

Supplementary material

Article title: Application of Exogenous Jasmonic Acid Enhances the Resistance of Pak Choi (*Brassica chinensis* L.) to High Levels of Selenium-Toxicity in the Soil

Journal name: Plant and Soil

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Supplementary Methods 1

Extraction, identification, and data analysis of metabolites

1. Metabolites Extraction

Tissues (100 mg) were individually grounded with liquid nitrogen and the homogenate was resuspended with prechilled 80% methanol and 0.1% formic acid by well vortex. The samples were incubated on ice for 5 min and then were centrifuged at 15,000 rpm, 4 °C for 5 min. Some of supernatant was diluted to final concentration containing 53% methanol by LC-MS grade water. The samples were subsequently transferred to a fresh Eppendorf tube and then were centrifuged at 15000 g, 4 °C for 10 min. Finally, the supernatant was injected into the LC-MS/MS system analysis ^[1]. PS: Liquid sample (100 µL) and prechilled methanol (400 µL) were mixed by well vortexing ^[2-3]. Cell sample and 4 times prechilled 80% methanol were mixed by well vortexing, and then sonicated for 6 min. Repeating this step once again and then operating the same steps as above ^[4-5].

2. UHPLC-MS/MS Analysis

UHPLC-MS/MS analyses were performed using a Vanquish UHPLC system (Thermo Fisher, Germany) coupled with an Orbitrap Q ExactiveTM HF mass spectrometer (Thermo Fisher, Germany) in Biozeron Co., Ltd. (Shanghai, China). Samples were injected onto a Hypesil Gold column (100×2.1 mm, 1.9µm) using a 17-min linear gradient at a flow rate of 0.2 mL/min. The eluents for the positive polarity mode were eluent A (0.1% FA in Water) and eluent B (Methanol). The eluents for the negative polarity mode were eluent A (5 mM ammonium acetate, pH 9.0) and eluent B (Methanol). The solvent gradient was set as follows: 2% B, 1.5 min; 2-100% B, 12.0 min; 100% B, 14.0 min; 100-2% B, 14.1 min; 2% B, 17 min. Q ExactiveTM HF mass spectrometer was operated in positive/negative polarity mode with spray voltage of 3.2

kV, capillary temperature of 320 °C, sheath gas flow rate of 40 arb and aux gas flow rate of 10 arb.

3. Data processing and metabolite identification

The raw data files generated by UHPLC-MS/MS were processed using the Compound Discoverer 3.1 (CD3.1, Thermo Fisher) to perform peak alignment, peak picking, and quantitation for each metabolite. The main parameters were set as follows: retention time tolerance, 0.2 minutes; actual mass tolerance, 5ppm; signal intensity tolerance, 30%; signal/noise ratio, 3; and minimum intensity, 100, 000. After that, peak intensities were normalized to the total spectral intensity. The normalized data was used to predict the molecular formula based on additive ions, molecular ion peaks and fragment ions. And then peaks were matched with the mzCloud (<https://www.mzcloud.org/>), mzVault and MassList database to obtain the accurate qualitative and relative quantitative results. Statistical analyses were performed using the statistical software R (R version R-3.4.3), Python (Python 2.7.6 version) and CentOS (CentOS release 6.6), When data were not normally distributed, normal transformations were attempted using of area normalization method.

4. Data Analysis

These metabolites were annotated using the KEGG database (<https://www.genome.jp/kegg/pathway.html>), HMDB database([https://hmdb.ca/ metabolites](https://hmdb.ca/metabolites)) and LIPID Maps database (<http://www.lipidmaps.org/>). Principal components analysis (PCA) and Partial least squares discriminant analysis (PLS-DA) were performed at metaX (a flexible and comprehensive software for processing metabolomics data). We applied univariate analysis (t-test) to calculate the statistical significance (P-value). The metabolites with $VIP > 1$ and $P\text{-value} < 0.05$ and fold change ≥ 2 or $FC \leq 0.5$ were considered to be differential metabolites. Volcano plots were used to filter metabolites of interest which based on $\log_2(\text{FoldChange})$ and $-\log_{10}(p\text{-value})$ of metabolites. For clustering heat maps, the data were normalized using z-scores of the intensity areas of differential metabolites and were plotted by Pheatmap package in R language. The

correlation between differential metabolites were analyzed by `cor ()` in R language (method=pearson). Statistically significant of correlation between differential metabolites were calculated by `cor.mtest ()` in R language. P-value < 0.05 was considered as statistically significant and correlation plots were plotted by `corrplot` package in R language. The functions of these metabolites and metabolic pathways were studied using the KEGG database. The metabolic pathways enrichment of differential metabolites was performed, when ratio were satisfied by $x/n > y/N$, metabolic pathway were considered as enrichment, when P-value of metabolic pathway < 0.05, metabolic pathway were considered as statistically significant enrichment.

Reference

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