

IPD Project Details

Project ID: IPD6899

Project Title: Gel based extracellular matrix proteome of jute

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. In order to develop ECM proteome of *C. olitorius* var. O-4, ECM fraction was isolated by mechanical disruption, CaCl_2 extraction and enrichment. ECM fraction purity was assessed by 1-DE profiling in which the ECM fraction was found to be discrete from the crude extract and supernatant recovered during purification steps. Three biological replicates were performed for gel-based jute ECM proteomic analysis. MS/MS analysis of total extract and supernatants were identified as Rubisco or other chloroplastic proteins which were depleted in consecutive steps of purification depicting high degree of ECM purity. Fourteen bands of ECM enriched fraction were identified as canonical ECM localized proteins, e.g., beta-glucosidase, beta-d xylosidase, peroxidase, glycoside hydrolase and peroxiredoxin, confirming jute ECM protein purity and enrichment.

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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) polyvinylpolypyrrolidone (PVPP). Ground tissue was homogenized three times in homogenizing buffer (5 mM K_3PO_4 , pH 6.0, 5 mM DTT, 1 mM PMSF) for 2 min and centrifuged at $1000 \times g$ 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 CaCl_2 , 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 $10\,000 \times g$ for 10 min at 4 °C. The fraction was then filtered with 0.45 μm filter, concentrated using Centricon YM3 and dialyzed overnight against 1000 volumes of deionized water. During ECM fraction isolation, supernatant,

pellet after first centrifugation, and purified ECM proteins (150 µg) were collected and resolved on 13 cm 1DE. In brief, proteins loaded on stacking gel of 4% acrylamide/bisacrylamide (40%/2%), and resolving gel of 12.5% acrylamide/bisacrylamide (40%/2%) were run initially on 50 V for 30 min and then at constant 80 V. Resolved proteins were stained with Coomassie Blue R250. Gel images were scanned with Bio-Rad FluorS system (GE, Healthcare, Japan).

Peptide Separation: In gel trypsin digestion of the gel bands were performed in three biological replicates of ECM enriched fraction. Gel bands were cut and chopped into 2 X 2 mm pieces and thoroughly washed with sterile water several times. Gel pieces were then destained in 50 mM ammonium bicarbonate in 50% acetonitrile (ACN) with shaking at 37°C. Destained gel pieces were shrunk in 100% ACN for 10 min followed by reduction and alkylation in 10 mM Dithiothreitol (DTT) and 55 mM iodoacetamide, respectively. Gels were again destained for 15 minutes, shrunk in acetonitrile at room temperature, dried and trypsin (0.01 µg/ µl) digested at 30°C overnight with shaking. Samples were then concentrated to dryness by speedvac and kept at -20°C till further analysis. LC-MS/MS Analysis was performed on Nanospray III and TripleTOF 6600 (AB SCIEX) in information-dependent acquisition (IDA) mode. Peptides were desalted and resuspended in 0.1% formic acid and loaded into Eksigent nanoLC with nanoLC trap (ChromXP C18-CL 3 µm 120 Å, 350 µm × 0.5 mm) with flow rate of 300 nL/min on nanoLC column (75 µm × 15 cm, 3C18-CL-120, 3 µm, 120 Å). Mobile phases consisted of 0.1% FA and water (A), and 0.1% FA and ACN (B). Nonlinear gradient was employed and mass tolerance was set to 50 mDa, and detection limit was 120 cps. MS spectra were acquired for 1s with mass range of 400-1800 m/z in which survey scan for peptides of charge state 2+-5+ was performed in positive ion mode.

Protein Characterization: Raw data generated by Analyst TF1.6 were converted to Mascot generic files by MS Converter and searched against database of *Corchorus olitorius* O4 (35489 proteins). Database search parameters were: peptide tolerance, 100 ppm; fragment mass tolerance, 0.4 Da; maximum allowed missed cleavage 1; fixed amino acid modification as carbamidomethyl and variable amino acid modifications; oxidation (M) and deamidated with targeted decoy search in within ProteinPilot Software with FDR< 0.01. Gel-based proteomic analysis was conducted using three biological replicates; each consisting of 25 seedlings.

Experiment Type: Gel-based experiment, Bottom-up proteomics

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

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