Metabolomics metadata

Fukushima, Kusano et al., in preparation

The metadata stored in MeKO are based on the Metabolomics Standards Initiative (MSI; Fiehn et al., 2007) and the Minimal Information for a Metabolomic Experiment standards (Bino et al., 2004).

1.1 BioSource

1.1.1 Species

Arabidopsis thaliana (ecotype: Col-0 or Col)

The mutants used in this study are as follows. All the mutants were purchased from ABRC.

Line	AGI or ID	Background	Link
aat2-2	AT5G19550	Col	CS3971
aba1-5	AT5G67030	Col	CS155
aba1-6	AT5G67030	Col-0	CS3772
aba2-1	AT1G52340	Col	CS156
aba2-3	AT1G52340	Col	CS156
aba3-1	AT1G16540	Col	CS157
amt1-1	AT4G13510	Col	CS6168
cim10	CIM10	Col	CS6574
cim11	CIM11	Col	CS6575
cim13	CIM13	Col	CS6576
cim14	CIM14	Col	CS6577
cim6	CIM6	Col	CS6571
cim7	CIM7	Col	CS6572
cim9	CIM9	Col	CS6573
cla-1S	AT4G15560	Col	CS16003
cob-2	AT5G60920	Col	CS8542
eto1-1	AT3G51770	Col	CS3072
eto3	AT3G49700	Col	CS8060
fad5-1	AT3G15850	Col	CS206
fad6-1	AT4G30950	Col	CS207
fah1-2	AT4G36220	Col	CS6172
fur1-1	AT4G05120	Col	CS3729

fus6-1S	AT3G61140	Col	CS16057
gsr1-1	GSR1	Col	CS6391
ixr1-1	AT5G05170	Col	CS6201
ixr1-2	AT5G05170	Col	CS6202
mur1-1	AT3G51160	Col	CS6243
mur1-2	AT3G51160	Col	CS6244
mur2-1	AT2G03220	Col	CS8565
mur3-2	AT2G20370	Col	CS8567
mur4-2	AT1G30620	Col	CS8569
mur5-1	MUR5	Col	CS8572
mur6-1	MUR6	Col	CS8573
mur7-1	MUR7	Col	CS8574
mur8-1	MUR8	Col	CS8575
mur9-1	MUR9	Col	CS8576
mur11-1	MUR11	Col	CS8579
pac-1S	AT2G48120	Col	CS16058
pad2-1	AT4G23100	Col	CS3804
pad3-1	AT3G26830	Col	CS3805
pad4-1	AT3G52430	Col	CS3806
pap1-D	AT1G56650	Col	CS3884
phyB-9	AT2G18790	Col	CS6217
prc1-1	AT5G64740	Col	CS297
rsw1-1	AT4G32410	Col	CS6554
rsw2-1	AT5G49720	Col	CS6555
rsw3-1	AT5G63840	Col	CS6556
sng1-1	AT2G22990	Col	CS3737
tbr-1	AT5G06700	Col	CS3741
vtc1-1	AT2G39770	Col-0	CS8326

1.1.2 Organ

Aerial parts

1.1.3 Amount

The harvested samples with fresh weight (FW) over 7 mg were used for GC-MS-based metabolite profiling. An equivalent of 55.6 μg of the derivatized samples were injected.

1.1.4 Growth condition

The sterilized seeds were stratified at 5°C for 2 days, and were sown on Murashige and Skoog (MS) medium containing 1% sucrose. Seedlings of *Arabidopsis* Col-0 and the mutants were cultivated in controlled growth chambers at 22°C in the 16-h light and 8-h dark condition for 18 days (Light strength, approx. 80 PPF).

1.1.5 Experimental condition

Same as section 1.1.4.

1.1.6 Sampling and sampling date

The leaves were harvested in September-December in 2008.

1.1.7 Metabolism quenching method

Samples were grown under the condition shown in section 1.1.4. Then, the aerial part of each sample was harvested. All the plant materials were frozen immediately in liquid nitrogen to quench the enzymatic activity.

1.2 Chemical analysis metadata

1.2.1 Sample processing and extraction

Each sample was extracted with a concentration of 5 mg flesh weight (FW) of tissues per ml extraction medium (methanol / chloroform/water [3:1:1 v/v/v]) containing 10 stable isotope reference compounds:

- [$^{2}H_4$]-succinic acid,
- [$^{13}C_5$,^{15}N]-glutamic acid,
- [$^{2}H_7$]-cholesterol,
- [$^{13}C_3$]-myristic acid,
- [$^{13}C_5$]-proline,
- [$^{13}C_{12}$]-sucrose,
- [$^{13}C_4$]-hexadecanoic acid,
- [$^{2}H_4$]-1,4-butanediamine,
- [$^{2}H_6$]-2-hydoxybenzoic acid and
- [$^{13}C_6$]-glucose

using a Retsch mixer mill MM310 at a frequency of 30 Hz for 3 min at 4°C. Each isotope compound was adjusted to a final concentration of 15 ng μl^{-1} for each 1-μl

injection. After centrifugation for 5 min at 15,100 × g, a 100-μl aliquot of the supernatant was drawn and transferred into a glass insert vial. The extracts were evaporated to dryness in an SPD2010 SpeedVac® concentrator from ThermoSavant (Thermo electron corporation, Waltham, MA, USA). For methoximation, 30 µl of methoxyamine hydrochloride (20 mg/ml in pyridine) was added to the sample. After 24 h of derivatization at room temperature, the sample was trimethylsilylated for 1 h using 30 µl of MSTFA with 1% TMCS at 37°C with shaking. For methoximation, 30 µl of methoxyamine hydrochloride (20 mg ml^{-1} in pyridine) was added to the sample. After 24 h of derivatization at room temperature, the sample was trimethylsilylated for 1 h using 30 μl of MSTFA with 1% TMCS at 37°C with shaking. Thirty μl of *n*-heptane was added following silylation. All the derivatization steps were performed in the vacuum glove box VSC-100 (Sanplatec, Japan) filled with 99.9995% (G3 grade) of dry nitrogen.

1.2.2 GC-TOF/MS conditions

One microliter of each sample was injected in the splitless mode by an CTC CombiPAL auto-sampler (CTC analytics, Zwin-gen, Switzerland) into an Agilent 6890N gas chromatograph (Agilent Technologies, Wilmingston, USA) equipped with a 30 m × 0.25 mm inner diameter fused-silica capillary column with a chemically bound 0.25-μl film Rtx-5 Sil MS stationary phase (RESTEK, Bellefonte, USA) for metabolome analysis. Helium was used as the carrier gas at a constant flow rate of 1 ml min^{-1}. The temperature program for metabolome analysis started with a 2-min isothermal step at 80 °C and this was followed by temperature ramping at 30 °C to a final temperature of 320 °C, which was maintained for 3.5 min. The transfer line and the ion source temperatures were 250 and 200 °C, respectively. Ions were generated by a 70-eV electron beam at an ionization current of 2.0 mA. Data acquisition was performed on a Pegasus IV TOF mass spectrometer (LECO, St. Joseph, MI, USA) with an acquisition rate of 30 spectra s^{-1} in the mass range of a mass-to-charge ratio of m/z = 60-800.

Alkane standard mixtures (C8-C20 and C21-C40) were purchased from Sigma-Aldrich (Tokyo, Japan) and were used for calculating the retention index (RI). The normalized response for the calculation of the signal intensity of each metabolite from the mass-detector response was obtained by each selected ion current that was unique in each metabolite MS spectrum to normalize the peak response. For quality control, we injected methylstearate in every 6 samples. Quality control samples were prepared by mixing 100 µl of extracts of each sample.

1.2.3 Data processing

Non-processed MS data from GC-TOF/MS analysis were exported in NetCDF format generated by chromatography processing and mass spectral deconvolution software, Leco ChromaTOF version 3.22 (LECO, St. Joseph, MI, USA) to MATLAB 6.5 (Mathworks, Natick, MA, USA), where all data-pretreatment procedures, such as smoothing, alignment, time-window setting, and peak deconvolution, were carried out by using hyphenated data analysis (HDA) (Jonsson et al., 2004; Jonsson et al., 2006). The resolved MS spectra were matched against reference mass spectra using the NIST mass spectral search program for the NIST/EPA/NIH mass spectral library (version 2.0) and our custom software for peak annotation written in JAVA. Peaks were identified or annotated based on RIs and the reference mass spectra comparison to the Golm Metabolome Database (GMD) and our in-house spectral library. The metabolites were identified by comparison with RIs from the library databases (GMD and our own library) and with those of authentic standards, and the metabolites were defined as annotated metabolites on comparison with mass spectra and RIs from these two libraries. The five batches of metabolite profiles were combined using the HDA method (Jonsson et al., 2004; Jonsson et al., 2006). To correct the “batch effect” we used COMBAT normalization (Johnson et al., 2007) with our quality samples consist of Col-0 wild-type plants for each batch. Data was normalized using the CCMN algorithm (Redestig et al., 2009).

References

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