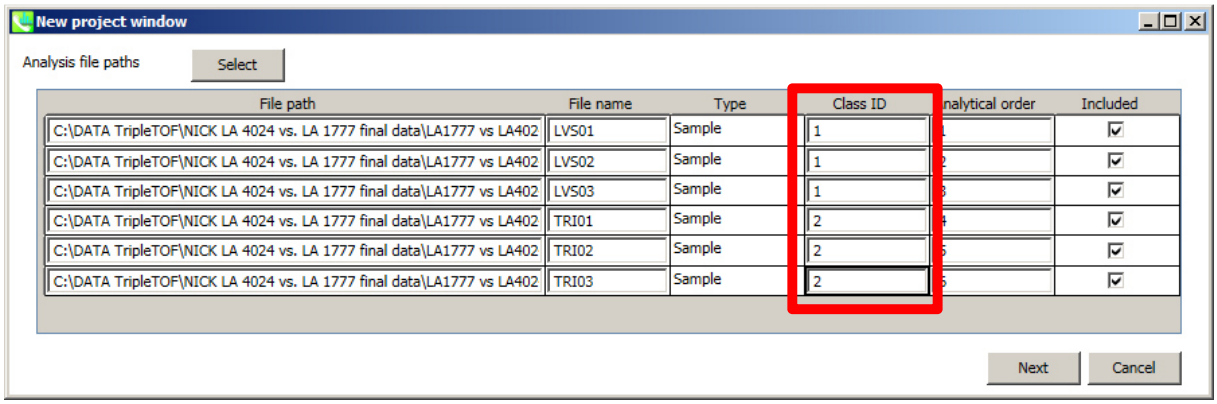
Supplementary Note 1 - Showcase Protocol

Using MS-Dial, we processed six UPLC-(-)ESI-SWATH-MS/MS runs of triplicate extracts from tomato leaf and trichome matter for feature detection, ion species annotation, peak alignment, and compound spectra purification.

For later analysis in MetFamily, class IDs are assigned during raw data upload in MS-DIAL (Supplementary Note Fig. 1).



Supplementary Note 1 – Figure 1. Hierarchical Cluster Dendrogram of LA1777

After pre-processing the data in MS-DIAL, we exported the resulting MS^1 metabolite profile to file 'Metabolite_profile_showcase.txt' and we exported the extracted purified MS/MS spectra to the MS/MS library in file 'MSMS_library_showcase.msp' (Supplementary Data 1 and 2). In order to compile a set of regulated metabolite families as contained in the MetFamily project file (Supplementary Data 4), we imported and processed the data of both files with MetFamily as follows.

1. Import MS^1 and MS/MS data in tab 'Input' under 'Import data'
    a. MS^1 data file 'MS_abundances_showcase.txt' (Supplementary Data 1)
    b. MS/MS-library 'MSMS_library_showcase.msp' (Supplementary Data 2)
    c. Default import parameters (Supplementary Table 2)
    d. Press button 'Import MS^1 and MS/MS data'

This process takes several minutes. During this time the fragment matrix is fused from both files. This resulting data can be exported in the tab 'Export' under 'Export project'.

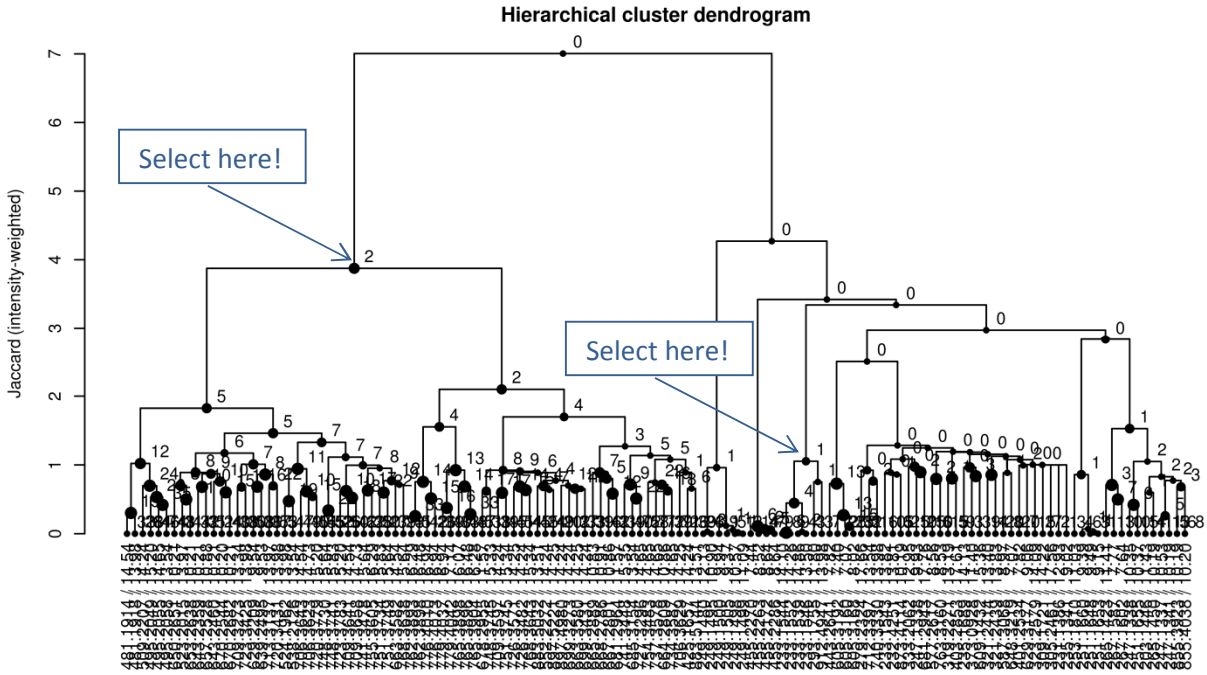
2. Perform hierarchical cluster analysis in tab 'HCA'

a. Apply MS^1 abundance filter
   i. Average MS^1 abundance: 20,000 counts
   ii. MS^1 log_2-fold change: 2
   iii. Group 1: TRI
   iv. Group 2: LVS
   v. Press 'Apply filter'

   The reasoning behind this was suppress noise and to focus on highly abundant metabolite families in GT.

b. Calculate hierarchical cluster dendrogram
   i. Default HCA properties
   ii. Press 'Draw hierarchical cluster'

This process takes a few seconds. On the right pane a hierarchical cluster dendrogram appears showing two main clusters (Supplementary Note 1 - Fig. 2).



Supplementary Note 1 – Figure 2. Hierarchical Cluster Dendrogram of LA1777

Each leaf corresponds to one MS^1 feature and each branch corresponds to one putative cluster of MS^1 features. The number adjacent to each branch indicates the number of highly common fragments or neutral losses amongst the MS^1 features of the corresponding cluster.

c. Select the left main branch as a click of the left mouse button and examine common fragments.

There are eight common MS/MS fragments which are characteristic to this clade of which two MS/MS-fragments (101.0603, 305.0864) are present in at least 75% of all spectra in this clade. According to own MS/MS analyses and reference data shown previously (Gosh et al. 2014) we annotate the clade as acyl sugars 'AS' in blue.

d. Annotate the selected clade as 'AS'.
   i. Scroll to the lower part of the panel to 'MS feature selections'
   ii. Select 'Selections by analysis'
   iii. Select tab 'Annotation'
   iv. In section 'Add new annotation', type 'AS' into the field below 'Type new annotation'
   v. Select the colour blue
   vi. Press button 'Add new annotation'

The updated hierarchical cluster dendrogram can be examined by scrolling up again and clicking the white space around the dendrogram with the left mouse button to de-select the left main clade.

e. Annotate further clades analogously as listed below and shown in Figure Supplementary Note 1 – Fig. 2:

Supplementary Note 1 - Table 1: Overview of MS^1 feature clusters which correspond to metabolite families.

metabolite family	colour	# of MS^1	characteristic fragments

AS	blue	73	87.0451, 101.0603, 161.0443, 305.0684, 323.0957, 393.1394, 411.1496, 425.1652
SQT-glycosides	red	4	-504.2939, -492.2943 -444.2704, -204.0640, -162.0632, -42.0100, 101.0242, 113.0234, 401.2548, 443.2611, 563.3051, 605.3176

3. Perform principal component analysis in tab 'PCA'

a. Use blank MS abundance filter
b. Calculate principal components
   i. Default PCA properties
   ii. Press 'Draw principal components'

On the right pane the PCA scores plots and the PCA loadings plot appear. The PC1 scores indicate a clear separation between the replicates of both groups. The PCA loadings now carry the colour codes for MS^1 features which were selected during previous HCA.

4. Export project file in tab 'Export' pressing button 'Export project'

This will download the current state of the project as illustrated in Supplementary Data 4. This file can be reloaded into MetFamily in tab 'Input' under 'Load project' for further data analysis.