https://doi.org/10.17221/110/2022-HORTSCI

UPLC-MS/MS-based widely-targeted metabolic profiling reveals leaf metabolite changes in sweet cherry under rain-shelter cultivation

Huimin Zhang[1,2], Yanhue Jiang[1,2], Haozhang Han[1,2], Yu Liu[1,2], Quan Li[3]**

[1]*School of Biology and Materials Engineering, Suqian University, Jiangsu, P. R. China*
[2]*Key Laboratory of Garden Plants and Ornamental Horticulture of Suqian, Suqian University, Jiangsu, P. R. China*

**Corresponding author: liquan-8@163.com*

Citation: Zhang H., Jiang Y.H., Han, H.Z., Liu Y. (2024): UPLC-MS/MS-based widely-targeted metabolic profiling reveals leaf metabolite changes in sweet cherry under rain-shelter cultivation. Hort. Sci. (Prague), 51: 29–38.

Abstract: Metabolomics analysis based on UPLC-MS/MS was used to investigate the influence of rain shelter (RS) conditions on metabolites of sweet cherry leaves. It was found that there were 134 differential metabolites. These differential metabolites were enriched in 40 metabolic pathways. Studies on the biosynthetic pathways and regulatory mechanisms of metabolites in sweet cherry leaves showed that low-light and drought stresses in RS plants were related to the amino acid biosynthesis metabolic pathway and that of flavone and flavonol biosynthesis. Sweet cherry trees exhibited improved tolerance to drought stress by regulating the increase in the content of metabolites, such as proline in the amino acid metabolic pathway and the content of flavonoids in the phenylpropane metabolic pathway. To cope with low-light stress, sweet cherry leaves can increase their photosynthetic efficiency by regulating the flavonol content in the flavone and flavonol biosynthetic pathway under the catalysis of a series of enzymes.

Keywords: *Prunus avium* L.; metabolites; pathway; stress; molecular biology

To prevent the flowers and fruits of fruit trees from being damaged by excessive rainfall and hail, sheltered cultivation, including rain-shelter cultivation, has been widely used for sweet cherry tree cultivation in southern China. However, the changes in the environment caused by mulch with rain-shelter cultivation was shown to affect the photosynthesis and fruit quality of fruit trees (Chockchaisawasdee et al. 2016). Fruit tree leaves have a determinant role in plant growth and development and synthesize nutrients for fruit development. The fruits gradually mature by absorbing these nutrients and eventually play a role in reproduction for the plant. Therefore, it is necessary to confirm the differences in metabolites between the leaves of sweet cherry trees cultivated under open-field cultivation (CK) and rain-shelter cultivation (RS) conditions. Research on rain-shelter cultivation has mainly focused on the detection and analysis of the photosynthetic and fluorescence

Supported by the Jiangsu Province University Philosophy and Social Science Research Project (2023SJYB2346), Research Project on Quality Assurance and Evaluation of Higher Education in Jiangsu Province (2023-C68).

https://doi.org/10.17221/110/2022-HORTSCI

parameters, anatomical characteristic, chlorophyll (Chl) content, superoxide dismutase (SOD) activity, peroxidase (POD) activity, catalase (CAT) activity, malondialdehyde (MDA) content and mineral element (P, K, Mg) contents of fruit tree leaves (Correia et al. 2017; Mirto et al. 2018; Michailidis et al. 2020). There are obvious effects of rain shelter or shading on plant growth and antioxidant systems. In general, the shading treatment could alter the content of Chl and MDA, while SOD and POD activities could significantly adapt to the low-light environment (Miao et al. 2016). Recent studies have shown that rain-shelter cultivation may substantially contribute to the total photosynthetic accumulation and increased fruit yield and has no obvious negative effects on the vegetative growth or fruit quality of Chinese cherry trees in southwest China (Tian et al. 2019). The main reason is that the rain-shelter system could increase leaf chlorophyll contents and rates of CO_2 assimilation (Gimã Nez et al. 2017; Martini et al. 2017). Scholars have found that rain-shelter cultivation can change the accumulation of metabolites in the fruits of fruit trees, leading to alterations in the polyphenolic and volatile organic compound profiles. For example, rain-shelter cultivation stimulated the accumulation of dihydroquercetin-3-O-rhamnoside in grape skins during grape maturation (Meng et al. 2013; Gao et al. 2016). Detection and analysis can determine the composition and content of metabolites under rain-shelter cultivation, but the mechanism of coregulation between secondary metabolite biosynthetic pathways has rarely been studied.

Metabolomics is a science that systematically studies the change in metabolites in the dynamic process of metabolism, analyzes the change in total metabolites in a given sample, cell or tissue, and integrates the data under the context of functional genomics to reveal the metabolic nature of organismal activities (Hong et al. 2016; Duan et al. 2019). Metabolites are the end products of gene expression. Very small changes in gene expression can lead to large changes in metabolites. Previously, it took a long time to determine the changes in gene expression through visible phenotypic changes, and sometimes gene expression changes do not cause phenotypic changes; differential gene expression can also change the content of certain metabolites in plants. Using metabolomics to detect changes in metabolites can determine changes in gene expression levels, thereby inferring the function of genes and their effects on metabolites (Okazaki, Saito 2012; Li et al. 2018). The RS conditions is different from the CK condition in terms of environmental factors, and the metabolites in sweet cherry tree leaves will inevitably change to respond. Plants use complex mechanisms to maintain relatively stable levels of major metabolites in the body. Plants produce a wide variety of compounds, far exceeding those produced by animals and even microorganisms, so plant metabolomics research is particularly necessary (Jorge et al. 2016; Guo et al. 2019). In this study, UPLC-MS/MS combined with multivariate statistical analysis was used to explore the effect of rain-shelter cultivation on metabolite accumulation in sweet cherry leaves. The mechanism of metabolite accumulation and regulation in sweet cherry leaves was analyzed.

MATERIAL AND METHODS

Plant materials. The leaves of the sweet cherry (*Prunus avium* L.) variety 'Summit' were collected from the Baiyi fruit tree experimental base of Guiyang Wudang, Guizhou Institute of Fruit Tree Science (27°03'3.89"N and 106°25'47.23"E), and collection was performed in July 2019. The rain-shelter facilities were 4 clear rain shelters built in April 2014. The 'Summit' trees were 5 years old and were grafted onto 'Gisela 6' root stock and planted under rain-shelter and open field conditions in March 2015. All trees were managed with the same practical techniques. Three trees with normal growth and relatively consistent growth potential were selected randomly from the rain-shelter cultivation and open field cultivation conditions, and leaves (the well-developed leaves from the base of the shoots) from the same location were harvested from each tree. After being washed with distilled water, the fresh leaves were immediately frozen in liquid nitrogen and stored at –80 °C.

Sample preparation and extraction. Freeze-dried leaves were crushed using a mixer mill (MM 400, Retsch) with a zirconia bead for 1.5 minutes at 30 Hz. One hundred milligrams of powder was weighed and extracted overnight at 4 °C with 0.6 mL 70% aqueous methanol. Following centrifugation at 10 000 g for 10 minutes, the extracts were enriched with solid-phase extraction (CNWBOND Carbon-GCB SPE Cartridge, 250 mg, 3 mL; ANPEL, Shanghai, China, www.anpel.com.cn/cnw) and filtered (SCAA-104, 0.22 μm pore size; ANPEL, Shanghai, China) before UPLC-MS/MS analysis.

https://doi.org/10.17221/110/2022-HORTSCI

UPLC conditions. The sample extracts were analyzed using a UPLC-ESI-MS/MS system (UPLC, Shim-pack UFLC SHIMADZU CBM30A system, www.shimadzu.com.cn; MS, Applied Biosystems.

4 500 Q TRAP, www.appliedbiosystems.com.cn). A Waters ACQUITY UPLC HSS T3 C18 column (1.8 µm, 2.1 mm × 100 mm) was used for separation. The mobile phase consisted of pure water with 0.04% acetic acid (solvent A) and acetonitrile with 0.04% acetic acid (solvent B). Sample measurements were performed with a gradient program that employed the starting conditions of 95% A, 5% B. Within 10 minutes, a linear gradient to 5% A, 95% B was programmed, and a composition of 5% A, 95% B was maintained for 1 minute. Subsequently, a composition of 95% A, 5.0% B was achieved within 0.10 minute and maintained for 2.9 minutes. The column oven was set to 40 °C; the injection volume was 4 µL. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS.

ESI-Q TRAP-MS/MS. LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (Q TRAP, API 4 500 Q TRAP UPLC/MS/MS System) equipped with an ESI Turbo Ion-Spray interface operating in positive and negative ion modes and controlled by Analyst 1.6.3 software (AB Sciex). The ESI source operation parameters were as follows: ion source, turbo spray; source temperature, 550 °C; ion spray (IS) voltage, 5 500 V (positive ion mode)/–4 500 V (negative ion mode); ion source gas I (GSI), gas II (GSII), and curtain gas (CUR), 50, 60, and 30.0 psi, respectively; and collision gas (CAD), high. Instrument tuning and mass calibration were performed with 10 and 100 µmol/L polypropylene glycol solutions in QQQ and LIT modes, respectively. QQQ scans were acquired as MRM experiments with the collision gas (nitrogen) set to 5 psi. The DP and CE values for individual MRM transitions were individually optimized. A specific set of MRM transitions was monitored for each period according to the metabolites eluted within this period.

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

The HCA (hierarchical cluster analysis) results of samples and metabolites are presented as heatmaps with dendrograms, while Pearson correlation coefficients (PCCs) between samples were calculated by the cor function in R and presented only as heatmaps. Both HCA and PCC were carried out with the R package pheatmap. For HCA, normalized signal intensities of metabolites (unit-variance scaling) were visualized as a color spectrum.

Significantly different metabolites between groups were determined by VIP ≥ 1 and absolute $\log_2$FC (fold change) ≥ 1. VIP values were extracted from the OPLS-DA results, which were also used to generate score plots and permutation plots using the R package MetaboAnalystR. The data were log-transformed ($\log_2$) and mean-centered before OPLS-DA. To avoid overfitting, a permutation test (200 permutations) was performed.

Identified metabolites were annotated using the KEGG compound database, and annotated metabolites were then mapped to the KEGG pathway database. Pathways mapped with significantly regulated metabolites were then utilized for MSEA (metabolite set enrichment analysis), and their significance was determined by p-values of hypergeometric tests.

RESULTS

Multivariate statistical analysis of metabolomics data. The PCA score results (Figure 1) show that the control group (CK) and the test group (RS) had a tendency to separate in the overall distribution, indicating that the metabolites of each group had significant differences.

To obtain a better separation model, orthogonal partial least squares discriminant analysis (OPLS-DA) was used to further analyze the data (Lu et al. 2019). The metabolomic data were analyzed according to the OPLS-DA model, a score chart was generated for each group, and the differences between each group were further illustrated. During OPLS-DA modeling, the X matrix information was decomposed into two types of information that were either related to Y or unrelated to Y. Among them, the variable information related to Y was the predicted principal component, and the variable information not related to Y was the orthogonal principal component (Figure 2A) (Lin et al. 2020). OPLS-DA was subjected to permutation verification (n = 200), and 200 permutation tests were performed to verify the model (Figure 2B). It can be seen that R2Y' and Q2' were both smaller than

https://doi.org/10.17221/110/2022-HORTSCI

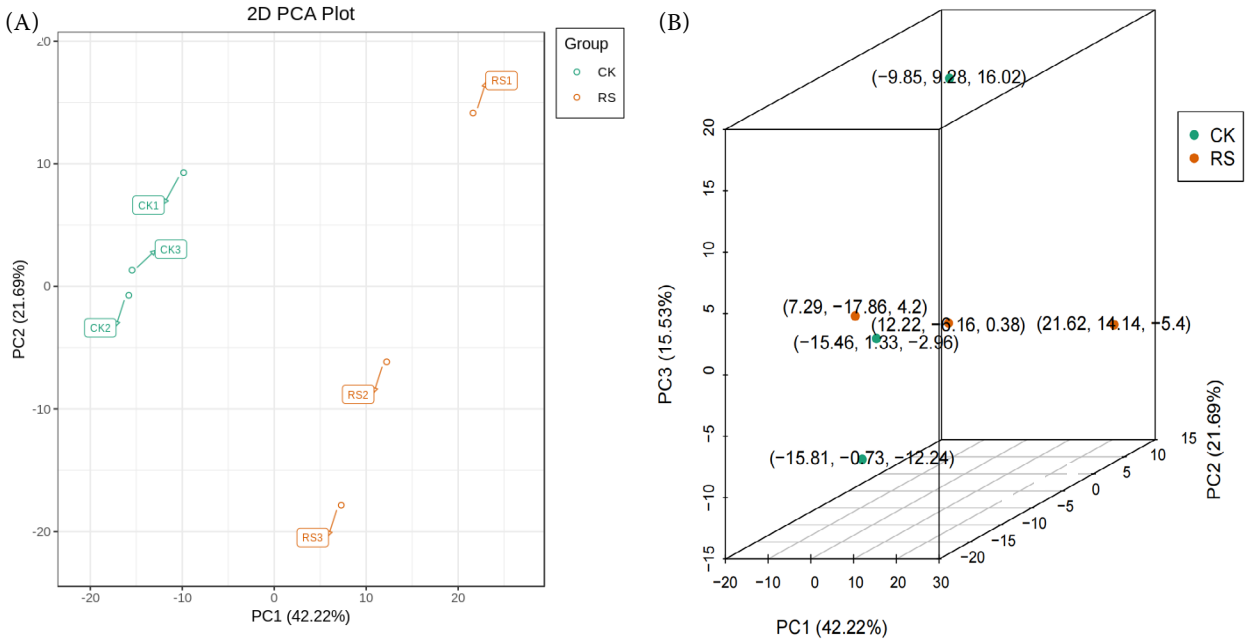

Figure 1. Principal component analysis
(A) 2D PCA plot; (B) 3D PCA plot; CK – open-field cultivation; RS – rain-shelter cultivation

the original model's R2Y and Q2 values. The R2Y and Q2 in this study were 0.999 and 0.831, respectively. There was no overfitting phenomenon, indicating the stability and predictability of the model and its good fitting of the data, with high interpretation and prediction ability. The model developed in this study was obviously effective and had a statistical basis for screening differential metabolites.

Screening of differential metabolites. Metabolomics data are 'high-dimensional and massive'. Therefore, it is necessary to combine univariate statistical analysis and multivariate statistical analysis to analyze them

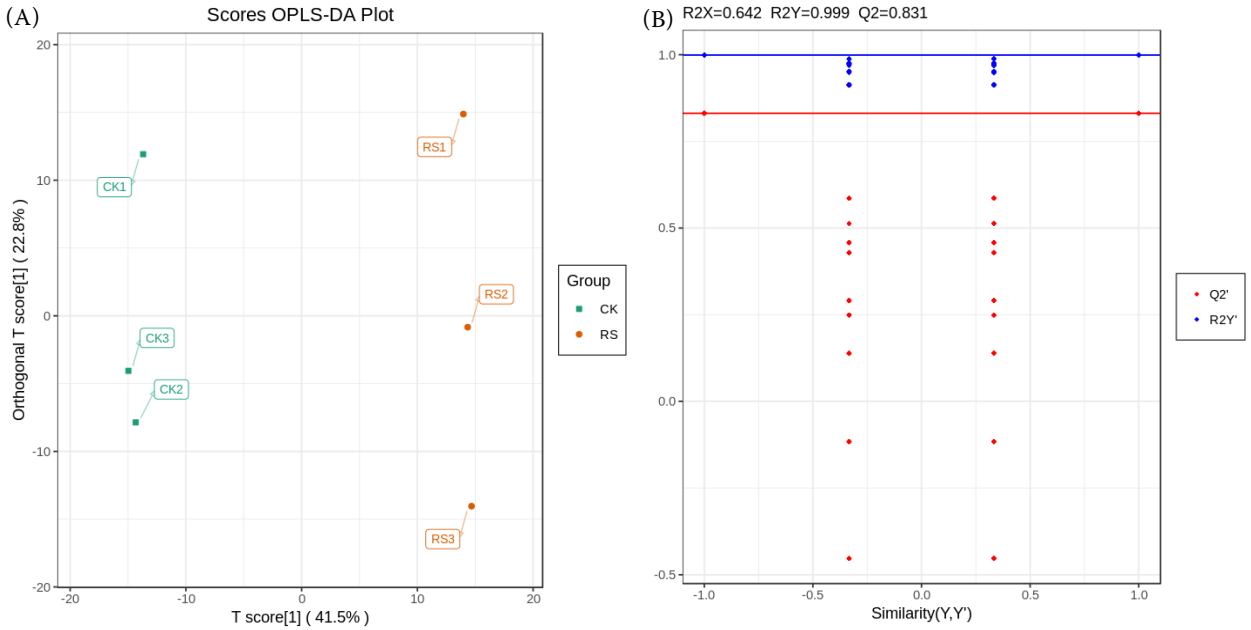

Figure 2. Orthogonal partial least-squares discriminant analysis (OPLS-DA) of the metabolomic data
(A) OPLS-DA score; (B) OPLS-DA permutation; CK – open-field cultivation; RS – rain-shelter cultivation; Q2 – Indicates the predictive power of the model; R2Y – Explanatory rate of the constructed model for the Y matrix

https://doi.org/10.17221/110/2022-HORTSCI

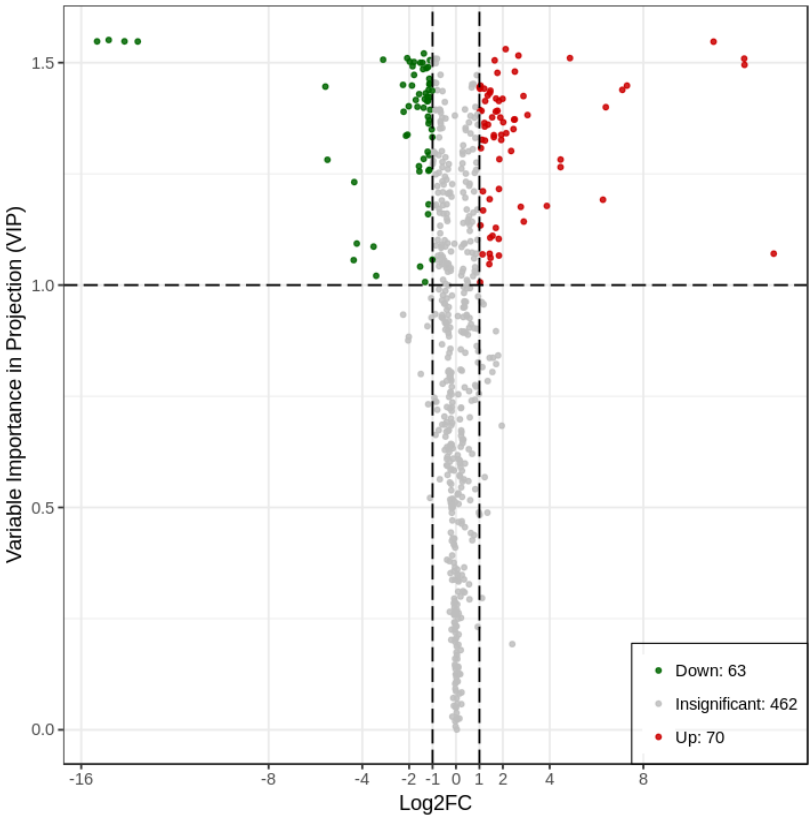

Figure 3. Volcano plot of differentially expressed metabolites

from multiple perspectives according to the characteristics of the data and finally determine the differential metabolites accurately. The OPLS-DA model was superior to the PCA, so differential metabolites were analyzed using the OPLS-DA model. Based on the OPLS-DA results, the variable importance in pro-

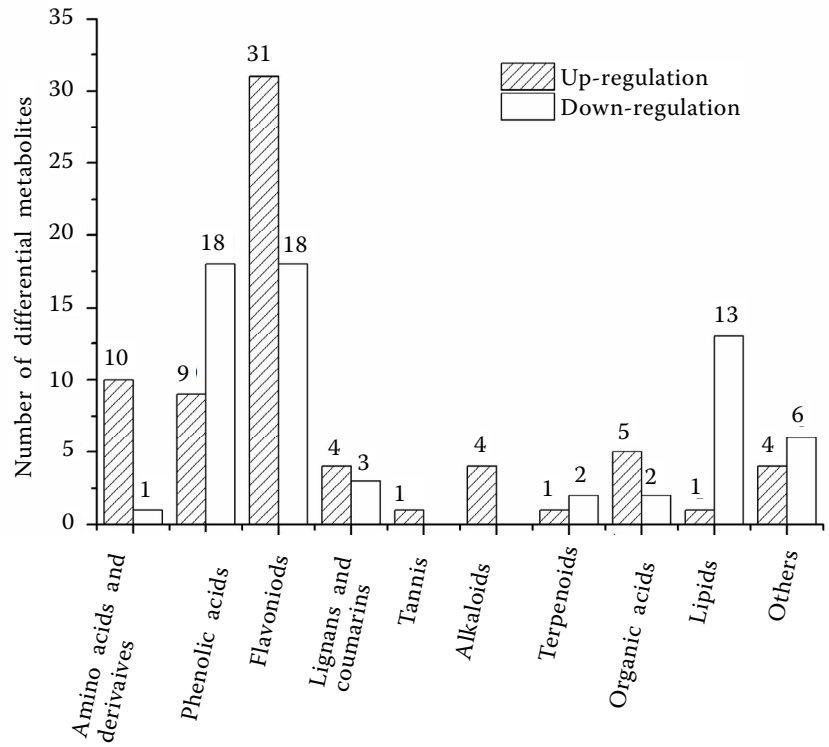

Figure 4. Categories of differential metabolites

https://doi.org/10.17221/110/2022-HORTSCI

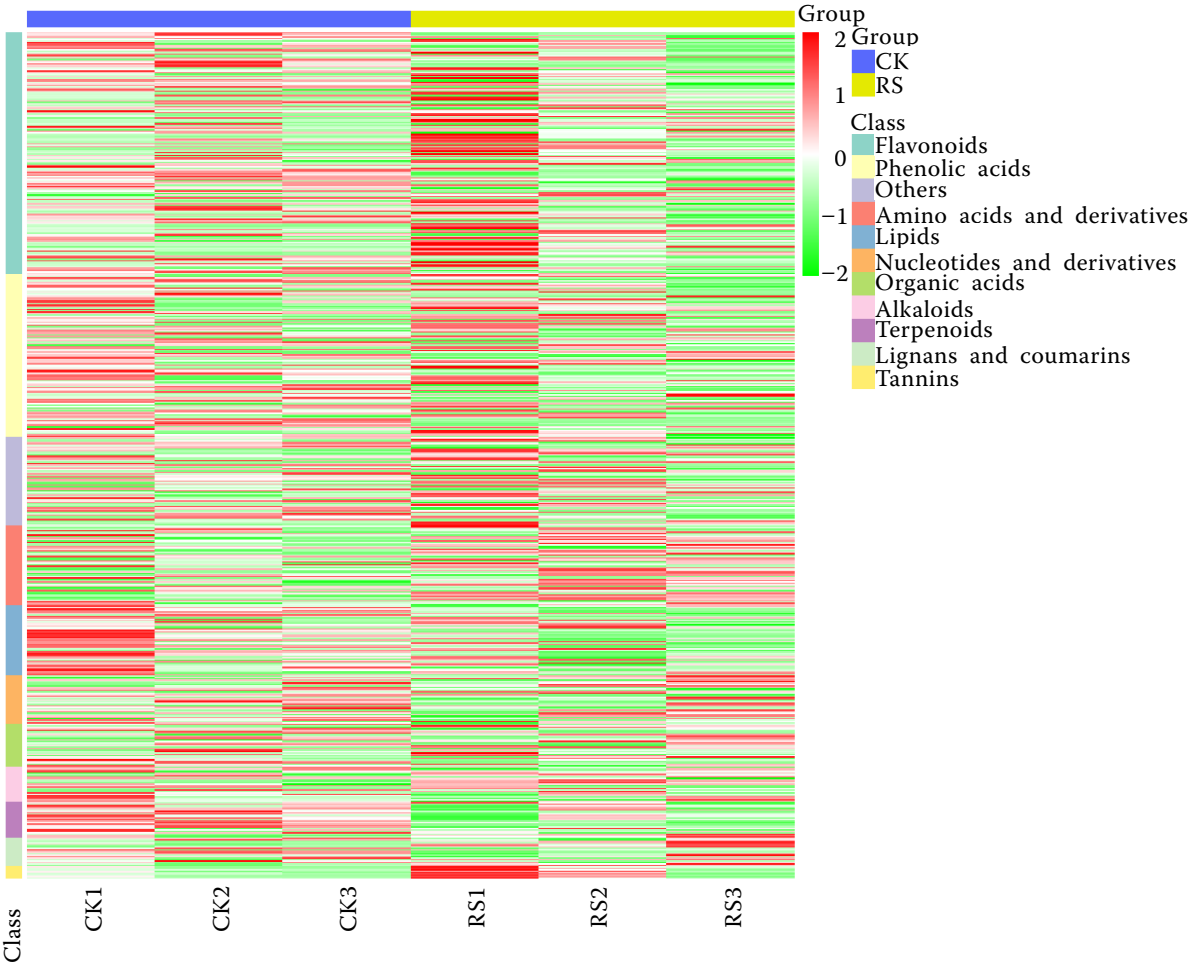

Figure 5. Heatmap analysis of the differential metabolites in sweet cherry leaves of trees cultivated under different conditions

jection (VIP) values of the OPLS-DA model were evaluated for preliminary screening of differential metabolites between the CK and RS groups (Wang et al. 2019). At the same time, the p-value or fold change values from the univariate analysis were combined to further screen for differential metabolites. A total of 595 metabolites were detected, 133 differential metabolites were screened, and 462 metabolites were not significantly different. The difference analysis results are shown in Table S1 Electronic Supplementary Material (ESM). Compared with those in the CK group, some RS metabolites, such as flavonoids, were upregulated the most, and phenolic acids and other metabolites were downregulated significantly, indicating that the differences in metabolites in sweet cherry leaves were impacted by cultivation patterns. From the volcano plot of the differential metabolites between the treatment groups (Figure 3), it could be found that there were 70 upregulated and 63 downregulated metabolites.

In Figure 4, there are 134 differential metabolites, including 11 amino acids and their derivatives, 27 phenolic acids, 49 flavonoids (including 9 flavonols, 5 dihydroflavones, 3 dihydroflavonols, 3 anthocyanins, 17 flavonoids, 2 flavonoid carbonosides, 4 flavanols, and 6 isoflavones), 7 lignans and coumarins, 1 tannin, 4 alkaloids, 3 terpenes, 7 organic acids, 14 lipids, and 10 other metabolites.

Cluster analysis of differential metabolites. To facilitate the observation of the metabolic changes, unit-variance (UV) scaling normalization was used for the metabolites with significant differences, and a heatmap was generated by R software to evaluate the rationality of the selected metabolites. When the candidate screened metabolites were determined to be reasonable and accurate, groups of samples converged in the same cluster, and the metabolites clustered according to similar expression patterns and were regarded as potentially being close in terms of reaction step in the metabolic process. Hierarchical

https://doi.org/10.17221/110/2022-HORTSCI

clustering heatmap (Figure 5) analysis clearly showed the up- and downregulation relationship of these differential metabolic components, as well as the significant differences between CK and RS metabolites.

DISCUSSION

Effects of rain-shelter cultivation on amino acid metabolism in sweet cherry leaves. After the growth process of plants is stimulated by some internal or external factors, the specific stress changes that occur for their survival under adverse conditions will eventually manifest itself in metabolic changes (Fürtauer et al. 2019; Xin et al. 2019). In addition to playing an indispensable role in protein synthesis, amino acids also play important roles in the primary and secondary metabolism of plants. Usually, related to plant osmoregulation and protein synthesis, some amino acids act on assimilation and source-sink transportation of nitrogen sources, while others are precursors of secondary metabolites (such as hormones and plant defense-related substances). Some intermediate metabolites can be used as signal factors for biotic and abiotic stress in plants (Zhu et al. 2018; Brosnan, Brosnan 2020). According to the biosynthetic pathway of amino acids (ko01230), the contents of primary and secondary metabolites, including proline, shikimic acid, arginine, homoserine, glutamine, 2,6-diaminoheptanedioate, and lysine, were all upregulated. Osmotic regulation in sweet cherry is a key mechanism for maintaining cell swelling when water is scarce. Proline is one of the major osmotic regulators in plants in response to adverse stress and is a signaling factor for plants under biotic and abiotic stress (Per et al. 2017). Under stress conditions, RS can enhance resistance to drought stress by regulating the increase in proline content in the plant. Osmotic potential and the osmotic pressure in and out of protoplasts must be balanced to prevent the loss of plant cell water to improve the stability of plant protoplast colloids (Antoniou et al. 2017).

The carbon skeleton required for plant amino acid biosynthesis comes from glycolysis, the carbon reduction reaction in photosynthesis, the oxidized pentose phosphate pathway and the citric acid cycle (Anwar et al. 2018). Amino acid synthesis directly or indirectly affects all aspects of plant growth and development. In this study, the increase in the content of proline and other metabolites in the RS group indicated that RS may increase plant tolerance to drought stress by regulating osmotic regulators and signal factor substances in amino acid metabolism. Glutamine is an amino acid that builds proteins. It is regulated

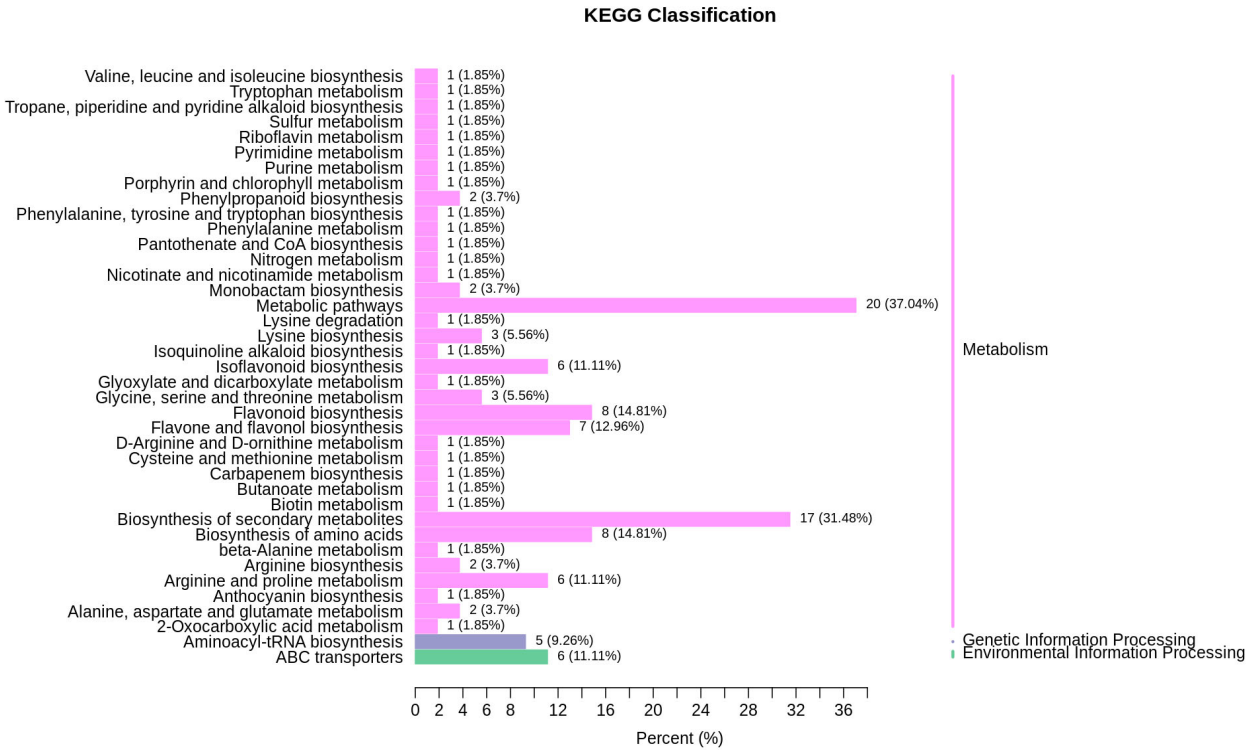

Figure 6. KEGG pathway bar chart

https://doi.org/10.17221/110/2022-HORTSCI

by light and other factors, causing its concentration to not be constant (Eelen et al. 2018). Plants assimilate inorganic nitrogen into a class of N-transport amino acids: glutamic acid, glutamine, aspartic acid, and asparagine. Glutamine and asparagine are forms of nitrogen transport in plants (Pavlova et al. 2018). Glutamate was upregulated in RS plants in this study, indicating that glutamate nitrogen enters plant metabolism and provides a nitrogen source for the synthesis of nucleic acids, other amino acids, and nitrogen-containing compounds. When sweet cherry leaves contain elevated levels of ammonia, ammonia will form glutamine or asparagine, which will release the toxic free ammonia. Nitrogen needs to be stored in an adequate nitrogen source for sweet cherry growth, defense and reproduction.

Flavonoid biosynthetic pathway analysis of sweet cherry leaves. Fourteen metabolites were annotated into the flavonoid biosynthetic pathway (ko00941), 6 metabolites were upregulated, and 2 metabolites were downregulated. RS plants respond to drought stress by regulating the flavonoid content in their leaves. The synthesis of flavonoids is affected by the supply of substrates, the competition of intermediates in the synthesis pathway, the activity of key enzymes, etc. The downregulation of pinocembrin increased the chrysin content under the catalysis of flavone synthase I, and the catalysis of flavanone 3-dioxygenase increased the content of pinobanksin. It can also be seen from the upstream and downstream relationships of the metabolic pathway (ko00941) that the increase in naringenin content under the catalysis of a series of enzymes led to the contents of downstream eriodictyol, luteolin, and catechin increasing and that of epigallocatechin decreasing (Xiu et al. 2017). Drought stress can produce many reactive oxygen species in sweet cherry and induce and promote the synthesis of flavonoids in the leaves of sweet cherry to avoid oxidative damage to the cells (Gharibi et al. 2019). Flavonoids have important physiological significance in plants and play an important role in the pollination of plants, resistance to pests and disease-causing microorganisms, reduction of ultraviolet radiation damage, and signal transmission to microorganisms (Acero et al. 2019).

Flavonol biosynthesis is a branch of the metabolism pathway of flavonoids. Flavonols are secondary metabolites that plants produce to resist stress. The related research on the regulation of flavonol metabolism is mainly focused on quercetin and kaempferol, and the environmental regulation factors of the biosynthesis of flavonol are mainly based on light (Aires et al. 2017; Matsui et al. 2018). From the flavone and flavonol biosynthetic pathway (ko00944), it can be found that the content of acacetin increased, which led to an increase in the contents of downstream cosmosiin, isovitexin, luteolin, 3,7-Di-O-methylquercetin and syringetin under the catalysis of enzymes and reduced the afzelin content. Flavonols are important secondary metabolites in plants adapting to low-light stress, and they are highly effective free radical scavengers. Different light treatments have an important effect on flavonol biosynthesis (Shoeva et al. 2016; Wu et al. 2018). Because RS plants were subjected to low-light stress, they could increase the overall flavonol content by regulating the content of acacia, apigenin, isovitexin and luteolin in the flavone and flavonol biosynthetic pathway to improve their photosynthetic efficiency and reduce the harm of low-light stress.

CONCLUSION

In this study, metabolomic techniques and multivariate statistical methods based on UPLC-MS/MS were used to explore the differential metabolites of sweet cherry leaves of trees grown under open-field cultivation (CK) and rain-shelter cultivation (RS) conditions. RS plants exhibited increased tolerance to drought stress by regulating osmotic regulators and signal factor substances in the amino acid metabolic pathway. In addition, regulating phenylpropane metabolic pathways could also improve plant stress resistance. RS plants responded to drought stress by producing a large number of flavonoids. Increasing the content of flavonols can increase the photosynthetic efficiency of sweet cherry to reduce the harm of low-light stress. These results provide a theoretical basis for further elucidating the mechanism of the corresponding genes expressing the regulated metabolites in sweet cherry leaves.

REFERENCES

Acero N., Gradillas A., Beltran M., García A., Mingarro D.M. (2019): Comparison of phenolic compounds profile and antioxidant properties of different sweet cherry (*Prunus avium* L.) varieties. Food Chemistry, 279: 260–271.

Aires A., Dias C., Carvalho R., Saavedra M.J. (2017): Analysis of glycosylated flavonoids extracted from sweet-cherry

https://doi.org/10.17221/110/2022-HORTSCI

stems, as antibacterial agents against pathogenic *Escherichia coli* isolates. Acta Biochimica Polonica, 64: 265–271.

Antoniou C., Chatzimichail G., Xenofontos R., Pavlou J.J., Panagiotou E., Christou A., Fotopoulos V. (2017): Melatonin systemically ameliorates drought stress-induced damage in *Medicago sativa* plants by modulating nitro-oxidative homeostasis and proline metabolism. Journal of Pineal Research, 62: e12401.

Anwar S., Wieczorek K., Inselsbacher E. (2018): Analysis of Arabidopsis amino acid metabolism in response to *Heterodera schachtii* infection. Pakistan Journal of Nematology, 36: 131–150.

Brosnan J.T., Brosnan M.E. (2020): Formate and its role in amino acid metabolism. Current Opinion in Clinical Nutrition and Metabolic Care, 23: 23–28.

Chockchaisawasdee S., Golding J.B., Vuong Q.V., Papoutsis K., Stathopoulos C.E. (2016): Sweet cherry: Composition, postharvest preservation, processing and trends for its future use. Trends in Food Science and Technology, 55: 72–83.

Correia S., Schouten R., Silva A.P., Gonçalves B. (2017): Factors affecting quality and health promoting compounds during growth and postharvest life of sweet cherry (*Prunus avium* L.). Frontiers in Plant Science, 8: 2166.

Duan S., Wu Y., Fu R., Wang L., Chen Y., Xu W., Wang S. (2019): Comparative metabolic profiling of grape skin tissue along grapevine berry developmental stages reveals systematic influences of root restriction on skin metabolome. International Journal of Molecular Sciences, 20: 534.

Eelen G., Dubois C., Cantelmo A.R., Goveia J., Brüning U., DeRan M., Jarugumilli G., van Rijssel J., Saladino G., Comitani F. (2018): Role of glutamine synthetase in angiogenesis beyond glutamine synthesis. Nature, 561: 63–69.

Fürtauer L., Weiszmann J., Weckwerth W., Nägele T. (2019): Dynamics of plant metabolism during cold acclimation. International Journal of Molecular Sciences, 20: 5411.

Gao Y., Li X.X., Han M.M., Yang X.F., Li Z., Wang J., Pan Q.H. (2016): Rain-shelter cultivation modifies carbon allocation in the polyphenolic and volatile metabolism of *Vitis vinifera* L. Chardonnay Grapes. PLoS ONE, 11: e0156117.

Gharibi S., Tabatabaei B.E.S., Saeidi G., Talebi M., Matkowski A. (2019): The effect of drought stress on polyphenolic compounds and expression of flavonoid biosynthesis related genes in *Achillea pachycephala* Rech. f. Phytochemistry, 162: 90–98.

Giménez M.J., Serrano M., Valverde J.M., Martínez-Romero D., Castillo S., Valero D., Guillén F. (2017): Preharvest salicylic acid and acetylsalicylic acid treatments preserve quality and enhance antioxidant systems during postharvest storage of sweet cherry cultivars. Journal of the Science of Food and Agriculture, 97: 1220–1228.

Guo H., Guo H., Zhang L., Tang Z., Yu X., Wu J., Zeng F. (2019): Metabolome and transcriptome association analysis reveals dynamic regulation of purine metabolism and flavonoid synthesis in transdifferentiation during somatic embryogenesis in cotton. International Journal of Molecular Sciences, 20: 2070.

Hong J., Yang L., Zhang D., Shi J. (2016): Plant metabolomics: an indispensable system biology tool for plant science. International Journal of Molecular Sciences, 17: 767.

Jorge T.F., Rodrigues J.A., Caldana C., Schmidt R., van Dongen J.T., Thomas-Oates J, António C. (2016): Mass spectrometry-based plant metabolomics: Metabolite responses to abiotic stress. Mass Spectrometry Reviews, 35: 620–649.

Li Y., Fang J., Qi X., Lin M., Zhong Y., Sun L., Cui W. (2018): Combined analysis of the fruit metabolome and transcriptome reveals candidate genes involved in flavonoid biosynthesis in *Actinidia arguta*. International Journal of Molecular Sciences, 19: 1471.

Lin W., Li Y., Lu Q., Lu H., Li J. (2020): Combined analysis of the metabolome and transcriptome identified candidate genes involved in phenolic acid biosynthesis in the leaves of *Cyclocarya paliurus*. International Journal of Molecular Sciences, 21: 1337.

Lu F., Zhang N., Yu D., Zhao H., Pang M., Fan Y., Liu S. (2019): An integrated metabolomics and 16S rRNA gene sequencing approach exploring the molecular pathways and potential targets behind the effects of *Radix Scrophulariae*. RSC Advances, 9: 33354–33367.

Martini S., Conte A., Tagliazucchi D. (2017): Phenolic compounds profile and antioxidant properties of six sweet cherry (*Prunus avium*) cultivars. Food Research International, 97: 15–26.

Matsui K., Oshima Y., Mitsuda N., Sakamoto S., Nishiba Y., Walker A.R., Takami H. (2018): Buckwheat R2R3 MYB transcription factor FeMYBF1 regulates flavonol biosynthesis. Plant Science, 274: 466–475.

Meng J.F., Ning P.F., Xu T.F., Zhang Z.W. (2013): Effect of rain-shelter cultivation of *Vitis vinifera* cv. *Cabernet gernischet* on the phenolic profile of berry skins and the incidence of grape diseases. Molecules, 18: 381–397.

Miao L., Zhang Y., Yang X., Jiang G. (2016): Effect of rain shelter and shading on plantlets growth and antioxidant contents in strawberry. Indian Journal of Horticulture, 73: 433–436.

Michailidis M., Karagiannis E., Tanou G., Samiotaki M., Sarrou E., Karamanoli K., Molassiotis A. (2020): Proteomic and metabolic analysis reveals novel sweet cherry fruit development regulatory points influenced by girdling. Plant Physiology and Biochemistry, 149: 233–244.

Mirto A., Iannuzzi F., Carillo P., Ciarmiello L.F., Woodrow P., Fuggi A. (2018): Metabolic characterization and antioxi-

https://doi.org/10.17221/110/2022-HORTSCI

dant activity in sweet cherry (*Prunus avium* L.) Campania accessions: Metabolic characterization of sweet cherry accessions. Food Chemistry, 240: 559–566.

Okazaki Y., Saito K. (2012): Recent advances of metabolomics in plant biotechnology. Plant Biotechnology Reports, 6: 1–15.

Pavlova N.N., Hui S., Ghergurovich J.M., Fan J., Intlekofer A.M., White R.M., Zhang J. (2018): As extracellular glutamine levels decline, asparagine becomes an essential amino acid. Cell Metabolism, 27: 428–438.

Per T.S., Khan N.A., Reddy P.S., Masood A., Hasanuzzaman M., Khan M.I.R., Anjum N.A. (2017): Approaches in modulating proline metabolism in plants for salt and drought stress tolerance: Phytohormones, mineral nutrients and transgenics. Plant Physiology and Biochemistry, 115: 126–140.

Shoeva O.Y., Mock H.P., Kukoeva T.V., Börner A., Khlestkina E.K. (2016): Regulation of the flavonoid biosynthesis pathway genes in purple and black grains of *Hordeum vulgare*. PLoS ONE, 11: e0163782.

Tian T., Qiao G., Deng B., Wen Z., Hong Y., Wen X. (2019): The effects of rain shelter coverings on the vegetative growth and fruit characteristics of Chinese cherry (*Prunus pseudocerasus* Lindl.). Scientia Horticulturae, 254: 228–235.

Wang J., Lv J., Liu Z., Liu Y., Song J., Ma Y., Juntawong N. (2019): Integration of transcriptomics and metabolomics for pepper (*Capsicum annuum* L.) in response to heat stress. International Journal of Molecular Sciences, 20: 5042.

Wu Y., Guo J., Zhou Q., Xin Y., Wang G., Xu L.A. (2018): De novo transcriptome analysis revealed genes involved in flavonoid biosynthesis, transport and regulation in Ginkgo biloba. Industrial Crops and Products, 124: 226–235.

Xin W., Zhang L., Zhang W., Gao J., Yi J., Zhen X., Chen L. (2019): An integrated analysis of the rice transcriptome and metabolome reveals root growth regulation mechanisms in response to nitrogen availability. International Journal of Molecular Sciences, 20: 5893.

Xiu Y., Jang S., Jones J.A., Zill N.A., Linhardt R.J., Yuan Q., Koffas M.A. (2017): Naringenin-responsive riboswitch-based fluorescent biosensor module for *Escherichia coli* co-cultures. Biotechnology and Bioengineering, 114: 2235–2244.

Zhu G., Xiao H., Guo Q., Zhang Z., Zhao J., Yang D. (2018): Effects of cadmium stress on growth and amino acid metabolism in two compositae plants. Ecotoxicology and Environmental Safety, 158: 300–308.

Received: September 1, 2022
Accepted: July 25, 2023
Published online: March 15, 2024