



Comparative leaf proteomic profiling of salt-treated natural variants of *Imperata cylindrica*

Yun-Jhih SHIH^{1,5}, Hui-Chun CHANG^{1,5}, Min-Chieh TSAI^{1,5}, Ting-Ying WU², Tai-Chung WU³, Ping KAO², Wen-Yuan KAO³, Ing-Feng CHANG^{1,2,4*}

1. Institute of Plant Biology, National Taiwan University, Taipei, Taiwan.

2. Department of Life Science, National Taiwan University, Taipei, Taiwan.

3. Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei, Taiwan.

4. Genome and Systems Biology Degree Program, National Taiwan University and Academia Sinica, Taipei, Taiwan.

5. These people contributed equally to this work.

*Corresponding author's phone number: +886-2-33662534; Fax number: +886-2-23918940; E-mail: ifchang@ntu.edu.tw

(Manuscript received 1 February 2018; accepted 1 June 2018; online published 13 June 2018)

ABSTRACT: Cogon grass (*Imperata cylindrica* (L.) Beauv. var. *major* (Nees) Hubb.) is one of the top-ten weeds worldwide. It is also a C₄ medicinal plant. In particular, an ecotype from Chuwei (CW) mangrove forest was found to be salt tolerant. Comparative proteomic analysis using two-dimensional (2D)-difference in gel electrophoresis coupled with liquid chromatography-mass spectrometry (LC-MS) was carried out to identify responsive leaf proteins in the CW ecotype and salt-intolerant Sarlun (SL) population following three days of 150 mM sodium chloride salt stress treatment. We identified five photosynthesis proteins including Rubisco small subunit, uncharacterized protein LOC100194054, Cyt b6-f, oxygen-evolving enhancer 2, and photosystem I reaction center subunit IV which were significantly up- or down-regulated by salt stress in CW ecotype but not SL population. Gene ontology enrichment analysis showed that photosynthesis was over-represented. The mass spectrometry proteomics data were deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD008482. Taken together, our proteomic study identified differentially accumulated proteins which provide additional evidence of ecophysiological variation in two natural variants of *I. cylindrica*.

KEY WORDS: 2D-DIGE, C₄ Plant, Cogon grass, *Imperata cylindrica* var. *major*, Photosynthesis, Salt stress.

INTRODUCTION

Abiotic stresses that include salt, heat, cold, and flooding all limit plant growth and reduce crop yields. Among these, salt stress affects plants the most because of two affective factors, osmotic stress and ion toxicity. Plants resolve the challenge of salt stress in different ways. One strategy is salt exclusion by reducing salt content in the cytosol through sodium proton antiporters. For example, Salt Overly Sensitive 1 (SOS1) transports sodium ions out of cells by the regulation of SOS2 and SOS3 (Zhu, 2003). Another strategy is to adjust osmotic potential by accumulation of compatible solutes *i.e.* proline (Slama *et al.*, 2015). Plants also activate kinase-mediated signal transduction pathways in response to salt stress. This usually accompanies downstream transcriptional activation of a series of stress-related genes (Shinozaki and Yamaguchi-Shinozaki, 2007). Moreover, antioxidants may play important roles in reactive oxygen species (ROS) homeostasis in halophytes (Bose *et al.*, 2014).

Photosynthesis in C₄ plants differs greatly from that in C₃ plants in that the C₄ pathway (C₄ cycle) is also involved in photosynthesis. The C₄ pathway includes key enzymes phosphoenolpyruvate carboxylase (PEPC),

malic enzyme, malate dehydrogenase, and pyruvate phosphate dikinase (PPDK) which increase the efficiency of photosynthesis in C₄ plants (Sage *et al.*, 2012). A possible correlation between salt tolerance and the C₄ pathway was proposed (Bromham and Bennett, 2014); however, how C₄ plants respond to salt stress is largely unknown. Studies have utilized a transcriptomic approach to identify salt-responsive genes and proteins in C₄ plants. For example, expressions of genes in response to salt stress changes in maize (*Zea mays*) kernels were profiled (Andjelkovic and Thompson, 2006). A late embryogenesis abundant (LEA) protein was up-regulated in response to dehydration. A transcriptomic study in *Sorghum bicolor* in response to dehydration, high salinity, and abscisic acid was carried out (Buchanan *et al.*, 2005). They found clusters of genes with sequence similarity to dehydrins or LEA proteins were up regulated in response to abscisic acid (ABA), NaCl or polyethylene glycol (PEG). Expression of the gene for glycine-rich RNA binding protein is regulated by salinity in *Sorghum bicolor* (Aneeta *et al.*, 2002).

In addition to studies on transcriptional regulation, many studies have used proteomic approach and identified proteins responsive to salt stress in C₄ plants (Zhao *et al.*, 2013a, 2013b). In maize, salt-responsive



proteins were identified using proteomic tools (Zörb *et al.*, 2009, 2010). These include proteins involved in primary metabolism, energy, protein synthesis, secondary metabolism, disease/defence, and signal transduction. In *Sorghum bicolor* seedlings, a proteomic study found salt-responsive proteins (Ngara *et al.*, 2012). In a C₄ dicot, *Amaranthus cruentus*, differentially accumulated proteins were identified in a proteomic study (Joaquin-Ramos *et al.*, 2014). They found that *A. cruentus* var. *Amaranteca* could be better classified as a C₃-C₄ photosynthetic plant. A sensitive gel-based technique, 2D-difference in-gel electrophoresis (2D-DIGE), is based on labeling of two biologically different samples by Cy3 and Cy5 dyes individually, followed by combination of the samples for 2D-polyacrylamide gel electrophoresis (PAGE) analysis (Arruda *et al.*, 2011; Nanjo *et al.*, 2011). The differentially detected fluorescence of proteins indicates protein abundance in the two independent samples. The 2D-DIGE has been utilized in quantitative analysis of proteomes of salt-stressed plants, including C₃ plants - *Arabidopsis* (*Arabidopsis thaliana*) (Ndimba *et al.*, 2005), rice (*Oryza sativa*) (Song *et al.*, 2011), and wheat (*Triticum aestivum*) (Gao *et al.*, 2011), but not in C₄ plants.

Imperata cylindrica is a top-ten weed worldwide and a C₄ plant (Holm *et al.*, 1977). It is widely used as a medicinal plant in Asia (Matsunaga *et al.*, 1994; Kumar *et al.*, 2011). The medical uses of *I. cylindrica* relies on its rhizomes (Sripanidkulchai *et al.*, 2001; Yoon *et al.*, 2006). *Imperata cylindrica* (L.) Beauv. var. *major* (Nees) Hubb. is widely distributed in Taiwan (Hsu, 1975) and also grows in the Chuwei (CW) mangrove salt marsh wetland. The leaf structure of *I. cylindrica* from CW examined by scanning electron microscopy (SEM) differed from other populations. The stele was empty and the surface of the lower stem was covered with epicuticular wax instead of trichomes (Cheng and Chou, 1997a). The polymorphism among populations was also analyzed by random amplified polymorphic DNA (RAPD) (Cheng and Chou, 1997b) and restriction fragment length polymorphism (RFLP) (Chou and Tsai, 1990) on ribosomal DNA (rDNA) (Chiang *et al.*, 1998; Tsai and Chou, 1999). The CW population was therefore identified as a unique ecotype of *I. cylindrica* in Taiwan based on molecular classification. Moreover, the CW ecotype was shown to be salt and flood-tolerant in a hydroponic system (Chang and Chou, 2006). For unknown reasons, the population size of the CW ecotype was found to be decreasing (Kao *et al.*, 2011). A proteomic approach showed that three proteins were differentially expressed in CW and a salt-intolerant Sarlun (SL) population: enolase, chloroplast ferredoxin-NADP(H) oxidoreductase, and mitochondrial malate dehydrogenase (Chang, 2008). However, the salt tolerance mechanism of the CW ecotype is unknown.

To date, proteomic studies of salt stress response in C₄ plants are limited. To investigate salt-responsive proteins in the salt-tolerant CW ecotype, proteomic analyses on salt-treated *I. cylindrica* were carried out in the present study. The CW ecotype and salt-intolerant SL population were used as plant materials for comparison. By use of 2D-DIGE, we identified several salt-responsive proteins. We found that the identified proteins are highly represented in the photosynthesis pathway.

MATERIALS AND METHODS

The study area: Sampling site and plant materials for salt stress treatment

Imperata cylindrica (L.) Beauv. var. *major* (Nees) Hubb, cogon grass, was used as plant materials in this study. The CW mangrove salt-marsh wetland in Taipei, Taiwan, was selected as the first sampling site. *Imperata cylindrica* collected from SL sandy beach in Taipei was used as a control. Plants collected from the field were grown in pots in a greenhouse. For hydroponic culture, plants were transplanted to Kimura's culture solution (Chang and Chou, 2006) aerated with an air pump for acclimation for 2 weeks. The culture solution was refreshed every week for hydroponic culture. After 2 weeks, salt treatment was conducted with fresh culture solution containing 150 mM sodium chloride (NaCl) for three days. As a control, no NaCl was added. Leaf tissues were harvested for further proteomic analyses. Four biological replicates (R1–R4) were prepared and analyzed for the CW ecotype and the SL population (Fig. 1).

Extraction of proteins

Proteins were extracted based on a phenol-based method as described by Torabi *et al.* (2009). One gram of leaf sample of *I. cylindrica* was ground to fine powder in liquid nitrogen with a mortar and pestle. The powder was resuspended directly in 2.5 mL of Tris pH 8.8 buffered phenol and an equal volume of extraction buffer containing 0.1 M Tris-HCl pH 8.8, 10 mM EDTA, 0.4% 2-mercaptoethanol, and 0.9 M sucrose. The homogenate was mixed for 30 min at 4°C and centrifuged afterwards at 5000 g at 4°C for 15 min. After removal of the phenol phase, proteins were precipitated with five volumes of ice-cold 0.1 M ammonium acetate in 100% methanol at –20°C overnight. The homogenate was centrifuged at 5000 g at 4°C for 10 min. The protein pellet was transferred into a 1.5-mL microfuge tube after washing twice in 5 mL of ice-cold 0.1 M ammonium acetate in 100% methanol. The protein pellet was further washed twice in 1 mL of 80% ice-cold acetone with 10 mM DTT and washed in 1 mL of 70% ethanol. Finally, the pellet was air-dried in a fume hood.

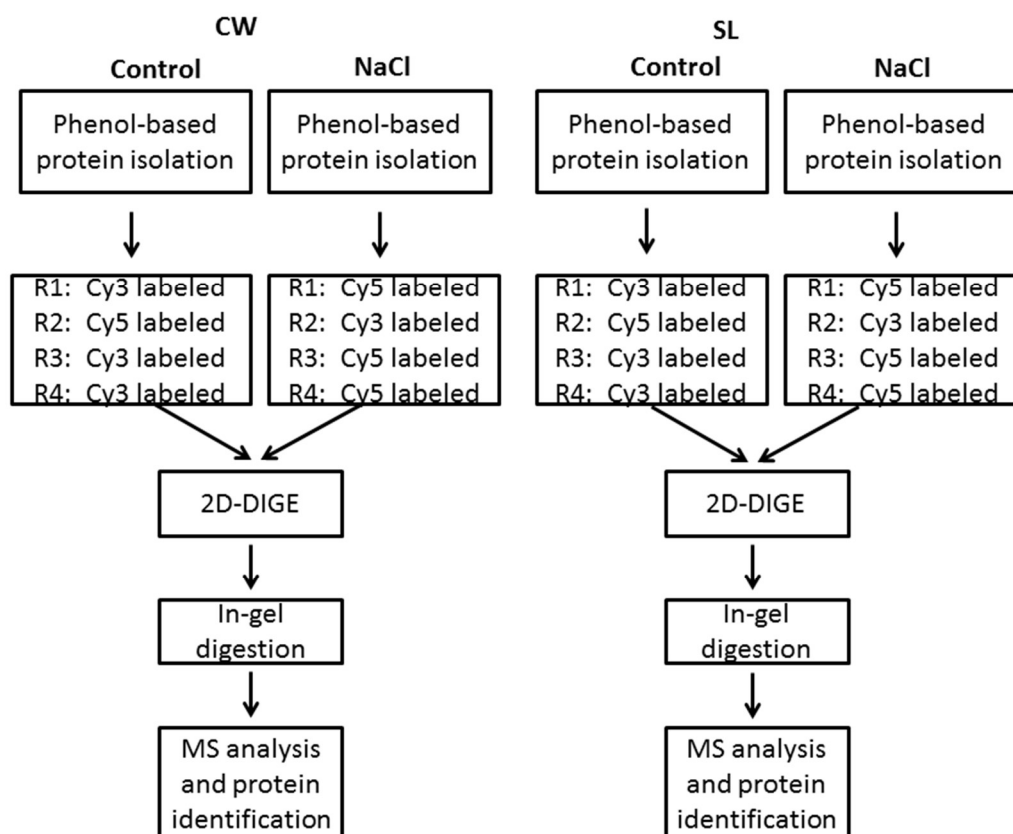


Fig. 1. Workflow of 2D-DIGE analysis of salt-stressed *Imperata cylindrica* leaf proteome.

Proteins labeling with cyanine dyes

A 1-mM stock of each CyDyes™ dye (GE Healthcare, USA) was diluted with anhydrous dimethylformamide to a working dye solution (400 pmol/μL) just prior to the labeling reaction. The extracted proteins were dissolved in lysis buffer containing 30 mM Tris pH 8.5, 7 M urea, 2 M thiourea, and 4% (w/v) CHAPS. Approximately 50 μg of proteins were mixed with 1 μL of Cy2, Cy3, or Cy5 (0.4 mM), vortexed, and incubated on ice for 30 min in darkness. The reactions were quenched by addition of 1 μL of 10 mM lysine, vortexed, and incubated on ice for 10 min in darkness.

Two-dimensional gel electrophoresis (2-DE)

After the protein samples were labeled by CyDye, an equal volume of 2× sample buffer containing 8 M urea, 130 mM DTT, 4% (w/v) CHAPS, and 2% (v/v) Pharmalyte™ 3-10 was added and left on ice for 10 min. Three prepared protein samples were combined. The volume of the combined labeled sample was adjusted to 250 μL with standard rehydration buffer containing 8 M urea, 4% (w/v) CHAPS, and 1% (v/v) Pharmalyte™ 3-10. For the first dimension, the adjusted labeled sample was subjected to isoelectric focusing (IEF) using a 13-cm IPG strip (pH 3–10 linear) in the IPGphor™ 3 system (GE Healthcare) with a total of 12,040 V h voltage-hours

applied. For the second dimension, the strip was equilibrated in the sodium dodecyl sulfate (SDS) equilibration buffer and resolved by 15% SDS-PAGE.

2D-DIGE imaging analysis

The Cy2-, Cy3-, and Cy5-labeled protein images were scanned by fluorescence scanner (Typhoon Trio+, GE Healthcare) using filters specific for each dye's excitation and emission wavelength. The excitation wavelengths were Cy2: 488 nm, Cy3: 532 nm, and Cy5: 633 nm; and corresponding emission wavelengths were 520 ± 20 , 580 ± 15 , and 670 ± 15 nm. The gel images were exported (in 16-bit tagged image file TIFF format) for analysis. The gel images were submitted to REDFIN 2D Gel Image Analysis server (Ludesi, Malmo, Sweden, www.ludesi.com) for spot detection, matching, and analysis.

Gel-based mass spectrometry (MS) sample preparation

Protein spots excised from the stained 2D gels were subjected to in-gel digestion using MS-grade Trypsin Gold (Promega, Madison, WI, USA) overnight at 37°C. Tryptic digested fragments were extracted using 10 μL of Milli-Q water initially, followed by two extractions with a total of 20 μL of solution containing 50% acetonitrile/0.1% trifluoroacetic acid. The combined extracts were dried in a vacuum concentrator at room temperature.



MS analysis and protein identification

For protein spots excised from 2D gels, the ESI-MS/MS mass spectrometers utilized for peptide analysis were a Thermo LTQ-Velos or LTQ-Orbitrap (Thermo Scientific, USA) in Taiwan. Peptide samples prepared from the CW ecotype were analyzed by LTQ-Velos. The tryptic peptide mixtures were injected into a nano-flow high-performance liquid chromatography system (Agilent Technologies 1200 series, Waldbronn, Germany) coupled to an LTQ-Velos mass spectrometer (Thermo Electron, Thermo Scientific, Waltham, MA, USA). The tryptic peptides were separated on an Thermo C18 column (100 × 0.075 mm, 2.1 μm particle size) with mobile phases of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 0.5 μL/min using a 30-min linear gradient of 5–35% solvent B. Peptide samples prepared from the SL population were analyzed by LTQ-Orbitrap. The tryptic peptide mixtures were injected into a nano-flow high-performance liquid chromatography system (Agilent Technologies 1200 series) coupled to an LTQ-Orbitrap Discovery™ hybrid mass spectrometer with a nanoelectrospray ionization source (Thermo Electron). The tryptic peptides were processed as previous described separated on an Agilent C18 column (100 × 0.075 mm, 3.5 μm particle size) with mobile phases of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 0.5 μL/min using a 30-min linear gradient of 5% to 35% solvent B. On both mass spectrometers, following each full scan (m/z range of 200–2000), a data-dependent acquired MS/MS scan for a series of precursor ions was selected on the basis of the conventional MS spectra (Survey Scan) triggered at high resolution (M/DM, 60,000 full-width half-maximum). The former acquired the spectrum (CID or MS/MS spectra) for the fragment ions generated by CID, whereas the latter examined the accurate mass and the charge state of the selected precursor ion.

The MS/MS fragmentation pattern was analyzed using the MASCOT v2.5 search engine (www.matrixscience.com) (Perkins *et al.*, 1999). The search parameters were defined as follows: Database, NCBI nr 20150912; Taxonomy, Viridiplantae (Green Plants); Enzyme, Trypsin; Variable modifications, phosphorylation; Peptide MS tolerance, ± 0.6 Da; Fragment MS tolerance, ± 0.6 Da and allowance of one missed cleavage site. Alternatively, CW- and SL-specific local databases (created by RNA-seq, data not shown) were also searched. Peptides were validated using Scaffold software (Proteome Software, USA). The mass spectrometry proteomics data were deposited in the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD008482.

Chlorophyll fluorescence measurement

To compare efficiency of excitation capture by open

photosystem II (the effective quantum yield of PSII or Y), we conducted chlorophyll fluorescence measurement with a portable, pulsed amplified modulation fluorometer (PAM-2000, Walz, Effeltrich, Germany) as described by Wu and Kao (2011). Before the measurement was taken, the plants were dark-adapted for 30 min. The Y was determined under steady-state illumination for 10 min: $Y = (F_m' - F_t)/F_m'$, where F_m' is maximal fluorescence and F_t is steady-state fluorescence of illuminated leaf.

RESULTS AND DISCUSSION

Five photosynthesis proteins were significantly up or down-regulated by salt stress in leaves of salt-treated CW ecotype, but not in SL population

A previous study showed that the CW ecotype was salt tolerant. In a hydroponic system, the CW ecotype survived but the SL population did not under 1% and 2% NaCl salt stress (Chang and Chou, 2006). Use of 1D Native gel-based analysis we identified three differentially expressed proteins among different *I. cylindrica* populations (Chang, 2008). However, the number of identified proteins remains limited. To systematically and quantitatively investigate the salt-responsive proteins, 2D-DIGE was introduced. We carried out salt treatment on the CW ecotype in 150 mM NaCl hydroponic culture for 3 days. Total proteins from the CW ecotype were isolated by phenol-based method. Proteins from control and salt-treated groups were labeled with either Cy3 or Cy5 dye. The mixture of the combined protein sample was subjected to 2DE followed by laser imaging. After analyses of gel images using REDFIN (Ludesi) software, fold change of protein spot intensity was calculated. By measuring Cy3-labeled protein spot intensity, Cy5-labeled protein spot intensity and each normalized to Cy2-labeled protein spot intensity, we further took Cy5/Cy3 ratio as fold change (DIGE ratio). Protein spots with differential abundance were excised from the gel followed by in-gel digestion and liquid chromatography tandem mass spectrometry (LC-MS/MS) analyses (Fig. 1). A total of 26 protein spots showed differential abundance in response to salt stress (intensity fold change >1.65 or <0.60). The DIGE ratios are shown in Table S1. Of these 26 spots, 20 showed significant differential accumulation of proteins between CW and SL (ANOVA, $p < 0.05$). These spots were subjected to in-gel digestion followed by LC-MS/MS analyses. Among these protein spots, five proteins significantly up- or down-regulated by salt stress (t -test, $p < 0.05$) were successfully identified by MASCOT searching against the NCBI database (Fig. 2, Figure S1, Table 1 and 2). These included uncharacterized protein LOC100194054 (thylakoid lumenal 17.4-kDa protein, spots # 52 and 70), ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) small subunit (spot #

**Table 1.** Proteins identified in 2D-DIGE of leaf proteins isolated from salt-treated CW ecotype

Spot #	Protein name	Accession number	Experimental pI/MW	Theoretical pI/MW	Mascot score	Unique peptide #	Organism	Fold Change (p value 1, 2)
Metabolism								
178	Plastidic aspartate aminotransferase	gi 633095	6.32/42.97	8.62/50.48	1129	16	<i>Panicum miliaceum</i>	1.66 (0.07, 0.6)
Signaling								
957	General regulatory factor (14-3-3)	gi 1345588	4.70/29.53	4.75/29.64	247	3	<i>Zea mays</i>	1.86 (0.28, 0.02)
Stress								
93	Superoxide dismutase [Mn] 3.1, mitochondrial precursor	gi 134668	6.40/20.77	6.71/25.21	248	2	<i>Zea mays</i>	5.16 (0.16, 0.045)
	Superoxide dismutase [Mn] 3.4, mitochondrial precursor	gi 212722004	6.03/23.89	6.71/25.21	157	4	<i>Zea mays</i>	
Photosynthesis								
61	Ribulose-1, 5-bisphosphate carboxylase/oxygenase small subunit	gi 3914607	4.70/13.29	9.04/19.25	227	2	<i>Sorghum bicolor</i>	0.5 (0.003, 0.02)
11	Hypothetical protein SORBIDRAFT_02g002690 (OEE2)	gi 242047384	6.23/23.38	8.63/27.72	725	5	<i>Sorghum bicolor</i>	2.07 (0.03, 0.0005)
27	Hypothetical protein SORBIDRAFT_02g002690 (OEE2)	gi 242047384	5.60/23.65	8.63/27.72	529	5	<i>Sorghum bicolor</i>	2.48 (0.38, 0.34)
52	Uncharacterized protein LOC100194054	gi 212721648	4.54/17.68	7.44/24.15	1459	5	<i>Zea mays</i>	1.76 (0.003, 0.00005)
70	Uncharacterized protein LOC100194054	gi 212721648	3.78/7.18	7.44/24.15	181	3	<i>Zea mays</i>	0.51 (0.02, 0.002)
957	Chloroplast oxygen-evolving enhancer protein 1, OEE1	gi 383511664	4.70/29.53	5.59/34.78	1072	12	<i>Saccharum hybrid cultivar</i>	1.86 (0.28, 0.02)
199	Unknown (Cyt b6-f)	gi 194702912	5.30/20.50	6.41/21.03	917	6	<i>Zea mays</i>	0.59 (0.01, 0.02)
208	Unknown (Cyt b6-f)	gi 194702912	6.23/12.52	6.41/21.03	217	3	<i>Zea mays</i>	0.25 (0.005, 0.00004)
279	ATP synthase CF1 beta subunit	gi 227786	4.98/28.60	8.62/38.68	272	10	<i>Sorghum bicolor</i>	1.57 (0.55, 0.76)
470	Photosystem I reaction center subunit IV		7.01/16.36	9.82/15.45	131	3	<i>Imperata cylindrica</i>	2.03 (0.03, 0.009)
666	Photosystem I reaction center subunit VII		5.06/7.50	6.69/9.52	88	2	<i>Imperata cylindrica</i>	1.89 (0.33, 0.3)
Others								
201	Cyclophilin	gi 242079005	4.24/41.25	4.83/46.69	497	14	<i>Sorghum bicolor</i>	1.77 (0.07, 0.03)

1. The first p value: p values of T-test for most proteins with significant differential abundance between control and salt-stressed plants are shown after fold change ratio.

2. The second p value: p values of ANOVA for proteins with significant differential abundance between CW ecotype and SL population are shown after fold change ratio.

61), photosystem I reaction center subunit IV (spot # 470), Cyt b6-f (spots # 199 and 208), and oxygen-evolving enhancer 2 (OEE2) (spot # 11 and #27). The identified peptides are shown in Table S2.

In addition, we identified proteins with significant differences in DIGE ratio between CW and SL, but not significantly up- or down-regulated by salt stress in CW: Mn superoxide dismutase (Mn-SOD3.4) (spot # 93) and cyclophilin (spot # 201) (Table 1 and 3). Moreover, we identified proteins with no significant difference in DIGE ratio between CW and SL: aspartate aminotransferase (spot # 178), ATP synthase beta subunit (spot # 279), and photosystem I reaction center

subunit VII (spot # 666) (Table 1 and 4). The identified peptides are shown in Table S2. However, oxygen-evolving enhancer 1 (OEE1) and 14-3-3 were identified in the same spot (# 957), and so the DIGE ratio is unknown for each protein. Both OEE2 and thylakoid luminal 17.4-kDa protein were identified in two individual spots. However, spot # 70 appeared to be the degraded product of thylakoid luminal 17.4-kDa protein. Photosystem I reaction center subunits IV (spot # 470) and VII (spot # 666) were identified by MASCOT searching against the local database. Interestingly, most of the proteins were in chloroplasts and involved in the photosynthesis pathway.

**Table 2.** Salt-regulated proteins with significant differences between salt-treated CW ecotype and SL population.

Spot # CW, SL	Protein name	Accession Number CW, SL	Mascot Score CW, SL	Unique peptide # CW, SL	Fold Change CW, SL (p value)
Photosynthesis					
61, 56	Ribulose-1, 5-bisphosphate carboxylase/oxygenase small subunit	gi 3914607, gi 164698711	227, 75	2, 2	0.5, 1.05 (0.02)
11	Hypothetical protein SORBIDRAFT_02g002690 (OEE2)	gi 242047384	725	5	2.07 (CW) (0.0005)
52, 755	Uncharacterized protein LOC100194054	gi 212721648, gi 212721648	1459, 213	5, 3	1.76, 1.18 (0.00005)
70	Uncharacterized protein LOC100194054	gi 212721648	181	3	0.51 (CW) (0.002)
199, 74	Unknown (Cyt b6-f)	gi 194702912, gi 194702912	917, 139	6, 4	0.59, 0.93 (0.02)
208	Unknown (Cyt b6-f)	gi 194702912	217	3	0.25 (CW) (0.00004)
470, 452	Photosystem I reaction center subunit IV		131, 177	3, 4	2.03, 0.99 (0.009)

P value: P values of ANOVA for proteins with significant differential abundance between CW ecotype and SL population are shown after fold change ratio.

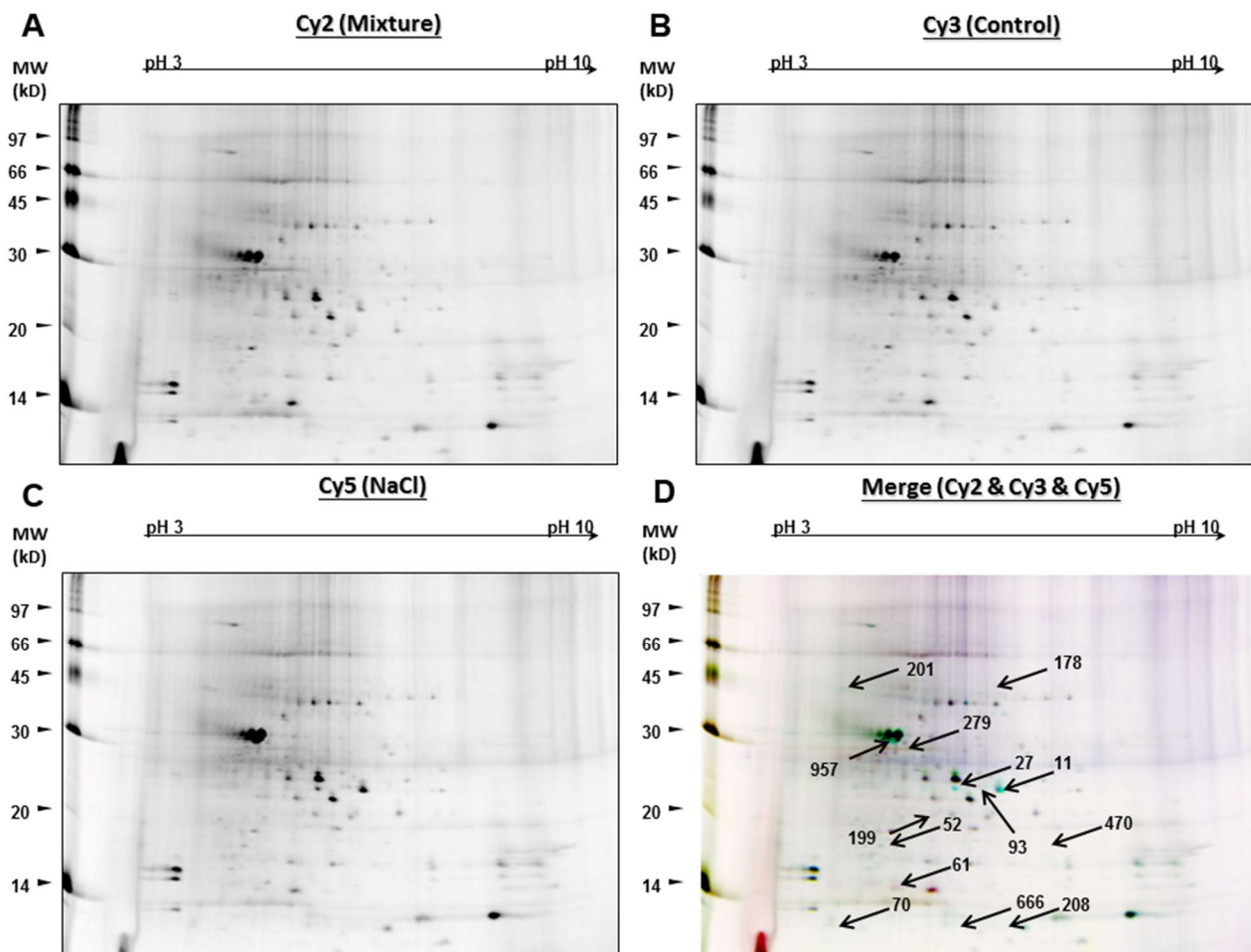


Fig. 2. Representative 2D-DIGE image of leaf proteins isolated from salt-treated CW ecotype. The gel image corresponds to one representative biological replicate. **A**, Loading control; **B**, control (no salt); **C**, salt-treated group; **D**, merged image. The image was analyzed using 2D gel image analysis software REDFIN.

**Table 3.** Proteins with significant differences between salt-treated CW ecotype and SL population but not regulated by salt stress

Spot # CW, SL	Protein name	Accession Number CW, SL	Mascot Score CW, SL	Unique peptide # CW, SL	Fold Change CW, SL (p value 1, 2)
Signaling					
957, 753	General regulatory factor (14-3-3)	gi 1345588, gi 242073380	247, 709	3, 36	1.86, 0.95 (0.02)
Metabolism					
612	Fructose-bisphosphate aldolase	gi 242059597	245	12	0.89 (SL) (0.048)
Protein degradation					
415	Proteasome beta subunit	gi 242079721	317	5	0.83 (SL) (0.045)
Stress					
91	Superoxide dismutase [Cu/Zn]	gi 1568639	104	2	1.05 (SL) (0.01)
93	Superoxide dismutase [Mn] 3.4, mitochondrial precursor	gi 212722004	157	4	5.16 (CW) (0.045)
Photosynthesis					
957, 753	Chloroplast oxygen-evolving enhancer protein 1, OEE1	gi 383511664, gi 383511664	1072, 477	12, 15	1.86, 0.95 (0.02)
470, 452	Photosystem I reaction center subunit IV		131, 177	3, 4	2.03, 0.99 (0.009)
Others					
201, 106	Cyclophilin	gi 242079005, gi 242079005	497, 304	14, 7	1.77, 0.96 (0.03)

P value: p values of ANOVA for proteins with significant differential abundance between CW ecotype and SL population are shown after fold change ratio.

Table 4. Proteins with no significant differences between salt-treated CW ecotype and SL population

Spot # CW, SL	Protein name	Mascot Score CW, SL	Unique peptide # CW, SL	Fold Change CW, SL (p value)
178, 214	Plastidic aspartate aminotransferase	1129, 507	16, 8	1.66, 0.95 (0.6)
666, 173	Photosystem I reaction center subunit VII	88, 189	2, 2	1.89, 1.71 (0.3)

P value: p values of ANOVA for proteins with significant differential abundance between CW ecotype and SL population are shown after fold change ratio.

In addition, we identified Cyt b6-f in two separate protein spots with decreased fold change (spots # 199 and 208). However, spot # 208 appeared to be the degraded product of Cyt b6-f. In an independent 2D-DIGE analysis, we identified one more isoform, Mn-SOD3.1, in CW. However, only Mn-SOD3.4 showed consistent up-regulation in CW in response to salt stress. Functional categories of the identified proteins were metabolism, signaling, stress, photosynthesis and others. The identification of these proteins was validated using Scaffold software v4.3 at probability >95% (Figure S2).

With the same approach as used in analyzing the salt-treated CW leaf proteome, we also aimed to identify salt-responsive proteins in the SL population (Fig. 1). A total of 26 protein spots corresponding to spots of the same position as in the CW protein gel were detected. These spots were subjected to in-gel digestion followed by LC-MS/MS analyses. The DIGE ratios are shown in Table S3. Of these spots, 20 showed differential accumulation of proteins between populations (significant difference

between CW and SL, by ANOVA at $p < 0.05$). In these spots, eight proteins with significant difference in DIGE ratio between CW and SL were successfully identified by MASCOT searching against the NCBI database (Fig. 3, Figure S3, Table 2, 3 and 5). However, none of these proteins were significantly up- or down-regulated by salt stress (t -test, $p < 0.05$). These proteins were fructose-bisphosphate aldolase (spot # 612), copper/zinc superoxide dismutase (Cu/Zn-SOD) (spot # 91), proteasome beta subunit (spot # 415), Cyt b6-f (spot # 74), Rubisco small subunit (spot # 56), photosystem I reaction center subunit IV (spot # 452), uncharacterized protein LOC100194054 (thylakoid lumenal 17.4-kDa protein, spot # 755), and cyclophilin (spot # 106). The identified peptides are shown in Table S4.

In addition, we identified proteins with no significant difference in DIGE ratio between CW and SL: aspartate aminotransferase (spot # 214) and photosystem I reaction center subunit VII (spot # 173) (Table 4 and Table 5). OEE1 and 14-3-3 were identified in the same

**Table 5.** Proteins identified in 2D-DIGE of leaf proteins isolated from salt-treated SL population

Functional category	Spot #	Protein name	Accession number	Experimental pI/MW	Theoretical pI/MW	Mascot score	Unique peptide #	Organism	Fold Change (p value 1, 2)
Metabolism									
	214	Plastidic aspartate aminotransferase	gi 514719430	6.60/40.55	8.62/50.48	507	8	<i>Setaria italica</i>	0.95 (0.62, 0.6)
	612	Fructose-bisphosphate aldolase	gi 242059597	6.32/38.68	6.96/38.99	245	12	<i>Sorghum bicolor</i>	0.89 (0.83, 0.048)
Signaling									
	753	General regulatory factor (14-3-3)	gi 242073380	5.09/28.71	4.76/29.74	709	36	<i>Sorghum bicolor</i>	0.95 (0.97, 0.02)
Stress									
	91	Superoxide dismutase [Cu/Zn]	gi 1568639	4.96/16.24	5.35/20.42	104	2	<i>Triticum aestivum</i>	1.05 (0.85, 0.01)
Protein degradation									
	415	Proteasome beta subunit	gi 242079721	6.36/19.81	5.71/26.31	317	5	<i>Sorghum bicolor</i>	0.83 (0.88, 0.045)
Photosynthesis									
	74	Unknown (Cyt b6-f)	gi 194702912	5.50/17.07	6.41/21.03	139	4	<i>Zea mays</i>	0.93 (0.68, 0.02)
	56	Ribulose-1, 5-bisphosphate carboxylase/oxygenase small subunit	gi 164698711	4.79/13.10	8.78/19.37	75	2	<i>Miscanthus xgiganteus</i>	1.05 (0.49, 0.02)
	173	Photosystem I reaction center subunit VII	gi 7524738	6.02/11.50	6.69/9.52	189	2	<i>Pinus thunbergii</i>	1.71 (0.43, 0.3)
	452	Photosystem I reaction center subunit IV		7.01/16.36	9.82/15.45	177	4	<i>Imperata cylindrica</i>	0.99 (0.49, 0.01)
	755	Uncharacterized protein LOC100194054	gi 212721648	4.78/15.91	7.44/24.15	213	3	<i>Zea mays</i>	1.18 (0.57, 0.00)
	753	Chloroplast oxygen-evolving enhancer protein 1, OEE1	gi 383511664	5.09/28.71	6.08/34.95	477	15	<i>Saccharum hybrid cultivar</i>	0.95 (0.97, 0.02)
Others									
	106	Cyclophilin	gi 242079005	4.36/40.14	4.83/46.69	304	7	<i>Sorghum bicolor</i>	0.96 (0.46, 0.03)

1. The first p value: p values of T-test for most proteins with significant differential abundance between control and salt-stressed plants are shown after fold change ratio.

2. The second p value: p values of ANOVA for proteins with significant differential abundance between CW ecotype and SL population are shown after fold change ratio.

spot (# 753) and so the DIGE ratio is unknown for each protein. Photosystem I reaction center subunit IV (spot # 452) was identified by MASCOT searching against the local database. The identified peptides are shown in Table S4.

Interestingly, by use of the local database, more peptides were identified for 14-3-3 proteins (Table S4). In addition, two phosphopeptides of thylakoid membrane phosphoprotein 14-kDa protein (ATpSGGEGATEEVPEIVK) and a chloroplast a and b binding protein CP29 (NEPGAVIGpTRFESSDVK) were identified from the data set of our previous study (Wu *et al.*, 2015). The phosphorylation sites are evolutionarily conserved compared with other plant species (Figure S4). These indicate that the local database helped improve peptide identification for *I. cylindrica*, a non-model organism with no genome sequence. Functional categories of the identified proteins were metabolism, stress, signaling, protein

degradation, photosynthesis, and others. The identification of these proteins was validated using Scaffold software v4.3 at probability >95% (Figure S5).

Gene ontology (GO) term enrichment analysis of the identified proteins

A total of five proteins were significantly up- or down-regulated by salt stress in CW: Rubisco small subunit, uncharacterized protein LOC100194054, Cyt b6-f, oxygen-evolving enhancer 2, and photosystem I reaction center subunit IV. All five proteins were from the photosynthesis pathway; however, their DIGE ratios varied in CW and SL. In CW, three of the five proteins (with the exceptions of Cyt b6-f and Rubisco small subunit) showed increased abundance; however, in SL they all had no significant change of abundance. The GO term enrichment analysis was carried out by searching the PANTHER GO database (<http://www.geneontology.org>). The GO biological process result showed that removal

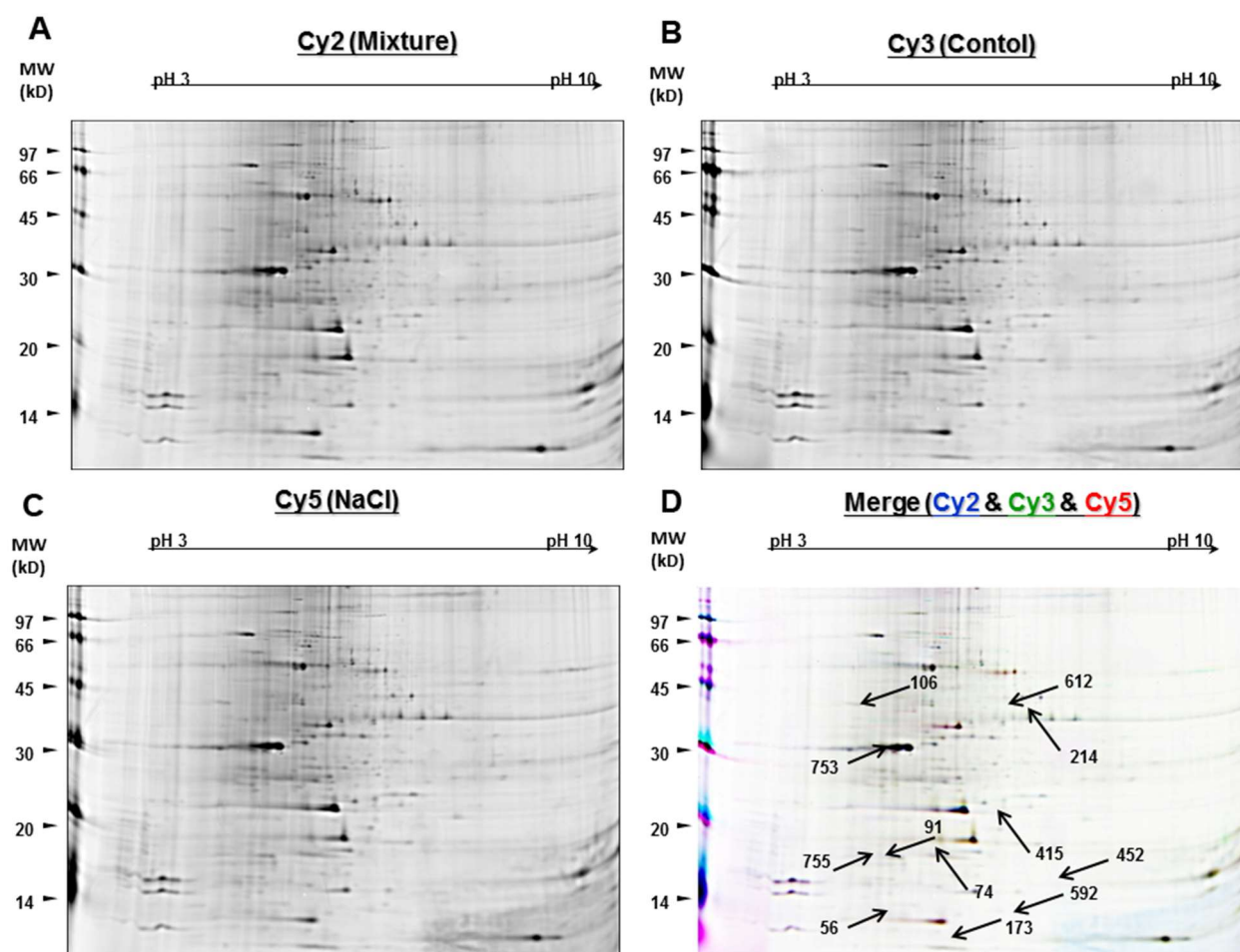


Fig. 3. Representative 2D-DIGE image of leaf proteins isolated from salt-treated SL population. The gel image corresponds to one representative biological replicate. **A**, Loading control; **B**, control (no salt); **C**, salt-treated group; **D**, merged image. The image was analyzed using 2D gel image analysis software REDFIN.

of superoxide radicals (p-value: $8.17E-03$, fold enrichment $> 100\%$) and photosynthesis were enriched (p-value: $5.76E-07$, fold enrichment $> 100\%$). The GO cellular component result showed enrichment of photosystem I (p-value: $9.53E-04$, fold enrichment $> 100\%$), thylakoid (p-value: $7.01E-05$, fold enrichment 83.43%), and thylakoid membrane (p-value: $3.99E-03$, fold enrichment 78.33%). Thus, GO term enrichment analysis showed that photosynthesis was over-represented.

Differentially accumulated photosynthesis proteins in salt stress response

Differentially accumulated of photosynthesis proteins under salt stress conditions have been reported in many plant proteomics studies (Zhang *et al.*, 2012; Kosová *et al.*, 2014; Nouri *et al.*, 2015; Silveira and Carvalho, 2016). A proteomic study showed that 20 chloroplast proteins were affected by salt stress treatment in the C_4 plant maize (Zörb *et al.*, 2009): 12 proteins increased their abundance and eight showed decreases. In our proteomic study, we identified five photosynthesis

proteins with differential accumulation patterns in response to salt stress in CW and SL (Tables 1, 2, and 5) and the GO enrichment result confirmed this. Our results support Zörb's and others findings. The differential accumulation of photosynthesis proteins may be the major difference between CW and SL. In fact, differential phosphorylation of C_4 -specific enzymes in the CW ecotype and SL population was previously reported (Wu *et al.*, 2015). Our results suggest that CW and SL may differ in the C_4 pathway in response to salt stress.

In particular in the present study, OEE2 (spots # 11 and 27) (Fig. 2) accumulated more in salt-treated plants than controls. The OEE2 is an extrinsic polypeptide in photosystem II involved in oxygen production (Seidler, 1996; Bricker *et al.*, 2012). Previous studies have shown a correlation between OEE abundance and salt stress. Abundance OEE1 or OEE2 were found to be changed in response to salt stress in *Bruguiera gymnorrhiza* (Sugihara *et al.*, 2000), maize (Zörb *et al.*, 2009), rice (Abbasi and Komatsu, 2004), cowpea (de Abreu *et al.*, 2014) tobacco, (Razavizadeh *et al.*, 2009), potato and



canola (Aghaei *et al.*, 2008; Bandehagh *et al.*, 2011), *Brachypodium* (Lv *et al.*, 2014), and *Kandelia candel* (Wang *et al.*, 2013). In mangrove species *Bruguiera gymnorrhiza*, *OEE1* transcripts were enhanced by salt stress (Sugihara *et al.*, 2000). In maize, *OEE2* abundance increased after salt exposure (Zörb *et al.*, 2009). In addition, *OEE1* and *OEE2* were found to be responsive to salt stress in rice leaf sheath (Abbasi and Komatsu, 2004). Moreover, there was increased abundance of *OEE2* in a salt-tolerant cultivar of cowpea following salt exposure (de Abreu *et al.*, 2014). In the present study, *OEE2* was up-regulated in response to salt stress based on our 2D-DIGE results (spots # 11 and 27). Overall, our results are consistent with these others. It appears that *OEE2* abundance may affect the PSII activity under salt stress in plants.

In contrast, we found that Rubisco small subunit and Cyt b6-f showed significantly decreased abundance under 150-mM salt stress. Similar results were reported by other groups. In a C_4 halophyte *Aeluropus lagopoides*, Rubisco small subunit was down-regulated by salt stress at the protein level (Sobhanian *et al.*, 2010). However, a proteomic study found Cyt b6-f was gradually up-regulated by salt stress in wheat (Kamal *et al.*, 2012).

Since most of the identified photosynthesis proteins showed increased abundance in CW, it is possible that CW had higher photosynthesis efficiency than SL under salt stress. This may help in tolerance to local salt stress in the CW mangrove forest. In fact, we found the chlorophyll fluorescence value of salt-treated *I. cylindrica* (both populations) decreased with stress but slightly recovered after acclimation (Figure S6); however, the chlorophyll fluorescence value was higher for CW than SL. Our results suggest a higher photosynthetic efficiency for CW than SL under salt stress. This phenomenon was also observed in other plant species. In *Arabidopsis* transformed with a *Lepidium crassifolium* gene conferring salt tolerance, the Fv/Fm value was less affected than in *Arabidopsis* wild type (Rigó *et al.*, 2016). Since our results showed that Rubisco protein abundance decreased in both populations with salt stress, this is highly possible due to differences in protein levels of photosystem components.

Identification of Mn-SOD

In addition to photosynthetic proteins, an antioxidant protein Mn-SOD was identified in spot # 93 (Fig. 2) in salt-treated CW leaves. Differential accumulation of ROS scavengers under salt stress has been reported in many plant proteomic studies (Zhang *et al.*, 2012; Kosová *et al.*, 2014). Abbasi and Komatsu (2004) showed that SOD increased in abundance in response to salt stress in rice leaf sheath. In *Suaeda aegyptiaca* leaves, Cu/Zn-SOD was responsive to salt stress in a proteomic study (Askari *et al.*, 2006). In pea, Cu/Zn-SOD increased in response to salt stress (Hernhdeza *et*

al., 1995). In rice, overexpression of yeast Mn-SOD and rice Cu/Zn-SOD increased salt tolerance (Tanaka *et al.*, 1999; Guan *et al.*, 2017). The increased abundance of SOD is believed to be a mechanism to deal with oxidative stress under salt stress conditions in plants. Our results concerning *OEE2* and SOD are consistent with previous results. This suggests an evolutionarily conserved mechanism for salt stress response of these proteins. However, we did not identify the proteins in the SL population. Our results suggest that Mn-SOD may be involved in salt stress tolerance of CW as a ROS scavenger. Whether enzyme activity of Mn-SOD is consistently up-regulated in the CW ecotype under salt stress is unknown and requires further study.

Identification of cyclophilin

In leaves of CW and SL, cyclophilin was identified as a protein with differential DIGE ratio. Cyclophilin is encoded by a multigene family. It was reported that cyclophilin gene expression in bean (*Phaseolus vulgaris*) is differentially regulated by salt stress (Marivet *et al.*, 1994). A proteomic study identified a rice cyclophilin OsCYP2 and its overexpression increased salt tolerance (Ruan *et al.*, 2011). Moreover, overexpression of a pigeonpea cyclophilin gene increased salt tolerance of *Arabidopsis* (Sekhar *et al.*, 2010). Our findings suggest that cyclophilin may be involved in salt stress response in the CW ecotype but with an unknown mechanism.

Possible salt tolerance mechanism of CW ecotype

In summary, we identified differentially accumulated proteins in leaves of two natural variants of *I. cylindrica* in response to salt stress using 2D-DIGE. These proteins included SODs, cyclophilin, and many photosynthesis proteins, which suggests that the CW ecotype may have higher antioxidant activity and photosynthesis efficiency, and so can deal with oxidative stress damage and energy limitation resulting from salt stress in mangrove forest. Low accumulation of sodium in leaves of the CW ecotype under salt stress was previously reported (Chang and Chou, 2006). It is highly likely that an unknown transporter is involved in avoiding salt accumulation in leaves. This would prevent salt damage to enzyme stability and activity in leaves. However, determining which transporter may be involved will require further study.

ACKNOWLEDGEMENTS

We appreciate funding support from the Ministry of Science and Technology, Taiwan (MOST# 104-2311-B-002-034, MOST# 106-2311-B-002-014, and MOST# 106-2313-B-002-004) and the National Taiwan University (Grant # 105R892002). We thank Mr. C.S. Wang and Mr. C.H. Wang (Tamsui Farm) for help with *Imperata cylindrica* sampling. Many thanks to BioMed Proofreading, LLC for English editing. We appreciate the technical support from Visual Protein,



Taipei, Taiwan. We also thank Technology Commons, College of Life Science, National Taiwan University, for technical help.

LITERATURE CITED

- Abbasi, F.M. and S. Komatsu. 2004. A proteomic approach to analyze salt-responsive proteins in rice leaf sheath. *Proteomics* **4**(7): 2072-2081.
- Aghaei, K., A.A. Ehsanpour and S. Komatsu. 2008. Proteome analysis of potato under salt stress. *J. Proteome Res.* **7**(11): 4858-4868.
- Andjelkovic, V. and R. Thompson. 2006. Changes in gene expression in maize kernel in response to water and salt stress. *Plant Cell Rep.* **25**(1): 71-79.
- Aneeta, N. Sanan-Mishra, N. Tuteja and S. Kumar Sopory. 2002. Salinity- and ABA-induced up-regulation and light-mediated modulation of mRNA encoding glycine-rich RNA-binding protein from *Sorghum bicolor*. *Biochem. Biophys. Res. Commun.* **296**(5): 1063-1068.
- Arruda, S.C., S. Barbosa Hde, R.A. Azevedo and M.A. Arruda. 2011. Two-dimensional difference gel electrophoresis applied for analytical proteomics: Fundamentals and applications to the study of plant proteomics. *Analyst* **136**(20): 4119-4126.
- Askari, H., J. Edqvist, M. Hajheidari, M. Kafi and G.H. Salekdeh. 2006. Effects of salinity levels on proteome of *Suaeda aegyptiaca* leaves. *Proteomics* **6**(8): 2542-2554.
- Bandehagh, A., G.H. Salekdeh, M. Toorchi, A. Mohammadi and S. Komatsu. 2011. Comparative proteomic analysis of canola leaves under salinity stress. *Proteomics* **11**(10): 1965-1975.
- Bose, J., A. Rodrigo-Moreno and S. Shabala. 2014. ROS homeostasis in halophytes in the context of salinity stress tolerance. *J. Exp. Bot.* **65**(5): 1241-1257.
- Bricker, T.M., J.L. Roose, R.D. Fagerlund, L.K. Frankel and J.J. Eaton-Rye. 2012. The extrinsic proteins of photosystem II. *Biochim. Biophys. Acta* **1817**(1): 121-142.
- Bromham, L. and T.H. Bennett. 2014. Salt tolerance evolves more frequently in C4 grass lineages. *J. Evol. Biol.* **27**(3): 653-659.
- Buchanan, C.D., S. Lim, R.A. Salzman, I. Kagiampakis, D.T. Morishige, B.D. Weers, R.R. Klein, L.H. Pratt, M. M. Cordonnier-Pratt, P.E. Klein and J.E. Mullet. 2005. *Sorghum bicolor*'s transcriptome response to dehydration, high salinity and ABA. *Plant Mol. Biol.* **58**(5): 699-720.
- Chang, I.F. 2008. Ecotypic variation of a medicinal plant *Imperata cylindrica* populations in Taiwan: Mass spectrometry-based proteomic evidence. *J. Med. Plants Res.* **2**: 71-76.
- Chang, I.F. and C.H. Chou. 2006. Ecotypic variation of *Imperata cylindrica* populations in Taiwan: II. Physiological and biochemical evidence. *Bot. Stud.* **47**: 175-184.
- Cheng, K.T. and C.H. Chou. 1997a. Ecotypic variation of *Imperata cylindrica* populations in Taiwan: I. Morphological and molecular evidences. *Bot. Bull. Acad. Sinica* **38**: 215-223.
- Cheng, K.T. and C.H. Chou. 1997b. Specific RAPD Markers of *Imperata cylindrica* populations in Taiwan. *J. Genet. Mol. Biol.* **8**(3): 41-54.
- Chiang, Y. C., T.Y. Chiang, I.F. Chang and C.H. Chou. 1998. Sequence announcement: rDNA IGS of *Imperata cylindrica* (L.) Beauv. Var. *major* (Ness) C E Hubb. *Plant Mol. Biol.* **39**: 391-392.
- de Abreu, C.E., S. Araújo Gdos A.C. Monteiro-Moreira, J. H. Costa, B. Leite Hde, F. B. Moreno, J.T. Prisco and E. Gomes-Filho. 2014. Proteomic analysis of salt stress and recovery in leaves of *Vigna unguiculate* cultivars differing in salt tolerance. *Plant Cell Rep.* **33**(8): 1289-1306.
- Gao, L., X. Yan, X. Li, G. Guo, Y. Hu, W. Ma and Y. Yan. 2011. Proteome analysis of wheat leaf under salt stress by two-dimensional difference gel electrophoresis (2D-DIGE). *Phytochemistry* **72**(10): 1180-1191.
- Guan, Q., X. Liao, M. He, X. Li, Z. Wang, H. Ma, S. Yu and S. Liu. 2017. Tolerance analysis of chloroplast OsCu/Zn-SOD overexpressing rice under NaCl and NaHCO₃ stress. *PLoS One* **12**(10): e0186052
- Hernández, J.A., E. Olmos, F.J. Corpas, F. Sevilla and L.A. del Río. 1995. Salt-induced oxidative stress in chloroplasts of pea plants. *Plant Sci.* **105**: 151-167.
- Holm, L.G., D.L. Plucknett, J.V. Pancho and J.P. Herberger. 1977. The world's worst weeds: Distribution and biology. University Press, Honolulu, Hawaii. pp. 609.
- Hsu, C. C. 1975. Gramineae. *In*: Li, H. L. et al. (eds.), *Flora of Taiwan*, 661-662, Vol. 6, Taiwan. pp. 661-662.
- Joaquin-Ramos, A., J.A. Huerta-Ocampo, A. Barrera-Pacheco, A. De Leon-Rodriguez, S. Baginsky, S. and A.P. Barba de la Rosa. 2014. Comparative proteomic analysis of amaranth mesophyll and bundle sheath chloroplasts and their adaptation to salt stress. *J. Plant Physiol.* **171**(15): 1423-1435.
- Kamal, A.H., K. Cho, D.E. Kim, N. Uozumi, K.Y. Chung, S.Y. Lee, J.S. Choi, S.W. Cho, C.S. Shin and S.H. Woo. 2012. Changes in physiology and protein abundance in salt-stressed wheat chloroplasts. *Mol. Biol. Rep.* **39**(9): 9059-9074.
- Kao, P., T.Y. Wu, C.L. Chang, C.H. Chou and I.F. Chang. 2011. Decreasing of population size of *Imperata cylindrica* mangrove ecotype & sea-level rising. *In*: Casalegno, S. (ed.), *Global warming impacts - case studies on the economy, human health, and on urban and natural environments*. InTech.
- Kosová, K., P. Vitamvás and I.T. Prášil. 2014. Proteomics of stress responses in wheat and barley-search for potential protein markers of stress tolerance. *Front. Plant Sci.* **5**: 711.
- Kumar, M., M.A. Sheikh and R.W. Bussmann. 2011. Ethnomedicinal and ecological status of plants in Garhwal Himalaya, India. *J. Ethnobiol. Ethnomed.* **7**(1): 32.
- Lv, D.W., S. Subburaj, M. Cao, X. Yan, X. Li, R. Appels, D. F. Sun, W. Ma and Y.M. Yan. 2014. Proteome and phosphoproteome characterization reveals new response and defense mechanisms of *Brachypodium distachyon* leaves under salt stress. *Mol. Cell. Proteomics* **13**(2): 632-652.
- Marivet J., M. Margis-Pinheiro, P. Frendo and G. Burkard. 1994. Bean cyclophilin gene expression during plant development and stress conditions. *Plant Mol. Biol.* **26**(4): 1181-1189.
- Matsunaga, K., M. Shibuya and Y. Ohizumi. 1994. Cylindrene, a novel sesquiterpenoid from *Imperata cylindrica* with inhibitory activity on contractions of vascular smooth muscle. *J. Nat. Prod.* **57**(8): 1183-1184.
- Nanjo, Y., M.Z. Nouri and S. Komatsu. 2011. Quantitative proteomic analyses of crop seedlings subjected to stress



- conditions; a commentary. *Phytochemistry* **72(10)**: 1263-1272.
- Ndimba, B.K., S. Chivasa, W.J. Simon and A.R. Slabas.** 2005. Identification of Arabidopsis salt and osmotic stress responsive proteins using two-dimensional difference gel electrophoresis and mass spectrometry. *Proteomics* **5(16)**: 4185-4196.
- Ngara, R., R. Ndimba, J. Borch-Jensen, O.N. Jensen and B. Ndimba.** 2012. Identification and profiling of salinity stress-responsive proteins in *Sorghum bicolor* seedlings. *J. Proteomics* **75(13)**: 4139-4150.
- Nouri, M. Z., A. Moumeni and S. Komatsu.** 2015. Abiotic stresses: Insight into gene regulation and protein expression in photosynthetic pathways of plants. *Int. J. Mol. Sci.* **16(9)**: 20392-20416.
- Perkins, D.N., D.J.C. Pappin, D.M. Creasy and J.S. Cottrell.** 1999. Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis* **20(18)**: 3551-3567.
- Razavizadeh, R., A.A. Ehsanpour, N. Ahsan and S. Komatsu.** 2009. Proteome analysis of tobacco leaves under salt stress. *Peptides* **30**: 1651-1659.
- Rigó, G., I. Valkai, D. Farago, E. Kiss, S. Van Houdt, N. Van de Steene, M.A. Hannah and L. Szabados, L.** 2016. Gene mining in halophytes: Functional identification of stress tolerance genes in *Lepidium crassifolium*. *Plant, Cell Environ.* **39(9)**: 2074-2084.
- Ruan, S.L., H.S. Ma, S.H. Wang, Y.P. Fu, Y. Xin, W.Z. Liu, F. Wang, J.X. Tong, S.Z. Wang and H.Z. Chen.** 2011. Proteomic identification of OsCYP2, a rice cyclophilin that confers salt tolerance in rice (*Oryza sativa* L.) seedlings when overexpressed. *BMC Plant Biol.* **11(1)**:34.
- Sage, R.F., T.L. Sage and F. Kocacinar.** 2012. Photorespiration and the evolution of C4 photosynthesis. *Annu. Rev. Plant Biol.* **63(1)**: 19-47.
- Seidler, A.** 1996. The extrinsic polypeptides of photosystem II. *Biochim. Biophys. Acta* **1277(1-2)**: 35-60.
- Sekhar, K., B. Priyanka, V.D. Reddy and K.V. Rao.** 2010. Isolation and characterization of a pigeonpea cyclophilin (CcCYP) gene, and its over-expression in Arabidopsis confers multiple abiotic stress tolerance. *Plant Cell Environ.* **33**: 1324-1338.
- Shinozaki, K. and K. Yamaguchi-Shinozaki.** 2007. Gene networks involved in drought stress response and tolerance. *J. Exp. Bot.* **58(2)**: 221-227.
- Silveira, J.A.G. and F.E.L. Carvalho.** 2016. Proteomics, photosynthesis and salt resistance in crops: An integrative view. *J. Proteomics* **143**: 24-35.
- Silveira, J. A.G., A.R.B. Melo, R.A. Viegas and J.T.A. Oliveira** 2001. Salinity-induced effects on nitrogen assimilation related to growth in cowpea plants. *Environ. Exp. Bot.* **46(2)**: 171-179.
- Slama, I., C. Abdelly, A. Bouchereau, T. Flowers and A. Savoure.** 2015. Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Ann. Bot.* **115(3)**: 433-447.
- Song, Y., C. Zhang, W. Ge, Y. Zhang, A.L. Burlingame and Y. Guo.** 2011. Identification of NaCl stress-responsive apoplastic proteins in rice shoot stems by 2D-DIGE. *J. Proteomics* **74(7)**: 1045-1067.
- Sripanidkulchai, B., V. Wongpanich, P. Laupattarakasem, J. Suwansaksri and D. Jirakulsomcho.** 2001. Diuretic effects of selected Thai indigenous medicinal plants in rats. *J. Ethnopharmacol.* **75(2-3)**: 185-190.
- Sugihara, K., N. Hanagata, Z. Dubinsky, S. Baba and I. Karube.** 2000. Molecular characterization of cDNA encoding oxygen evolving enhancer protein 1 increased by salt treatment in the mangrove *Bruguiera gymnorrhiza*. *Plant Cell Physiol.* **41(11)**: 1279-1285.
- Tanaka, Y., T. Hibino, Y. Hayashi, A. Tanaka, S. Kishitani, T. Takabe, S. Yokota and T. Takabe.** 1999. Salt tolerance of transgenic rice overexpressing yeast mitochondrial Mn-SOD in chloroplasts. *Plant Sci.* **148(2)**: 131-138.
- Torabi, S., M. Wissuwa, M. Heidari, M.R. Naghavi, K. Gilany, M.R. Hajirezaei, M. Omid, B. Yazdi-Samadi, A.M. Ismail and G.H. Salekdeh.** 2009. A comparative proteome approach to decipher the mechanism of rice adaptation to phosphorous deficiency. *Proteomics* **9(1)**: 159-170.
- Tsai, C.C. and C.H. Chou.** 1999. Sequence of a 5.8s rRNA gene and internal transcribed spacer (ITS) from *Imperata cylindrica* var. *major*. *J. Genet. Mol. Biol.* **10(2)**: 41-47.
- Wadsworth, G.J.** 1997. The plant aspartate aminotransferase gene family. *Physiol. Plant.* **100(4)**: 998-1006.
- Wang, L., W. Liang, J. Xing, F. Tan, Y. Chen, L. Huang, C.L. Cheng and W. Chen.** 2013. Dynamics of chloroplast proteome in salt-stressed mangrove *Kandelia candel* (L.) Druce. *J. Proteome Res.* **12(11)**: 5124-5136.
- Wu, T.C. and W.Y. Kao.** 2011. Ecophysiological traits of leaves of three *Marsilea* species distributed in different geographical regions. *Taiwana* **56(4)**: 279-286.
- Wu, T.Y., P. Kao, C.L. Chang, P.H. Hsu, C.H. Chou and I.F. Chang.** 2015. Phosphoproteomic profiling of microsomal fractions in leaves of Cogon grass (*Imperata cylindrica*). *Plant OMICS J.* **8**: 595-603.
- Yoon, J.S., M.K. Lee, S.H. Sung and Y.C. Kim.** 2006. Neuroprotective 2-(2-phenylethyl) chromones of *Imperata cylindrica*. *J. Nat. Prod.* **69(2)**: 290-291.
- Zhang, H., B. Han, T. Wang, S. Chen, H. Li, Y. Zhang and S. Dai.** 2012. Mechanisms of plant salt response: Insights from proteomics. *J. Proteome Res.* **11(1)**: 49-67.
- Zhao, Q., S. Chen and S. Dai.** 2013a. C4 photosynthetic machinery: Insights from maize chloroplast proteomics. *Front. Plant Sci.* **4**: 85.
- Zhao, Q., H. Zhang, T. Wang, S. Chen and S. Dai.** 2013b. Proteomics-based investigation of salt-responsive mechanisms in plant roots. *J. Proteomics* **82**: 230-253.
- Zhu, J.K.** 2003. Regulation of ion homeostasis under salt stress. *Curr. Opin. Plant Biol.* **6**: 441-445.
- Zörb, C., R. Herbst, C. Forreiter and S. Schubert.** 2009. Short-term effects of salt exposure on the maize chloroplast protein pattern. *Proteomics* **9(17)**: 4209-4220.
- Zörb, C., S. Schmitt and K.H. Muhling.** 2010. Proteomic changes in maize roots after short-term adjustment to saline growth conditions. *Proteomics* **10(24)**: 4441-4449.

More supplementary data is shown in electronic form.



SUPPLEMENTARY DATA

Table S1. Proteins identified in 2D-DIGE of leaf proteins isolated from salt-treated CW ecotype.

Spot #	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Average (p value)
	R1 control	R1 NaCl	R1 DIGE Ratio	R2 control	R2 NaCl	R2 DIGE Ratio	R3 control	R3 NaCl	R3 DIGE Ratio	R4 control	R4 NaCl	R4 DIGE Ratio	
11	2606.12	9320.89	3.57653694	2505.63	1076.50	0.429632328	2352.45	5200.82	2.21080929	665.79	6901.60	10.3660937	2.072666667 (0.03, 0.0005)
27	821.23	3881.63	4.726616397	405.49	0.00	0	758.45	2068.46	2.72721881	33375.14	85737.34	2.56889782	2.484666667 (0.38, 0.34)
52	1005.89	1783.18	1.772739447	941.56	1785.15	1.895952967	1149.32	1838.48	1.59961654				1.756333333 (0.003, 0.00005)
61	4259.56	1858.45	0.436301059	4295.43	2143.23	0.498957148	4830.62	2701.25	0.55919312				0.498 (0.003, 0.02)
70	1460.09	1229.76	0.842248416	1313.71	348.11	0.264986932	1404.61	576.96	0.41076241	2186.77	811.89	0.37127478	0.506 (0.02, 0.002)
83	1159.00	634.04	0.547061952	1729.46	360.56	0.208482516	1351.63	473.04	0.34997824	2949.55	127.76	0.04331656	0.368333333 (0.04, 0.0007)
93	77.24	1017.54	13.17312884	123.28	0.00	0	0.00	625.74	#DIV/0!	126.67	291.97	2.3049637	5.159321333 (0.16, 0.045)
96	512.48	431.29	0.841573677	472.55	79.15	0.167500038	537.74	115.33	0.2144641				0.408 (0.11, 0.01)
123	1275.54	1210.83	0.94927326	1208.52	435.65	0.360481568	1185.15	486.64	0.41060907				0.573333333 (0.18, 0.01)
125	907.20	682.71	0.752543401	1240.87	379.49	0.305824539	1210.90	628.78	0.51927092				0.526 (0.02, 0.04)
150	750.23	583.59	0.777887002	783.61	16.21	0.020683334	698.21	256.30	0.36707989				0.388666667 (0.11, 0.04)
178	210.54	300.12	1.42550449	298.99	454.40	1.519796246	217.59	444.61	2.04339308				1.663 (0.07, 0.6)
182	54.17	381.58	7.044460573	42.10	383.73	9.11431732	76.70	490.54	6.39518367				7.517666667 (0.01, <0.00001)
189	842.94	543.84	0.645165205	812.02	345.19	0.425095735	743.44	533.94	0.71820322	701.24	514.70	0.73397715	0.596 (0.02, 0.004)
199	1196.38	882.06	0.737269475	1254.90	612.18	0.487830848	1414.56	784.06	0.55427769				0.593 (0.01, 0.02)
201	475.79	957.08	2.011559033	513.90	697.15	1.356598352	531.24	1025.51	1.93039639	960.51	1370.51	1.42686253	1.766 (0.07, 0.03)
208	765.84	214.23	0.279724954	739.35	153.81	0.208028923	729.38	194.08	0.26608334	773.44	465.19	0.60146081	0.251333333 (0.005, 0.00004)
212	543.32	253.23	0.466081371	710.14	302.71	0.426270762	599.41	254.31	0.42426813				0.438666667 (0.01, 0.0007)
279	429.51	337.19	0.785057014	1308.12	3748.40	2.865481252	503.68	534.79	1.06176757				1.570666667 (0.55, 0.76)
315	270.59	293.51	1.084706513	74.14	455.15	6.139233109	262.75	354.77	1.35021193				2.858 (0.11, 0.05)
378	557.34	330.45	0.592896508	775.75	362.59	0.467407913	514.27	274.73	0.53421174				0.531333333 (0.06, 0.3)
440	261.50	1283.05	4.906401101	249.36	423.35	1.697775023	195.41	395.34	2.0231673				2.875666667 (0.25, 0.19)
441	11.80	82.34	6.976285896	5.29	139.43	26.34717534	0.00	1351.26	#DIV/0!				16.6615 (0.34, 0.19)
470	454.16	820.49	1.806632791	491.52	793.84	1.615086394	402.62	1076.12	2.67280324				2.031333333 (0.03, 0.009)
666	295.44	755.89	2.558539947	0.00	156.27		380.48	467.01	1.22742629				1.893 (0.33, 0.3)
957	1935.94	5686.02	2.937080548	2005.21	1849.33	0.922262587	1864.68	3190.75	1.71115236				1.856666667 (0.28, 0.02)

**Table S2.** Proteins identified in 2D-DIGE of leaf proteins isolated from CW ecotype

Spot #	Accession number	Experimental pI/MW	Theoretical pI/MW	Mascot score	Unique peptide #	Peptide score	Experimental pI/MW	Theoretical pI/MW	Peptide Sequence (Scaffold probability)
Metabolism									
178	gi 633095	6.32/42.97	8.62/50.48	1129	20	46	888.4385	888.5181	LAAAFIQK (97%)
Plastidic aspartate aminotransferase						45	911.6042	911.46	LYDSISSK
						41	961.4454	961.5345	LNLGVGAYR (100%)
						69	971.5229	971.6015	ISLAGLSLAK (100%)
						28	1008.3218	1008.4182	AQSDNMTDK (99%)
						54	1079.318	1079.4375	QEMEEMAGR
						42	1095.8918	1095.4325	QEMEEMAGR.I + Oxidation (M)
						34	1337.5794	1337.7092	VLISSPTWGNHK (100%)
						90	1344.5218	1344.6384	GMEVFVAQSYK
						30	1357.549	1357.67	QIGMFSYTLNKK (99%)
						90	1360.479	1360.6333	GMEVFVAQSYK.N + Oxidation (M)
						50	1373.5414	1373.6649	QIGMFSYTLNKK + Oxidation (M)
						43	1463.6104	1463.766	EYLPPIEGLAANFK (100%)
						51	1500.615	1500.7395	RGMEVFVAQSYK (100%)
						36	1516.5657	1516.7344	RGMEVFVAQSYK.N + Oxidation (M)
						91	1523.7066	1523.8559	ATAELLLGADNPVIK (100%)
						85	1530.7016	1530.8293	TEELQPYVLNVK (100%)
						75	1669.8254	1669.8457	IGAINVCSAPEVADR.V + Carbamidomethyl (C)
						108	1686.8	1686.9265	QGLVATLQSLSGTGLR (100%)
						103	1761.7211	1761.876	IVANVVDPTMFGGEWK (100%)
						37	1777.7211	1777.8709	IVANVVDPTMFGGEWK.Q + Oxidation (M)
						78	1891.7951	1891.9679	GENKEYLPPIEGLAANFK
						16	1953.668	1953.8713	AQSDNMTDKWHVYMTK
						65	2099.8473	2100.0602	FEGVPMAPPDILGVSEAFK (100%)
						75	2115.832	2116.0551	FEGVPMAPPDILGVSEAFK.A + Oxidation (M)
						25	2839.0474	2839.2979	IVANVVDPTMFGGEWKQEMEEMAGR.I + Oxidation (M)
Signaling									
957	gi 1345588	4.70/29.53	4.75/29.64	247	5	45	817.39	817.4368	ICDGILK.L + Carbamidomethyl (C)
General regulatory factor (14-3-3)							906.4369	906.5174	NLLSVAYK (98%)
							1405.5048	1405.6573	TVDSEELTVEER (100%)
							1693.7479	1693.8886	LLESHLVPSSTAPESK (100%)
							1785.8167	1785.9737	AAQDIALAELAPTHPIR (100%)
Stress									
Superoxide dismutase [Mn] 3.1, mitochondrial precursor	gi 134668	6.40/20.77	6.71/25.21	248	5	74	1316.6267	1316.6262	HHATYVANYNK (100%)
						43	1226.4512	1226.5819	YAGEVYENVLA (100%)
						66	1530.6674	1530.7943	NVRPDYLNIIWK (100%)
						26	1598.7729	1598.7743	FNGGGHVNHSIFWK (94%)
						64	1615.831	1615.8318	NLKPISSEGGEPHKG (100%)
93	gi 212722004	6.03/23.89	6.71/25.21	157	6	74	1127.6175	1127.6186	ALEQLDAAVAK (100%)
Superoxide dismutase [Mn] 3.4, mitochondrial precursor						96	1355.7405	1355.7409	GDASAVVQLQGAIK (100%)
						41	1530.7938	1530.7943	NVRPDYLNIIWK (100%)
						79	1614.8463	1614.8465	LSVETTANQDPLVTK (100%)
						33	1615.831	1615.8318	NLKPISSEGGEPHKG
						113	1742.9452	1742.9414	KLSEVETTANQDPLVTK
Photosynthesis									
61	gi 3914607	4.70/13.29	9.04/19.25	227	2	63	1447.4446	1447.5674	ENSTSPCYDGR.Y + Carbamidomethyl (C)
Ribulose-1, 5-bisphosphate carboxylase/oxygenase small subunit						35	2018.9003	2019.0928	FETLSYLPPLTQEQLLK (100%)
						85	2147.0061	2147.1878	KFETLSYLPPLTQEQLLK (100%)
						39	944.473	944.508	EFPQGVLR (95%)
11	gi 242047384	6.23/23.38	8.63/27.72	725	5	54	1229.6442	1229.6517	ERFPGQVLR (100%)
Hypothetical protein SORBIDRAFT_02g002690 (OEE2)						80	1268.5908	1268.6725	HQLITATVSDGK (100%)
						80	2098.8688	2099.0131	TADGDEGGKHQLITATVSDGK (100%)
						125	2239.8898	2240.0597	YEDNFDANSNVSVIIQPTSK (100%)
						84	2500.0919	2500.2737	KTITEYGSPEEFLSQVDFLLGK (100%)
						38	790.9552	790.4589	VDFLLGK
						34	944.4306	944.508	EFPQGVLR (99%)
						51	1229.6032	1229.6517	ERFPGQVLR (100%)
27	gi 242047384	5.60/23.65	8.63/27.72	529	6	54	1268.5737	1268.6725	HQLITATVSDGK (100%)
Hypothetical protein SORBIDRAFT_02g002690 (OEE2)						90	2098.8606	2099.0131	TADGDEGGKHQLITATVSDGK (100%)
						62	2239.8467	2240.0597	YEDNFDANSNVSVIIQPTSK (100%)
						132	2372.2294	2372.1788	TITEYGSPEEFLSQVDFLLGK (100%)
						62	2500.0743	2500.2737	KTITEYGSPEEFLSQVDFLLGK (100%)
						37	912.3221	912.4705	AYAVGASFK (96%)
						65	934.4428	934.5124	ADLTGAIFK (97%)
						48	1075.3442	1075.4281	FCDYTNEK.T + Carbamidomethyl (C)
						101	1090.4656	1090.5692	SLAAALMSEAK (100%)
						98	1106.4468	1106.5641	SLAAALMSEAK.F + Oxidation (M)
						101	1207.5175	1207.5833	GTDFTNVIDR (100%)
52	gi 212721648	4.54/17.68	7.44/24.15	1459	6	92	1414.5043	1414.6109	FDGADMSEVMSK (100%)
Uncharacterized protein LOC100194054						107	1430.5184	1430.6058	FDGADMSEVMSK.A + Oxidation (M)
						80	1446.4746	1446.6007	FDGADMSEVMSK.A + 2 Oxidation (M)
						46	934.452	934.5124	ADLTGAIFK (100%)
						64	1090.4809	1090.5692	SLAAALMSEAK (100%)
						89	1414.4982	1414.6109	FDGADMSEVMSK (100%)
						55	949.4752	949.5637	VPFLFTVK (99%)
70	gi 212721648	3.78/7.18	7.44/24.15	181	3	46	934.452	934.5124	ADLTGAIFK (100%)
Uncharacterized protein LOC100194054						64	1090.4809	1090.5692	SLAAALMSEAK (100%)
						89	1414.4982	1414.6109	FDGADMSEVMSK (100%)
						55	949.4752	949.5637	VPFLFTVK (99%)
957	gi 383511664	4.70/29.53	5.59/34.78	1072	14	55	949.4752	949.5637	VPFLFTVK (99%)



Table S2. continued

Spot #	Accession number	Experimental pI/MW	Theoretical pI/MW	Mascot score	Unique peptide #	Peptide score	Experimental pI/MW	Theoretical pI/MW	Peptide Sequence (Scaffold probability)
Chloroplast oxygen-evolving enhancer protein 1, OEE1						57	1095.4492	1095.5448	LTIDEIQSK (100%)
						49	1251.5808	1251.6459	RLTYDEIQSK (100%)
						69	1421.6535	1421.7588	KLCLEPTSFTVK.A + Carbamidomethyl (C)
						53	1430.5731	1430.6889	GDEEELQKENIK (100%)
						112	1462.6138	1462.7627	NAASSTGNITLSVTK (100%)
						100	1561.6072	1561.7485	GGSTGYDNAVALPAGGR (100%)
						77	1759.7137	1759.8741	DGIDYAAVTVQLPGGER (100%)
						48	1774.7089	1774.8711	GRGGSTGYDNAVALPAGGR (100%)
						114	2181.809	2182.0001	GTGTANQCPTIDGGVESFPFK.A + Carbamidomethyl (C)
						64	2249.0126	2249.1845	QLVATGKPESEFGGPFVPSYR (100%)
						69	2292.9453	2293.1226	FEEKDGIDYAAVTVQLPGGER (100%)
						60	2489.9193	2490.1623	GGSTGYDNAVALPAGGRGDEEELQK (100%)
						75	2575.0071	2575.229	SNPETGEVIGVFESVQPSDTDLGAK (100%)
199	gij194702912	5.30/20.50	6.41/21.03	917	7	67	1088.3727	1088.4927	TGEDPWWKA (100%)
Unknown (Cyt b6-f)						90	1362.5254	1362.6667	GDPTYLVVEQDK (100%)
						47	1413.6151	1413.7504	LGNDILVEDWLK
						83	1506.6421	1506.7871	VLFPVWVETDFR (100%)
						61	1656.7038	1656.8723	DKLGNLVEDWLK
						121	1773.7377	1773.9261	GPAPLSLALVHADVDDGK (100%)
						52	1808.6325	1808.7723	FICPCHGSGYNNQGGK.V + 2 Carbamidomethyl (C)
208	gij194702912	6.23/12.52	6.41/21.03	217	3	83	1362.5067	1362.6667	GDPTYLVVEQDK (100%)
Unknown (Cyt b6-f)						83	1506.6643	1506.7871	VLFPVWVETDFR (100%)
						65	1773.745	1773.9261	GPAPLSLALVHADVDDGK (100%)
279	gij227786	4.98/28.60	8.62/38.68	272	17	59	974.506	974.5549	IGLFGGAGYK (100%)
ATP synthase CF1 beta subunit						53	1031.4001	1031.5135	AINLEESK (100%)
						37	1044.4845	1044.5968	VVDLLAPYR (100%)
						27	1172.5617	1172.6554	VVDLLAPYR
						65	1190.4984	1190.6183	SAPAFIELDTK (100%)
						47	1221.4738	1221.5846	AVAMSATDGLMR (100%)
						27	1261.4943	1261.6336	TIAMDGTEGLVR
						33	1277.5054	1277.6286	TIAMDGTEGLVR.G + Oxidation (M)
						41	1327.5855	1327.6633	AHGGVSVFGGVGER (100%)
						29	1398.676	1398.762	VGLTGLTVAEHFR (100%)
						24	1408.648	1408.8038	VLNTGSPITVPVGR
						57	1415.5173	1415.6793	IVGNEHYETAQR (100%)
						47	1432.6444	1432.7344	MVQAGSEVSALLGR.M + Oxidation (M)
						94	1433.3454	1432.7674	FVQAGSEVSALLGR (100%)
						98	1470.6003	1470.7541	VGLTALTMAEYFR
						59	1486.6275	1486.749	VGLTALTMAEYFR.D + Oxidation (M)
						22	1491.6424	1491.7681	FTQANSEVSALLGR
						29	2059.8438	2060.0248	GIYPAVDPLDSTMLQPR (100%)
						42	2184.9207	2185.1379	IPSAVGYQPTLATDLGGLQER
						50	2280.9025	2281.0719	MPSAVGYQPTLSTEMGSLQER (100%)
						21	2296.8617	2297.0668	MPSAVGYQPTLSTEMGSLQER.I + Oxidation (M)
470		7.01/16.36	9.82/15.45	131	5	33	731.4335	731.433	YPVVVR
Photosystem I reaction center subunit IV						103	1869.9116	1869.9108	VNYAGVSTNNYALDEIK
						20	1018.49	1018.5811	ADKPPPIGPK
						53	2083.817	2083.9909	AEDATAEPAEGEVVATKPK
						30	2425.8851	2426.1139	ESYWYNGIGNVVTVDQDPNTR
666		5.06/7.50	6.69/9.52	88	2	53	1054.3881	1054.5295	VYLSSETTR
Photosystem I reaction center subunit VII						49	1540.4911	1540.665	CESACPTDFLSVR.V + 2 Carbamidomethyl (C)
Others									
201	gij242079005	4.24/41.25	4.83/46.69	497	14	59	788.4033	788.4028	ALDSVER (90%)
Cyclophilin						52	932.4961	932.4967	YALPIDNK (100%)
						51	986.5029	986.5032	ANGEELLNK (100%)
						82	1110.6626	1110.6648	SLILAGLAEPK (100%)
						93	1126.6339	1126.6346	LAVGLEELQR (100%)
						54	1142.6033	1142.6043	RANGEELLNK (100%)
						79	1157.5164	1157.5175	FYDGMIEQR (100%)
						73	1285.6136	1285.6125	KFYDGMIEQR (100%)
						78	1315.6694	1315.6694	TVPLEIMVDGDK (100%)
						51	1377.5978	1377.5983	DNPNIEDCVFR.I + Carbamidomethyl (C)
						97	1425.6896	1425.6897	LPFNAFGTMAMAR (100%)
						101	1432.7199	1432.7198	APVYGETLEELGR (100%)
						88	1613.8525	1613.8512	EVQKPLEDITDSLK (100%)
						100	1614.7861	1614.7849	ESELTPSANILDGR (100%)
						80	1618.7779	1618.7773	IKDNPNIEDCVFR.I + Carbamidomethyl (C)
						77	2490.1548	2490.1551	ADGFVVQTDGPEGPAEGFIDPSTGK (100%)



Table S3. DIGE ratio of leaf proteins identified from SL population

Spot #	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Average DIGE Ratio (p value)
	R1 control	R1 NaCl	R1 DIGE Ratio	R2 control	R2 NaCl	R2 Ratio	R3 control	R3 NaCl	R3 Ratio	R4 control	R4 NaCl	R4 Ratio	
99	611.93	791.15	1.29286605	353.57	260.24	0.736043932	320.74	264.40	0.824357642	2399.17	2969.42	1.237689567	0.951 (0.86, 0.0005)
434	286.87	248.87	0.8675393	348.20	467.06	1.341344649	440.07	273.06	0.620485774	698.41	535.44	0.766658886	0.943 (0.61, 0.34)
755	439.45	350.51	0.797619821	311.94	446.54	1.431503684	664.07	862.83	1.299315868				1.176333333 (0.57, 0.00005)
56	5012.29	2860.57	0.570711131	2882.18	4881.42	1.693655994	4638.30	4088.68	0.881503202	5653.75	4224.53	0.747208497	1.049 (0.49, 0.02)
537	82.19	88.90	1.081737604	11.33	25.13	2.217162584	14.01	32.49	2.318542424	968.04	868.38	0.897051182	1.872666667 (0.96, 0.002)
621	94.70	52.27	0.551984903	32.25	83.96	2.603565285	57.82	90.92	1.572624749	758.55	272.43	0.359145023	1.576333333 (0.58, 0.0007)
415	19.84	23.51	1.185487796	9.46	4.49	0.474598047	18.21	15.13	0.830943856	119.66	152.22	1.272032526	0.830333333 (0.88, 0.045)
91	1238.91	1331.20	1.074497183	1191.75	1317.68	1.105668386	1679.54	1637.11	0.974733919	542.84	622.83	1.147356479	1.051666667 (0.85, 0.01)
559	94.89	129.65	1.366343464	21.95	73.57	3.351283641	27.26	55.83	2.04771984	834.18	699.23	0.838226477	2.255 (0.99, 0.01)
59	3092.78	2744.38	0.887351632	2774.69	3042.02	1.096348687	3454.77	2377.93	0.688303204	901.60	677.44	0.751374516	0.889333333 (0.67, 0.04)
272	53.49	57.70	1.078687883	0.00	1.37	#DIV/0!	34.56	29.64	0.857541269	437.68	667.78	1.525741155	1.154247 (0.77, 0.04)
214	392.23	460.30	1.173539856	611.65	375.96	0.614670353	230.45	241.41	1.047563396	152.66	19.82	0.129849756	0.945666667 (0.62, 0.6)
723	0.23	0.23	0.998264708	8.64	27.60	3.194981229	3.38	6.84	2.024976031	29.61	113.49	3.832427833	2.072666667 (0.39, <0.00001)
656	248.05	72.13	0.290782993	126.88	331.57	2.613261045	229.21	202.03	0.88141365	469.61	568.23	1.210025421	1.261666667 (0.85, 0.004)
74	3120.87	2692.13	0.862620502	2707.27	3220.01	1.189391898	3408.23	2478.42	0.727185244	1342.26	1043.04	0.777072449	0.926333333 (0.68, 0.02)
106	1093.39	1045.88	0.956545116	1125.58	1013.39	0.900332924	908.16	918.09	1.010934894	813.13	493.96	0.607478224	0.956 (0.46, 0.03)
592	195.16	7.82	0.040062163	163.55	273.89	1.674617947	315.09	306.39	0.972375913	394.09	196.81	0.499400819	0.895666667 (0.44, 0.00004)
504	418.48	259.78	0.620768697	452.61	262.97	0.580998543	462.54	484.40	1.047259974	471.62	247.89	0.525615438	0.749666667 (0.09, 0.0007)
188	714.17	726.94	1.017881051	1449.85	1221.04	0.842184761	1273.66	1192.28	0.936101541	1019.65	1029.14	1.0093113	0.932 (0.73, 0.76)
612	129.57	196.42	1.515956208	189.73	39.80	0.209761766	107.77	101.10	0.938176076	170.85	318.25	1.862713743	0.888 (0.83, 0.048)
589	425.10	546.59	1.285798279	325.77	659.46	2.024282133	631.50	1271.96	2.01420197	296.87	231.52	0.77985345	1.775333333 (0.33, 0.3)
427	188.80	192.41	1.019109661	536.96	645.53	1.202205019	202.29	177.30	0.876450833	93.82	199.04	2.12150493	1.032333333 (0.76, 0.19)
566	9.96	15.72	1.57864553	55.12	94.27	1.71004735	35.51	42.94	1.209140018	3.20	3.33	1.042782462	1.499333333 (0.6, 0.19)
452	484.67	403.76	0.833051921	423.09	303.30	0.716868564	535.51	765.16	1.428837611	214.69	553.75	2.579307044	0.993 (0.49, 0.009)
173	198.57	204.48	1.029798049	213.89	255.50	1.194542289	153.45	443.52	2.890235709	187.62	109.95	0.586006167	1.705 (0.43, 0.3)
753	4005.60	3138.37	0.783495357	2025.79	1966.88	0.970920806	2285.03	2490.18	1.089781756	3306.89	4121.66	1.246382791	0.948 (0.97, 0.02)
99	611.93	791.15	1.29286605	353.57	260.24	0.736043932	320.74	264.40	0.824357642	2399.17	2969.42	1.237689567	0.951 (0.86, 0.0005)
434	286.87	248.87	0.8675393	348.20	467.06	1.341344649	440.07	273.06	0.620485774	698.41	535.44	0.766658886	0.943 (0.61, 0.34)
755	439.45	350.51	0.797619821	311.94	446.54	1.431503684	664.07	862.83	1.299315868				1.176333333 (0.57, 0.00005)
56	5012.29	2860.57	0.570711131	2882.18	4881.42	1.693655994	4638.30	4088.68	0.881503202	5653.75	4224.53	0.747208497	1.049 (0.49, 0.02)
537	82.19	88.90	1.081737604	11.33	25.13	2.217162584	14.01	32.49	2.318542424	968.04	868.38	0.897051182	1.872666667 (0.96, 0.002)
621	94.70	52.27	0.551984903	32.25	83.96	2.603565285	57.82	90.92	1.572624749	758.55	272.43	0.359145023	1.576333333 (0.58, 0.0007)
415	19.84	23.51	1.185487796	9.46	4.49	0.474598047	18.21	15.13	0.830943856	119.66	152.22	1.272032526	0.830333333 (0.88, 0.045)
91	1238.91	1331.20	1.074497183	1191.75	1317.68	1.105668386	1679.54	1637.11	0.974733919	542.84	622.83	1.147356479	1.051666667 (0.85, 0.01)
559	94.89	129.65	1.366343464	21.95	73.57	3.351283641	27.26	55.83	2.04771984	834.18	699.23	0.838226477	2.255 (0.99, 0.01)
59	3092.78	2744.38	0.887351632	2774.69	3042.02	1.096348687	3454.77	2377.93	0.688303204	901.60	677.44	0.751374516	0.889333333 (0.67, 0.04)
272	53.49	57.70	1.078687883	0.00	1.37	#DIV/0!	34.56	29.64	0.857541269	437.68	667.78	1.525741155	1.154247 (0.77, 0.04)
214	392.23	460.30	1.173539856	611.65	375.96	0.614670353	230.45	241.41	1.047563396	152.66	19.82	0.129849756	0.945666667 (0.62, 0.6)
723	0.23	0.23	0.998264708	8.64	27.60	3.194981229	3.38	6.84	2.024976031	29.61	113.49	3.832427833	2.072666667 (0.39, <0.00001)
656	248.05	72.13	0.290782993	126.88	331.57	2.613261045	229.21	202.03	0.88141365	469.61	568.23	1.210025421	1.261666667 (0.85, 0.004)
74	3120.87	2692.13	0.862620502	2707.27	3220.01	1.189391898	3408.23	2478.42	0.727185244	1342.26	1043.04	0.777072449	0.926333333 (0.68, 0.02)
106	1093.39	1045.88	0.956545116	1125.58	1013.39	0.900332924	908.16	918.09	1.010934894	813.13	493.96	0.607478224	0.956 (0.46, 0.03)
592	195.16	7.82	0.040062163	163.55	273.89	1.674617947	315.09	306.39	0.972375913	394.09	196.81	0.499400819	0.895666667 (0.44, 0.00004)



Table S3. Continued

Spot #	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Average DIGE Ratio (p value)
	R1 control	R1 NaCl	R1 DIGE Ratio	R2 control	R2 NaCl	R2 Ratio	R3 control	R3 NaCl	R3 Ratio	R4 control	R4 NaCl	R4 Ratio	
504	418.48	259.78	0.620768697	452.61	262.97	0.580998543	462.54	484.40	1.047259974	471.62	247.89	0.525615438	0.749666667 (0.09, 0.0007)
188	714.17	726.94	1.017881051	1449.85	1221.04	0.842184761	1273.66	1192.28	0.936101541	1019.65	1029.14	1.0093113	0.932 (0.73, 0.76)
612	129.57	196.42	1.515956208	189.73	39.80	0.209761766	107.77	101.10	0.938176076	170.85	318.25	1.862713743	0.888 (0.83, 0.048)
589	425.10	546.59	1.285798279	325.77	659.46	2.024282133	631.50	1271.96	2.01420197	296.87	231.52	0.77985345	1.775333333 (0.33, 0.3)
427	188.80	192.41	1.019109661	536.96	645.53	1.202205019	202.29	177.30	0.876450833	93.82	199.04	2.12150493	1.032333333 (0.76, 0.19)
566	9.96	15.72	1.57864553	55.12	94.27	1.71004735	35.51	42.94	1.209140018	3.20	3.33	1.042782462	1.499333333 (0.6, 0.19)
452	484.67	403.76	0.833051921	423.09	303.30	0.716868564	535.51	765.16	1.428837611	214.69	553.75	2.579307044	0.993 (0.49, 0.009)
173	198.57	204.48	1.029798049	213.89	255.50	1.194542289	153.45	443.52	2.890235709	187.62	109.95	0.586006167	1.705 (0.43, 0.3)
753	4005.60	3138.37	0.783495357	2025.79	1966.88	0.970920806	2285.03	2490.18	1.089781756	3306.89	4121.66	1.246382791	0.948 (0.97, 0.02)

The first p value: p values of T-test for most proteins with significant differential abundance between control and salt-stressed plants are shown after fold change ratio.

2. The second p value: p values of ANOVA for proteins with significant differential abundance between CW ecotype and SL population are shown after fold change ratio.

Table S4. Proteins identified in 2D-DIGE of leaf proteins isolated from SL population

Spot #	Accession number	Experimental pI/MW	Theoretical pI/MW	Mascot score	Unique peptide #	Peptide score	Experimental pI/MW	Theoretical pI/MW	Peptide Sequence (Scaffold probability)
Metabolism									
214	gi 514719430	6.60/40.55	8.62/50.48	507	7	58	914.5345	914.5073	IADVIQEK (99%)
Plastidic aspartate aminotransferase									
						35	935.4753	935.4501	VPWSEYR
						34	950.5081	950.4821	NLGLYSER
						43	961.5606	961.5345	LNLGVGAYR (90%)
						63	971.6303	971.6015	ISLAGLSLAK (100%)
						91	1360.6502	1360.6333	GMEVFVAQSYSK.N + Oxidation (M)
						68	1523.8794	1523.8559	ATAELLGADNPVIK (100%)
						99	1530.8434	1530.8293	TEELQPYVLNVVK (100%)
612	gi 242059597	6.32/38.68	6.96/38.99	245	15	43	647.3753	647.3755	AAFLAR
Fructose-bisphosphate aldolase									
						48	830.4861	830.4861	ALQASTLK (99%)
						41	989.5021	989.5029	GTIEVAGTDK
						55	990.5134	990.5134	NAAYIGTPGK (100%)
						47	1116.6174	1116.6179	DGKPFVDVLK (99%)
						43	1153.5617	1153.5615	ANSEATLGTYK (100%)
						51	1210.6914	1210.6921	EGGVLPGIKVDK (99%)
						59	1243.6596	1243.6561	AWAGKVENIEK (100%)
						78	1312.6268	1312.6259	GDAAADTESLHVK (100%)
						80	1331.6934	1331.6933	GILAADESTGTIGK (100%)
						67	1375.7326	1375.7347	VTPEVIAEYTVR (100%)
						73	1487.7956	1487.7944	GILAADESTGTIGKR (100%)
						66	1501.7371	1501.7372	LSSINVENVEENR (100%)
						42	1503.8318	1503.8297	KVTPVIAEYTVR (97%)
						27	1657.8404	1657.8383	LSSINVENVEENRR
Signaling									
753	gi 242073380	5.09/28.71	4.76/29.74	709	36	20	769.4015	769.401	YLAEFK
General regulatory factor (14-3-3)									
						50	815.4122	815.4137	LAEQAER (90%)
						40	817.4366	817.4368	ICDGILK.L + Carbamidomethyl (C) (81%)
						38	902.5083	902.5073	IVSSIEQK
						34	906.5177	906.5174	NLLSVAYK (100%)
						25	931.4229	931.4222	MKGDYYR
						14	1006.5824	1006.5811	VTLIKDYR
						29	1024.5918	1024.5917	LVPAAAADDAK
						30	1143.6269	1143.6248	GNEDRVTLIK
						71	1188.6542	1188.6536	DSTLIMQLLR (100%)
						53	1194.6356	1194.6357	KNEEHVAQIK (98%)
						74	1199.5135	1199.5128	DAAESTMNAYK (100%)
						72	1204.6488	1204.6485	DSTLIMQLLR.D + Oxidation (M)
						76	1211.5496	1211.5492	DAAENTMVAYK (100%)
						33	1214.5508	1214.5489	ESAESTMVAYK
						49	1215.5078	1215.5077	DAAESTMNAYK.A + Oxidation (M)
						66	1327.6094	1327.6078	KDAAESTMNAYK (100%)
						34	1333.5566	1333.557	YEEMVEFMEK (99%)
						101	1339.6436	1339.6442	KDAAENTMVAYK
						48	1349.5507	1349.5519	YEEMVEFMEK.V + Oxidation (M)
						107	1355.6392	1355.6391	KDAAENTMVAYK.A + Oxidation (M)
						96	1387.7299	1387.7307	IISIEQKEEGR
						77	1403.724	1403.7256	IVSSIEQKEESR
						90	1405.6573	1405.6573	TVDSEELTVEER
						71	1417.693	1417.6936	TVDVEELTVEER (100%)
						80	1426.7459	1426.7416	IVSSIEHKEETR (94%)
						71	1486.7779	1486.7813	VEAELSGICAGILR + Carbamidomethyl (C)



Table S4 Continued.

Spot #	Accession number	Experimental pI/MW	Theoretical pI/MW	Mascot score	Unique peptide #	Peptide score	Experimental pI/MW	Theoretical pI/MW	Peptide Sequence (Scaffold probability)
						33	1495.7125	1495.7127	EEGRGNEAHAASIR
						106	1503.6801	1503.6801	SAGGAGGEEELSVEER (100%)
						30	1516.8722	1516.8725	NLLSVAYKNVIGAR
						81	1573.8039	1573.8021	IEAELSNICDGLK + Carbamidomethyl (C) (100%)
						51	1637.9163	1636.9148	LLDRLVPAAAAVDAK
						46	1653.8592	1653.8573	LLDHLVPSSTAAESK (100%)
						70	1669.8534	1669.8523	LLDHLVPSSTASESK
						68	1693.8908	1693.8886	LLESHLVPSSTAPESK
						21	1709.7929	1709.793	SPTEPTREESVYMAK (100%)
						56	1771.9583	1771.9581	AAQDIALADLAPTHPIR
						100	1785.9738	1785.9737	AAQDIALAELAPTHPIR (100%)
						85	1920.9446	1920.9429	DAADSTLAAYQAAQDIIVK
						36	2049.9794	2049.0378	KDAADSTLAAYQAAQDIIVK
Stress									
91	gij1568639	4.96/16.24	5.35/20.42	104	3	17	1098.61	1098.6107	LACGVVGLTPL- + Carbamidomethyl (C)
	Superoxide dismutase [Cu/Zn]					94	1411.6814	1411.6804	GGHELSTLSTGNAGGR (100%)
						66.2	1086.53	1086.5378	EDGPTTVNVVR (100%)
Protein degradation									
415	gij242079721	6.36/19.81	5.71/26.31	317	5	36	755.4887	755.4653	VAANLIR (90%)
	Proteasome beta subunit					43	845.5052	845.4793	VVSLAMAR (99%)
						38	1016.5606	1016.5502	TVTINADGVK (98%)
						49	1098.5348	1098.5128	TSTGMVYANR (100%)
						60	1114.5304	1114.5077	TSTGMVYANR.A + Oxidation (M)
						111	1567.7608	1567.7478	SGSAADTQVISDYVR (100%)
Photosynthesis									
74	gij194702912	5.50/17.07	6.41/21.03	139	3	66	1088.4925	1088.4927	TGEDPWWKA (100%)
	Unknown (Cyt b6-f)					79	1362.6678	1362.6667	GDPTYLVVEQDK (100%)
						56	1808.7704	1808.7723	FICPCHGSQYNNQK.V + 2 Carbamidomethyl (C) (100%)
56	gij164698711	4.79/13.10	8.78/19.37	75	4	15	769.3757	769.3759	EGFVYR
	Ribulose-1, 5-bisphosphate carboxylase/oxygenase small subunit					31	905.4966	905.4971	QVDYLLR
						22	1392.6498	1392.6496	NNWVPCLEFSK.E + Carbamidomethyl (C) (100%)
						48	1447.5664	1447.5674	ENSTSPCYDGR.Y + Carbamidomethyl (C) (100%)
173	gij7524738	6.02/11.50	6.69/9.52	189	3	23	967.3716	967.3739	TEDCVGCKR + 2 Carbamidomethyl (C)
	Photosystem I reaction center subunit VII					61	1540.6612	1540.665	CESACPTDFLSVR.V + 2 Carbamidomethyl (C) (100%)
						90	1644.7068	1644.7059	IYDTCIGCTQCVR.A + 3 Carbamidomethyl (C) (100%)
452		7.01/16.36	9.82/15.45	177	3	27	731.4555	731.4330	YPVVVR
	Photosystem I reaction center subunit IV					28	1018.6050	1018.5811	ADKPPPIGPK (91%)
						46	2054.0008	2053.9804	ADEDEAAEPAEGEGVVATKPK (100%)
755	gij212721648	4.78/15.91	7.44/24.15	213	3	69	1090.5704	1090.5692	SLAALMSEAK (100%)
	Uncharacterized protein LOC100194054					61	1106.5644	1106.5641	SLAALMSEAK.F + Oxidation (M)
						95	1207.5826	1207.5833	GTDFTNVIDR (100%)
						77	1446.5949	1446.6007	FDGADMSEVMSK.A + 2 Oxidation (M)
753	gij383511664	5.09/28.71	6.08/34.95	477	19	24	849.427	849.4232	GSSFDPK
	Chloroplast oxygen-evolving enhancer protein 1, OEE1					26	929.4605	929.4607	NAPPEFQK
						48	949.5638	949.5637	VPFLFTVK (98%)
						49	1095.5448	1095.5448	LTYDEIQSK (99%)
						11	1219.6234	1219.6237	IQGIWYAQLE
						54	1251.6462	1251.6459	RLTYDEIQSK (100%)
						72	1285.59	1285.5907	ANLGMVMHER
						46	1293.6646	1293.6639	LCLEPTSFTVK.A + Carbamidomethyl (C) (100%)
						64	1301.5846	1301.5856	ANLGMVMHER.N + Oxidation (M)
						59	1313.7102	1313.7092	VINTWADIINR
						76	1421.7594	1421.7588	KLCLEPTSFTVK.A + Carbamidomethyl (C) (100%)
						34	1427.6318	1427.614	FCDWITSTENR.L + Carbamidomethyl (C)
						59	1430.6904	1430.6889	GDEELQKENIK
						59	1458.7256	1458.7255	LIFQYASFNSR
						96	1462.7625	1462.7627	NAASSTGNITLSVTK (100%)
						58	1463.7504	1463.8195	SVASSSGKITLSVTK (100%)
						81	1498.617	1498.6172	ETTENESANEGYR
						74	1561.7509	1561.7485	GGSTGYDNAVALPAGGR (100%)
						83	1759.8731	1759.8741	.DGIDYAAVTQLPGGER (100%)
						95	1774.8731	1774.8711	GRGGSTGYDNAVALPAGGR (100%)
						54	2491.1665	2491.1463	GGSTGYDNAVALPAGGRGDEEELEK
106	gij242079005	4.36/40.14	4.83/46.69	304	13	20	630.334	630.3337	IVEDR
Cyclophilin						52	788.4021	788.4028	ALDSVER (90%)
						49	986.5035	986.5032	ANGEELLNK (100%)
						58	1110.6648	1110.6648	SLILAGLAEPK (100%)
						73	1126.6378	1126.6346	LAVGLEELQR (100%)
						43	1142.6036	1142.6043	RANGEELLNK (98%)
						37	1157.5168	1157.5175	FYDGMIEQR (98%)
						67	1266.7644	1266.7659	SLILAGLAEPKR (100%)
						60	1285.6138	1285.6125	KFYDGMIEQR (100%)
						46	1315.6688	1315.6694	TVPLEIMVDGDK
						19	1377.5966	1377.5983	DNPNEEDCVFR.I + Carbamidomethyl (C)
						64	1425.6902	1425.6897	LPFNAFGTAMAMAR (100%)
						71	1613.8514	1613.8512	EVQKPLEDITDSLK (100%)
						61	1614.789	1614.7849	ESELTPSNANILDGR (100%)
						77	1618.7792	1618.7773	IKDNPNEEDCVFR.I + Carbamidomethyl (C)

Table S5. MASCOT search results deposited to ProteomeXchange consortium.



Peak list file	Mascot search result file
12_final spot#11	F011716.dat
32_final spot#27	F011717.dat
52_final spot#61	F011718.dat
56	F011745.dat
58_final spot@52	F011719.dat
64_final spot#70	F011720.dat
97_final spot#93	F011721.dat
111_new spot#755	F011735.dat
112_final spot#957	F011722.dat
157_new spot#91	F011736.dat
157_new spot#91	F011737.dat
162_final spot#178	F011723.dat
165_final spot#199	F011724.dat
173	F011747.dat
183_final spot#208	F011725.dat
220_new spot#106	F011738.dat
220_new spot#106	F011739.dat
259_final spot#201	F011726.dat
284_new spot#74	F011740.dat
385_final spot#279	F011727.dat
415	F011746.dat
452	F011748.dat
457_new spot#612	F011741.dat
506_final spot#470	F011728.dat
515_final spot#666	F011731.dat
597_final spot#470	F011730.dat
707_new spot#173	F011742.dat
835_new spot#753	F011743.dat

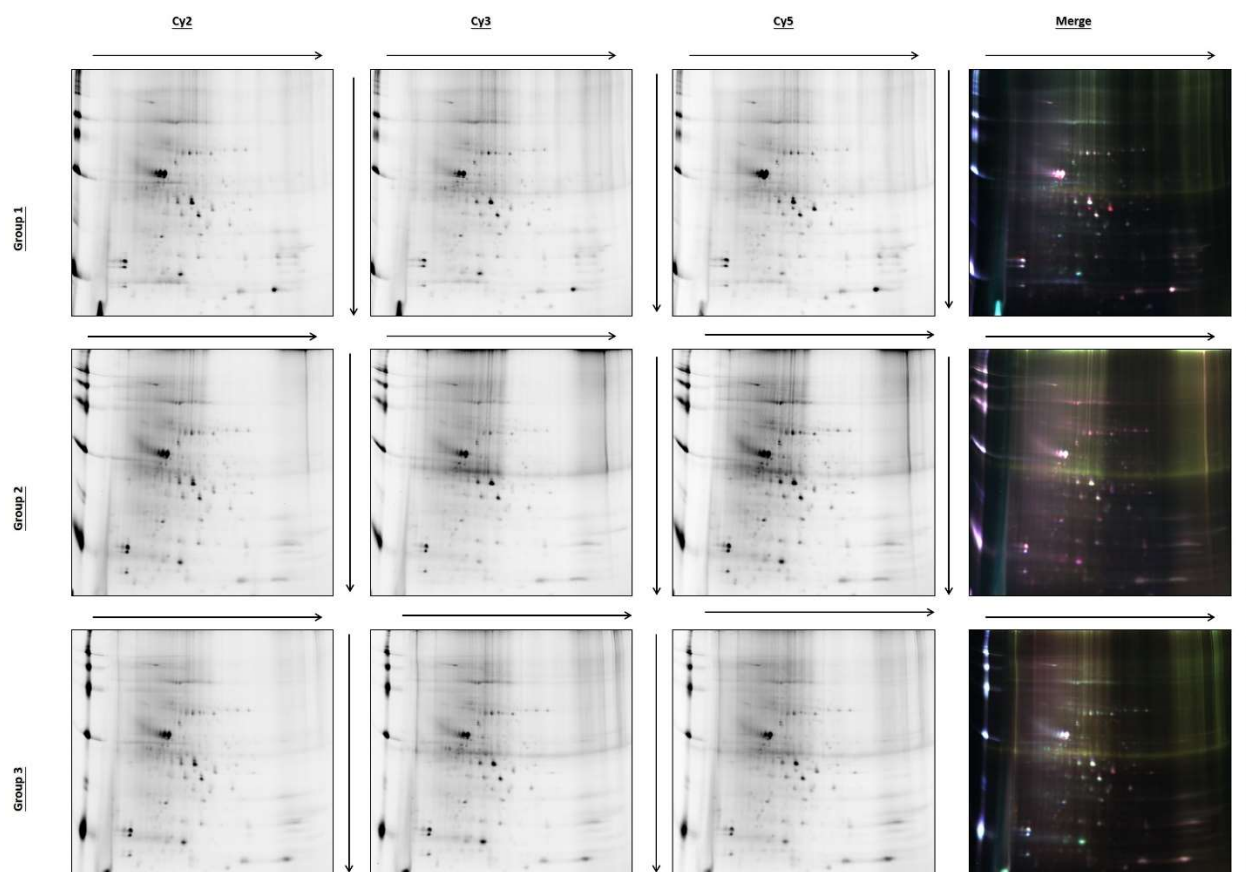


Fig. S1. 2D-DIGE images of leaf proteins isolated from salt-treated CW ecotype. The gel images correspond to three representative biological replicates.



Spot # 11

Table with columns: Valid, Sequence, Prob, Masc..., Misco..., Misco..., NTT, Modifications, Observed, Actual Mass, Charge, Delta Da, Delta..., Retent..., Intensity, TIC, Start, Stop, # Oth..., Other Protei..., Spectrum ID. Contains multiple rows of peptide data.

gj|242047384 (100%), 27,563.8 Da

hypothetical protein SORBIDRAFT_02g002690 [Sorghum bicolor]

7 exclusive unique peptides, 10 exclusive unique spectra, 51 total spectra, 73/261 amino acids (28% coverage)

MASTSCFLHQ STARLGASPR VAAAPRSAQL FVCKAQQKQQ QDAAAVDQSD VSRRAALALF
AGVAAVGAKV SPAAAAAYGEA ANVFGKPKTN TEYIAYNGDG FKLLIPSKWN PSKEREFPGQ
VLR YEDNFD A NSNVSVIIQP TSKKTTIEYG SPEEFLSQVD FLLGKQAFGG STDSEGGFET
GAVATANVLE SSTPVVDGKQ YYSVSVLTRT ADGDEGGKHQ LITATVSDGK LYICKAQAGD
KRWFKGARKG VEKAAASFV A

Spot # 61

gj|3914607 (100%), 19,036.4 Da

RecName: Full=Ribulose biphosphate carboxylase small chain, chloroplastic; Short=RuBisCO small subunit; Flags: Precu

3 exclusive unique peptides, 5 exclusive unique spectra, 15 total spectra, 30/168 amino acids (18% coverage)

MAL TVMASSA TAAAPFQGLK STASLPVARR STTSLAKVSN GGRIRCMQVW
PAYGNKKFET LSYLPPLTQE QLLKQVDYLL RNNWVPCLEF SKEGFVYREN
STSPCYDGR YWTMWKLP MF GCTDASQVYK ELQEA IASYP ELRAILGFDN
IRQTQWLTFI AYKPAGSE



Spot # 70

Valid	Sequence	Prob	Mass	Mass	Mass	NTT	Modifications	Observed	Actual Mass	Charge	Delta Da	Delta	Retent.	Intensity	TIC	Start	Stop	# Oth.	Other Protei.	Spectrum ID
✓	1.0 QLSLAALMSEAK(F)	100%	63.9	52.2	31.3	2		546.25	1,090.48	2	-0.088	-81	1460	1175	119	129	0		1416 Scan 8846 [..]	
✓	1.0 QLSLAALMSEAK(F)	100%	59.4	52.2	32.6	2		546.25	1,090.48	2	-0.087	-80	1430	1164	119	129	0		1406 Scan 8796 [..]	
✓	1.0 QLSLAALMSEAK(F)	100%	58.3	52.2	16.9	2		546.25	1,090.49	2	-0.079	-72	1430	1098	119	129	0		1388 Scan 8682 [..]	
✓	1.0 QLSLAALMSEAK(F)	100%	57.2	52.2	26.5	2		546.25	1,090.49	2	-0.083	-76	1440	1193	119	129	0		1398 Scan 8744 [..]	
✓	1.0 KFDGADMSEVVMSE(A)	100%	88.7	51.5	68.4	2		708.26	1,414.50	2	-0.11	-80	1360	651.7	130	142	0		1306 Scan 8352 [..]	
✓	1.0 KFDGADMSEVVMSE(A)	100%	68.8	51.5	52.7	2		708.26	1,414.50	2	-0.11	-81	1370	722.5	130	142	0		1314 Scan 8297 [..]	
✓	1.0 KADLTDAIR(Q)	100%	46.3	52.7	2.1	2		468.23	934.45	2	-0.061	-65	1400	1601	168	176	0		1348 Scan 8494 [..]	

gil212721648 (100%), 23,654.3 Da

uncharacterized protein LOC100194054 [Zea mays]

3 exclusive unique peptides, 3 exclusive unique spectra, 7 total spectra, 33/225 amino acids (15% coverage)

MASSSCLASP SGATLCRPRR PRCRVACSAA DAGGSTGPAW AKGAGRLACG VLAAWSVASA
 SNPVI AASQR LPPLSTEPNR CERAFVGN TI GQANGVYDKP LDLRFCDYTN EKTNLKGGK **SL**
AAALMSEAKF DGADMSEVVM SKAYAVGASF KGTDFTN AVI DRVNF EKADL TGAIFKNTVL
 SGSTFDDAKM DDVVFEDTII GYIDLQKLC T NTSISPDARL ELGCR

Spot # 93

Valid	Sequence	Prob	Mass	Mass	Mass	NTT	Modifications	Observed	Actual Mass	Charge	Delta Da	Delta	Retent.	Intensity	TIC	Start	Stop	# Oth.	Other Protei.	Spectrum ID
✓	1.0 QALBQLDAAYAK(G)	100%	77.1	51.5	16.5	2		564.76	1,127.50	2	-0.12	-100	1250	4939	72	82	0		1212 Scan 7572 [..]	
✓	1.0 QALBQLDAAYAK(G)	99%	32.5	51.5	10.2	2		564.74	1,127.48	2	-0.14	-130	2390	473.0	72	82	0		2719 Scan 14576 [..]	
✓	1.0 QGDASAVYVQIQAK(F)	100%	98.4	51.3	49.8	2		678.82	1,355.62	2	-0.12	-89	1370	1418	82	96	0		1249 Scan 8315 [..]	
✓	1.0 QLSVETLANQDPLYTE(G)	100%	63.2	50.9	0.0	2		808.35	1,614.69	2	-0.15	-94	1260	823.8	169	183	0		1228 Scan 7656 [..]	
✓	1.0 QLSVETLANQDPLYTE(G)	100%	51.8	50.9	0.0	2		539.22	1,614.65	3	-0.20	-120	1270	1364	169	183	0		1239 Scan 7717 [..]	
✓	1.0 QLSVETLANQDPLYTE(G)	87%	29.0	50.9	0.0	2		539.23	1,614.68	3	-0.17	-100	1250	922.1	169	183	0		1218 Scan 7608 [..]	
✓	1.0 QNVYREDYLNHWK(V)	100%	43.4	51.1	8.4	2		511.23	1,330.67	3	-0.13	-83	1480	1839	206	217	0		1466 Scan 8649 [..]	
✓	1.0 QNYAGEVYENYLA(-)	100%	36.8	51.5	15.2	2		614.23	1,226.45	2	-0.13	-110	1420	922.7	223	233	0		1414 Scan 8594 [..]	
✓	1.0 QNYAGEVYENYLA(-)	97%	30.1	51.5	14.2	2		614.22	1,226.42	2	-0.16	-130	1400	683.9	223	233	0		1391 Scan 8473 [..]	

gil1174391 (100%), 25,239.0 Da

RecName: Full=Superoxide dismutase [Mn] 3.4, mitochondrial; Flags: Precursor

5 exclusive unique peptides, 6 exclusive unique spectra, 9 total spectra, 63/233 amino acids (27% coverage)

MALRTLASKN ALSFALGGAA RPSAASARGV TTVALPDL SY DFGALEPAIS GEIMRLHHQK
 HHAATYVGNYN **KALEQLDAAY AKGDASAVVQ LQGAIKFN**GG GHVNHSIFWK NLKPISEGGG
 EPPHGKLGWA IDEDFGSFEA LVKRMNAEGA ALQGS GWVWL ALDKPKK **LS VETTANQDPL**
VTKGASLVPL LGIDVWEHAY YLQYK NVRPD YLNNIWKVMN WKYAGEVYEN VLA



Spot # 199

Table with columns: Valid, Sequence, Frob, Mass, Mono, N/T, Modifications, Observed, Actual Mass, Charge, Delta Da, Delta, Retent., Intensity, TIC, Start, Stop, # Other, Other Protei., Spectrum ID. Contains 100 rows of protein identification data for Spot # 199.

gi|194702912 (100%), 20,753.4 Da

unknown [Zea mays]

4 exclusive unique peptides, 5 exclusive unique spectra, 73 total spectra, 66/193 amino acids (34% coverage)

MGRERAQRSI VCAASSISA DRVPDMEKRR LMNLLLLGAI SLPTVGMVVP YGAFFVPAGS
GNAGGGTYAK DKLGN DITVE AWLNTHGPNDR TLAQGLKGD PTYLVVEQDK TLATYGINAV
CTHLGGVYAW NGAENK FICP CHGSQYNNQG KVVVRGPAPLS LALVHADVDD GKVLVFPWVE
TDFRTGEDPW WKA

Spot # 957

gi|1345588 (100%), 29,637.6 Da

RecName: Full=14-3-3-like protein GF14-12

2 exclusive unique peptides, 2 exclusive unique spectra, 10 total spectra, 60/261 amino acids (23% coverage)

MASAELSREE NYMAKLAEQ AERYEEMVEF MEKVAK TVDS EELTVEERNL LSVAYKNVIG
ARRASWRIIS SIEQKEEGRG NEDRVTLIKD YRGTIETELT KIGD GILKLL ESHLPVSSTA
PEKSVFYLKM KGDYRYLAE FKTGYAERKDA ENKTMVAYKA AQDIALAELLA PTHPIRLGLA
LNFSVFYFEI LNSPDRACSL AKQAFDEAIS ELDTLSEESY KDSTLIMQLL HDNLTLLWTS D
I SEDPAEEIR EAPKHDLSEG Q

Table with columns: Valid, Sequence, Frob, Mass, Mono, N/T, Modifications, Observed, Actual Mass, Charge, Delta Da, Delta, Retent., Intensity, TIC, Start, Stop, # Other, Other Protei., Spectrum ID. Contains 10 rows of protein identification data for Spot # 957.



Spot # 178

gjl633095 (100%), 50,282.1 Da

plastidic aspartate aminotransferase [Panicum miliaceum]

7 exclusive unique peptides, 15 exclusive unique spectra, 95 total spectra, 186/457 amino acids (41% coverage)

Sequence alignment showing unique peptides in yellow: MASAAAFALTS, PMAAPPDPILG, LPIEGLAAFN, SSPTWGNHKN, GIDPTPEQWE, SKNLGLY AER, GEWKQEMEQD, KWHVYMTQDG, VAAEPALTAARS, PSEAFKADKN, KATAELLGGA, KIFNDARVPWS, KIADVIQEKK, IGAINVVC SA, AGRIKNVQRK, RISLAGLSLA, KVLGWGKNGQ, DLKLNLVGVA, DNPVIKQGLV, EYRYVDPKYV, HMPFFDVAYQ, PEVADRVK SQ, LYDLSLSAKDK, KCDYLADAI I, RNGCRVGITR, YRTEELQPYV, ATLQSLSGTG, GLDFEGMI AD, GFASGSLDED, LKRLARPMYS, DSKDWSVILR, SDFHNVS, KNFGRVLMAL, LNVVKKAENL, SLRLAAAFIQ, IEAAPEGSFV, AFSVRLFVKR, NPPIHGARI V, QIGMFSYTG, AVDSRFEQV, MLEKGENKEY, RYFPEAKVLI, LLHGCAHNPT, GMVFVAQSY, ANIVGDPTMF, NKAQSDNMTD

Table with columns: Peptide, Sequence, Pmob, Mass, Mass, Mass, NIT, Modifications, Observed, Actual Mass, Charge, Delta Da, Delta, Retent., Intensity, TIC, Start, Stop, # Obs., Other Peaks, Spectrum ID. Contains 186 rows of peptide data.

Spot # 208

gjl194702912 (100%), 20,753.4 Da

unknown [Zea mays]

2 exclusive unique peptides, 2 exclusive unique spectra, 9 total spectra, 42/193 amino acids (22% coverage)

Sequence alignment showing unique peptides in yellow: MGRERAQRSI, GNAGGGTYAK, CTHLGCVV PW, TDFRTGEDPW, VCAQAISSISA, DKLGNIDITVE, NGAENKFCIP, WKA, DRVPDMEKRK, AWLNTHGPN D, CHGSQYNNQG, KVLVGRAPLS, LMNLLLLLGA I, RTLAQGLKGD, KVLVVEQDK, YGAFVFPAGS, TLATYGINAV, LALVHADVDD, GKVLFPVWWE

Table with columns: Peptide, Sequence, Pmob, Mass, Mass, Mass, NIT, Modifications, Observed, Actual Mass, Charge, Delta Da, Delta, Retent., Intensity, TIC, Start, Stop, # Obs., Other Peaks, Spectrum ID. Contains 9 rows of peptide data.



Spot # 279

gij118614499 (100%), 54,014.4 Da

ATP synthase CF1 beta subunit (chloroplast) [*Sorghum bicolor*]

1 exclusive unique peptides, 1 exclusive unique spectra, 23 total spectra, 145/498 amino acids (29% coverage)

```

MRTNPTTSRP  GVSTIEEKS  V  GRIDQIIGPV  LDITFPPGKL  PYIYNALIVK  SRDTADKQIN
VTCEVQQLLG  NNRVRAVAMS  ATDGLMRGME  VIDTGTPLSV  PVGGATLGRV  FNVLGEPIDN
LGPVDTSATF  PIHRSAPAFI  ELDTKLSIFE  TGIKVVDLLA  PYRRGGKIGL  FGGAGVGVKTV
LIMELINNJIA  KAHGGVSVFG  GVGERTREGN  DLYMEMKESG  VINEKNIEES  KVALVYGMQN
EPPGARMRVG  LTALTMAEYF  RDVKNQDVLL  FIDNIFRFVQ  AGSEVSALLG  RMPSAVGYQP
TLSTEMGSLQ  ERITSTKKGS  ITSIAQAVVP  ADDLTDPAPA  TTFAHLDATT  VLSRGLASKG
IYPAVDPLDS  TSTMLQPRIV  GNEHYETAQR  VKETLQRYKE  LQDIIAAILGL  DELSEEDRLT
VARARKIERF  LSQPFFVAEV  FTGSPGKYVG  LAETIRGFQL  ILSGELDGLP  EQAFYLVGNI
DEASTKAINL  EEESKLKK
    
```

Valid	Sequence	Prob	Misco...	Misco...	Misco...	NTT	Modifications	Observed	Actual Mass	Charge	Delta Da	Delta...	Retent...	Intensity	TIC	Start	Stop	# Oth...	Other Protei...	Spectrum ID
✓	(R)AVAMSAIDGLMR(G)	100%	47.0	51.7	0.0	2		611.74	1,221.47	2	-0.11	-91	1280	978.8	75	87	2	gij1328785...	1291 Scan 7729 [...]	
✓	(R)SAPAFIEDTK(L)	100%	64.8	51.8	0.0	2		596.26	1,190.50	2	-0.12	-100	1430	2427	135	145	1	gij10915642	1515 Scan 8671 [...]	
✓	(R)VVDLLAFYR(R)	99%	37.4	52.2	0.0	2		523.25	1,044.48	2	-0.11	-110	1500	2066	155	163	1	gij10915642	1629 Scan 9082 [...]	
✓	(R)VVDLLAFYR(R)	96%	31.9	52.2	0.0	2		523.26	1,044.50	2	-0.100	-95	1490	1644	155	163	1	gij10915642	1610 Scan 9024 [...]	
✓	(R)VVDLLAFYR(R)	88%	28.4	52.2	0.0	2		523.26	1,044.50	2	-0.095	-91	1520	1596	155	163	1	gij10915642	1643 Scan 9155 [...]	
✓	(R)VVDLLAFYR(R)	80%	27.0	52.2	0.0	2		523.26	1,044.50	2	-0.099	-95	1480	1300	155	163	1	gij10915642	1592 Scan 8958 [...]	
✓	(R)KGLPFGAGYVK(T)	100%	59.0	52.6	0.0	2		488.26	974.51	2	-0.049	-50	1420	2325	168	178	17	gij85909168...	1485 Scan 8593 [...]	
✓	(R)KGLPFGAGYVK(T)	100%	52.5	52.6	0.0	2		488.25	974.49	2	-0.070	-72	1410	1771	168	178	17	gij85909168...	1475 Scan 8515 [...]	
✓	(R)AHGGVSVFGVGER(T)	100%	40.9	51.5	0.0	2		664.80	1,327.59	2	-0.078	-59	1230	571.6	192	205	2	gij1328785...	1225 Scan 7444 [...]	
✓	(R)VGTLTALIMAEYFR(D)	100%	97.8	51.8	0.0	2		736.31	1,470.60	2	-0.15	-100	1770	1339	249	261	3	gij1328785...	2014 Scan 10889 [...]	
✓	(R)VGTLTALIMAEYFR(D)	100%	66.3	51.8	0.0	2		736.31	1,470.61	2	-0.15	-100	1750	801.0	249	261	3	gij1328785...	1593 Scan 10592 [...]	
✓	(R)VGTLTALIMAEYFR(D)	100%	59.5	51.7	0.0	2	Coxidation (+16)	744.32	1,466.63	2	-0.12	-82	1590	941.7	249	261	3	gij1328785...	1763 Scan 9625 [...]	
✓	(R)VGTLTALIMAEYFR(D)	100%	45.5	51.8	0.0	2		736.32	1,470.63	2	-0.13	-87	1760	913.8	249	261	3	gij1328785...	2002 Scan 10635 [...]	
✓	(R)VGTLTALIMAEYFR(D)	97%	31.9	51.7	0.0	2	Coxidation (+16)	744.32	1,466.62	2	-0.13	-87	1580	810.2	249	261	3	gij1328785...	1737 Scan 9519 [...]	
✓	(R)FYQAGSEVSALLGR(M)	100%	93.8	52.8	0.0	2		717.68	1,433.35	2	0.58	-300	1540	1513	278	291	3	gij1328785...	1685 Scan 9319 [...]	
✓	(R)FYQAGSEVSALLGR(M)	100%	67.3	51.5	0.0	2		717.32	1,432.63	2	-0.14	-95	1520	1036	278	291	3	gij1328785...	1647 Scan of 2 ses...	
✓	(R)FYQAGSEVSALLGR(M)	100%	79.7	54.3	0.0	2		717.13	1,432.25	2	-0.52	340	1530	1039	278	291	3	gij1328785...	1668 Scan 9254 [...]	
✓	(R)FYQAGSEVSALLGR(M)	100%	45.2	51.5	0.0	2		717.33	1,432.64	2	-0.12	-85	1550	938.5	278	291	3	gij1328785...	1697 Scan 9271 [...]	
✓	(R)MSAYGYQIILSTEMGSLGR	100%	50.4	50.1	0.0	2		761.31	2,280.90	3	-0.17	-74	1480	1172	292	312	3	gij1328785...	1591 Scan 8655 [...]	
✓	(R)IYPAVDPLDSITMLQPR(D)	94%	28.7	50.3	0.0	2		1,030.53	2,059.84	2	-0.18	-85	1620	699.7	360	378	3	gij1328785...	1500 Scan 9766 [...]	
✓	(R)GNEHYETAQR(Y)	100%	56.7	51.3	0.0	2		708.77	1,415.52	2	-0.16	-110	849	1151	379	350	1	gij10915642	779 Scan of 2 ses...	
✓	(R)KAINLEESK(L)	100%	53.4	52.2	0.0	2		516.71	1,031.40	2	-0.11	-110	1040	2731	487	495	0	985 Scan 6298 [...]		
✓	(R)KAINLEESK(L)	100%	45.7	52.2	0.0	2		516.71	1,031.40	2	-0.12	-110	1030	2111	487	495	0	976 Scan 6243 [...]		

Spot # 470

gij131176 (100%), 15,456.6 Da

RecName= Full=Photosystem I reaction center subunit IV, chloroplastic; Short=PSI-E; AltName= Full=Photosystem I 10.8 kDa polypeptide; Flags: Precursor

1 exclusive unique peptides, 1 exclusive unique spectra, 2 total spectra, 171/147 amino acids (12% coverage)

```

MASTNMASAT  SRFMLAAGIP  SGANGGVSSR  VSFLPSNRLG  LKLVARAEPP  TAAAPAEPAP
AADEKPEAAV  ATKEPAKAKP  PPRGPKRGTK  VKILRRESYW  YNGTGSVVTV  DQDPNTRYPV
VVRFAKVNYA  GVSTNNYALD  EIKEVAA
    
```

Valid	Sequence	Prob	Misco...	Misco...	Misco...	NTT	Modifications	Observed	Actual Mass	Charge	Delta Da	Delta...	Retent...	Intensity	TIC	Start	Stop	# Oth...	Other Protei...	Spectrum ID
✓	(K)YNYAGYFSTNNYALDEIK(E)	100%	47.7	50.5	27.8	2		935.88	1,869.74	2	-0.17	-91	1370	749.3	127	143	0		1047 Scan 8689 [...]	
✓	(K)YNYAGYFSTNNYALDEIK(E)	100%	42.7	50.5	22.1	2		935.89	1,869.77	2	-0.14	-76	1410	475.1	127	143	0		1099 Scan 8900 [...]	

Spot # 666

gij108773054 (100%), 8,960.2 Da

photosystem I subunit VII (chloroplast) [*Acutodesmus obliquus*]

1 exclusive unique peptides, 1 exclusive unique spectra, 3 total spectra, 9/81 amino acids (11% coverage)

```

MSHIVKIYDT  CIGCTQCVR  CPLDVLEMVP  WNGCKANQMA  SAPRTEDCVG  CKRCETACPT
DFLSIRVYLS  SETTRSMGLS  Y
    
```

Valid	Sequence	Prob	Misco...	Misco...	Misco...	NTT	Modifications	Observed	Actual Mass	Charge	Delta Da	Delta...	Retent...	Intensity	TIC	Start	Stop	# Oth...	Other Protei...	Spectrum ID
✓	(R)YVLSSEITR(S)	100%	53.3	51.3	19.4	2		528.20	1,054.39	2	-0.14	-130	947	1682	67	75	0		799 Scan 5651 [...]	
✓	(R)YVLSSEITR(S)	100%	40.5	51.3	1.2	2		528.21	1,054.40	2	-0.13	-130	957	2138	67	75	0		810 Scan 5712 [...]	
✓	(R)YVLSSEITR(S)	97%	38.4	53.5	4.5	2		528.57	1,055.12	2	0.59	-390	938	1073	67	75	0		793 Scan 5602 [...]	



Spot # 666

gj108773244 (100%), 10,853.8 Da

photosystem I subunit VII [Chara vulgaris]

1 exclusive unique peptides, 1 exclusive unique spectra, 2 total spectra, 13197 amino acids (13% coverage)

MEK L K L S Y F K T N L E S L M S H K I K I Y D T C I G C T Q C V R A C P T D V L E M I P W Q G C K A K Q I A S A P R
T E D C V G C K R **C** **E S A C P T D F L S** **V R** V Y L G S E T T R S M G L A Y

Val#	Sequence	Prob	Misco...	Misco...	Misco...	NTI	Modifications	Observed	Actual Mass	Charge	Delta Da	Delta...	Retent...	Intensity	TIC	Start	Stop	# Othe...	Other Protei...	Spectrum ID
10	R(C)E S A C P T D F L S R(V)	100%	43.9	51.2	17.5	2	Carbamalcomethyl...	771.25	1,540.49	2	-0.17	-110	1440	972.3	70	82	0		1236: Scan 8539 [...]	
10	R(C)E S A C P T D F L S R(V)	100%	35.8	51.2	19.9	2	Carbamalcomethyl...	771.25	1,540.49	2	-0.18	-120	1430	633.8	70	82	0		1234: Scan 8553 [...]	

SODM4_MAIZE (100%), 25,239.0 Da

Superoxide dismutase [Mn] 3.4, mitochondrial OS=Zea mays GN=SODA.3 PE=2 SV=1

4 exclusive unique peptides, 6 exclusive unique spectra, 16 total spectra, 94/233 amino acids (40% coverage)

M A L R T L A S K N A L S F A L G G A A R P S A A S A R G V T T V A L P D L S Y D F G A L E P A I S
G E I M R L H H Q K H H A T Y V G N Y N K A L E Q L D A A V A K G D A S A V V Q L Q G A I K F N G G
G H V N H S I F W K N L K P I S E G G E P P H G K L G W A I D E D F G S F E A L V K R M N A E G A
A L Q G S G W V W L A L D K E P K K L S V E T T A N Q D P L V T K G A S L V P L L G I D V W E H A Y
Y L Q Y K N V R P D Y L N N I W K V M N W K Y A G E V Y E N V L A

SODM1_MAIZE (100%), 25,545.4 Da

Superoxide dismutase [Mn] 3.1, mitochondrial OS=Zea mays GN=SODA.4 PE=2 SV=1

1 exclusive unique peptides, 2 exclusive unique spectra, 9 total spectra, 64/235 amino acids (27% coverage)

M A L R T L A S K K V L S F P F G G A G R P L A A A A S A R G V T T V T L P D L S Y D F G A L E P A
I S G E I M R L H H Q K H H A T Y V G N Y N Y N K A L E Q L E T A V S K G D A S A V V Q L Q A A I K F N
G G G H V N H S I F W K N L K P I S E G G E P P H G K L G W A I D E D F G S F E A L V K K M N A E
G A A L Q G S G W V W L A L D K E A K K V S V E T T A N Q D P L V T K G A S L V P L L G I D V W E H
A Y Y L Q Y K N V R P D P D Y L N N I W K V M N W K Y A G E V Y E N V L A

SODM4_MAIZE (100%), 25,239.0 Da

Superoxide dismutase [Mn] 3.4, mitochondrial OS=Zea mays GN=SODA.3 PE=2 SV=1

4 exclusive unique peptides, 6 exclusive unique spectra, 16 total spectra, 94/233 amino acids (40% coverage)

M A L R T L A S K N A L S F A L G G A A R P S A A S A R G V T T V A L P D L S Y D F G A L E P A I S
G E I M R L H H Q K H H A T Y V G N Y N K A L E Q L D A A V A K G D A S A V V Q L Q G A I K F N G G
G H V N H S I F W K N L K P I S E G G E P P H G K L G W A I D E D F G S F E A L V K R M N A E G A
A L Q G S G W V W L A L D K E P K K L S V E T T A N Q D P L V T K G A S L V P L L G I D V W E H A Y
Y L Q Y K N V R P D Y L N N I W K V M N W K Y A G E V Y E N V L A

SODM1_MAIZE (100%), 25,545.4 Da

Superoxide dismutase [Mn] 3.1, mitochondrial OS=Zea mays GN=SODA.4 PE=2 SV=1

1 exclusive unique peptides, 2 exclusive unique spectra, 9 total spectra, 64/235 amino acids (27% coverage)

M A L R T L A S K K V L S F P F G G A G R P L A A A A S A R G V T T V T L P D L S Y D F G A L E P A
I S G E I M R L H H Q K H H A T Y V G N Y N Y N K A L E Q L E T A V S K G D A S A V V Q L Q A A I K F N
G G G H V N H S I F W K N L K P I S E G G E P P H G K L G W A I D E D F G S F E A L V K K M N A E
G A A L Q G S G W V W L A L D K E A K K V S V E T T A N Q D P L V T K G A S L V P L L G I D V W E H
A Y Y L Q Y K N V R P D P D Y L N N I W K V M N W K Y A G E V Y E N V L A

**SODM2_MAIZE (100%), 25,357.0 Da****Superoxide dismutase [Mn] 3.2, mitochondrial OS=Zea mays GN=SODA.1 PE=2 SV=1****1 exclusive unique peptides, 1 exclusive unique spectra, 1 total spectra, 14/232 amino acids (6% coverage)**

```

MALRTLASKN  ALSFALGGAA  RPSAASARGV  TTVALPDLSY  DFGALEPVIS
GEIMRLHHQK  HHATYVVNYN  KALEQLDAVV  VKGDASAVVQ  LQGAIKFNGG
GHFNHSIFWE  NLKPISEGGE  PPHGKLGWAI  DEDFGSFEAL  VKRMNAEGAA
LQGS GWVWLA  LDKEPKKLSV  ETTANQDPLV  TKGASLVPLL  GIDVWEHAYY
LQYKNVRPDY  LNNIWKVMNW  KYAGEVYENV  LA

```

Spot # 110

gi|1174391 (100%), 25,239.0 Da

RecName: Full=Superoxide dismutase [Mn] 3.4, mitochondrial; Flags: Precursor

5 exclusive unique peptides, 6 exclusive unique spectra, 6 total spectra, 53/233 amino acids (23% coverage)

```

MALRTLASKN  ALSFALGGAA  RPSAASARGV  TTVALPDLSY  DFGALEPAIS  GEIMRLHHQK
HHATYVGNYN  KALEQLDAAV  AKGDASAVVQ  LQGAIKFNGG  GHVNHSIFWK  NLKPISEGGE
EPPHGKLGWA  IDEDFGSFEA  LVKRMNAEGA  ALQSGWVWL  ALDKEPKKLS  VETTANQDPL
VTKGASLVPL  LGIDVWEHAY  YLQYKNVRPD  YLNNIWKVMN  WKYAGEVYEN  VLA

```

Yield	Sequence	Prob	Misco..	Misco..	Misco..	NTI	Modifications	Observed	Actual Mass	Charge	Delta Da	Delta Da	Revert..	Intensity	TIC	Start	Stop	# Oth...	Other Protei...	Spectrum ID
10	(K)ALQLDAAVAK(G)	100%	74.0	51.5	20.0	2		564.82	1,127.62	2	-0.0012	-1.1	1030	1328	72	82	0		207: Sum of 2 sca...	
10	(K)GDASAVVQLQGAIK(G)	100%	95.9	51.3	51.3	2		678.88	1,355.74	2	-0.0060	-0.44	1150	174.7	83	96	0		266: Sum of 2 sca...	
10	(K)LSVETTANQDELPTK(G)	100%	113.1	50.9	16.2	2		872.48	1,742.94	2	0.0014	0.80	995	197.8	168	183	0		191: Sum of 2 sca...	
10	(K)LSVETTANQDELPTK(G)	100%	60.8	50.9	0.0	2		581.99	1,742.94	3	-0.0011	-0.61	990	677.0	168	183	0		188: Sum of 2 sca...	
10	(K)LSVETTANQDELPTK(G)	100%	78.8	50.9	0.0	2		808.49	1,614.85	2	-0.0034	-0.21	1070	1190	168	183	0		221: Sum of 2 sca...	
10	(K)NVRPDYLNK(G)	89%	41.3	51.1	0.2	2		511.27	1,530.79	3	-0.0056	-0.36	1290	119.9	206	217	0		300: Sum of 2 sca...	

Spot # 201

gi|242079005 (100%), 46,381.9 Da

hypothetical protein SORBIDRAFT_07g019320 [*Sorghum bicolor*]**4 exclusive unique peptides, 4 exclusive unique spectra, 16 total spectra, 148/426 amino acids (35% coverage)**

```

MAALLASSRC  CCSRPSLPPL  PTRGRRSVAR  CALSGGEKRN  SFSWKECAVS
VALSVGLING  APTLGGPAYA  SPLEPVLPDV  SVLISGPPIK  DPGALLRYAL
PIDNKAIREV  QKPLEDITDS  LKVAGVREALD  SVERNIKQAS  RALNNGRSLI
LAGLAEPKRA  NGEELLNKLA  VGLEELQRIV  EDRNRDAVAP  KQKELLQYVG
TVEEDMVDGF  PYEIPPEEYS  MPLLKGRATV  DMKVKIKDNP  NVEDCVFRIV
LDGYNAPVTA  GNFIDLVERK  FYDGMEIQRA  DGFVVQTGDP  EGPAEGFIDP
STGKIRTVPL  EIMVDGDKAP  VYGETLEELG  RYKAQTKLPF  NAFGTMAMAR
EEFDDNSASS  QIFWLLKESE  LTPSNANILD  GRYAVFGYVT  ENEDYLADVK
VGDVIESIQV  VSGLDNLVNP  SYKIVG

```

Spot # 61

gi|3914607 (100%), 19,036.4 Da

RecName: Full=Ribulose biphosphate carboxylase small chain, chloroplastic; Short=RuBisCO small subunit; Flags: Precu

3 exclusive unique peptides, 5 exclusive unique spectra, 15 total spectra, 30/168 amino acids (18% coverage)

```

MALTVMASSA  TAAAPFQGLK  STASLPVARR  STTSLAKVSN  GGRIRCMQVW
PAYGNKKFET  LSYLPPLTQE  QLLKQVDYLL  RNNWVPCLEF  SKEGFVYREN
STSPCYDGR  YWTMWKLPF  GCTDASQVYK  ELQEAIASYP  ELRAILGFDN
IRQTQWLTFI  AYPKAGSE

```

Fig. S2. Validation of identified proteins in leaves of salt-treated CW ecotype by Scaffold.

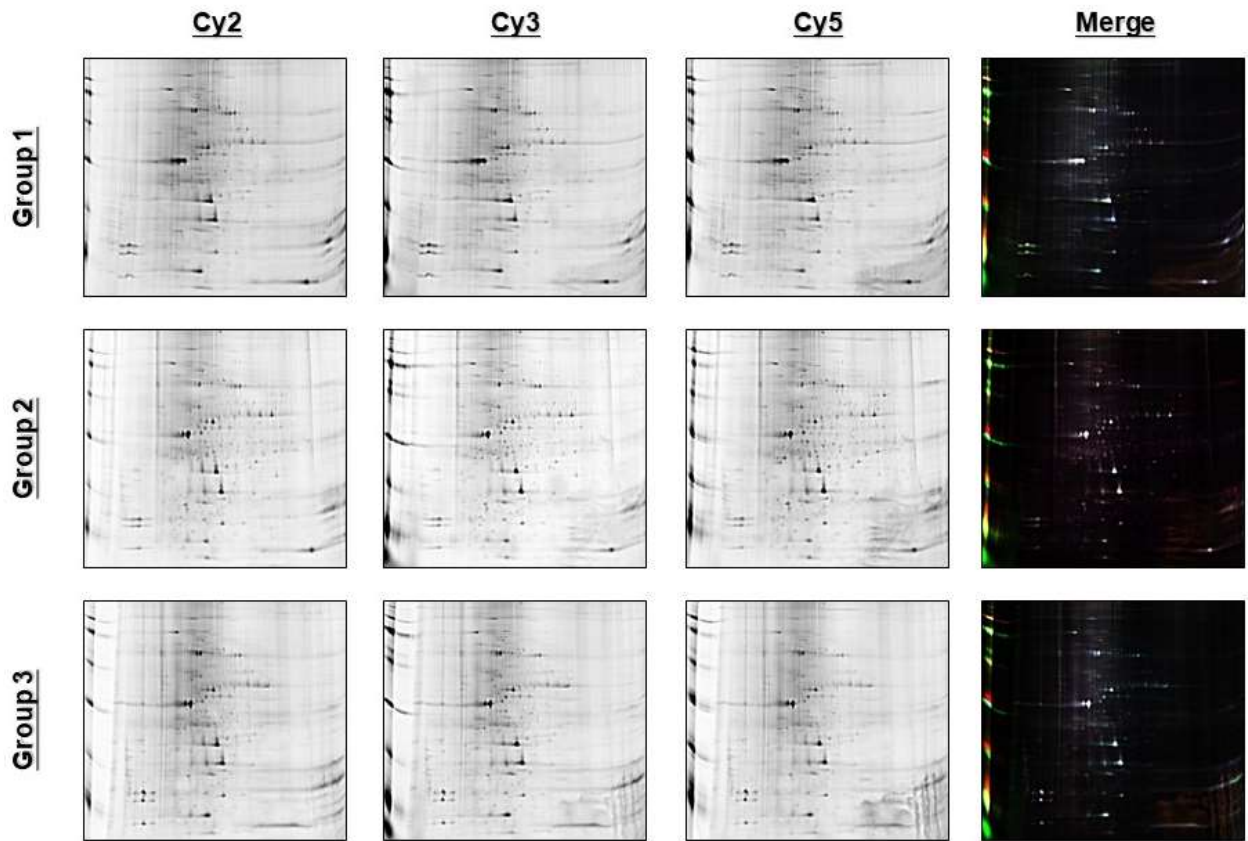


Fig. S3. 2D-DIGE images of leaf proteins isolated from salt-treated SL population. The gel images correspond to three representative biological replicates.



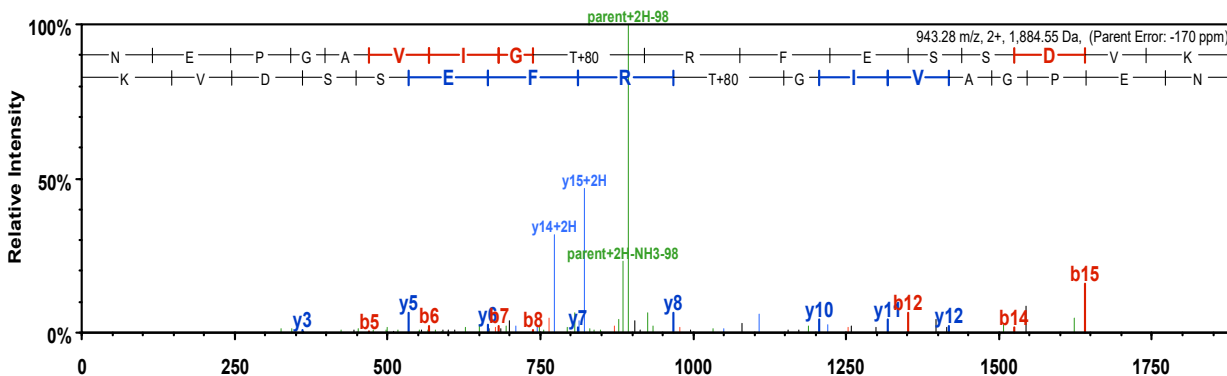
A.

Unigene22380_SL (100%), 28,149.0 Da

211 981 hypothetical protein SORBIDRAFT_02g036260 [Sorghum bicolor] >gj|241926393|gb|EER99537.1| hypothetical protein SORBI_002G338000 [Sorghum bicolor]

1 exclusive unique peptides, 2 exclusive unique spectra, 2 total spectra, 17/257 amino acids (7% coverage)

G S E R Q K K P A G P K K A T K I S S D R P L W F P G A V A P D Y L D G S L V G D Y G F D P F G L G K P V E Y L Q F E L
 D S L D Q N L A K N E P G A V I G T R F E S S D V K S T P L Q P Y S E V F G L Q R F R E C E L I H G R W A M L A T L G A
 L S V E W L T G V T W Q D A G K V E L V D G S S Y L G Q P L P F S I S T L I W I E V L V I G Y I E F Q R N A E L D P E K
 R L Y P G G S Y F D P L G L A A D P E K K E R L Q L A E I K H A R L A M V A F L G F A V Q A A A T G K G P L N N W A T H
 L S D P L H T T I F D T F G G S S



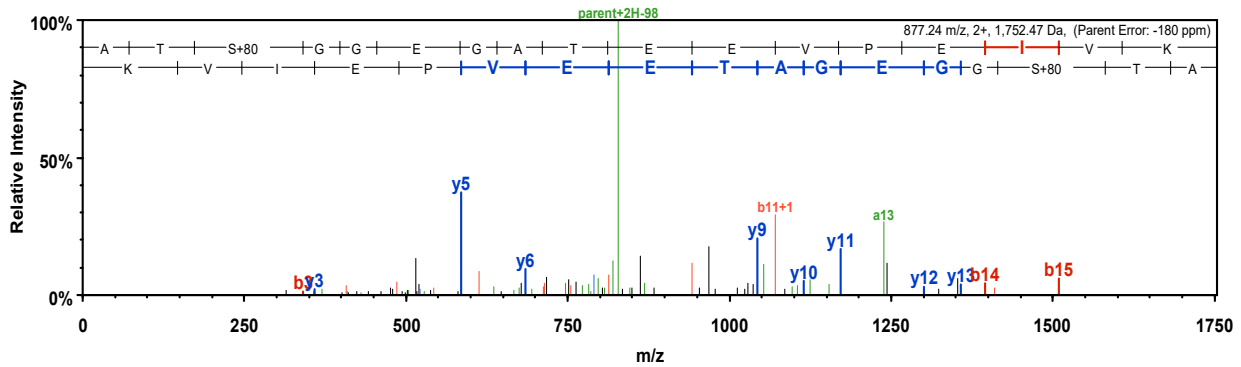
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	115.1		98.0		N	1,885.9	943.4	1,868.8	1,867.9	17
2	244.1		227.1	226.1	E	1,771.8	886.4	1,754.8	1,753.8	16
3	341.1		324.1	323.1	P	1,642.8	821.9	1,625.8	1,624.8	15
4	398.2		381.1	380.2	G	1,545.7	773.4	1,528.7	1,527.7	14
5	469.2		452.2	451.2	A	1,488.7	744.9	1,471.7	1,470.7	13
6	568.3	284.6	551.2	550.3	V	1,417.7	709.3	1,400.6	1,399.7	12
7	681.4	341.2	664.3	663.3	I	1,318.6	659.8	1,301.6	1,300.6	11
8	738.4	369.7	721.4	720.4	G	1,205.5	603.3	1,188.5	1,187.5	10
9	919.4	460.2	902.4	901.4	T+80	1,148.5	574.8	1,131.5	1,130.5	9
10	1,075.5	538.3	1,058.5	1,057.5	R	967.5	484.2	950.5	949.5	8
11	1,222.6	611.8	1,205.5	1,204.6	F	811.4	406.2	794.4	793.4	7
12	1,351.6	676.3	1,334.6	1,333.6	E	664.3	332.7	647.3	646.3	6
13	1,438.6	719.8	1,421.6	1,420.6	S	535.3		518.2	517.3	5
14	1,525.7	763.3	1,508.6	1,507.7	S	448.2		431.2	430.2	4
15	1,640.7	820.9	1,623.7	1,622.7	D	361.2		344.2	343.2	3
16	1,739.8	870.4	1,722.7	1,721.8	V	246.2		229.2		2
17	1,885.9	943.4	1,868.8	1,867.9	K	147.1		130.1		1

Unigene30507_SL (100%), 16,044.8 Da

150 614 TPA: thylakoid membrane phosphoprotein 14 kDa [Zea mays]

1 exclusive unique peptides, 1 exclusive unique spectra, 1 total spectra, 17/155 amino acids (11% coverage)

A C S S C S F P M A T A C R L A A P L G L A P L P R V R A S A G V V A V A A Q C G S K I P R G V A V R A T S G G E G A T
 E E V P E I V K A A Q D A W D K V E D K Y A V A T I G V A A I V A L W T V V G A I K A I D K L P L L P G V L E I V G I G
 Y T G W F T Y R N L I F Q P D R E A L I G K I K S T Y K E I T G S S S



B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	72.0				A	1,753.8	877.4	1,736.8	1,735.8	17
2	173.1			155.1	T	1,682.8	841.9	1,665.7	1,664.7	16
3	340.1			322.1	S+80	1,581.7	791.4	1,564.7	1,563.7	15
4	397.1			379.1	G	1,414.7	707.9	1,397.7	1,396.7	14
5	454.1			436.1	G	1,357.7	679.3	1,340.7	1,339.7	13
6	583.2	292.1		565.2	E	1,300.7	650.8	1,283.6	1,282.7	12
7	640.2	320.6		622.2	G	1,171.6	586.3	1,154.6	1,153.6	11
8	711.2	356.1		693.2	A	1,114.6	557.8	1,097.6	1,096.6	10
9	812.3	406.6		794.3	T	1,043.6	522.3	1,026.5	1,025.6	9
10	941.3	471.2		923.3	E	942.5	471.8	925.5	924.5	8
11	1,070.4	535.7		1,052.4	E	813.5	407.2	796.4	795.5	7
12	1,169.4	585.2		1,151.4	V	684.4	342.7	667.4	666.4	6
13	1,266.5	633.7		1,248.5	P	585.4		568.3	567.4	5
14	1,395.5	698.3		1,377.5	E	488.3		471.3	470.3	4
15	1,508.6	754.8		1,490.6	I	359.3		342.2		3
16	1,607.7	804.3		1,589.7	V	246.2		229.2		2
17	1,753.8	877.4	1,736.8	1,735.8	K	147.1		130.1		1

B. Partial sequence alignment of thylakoid membrane phosphoprotein 14 kDa protein orthologs in other plant species. Protein sequences from different plant species include XP_015613093.1 (*Oryza sativa Japonica Group*); XP_021314085.1 (*Sorghum bicolor*); XP_021314075.1 (*Sorghum bicolor*); NP_001130557.1 (*Zea mays*); NP_566086.1 (*Arabidopsis thaliana*); XP_015645395.1 (*Oryza sativa Japonica Group*); XP_015645396.1 (*Oryza sativa Japonica Group*); NP_001150124.1 (*Zea mays*); Unigene30507_SL (*Imperata cylindrica*); XP_021308012.1 (*Sorghum bicolor*). Sequence alignment was performed using ClustalW2. The phosphopeptides (ATpSGGEGATEEVPEIVK) identified in leaves of SL population is underline. Conserved phosphorylation sites among different plant species are in gray box.

```

XP_015613093.1   SFCKRLARNVVAMAAGEA----PAAPLAANAEITEFINALKQEWDRIEDKYAVTTLAVAA
XP_021314085.1   C--KRLARNVVAMATGEP----TAAPVADNEELTEFVNALKKEWDRIEDKYAVTTLAVAA
XP_021314075.1   CECKRLARNVVAMATGEP----TAAPVADNEELTEFVNALKKEWDRIEDKYAVTTLAVAA
NP_001130557.1   SLYKRLARDVVAMAAGEP----A-APQAANEELTEFVDALKKEWDRIEDKYAVTTLAVAA
NP_566086.1      AYCRKIVRNVVTRATTEVGEAPATTTEAETTELPEIVKTAQEAWEKVDDKYAIGSLAFAG
XP_015645395.1   ATGIKYV---GLRASRGVAIRAAD-GTGSETEVPEVVKAAQDAWAKVEDKYAVTAIGVAA
XP_015645396.1   ATGIKY----GLRASRGVAIRAAD-GTGSETEVPEVVKAAQDAWAKVEDKYAVTAIGVAA
NP_001150124.1   VRCARSS---GSKI PRGVAVRATSGGEGATEEVPEIVKAAQDAWDKVEDKYAVATIGVAA
Unigene30507_SL  ---AAQC---GSKI PRGVAVRATSGGEGATEEVPEIVKAAQDAWDKVEDKYAVATIGVAA
XP_021308012.1   ---AQC---GSKI PRGVAVRATSGGEGATEDVPEIVKAAQDAWDKVEDKYAVATIGVAA

```

. : * : : : . : * : : : * : : : * : : : . : : : *



Partial sequence alignment of chloroplast a/b binding protein CP29 orthologs in other plant species. Protein sequences from different plant species include XP_015646910.1 (*Oryza sativa Japonica Group*); EAZ40279.1 (*Oryza sativa Japonica Group*); NP_001105502.1 (*Zea mays*); NP_001136606.1 (*Zea mays*); Unigene22380_SL (*Imperata cylindrica*); XP_002463016.1 (*Sorghum bicolor*); OAP04488.1 (*Arabidopsis thaliana*); NP_187506.1 (*Arabidopsis thaliana*); AAK82524.1 (*Arabidopsis thaliana*); AAK43851.1 (*Arabidopsis thaliana*); AAM12979.1 (*Arabidopsis thaliana*); pdb|5MDX|R (*Arabidopsis thaliana*); NP_195773.1 (*Arabidopsis thaliana*). Sequence alignment was performed using ClustalW2. The phosphopeptides (NEPGAVIGpTRFESSDVK) identified in leaves of SL population is underline. Conserved phosphorylation sites among different plant species are in gray box.

```

XP_015646910.1      WFPGAVAPDYLDGSLVGDYGFDPFGLGKPAEYLQFELDSLQNLAKNNAGE I I GTRFETG
EAZ40279.1          WFPGA-----PAEYLQFELDSLQNLAKNNAGE I I GTRFETG
NP_001105502.1      WFPGAVAPDYLDGSLVGDYGFDPFGLGKPV EYLQFELDSLQNLAKNEAGGI I GTRFESS
NP_001136606.1      WFPGAVAPDYLDGSLVGDYGFDPFGLGKPV EYLQFELDSLQNLAKNEAGGI I GTRFESS
Unigene22380_SL      WFPGAVAPDYLDGSLVGDYGFDPFGLGKPV EYLQFELDSLQNLAKNEPGAV I GTRFESS
XP_002463016.1      WFPGAVAPDYLDGTLVGDYGFDPFGLGKPV EYLQFDLDSLQNLAKNEPGVV I GTRFESS
OAP04488.1          WFPGAKSPEYLDGSLVGDYGFDPFGLGKPAEYLQFDLDSLQNLAKNLYGEV I GTRTEAV
NP_187506.1          WFPGAKSPEYLDGSLVGDYGFDPFGLGKPAEYLQFDLDSLQNLAKNLYGEV I GTRTEAV
AAK82524.1          WYPGAI SPDWLDGSLVGDYGFDPFGLGKPAEYLQFDIDSLDQNLAKNLAGDV I GTRTEAA
AAK43851.1          WYPGAI SPDWLDGSLVGDYGFDPFGLGKPAEYLQFDIDSLDQNLAKNLAGDV I GTRTEAA
AAM12979.1          WYPGAI SPDWLDGSLVGDYGFDPFGLGKPAEYLQFDIDSLDQNLAKNLAGDV I GTRTEAA
pdb|5MDX|R          WYPGAI SPDWLDGSLVGDYGFDPFGLGKPAEYLQFDIDSLDQNLAKNLAGDV I GTRTEAA
NP_195773.1          WYPGAI SPDWLDGSLVGDYGFDPFGLGKPAEYLQFDIDSLDQNLAKNLAGDV I GTRTEAA
*:.***                               * .***** :.***** * :.***** *:
    
```

```

XP_015646910.1      EVKSTPFQPYTEV FGLQRFRECELI HGRWAMLATLGALSVEWLTGVTWQDAGKVELVDGS
EAZ40279.1          EVKSTPFQPYTEV FGLQRFRECELI HGRWAMLATLGALSVEWLTGVTWQDAGKVELVDGS
NP_001105502.1      EVKSTPLQPYSEV FGLQRFRECELI HGRWAMLATLGALSVEWLTGVTWQDAGKVELVDGS
NP_001136606.1      DVKSTPLQPYSEV FGLQRFRECELI HGRWAMLATLGALTVEWLTGVTWQDAGKVELVDGS
Unigene22380_SL      DVKSTPLQPYSEV FGLQRFRECELI HGRWAMLATLGALSVEWLTGVTWQDAGKVELVDGS
XP_002463016.1      DVKSTPLQPYSEV FGLQRFRECELI HGRWAMLATLGALSVEWLTGVTWQDAGKVELVDGS
OAP04488.1          DPKSTPFQPYSEV FGLQRFRECELI HGRWAMLATLGAITVEWLTGVTWQDAGKVELVDGS
NP_187506.1          DPKSTPFQPYSEV FGLQRFRECELI HGRWAMLATLGAITVEWLTGVTWQDAGKVELVDGS
AAK82524.1          DAKSTPFQPYSEV FGIQRFRECELI HGRWAMLATLGALSVEWLTGVTWQDAGKVELVDGS
AAK43851.1          DAKSTPFQPYSEV FGIQRFRECELI HGRWAMLATLGALSVEWLTGVTWQDAGKVELVDGS
AAM12979.1          DAKSTPFQPYSEV FGIQRFRECELI HGRWAMLATLGALSVEWLTGVTWQDAGKVELVDGS
pdb|5MDX|R          DAKSTPFQPYSEV FGIQRFRECELI HGRWAMLATLGALSVEWLTGVTWQDAGKVELVDGS
    
```

Fig. S4. Identification of two phosphopeptides from data set of our previous study. The study identified phosphopeptides in leaves of CW ecotype and SL population (Wu *et al.*, 2015). **A**, MS/MS fragmentation pattern of the identified phosphopeptides of thylakoid membrane phosphoprotein 14 kDa protein (ATpSGGEGATEEVPEIVK) and a chloroplast a/b binding protein CP29 (NEPGAVIGpTRFESSDVK); **B**, sequence alignment show evolutionarily conserved phosphorylation sites among different plant species.



Spot # 111(755)

Table with columns: Vahl, Sequence, Prob, Missco..., NTT, Modifications, Observed, Actual Mass, Charge, Delta Da, Delta..., Retent..., Intensity, TIC, Start, Stop, # Othe..., Other Protei..., Spectrum ID. Contains 8 rows of protein identification data.

gij212721648 (100%), 23,654.3 Da

uncharacterized protein LOC100194054 [Zea mays]

3 exclusive unique peptides, 4 exclusive unique spectra, 7 total spectra, 35/225 amino acids (16% coverage)

Mass spectrometry peptide sequence: MASSSCLASP SGATLCRRR PRCRVACSAA DAGGSTGPAW AKGAGRLACG VLAAWSVASA SNPVIAASQR LPPLSTENR CERAFVGTI GQANGVYDKP LDLRFGDYTN EKTNLKGS L AAALMSEAKF DGADMSEVVM SKAYAVGASF KGTDFNAVI DRVNF EKADL TGAIFKNTVL SGSTFDDAKM DDVVFEDTII GYIDLQKLCNTSISPDARL ELGCR

Spot # 835(753)

Table with columns: Vahl, Sequence, Prob, Missco..., NTT, Modifications, Observed, Actual Mass, Charge, Delta Da, Delta..., Retent..., Intensity, TIC, Start, Stop, # Othe..., Other Protei..., Spectrum ID. Contains 40 rows of protein identification data.

gij242073380 (100%), 29,649.7 Da

hypothetical protein SORBIDRAFT_06g019100 [Sorghum bicolor]

5 exclusive unique peptides, 10 exclusive unique spectra, 32 total spectra, 117/261 amino acids (45% coverage)

Mass spectrometry peptide sequence: MASAELSR EE NVYMAK LAEQ AERYEEMVEF MEKVAKTVD S EELTVEERNL LSVAYKNVIG ARRASWR IIS SIEQKEEGRG NEDRVTLIK D YRDKIETELT KICDGIKLL ESHLVPSS TA PESKVFY LKM KGDYRYLAE FKTGAERKDA AENTMVAYKA AQDIALAELA PTHPIRLGLA LNFSVFY YEI LNSPDRACSL AKQAFDEAIS ELDTLSEESY KDSTLIMQLL RDNLTLWTSD ISEDPAAEIR EAPKRDSSEG Q



Spot # 284(74)

Valid	Sequence	Prob	Masso...	Masso...	Masso...	NTT	Modifications	Observed	Actual Mass	Charge	Delta Da	Delta...	Retent...	Intensity	TIC	Start	Stop	# Othe...	Other Protei...	Spectrum ID
✓	1.0 (K)GDPTLYVVEQDK(T)	100%	78.9	51.6	0.0	2		682.34	1,362.67	2	0.00088	0.64	671	2563	99	110	0		index=10241	
✓	1.0 (K)GDPTLYVVEQDK(T)	100%	64.3	51.6	0.0	2		682.34	1,362.67	2	-0.00012	-0.089	672	4286	99	110	0		index=10242	
✓	1.0 (K)YFCPCCHGSSQYNNQGR(T)	100%	55.9	52.0	0.0	2	Carbamidomethyl ...	905.39	1,808.77	2	-0.0020	-1.1	376	4527	137	151	0		index=10012	
✓	1.0 (R)YGEDPWWK(-)	87%	33.5	52.3	4.1	2		509.73	1,017.45	2	-0.0013	-1.2	753	3969	185	192	0		index=10281	
✓	1.0 (R)YGEDPWWKA(-)	100%	65.5	51.9	35.7	2		545.25	1,088.49	2	-0.00034	-0.31	809	13690	185	193	0		index=10294	

gij194702912 (100%), 20,753.4 Da

unknown [*Zea mays*]

4 exclusive unique peptides, 4 exclusive unique spectra, 5 total spectra, 36/193 amino acids (19% coverage)

MGRERAQRS I VCQAASSISA DRVPDMEKRK LMNLLLLGAI SLPTVGMVVP YGAFFVPAGS
 GNAGGGTYAK DKLGN DITVE AWLNTHGPN D RTLAQGLK **GD** **PTYLVVEQDK** TLATYGINAV
 CTHLGCVVPW NGAENK **FICP** **CHGSQYNNQG** **KVVRGPAPLS** LALVHADVDD GKVLFPVWE
 TDFR **TGEDPW** **WKA**

Spot # 707(173)

Valid	Sequence	Prob	Masso...	Masso...	Masso...	NTT	Modifications	Observed	Actual Mass	Charge	Delta Da	Delta...	Retent...	Intensity	TIC	Start	Stop	# Othe...	Other Protei...	Spectrum ID
✓	0.5 (K)YDTCIGCTQCYRA(A)	100%	89.6	51.1	62.5	2	Carbamidomethyl ...	823.36	1,644.71	2	0.00078	0.48	604	7517	23	35	160	gi34501452...	index=17328	
✓	1.0 (R)YVLSSETTR(S)	100%	46.9	51.3	10.1	2		528.27	1,054.53	2	-0.00032	-0.31	361	8928	67	75	0		index=17257	
✓	1.0 (R)YVLSSETTR(S)	98%	38.8	51.3	9.0	2		528.27	1,054.53	2	0.00066	0.62	359	5058	67	75	0		index=17256	

gij108773054 (100%), 8,960.2 Da

photosystem I subunit VII (chloroplast) [*Acutodesmus obliquus*]

1 exclusive unique peptides, 1 exclusive unique spectra, 3 total spectra, 22/81 amino acids (27% coverage)

MSHIVK **IYDT** **CIGCTQCVRA** CPLDVLEMVP WNGCKANQMA SAPRTEDCVG CKRCETACPT
 DFLSIR **VYLS** **SETTR** SMGLS Y

Spot # 214

Valid	Sequence	Prob	Masso...	Masso...	Masso...	NTT	Modifications	Observed	Actual Mass	Charge	Delta Da	Delta...	Retent...	Intensity	TIC	Start	Stop	# Othe...	Other Protei...	Spectrum ID
✓	0.8 (K)NLNGVGYR(T)	90%	43.1	52.1	0.0	2		481.79	961.56	2	0.026	27	1450	842.2	82	90	1	gi357137439	index=288	
✓	1.0 (R)TEELQPTLYLVVYK(K)	100%	99.2	50.1	0.0	2		766.43	1,530.84	2	0.014	9.1	1840	3055	94	106	0		index=248	
✓	1.0 (K)ATAELLGADNPVIK(Q)	100%	68.2	49.9	36.0	2		762.95	1,523.88	2	0.023	15	1860	1447	133	147	0		index=352	
✓	0.8 (K)ADVIQEK(K)	99%	57.8	51.9	0.0	2		458.27	914.53	2	0.027	30	1040	1524	253	260	1	gi357137439	index=206	
✓	1.0 (R)ISLAGLSLAK(C)	100%	62.7	51.4	22.8	2		486.82	971.63	2	0.029	29	1780	688.1	433	442	0		index=337	

gij514719430 (100%), 50,192.7 Da

PREDICTED: aspartate aminotransferase, chloroplastic [*Setaria italica*]

3 exclusive unique peptides, 3 exclusive unique spectra, 5 total spectra, 55/458 amino acids (12% coverage)

MASTAAFAVS SPAASAVAAR SKVHGGGKNA GRIGCRVGIT RKNFGRVMMALAVDVSRLFEG
 VPMAPPDPI LGVSEAFKADK SDLK **LN LGV G** **AYRTEELQPY** **VLNVVK** KAEN LMLEKGEYKE
 YLPIEGLAAF NK **ATAELLLG** **ADNPVIK** QGL VATLQSLSGT GSLRLAAAFI QRYFPEAKVL
 ISSPTWGNHK NIFNDARVPW SEYRYDPKT VGLDFEGMIA DIEAAPEGSF VLLHGCAHNP
 TIGDPTPEQW EK **IADVIQEK** **KHMPFFDVAY** QGFASGLDE DAFSVRLFVK RGMVFVAQS
 YSKNLGLYSE RIGAINVCS APGVADRVS KQLKRLARPMY SNPPIHGARI VANVVDPTM
 FGEWKQEMEQ MAGRIKNVRQ KLYDSL SVKD KSGKDW SFIL RQIGMFSYTG LNKAQSDNMT
 DKWHVYMTKD GR **ISLAGLSL** **AK** CDYLADA I IDSFHNVN



Spot # 173

Valid	Sequence	Prob	Masso...	Masso...	Masso...	NTT	Modifications	Observed	Actual Mass	Charge	Delta Da	Delta...	Retent...	Intensity	TIC	Start	Stop	# Othe...	Other Protei...	Spectrum ID
✓	0.5 (K)IYDTCIGCTQCVR(A)	100%	89.4	50.0	66.5	2	Carbamidomethyl...	823.37	1,644.73	2	0.023	14	1320	9739	23	35	176	gi34501452...	index=1102	
✓	0.5 (K)IYDTCIGCTQCVR(A)	100%	48.3	50.0	24.7	2	Carbamidomethyl...	549.25	1,644.73	3	0.027	17	1330	1139	23	35	176	gi34501452...	index=1104	
✓	1.0 (R)VYLSSETTR(S)	100%	76.2	50.5	38.3	2		528.29	1,054.56	2	0.028	27	969	2092	67	75	0		index=1074	

gij108773054 (100%), 8,960.2 Da

photosystem I subunit VII (chloroplast) [Acutodesmus obliquus]

1 exclusive unique peptides, 1 exclusive unique spectra, 3 total spectra, 22/81 amino acids (27% coverage)

M S H I V K I Y D T C I G C T Q C V R A C P L D V L E M V P W N G C K A N Q M A S A P R T E D C V G C K R C E T A C P T
D F L S I R V Y L S S E T T R S M G L S Y

Spot # 452

Valid	Sequence	Prob	Masso...	Masso...	Masso...	NTT	Modifications	Observed	Actual Mass	Charge	Delta Da	Delta...	Retent...	Intensity	TIC	Start	Stop	# Othe...	Other Protei...	Spectrum ID
✓	1.0 (K)AEDAAAEAFARFGGVVATPK	100%	46.5	28.7	37.4	2		685.67	2,054.00	3	0.020	9.8	1090	13730	78	98	0		index=2226	
✓	1.0 (K)ADKPPPIGPK(R)	90%	28.4	30.4	12.5	2		510.31	1,018.61	2	0.024	23	837	1140	99	108	0		index=2202	

CL6460.Contig1_All (100%), 17,924.2 Da

26 529 PREDICTED: photosystem I reaction center subunit IV, chloroplastic-like [Setaria italica]

2 exclusive unique peptides, 2 exclusive unique spectra, 2 total spectra, 31/168 amino acids (18% coverage)

A R T P H P L T L S S P S A R A R P T Q H P R Q G E F T P M A S T N M A S A T S R F M L A A G V P T A G S G S G I S G R
V S F A P A P N R L G R R L V V R A D E D A A A E P A E G E G V V A T K P K A D K P P P I G P K R G A K V K I L R R E S
Y W Y N G I G N V V T V D Q D P N T R Y P V V V R F S K V N Y A G V S T N N Y A L D E I K E V K

Spot # 415

Valid	Sequence	Prob	Masso...	Masso...	Masso...	NTT	Modifications	Observed	Actual Mass	Charge	Delta Da	Delta...	Retent...	Intensity	TIC	Start	Stop	# Othe...	Other Protei...	Spectrum ID
✓	1.0 (R)TSTGMYYANR(A)	100%	60.2	51.7	36.3	2	Oxidation (+16)	558.27	1,114.53	2	0.023	20	828	1511	44	53	0		index=2930	
✓	1.0 (R)TSTGMYYANR(A)	100%	48.8	51.6	31.9	2		550.27	1,098.53	2	0.022	20	955	1611	44	53	0		index=2940	
✓	1.0 (R)SGSAADTQVSDVVR(Y)	100%	111.4	51.0	34.6	2		784.89	1,567.76	2	0.013	8.3	1570	5409	70	84	0		index=2009	
✓	1.0 (K)VAANLIR(L)	90%	35.6	50.9	1.3	2		378.75	755.49	2	0.023	31	1000	616.7	102	108	0		index=2950	
✓	1.0 (K)VVSLAMAR(D)	99%	43.4	52.9	14.8	2		423.76	845.51	2	0.026	31	1260	1085	183	190	0		index=2986	
✓	1.0 (R)VTINADGVK(R)	99%	37.8	52.1	5.8	2		509.29	1,016.56	2	0.010	10	1120	1943	200	209	0		index=2967	

gij242079721 (100%), 26,207.5 Da

hypothetical protein SORBIDRAFT_07g025030 [Sorghum bicolor]

5 exclusive unique peptides, 6 exclusive unique spectra, 6 total spectra, 50/245 amino acids (20% coverage)

M D A S H T G S S S A A G E A S T T G E H R M G T T I V G V C Y D G G V I L G A D S R T S T G M Y V A N R A S D K I T Q
L T D N V Y V C R S G S A A D T Q V I S D Y V R Y F L H Q H T I Q L G Q P A T V K V A A N L I R L L A Y Q N K N M L Q A
G M I V G G W D K Y E G G Q I F S V P L G G T I L K Q P F A I G G S G S S Y L Y A L L D H E W K E G M S Q E E A E K F V
V K V V S L A M A R D G A S G G V V R T V T I N A D G V K R N F Y P G D K L P L W H D E L E P H S L L D I L A A G N P
D P M V Q

Spot # 56

gij164698713 (100%), 19,198.3 Da

chloroplast ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit [Miscanthus x giganteus]

2 exclusive unique peptides, 2 exclusive unique spectra, 7 total spectra, 20/169 amino acids (12% coverage)

M A P T V M A S S A T A V A P F Q W L K S T A S L P V A R R S T T S L A K V S N G G R I R C M Q V W
P A Y G N K K F E T L S Y L P P L T E E Q L L K Q V D Y L L R N N W V P C L E F S K E G F V Y R E N
S T S P C Y Y D G R Y W T M W K L P M F G C T D A S Q V Y K E P Q E A I A S Y P D A Y V R I L G F D
N I R Q T Q C V S F I A Y K P P G S D

**Spot # 91**

gi|701216950 (100%), 20,781.5 Da

superoxide dismutase [*Saccharum spontaneum*]

2 exclusive unique peptides, 2 exclusive unique spectra, 2 total spectra, 25/207 amino acids (12% coverage)

```

MAAQSFL LAA TATATTAALF AAPYSSARPF HSAHFVAGPG GAAAAARVLV
VADASKKAVA VLKGTSEVEG VVTLTQD EDG PTTVNVR ITG LTPGLHGFHL
HEFGDTTNGC ISTGPHLNPN NLTHGAPED VRHAGDLGNI VANAEGVAEA
TIVDTQIPLS GPNSVVGRAF VVHELEDDLK GGHELST GNAGGR LACG
VVGLTPL

```

Spot # 612

gi|162462282 (100%), 38,605.0 Da

fructose-bisphosphate aldolase, cytoplasmic isozyme [*Zea mays*]

2 exclusive unique peptides, 2 exclusive unique spectra, 23 total spectra, 123/355 amino acids (35% coverage)

```

MSAYCGK YKD ELIKNAAYIG TPGKGILAAD ESTGTIGKRL SSINVENVEE
NRRALRELLF CCPGALQYIS GVILFEETLY QKTK DGKPFV DVLKEGGVLP
GIKVDKGTIE VVGTDKETT QGHDDLKRC AKYYEAGARF AKWRAVLKIG
PNEPSQLAID LNAQGLARYA IICQENGLVP IVEPEILVDG PHDIDRCAYV
TETVLAACYK ALNEHHVLE GTLLKPNMVT PGSDSK KVTP EVIAEYTVRT
LQRTVPAAVP AVLFLSGGQS EEEATRNLNA MNKLSTKKPW SLSFSFGRAL
QASTLKAWAG KVENLEKARA AFLARCK ANS EATLGTYKGD AAADTESLHV
KDYKY

```

Fig. S5. Validation of identified proteins in leaves of salt-treated SL population by Scaffold.

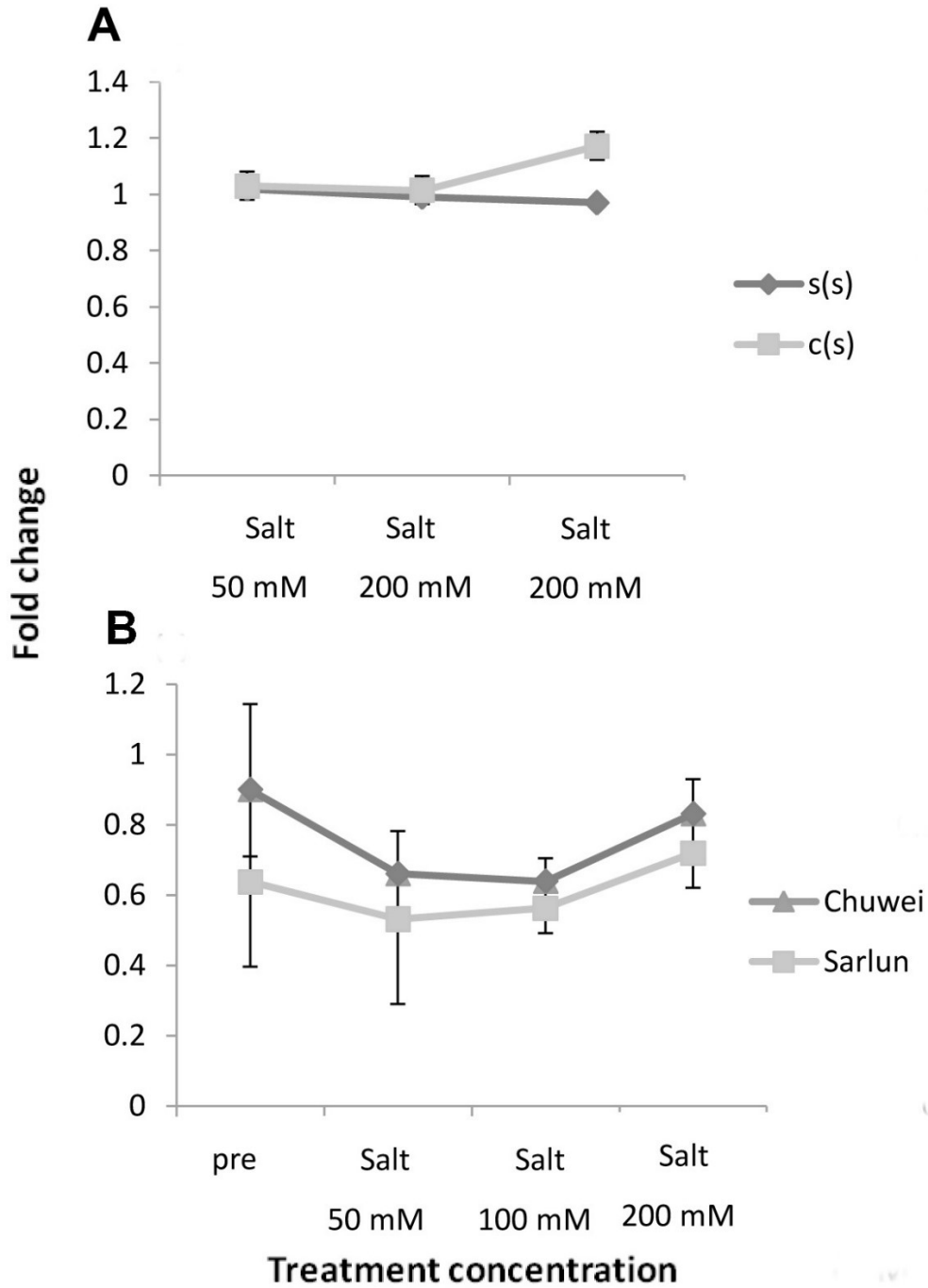


Fig. S6. Chlorophyll fluorescence yield of leaves from salt-treated Chuwei ecotype and Sarlun population. **(A)** and **(B)** shows results of the first and second water culture repeat, respectively; X axis indicates salt treatment by 50 mM, 100 mM, and 200 mM NaCl. Y axis indicates fold ratio against control Kimura's solution only. Pre, the day before treatment. C or Chuwei, samples from Chuwei ecotype; S or Sarlun, samples from Sarlun population. The treatment concentrations were changed every three days, and the last concentration was prolonged for one week. Data are mean \pm S.D, n =10.