IPD Project Details

Project ID: IPD2566

Project Title: iTRAQ based disease responsive extracellular matrix proteome of jute infected with Macrophomina Phaseolina

Description: Extracellular matrix (ECM) is the first line of defense which is an inimitable organelle that perceives biotic and abiotic stresses and reprograms biological processes of host. It also activates innate immune responses in temporal and spatial manner and acts as physical scaffold that prevents the entry of pathogen and microbes in response to stress and hormonal signals. Stem rot, caused by Macrophomina phaseolina adversely affects fiber production in jute. However, how wall related susceptibility affects ECM proteome remains undetermined in bast fiber crops. Here, stem rot responsive quantitative temporal ECM proteome was developed in jute upon M. phaseolina infection. Using isobaric tags for relative and absolute quantitative proteomics and MS/MS analysis, we identified 415 disease responsive proteins (DRPs), involved in wall integrity, acidification, proteostasis, hydration and redox homeostasis. Disease-related correlation network identified functional hubs related to wall degrading enzymes, structural carbohydrates and signaling govern rot responsive wall-susceptibility.

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Sample Preparation: ECM fraction was isolated and purified from jute seedlings by grinding 25.0g seedling tissue in liquid nitrogen with 0.45% (w/w) polyvinylpolypyrollidone (PVPP). Ground tissue was homogenized three times in homogenizing buffer (5 mM K3PO4, pH 6.0, 5 mM DTT, 1 mM PMSF) for 2 min and centrifuged at 1000 × g for 10 min at 4 °C. Pellet was washed ten times with water to obtain purified ECM fraction. Three volumes of extraction buffer (200 mM CaCl2, 5 mM DTT, 1 mM PMSF, 0.3% (w/w) PVPP) was used to resuspend ECM fraction and kept on shaker for 2 h and centrifuged at 10 000 × g for 10 min at 4 °C. The fraction was then filtered with 0.45 ?m filter, concentrated using Centricon YM3 and dialyzed overnight against 1000 volumes of deionized water. ECM proteins were extracted, diluted, boiled and centrifuged at 4 °C. Pellets were washed twice with 80% acetone, air dried, and resuspended in 0.5 m TEAB at 25 °C. Protein concentration was determined by Bradford assay.

Peptide Separation: 85?g of ECM protein from different time points were trypsin digested overnight in the ration of 1:20 (trypsin:protein) at 37°C. Peptide digest was then dried in a speedvac and labeled for 2 h at room temperature using iTRAQ4-plex labelling kit by adding one reagent vial of an isobaric tag to each peptide digest of different time points according to the manufacturer's manual (AB Sciex, USA). The control sample was labeled with iTRAQ tag 114, and M. phaseolina treated jute samples was tagged with 115, 116, 117 for 24, 48, 96hpi, respectively. Excess iTRAQ reagent was quenched with 100ul water, followed by pooling of samples. Pooled samples were then SCX fractionated on an Agilent 1260 HPLC system (Agilent Technologies, Palo Alto, CA, USA) using a PolySulfoethyl column (4.6–100 mm, 5 ?m, 300 A) and eluted with a linear gradient of Buffer B (500 mMKCl, 10% ACN and 10 mM KH2PO4, pH 3.0). A total of 48 fractions were collected and pooled into eight fractions, desalted and dried before LC-MS/MS analysis. Experiments were performed in four biological replicates. Following SCX fractionation, 5?g of each fraction was injected into nano HPLC system. Peptides were resolved with a gradient of 10–40% acetonitrile (0.1% formic acid) over 110 min and analyzed on a TripleTOF6600 mass spectrometer (AB Sciex). Data were acquired using an ion spray voltage of 2.3 kV, curtain gas of 24, nebulizer gas of 20, and an interface heater temperature of 150°C. The MS was operated with a resolution of 30 000 fwhm for TOF MS scans. For IDA, survey scans were acquired in 250 msec, and 35 product ion scans were collected if they exceeded a threshold of 120 counts sec?1 with a +2 to +5 charge state. Total cycle time was 2.5 sec. A rolling collision energy setting was applied to precursor ions for collision-induced dissociation.

Protein Characterization: Raw data files (.wiff) acquired from TripleTOF 6600 System were converted into .mgf file format by ProteinPilotTM software (AB SCIEX, USA). Protein identification was performed by searching MS/MS spectra against a combined database of M. phaseolina (13803 proteins) and Corchorus olitorius O4 (35489 protein), and the FDR was estimated using integrated PSPEP tool. The database search search parameters included iTRAQ4-plex quantification, carbamidomethyl (C) as fixed modification, oxidation (M) and trypsin digestion. The screening criteria for DRPs included a fold change > 1.5 or < 0.67 and p< 0.05 in each replicate, FDR ? 1% and a peptide ? 1 based on protein abundance and comparison among groups. Differentially expressed proteins were identified and quantified with at least three unique peptides.

Experiment Type: Bottom-up

Species: Corchorus olitorius-93759

Tissue: Seedling (bto:0001228)

Cell Type:

Disease: Unknown

Instrument Details: TripleTOF 6600 (MS:1002533)

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue, iTRAQ4plex-116 reporter+balance reagent acylated residue

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