Metabolite extraction and metabolite profiling analysis

Metabolites were extracted according to Lisec et al., (2006) with some modifications. Briefly, about 100 mg of each sample was taken and crushed in 100% methanol and the mixture was vortexed for 15 min. Subsequently, the tubes containing the homogenate were centrifuged at 14, 000 g at 4 °C for 10 min. The supernatant was taken and mixed with 750 µL chloroform and 1400 µL of Milli Q. Afterwards, the mixture was centrifuged for 15 min at 2, 200 g at 4 °C.

Finally, the supernatant was taken and dried in vacuum concentrator and stored at -80 °C till use. Prior to GC-MS analysis, the dried samples were dissolved and derivatized in a two-step procedure. First, 40 µL of methoximation mixture of methoxylamine hydrochloride dissolved in pyridine (20 mg mL−1) was added and placed in a thermomixer for 2 h at 37 °C. This was followed by trimethylsilylation with 40 µL of N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) at 37 °C for 30 min and cooled to room temperature before injection. A 1 µl aliquot of the analyte was injected in splitlessmode in an ISQ 7000 single quadrupole GC-MS system (Thermo Scientific, San José, CA, USA).

Helium was used as the carrier gas, the front inlet purge flow was 3 ml_min−1, and the gas flow rate through the column was 1 mL_min−1. The initial temperature was kept at 90°C for 0. 25 min, then raised to 180°C at a rate of 10°C_min−1, then raised to 240°C at a rate of 5°C_min−1, and finally to 285°C at a rate of 20°C_min−1 for 11. 5 min. The injection, transfer line, and ion source temperatures were 280, 270 and 220°C, respectively. The energy was -70 eV in electron impact mode. The mass spectrometry data were acquired in full-scan mode 5. Analysis of metabolomics data to

elucidate the PGPR induced metabolites and pathways responsible for nutrient stress tolerance Raw GC-MS data files obtained from acquisition were aligned and processed using XCMS online package (www. xcmsonline. scripps. edu). Metabolites were identified by comparing the mass spectra and fragmentation pattern of individual metabolites with those of the NIST-Wiley Mass Spectra Library. Then, data normalization for each sample was done by the total sum of the signal integration area.

Next, a principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed by MetaboAnalyst online software (www. metaboanalyst. ca/) using normalized data. Also, Student’s t test (P <0. 01) was performed to find differential metabolites. Subsequently, a metabolic pathway was constructed according to pathway analysis on the MetaboAnalyst online software (www. metaboanalyst. ca/) and KEGG (http://www. genome. jp/kegg/) based on the changes in metabolite concentrations compared with those of the respective controls.