IPD Project Details

Project ID: IPD5253

Project Title: Molecular analysis of somatic embryogenesis through proteomic approach and optimization of protocol in recalcitrant Musa spp.

Description: Somatic embryogenesis (SE) is a complex stress related process regulated by numerous biological factors. SE is mainly applicable to mass propagation and genetic improvement of plants through gene transfer technology and mutation breeding. In banana, SE is highly genome dependent as the efficiency varies with cultivars. To understand molecular mechanism of SE, proteomics approach was carried out to identify genes responsible for embryogenic calli (EC) induction, regeneration and germination of somatic embryos (se) in cv. Rasthali (AAB). In total, 70 spots were differentially expressed in various developmental stages of SE. Of which, 16 were uniquely expressed and 17 were highly abundant in EC than nonembryogenic calli and explant and four spots were also uniquely expressed in germinating se. Functional annotation of identified proteins revealed that calcium signaling along with stress and endogenous hormones related proteins played a vital role in EC induction and germination of se. Thus based on the outcome, callus induction media was modified and tested in five cultivars. In cv. Grand Naine (AAA), increased concentration of 3- IAA and tryptophan recorded highest EC induction of 24.28% while Red Banana with similar genome showed 18.96% in kinetin supplemented media. Similarly, in cultivars Monthan and Karpuravalli with ABB genome showed maximum EC induction in tryptophan supplemented media (8.54%) and CaCl2 enriched media (17.34%) respectively. In cv. Neypoovan (AB), higher concentration of tryptophan induced more EC. These results illustrated that EC formation is genome as well as cultivar dependent. Simultaneously, germination media was modified to induce proteins responsible for germination. In cv. Rasthali, media supplemented with 10 mM CaCl2 showed maximum increase in germination (51.79%) over control. Thus present study revealed that media modification based on proteomic studies can induce SE in recalcitrant cultivars and also enhance germination in cultivars amenable for SE.

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Sample Preparation: The excised gel spots were minced into small pieces (1mm3) and destained with 500 µl of wash solution containing 50 mM ammonium bicarbonate in 50%

acetonitrile and incubated at room temperature (RT) for 15 min with gentle vortexing. After removing the wash solution, the gel pieces were again washed twice with 500 µl of wash solution until the complete removal of coomassie dye. The gels were dehydrated with 100% acetonitrile (ACN) for 5 min until the gel turns to opaque white. ACN was removed and gels were completely dried at RT for 10 - 20 min using centrifugal evaporator. Around 150 µl of reduction solution containing 10 mM DTT and 100 mM ammonium bicarbonate was used to rehydrate the gel for 30 min at 56?C. Then reduction solution was discarded and 100 µl of alkylation solution containing 50 mM iodoacetamide and 100 mM ammonium bicarbonate was added and incubated for 30 min at RT in dark condition. Then alkylation solution was removed and again 500 µl of wash solution was added and incubated for 15 min at RT with gentle vortexing. After discarding the wash solution, the gel was dehydrated in 100 µl of 100% acetonitrile for 5 min.

Peptide Separation: ACN was discarded and the gel pieces were completely dried in centrifugal evaporator at RT. Finally rehydrated the gel with 20-30 µl of protease digestion solution made up of modified sequencing grade Trypsin (Product no V 5111, Promega, Madison, WI) which was resuspended in 1 mL of 50 mM ammonim bicarbonate. Overnight digestion was carried out at 37? C. The content was centrifuged at 12 kg for 30 sec and transferred the supernatant to sterile microfuge tube. Around 25-50 µl of extraction solution (60% ACN and 0.1% Tri fluroacetic acid - TFA) was added and sonicated in ultrasonic waterbath for 10 min. The tube was again centrifuged at 12 kg for 30 sec and the supernatant was transferred to new microfuge tube. The pooled extracted peptides were dried by centrifugal evaporation. Then 5 µl of resuspension solution (50% ACN and 0.1% TFA) was added and sonicated in water bath. Spined down the sample in same centrifugation condition and placed 0.5 µl on MALDI plate followed by 0.5 µl of alpha-cyano-4-hydroxycinnamic acid matrix (10 mg/mL in 50% acetonitrile, 0.1% TFA). Allowed the samples to dry completely and subjected the plate into voyager of MALDI TOF-TOF instrument (UltrafleXtreme, Bruker Daltonics, Germany) at Indian Institute of Science (Molecular Biophysics Unit, IISc, Bengaluru). The condition was calibrated using internal tryptic peaks of 842.5 and 2211.1 Da. Mass spectra were acquired using Flex control software (Bruker). The MS/MS data were acquired using smart beam laser.

Protein Characterization: The PMF and MS/MS data were analysed using Flex analysis 3.1 software. The proteins were initially identified using MASCOT tool (www.matrixscience.com) and the search parameters were followed according to Kumaravel et al. (2016). Finally the MS/MS analysis was carried and proteins were identified by performing by 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 500 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.

Experiment Type: Gel-based experiment

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: 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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