IPD Project Details

Project ID: IPD1819

Project Title: Proteomic analysis of somatic embryo development in Musa spp. cv. Grand Naine (AAA)

Description: Somatic embryos are very much similar to zygotic counterparts in many morphological aspects and the somatic embryos are derived from somatic cells by undergoing various metabolic regulations. The somatic embryos have been used in artificial seed technology, genetic engineering and germplasm conservation. Though somatic embryo development is an important topic in growth and developmental studies, the molecular mechanism underlying the developmental process remains unclear. Therefore, understanding the molecular basis behind somatic embryo development can provide insight on the signaling pathways integrating this process. Proteomic analysis of somatic embryo development in cv. Grand Naine (AAA) was carried out to identify the differentially accumulated protein using two dimensional gel electrophoresis coupled with mass spectrometry. In total, 25 protein spots were differentially accumulated in different developmental stages of somatic embryos. Among them, three proteins were uniquely present in 30 days globular stage somatic embryos and six proteins were uniquely present in 60 days matured somatic embryo. Functional annotation of identified spots showed that major proteins are involved in growth and developmental process (17 %) followed by defense response (12%) and signal transportation events (12 %). In early stage, cell division and growth related proteins were involved in the induction of somatic embryos whereas in late developmental stage, cell wall modification proteins along with stress related proteins like played a defense role against dehydration and osmotic stress and resulted in maturation of somatic embryo. Alongside some identified stage specific proteins are valuable indicators and have been used as genetic markers.

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Sample Preparation: Plant materials and sample collection The embryogenic cell suspension (NGFB0189) generated from male flower bud of cv. Grand Naine (AAA) was used as an initiating material. After checking the viability of six month old embryogenic cell suspension using fluroscein diacetate (FDA) stain, one mL settled cell volume (SCV) of fine yellowish white homogenous suspension was plated on the somatic embryo regeneration medium supplemented with 80 µg/L kinetin , 200 µg/L napthaleneacetic

acid and 40 µg/L zeatin as suggested in INIBAP technical guidelines (Strosse et al., 2003) and maintained in complete darkness at 25±2 ?C. In total five plates were plated as replication. Embryos were collected in triplicates at 0, 30, 45 and 60 days after initiation of embryogenic cells on regeneration medium. All the collected samples were weighed, frozen with LN2 and stored in deep freezer for proteomic analysis. Protein extraction and 2DE Proteins were extracted from three biological replicates each with 200 mg of sample by following phenol ammonium acetate method as described by Kumaravel et al. (2016). The extracted protein pellets were resuspended in lysis buffer [7M Urea, 2M Thiourea, 4% CHAPS, 40mM DTT and 2% IPG buffer (pH 4-7)]. The protein mixture was vortexed for 1 hr at room temperature. The samples were then quantified using 2D quantification kit (GE Healthcare Bio- Science Crop, USA) at 480nm using Bovine serum albumin (BSA) as standard and purified using 2D clean up kit (GE Healthcare Bio- Science Crop, USA) before applying on immobiline pH gradient (IPG) strips. A total of 200 µg of each sample protein extracts were loaded on 13cm IPG strips (pH 4-7) (GE Healthcare Bio-Sciences AB, Sweden) for overnight rehydration. After 12-14 h, the rehydrated strips were subjected to first dimensional separation of proteins using Iso electric focusing (IEF) 100 unit (Hoefer Inc, San Francisco, USA) adopting the IEF conditions followed by Kumaravel et al. (2016). The focused strips were then subjected to two equilibration process as described by Sherifi et al., 2012. Then the equilibrated strips were placed on top of SDS - polyacrylamide gel (12%) for second dimensional separation of proteins using SE 600 unit (Hoefer Inc, San Francisco, USA) at 80 V for 30 min followed by 150 V for 5 h. Three biological replicates were performed for all the samples. Staining and Image analysis The gels were stained for overnight with colloidal coomassie brilliant blue (CBB) stain. Then the gels were destained with distilled water for several wash. The destained gels were documented using EPSON scanner (EPSON PERFECTION V 750 PRO, Seiko Epson Corp, Japan) at 800 dpi and images were documented. The documented images were analysed using Hoefer- 2D software (Hoefer Inc, San Francisco, USA) and spots with more than 1.5 folds were selected for further analysis. The volumes of reproducible spots from replicate gels were normalized against total spot volume.

Peptide Separation: In gel digestion and protein identification The excised gel spots were sent to Indian Institute of Science (Molecular Biophysics Unit, IISc, Bengaluru) for in gel digestion and processed for MALDI TOF- TOF instrument (UltrafleXtreme, Bruker Daltonics, Germany). The steps were followed based on the instructions provided on the website (http://proteomics.mbu.iisc.ac.in/). The PMF and MS/MS data were analysed using Flex analysis 3.1 software. The proteins were identified using MASCOT tool (www.matrixscience.com) and the search parameters were set as suggested by Kumaravel et al. (2016). Finally the MS/MS analysis was carried and proteins were identified by performing the following search criteria: Swissprot/ NCBInr for database, Trypsin for enzyme, no. 1 for missed cleavage, viridiplantae for taxonomy, carbamidomethyl (C) for fixed modification, oxidation (M) for variable modification, 5 to 200 ppm for peptide tolerance, + 1 for peptide charge, default for data format, error tolerant, decoy and precursor, MALDI-TOF-TOF for instrument and auto for report top.

Protein Characterization: The homologous protein spots were once again blast with Banana Genome Hub- South Green (https://banana-genome-hub.southgreen.fr/) to get protein identity. The identified proteins were successfully annotated through BLAST2GO and Uniprot analysis and functionally categorized based on their specific role during embryo development process.

Experiment Type: Gel-based experiment

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Early embryonic cell (metazoa) (cl:0000007), Plant cell (PO:0009002) No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue

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