IPD Project Details

Project ID: IPD6869

Project Title: Integrated seed proteome and phosphoproteome analyses reveal interplay of nutrient dynamics, carbon-nitrogen partitioning and oxidative signaling in chickpea, part 3

Description: To understand nutrient dynamics during embryonic and cotyledonary photoheterotrophic transition to mature and germinating autotrophic seeds, TiO2-based phosphoproteomics study in three sequential seed developmental phases in chickpea was performed.

Principal Investigator: Dr Subhra Chakraborty

PI Affiliation: National Institute of Plant Genome Research, JNU Campus, Aruna Asaf Ali Marg, New Delhi-110067, India

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 8 M urea, 4% CHAPS, 20 mM dithiothreitol and quantified. Phosphopeptides were enriched using ProteoExtract phosphopeptide enrichment TiO2 kit. Seed proteins extracted from nutrient synthesis, accumulation and utilization phases were in-solution digested by TFE protein digestion protocol. Digestion procedure involved resuspension, denaturation, and reduction of extracted protein in 100mM ammonium bicarbonate, trifluroethanol and 200mM DTT for 1h at 60 °C followed by alkylation with 200 mM iodoacetamide for 1h in dark. Quenching of excess iodoacetamide was performed with 200mM DTT and pH of the solution was adjusted with 100mM ammonium bicarbonate for trypsin digestion.

Peptide Separation: Trypsin digestion was performed in 1:30 (enzyme to substrate

ratio) overnight at 37 °C. The digested protein solution was dried in vacuo and resuspended in 0.1% formic acid and diluted in 1:4 ratio with TiO2 Phosphobind Buffer containing 2,5-dihydroxybenzoic acid (DHB) as per user manual of phosphopeptide enrichment kit. The diluted peptide mixtures were then mixed with TiO2 Phosphobind resin vortexed for 10min and incubated overnight at 4ºC. The peptide-TiO2 Phosphobind resin mixture was washed twice with Wash Buffer 1 and Wash Buffer 2 provided in the kit, respectively. Finally, the phosphopeptides were eluted from the TiO2 Phosphobind Resin by elution buffer. The collected phosphopeptide solutions were dried in vacuo for LC-MS/MS analysis.

Protein Characterization: LC–MS analysis was performed on a Nanospray III source and a TripleTOF 6600 (AB SCIEX) mass spectrometer in information-dependent acquisition mode. The phosphopeptides were resuspended in 0.1% formic acid (FA) and loaded separately into an EkspertTM nanoLC (AB SCIEX) with a nanoLC trap (ChromXP C18-CL 3 ?m 120 Å, 350 ?m × 0.5 mm), before being washed for 15 min with a flow rate of 300 nL/min on the nanoLC trap and the nanoLC column (75 ?m × 15 cm, 3C18-CL-120, 3 ?m, 120 Å) was used for MS analysis. The mobile phases consisted of 0.1% FA and water (A), and 0.1% FA and ACN (B). A nonlinear gradient was employed, mass tolerance was set to 30 ppm, and the detection limit was 120 cps. The spray voltage was set to 2.3 kV, and the temperature of the heated capillary was set to 75 °C. MS spectra (250 msec) followed by 10 MS/MS spectra (100 msec each) were acquired in data-dependent mode. The dynamic exclusion time was set at 12 sec. Autocalibration using 50 fmol of tryptic peptides of beta-galactosidase was performed every four samples. The raw data generated by Analyst TF 1.6 (Build 6211) were converted to Mascot generic files by MS Converter (Sciex) and searched against MSPnr100 Q315 database (75925788 sequences; 27045014025 residues) using the ProteinPilot 2.0 (Sciex) using the Paragon algorithm. The database search criteria were as follows: taxonomy, Viridiplantae; peptide tolerance, 50 ppm; MS/MS tolerance, ±0.2 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. The significance threshold was set at $p < 0.01$, which gave an FDR of < 0.01.

Experiment Type: Shotgun proteomics

Species: Cicer arietinum-3827

Tissue: Seed (bto:0001226)

Cell Type:

Disease: Unknown

Instrument Details: TripleTOF 6600 (MS:1002533)

Protein Modifications: monohydroxylated residue, phosphorylated residue, iodoacetamide derivatized residue

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