IPD Project Details

Project ID: IPD5373

Project Title: TOMATO SEEDLING LIGHT INDUCED PROTEOMICS ALTERATON

Description: We use quantitative discovery proteomics along with metabolomics to unravel the role of Phytochrome A in regulating central metabolism.

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Sample Preparation: Total protein was extracted from tomato seedlings grownunder FR light and dark using Urea extraction protocol (Zhang, H. et al. 2016) with slight modification. The powdered seedling samples (?100 mg each) extracted with a urea-based lysis buffer . Tissue lysis was performed with sonication and bead milling. Clear supernatant was collected after centrifugation.

Peptide Separation: Sample was digested into peptides with trypsin .Peptides were subsequently cleaned up with C18 desalting columns and then iTRAQ labelling was performed according to the manufacturer’s instructions for the iTRAQ reagents 4-plex kit (AB Sciex Inc., Foster City, California, USA).After labelling, samples from the same set were combined together and lyophilized. The peptide mixtures were dissolved in 0.1% TFA. The samples were fractionated using a gradient of ACN and triethylamine by eluting the peptide with a linear gradient of 10–90% ACN in triethylamine. 7 fractions were collected and lyophilized for LC?MS/MS analysis. Each fraction was resuspended in 0.1% formic acid. MS/MS analysis was performed on a hybrid quadrupole Orbitrap (Q Exactive) mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) with high energy collision dissociation (HCD). The MS system was equipped with an automated Easy-nLC 1000 system (Thermo Fisher Scientific, Germerling, Germany). A linear gradient of solvent A (0.1% formic acid) to solvent B (0.1% formic acid, 99.9% acetonitrile) was run for 95 min, followed by a ramp to 98% B for 5 min.

Protein Characterization: Proteome Discoverer 1.4 with SEQUEST algorithm (Thermo Scientific Inc., Bremen, Germany) was used to search the original MS/MS protein data in the specified non-redundant databases. Protein identification was executed against the SGN tomato database ITAG3.0.Proteome Discoverer nodes for spectrum grouper and

spectrum selector were set using default parameters. Tolerances were set to a 10 ppm precursor mass tolerance and a 0.05 Da fragment mass tolerance. 1 maximum missed cleavage sites of trypsin digestion were allowed. Percolator was used for protein identification with parameters set with a strict target fale discovery rate (FDR) at 0.01 and a relaxed target FDR at 0.05.

Experiment Type: Shotgun proteomics

Species: Solanum lycopersicum -4081

Tissue: Seedling (bto:0001228)

Cell Type:

Disease: Unknown

Instrument Details: Other Q Exactive (MS:1001911), Q Exactive HF (MS:1002523)

Protein Modifications: acetylated residue, iodoacetamide derivatized residue

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