IPD Project Details

Project ID: IPD1218

Project Title: Integrated seed proteome and phosphoproteome analyses reveal interplay of nutrient dynamics, carbon-nitrogen partitioning and oxidative signaling in chickpea, part 2

Description: To understand nutrient dynamics during embryonic and cotyledonary photoheterotrophic transition to mature and germinating autotrophic seeds, 2-DE based phosphoproteomics study in six sequential seed developmental stages in chickpea was performed.

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Sample Preparation: Seed tissue was ground with mortar-pestle in liquid nitrogen with 0.3% (w/w) polyvinylpolypyrollidone (PVPP). Immediately, tissue powder was mixed with low ionic strength homogenizing buffer (30% sucrose, 2% SDS, 0.1 M Tris-HCl, pH 8.0, 5% 2-mercaptoethanol, 1 mM PMSF) for 2 min. The extract was then resuspended in equal volume of Tris-phenol (pH 8.0). The mixture was vortexed for 5 min and homogenized using homogenizer for 30 s three times followed by centrifugation at 10,000g for 10 min. The upper phenol phase was transferred to fresh tubes and at least 5 volumes of cold methanol in 0.1 M ammonium acetate was added and stored at -20ºC overnight. Precipitated proteins were recovered by centrifuging at 10,000 g for 5 min. Protein pellet was then washed twice with cold methanolic ammonium acetate and cold 80% acetone, respectively. The final pellet was dried and dissolved in 2-DE rehydration buffer (8 M urea, 4% CHAPS, 2% IPG buffer, 20 mM dithiothreitol) and quantified. Extracted proteins were separated by isoelectric focusing (IEF) using immobilized pH gradient gel strips (ImmobilineDryStrip, pH 4?7, 24 cm; GE Healthcare) followed by SDS-PAGE using 12.5% polyacrylamide gels. For phosphoproteins detection, 2-D gels were fixed overnight in fixation solution (50% methanol, 10% glacial acetic acid) and washed thrice with deionized water for 30 min with shaking. The gels were then stained with Pro-Q Diamond phosphoprotein gel stain solution (Invitrogen, Carlsbad, CA, USA) for 2 h with shaking followed by destaining with 20% acetonitrile, sodium acetate (pH 4), three times each for 30 min followed by washing with water thrice each for 10 min. After imaging, the same gels were then stained with a silver stain plus kit (Bio-Rad) to detect

total proteins.

Peptide Separation: Protein spots were excised from silver-stained gels and subjected to trypsin digestion as described by Ghosh et al., 2016. For each liquid chromatography coupled to tandem mass spectrometry run, trypsin-digested and eluted peptides were loaded onto a C18 Pep-Map100 column using a linear gradient of water/acetonitrile/0.1% formic acid (v/v) and analyzed by ESI using the Ultimate 3000 Nano HPLC system coupled to a QSTAR Elite mass spectrometer and peptides were eluted using a 33 min 10-40% acetonitrile gradient.

Protein Characterization: The MS/MS data was extracted using Analyst software version 1.5.1. Peptides were identified by searching the peak list against MSPnr100 Q315 database (75925788 sequences; 27045014025 residues) using the Mascot version 2.1 search engine. The database search criteria were as follows: taxonomy, Viridiplantae; peptide tolerance, 100ppm; MS/MS tolerance, ±0.4 D; peptide charge, +1, +2, or +3; maximum allowed missed cleavage, 1; fixed modification, carbamidomethylation; variable modification, oxidation (M), phospho (ST), phospho (Y) for phosphoproteins; and instrument type, ESI-QUAD-TOF. Protein scores were derived from ion scores as a nonprobabilistic basis for ranking protein hits and as the sum of a series of peptide scores. The score threshold to achieve $p < 0.05$ was set by the Mascot algorithm and was based on the size of the database used in the search.

Experiment Type: Gel-based experiment

Species: Cicer arietinum-3827

Tissue: Seed (bto:0001226)

Cell Type:

Disease: Unknown

Instrument Details: QSTAR (MS:1000190)

Protein Modifications: monohydroxylated residue, phosphorylated residue, Phospho, iodoacetamide derivatized residue, Carbamidomethyl, Oxidation

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