Supplementary Material

Fine-tuning and remodeling of pectins play a key role in the maintenance of cell adhesion

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Supporting Experimental procedures:

Monosaccharides quantification by GC/MS

The monosaccharides of the cell wall were analyzed with the protocol according to (Clement et al., 2018). Four biological replicates of approximately 300 seedlings cultivated in the dark over 4 days were harvested in a dark room and placed directly in the 96 ° ethanol for 1 h at 80 ° C to fix the sample. After removing the ethanol, the first step is repeated for 20 minutes. 1 ml of acetone is added and the tubes are placed in a thermomixer for 20 min at 25 ° C, this step is repeated twice. The cell walls are then dried in a high vacuum concentrator at room temperature overnight. 1 mg dry cell wall previously weighed is resuspended in 400 µL of TFA (2 M, freshly prepared), and incubate at 120 ° C for 1 h in a heat block in 1.5 mL screwcap tubes. Then samples were centrifugated for 10 min. The supernatant was transferred and dried in a speed vacuum concentrator at room temperature.

Derivatization: after adding 10 µL of 20 mg/ml methoxamine in pyridine to the samples, the reaction was performed for 90 min at 28 °C under continuous shaking in an Eppendorf thermomixer. 50 µL of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) (Sigma M7891-10x1 mL) were then added and the reaction continued for 30 min at 37 °C. After cooling, 45 µL were transferred to an Agilent vial for injection.

Data processing: Raw Agilent datafiles were converted in NetCDF format and analyzed with AMDIS http://chemdata.nist.gov/mass-spc/amdis/. A home retention indices/ mass spectra library built from the NIST, Golm, http://gmd.mpimp-golm.mpg.de/ and Fiehn databases and standard compounds were used for metabolites identification. Peak areas were also determined with the Targetlynx software (Waters) after conversion of the NetCDF file in masslynx format. AMDIS, Target Lynx in splitless and split 30 modes were compiled in one single Excel file for

comparison. After blank mean subtraction, peak areas were normalized to Ribitol and dry weight.

Absolute quantification: A response coefficient was determined for 4 ng each of a set of monosaccharides, respectively to the same amount of ribitol. This factor was used to give an estimation of the absolute concentration of the metabolite in what we may call a “one point calibration”.

High-performance size exclusion chromatography (HP-SEC) coupled with mass spectrometry analysisChromatographic separation was conducted using an ACQUITY UPLC Protein BEH SEC Column (125Å, 1.7 µm, 4.6 mm × 300 mm, Waters Corporation, Milford, MA, USA) coupled with a guard Column BEH SEC Column (125Å, 1.7 µm, 4.6 mm × 30 mm). Elution was performed with a flow rate of 400 µl/min at a column oven temperature of 40 °C using 50 mM ammonium formate containing 0.1 % formic acid. The injection volume was set to 10 µl. ESI MS-detection was carried out in negative mode on a Bruker impact II QTOF with the following parameters: end plate offset set voltage of 500 V, capillary voltage of 4000 V, Nebulizer at 40 psi, dry gas flow at 8 l/min, and dry temperature at 180 °C. Data acquisition was performed using Compass 1.8 software (Bruker Daltonics). Data analysis was conducted using Mzmine 2.53 software, as described by Pluskal et al. (2010). The integration process involved the following steps: mass detection with a filter noise level set to 500, ADAP Chromatogram Builder using the parameters range of 6 - 9.60 min, minimum group size of scan of 10, group intensity threshold of 1500, minimum highest peak of 1000, and m/z tolerance of 0.01 or 10 ppm. The chromatogram peaks were deconvoluted using baseline cut-off with the baseline level set at 300. Chromatograms were deisotoped with an m/z tolerance of 0.01 or 5 ppm, retention time tolerance of 0.1, maximum charge of 2, and representative isotope as the most intense. Subsequently, peaks were aligned with an m/z tolerance of 0.01 or 5 ppm, retention time tolerance of 0.1, and equal weight for m/z and retention time (weight=1). Finally, a gap-filled peak finder was applied with an m/z tolerance of 0.01 or 5 ppm, retention time tolerance of 0.1, and intensity tolerance of 20 %. The peak areas were exported at the end.

Protein expression and application on seedlings

The coding sequence of *PME53, PAE7 &12* without their signal peptide and codon optimization was synthetized and cloning by PROTEOGENIX company (Oberhausbergen, France,

http://www.proteogenix.fr) into pPICZαB α-factor (Invitrogen) with EcoRI and NotI restriction enzymes (marque des enzymes) Otherwise, for the other genes (PME 41, 35 & PMEI 4) the cloning protocol was performed according to (Lemaire et *al.*, 2020) with the appropriate primers. After E. coli TOP10 (Invitrogen) transformation, recombinant plasmids were linearized with PmeI NEW England Biolabs enzyme and were inserted into X-33 P. *pastoris* (Invitrogen) for protein expression. The culture media were prepared according to the protocol from the Pichia Protein Expression Kit (Invitrogen). The transformed *P. pastoris* strain was grown overnight at 30 °C in baffled flasks in 10 ml of buffered glycerol–complex medium. Cells were then collected by centrifugation and resuspended to an OD600 of 1.0 in 100 ml of buffered methanol complex medium. A final concentration of 0.5 % (v/v) methanol was maintained every 24 h for induction. Supernatants are recovered after centrifugation (1 500×g, 8 min, 4 °C) and filtered with GD/X0.45 µm PES filter Media (Whatman, Maidstone, United Kingdom). 50 mL were concentrated to 500 µL with an Amicon Ultra Centrifugal filter with a 10 or 3 kDa cut-off (Merck Millipore, Burlington, Massachusetts, United States). Buffer exchange of concentrated protein was performed using PD Spintrap G-25 column (GE Healthcare). The buffer used was H_2O buffered to pH 5.7 with MES.

20 µL supernatant containing the enzyme was applied at 48 h after the start of the culture on 2 biological replicates of approximately 50 seedlings cultivated in the dark over 4 days. Seedlings were stained by ruthenium red (0.5 mg/mL) during 2 minutes and observed, after rinsing with water, using binocular loupe.

Enzyme effects on cell adhesion were phenotyping by ruthenium red staining.