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Research Article

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LC-MS/MS Based Label Free Quantitative Shotgun Proteomics Revealed Contrasting Responses of Rice Germplasms towards Salinity and Identified Expression of Redox-Regulatory Proteome

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Abstract

Background: *High throughput proteomic studies are extremely important in investigating reprogramming of gene expression in plants grown under environmental stress. Though there are plenty of evidences of salt inducible proteins characterized through different proteomic investigations in rice, but scant attentions are paid in identifying salt-responsive proteomes in rice that are involved in redox regulation necessary for combating oxidative stress induced by salinity, and hence is the objective of the present investigation.*

Results: *Imposition of post-imbibitional salinity stress (PISS) to the experimental rice cultivars differing in sensitivity towards salinity (Oryza sativa L., Cultivars Patnai and IR29) in general revealed strong correlation between the parameters of oxidative damages (lipid peroxidation, protein oxidation) and redox status (endogenous level of total ROS, H2O2, and total radical scavenging properties). Cultivar Patnai with better redox-regulatory attributes at metabolic interface under PISS, exhibited better germination phenotypes (T50 value of germination). The level free quantitative shotgun proteomic analysis through LC-MS/MS identified a number of salinity-responsive proteins, whose abundance changes significantly in response to PISS, particularly for the redox competent and tolerant germplasm Patnai. The greater abundance of expressed proteins is associated with the biological, cellular and molecular processes for the tolerant germplasm Patnai grown under PISS in contrast to its counterpart IR29. Comparative GO analysis of separated proteins revealed an abundance of expressed proteins involved in the regulation of redox homeostasis, like ascorbate-glutathione cycle, hydrogen peroxide metabolic process, hydrogen peroxide –responsive proteins, cellular redox homeostasis, hydrogen peroxide signalling proteins etc.*

Conclusion: *Comparative proteomic investigation through LC-MS/MS identified a number of salinity-responsive proteins whose abundance changes significantly under PISS, particularly for the tolerant rice cultivar Patnai, confirming the role of redox-regulatory proteins in redox–regulation at metabolic interface necessary for salinity tolerance.*

Keywords: Comparative Proteomics, Salinity Stress, LC- MS/MS, Redox Regulatory Proteins, Gene Ontology

Background

Salinity stress is one of the most important adverse environmental constraints that has significant negative impact on plant performance, limiting the distribution and productivity of crops and thus reducing yield potential [1- 3]. Plants subjected to salinity stress face significant osmotic stress, ionic stress, nutrient deficiency and oxidative threat [4, 5] and evolved diverse strategies like exclusion and compartmentalization of toxic ions, accumulation of compatible cell cytosolutes and osmolytes, reprogramming of energy and photosynthetic metabolism, hormonal functioning and restoration of redox homeostasis to withstand this environmental constraint that possess a severe threat to plant survival. Rice is the third highest globally produced crop with worldwide production of more than 700 million tonnes from 120 countries, major being

China, Africa and India with approximately 158 million hectares dedicated to its production [6]. It accounts for the fairly significant portion of staple diet in Asian countries, particularly rural areas. Salinity also possesses a threat to rice, reduces its yield and production [7-12].

Proteomics emerged as an important technology in the field of experimental plant science, helping us to unfold the mechanistic aspect of stress responses of plants. In fact, in the post-genomic era techniques associated with proteomic studies have been explored widely in studying altered protein profiles associated with environmental stress. Several recent works suggest the significant roles played by salt inducible proteomes in the complex events of salt response and tolerance of the crop. [13-15]. Literature survey revealed characterization of more than 2100 salinity induced proteins in 34 species in unicells including the cereals crop like rice. [16-19]. The proteomic data gathered so far helped us to unfold the diverse strategies that plants adopt for reprogramming their physiology necessary for combating salt stress.

Moreover, for the identification of salt-responsive genes, proteomic studies seem to be the most dependable and reproducible molecular tool to unfold the functionality of salt-responsive genes having a putative role in salt tolerance. With the increasing intensity of salinity due to the inundation of coastal areas associated with potential climate change, we need to have molecular breeding not only for the cultivation of rice in those marginal salt-affected areas but also to enhance the productivity of rice. The significantly greater number of genes associated with salinity response and their variable expression patterns associated with severity, further magnify the challenge of rice production in the salt-affected areas. These necessitates characterization of salt-responsive inducible genes based on the most reliable molecular tool, that is comparative proteomics. In the present study, we have explored comparative proteomic investigation with two indica rice cultivars differing in sensitivity towards NaCl salinity (*Oryza sativa* L., Cultivars Patnai, IR29) for characterizing the salt-induced redox proteomes necessary for salinity tolerance.

Methods

Seeds of each rice cultivars (*Oryza sativa* L., Cultivar IR29 & Patnai) were washed with distilled water and surface sterilized with 0.2% $HgCl_2$ solution for five minutes. The sterilized seeds were washed thrice in distilled water and imbibed in sterile distilled water in darkat 25º±2 ºC, for 24h. Thereafter, imbibed seeds were plated and post-imbibitional salinity (NaCl) stress (PISS) of magnitude 250mM was imposed, for 24 hours at 25 °C with 14-hphotoperiod (270 µm $m^{-1}s^{-1}$) and 65±2% relative humidity (RH) in stability chamber cum seed germinator (LAB-X, India). After the imposition of PISS, germinating seeds were allowed to grow for next 72 h in environmental chamber (LAB-X, India) maintained at temperature 25°C ± 2°C, RH 65±2% and 14-hour photoperiod with 270 µm m-1 s-1 illumination. These 72-h old PISS of 250mM NaCl -raised germinating seedlings of both the cultivars were used for proteomics study for identifying redox regulated proteins that confers salinity tolerance.

Extraction and Estimation of Thiobarbituric Acid Reactive substances (TBARS)

The membrane lipid peroxidations of tissues were estimated in terms of malondialdehyde accumulation. To estimate MDA content, the TBA (thiobarbituric acid) test was performed using the procedure of Heath and Packer [20].

Extraction and Estimation of Free Carbonyl Content

Oxidative damage to protein was estimated as the content of carbonyl groups following the procedures of Jiang and Zhang and Bhattacharjee [21-22].

Estimation of "Total ROS" generation

For the estimation of total ROS, an in vivo assay was performed spectroflurometrically following the process of Simontacchi et al [23].

Estimation of Radical Scavenging Property [DPPH (2, 2/-diphenyl-1-pycryl hydrazyl)]

For determination of DPPH+ free radical scavenging activity, the process of Mensor et alwas followed [24].

Estimation of H_2O_2 Generation

Hydrogen peroxide was extracted and estimated following the procedure of MacNevin and Uron [25].

Determination of T_{50} value of Germination

T_{50} Value of germination is done according to the following formula T_{50}= the time in hour to reach 50% germination (Rubio-Casal et al.and Bhattacharjee [26, 27]

Protein Extraction and Protein Assay

For the extraction of protein, the process of Hamzelou et al. was followed. Seedlings were ground to a fine powder in liquid N2 using a mortar and pestle and the protein was extracted from 50mg of leaf powder using the trichloroacetic acid- acetone method [28]. Seedling powder was suspended in 1.5 ml of 10% trichloroacetic acid in acetone, 2% β-mercaptoethanol, vortexed for 30min at 4°C and incubated at -20°C for 45min. After centrifugation of the extract, the pellet was collected and washed three times with 100% ice-cold acetone, followed by centrifugation after each washing step at 16000rpm for 30min. The protein pellet was lyophilized in a vacuum centrifuge and resuspended in 3% SDS in 50mM Tris-HCl (PH- 8.8). Samples were then methanol-chloroform precipitated. The protein pellet was suspended in 8M urea in 100mM Tris-HCl (PH-8.8).

Digestion of Purified Protein in Solution and Peptide Extraction

Protein pellet was suspended in 8M Urea in 100mM Tris HCl (PH-

8.7) and the concentration was determined using Bradford dye binding method [29]. 100µg of protein sample was taken for digestion. The sample was diluted with 50mM NH_4HCO_3 and treated with 100mM DTT at 37°C for 1h followed by alkylation with 20mM iodo acetamide at room temperature in dark for 45min. Finally, the sample was digested with trypsin at 37°C overnight with protein enzyme ratio 100:1. The resulting sample was vacuumed dried and dissolved in 20µl of 0.1% formic acids in water. After centrifugation at 10,000g (at 4°C) the supernatant was collected into separated tube. The samples desalted using a stage-tip. The samples were then eluted from stage-tip using 200 µl of 80% acetonitrile. Desalted proteins were dried in vacuum centrifuged and redissolved in 0.1% formic acid (Mobile phase A). 10µl injection volume was used on BEH C18 UPLC column in waters UPLC (Model UPLC- Aquity system, 50mm x 4.6 mm ,5 µm) for the separation of peptides. 100 % acetonitrile is used as mobile phase B. The total run time was 60 min with flow rate of 0.3 ml/min. The column oven temperature was maintained at 40 °C and sampler cooler temperature was kept at 4 °C. Waters acuity (Waters Co. USA) interfaced to a Water Q-Tof-LCMS (waters Co., USA) was used for mass spectrometric investigation. Electro spray ionization (ESI-MS) analysis was performed in both positive ion mode and full scan mass spectra was acquired over a mass range of m/z 50-2000. With detection of ions at a resolution of 70000 HCD fragmented ions. Tandem mass spectrometry was done in DDA mode (Data Dependent acquisition) with ESI-MS of top 10most abundance precursor ions at HCD normalised collision energy of 35%.

Statistical Analysis

Each biochemical experiment was carried out twice at different times and had three replicates for each treatment. Results calculated as mean of three replicates ± standard error. Statistical analysis for the data significance and the t-test was done using Microsoft Excel 2010.

Results

Oxidative Stress Biomarkers as Sensitive Quality Parameters for the Assessment of Redox Status of PISS Raised Seedlings of Experimental Rice Cultivars

Figures 1 and 2 show relationship between PISS induced oxidative lipid peroxidation product (TBARS) & protein oxidation product (free carbonyl content) with redox status (ROS and H_2O_2 accumulation), total antioxidant capacity and growth parameters in experimental rice cultivars. The overall results exhibited a positive correlation between the accumulation of lipid peroxidation products with redox status of germinating seedling, where the accumulation of pro-oxidant seems to be directly associated with elevation of lipid peroxidation and protein oxidation.

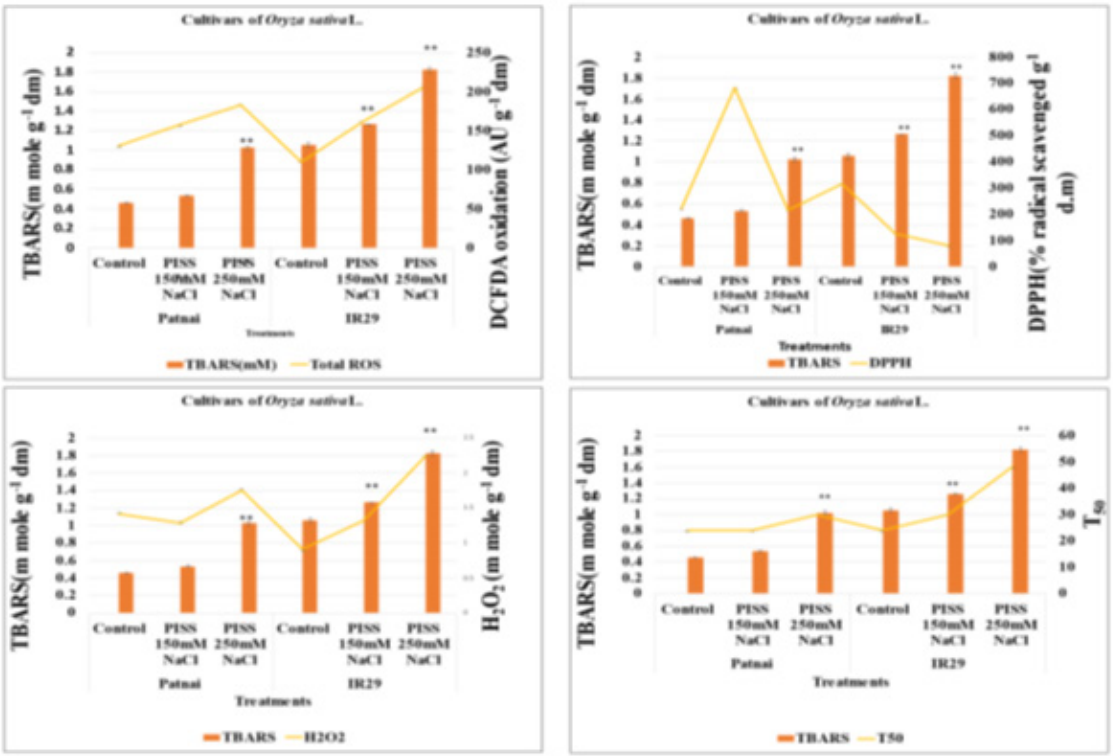


Figure 1: Relationship between PISS induced oxidative lipid peroxidation product TBARS and redox status [ROS (A), total antioxidant capacity (B) and H_2O_2 accumulation (C)] and growth parameter [t50 value (D)] in experimental rice cultivars (Oryza sativa L., Cultivars Patnai & IR29). Results are mean of three replicates ± standard error. *Significant from control at 0.05 level (t-test). **Significant from control at 0.01 level (t-test).

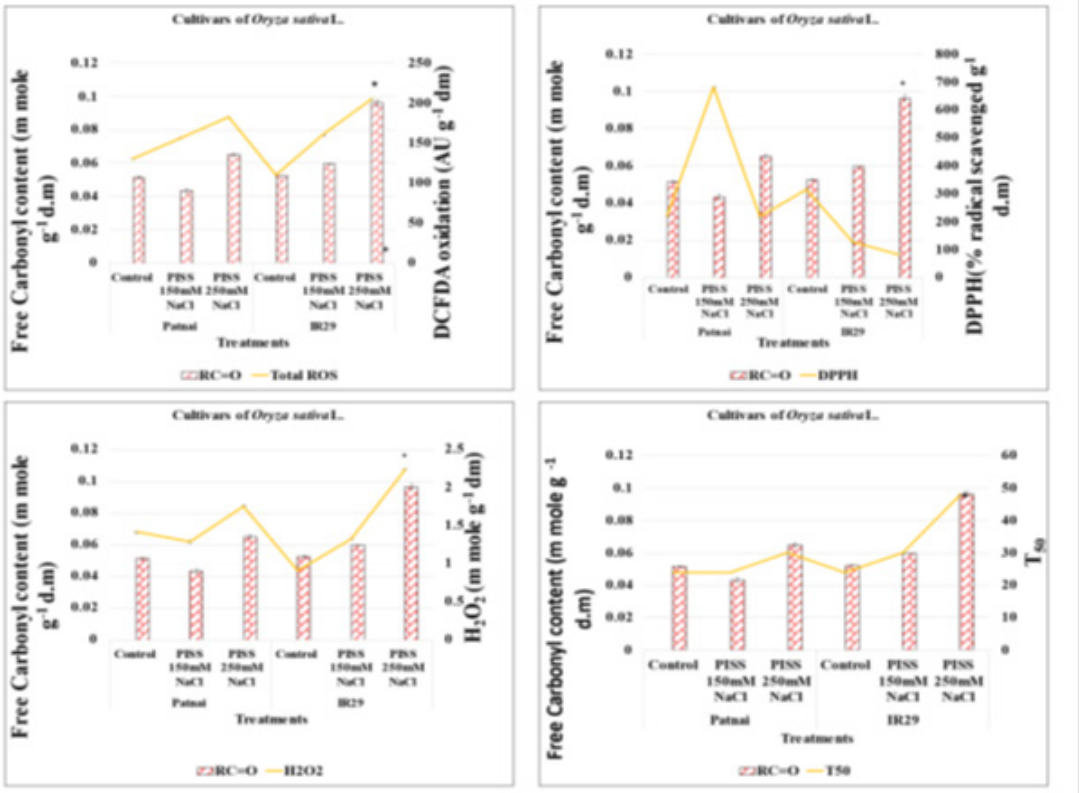


Figure 2: Relationship between PISS induced protein oxidation product free carbonyl content and redox status [ROS (A), total antioxidant capacity (B) and H_2O_2 accumulation (C)] and growth parameter [t_{50} value (D)] in experimental rice cultivars (*Oryza sativa* L., Cultivars Patnai & IR29). Results are mean of three replicates ± standard error. *Significant from control at 0.05 level (t-test). **Significant from control at 0.01 level (t-test).

Comparative proteomics involving LC-MS/MS (label-free quantitative shotgun procedure) for characterization of proteomic responses in PISS-raised seedlings of experimental rice cultivars

To characterize further the salinity-induced proteomic responses of two experimental cultivars of rice differing in sensitivity towards salinity stress, label-free quantitative shotgun proteomic analysis was done. Water imbibed seeds of both the experimental rice cultivars were grown under the same magnitude of PISS salinity stress (250mM NaCl, EC-18.10 ds m^{-1}) for 24h and the soluble protein extracted were subjected to label-free quantitative shotgun proteomic analysis. The analysis enabled high confidence identification of several non-redundant proteins across both the genotypes (*Oryza sativa* L., cultivars IR29 and Patnai) grown under NaCl salinity stress (Supplementary Table 1 and 2).The range of molecular weight of characterized proteins was found to be greater for identified rice proteins. The isoelectric points range from 3.5 to 12.5 for most of the rice proteins (Supplementary Tables 1 and 2 identified rice protein in two experimental rice cultivars). For the said identification, all quantitative data of peptides characterized from the soluble fractions (Fig. 3 and 4) were explored to identify proteins.

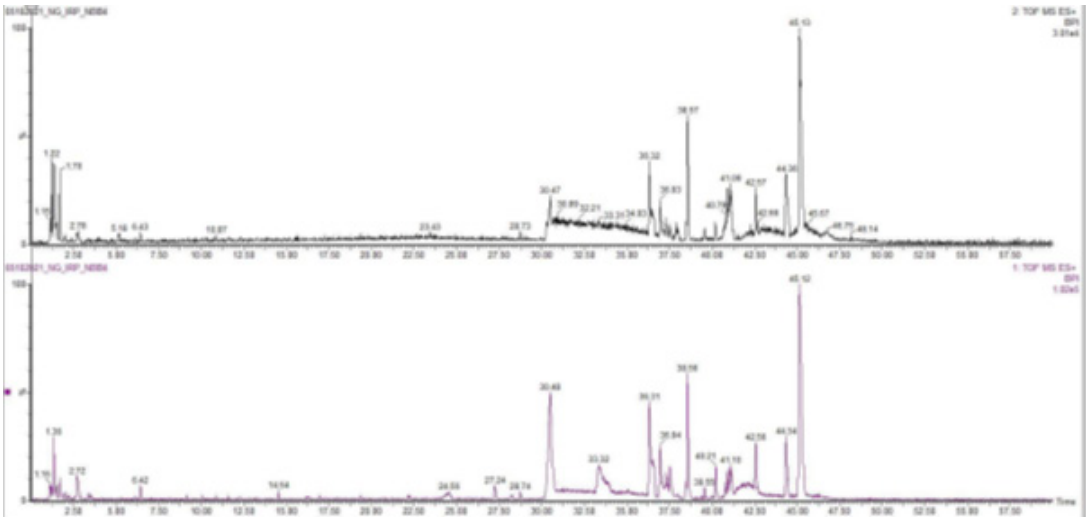


Figure 3: HPLC based chromatogram of separated protein (B) and mass spectra(A) of PISS-raised seedlings of salt sensitive rice cultivar *Oryza sativa* L., Cultivar IR29.

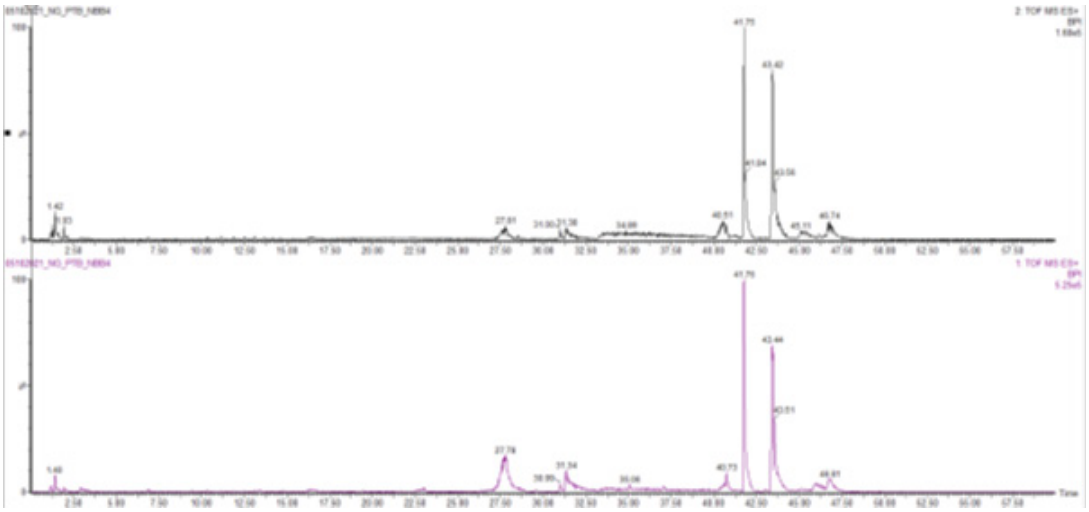


Figure 4: HPLC based chromatogram of separated protein(B) and mass spectra (A) of PISS-raised seedlings of salt-tolerant rice cultivar *Oryza sativa* L., Cultivar Patnai.

The MS-MS spectra obtained (Fig. 5 and 6) for both the rice cultivar were matched to the known peptide sequences for identification of the peptides of both the rice cultivars grown under post imbibitional salinity stress. Overall, the present effort identified 1338 & 842 peptide bands in both the experimental rice cultivars (Supplementary Tables 1 and 2).

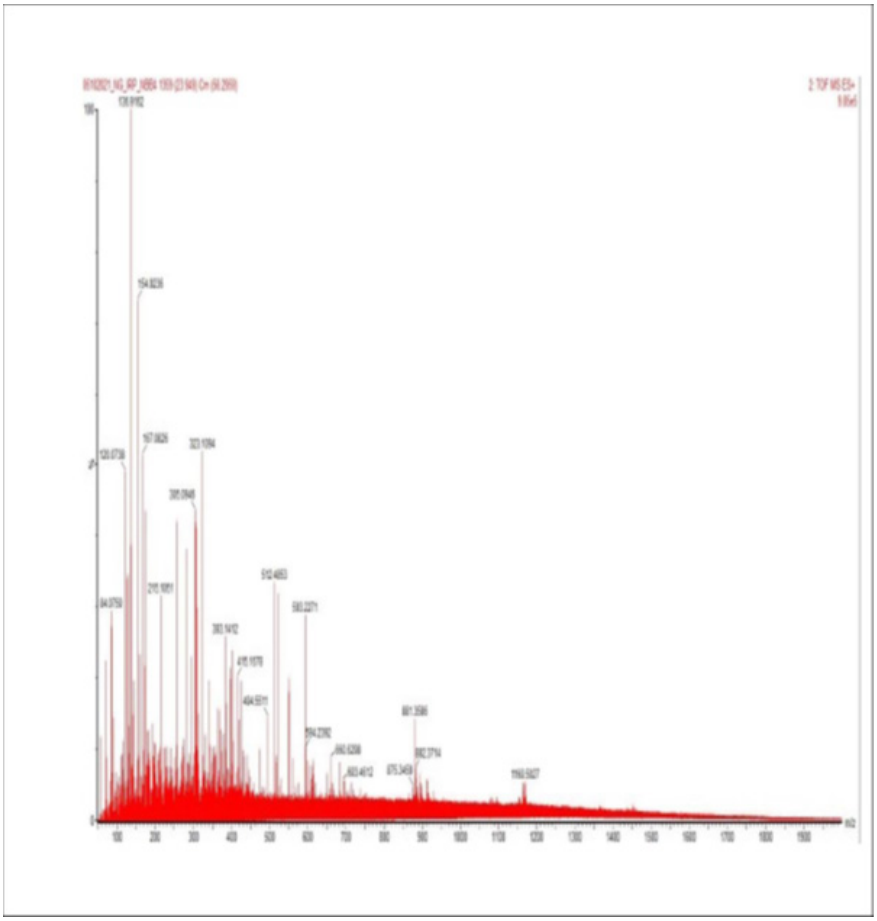


Figure 5: MS/MS spectra of separated protein of PISS raised seedlings of salt-sensitive rice cultivar (*Oryza sativa* L., Cultivar IR29).

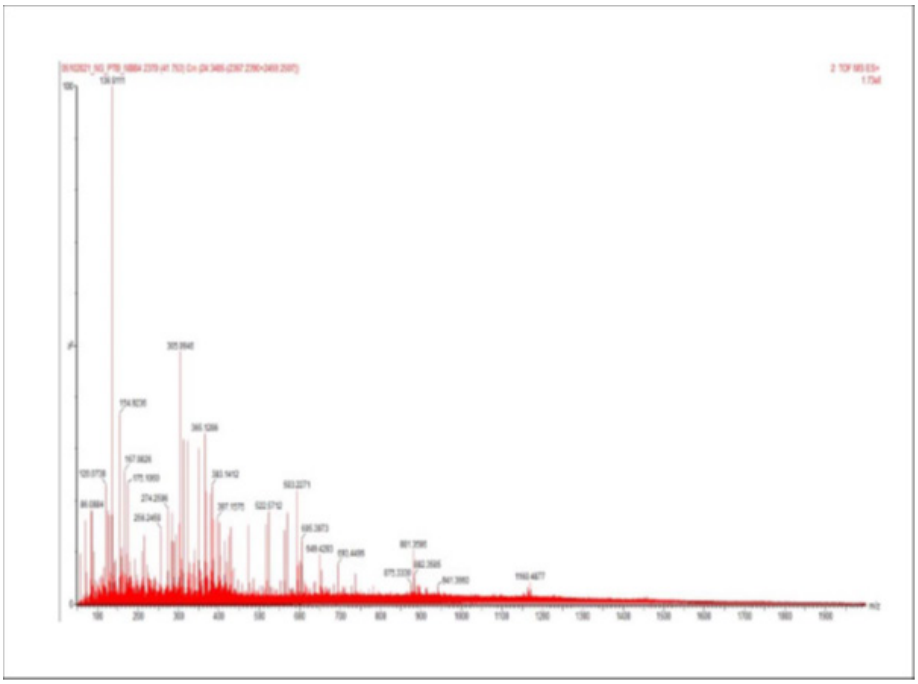
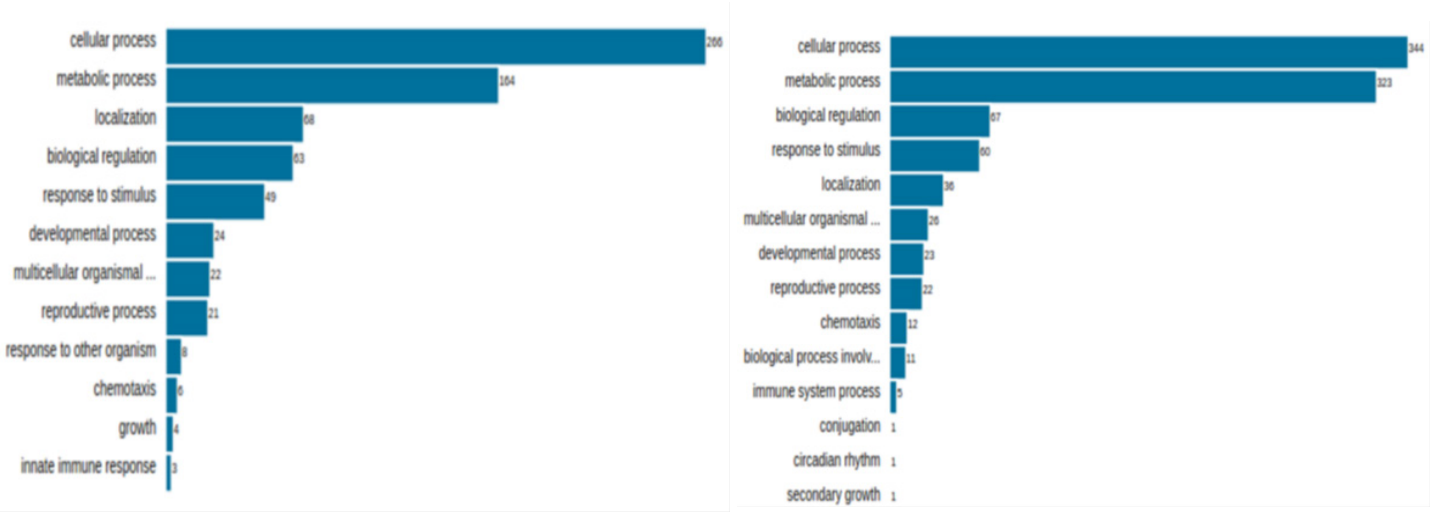


Figure 6: MS/MS spectra of separated protein of PISS-raised seedlings of salt tolerant rice cultivar (*Oryza sativa* L., Cultivar Patnai).

The Functional Analysis of the Identified Proteins

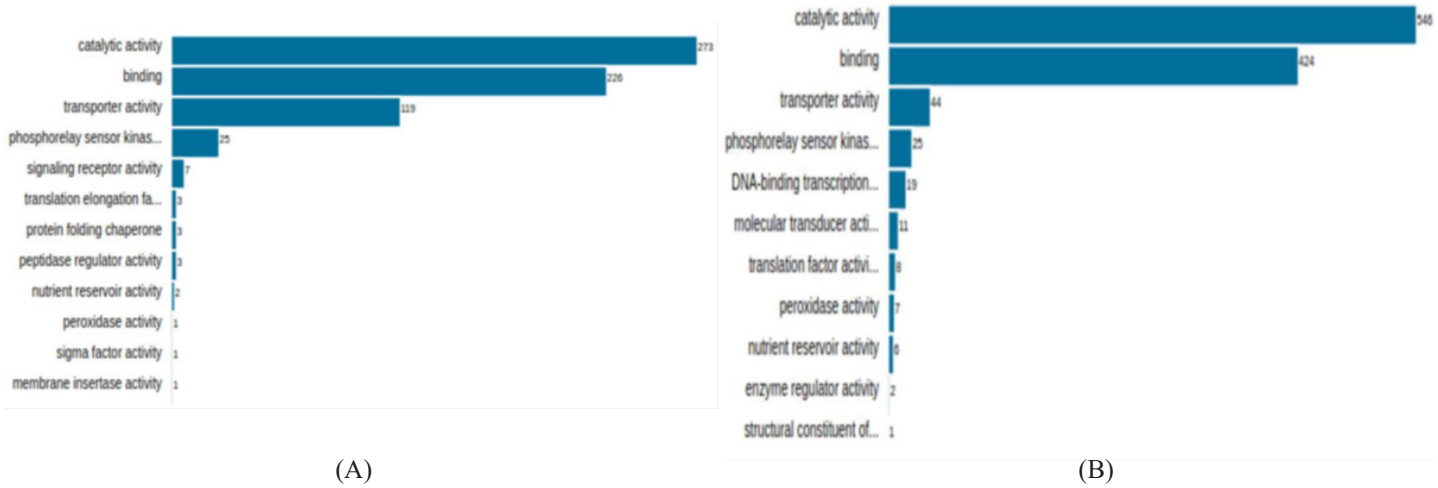
The identified sequences of proteins were blasted by BLAST P in NCBI database and identified proteins of both the experimental rice cultivars were classified into different functional groups based on gene ontology (GO) prediction. It is being divided broadly into biological, cellular, and molecular categories of protein. The biological category of protein includes mainly cellular processes, metabolic process and biological regulation, response to stimulus, localization, developmental process, reproductive process. Whereas the cellular processes mainly include proteins associated with membrane, cytoplasm, DNA-directed RNA polymerase, nuclear, and signal recognition particles. In the molecular category, the protein associated with catalytic activity, binding, transporter activity, phosphorelay sensor kinase activity, DNA binding transcription, molecular transducer, translation factor and peroxidase activity is induced. When we compared, the functional groups of proteins under the category of biological proteins based on GO prediction (Figs.7A, B) between the salinity tolerant rice cultivars Patnai and susceptible rice cultivars IR29, significant differences in the abundance of proteins involved in cellular process, metabolic process, and biological regulation has been noticed (Fig. 7A, B).



(A) (B)

Figure 7: GO of biological protein separated from PISS-raised seedlings of two experimental rice cultivars Oryza sativa L., Cultivar IR29 (A) and Patnai (B) separated on the basis of LC-MS/MS.

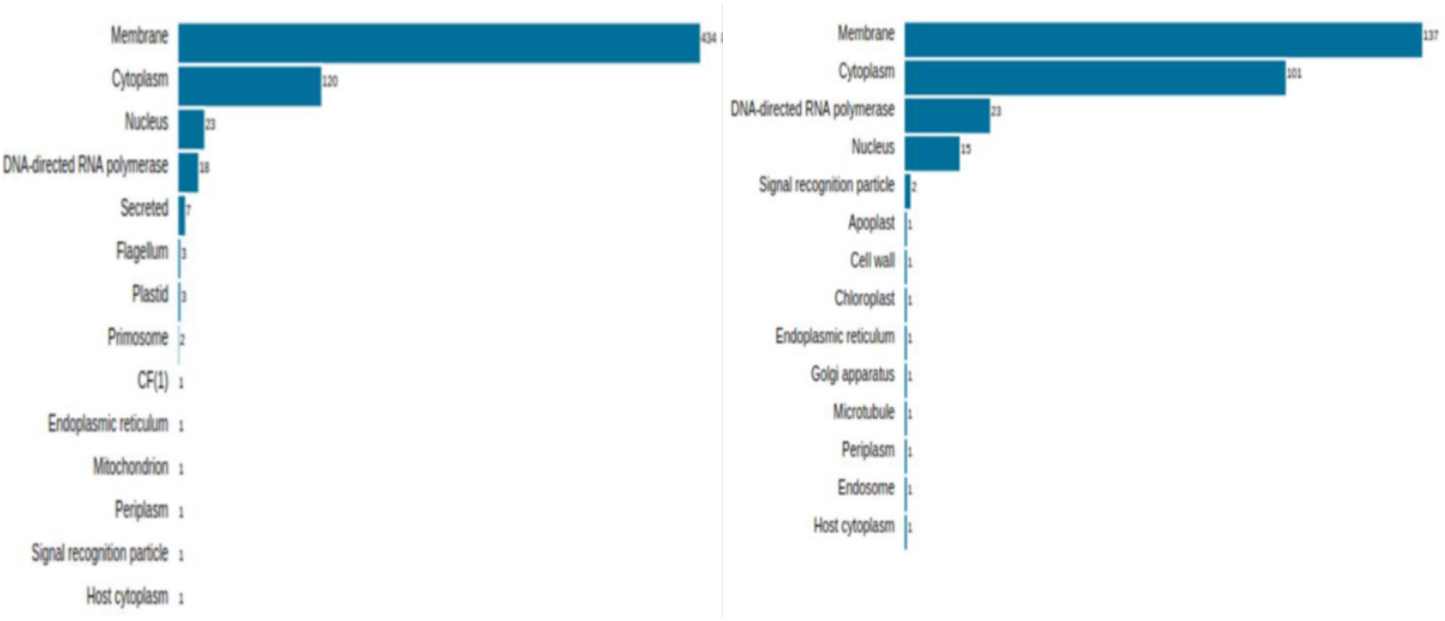
The salt-resistant cultivars Patnai showed 344, 323 and 67 expressed identified proteins as against 266, 164 and 63 numbers for the salinity susceptible cultivar IR29 confirming the severe loss of metabolic homeostasis and cellular processes for the salinity susceptible cultivar Patnai as compared to the salinity tolerant one. Similarly, the proteins associated with the reproductive and other biological processes are also found to be significantly higher for the cultivar Patnai than that of IR29. When we compared, the functional groups of proteins under the category of proteins with molecular function, we found significant differences between the salt-resistant cultivar Patnai and salt-sensitive cultivar IR29. In the molecular category of proteins, we have characterized, the protein associated with catalytic activity, binding, transporter activity, phosphorelay sensor kinase activity, DNA binding transcription factor, molecular transducer activity translation factor activity, enzyme regulator activity, etc. A comparison of protein abundance associated with catalytic and binding activity revealed significantly higher abundance of the same for the cultivar Patnai as compared to IR29 (Fig. 8A, B). Similarly, other subcategories of proteins like molecular transducer activity, DNA binding transcriptional proteins are found to be absolutely confined to the salinity resistant cultivar Patnai. Though there are no differences in the expression of phosphorelay sensor kinase protein abundance the level of molecular transducer protein is found to be significantly higher for the cultivar Patnai.



(A) (B)

Figure 8: GO of molecular proteins separated from PISS-raised seedlings of two experimental rice cultivars *Oryza sativa* L., Cultivar IR29 (A) and Patnai (B)separated on the basis of LC MS/MS

When we compared the expression of antioxidant protein peroxidase it showed a significant difference with Patnai showing 7 proteins exhibiting peroxidase activity vis-à-vis only one protein having peroxidase activity for the cultivar IR29. The data of cellular protein assessed (Figs. 9A, B) in terms of abundance of membrane, cytoplasm, and nucleus associated proteins revealed an otherwise picture, showing a greater abundance of the membrane, cytoplasm and nucleus associated proteins for the salt susceptible cultivar IR29, as compare to salt-resistant cultivar Patnai. The signal recognition protein which has been identified in the salt tolerant cultivar Patnai was found to be absolutely lacking in salt susceptibleIR29 and the data associated with the cell wall, chloroplast, endoplasmic reticulum, golgi bodies apparatus associated protein seems to have no significant difference between the two cultivars.



(A) (B)

Figure 9: Go of cellular protein separated from PISS-raised seedlings of two experimental rice cultivar *Oryza sativa* L., Cultivar IR29 (A) and Patnai (B)on the basis of LC MS/MS.

Discussion

The label-free quantitative shotgun proteomic analysis of the soluble proteins of PISS-raised seedlings of both the experimental rice cultivars provides a detailed proteomic profile. The two contrasting rice genotypes differing in sensitivity towards salinity have been chosen for making an insight into the qualitative changes in protein profile associated with salinity stress during early post imbibitional phase of germination. In fact, the germplasm diversity associated with salinity tolerance was expected to be reflected in the variation of proteome label when each experimental germplasm was exposed to PISS. The altered physiological phenotypes under PISS during early germination which largely include a redox metabolic shift and altered hormonal homeostasis (ABA and GA) as a result of salinity largely determine the contrasting germination ability of both the experimental germplasms. The salinity-resistant cultivar Patnai fine-tune redox homeostasis through tandem action of enzymatic and non-enzymatic antioxidative defense, whereas the sensitive cultivar IR29 is largely incapable of maintaining redox homeostasis, exhibiting oxidative deterioration. Overall, the redox and energy homeostasis as well as maintenance of the hormonal status found to be associated with survival and germinability. And this might be expected to be acquired significantly, at least partially through the altered abundance of major key proteins.

The present experimental design involving LC-MS/MS enabled us to identify a group of stress proteins in both the germplasms under salinity (regardless of their sensitivity towards NaCl salinity). The experiment also helped us to identify proteins induced under PISS for the salt-tolerant cultivar vis-à-vis the salt-sensitive one. Moreover, the present proteomic strategy also enabled us to identify a number of salinity-responsive proteins whose abundance changes significantly in response to PISS, particularly for the tolerant germplasm. The greater abundance of expressed protein associated with the biological, cellular and molecular process for the tolerant germplasm Patnai in response to PISS in contrast to a significantly repressed abundance of the same categories of proteins for the sensitive cultivar IR29 is the prime outcome of proteomic investigation. And most of these proteins seems to have a vital role in the regulation of hormonal status as well as metabolic homeostasis, particularly the redox tuning necessary for the progression of germination [30 - 32].

Comparative GO analysis of separated proteins revealed an abundance of several redox proteins identified in PISS-raised seedlings of the salt-resistant cultivar Patnai (Supplementary Table 2). These are Protein GO:0033355 (ascorbate-glutathione cycle), GO: 0051776 (detection of redox state), GO:0042743 (hydrogen peroxide metabolic process), GO:0042744 (hydrogen peroxide catabolic process), GO:0042542 (response to hydrogen peroxide), GO:0070301 (cellular response to hydrogen peroxide), GO:0072593 (reactive oxygen species metabolic process), GO:0006560(proline metabolic process),GO:0004735(pyrroline-5-carboxylate reductase activity),GO:0004657 (proline dehydrogenase activity),GO:0051776(detection of redox state),GO:0051775 (response to redox state), and GO:1903409 (reactive oxygen species biosynthetic process). The abundance of these proteins under PISS has been previously suggested to play a significant role in redox homeostasis and integrity of cellular and mitochondrial membrane under stress conditions [28, 33]. The expression of the proteins associated with ascorbate-glutathione cycle protein, hydrogen peroxide metabolic process, hydrogen peroxide responsive protein, cellular redox homeostasis, detection of redox states and proline metabolism are largely considered with ROS processing and detoxification as well as regulation of proline biosynthesis necessary for amelioration for salinity stress [30, 34, 35]. Through most of these proteins are expressed in the sensitive cultivar IR29 under PISS but when compared between the two experimental rice cultivars a general increase in abundance of the redox proteins have been noticed for the cultivar Patnai, confirming the data of redox metabolic investigation and the role of redox regulation under PISS during early germination (Supplementary Table 1 and 2). Out of the several redox proteins detected, the protein GO: 0033355 (associated with Ascorbate-Glutathione cycle) which takes an active role in fine-tuning the endogenous H2O2 concentration, formed during early germination under PISS seems to be a significant finding necessary for survival under salinity for both the experimental germplasms.

So, the GO data in general exhibited that the stress-inducible protein especially those associated with redox regulation and management of oxidative stress increases in response to salinity. In fact, salinity triggers a cascade of cellular processes that eventually caused reprogramming of metabolism in favour of the conservation of metabolic energy [36]. The germinating tissue exposed to a high concentration of salinity caused a redox shift towards the accumulation of pro-oxidant. And if not mitigated the enhanced ROS titer causes oxidative deterioration of biomolecules including proteins [37, 38]. Therefore, the germinating tissue regulates this hostile condition by up-regulating the synthesis of proteins that play a significant role in scavenging ROS and systematically degrading oxidized protein. Several ROS scavengers were found to be enhanced in abundance in both genotypes and these include glutathione dehydrogenase (GO: 0045174), glutathione reductase (GO: 0004362), peroxiredoxin (GO: 001920), peroxidase (GO: 0004601), thioredoxin (GO: 0004791) and SOD (GO: 0004784). So, elaborate antioxidative defense mechanisms along with several redox homeostatic proteins are expressed under PISS for redox regulation and progression of germination [30, 39]. The proteins are mostly involved in SOD, ascorbate-glutathione/catalase and glutaredoxin pathways as proposed from the finding of our redox metabolic investigation. The most significant outcome of this comparative proteomic investigation involving LC-MS/MS is the characterization of the proteins associated with the ascorbate-glutathione cycle and integrated H_2O_2 signaling pathway (GO: 0071588, GO: 007165, GO: 0023051). Ascorbate-glutathione pathway being the most important significant ROS processing system in the germinating tissue responsible for regulating the endogenous titer of ROS can be strongly vouched from the present

experiment [40, 41]. All the important enzymes of ascorbate-glutathione/catalase/peroxiredoxin pathway like ascorbate peroxidase [42 - 44].Glutathione reductase glutathione dehydrogenase [35, 45]. Peroxiredoxin and thioredoxin are also found in similar proteomic studies in plants exposed to salinity [46]. Several previous workers through their proteomic investigation also characterized the salinity-induced expression of ascorbate peroxidase, DHAR, GRase, thioredoxin in rice [42, 47 and 48]. Similarly, the induction of the catalase pathway as a redox regulatory mechanism in rice is also characterized by the proteomic investigation [18, 44]. Thioredoxin and glutaredoxin pathway which is found to be a central pathway of antioxidative defense mechanism is also identified in Zea maize and in other plants [43]. The role of peroxidase has also been identified by several workers under salinity through proteomic investigation in rice [49-52]. Through several other proteins have been found to be expressed under salinity in the salt-tolerant cultivar Patnai vis-à-vis the salt-sensitive cultivar IR29, the most significant one associated with redox regulation seems to be signaling protein involved in H2O2 signaling, (GO: 0071588), receptor signal protein (GO: 0140626) and signal recognition protein (GO: 0023051). Since signal transduction under salinity is a very important avenue of research, several workers identified different salt-responsive pathways involving SOS, ABA, Ca2+, and ethylene, JA with the help of different proteomic approaches [9, 53, and 54]. our present investigation also confirms the active participation of different signaling pathways as several proteins associated with growth regulator function protein have been found to be expressed in the cultivar Patnai (Signaling protein GO: 0023052, GO: 0046883).

Conclusion

So, proteomic label-free quantitative shotgun proteomic study (LC-MS/MS) helped us to characterize the low abundant proteomes (cellular, metabolic and molecular) and identified noble redox-regulatory proteins that are expressed under salt stress particularly in the salt-tolerant germplasm Patnai under PISS which is largely involved with signalling and metabolic reprogramming necessary for conferring salinity tolerance.

Abbreviations

APOX: Ascorbate peroxidase; AsA: Ascorbic acid; BEH C18 UPLC:Ethylene Bridged Hybrid C18 Ultra Performance Liquid Chromatography; CA: Catalase;CaCl2: Calcium chloride;COX6b-1: Cytochrome C Oxidase Subunit 6B1;CP47: Photosystem II CP47 reaction centre protein;DHAR: Dehydroascorbate reductase;DTT: Dithiothreitol;GR: Glutathione reductase; GR-RBP: glycine-rich RNA-binding proteins;HCD:higher energy collisional dissociation; HSP70: Heat shock protein-70;H2O2:Hydrogen peroxide; KNO3:Potassium nitrate; LC-MS/MS: Liquid Chromatography mass spectrometry; LEA:Late embryogenesis abundant; NaCl: Sodium chloride;OEE2: 3 KDa protein of the oxygen-evolving complex; ROS: Reactive oxygen species; SDS: Sodium dodecyl sulphate; SOD: Superoxide dismutase; UGPase:UDP-glucose pyrophosphorylase

Declarations

Ethics approval and consent to participate
Not applicable

Consent for publication

Not applicable

Availability of data and material

The materials were collected from Central Rice Research Institute (CRRI) Bhubaneswar Orissa, India and are grown each year at Crop Research and Seed Multiplication Farm (CRSMF), The University of Burdwan, West Bengal. All the data generated in this study are available in this article and supplementary files

Competing interests

The authors have no competing interests

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Author's contribution

Conceptualization, Designing, data collection, data analysis, manuscript drafting and editing was done by SB. Material preparation, data collection, analysis, manuscript editing was performed by NB. Manuscript editing was done by DK and UKR. All authors read and approved the final manuscript.

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