IPD Project Details

Project ID: IPD1935

Project Title: Dehydration-induced mitochondrial proteome dynamics and defense response in rice

Description: Water-deficit or dehydration greatly influences plant development and reduces crop productivity, particularly in rain-fed areas across the world. Despite extensive research over the past several decades, little is known about dehydration management of mitochondria and cellular energy metabolism. We, for the first time, mapped dehydration-induced global changes in protein expression of the mitochondrial landscape to dissect the molecular mechanism, which might enable crop species to survive. Toward this, four-week-old rice seedlings were subjected to progressive dehydration by withholding water, and the stress severity was assessed by physicochemical reactions and mitochondrial architecture. The comparative mitochondrial proteomics analysis led to the identification of an array of stress-responsive proteins, presumably involved in a variety of cellular functions that includes energy production, organelle protein transportation and ROS detoxification, among others. The proteomics profile demonstrated that the alteration of tricarboxylic acid cycle intermediates is crucial for fuelling ATP production. Collectively, our results demonstrate that the global regulation of mitochondrial proteins is linked to dehydration response, which would favour genetic manipulation of crop species for better adaptation.

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Sample Preparation: [4] Sample processing protocol Aliquots of ~100 ?g rice mitochondrial enriched proteins from the unstressed control and dehydrated (3-d, 6-d and 9-d) samples were subjected to reduction and alkylation, followed by in-solution tryptic digestion (1:10 enzyme-substrate ratio) at 37°C.

Peptide Separation: The peptides were labelled by iTRAQ 4-plex Kit (AB Sciex, USA). The experiment was performed with three biological replicates. Unstressed control and 3-, 6- and 9-d dehydrated samples were labelled with 114, 115, 116 and 117 iTRAQ tags, respectively.

Protein Characterization: Protein identification and relative quantification were

performed by ProteinPilot software (v 4.5; AB Sciex, USA) using Paragon algorithm. The search parameters were: (i) sample type, iTRAQ 4-plex (peptide-labelled); (ii) cysteine alkylation, methyl methanethiosulfonate (MMTS); (iii) digestion, trypsin; (iv) instrument, TripleTOF 5600; (v) special factors, none; (vi) species, Oryza sativa; (vii) ID focus, biological modifications; and (viii) database, NCBInr.fasta (protein sequence). Proteins were identified with 1% FDR, and the results were exported to Excel for manual data interpretation. Among the identified proteins, those having unused score ? 1.3 with, at least, two peptides were considered for quantitative analysis. The variation among three replicates (control vs 9-d) was determined by plotting frequencies and percentage variations of the identified proteins. The proteins having missing value, in any of the replicates, were not considered for further analysis. A cut-off of 1.5-fold change was set to define differentially abundant status.

Experiment Type: Shotgun proteomics

Species: Oryza sativa

Tissue: Plant cell

Cell Type: Plant cell

Disease: Unknown

Instrument Details: TripleTOF 5600 (MS:1000932)

Protein Modifications: iodoacetamide derivatized residue

PubMed ID: