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ORIGINAL ARTICLE

# Differential proteomic analysis reveals the mechanism of *Musa paradisiaca* responding to salt stress

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## Abstract

Salinity is one of the most important abiotic stresses, which affects the yield and quality of banana (*Musa paradisiaca*). To understand the salinity tolerance mechanisms of banana, the iTRAQ technique is employed to reveal the proteomic response of Brazil banana under different durations of 60 mmol/L NaCl stress. We have identified 77 DEPs and classified them into nine functional categories, compared with control (0 mmol/L NaCl treatment). The four major categories involve protein synthesis and degradation, photosynthesis, defense response, and energy and carbohydrate metabolism. The results indicate that photosynthesis, protein synthesis and degradation, lipid metabolism and secondary metabolism are promoted to limit damage to a repairable level. The accumulation of ROS under salt stress is harmful to cells and causes up-regulation of antioxidant systems. Furthermore, to cope with cells injured by salt stress, PCD is used to remove the damaged. Additionally, the cytoskeleton can play an important role in maintaining cellular and redox homeostasis. Different categories of functional proteins by changing the abundance ratio shows that plants have different mechanisms of response to salinity. Conclusively, Function of the observed changes in protein expression objective is to establish a new metabolic process of steady-state balance. To my knowledge, this is the first report that investigates responses of *M. paradisiaca* to salt stress by proteomic analysis.

**Keywords** *M. paradisiaca* · Salt stress · Proteomics · iTRAQ · Functional categories

## Introduction

Soil salinity is a major abiotic stress, which seriously impacts crop quality and productivity in the world [1, 2]. Salt stress causes many problems, such as ion toxicity, nutrient imbalance, water deficiency and oxidative stress, etc, resulting in plant cellular damage, growth reduction, even death [1, 3, 4]. Thus, improving responses to salt stress tolerance in plants and increase plant production has become urgent goal of plant breeders. The response mechanisms of plant stress are divided into stress tolerance and stress avoidance, stress tolerance mechanism is used when the stress is serious [5]. Under serious osmotic stress, with the increase of $Cl^-$ and $Na^+$ ion toxicity, salt stress affects plants far more seriously [6]. The salt stress response mechanism of plant has become a heated debate for those who are interested in studying salt tolerance mechanism of plant, and the tolerance of plant to salinity. Through exploring mechanism of salt tolerance in plants on the basis of molecular and biochemical response

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to salt stress in plants, we can have a better understanding of plants responding to salt stress.

Banana is a large monocotyledonous herbaceous plant widely distributed in subtropical and tropical regions. It is also the most popular fruit as well as the largest fruit crop, vital for thousands of people in the world [7, 8]. Compared with other fruits, banana research has developed slowly, the reason being that banana is widely cultivated in Africa [7]. Most banana cultivars are salt sensitive, hence, a better understanding of genetic regulation of the salt induced stress responses in banana can strengthen future banana management and improve the soil salinity related to irrigation and climate change [9]. Soil salinization seriously affects banana production and restricts the development of banana industry. Therefore, it is important to explore the salt tolerance mechanism of banana [10]. However, few people have used molecular biological methods to study the banana differentially expressed proteins (DEPs) in response to salt stress. Once we are clear about the molecular mechanisms of banana response to salt stress, it has a great potential for developing salt-tolerated banana cultivars. The investigation of banana protein expression patterns in response to salt stress will pave the way for further understanding the regulatory networks of salt stress acclimation in banana and help to select candidate proteins for manipulation to improve salt stress tolerances.

Now, as proteomic technology develops rapidly, if we combine this technology with the genome sequence information of most plants, it will provide a good opportunity for banana proteomic analysis [11, 12]. Proteomics is beneficial in studying DEPs of plants response to salt stress since it analyzes the salt stress induced proteome changes of many plant species, including *Arabidopsis* [13], rice [14, 15], plasma membrane [16], wheat [17] and *Suaeda* [18] et al. The DEPs in different tissue of plants have a synergistic effect when plants are subjected to salt stress [19]. Previous studies have shown that 2-DE (two-dimensional electrophoresis) technology are ineffective in identifying the low abundant proteins, i.e. basic or acidic proteins and hydrophobic proteins [20]. In recent years, with the development of non-gel-based quantitative proteomics techniques, disadvantages from the above mentioned technology has overcome. iTRAQ (isobaric tags for relative and absolute quantification) is the mass spectrometry proteomics technique and it can be used to evaluate cell metabolic differences. Meanwhile, iTRAQ is widely used in plant quantitative proteomics [21, 22]. Furthermore, it is revealed that this technology is used to demonstrate the functional differentiation of the mesophyll cells and *Brassica napus* guard cells [23]; and can successfully analyze protein profile of plant responses to deficient or excess mineral nutrients, such as *Citrus sinensis* roots response to boron deficiency [24]. iTRAQ protein profile analysis is used to identify many DEPs in tomato [25], *Arabidopsis thaliana* and *Brassica juncea* [26], respectively, subject to alkali stress and salt stress. In conclusion, molecular mechanism of plant response to abiotic stress by iTRAQ will be widely used in the future.

In this study, the researchers have used the iTRAQ-based quantitative proteomic analysis to identify the DEPs in banana leaves, which responds to 60 mmol/L NaCl stress by using hydroponic test. Based on enrichment analysis of gene ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG), the researchers carry out the differential protein function to realize the salt-related proteins of banana.

In short, by proteomic analysis of molecular mechanism of banana response to salt stress and by filtering out the salt tolerance-related protein, the result has shown that banana has a certain amount of salt tolerance. The result is significant because it has paved the way for theoretical basis for studies on new type of banana varieties of salt tolerance and the mechanism of salt tolerance.

## Materials and methods

### Plants and stress treatment

The was tissue culture plantlet of Brazil banana *(Musa paradisiacal*. AAA Group cv. Brazil) of experimental material is provided by the Chinese academy of tropical agricultural sciences. Banana plantlets are about 25 cm high, the growth of seedlings is basically consistent, five leaves with one leave in the center without pests and diseases. Seedlings are removed from their culture soil, then they are cultured in 1/2 Hoagland nutrient solution in pot culture under temperature of 27 °C/21 °C (day/night), a relative humidity of 85%, a 14-h photoperiod, and a photosynthetically active radiation of 75 μmol/$m^2$/s. The solution is renewed every 3 days. Banana seedlings are randomly divided into two groups including control (0 mmol/L NaCl) and treatment groups (60 mmol/L NaCl) after 3 days. The leaves of control group and the treatment group are sampled at 0, 12, 24 h, respectively. The leaflet samples are collected at different time intervals, frozen in liquid nitrogen, and stored at − 80 °C.

### Protein digestion and iTRAQ labeling

Leaf proteins of the banana samples are extracted with the help of the Borax/PVPP/Phenol (BPP) protocol [27]. Bicinchoninic acid (BCA) protein assay was used to determine the protein concentration of the supernatant. The 100 μg protein per condition was transferred into the new tube and adjusted to a final volume of 100 μL with 8 mol/L urea. 11 μL of 1 M DTT was added, and

samples were incubated at 37 °C for 1 h. Then 120 µL of the 55 mM iodoacetamide was added to the sample and incubated for 20 min at 25 °C.

Proteins were then tryptic digested with sequence-grade modified trypsin (Promega, Madison, WI, USA) at 37 °C the whole night. For each time point (i.e., 0 h, 12 h, 24 h), three samples were iTRAQ labeled. Peptides were labeled with respective isobaric tags (113 for 0 h; 115 for 12 h; 117 for 24 h). The labeled samples were combined and dried in vacuum.

### LC–MS/MS analysis

The fusion mass spectrometer was operated in the data dependent mode to switch automatically between the MS and MS/MS acquisition. Survey full scan MS spectra (m/z 350–1550) were acquired with a mass resolution of the 120K, followed by sequential high energy collision dissociation (HCD) MS/MS scans with a resolution of 30K. The isolation window was set as 1.6 Da. The AGC target was set as 400,000. MS/MS fixed first mass was set at 110. In all situations, one microscan was recorded using dynamic exclusion of 45 s.

### Data analysis

The obtained peptide fragment quality data are retrieved by way of the MASCOT software 2.3.02 online search (http://www.matrixscience.com), with NCBI database as search database. The researchers have used GO database (http://www.geneontology.org/) and KEGG database to determine the differential proteins of enrichment GO terms and the significant enrichment pathways respectively. Proteins with 1.5 fold change between samples and $p$ value $< 0.05$ are determined as DEPs.

## Results

### Overview of quantitative proteomics

Protein identification information of banana leaves is showed in Fig. 1. The basic information of chart proteome identification in banana plantlet leaf. A total of 237,424 spectra are obtained from the iTRAQ proteomic analysis of all banana samples. After data filtering to eliminate low-scoring spectra, a total of 36,705 unique spectra that meet the strict confidence criteria for identification are matched to 3105 unique proteins.

### Differential protein statistics

According to protein expression level requirements, DEPs with 1.5 fold change and $p < 0.05$ can meet the required criteria of DEPs. Based on the two criteria mentioned above, 77 differentially abundant proteins are identified in salt stress of banana leaves (Table 1). At 12 h and 24 h of salt stress, 38 and 20 proteins are up-regulated, while 7 and 17 proteins are down-regulated, respectively (Fig. 2). The bigger number (45) of DEPs is between 12 and 0 h, and the smaller number (37) of DEPs between 24 and 0 h, of which 5 DEPs are expressed in both groups.

### Functional categorization of the DEPs

DEPs are classified into nine categories based on their putative biological functions (Fig. 3). The majority of DEPs (81%) are classified into 4 categories: defense response (30%), energy and carbohydrate metabolism (21%), photosynthesis (17%), protein synthesis, processing and degradation (13%); the other categories are as follows: signal transduction (4%); cytoskeleton (4%); lipid metabolism (4%); secondary metabolism (3%) and hypothetical or unknown (6%).

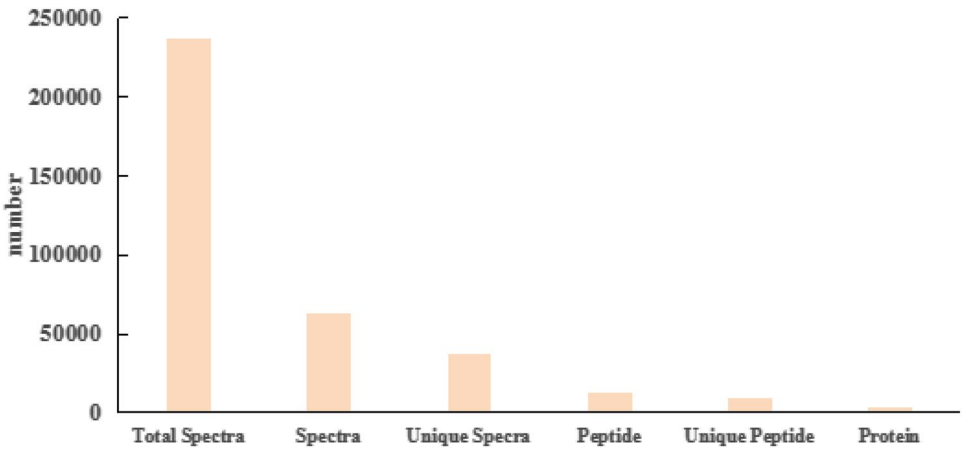


**Fig. 1** The basic information of chart proteome identification in banana plantlet leaf

**Table 1** List of DEPs in salt stress *M. paradisiaca* leaves

| Group ID | Accession | Function category protein name | Plant species | Score | Mass (Da) | Cov (%) | Ratios | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | 12 h/0 h | 24 h/0 h |
| Protein synthesis and degradation | | | | | | | | |
| 120 | Ma08_g14870 | Disulfide-isomerase | *M. acuminata* | 289 | 79,477 | 12.1 | 1.501 | 1.124 |
| 23 | Ma00_g03400 | 60S ribosomal protein L3 | *M. acuminata* | 118 | 61,402 | 12.3 | 0.819 | 0.403 |
| 23 | Ma11_g08620 | 60S ribosomal protein L3 | *M. acuminata* | 118 | 61,070 | 12.3 | 0.819 | 0.403 |
| 183 | Ma06_g16010 | Cysteine proteinase | *M. acuminata* | 579 | 61,143 | 16.7 | 2.426 | 0.782 |
| 28 | Ma01_g00800 | 50S ribosomal protein L35 | *M. acuminata* | 260 | 22,481 | 9.1 | 1.553 | 1.212 |
| 28 | Ma03_g13190 | 50S ribosomal protein L35 | *M. acuminata* | 260 | 22,166 | 9.9 | 1.553 | 1.212 |
| 15 | Ma04_g00840 | 40S ribosomal protein S30 | *M. acuminata* | 36 | 10,548 | 16.1 | 0.883 | 0.561 |
| 15 | Ma04_g08690 | 40S ribosomal protein S30 | *M. acuminata* | 36 | 10,895 | 16.1 | 0.883 | 0.561 |
| 15 | Ma06_g37630 | 40S ribosomal protein S30 | *Phoenix dactylifera* | 36 | 10,908 | 16.1 | 0.883 | 0.561 |
| 1 | Ma08_g02150 | 50S ribosomal protein L4 | *M. acuminata* | 257 | 36,290 | 11 | 1.555 | 1.304 |
| 135 | Ma08_g15350 | 50S ribosomal protein L19 | *M. acuminata* | 71 | 29,520 | 8.4 | 1.562 | 1.562 |
| Photosynthesis | | | | | | | | |
| 19 | Ma09_g26690 | Oxygen-evolving enhancer protein 2 | *M. acuminata* | 964 | 34,398 | 23.8 | 2.893 | 1.102 |
| 151 | Ma08_g03020 | Ribose-5-phosphate isomerase | *M. acuminata* | 823 | 34,184 | 31.8 | 1.503 | 1.176 |
| 12 | Ma06_g24480 | Uroporphyrinogen decarboxylase | *M. acuminata* | 702 | 50,109 | 15.1 | 1.981 | 0.889 |
| 26 | Ma03_g14780 | Protochlorophyllide reductase | *M. acuminata* | 62 | 51,441 | 8.1 | 2.702 | 0.831 |
| 1516 | Ma07_g04400 | Protochlorophyllide reductase-like | *M. acuminata* | 120 | 51,391 | 6.3 | 6.935 | 1.164 |
| 155 | Ma11_g01810 | Protochlorophyllide reductase-like | *M. acuminata* | 1056 | 51,427 | 26.5 | 2.384 | 1.202 |
| 124 | Ma06_g09580 | Glutamate-1-semialdehyde 2,1-amino-mutase | *M. acuminata* | 2654 | 58,364 | 32.5 | 1.641 | 1.099 |
| 49 | Ma11_g06010 | ruBisCO | *M. acuminata* | 4661 | 81,274 | 33.5 | 1.311 | 1.507 |
| 134 | Ma05_g08930 | Chlorophyll a/b binding protein | *M. acuminata* | 1050 | 32,889 | 12.7 | 1.738 | 0.819 |
| 133 | Ma09_g02760 | Chlorophyll a/b binding protein | *M. acuminata* | 352 | 36,965 | 16.7 | 2.461 | 0.977 |
| 9 | Ma09_g06640 | Chlorophyll a/b binding protein | *M. acuminata* | 906 | 32,314 | 13.2 | 2.007 | 0.909 |
| 2323 | Ma10_g30410 | Chlorophyll a/b binding protein | *M. acuminata* | 46 | 18,551 | 12.1 | 1.842 | 0.926 |
| 1192 | Ma06_g26790 | Ferredoxin | *M. acuminata* | 189 | 82,818 | 10.3 | 1.5 | 1.055 |
| Defense response | | | | | | | | |
| 142 | Ma06_g34810 | L-Ascorbate peroxidase | *M. acuminata* | 773 | 32,272 | 54.6 | 0.613 | 0.871 |
| 116 | Ma01_g10810 | Catalase | *M. acuminata* | 1943 | 64,985 | 33.1 | 0.93 | 1.546 |
| 159 | Ma04_g01420 | Thioredoxin-like protein | *M. acuminata* | 74 | 18,569 | 19.4 | 0.917 | 0.484 |
| 17 | Ma08_g27780 | Thioredoxin-like protein | *M. acuminata* | 43 | 23,838 | 16.1 | 0.946 | 0.663 |
| 11 | Ma09_g09320 | Thioredoxin-like protein | *M. acuminata* | 168 | 32,765 | 8.2 | 0.807 | 0.502 |
| 129 | Ma08_g09150 | Polyphenol oxidase | *M. acuminata* | 3561 | 79,886 | 34.7 | 0.919 | 1.611 |
| 51 | Ma08_g09160 | Polyphenol oxidase | *M. acuminata* | 2847 | 69,154 | 32.5 | 1.177 | 1.72 |
| 17 | Ma08_g09170 | Polyphenol oxidase | *M. acuminata* | 1275 | 81,347 | 21.6 | 0.995 | 2.195 |
| 82 | Ma06_g27520 | Abscisic stress-protei | *M. acuminata* | 135 | 21,741 | 9.6 | 1.636 | 0.926 |
| 141 | Ma09_g08750 | Stress-response protein | *Daucus carota* | 93 | 14,331 | 8.6 | 0.798 | 2.097 |
| 132 | Ma03_g03390 | Peroxidase P7 | *M. acuminata* | 399 | 36,072 | 30.1 | 1.508 | 1.113 |
| 6 | Ma04_g05290 | Peroxidase P7 | *M. acuminata* | 109 | 40,285 | 11.1 | 1.28 | 1.661 |
| 163 | Ma05_g22740 | Peroxidase 5 | *M. acuminata* | 1309 | 39,376 | 30.2 | 1.502 | 0.991 |
| 65 | Ma06_g24120 | Peroxidase P7 | *M. acuminata* | 446 | 35,793 | 34.4 | 1.921 | 0.744 |
| 70 | Ma10_g05350 | Peroxidase 21 | *M. acuminata* | 32 | 42,159 | 4.6 | 1.563 | 0.541 |
| 144 | Ma10_g27810 | Peroxidase P7 | *M. acuminata* | 170 | 35,706 | 23 | 1.531 | 1.06 |
| 99 | Ma01_g08410 | Glutathione S-transferase | *M. acuminata* | 71 | 14,234 | 20.2 | 0.504 | 0.634 |
| 36 | Ma03_g17130 | Allene oxide cyclase 3 | *M. acuminata* | 72 | 30,212 | 10 | 1.052 | 1.919 |
| 26 | Ma09_g10450 | Lectin | *M. acuminata* | 4748 | 17,048 | 44 | 0.89 | 2.546 |
| 7 | Ma09_g10470 | Lectin | *M. acuminata* | 5101 | 17,294 | 39 | 1.131 | 4.085 |
| 197 | Ma02_g20530 | Germin-like protein | *M. acuminata* | 1296 | 25,325 | 18.6 | 1.616 | 0.687 |

**Table 1** (continued)

| Group ID | Accession | Function category protein name | Plant species | Score | Mass (Da) | Cov (%) | Ratios | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | 12 h/0 h | 24 h/0 h |
| 73 | Ma07_g18510 | Germin-like protein | *M. acuminata* | 369 | 22,536 | 13.3 | 2.042 | 0.871 |
| Energy and carbohydrate metabolisms | | | | | | | | |
| 101 | Ma06_g16620 | Enolase 3 | *M. acuminata* | 107 | 63,947 | 10.1 | 1.5 | 1.5 |
| 19 | Ma11_g17540 | Glyceraldehyde-3-phosphate dehydrogenase | *M. acuminata* | 2982 | 45,950 | 35.8 | 1.104 | 1.519 |
| 121 | Ma04_g08470 | V-type proton ATPase | *M. acuminata* | 424 | 34,370 | 18.3 | 1.038 | 1.509 |
| 2 | Ma09_g23510 | V-type proton ATPase | *M. acuminata* | 2086 | 79,671 | 39.8 | 1.018 | 1.593 |
| 164 | mito2_g00070 | ATP synthase | *Capsicum annuum* | 1100 | 14,672 | 12.2 | 0.992 | 0.665 |
| 8 | Ma02_g18550 | beta-Galactosidase-like | *M. acuminata* | 261 | 95,286 | 4.5 | 3.45 | 1.127 |
| 184 | Ma04_g27470 | beta-Galactosidase 6 | *M. acuminata* | 356 | 109,029 | 8.4 | 1.606 | 1.237 |
| 8 | Ma07_g08780 | beta-Galactosidase-like | *M. acuminata* | 261 | 94,847 | 4.5 | 3.45 | 1.127 |
| 8 | Ma07_g08790 | beta-Galactosidase-like | *M. acuminata* | 261 | 94,368 | 4.5 | 3.45 | 1.127 |
| 8 | Ma07_g08800 | beta-Galactosidase-like | *M. acuminata* | 261 | 93,906 | 4.5 | 3.45 | 1.127 |
| 21 | Ma06_g01570 | Fructokinase-1 | *M. acuminata* | 282 | 40,905 | 30 | 1.793 | 0.811 |
| 84 | Ma06_g13970 | Fructokinase-1 | *M. acuminata* | 384 | 39,527 | 29.9 | 1.738 | 0.882 |
| 60 | Ma06_g29050 | Galactinol synthase 1 | *M. acuminata* | 153 | 43,132 | 4.3 | 0.696 | 0.55 |
| 33 | Ma03_g08680 | 4-alpha-Glucanotransferase | *M. acuminata* | 151 | 131,254 | 5.7 | 0.636 | 1.12 |
| 92 | Ma04_g36160 | NADH dehydrogenase | *M. acuminata* | 247 | 16,500 | 33 | 1.042 | 0.61 |
| 49 | Ma10_g00760 | Glucan endo-1,3-beta-glucosidase | *M. acuminata* | 48 | 38,862 | 9.1 | 1.126 | 2.73 |
| Lipid metabolism | | | | | | | | |
| 139 | Ma01_g01460 | Acyl-CoA binding protein | *M. acuminata* | 1116 | 13,495 | 61.5 | 1.935 | 0.955 |
| 41 | Ma04_g18960 | Acyl-CoA binding protein | *M. acuminata* | 213 | 13,468 | 34.4 | 1.718 | 0.777 |
| 119 | Ma08_g30750 | Phospholipase | *M. acuminata* | 115 | 66,031 | 7.5 | 0.728 | 1.763 |
| Cytoskeleton | | | | | | | | |
| 188 | Ma05_g00250 | Tubulin beta chain | *M. acuminata* | 4764 | 55,849 | 50.3 | 3.185 | 1.365 |
| 129 | Ma11_g22270 | Actin | *M. acuminata* | 1854 | 47,892 | 45.9 | 0.57 | 0.648 |
| Signal transduction | | | | | | | | |
| 119 | Ma08_g30750 | Phospholipase | *M. acuminata* | 115 | 66,031 | 7.5 | 0.728 | 1.763 |
| 139 | Ma03_g25530 | Calreticulin | *M. acuminata* | 1388 | 62,685 | 32.5 | 1.623 | 1.002 |
| 34 | Ma05_g29490 | Calreticulin-like | *M. acuminata* | 320 | 62,921 | 21.7 | 1.573 | 0.833 |
| Secondary metabolism | | | | | | | | |
| 20 | Ma06_g26840 | Linoleate 9S-lipoxygenase 4 | *M. acuminata* | 5431 | 86,437 | 36.4 | 1.42 | 1.64 |
| 21 | Ma10_g01130 | 1-Aminocyclopropane-1-carboxylate oxidase | *M. acuminata* | 351 | 42,006 | 13.6 | 0.644 | 0.898 |
| Hypothetical or unknown | | | | | | | | |
| 2126 | Ma08_g24690 | Uncharacterized protein | *M. acuminata* | 57 | 26,149 | 3.9 | 0.562 | 0.48 |
| 2028 | Ma09_g29940 | Uncharacterized protein | *M. acuminata* | 64 | 25,014 | 7.4 | 0.547 | 0.54 |
| 82 | Ma05_g19640 | Probable protein phosphatase | *M. acuminata* | 53 | 36,481 | 12 | 0.732 | 0.596 |
| 95 | Ma02_g09990 | Short-chain dehydrogenase | *M. acuminata* | 46 | 42,529 | 11.1 | 0.837 | 0.686 |
| 2392 | Ma06_g13990 | Predicted membrane protein | *M. acuminata* | 42 | 37,692 | 6.1 | 1.467 | 2.019 |

Based on hierarchical cluster analysis, we have grouped DEPs in the main categories during salt stress (Fig. 4). The protein of synthesis and degradation (Fig. 4a), several enzymes involved in protein synthesis are up-regulated, such as disulfide-isomerase, cysteine protease (CP), and ribosomal proteins (RP). For photosynthesis (Fig. 4b), most proteins have increased, including oxygen-evolving enhancer protein, RuBisCO, ribose-5-phosphate isomerase, and are involved in the formation of a Calvin cycle complex in photosynthetic organisms. For defense response-related proteins (Fig. 4c), many proteins are up-regulated, including allene oxide cyclase, lectin, germin-like protein. Finally, for energy and carbohydrate metabolism (Fig. 4d), several proteins that participate in carbohydrate metabolism are up-regulated, including glyceraldehyde-3-phosphate dehydrogenase, V-type

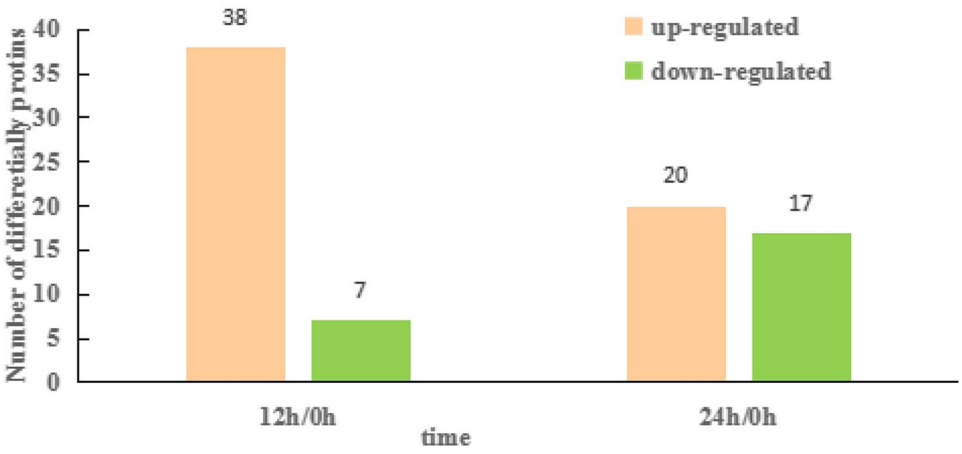


**Fig. 2** The comparison between two numbers of DEPs

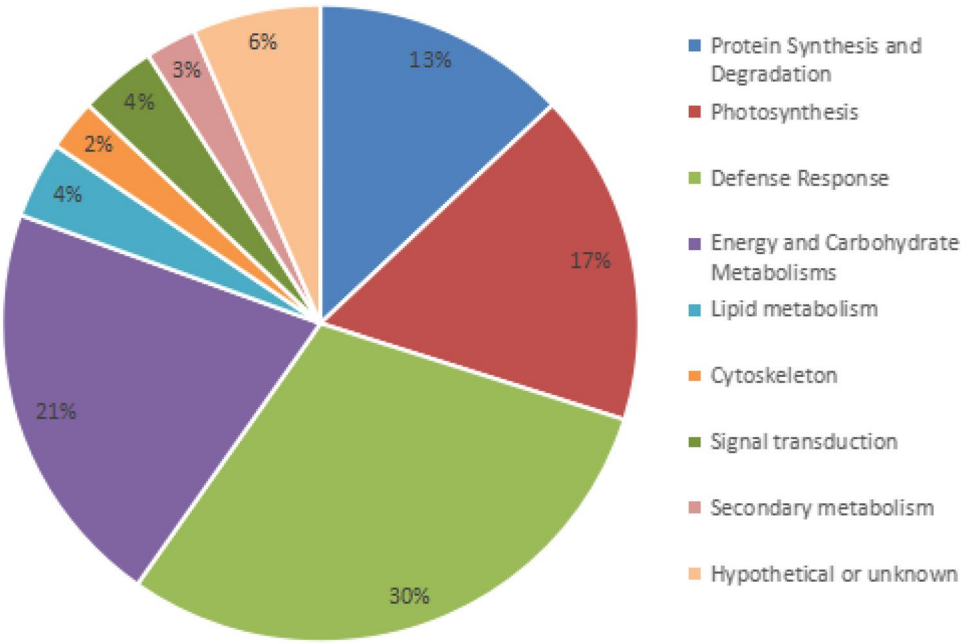


**Fig. 3** Functional categorization of DEPs

proton ATPase, beta-galactosidase, fructokinase, glucan endo-1,3-beta-glucosidase.

## Discussion

A great deal research has done in the area of differential proteomics of plant responses to salt stress. More salt stress DEPs of plant have been identified, which has laid a foundation for revealing banana responses to salt stress. However, little study is carried out to investigate banana proteomics under salt stress. In view of this, this paper has used the iTRAQ-based proteomic analysis to analyze DEPs under salt stress of banana leaves.

### Defense response

Under salt stress condition, plants produce a large amount of reactive oxygen species (ROS), the accumulation of which leads to plants oxidative stress. When plants are under salt stress, the clearance mechanism of ROS serves as an important part of the plant salt tolerance mechanism [28]. In this research, some antioxidant enzymes are identified involving thioredoxin (TRX), peroxidase (POD), catalase (CAT), gultathione S-transferases (GSTs) and allene oxide cyclase (AOC) (Table 1). In contrast to the down regulation of TRX, APX and GSTs, AOC, CAT and POD are up regulated under salt stress. As an antioxidant, POD enzyme overexpression in maize can increase the capacity of antioxidant [29]. Previous study shows that AOC enzyme overexpression in tomato

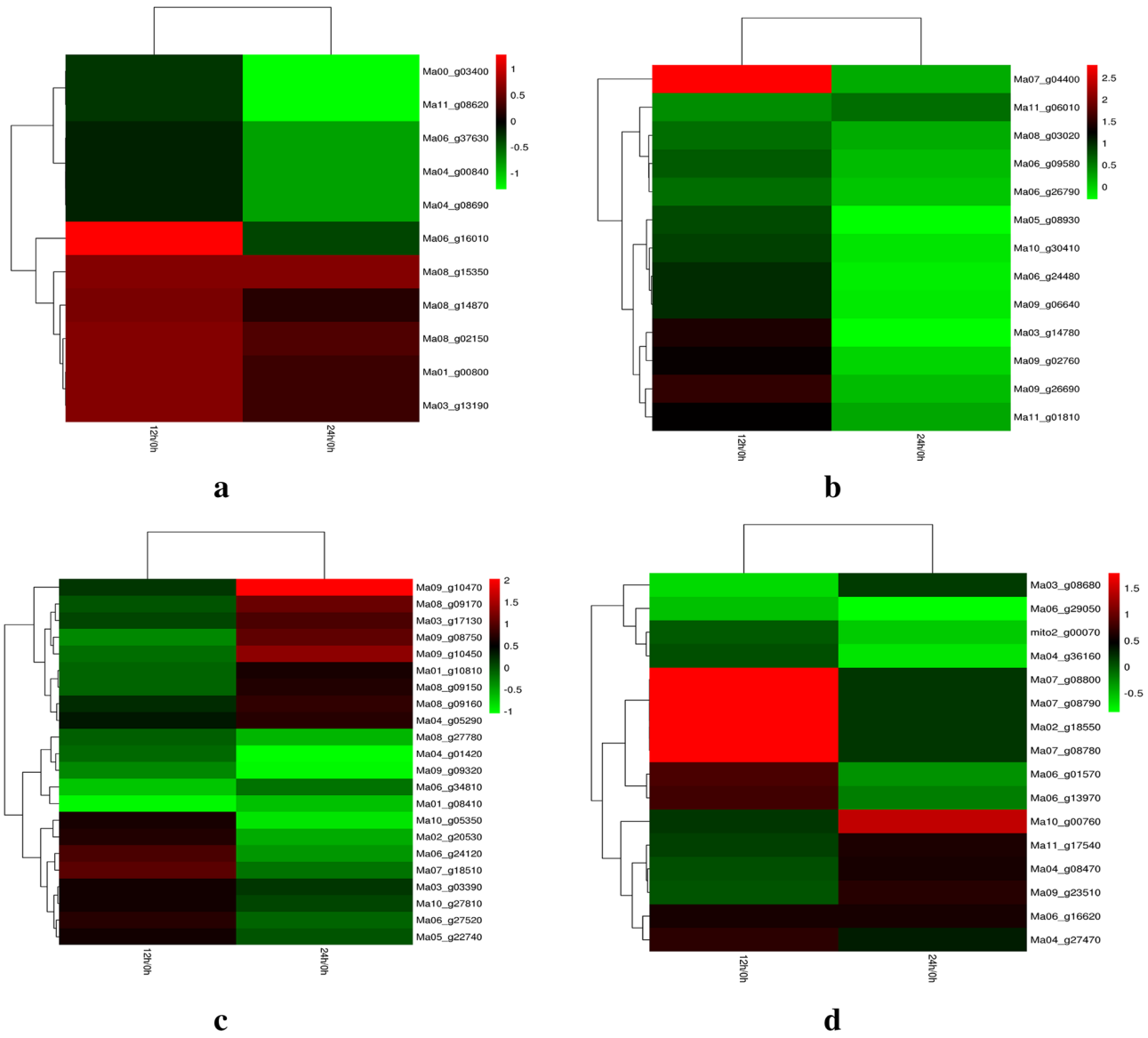


**Fig. 4** Hierarchical clustering of DEPs with similar functions under salt stress. **a** Protein synthesis and degradation-related proteins; **b** photosynthesis-related proteins; **c** defense response-related proteins; **d** energy and carbohydrate metabolism-related proteins

and *Arabidopsis* can strengthen salt tolerance [30, 31]. The result of this paper demonstrates that AOC facilitates survival of the banana under salt stress. Similarly, the enzyme overexpression of CAT equally functions as scavenging ROS. In this study, there are six peroxidase i.e. AOC and CAT that are up-regulated. This result shows that increasing the abundance of peroxidase, AOC and CAT enzymes can remove ROS and slow down salt damage. Besides, rice under hypoxia condition, the TRX acts as a negative regulator to participate in the regulation of response to salt stress [32]. There are three TRX down-regulated after 48 h of NaCl treatment, which illustrates that the antioxidant enzyme TRX is involved in the negative regulation of banana response to salt stress. Besides, germin-like protein (GLP) up-regulated is observed during salinity, GLP plays a role during embryogenesis in salt stress conditions [33]. The overexpression of GLP is reported in *Arabidopsis* and barley response to salt stress [18, 34].

Defense-related proteins are vital in the process of plant response to the salt stress [35]. Salt stress-related proteins such as polyphenol oxidase and stress-response proteins are up-regulated to tackle salt stress. These proteins are positive in salt stress responses in plants [36]. Moreover, the lectin family protein of rice is overexpression under salt stress [18, 37]. This study has revealed that seven lectins are up-regulated after 24 h of NaCl

treatment, indicating that lectin is involved in regulating the mechanism of *M. paradisiaca* response to salt stress.

Programmed cell death (PCD) is a crucial element of plant development and defense mechanisms [38]. PCD is caused by sequential activation of the CPs known as caspases, and the inactive precursors of caspases is induced by the release of electron carrier protein cytochrome c [39]. The results find that a meta caspase protein is up-regulated after 24 h of NaCl treatment. This may suggest that PCD is involved in *M. paradisiaca* response to salt stress.

In summary, the defense response of *M. paradisiaca* under salt stress condition is complex and involves antioxidant systems, some stress-related proteins and PCD. These proteins collaborate and maintain the redox homeostasis.

## Protein synthesis and degradation

Protein synthesis machinery is indispensible in salt stress adaptation [40]. RPs play important roles in synthesis proteins under salt stress. From the iTRAQ data, we have discovered that two 60S RPs L3 and three 40S RPs S30 are down-regulated under salt (Table 1). Previous studies show that the RP is down-regulated in *Arabidopsis thaliana* [18] and maize under salt stress [41]. This explains that *M. paradisiaca* responding to salt stress is through reducing irrelevant protein synthesis and better reducing salt harm. In addition, previous research shows that increasing the abundance of CP can enhance *Arabidopsis* tolerance to salt stress [42]. There is one CP up-regulated and this explains that CP may play an important role in regulating *M. paradisiaca* response to salt stress.

Misfolded proteins may accumulate in plant cells under salt stress conditions [26]. Plants can employ two strategies to deal with abiotic stress, one is to remove and the other is to refold [43]. Disulfide-isomerases is vital in folding and proper formation of disulfide bonds in protein folding [44]. It is discovered that the disulfide-isomerases is up-regulated after salt treatment. Moreover, some chaperones indispensible in repairing the potential damage caused by misfolding of proteins [45]. Many newly synthesized proteins can fold without chaperones, but it is a must for some of them. Chaperone protein is up-regulated in this study, indicating that protection of proteins by the chaperone in *M. paradisiaca* is very important to avoid misfolding of proteins under salt stress. Meanwhile, glycine cleavage system removing the misfolded and denatured proteins is up-regulated. This result suggests that *M. paradisiaca* reduces the production of proteins to avoid misfolding, and increases some enzymes to remove the misfolded and denatured proteins under salt stress.

## Cytoskeleton

In the plant cells, cytoskeleton is crucial in mediating intracellular signaling and controlling cell shape. And it can undergo profound changes when under salt stress [46]. Tubulin and actin dynamics have important functions in cellular homeostasis [18]. Actin has decreased in abundance of *Arabidopsis* under salt stress [47]. It is found that one actin protein is down-regulated following NaCl treatment. This observation is consistent with previously reported result. Moreover, tubulin plays an essential role in cell division and movement. In this study, two tubulin beta chain proteins are up-regulated after 12 h of NaCl treatment (Table 1). This concludes that the up-regulation of the tubulin beta chain in response to salt stress indicates that it has a function in *M. paradisiaca* cellular homeostasis.

## Energy and carbohydrate metabolism

Energy provision is necessary for plants to survive under salt stress [48]. Plants need to regulate different processes, such as scavenging ROS and synthesis osmolytes to reduce damage under salt stress. Glycolysis is the metabolic pathway that oxidizes glucose to generate ATP [49]. Glyceraldehyde-3-phosphate dehydrogenase and enolase of glycolysis related proteins are up-regulated. Glyceraldehyde-3-phosphate dehydrogenase is an important enzyme in glycolysis and it has been confirmed that it is involved in plant response to salt stress [50]. Moreover, fructokinase is the key enzyme in the gluconeogenesis pathway; fructokinase can catalyze the phosphorylation of fructose to form the 6-phosphate fructose, which is an important substrate for glucose metabolism, including the synthesis of starch and the degradation of sugars and the route of pentose metabolism [51]. In this study, there are two fructokinases that are up regulated after 12 h of NaCl treatment. This indicates that under short-time salt stress fructosekinase can catalyzes glucose metabolism to keep itself functioning. Apart from the above mentioned enzymes, ATP synthase, galactinol synthase galactinol synthase, 4-alpha-glucanotransferase and ADH-dehydrogenase are inhibited by salt stress. Furthermore, other proteins including V-type proton ATPase, beta-galactosidase and glucan endo-1,3-beta-glucosidase are up-regulated (Table 1). These proteins are the main members in carbohydrate and energy metabolism. From iTRAQ data, we find that proteins with different abundance profiles are identified. These results show that the leaves of *M. paradisiaca* require high energy levels to repair damage under salt stress.

## Photosynthesis

Photosynthesis is one of primary processes that are affected by environmental stresses such as salinity and drought, etc.

[52]. Thirteen proteins including a Rubisco, an oxygen-evolving enhancer 2, a ribose-5-phosphate isomerase, three chlorophyll a/b binding proteins, a glutamate-1-semialdehyde 2, 1-aminomutase, an uroporphyrinogen decarboxylase chloroplast precursor, four protochlorophyllide reductase chloroplast precursors and a ferredoxin show significant accumulation in response to salt stress in *M. paradisiaca* (Table 1).

The key enzyme of the Calvin cycle is Rubisco. The increased activating enzyme of Rubisco can increase the amount of Rubisco activity and the efficiency of photosynthesis [53]. It is noted that Rubisco is up-regulated when *M. paradisiacal* is under salt stress. But the abundance of Rubisco is decreased in *Arabidopsis* [26], while it is up-regulated in rice after salt stress [54]. The results mentioned above are consistent with the results of this study, which indicates that the abundance of Rubisco enzymes is significantly different after salt stress and which also illustrates that *M. paradisiaca* salt tolerance regulation mechanism is complex.

Chloroplast chlorophyll a/b binding protein is a member of light-harvesting complex protein family. It shows that the abundance of Chloroplast chlorophyll a/b binding protein is increased under salt stress and is most adaptable to salinity. Overexpression in oxygen-evolving enhancer protein 2 (OEE2) is observed during salinity [33]. OEE2 is important for $O_2$ evolution and photosystem II (PSII) stability [55]. OEE2 is also reportedly up-regulated in tobacco in response to biotic stress [56]. This paper has discovered that PSII OEE2 is up-regulated in response to salt stress, the result is consistent with the previous result.

Ribose-5-phosphate isomerases are involved in the Calvin cycle [57], including uroporphyrinogen decarboxylase, glutamate-1-semialdehyde 2,1-aminomutase and protochlorophyllide reductase chloroplast precursor which are increased under salt stress. Ferredoxin has increased as well. The overexpression of these proteins further suggests that salt stress promotes photosynthesis in *M. paradisiaca*. Based on the expression of proteins related to photosynthesis, it concludes that through increasing photosynthesis of *M. paradisiaca* under salt stress, damage is limited and can be repairable.

### Signal transduction

Plants respond to the abiotic stress by modifying complex signaling networks, which help them adapt to stress and consolidate their growth and development accordingly [58]. Calreticulin is an important calcium-binding protein with chaperone functions and regulates calcium homeostasis [59]. Previous studies show that calreticulin is down-regulated in rice under osmotic stress [60], but its up-regulation is correlated with the inhibition of the seedling growth [61]. Besides, under the stress of salt and cold, the regulating signaling pathways of calreticulin has a similarity [62]. In this study, there are two calreticulins that are up-regulated after 12 h of NaCl treatment. Phospholipase (PL) is indispensible to plant growth, development and environmental factors [63]. There is one PL up-regulated. It can be speculated that PL regulation *M. paradisiaca* respond to salt stress.

### Lipid metabolism

Lipids are important membrane components and linked to many cellular functions, such as storage for energy generation and membrane synthesis [64]. PL is involved in lipid metabolism and it is up-regulated in *Arabidopsis* to recover from the salt stress [26]. Study shows that PL is in abundance after 24 h of NaCl treatment. It indicates that PL is important for *M. paradisiaca* to recover from the salt stress. Acyl-CoA binding protein participates in fatty acid beta-oxidation and is up-regulated under salt stress. This expression reflects that when coping with salt stress *M. paradisiaca* can use lipids as an energy source.

### Secondary metabolism

Secondary metabolites of the plants often refer to compounds that have no fundamental part in the maintenance of life processes, but are vital for interaction with the environment for defense and adaptation [65]. Relative expression of linoleate 9S-lipoxygenase-4 is responsible for regio- and stereo- specific dioxygenation of the polyunsaturated fatty acids [66] and is up regulated under salt stress. This result suggests that the high rate of hydroperoxidation of lipids contains a *cis, cis*-1,4-pentadiene structure. This paper shows that the linoleate 9S-lipoxygenase-4 is in abundance after 24 h NaCl treatment. Previous study shows that 1-aminocyclo-propane-1-carboxylate synthase (ACC synthase) is the rate-limiting enzyme of ethylene biosynthesis in higher plants, which is down regulated under the salt stress [67]. We have discovered that the ACC synthase is down-regulated by NaCl treatment. Therefore, it can be explained that this enzyme plays an important role in salt stress.

## Conclusion

A significant number of salt stress responsive proteins is identified from the *M. paradisiaca* via iTRAQ. The expression of these proteins shows that there is a clear response to salt stress in *M. paradisiaca* (Fig. 5).

Under salt stress, photosynthesis, protein synthesis and degradation, lipid metabolism and secondary metabolism are promoted to limit damage to a repairable level. ROS accumulates under salt stress, which is harmful to cells

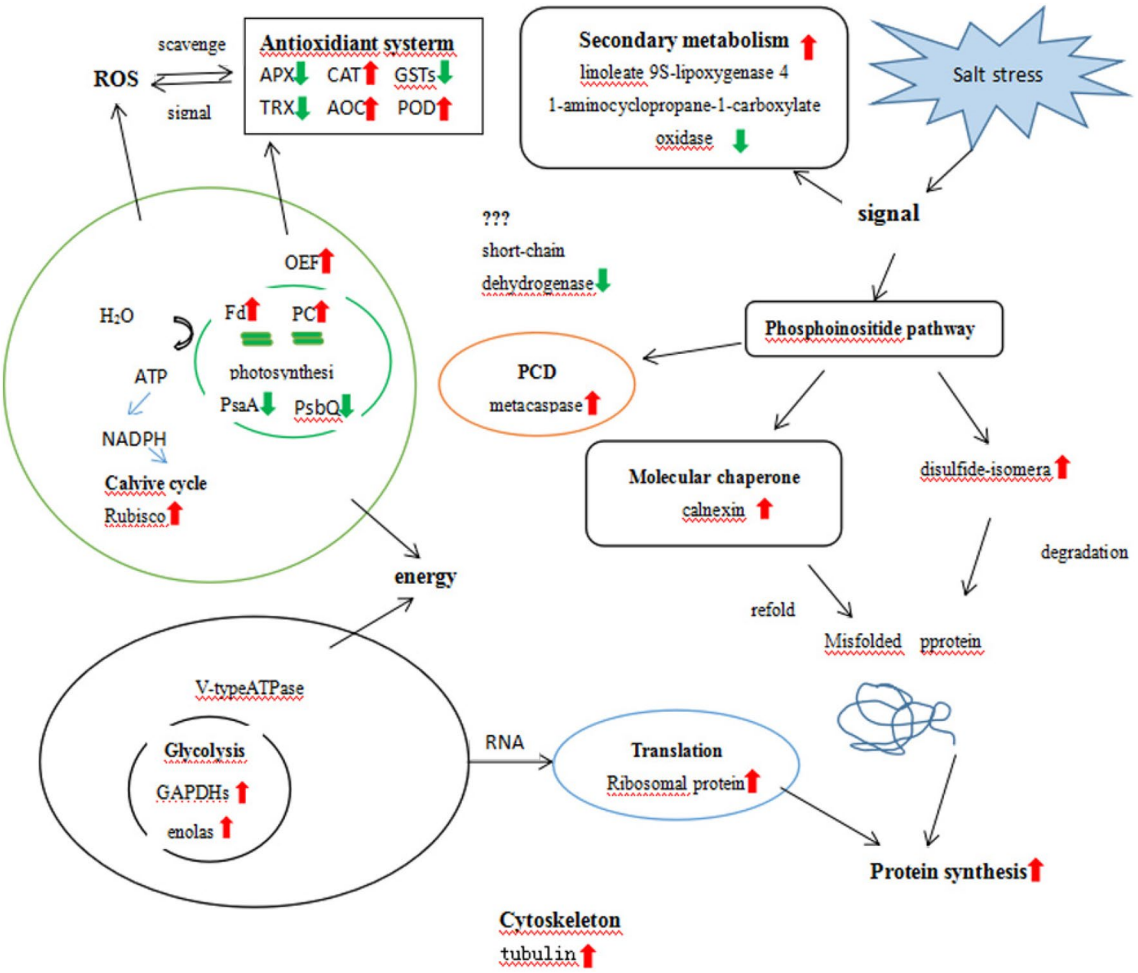


**Fig. 5** Cell diagram of *M. paradisiaca* mechanisms involved in salt stress tolerance. Down-regulated proteins are indicated by green arrow, whereas up-regulated proteins are indicated by red arrow, hypothetical or unknown proteins are indicated by ???

and leads to the up-regulation of antioxidant systems. This indicates that some cells are injured by salt stress and PCD aims to remove them. In addition, cytoskeleton can maintain cellular and redox homeostasis. Proteins with changed ratios of abundance belong to different functional categories and this demonstrates that *M. paradisiaca* has differential mechanisms to respond to salinity.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

**Research involving human and animal participants** This article does not contain any studies conducted on human or animal subjects.

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