for supplying the materials, facilities and equipment for conducting the experiments.

Authors' contributions

CDQ and NTN were responsible for project conceptualization and experiment design. CDQ, TLH, GBT, TTD, HNC, NNTL and NTN carried out investigation and data collection. Data analysis was performed by CDQ, ABL, TTD, TNH, TNHN and NTN. Proteomic analysis and data interpretation were conducted by CDQ, TLH, GBT, TNH and NTN. Data validation was undertaken by CDQ, TNHN, HNC, NNTL and NTN, while data visualization was handled by CDQ, ABL, TLH, HNC and NNTL. CDQ drafted the first version of the manuscript, which underwent thorough revisions by GBT and NTN. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Author do not have any conflict of interests to declare.

Ethical issues: None

References

1. Musse OSH, Echchabi A, Mohamud IH, Sharif MN. Empirical assessment of climate change effect on cereal crop production in the East African Community. Discov Sustain. 2025;6:1147. https://doi.org/10.1007/s43621-025-01998-w
2. Chen T, Niu Y, Yang C, Liang Y, Xu J. Screening of rice (*Oryza sativa* L.) genotypes for salinity tolerance and dissecting determinants of tolerance mechanism. Plants. 2024;13(7):1036. https://doi.org/10.3390/plants13071036
3. Ghosh B, Ali MN, Saikat G. Response of rice under salinity stress: A review update. J Rice Res. 2016;4(2):167-74. https://doi.org/10.4172/2375-4338.1000167
4. Arif YS, Priyanka, Siddiqui H, Bajguz A, Hayat S. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. Plant Physiol. Biochem. 2020;156:64-77. https://doi.org/10.1016/j.plaphy.2020.08.042

5. Goharrizi KJ, Hamblin MR, Karami S, Nazari M. Physiological, biochemical and metabolic responses of abiotic plant stress: salinity and drought. Turk J Bot. 2021;45(7):623-42. https://doi.org/10.3906/bot-2108-30
6. Kalita CK. Plant stress physiology-A comprehensive account. E-Zine Biol Sci. 2022:2456-7264.
7. Duong CQ, Bui AL, Le TH, Thanh TT, Ngoc TN. Differential biochemical and metabolic responses of contrast rice cultivars (*Oryza sativa* L.) under salt stress. Acta Agrobot. 2023;76. https://doi.org/10.5586/aa/172513
8. Hussain S, Cao X, Hong C, Zhu L, Khaskheli MA, Fiaz S, et al. Sodium chloride stress during early growth stages altered physiological and growth characteristics of rice. Chil J Agric Res. 2018;78(2):183-97. https://doi.org/10.4067/S0718-58392018000200183
9. Quoc CD, Lan AB, Nguyen NT, Le Hong T, Thanh TT, Tran G-B, et al. Variations in some metabolic compounds in the roots of rice varieties (*Oryza sativa* L.) with different salinity tolerance under salinity stress during the seedling stage. Plant Physiol Rep. 2024;29(3):660-77. https://doi.org/10.1007/s40502-024-00804-5
10. Huyen LTN, Cuc LM, Ham LH, Khanh TD. Introgression the Saltol QTL into Q5DB, the elite variety of Vietnam using marker-assisted selection (MAS). Am J BioSci. 2013;1(4):80-4. https://doi.org/10.11648/j.ajbio.20130104.15
11. Duong CQ, Bui AL, Trinh NN, Le TH, Tran TT, Tran G-B. Evaluation of some biochemical and biomolecular indicators in rice (*Oryza sativa* L.) during the seedling stage under NaCl stress. J Crop Sci Biotechnol. 2024:1-18. https://doi.org/10.2139/ssrn.4570825
12. Barkla BJ, Castellanos-Cervantes T, León JLDd, Matros A, Mock H-P, Perez-Alfocea F, et al. Elucidation of salt stress defence and tolerance mechanisms of crop plants using proteomics - current achievements and perspectives. Proteomics. 2013;13(12-13):1885-900. https://doi.org/10.1002/pmic.201200399
13. Fatehi F, Hosseinzadeh A, Alizadeh H, Brimavandi T, Struik PC. The proteome response of salt-resistant and salt-sensitive barley genotypes to long-term salinity stress. Mol Biol Rep. 2012;39:6387-97. https://doi.org/10.1007/s11033-012-1460-z
14. Passamani L, Barbosa RR, Reis RS, Heringer AS, Rangel PL, Santa-Catarina C, et al. Salt stress induces changes in the proteomic profile of micropropagated sugarcane shoots. PLoS One. 2017;12(4):e0176076. https://doi.org/10.1371/journal.pone.0176076
15. Rotilio D, Della Corte A, D'Imperio M, Coletta W, Marcone S, Silvestri C, et al. Proteomics: bases for protein complexity understanding. Thromb Res. 2012;129(3):257-62. https://doi.org/10.1016/j.thromres.2011.12.035
16. Frukh A, Siddiqi TO, Khan MIR, Ahmad A. Modulation in growth, biochemical attributes and proteome profile of rice cultivars under salt stress. Plant Physiol Biochem. 2020;146:55-70. https://doi.org/10.1016/j.plaphy.2019.11.011
17. Liu C-W, Chang T-S, Hsu Y-K, Wang AZ, Yen H-C, Wu Y-P, et al. Comparative proteomic analysis of early salt stress responsive proteins in roots and leaves of rice. Proteomics. 2014;14(15):1759-75. https://doi.org/10.1002/pmic.201300276
18. Wang F, Yang YSL, Zhu C. Comparative proteomic study and functional analysis of translationally controlled tumour protein in rice roots under Hg^{2+} stress. J Environ Sci. 2012;24(12):2149-58. https://doi.org/10.1016/S1001-0742(11)61062-0
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75. https://doi.org/10.1016/S0021-9258(19)52451-6
20. Ngoc NT, Tri PN, Le Hong T, Quoc CD. Biomolecular evaluation of three contrasting rice cultivars (*Oryza sativa* L.) in salt stress response at seedling stage. Plant Sci. Today. 2022;9:491-503. https://doi.org/10.14719/pst.1539
21. Dang TT, Tran TTT, Tran G-H, Pham SH, Nguyen TH. Cyclotides derived from *Viola dalatensis* Gagnep: A novel approach for enrichment and evaluation of antimicrobial activity. Toxicon. 2024;239:107606. https://doi.org/10.1016/j.toxicon.2024.107606
22. Ahn SH, Yang H-Y, Tran GB, Kwon J, Son K-Y, Kim S, et al. Interaction of peroxiredoxin V with dihydrolipoamide branched chain transacylase E2 in mouse kidney under hypoxia. Proteome Sci. 2015;13:1-9. https://doi.org/10.1186/s12953-014-0061-2
23. Liu Y, Wang B, Li J, Song Z-L, Chi M-Y, Liu J, et al. Salt-response analysis in two rice cultivars at seedling stage. Acta Physiol Plant. 2017;39:1-9. https://doi.org/10.1007/s11738-017-2514-6
24. Munns R, Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 2008;59:651-81. https://doi.org/10.1146/annurev.arplant.59.032607.092911
25. Padilla-Chacón D, Campos-Patiño L, Peña-Valdivia CB, García-Esteva A, Jiménez-Galindo JC, Pizeno-García JL. Proteomic profile of tepary bean seed storage proteins in germination with low water potential. Proteome Sci. 2024;22(1):1. https://doi.org/10.1186/s12953-023-00225-6
26. Wang D, Li J, Li S, Fu J, Liu B, Cao D. Multi-omics analysis of hexaploid triticale that show molecular responses to salt stress during seed germination. Front Plant Sci. 2025;15:1529961. https://doi.org/10.3389/fpls.2024.1529961
27. Natera SH, Ford KL, Cassin AM, Patterson JH, Newbigin EJ, Bacic A. Analysis of the *Oryza sativa* plasma membrane proteome using combined protein and peptide fractionation approaches in conjunction with mass spectrometry. J Proteome Res. 2008;7(3):1159-87. https://doi.org/10.1021/pr070255c
28. Chompa SS, Zuan ATK, Amin AM, Hun TG, Ghazali AHA, Sadeq BMA, et al. Growth and protein response of rice plant with plant growth-promoting rhizobacteria inoculations under salt stress conditions. Int Microbiol. 2024;27(4):1151-68. https://doi.org/10.1007/s10123-023-00469-4
29. Ruan S, Xiao W, Qiu J, Hu W, Ying WC, Tong J, et al. Proteomic analysis of 2-chloroethanol extracts of rice (*Oryza sativa* L.) seeds. Food Chem Mol Sci. 2020;1:100002. https://doi.org/10.1016/j.fochms.2020.100002
30. Wang Q. The role of forkhead-associated (FHA) domain proteins in plant biology. Plant Mol Biol. 2023;111(6):455-72. https://doi.org/10.1007/s11103-023-01338-4
31. Shi F, Liu Z, Pang Z, Feng H. The role of the FHA domain-containing protein family in pollen development of *Brassica rapa* L. Sci Hortic. 2021;288:110339. https://doi.org/10.1016/j.scienta.2021.110339
32. Zhang C-J, Zhao B-C, Ge W-N, Zhang Y-F, Song Y, Sun D-Y, et al. An apoplastic h-type thioredoxin is involved in the stress response through regulation of the apoplastic reactive oxygen species in rice. Plant Physiol. 2011;157(4):1884-99. https://doi.org/10.1104/pp.111.182808
33. Japelaghi RH, Haddad R, Ghasem-Ali M. Molecular characterization of an h-type thioredoxin gene from grape. Open Life Sci. 2011;6(6):1006-22. https://doi.org/10.2478/s11535-011-0051-7
34. Boubakri H, Saidi MN, Barhoumi F, Hanen J, Moez B, Faiçal B. Identification and characterization of thioredoxin h-type gene family in *Triticum turgidum* ssp. *durum* in response to natural and environmental factor-induced oxidative stress. Plant Mol Biol Rep. 2019;37:464-76. https://doi.org/10.1007/s11105-019-01176-z
35. Lin L, Wu J, Jiang M, Wang Y. Plant mitogen-activated protein kinase cascades in environmental stresses. Int J Mol Sci. 2021;22(4):1543. https://doi.org/10.3390/ijms22041543
36. Yang A, Dai X, Zhang W-H. A R2R3-type MYB gene, OsMYB2, is involved in salt, cold and dehydration tolerance in rice. J Exp Bot. 2012;63(7):2541-56. https://doi.org/10.1093/jxb/err431
37. Zhang H-C, Gong Y-H, Tao TL, Zhou W-Y, Xia HZ, Yang Q-Q, et al. Genome-wide identification of R2R3-MYB transcription factor subfamily genes involved in salt stress in rice (*Oryza sativa* L.). BMC Genomics. 2024;25(1):797. https://doi.org/10.1186/s12864-024-10693-5
38. Nie S, Huang W, He C, Wu B, Duan H, Ruan J, et al. Transcription factor OsMYB2 triggers amino acid transporter OsANT1 expression to regulate rice growth and salt tolerance. Plant Physiol. 2025;197(2):kiae559. https://doi.org/10.1093/plphys/kiae559
39. Qi P, Lin Y-S, Sun X-J, Huang J-B, Wei S, Zhang J-X, et al. The novel quantitative trait locus GL3.1 controls rice grain size and yield by

regulating Cyclin-T1;3. Cell Res. 2012;22(12):1666-80. https://doi.org/10.1038/cr.2012.151

40. Guo Y, You Y, Chen F, Liao Y. Identification of the histone acetyltransferase gene family in the *Artemisia annua* genome. Front Plant Sci. 2024;15:1389958. https://doi.org/10.3389/fpls.2024.1389958
41. Hou J, Ren R, Xiao, Huangzhuo C, Zhenfei Y, Jinfu Z, et al. Characteristic and evolution of HAT and HDAC genes in gramineae genomes and their expression analysis under diverse stress in *Oryza sativa*. Planta. 2021;253:1-22. https://doi.org/10.1007/s00425-021-03589-1
42. Xia L, Luo M, Zhang W, Zhao J, Zhang J, Wu K, et al. Histone acetyltransferases in rice (*Oryza sativa* L.): phylogenetic analysis, subcellular localization and expression. BMC Plant Biol. 2012;12:1-17. https://doi.org/10.1186/1471-2229-12-145
43. Brew-Appiah RATY, Zara BK, Vandhana R, Eric HS, A K Sanguinet. Genome-wide identification and analysis of the alternative oxidase gene family in diploid and hexaploid wheat. PLoS One. 2018;13(8):e0201439. https://doi.org/10.1371/journal.pone.0201439
44. Gunji S, Kawade K, Tabeta H, Horiguchi G, Oikawa A, Asaoka M, et al. Tissue-targeted inorganic pyrophosphate hydrolysis in a fugu5 mutant reveals that excess inorganic pyrophosphate triggers developmental defects in a cell-autonomous manner. Front Plant Sci. 2022;13:945225. https://doi.org/10.3389/fpls.2022.945225
45. Grzechowiak M, Ruszkowski M, Sliwiak J, Szpotkowski K, Sikorski M, Jaskolski M. Crystal structures of plant inorganic pyrophosphatase, an enzyme with a moonlighting autoproteolytic activity. Biochem J. 2019;476(16):2297-319. https://doi.org/10.1042/BCJ20190427
46. Singh A, Giri J, Kapoor S, Tyagi AK, Pandey GK. Protein phosphatase complement in rice: genome-wide identification and transcriptional analysis under abiotic stress conditions and reproductive development. BMC Genomics. 2010;11:1-18. https://doi.org/10.1186/1471-2164-11-435
47. Bheri M, Mahiwal S, Sanyal SK, Pandey GK. Plant protein phosphatases: what do we know about their mechanism of action? FEBS J. 2021;288(3):756-85. https://doi.org/10.1111/febs.15454
48. Farkas I, Dombradi V, Miskei M, Szabados L, Koncz C. *Arabidopsis* PPP family of serine/threonine phosphatases. Trends Plant Sci. 2007;12(4):169-76. https://doi.org/10.1016/j.tplants.2007.03.003

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.