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*Functional Plant Biology*

**Supplementary Material**

# Photosynthesis-related physiology and metabolomics responses of *Polygonum lapathifolium* in contrasting manganese environments

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**Details of metabolite analysis methods**

The raw MS data were acquired on the Q-Exactive using Xcalibur 4.1 (Thermo Scientific), and processed using Progenesis QI (Waters Corporation, Milford, USA). to identify features, deisotope, align features, and perform a gap-filling to fill in any features that may have been missed in the first alignment algorithm. Finally, a data matrix of retention time (r/t), mass to charge ratio (m/z), and peak strength is obtained. All adducts and complexes were identified and removed from the data set. The data matrix was searched at http://www.hmdb.ca/, https://metlin.scripps.edu/. And other public metabolite libraries and self-built metabolite libraries. Quantified data were output into excel format. Data were analyzed by R, where it was subjected to multivariate data analysis, including the unsupervised method's principal component analysis (PCA) and the supervised method's orthogonal partial least-squares discriminant analysis (OPLS-DA). The the7-fold cross-validation and response permutation testing were used to evaluate the robustness of the model. The variable importance in each variable's projection (VIP) value in the OPLS-DA model was calculated to indicate its contribution to the classification. Metabolites with the *VIP* value >1 were further applied to Student's t-test at the univariate level to measure the significance of each metabolite, the *P* values less than 0.05 were considered statistically significant.

PCA Unsupervised PCA (principal component analysis) was performed using the statistics function prcomp within R (www.r-project.org). The data was unit variance scaled before unsupervised PCA.

Differential metabolites selected Significantly regulated metabolites between groups were determined by $VIP \geq 1$ and $P < 0.05$. *VIP* values were extracted from the OPLS-DA result, which also contains score plots and permutation plots, and were generated using the R package MetaboAnalyst R. The data was log transform ($log_2$) and mean centering before OPLS-DA. To avoid overfitting, a permutation test (200 permutations) was performed. The Fold Change Analysis of the Significantly regulated metabolites was performed by R. If Fold change (Fc) > 1, it showed that the

concentration of this metabolite increased compared with the control. Results are the opposite if Fc < 1.

Hierarchical Cluster Analysis and Pearson Correlation Coefficients The HCA (hierarchical cluster analysis) results of samples and metabolites were presented as heatmaps with dendrograms, while Pearson correlation coefficients (PCC) between samples were calculated by the cor function in R and presented as only heatmaps. Both HCA and PCC were carried out by R package Complex Heatmap.

KEGG annotation and enrichment analysis Identified metabolites were annotated using the KEGG Compound database (http://www.kegg.jp/kegg/ compound/), and annotated metabolites were then mapped to the KEGG Pathway database (http://www.kegg.jp/ kegg/pathway.html). Pathways with significantly regulated metabolites mapped to were then fed into MSEA (metabolite sets enrichment analysis), and their significance was determined by the hypergeometric test's *P* values. For comparison between different pathways, differential metabolites were input into MetPa (https://www.metaboanalyst.ca/) for path topology analysis, the comprehensive score of each path is normalized to 1, and the important measurement of each biological molecule obtains a weighted score according to its relative position importance; The cumulative importance score of the current pathway was obtained by calculating the weighted score of matched metabolism.

**Supplementary Table S1.** Details of KEGG pathway analysis of the leaves of *P. lapathifolium* with and without additional Mn shown in Fig. 6

| **Pathway name** | **Class** | **Differentially accumulated metabolites and KEGG pathway ID** | **Enrichment factor** | ***P* value** |
|---|---|---|---|---|
| ABC transporters | Environmental Information Processing | C00062(L-arginine);C00492(raffinose);C00079(L-phenylalanine) | 0.02 | 0.01 |
| Aminoacyl-tRNA biosynthesis | Genetic Information Processing | C00062(L-arginine);C00079(L-phenylalanine) | 0.04 | 0.01 |

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|---|---|---|---|---|
| Biosynthesis of amino acids | Metabolism | C00062(l-arginine);C00079(L-phenylalanine) | 0.02 | 0.02 |
| D-Arginine and D-ornithine metabolism | Metabolism | C00062(L-arginine) | 0.09 | 0.02 |
| Arginine biosynthesis | Metabolism | C00062(L-arginine) | 0.04 | 0.04 |
| Phenylalanine, tyrosine and tryptophan biosynthesis | Metabolism | C00079(L-ahenylalanine) | 0.03 | 0.06 |
| Monobactam biosynthesis | Metabolism | C00062(L-arginine) | 0.03 | 0.06 |
| Cyanoamino acid metabolism | Metabolism | C00079(L-phenylalanine) | 0.02 | 0.07 |
| Galactose metabolism | Metabolism | C00492(raffinose) | 0.02 | 0.07 |
| Flavone and flavonol biosynthesis | Metabolism | C05623(isoquercitrin) | 0.02 | 0.08 |
| Isoflavonoid biosynthesis | Metabolism | C10503(medicarpin) | 0.02 | 0.10 |
| Phenylpropanoid biosynthesis | Metabolism | C00079(L-phenylalanine) | 0.01 | 0.10 |
| Tropane, piperidine and pyridine alkaloid biosynthesis | Metabolism | C00079(L-phenylalanine) | 0.01 | 0.11 |
| Phenylalanine metabolism | Metabolism | C00079(L-phenylalanine) | 0.01 | 0.11 |
| Glucosinolate biosynthesis | Metabolism | C00079(L-phenylalanine) | 0.01 | 0.12 |
| Arginine and proline metabolism | Metabolism | C00062(L-arginine) | 0.01 | 0.12 |
| Ubiquinone and another terpenoid-quinone | Metabolism | C05804(2-hexaprenyl-3-methyl-6-methoxy-1,4 benzoquinone) | 0.01 | 0.14 |

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|---|---|---|---|---|
| biosynthesis | | | | |
| Purine metabolism | Metabolism | C00366(uric acid) | 0.01 | 0.14 |
| Biosynthesis of secondary metabolites - unclassified | Metabolism | C00062(L-arginine) | 0.01 | 0.15 |
| 2-Oxocarboxylic acid metabolism | Metabolism | C00079(L-phenylalanine) | 0.01 | 0.19 |