HortScience 58(8):885–892. 2023. https://doi.org/10.21273/HORTSCI17221-23

Effects of Comparative Metabolism on Tomato Fruit Quality under Different Levels of Root Restriction

Yaqing Gao and Xinyuan Zhou
Vegetable Institute of Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China

Hao Liang, Yanhai Ji, and Mingchi Liu
Vegetable Institute of Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China; and North China Key Laboratory of Urban Agriculture, Ministry of Agriculture, Beijing 100097, China

Keywords. metabolomics, quality, root restriction, *Solanum lycopersicum*

Abstract. **In a soilless culture (perlite substrate), root restriction cannot only reduce production costs but also improve fruit quality. Therefore, this study used different levels of root restriction [T1: 0.5 L, T2: 4 L, nonrestriction treatment (CK): 35 L] on tomatoes to explore their impact on quality. Results showed that total soluble solids (TSS), glucose, fructose, and sucrose contents were increased, whereas L-tryptophan, L-tyrosine, and titratable acidity were decreased under two restriction treatments. Meanwhile, root restriction also promoted the accumulation of phenylalanine and proline. For lycopene and flavonoid biosynthesis (prunin, naringin, naringenin), the restriction groups were significantly higher than those in the control group. Overall, T1 and T2 treatment had a better effect than CK treatment. This study provided an idea for improving substrate use efficiency and tomato quality.**

Tomato (*Solanum lycopersicum* L) is one of the most important widely grown vegetables in the world (Dorais et al. 2008; Mekhled et al. 2020); has a particularly attractive flavor in carbohydrates, carotenoids, amino acids, vitamins, fiber, and minerals (Li et al. 2018; Mun et al. 2021); and plays an important role in certain human disease prevention (Martí et al. 2016). However, tomato growth, productivity, and nutritional quality are usually affected by abiotic stress factors such as drought, salinity, and chilling damage (Shinozaki et al. 2003; Zhang et al. 2021; Zhu 2002). In addition, root restriction is considered to be another abiotic stress method that has direct and indirect effects on morphology and physiology. It can improve plant quality and container efficiency by optimizing the container size. Today, root restriction has been well applied to various plants, such as pepper (Kharkina et al. 1999), apple (Webster et al. 2000), and grape (Wang et al. 1997). The taproot replaced the adventitious root to promote lateral root development by restricting the root system, which can improve the root absorption capacity and fruit quality (Wang et al. 2001; Zhu et al. 2006). Fruit quality mainly includes primary and secondary metabolites (Wahid and Ghazanfar 2006). It was reported that plant metabolites increase under abiotic stress (Naik et al. 2023). Root restriction is a kind of physical stress. When plant roots are exposed to this physical stress, the primary metabolites of soluble sugar, vitamin C, and titratable acidity (Xie et al. 2009) and the secondary biomass of carotenoids, flavonoids, phenolic acids, and alkaloids (Chen et al. 2019) increase. These metabolites are beneficial in improving the quality and active substances of fruits (Webster et al. 1996).

Recently, metabolomics technology has been widely applied in the field of agricultural food analysis (Uawisetwathana and Karoonuthaisiri 2019). With metabolomics, part of the metabolic composition of an organism or biological system could be studied, and the metabolic profile could be characterized using analytical and computational technologies (Bino et al. 2004; Moco et al. 2007). It was known that widely targeted metabolomics analysis explored the advantage of large-scale targeted metabolomics analyses together with comparative metabolomics. It provided an effective qualitative and quantitative method to determine the pathways governing the metabolism in a plant's response to stress (Li et al. 2021). At present, metabolic analysis has been successfully applied to discriminate different plant phenotypes and provide potential biomarkers to control food quality (Sumner et al. 2015). By characterizing the metabolic profiles of tomatoes grown in containers of different sizes, it will be possible to provide a mechanistic link between metabolic changes and phenotypes in tomatoes, similar to studies of grapes (Leng et al. 2021). Furthermore, key metabolites thought to be biomarkers associated with improved quality can be identified, leading to a better understanding of the genetic basis of the tomato response to root restriction.

In the past, most of the studies on root restriction were carried out in the soil, but less in soilless culture. Until now, there are also few studies on the improvement of tomato quality with different container sizes, particularly under the mode of soilless cultivation (recirculating nutrient solution). Therefore, the objective of this study was to investigate the metabolic changes in tomato fruit quality improvement as affected by root restriction.

Materials and Methods

Materials and experimental design. The experiment was carried out in a glass greenhouse at the Institute of Vegetables, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China (39°94′ N, 116°28′ E), from February to June 2022. The 'Rui fen 882' tomato was planted in containers with perlite as the substrate. The volume of root restriction was 0.5 L (10 cm in height, 9 cm in diameter) and 4 L (20 cm in height, 16 cm in diameter), and the CK was 35 L (28 cm in length, 45.5 cm in width, 27.5 cm in height). The nutrient solution containing nitrogen 10.5 $mmol{\cdot}L^{-1}$, phosphorus 3.56 $mmol{\cdot}L^{-1}$, potassium 8 $mmol{\cdot}L^{-1}$, calcium 3 $mmol{\cdot}L^{-1}$, magnesium 2.04 $mmol{\cdot}L^{-1}$, and sulfur 4.29 $mmol{\cdot}L^{-1}$ was supplied through fertigation. During the experiment, from 0800 to 1900 HR, plant nutrient solution was given eight times per plant for 6 min each time, and the total supply of nutrient solution was 1.7 $L{\cdot}d^{-1}$. The pH range of the nutrient solution was 6.2 ± 0.2, and the EC range was 2.3 ± 0.2. Each treatment was replicated three times, with 10 plants per replicate, and a total of 90 plants were grown. At the mature stage (2 months after flowering), nine tomato fruits with the same flowering date and maturity from the second ear were randomly selected from each treatment. Peel tissue was rapidly frozen in liquid nitrogen and stored at –80 °C for subsequent analysis.

Determination of tomato fruit quality. For determination of the TSS content, 5.0 g of tomato fruit samples were ground in a mortar and filtered, and determined by Portable Brix Meter (PAL-1; ATAGO, Tokyo, Japan); Titratable acidity (TA) by titration with 0.1 $mol{\cdot}L^{-1}$ NaOH and both expressed as %. The soluble sugar content was measured by anthrone colorimetry (Liu et al. 2018). The lycopene content was measured by high-performance liquid chromatography (Sathish et al. 2009). Glucose, fructose, and sucrose contents were determined by spectrophotometry using a reagents kit (obtained from Suzhou Keming Co., Ltd., Suzhou, China).

Widely targeted metabolites detection. The tomato sample extracts were analyzed using an ultra-performance liquid chromatography electrospray ionization tandem mass spectrometry

Received for publication 24 Apr 2023. Accepted for publication 23 May 2023.
Published online 14 Jul 2023.
This research was funded by the Beijing Academy of Agriculture and Forestry Science and Technology Innovation Capacity Building Project (KJCX20210437); The Ministry of Finance and the Ministry of Agriculture and Rural Affairs: National Modern Agricultural Industry Technology System Funding Project (CARS-23-G-06).
The data presented in this study are available in article and Supplementary Materials. The authors declare no conflict of interest.
Y.J. and M.L. are the corresponding authors. E-mail: jiyanhai@nercv.org or liumingchi@nercv.org.
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(UPLC-ESI-MS/MS) system (UPLC, Shim-pack UFLC SHIMADZU CBM30A system, www.shimadzu.com.cn/; MS, Applied Biosystems 4500 Q TRAP, www.appliedbiosystems.com.cn/). The analytical conditions were as follows. UPLC: column, waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm × 100 mm); The mobile phase consisted of solvent A, pure water with 0.04% acetic acid, and solvent B, acetonitrile with 0.04% acetic acid. Sample measurements were performed with a gradient program that used the starting conditions of 95%-A, 5%-B. Within 10 min, a linear gradient to 5%-A, 95%-B was programmed, and a composition of 5%-A, 95%-B was kept for 1 min. Subsequently, a composition of 95%-A, 5.0%-B was adjusted within 0.10 min and kept for 2.9 min. The column oven was set to 40 °C. The injection volume was 4 μL. The effluent was connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS. Linear ion trap (LIT) and triple quadrupole (QQQ) scans were acquired on a QQQ-LIT mass spectrometer (QTRAP), API 4500 Q TRAP UPLC/MS/MS system, equipped with an ESI turbo ion-spray interface, operating in positive and negative ion mode and controlled by Analyst 1.6.3 software (AB Sciex, Framingham, MA, USA). The ESI source operation parameters were as follows: ion source, turbo spray; source temperature 550 °C; ion-spray voltage (IS) 5500 V (positive ion mode)/ −4500 V (negative ion mode); ion source gas I (GSI), gas II (GSII), and curtain gas (CUR) were set at 50, 60, and 30.0 psi, respectively; the collision gas (CAD) was high. Instrument tuning and mass calibration were performed with 10 and 100 μm polypropylene glycol solutions in QQQ and LIT modes, respectively. QQQ scans were acquired as multiple-reaction monitoring (MRM) experiments with collision gas (nitrogen) set to 5 psi. Declustering potential (DP) and collision energy (CE) for individual MRM transitions were done with further DP and CE optimization. A specific set of MRM transitions was monitored for each period according to the metabolites eluted within this period.

Statistical analysis. All physiological and metabolism experiments were performed in three independent replicates. Statistical analysis and plotting of data were done using Origin 2021 software. One-way analysis of variance was performed using SPSS 20.0 IBM Corp, Armonk, NY, USA). Comparisons between means were performed using Duncan's multiple range test at a significance level of $P < 0.05$. Unsupervised principal component analysis (PCA) was performed using the statistics function prcomp within R × 64 v 3.6.1 (www.r-project.org). The data were unit variance scaled before unsupervised PCA. The hierarchical cluster analysis (HCA) results of samples and metabolites were presented as heatmaps with dendrograms. HCA was carried out using the R package, pheatmap. For HCA, normalized signal intensities of metabolites (unit variance scaling) were visualized as a color spectrum. Significantly regulated metabolites between groups were determined by variable importance in projection (VIP) ≥1 and absolute Log2FC (fold change) >1. VIP values were extracted from the orthogonal partial least-squares discriminant analysis (OPLS-DA) SIMCA-P 14.1 result, which also contained score plots and permutation plots, and were generated using the R package, MetaboAnalystR. The data were log transformed (log2) and mean centered before OPLS-DA. To avoid over fitting, a permutation test (200 permutations) was performed. Identified metabolites were annotated using the Kyoto Encyclopedia of Genes and Genomes (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 mapped to significantly regulated metabolites were then fed into metabolite sets enrichment analysis, and their significance was determined by *P* values from a hypergeometric test.

Results

Physiological indexes. As shown in Fig. 1, there were significant differences between the control group and the two root-restricted groups. T1 and T2 had better coloring effects on tomatoes. The results of the fruit quality index, including TSS, titratable acidity, lycopene, glucose, fructose, and sucrose contents are displayed in Table 1. We found that both T1 and T2 significantly increased lycopene content, and the effect of the T1 treatment was better than that of the T2 treatment. Compared with CK, T1 root restriction significantly increased the content of TSS and titratable acidity, whereas T2 root restriction treatment had no significant difference with CK. Compared with CK, T1 root restriction increased the content of glucose, fructose, and sucrose content. The T1 root restriction treatment increased the organic matter, total nitrogen, and total potassium content compared with the CK treatment, increasing by 43.69%, 36.82%, and 90.16%, respectively (Table 2), whereas the total phosphorus content significantly decreased. The average fruit weight of CK treatment reached 125.49 g, and decreased in the root restriction treatment. In addition, the fruit moisture content under root restriction treatment was the lowest, whereas the CK treatment had the highest, indicating that the difference in fruit size may be mainly caused by water content.

Data quality assessment. To more clearly understand the changes of metabolites in different root restriction treatments, the primary and secondary metabolites in the samples were identified by the UPLC-MS platform broad-targeted metabolomic technology. A total of 1006 metabolites were detected in nine tomato samples. They were divided into 12 classes, including 87 amino acids and derivatives, 160 phenolic acids, 56 nucleotides and derivatives, 175 flavonoids, 3 quinones, 24 lignans and coumarins, 109 others, 139 alkaloids, 36 terpenoids, 69 organic acids, 9 steroids, and 139 lipids (Fig. 2A). The accumulation pattern of metabolites among tomato samples could be visualized through a heatmap HCA (Fig. 2D). The heatmap showed that some metabolites of tomatoes were upregulated in T1 and T2 (restriction treatment), but downregulated in CK (nonrestriction treatment), suggesting that restriction treatment might undergo significantly different metabolic processes compared with nonrestriction treatment. As shown in Fig. 2D, the three biological replicates of each group were clustered together, indicating good homogeneity between replicates and high reliability of the data. The PCA (Fig. 2C) result of the three groups of samples showed that the tomatoes with different treatments were separated, which indicated that the metabolic differences were significant, corresponding to the physiological indexes observation of characteristics. The MIX was the quality control sample mentioned previously. The first component (PC1) accounted for 31.55% of the total change, and the second (PC2) explained 21.34% of the difference for the entire data set. The loading plot showed that the metabolites responsible for the discrimination included bartsioside, N-benzoyl-2-aminoethyl-β-D-glucopyranoside, L-citramalic acid, 1-O-p-coumaroyl-β-D-glucose*, guanosine, pyridoxine, lactobiose, L-methionine, 3-O-p-coumaroylquinic acid*, asperulosidic acid, eugenol, 2-linoleoylglycerol*, O-phospho-L-serine, and agmatine (Fig. 2B).

Identification of differential metabolites. OPLS-DA is a multivariate statistical analysis method with supervised pattern recognition that can maximize group differentiation and help to find differential metabolites. Pairwise comparisons were achieved by the OPLS-DA model, and the score plots are shown in Fig. 3. In this model, R2X and R2Y were used to represent the interpretation rate to the X and Y matrices, respectively, and Q2Y indicated the predictive ability of the model, which was whether the model can distinguish correct sample groups by metabolic expression. The closer R2Y and Q2Y in the indicator were to 1, the more stable and reliable the model was, that is, it can be used to screen differential metabolites. The replacement test was carried out and repeated many times. The

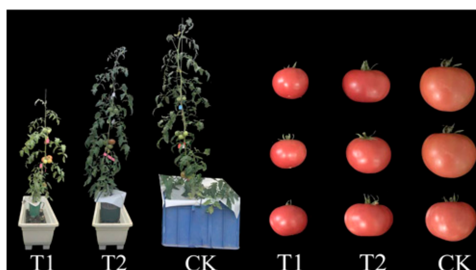

Fig. 1. Phenotypic map of tomatoes under different restriction treatments.

Table 1. Physiological index content of tomatoes after root restriction treatment. Data given in the form means ± *SE*.

Treatment	Lycopene ($\mu g \cdot g^{-1}$)	Total soluble solids (%)	Titratable acidity (%)	Glucose ($mg \cdot g^{-1}$)	Fructose ($mg \cdot g^{-1}$)	Sucrose ($mg \cdot g^{-1}$)
CK	56.02 ± 0.88 c[i]	6.20 ± 0.06 c	0.38 ± 0.02 b	14.70 ± 0.40 b	23.73 ± 0.49 a	1.77 ± 0.06 b
T1	200.33 ± 4.13 a	8.40 ± 0.06 a	0.45 ± 0.02 a	19.39 ± 1.53 a	25.05 ± 0.40 a	3.80 ± 0.07 a
T2	110.39 ± 3.06 b	6.50 ± 0.06 c	0.36 ± 0.03 b	13.80 ± 0.31 c	20.00 ± 0.45 b	1.56 ± 0.01 b

[i] Different letters indicate significant differences among restriction treatments according to Duncan's multiple range test at $P < 0.05$.

Table 2. Root essential nutrients and vegetative biomass production and fruit weight of tomato. Data given in the form means ± *SE*.

Treatments	OM ($mg \cdot g^{-1}$)	TN ($mg \cdot g^{-1}$)	TP ($mg \cdot g^{-1}$)	TK ($mg \cdot g^{-1}$)	Fruit moisture content	Avg fruit wt (g)	Vegetative biomass (g)
CK	415.79 ± 40.38 b[i]	17.00 ± 0.64 c	13.73 ± 0.43 a	16.36 ± 1.19 b	93.19 ± 0.06 a	125.49 ± 2.07 a	1198.33 ± 174.81 a
T1	597.43 ± 31.33 a	23.26 ± 0.58 a	6.72 ± 0.71 c	31.11 ± 1.24 a	89.83 ± 0.27 c	66.51 ± 2.39 c	853.33 ± 55.08 b
T2	513.29 ± 41.69 ab	18.40 ± 0.30 c	11.49 ± 0.63 b	19.69 ± 0.67 b	91.82 ± 0.29 b	88.76 ± 1.15 b	1143.33 ± 84.61 a

[i] Different letters indicate significant differences among restriction treatments according to Duncan's multiple range test at $P < 0.05$.
OM = organic matter; TN = total nitrogen; TP = total phosphorus; TK = total potassium.

results of the modeling were drawn many times into a scatter chart to check the reliability of the OPLS-DA model (Supplemental Fig. 1). The overall trend of the differences in the content of metabolites in the two groups could be visualized through volcanic maps (Supplemental Fig. 2). The fold change value, VIP, and *P* value were combined to screen the differentially expressed metabolites. Select fold change $\geq$ 1 and the metabolites of VIP $\geq$ 1 were combined with *P* value $<$ 0.05 screening differentiated metabolites.

As shown in Fig. 4A, for CK vs. T1, 278 differential metabolites were annotated. Among them, 169 metabolites were upregulated, and 145 differential metabolites were annotated for CK vs. T2, and 81 metabolites were upregulated among them, which indicated that the root restriction technology may activate some key physiological metabolism activity of improving tomato quality. As is shown in Fig. 4B, the number of upregulated secondary metabolites (including alkaloids and phenolic acids), nucleotides and derivatives, and amino acids and derivatives was higher than other metabolites. Secondary metabolites were essential for the interaction between the plant and root restriction (Leng et al. 2021). As shown in Fig. 4B, more upregulated phenolic acids were detected in tomatoes at T1 than in tomatoes at T2.

KEGG annotation and enrichment analysis of differential metabolites. The relative metabolic pathways according to the KEGG annotation and enrichment results are shown in Fig. 5. In CK vs. T1, differential metabolites that might relate to tomato quality were mainly annotated and enriched in the biosynthesis of plant secondary metabolites, biosynthesis of alkaloids derived from the shikimate pathway, biosynthesis of plant hormones, phenylpropanoid biosynthesis, tryptophan metabolism, purine metabolism, and so on (Fig. 5A). For CK vs. T2, the metabolic pathways of the differential metabolites contained biosynthesis of plant secondary metabolites, biosynthesis of phenylpropanoids, cyanoamino acid metabolism, cysteine and methionine metabolism, and so on (Fig. 5B). For T1 vs. T2, the metabolic pathways of the differential metabolites contained biosynthesis of plant secondary metabolites; D-amino acid metabolism; arginine and proline metabolism; and glycine, serine, and threonine metabolism, and so on. Furthermore, some metabolic pathways between these two comparisons overlap, mainly the biosynthesis of plant secondary metabolites (Fig. 5C).

Key metabolites and pathways related to root restriction. A Venn diagram was used to describe the differently expressed metabolites among T1 vs. T2 (Fig. 6A). Among the pairwise comparisons, 126 overlapping differential metabolites were considered as key metabolites in response to root restriction tomato (Supplemental Table 1). The classification is shown in Fig. 6B. In addition, based on the KEGG annotation and enrichment data, these metabolic pathways were mapped to these key metabolites, so that changes in restrictive metabolic regulation can be clearly outlined. There were many differentially expressed metabolites between CK and T1, indicating that the mechanisms of tomato expression under restricted and open roots were different. The metabolic network map further validated this hypothesis (Fig. 7). Responses of tomatoes to root restriction induced some functional substance accumulations, which included saccharides, such as lactobiose, D-maltose, and amino acids (Supplemental Table 2), such as proline and tyrosine, which helped to stabilize the cellular structure and remodel membrane fluidity. Meanwhile, lignin synthesis in tomatoes could be stimulated to protect the cell wall from disruption, and some lignin-related substances, such as p-coumaric acid, coniferin, prunin, naringenin chalcone, and naringenin were produced. Root restriction also promoted some metabolic processes, such as the decomposition of carbohydrates, leading to the increase in D-fructose-6p and D-glucose-6p (Fig. 7). It can be seen that the tomato was probably related to the biosynthesis of amino acids and carbohydrate metabolism.

Discussion

Root restriction is a cultivation technique that can improve the utilization efficiency of agricultural resources by restricting root growth within a certain volume (Kasai et al. 2012; Ray and Sinclair 1998). The identification of key metabolites related to tomato quality will contribute to the improved application of root restriction technology. It has been adopted in many fruits, such as strawberries (Giannina et al. 1998), peaches (Costa et al. 1992), and tomatoes (Bar-Tal et al. 1995). Root restriction treatment can increase total sugar, ascorbic acid, and lycopene content (Byers et al. 2000; Li et al. 2022; Lu et al. 2009). In this study, it was found that the flavor of tomatoes was mainly influenced by primary metabolites (sugars, titratable acids) and secondary metabolites (flavonoids, polyphenols, and amino acids).

Sugar contents, titratable acidity. Sugar accumulation was a comprehensive result of the physiological, metabolic, and genetic processes of tomato fruits during ripening (Carrari and Fernie 2006). The major sugars and sugar alcohols in tomato fruit were fructose, glucose, sucrose, inositol, and galactose (Osorio et al. 2020). Data analysis showed that the level of carbohydrates in the T1 groups was higher than in the CK groups. The increases of D-glucose-6p, D-fructose-6p, lactobiose, and D-maltose could be observed in the T1 treatment. This was consistent with the physiological indicators showing that the content of glucose and fructose in the T1 root restriction treatment was significantly higher than that in the CK treatment. In addition, the variation trend of monosaccharide contents was consistent with a previous study that reported a positive correlation between the content of glucose, fructose, and the degree of restriction (Xie et al. 2009). In tomato fruits, the primary organic acids were malate, citric acid, and tartaric acid, among which malate was a critical compound that contributes to fruit flavor and palatability (Ye et al. 2017). Enhanced malate concentrations lead to altered starch metabolism and soluble solid contents in tomatoes, which subsequently affect postharvest fruit softening (Centeno et al. 2011). Similarly, in this study, the contents of titratable acidity increased in the T1 restriction treatment, and the soluble solid contents of restricted tomatoes were higher than the unrestricted tomatoes.

Many previous studies reported that reducing irrigation amount was favorable for the accumulation of lycopene in tomato fruits (Kim et al. 2022; Mitchell et al. 1991). Water stress can occur almost every day because of the smaller amount of available water under root-zone restriction. Therefore, the increase in lycopene content under root restriction and

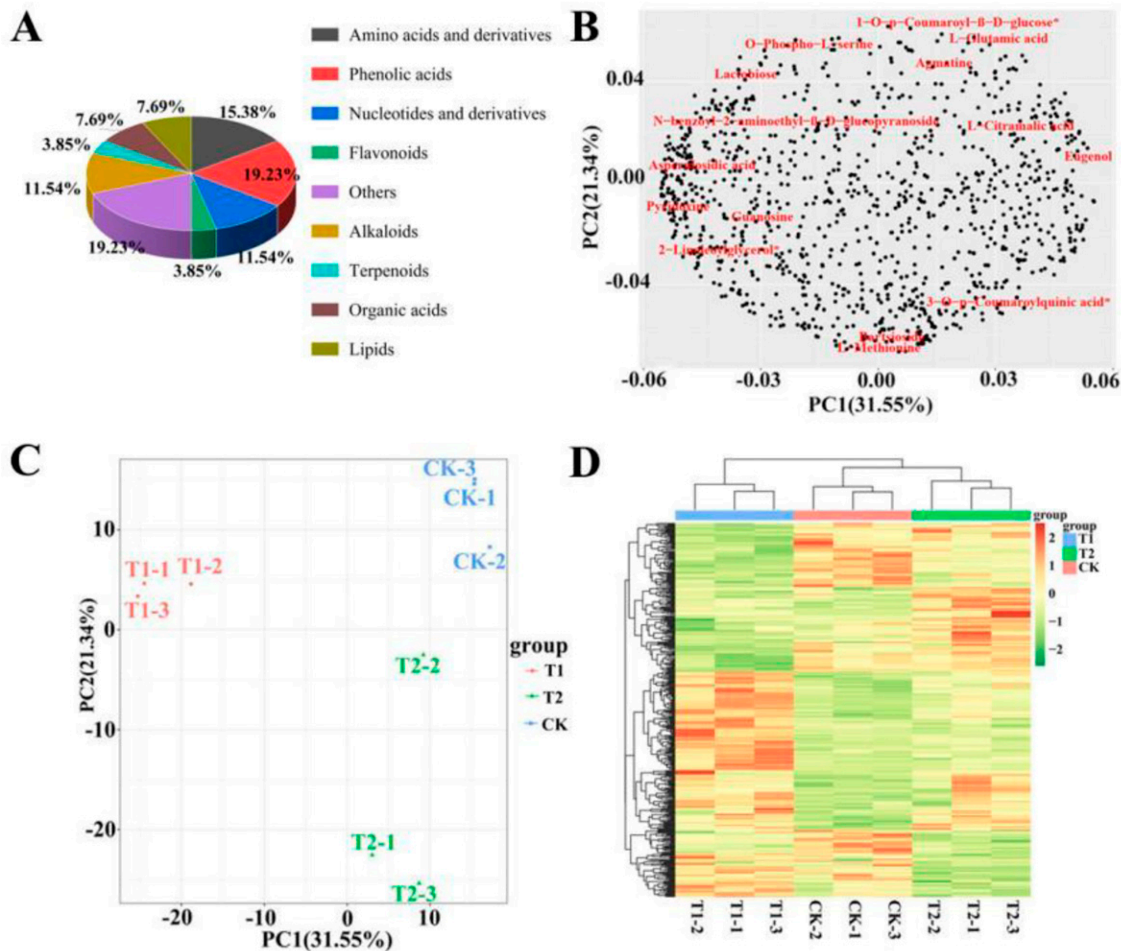

Fig. 2. Classification of the 1006 metabolites of tomato samples (**A**). Loading plot of principal component analysis (PCA) (**B**). PCA (**C**). Hierarchical cluster analysis (**D**).

water stress will have a similar physiological response, which was consistent with previous studies (Kim et al. 2020; Stefanelli et al. 2010).

Flavonoids biosynthesis, phenylpropanoid biosynthesis, amino acids. The phenylpropanoid biosynthesis pathway was one of the main secondary metabolic pathways of plants under abiotic or biological stress (Dixon et al. 2002). It was believed to produce a variety of antioxidants, including flavonoids, phenols, lignin, and their precursors, to protect themselves from attack and prevent electrolyte leakage to surrounding tissues (Xu et al. 2021). Flavonoids, a major secondary metabolite in plants, have various functions in plant development and in response to biotic and abiotic stress (Nakabayashi et al. 2014; Saito et al. 2013). In our study, especially in T1

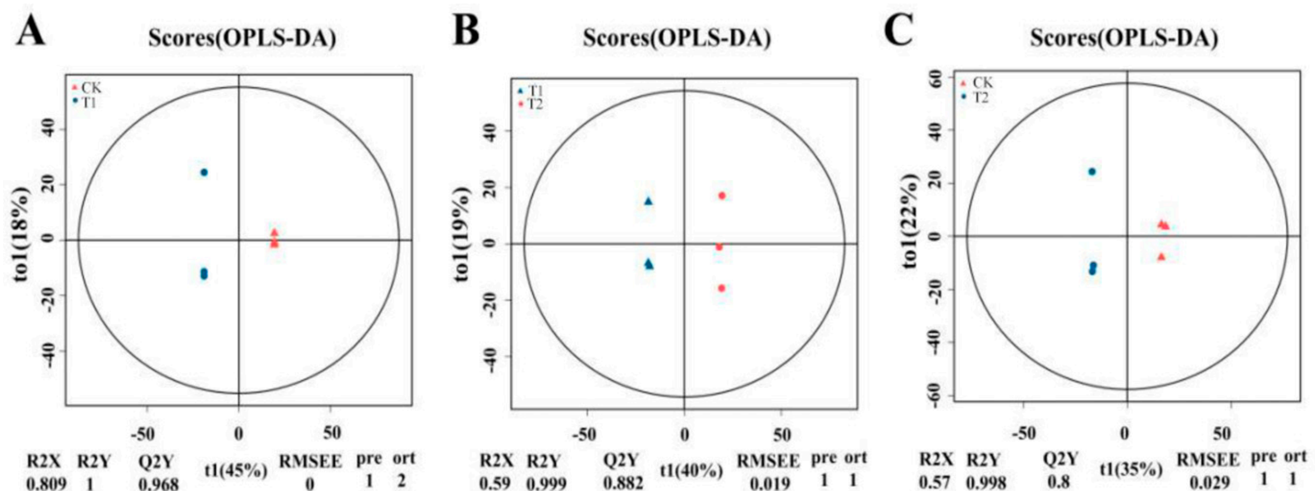

Fig. 3. The score plots of orthogonal partial least-squares discriminant analysis (OPLS-DA) pairwise comparisons of differential metabolites. CK vs. T1 (**A**); T1 vs. T2 (**B**); and CK vs. T2 (**C**). R2X and R2Y were used to represent the interpretation rate to the X and Y matrices, respectively, and Q2Y indicates the predictive ability of the model. RMSEE = root mean square errors of estimation.

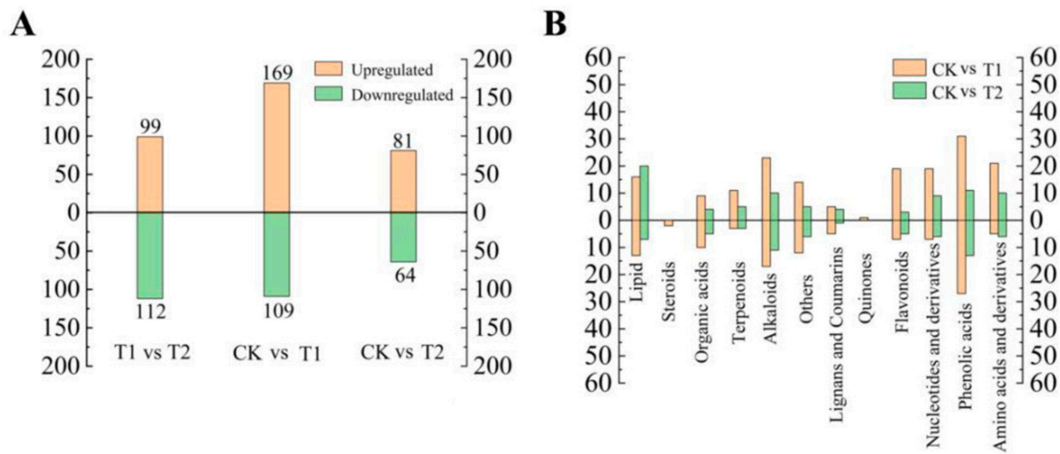

Fig. 4. The number of differentially expressed metabolites of each pairwise comparison of tomato (**A**) and classification of differentially expressed metabolites of two pairwise comparisons (**B**).

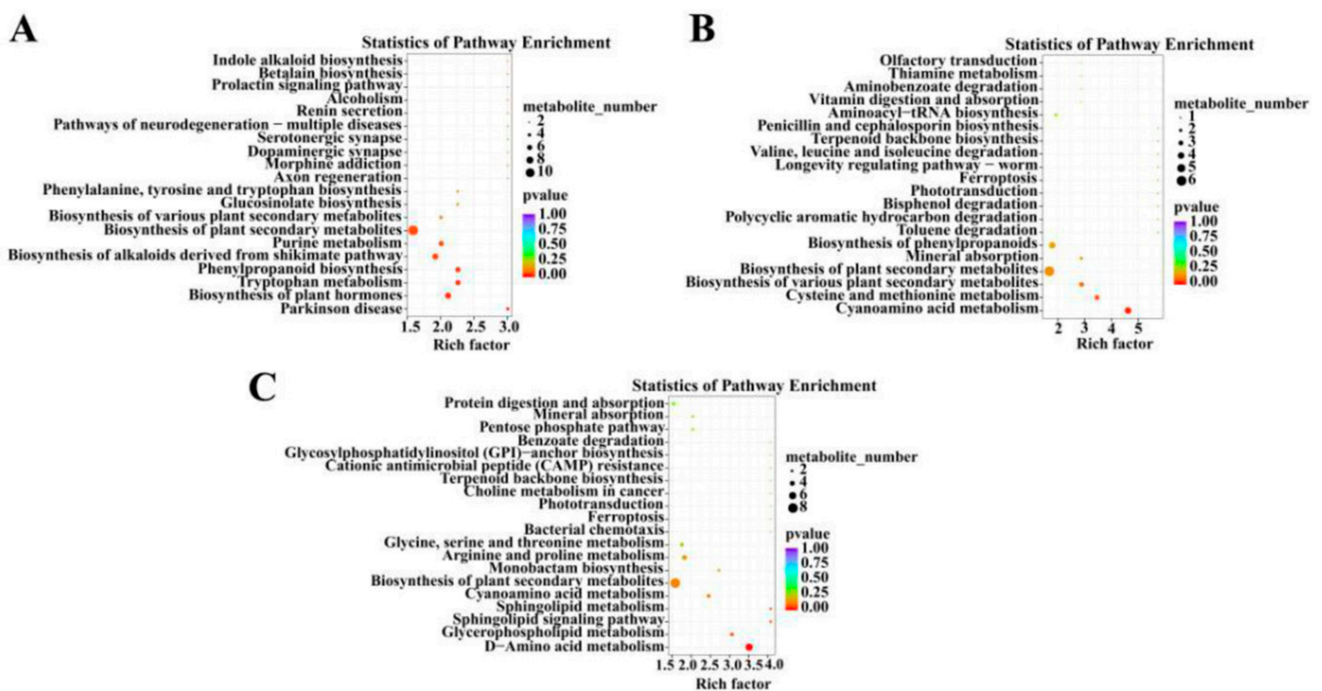

Fig. 5. Kyoto Encyclopedia of Genes and Genomes annotations and enrichment of differentially expressed metabolites of each pairwise comparison of tomato. CK vs. T1 (**A**); CK vs. T2 (**B**); and T1 vs. T2 (**C**).

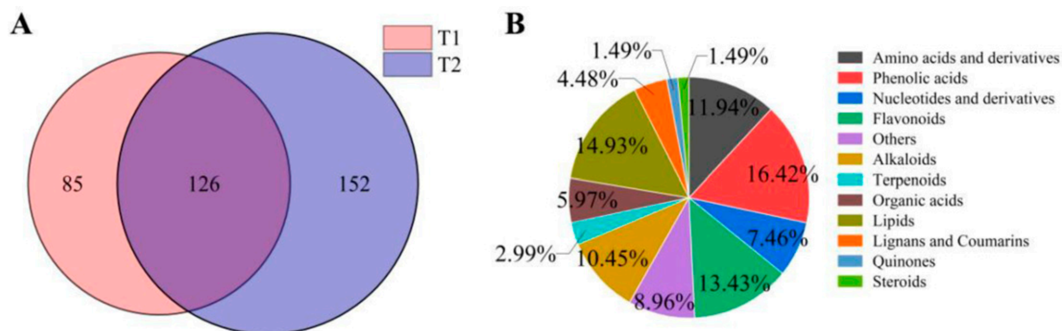

Fig. 6. Venn diagram between T1 vs. T2 (**A**) and the classification of the 126 key metabolites (**B**).

treatment, the flavonoid biosynthesis was significantly enhanced in restriction treatment. It was found that the contents of many flavonoid metabolites such as naringenin chalcone, prunin, and naringenin increased. Polyphenols were the major products of secondary metabolism, which were generated through the phenylpropanoid metabolism pathway, acting as scavengers of free radicals, such as reactive oxygen species

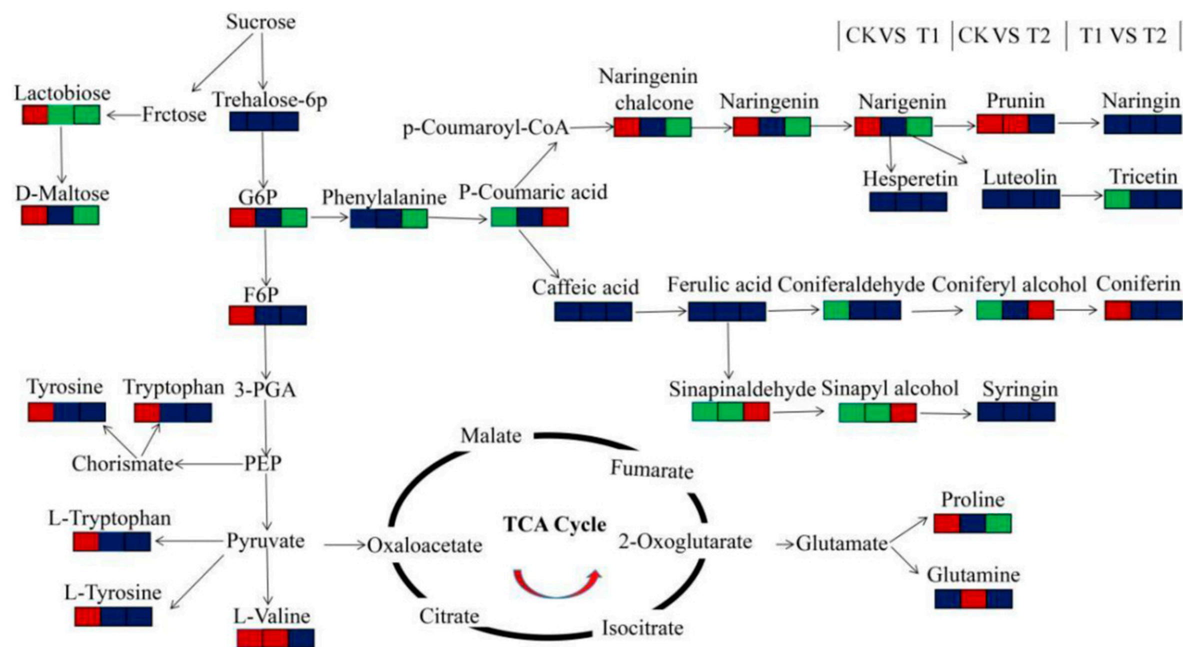

Fig. 7. The changes of key metabolites in the metabolic pathway in tomato samples were compared in pairs. Note: The small red rectangle indicates that the metabolite content was significantly upregulated; the small green rectangle indicates that metabolite content was significantly downregulated; the small blue rectangle indicates no significant difference in that metabolite content. G6P = glucose 6-phosphate; F6P = fructose 6-phosphate; 3-PGA = glyceraldehyde 3-phosphate; PEP = phosphoenolpyruvate; TCA = tricarboxylic acid.

(Perron and Brumaghim 2009; Valcic et al. 2000). Phenolic acids were a kind of small molecular metabolites that can be divided into two groups: hydroxycinnamic acids (e.g., coumaric acid, caffeic acid, ferulic acid, p-coumaric acid, and caftaric acid) and hydroxybenzoic acids (e.g., p-hydroxybenzoic acid, vanillic acid, syringic acid, protocatechuic acid, and gallic acid) (Leng et al. 2020). It can be inferred that root restriction treatment can increase the phenolic acid content of the fruit, and the more severe the root restriction, the more obvious the increase. In this study, the contents of many polyphenols-related metabolites, such as coniferin and p-coumaric acid, increased while coniferaldehyde decreased in tomatoes under restrictions. This result was consistent with previous findings in which flavonoids and polyphenols with protective functions in plants and their biosynthesis were upregulated under restriction stress aiming at scavenging free radicals (Wang et al. 2012).

Abiotic stress was found to have a significant effect on amino acid metabolism, especially in the biosynthesis or degradation of some amino acids. In this study, results revealed that the changes of amino acids during restriction root were mainly involved in the biosynthesis of alkaloids derived from the shikimate pathway, cyanoamino acid metabolism, cysteine, and methionine metabolism. It has been reported that many amino acids derived from these pathways are mainly involved in nitrogen storage and utilization (Sharma and Dietz 2006). Proline accumulation was positively correlated with plant stress resistance (Trovato et al. 2008). In this study, it was found that the content of proline in tomatoes was higher after root restriction. Proline was accumulated in response to environmental pressure sources, such as drought and salinity. The root restriction in this experiment was both spatial pressure stress and drought stress, which was an important penetrant protector to reduce cell osmotic stress (Knipp and Honermeier 2006; Slama et al. 2006).

Conclusion

In this study, widely targeted metabolomics analysis was carried out on tomatoes with three restriction degrees, 126 key differentially expressed metabolites were identified, and potential metabolic networks related to tomato restriction were established. We found restriction treatments (0.5 L) increased the main soluble sugars (glucose, fructose, and sucrose) and some amino acid contents (L-tryptophan, L-tyrosine acid, and L-proline), and decreased the main titratable acidity contents in tomatoes. At the same time, root restriction increased the contents of most alkaloids and flavonols, which contribute to the coloring of tomatoes, and also elevated the contents of tomato antioxidants. Moreover, restriction treatments could increase the contents of most phenolic acids, lactobiose, and D-maltose that have a delicious taste, thus promoting the flavor quality and nutritional value of tomatoes.

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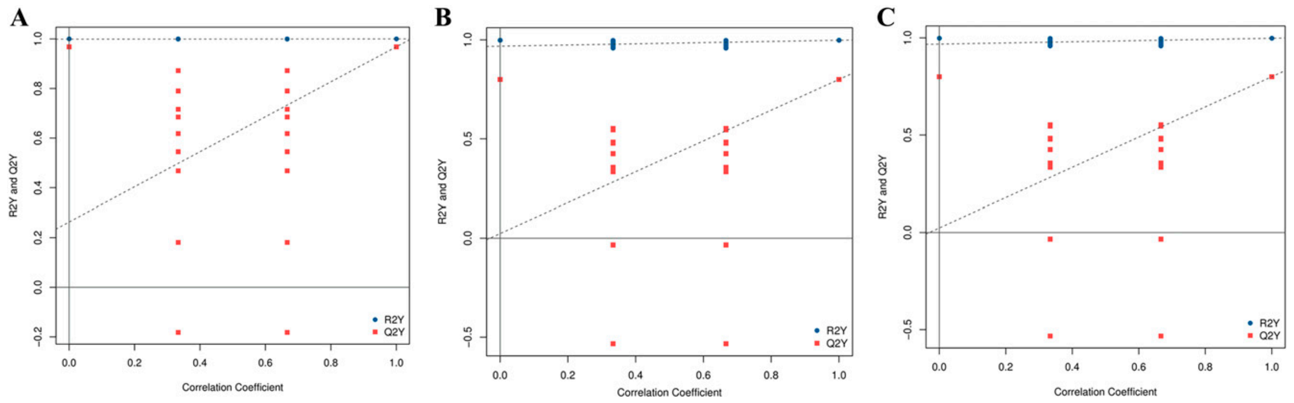
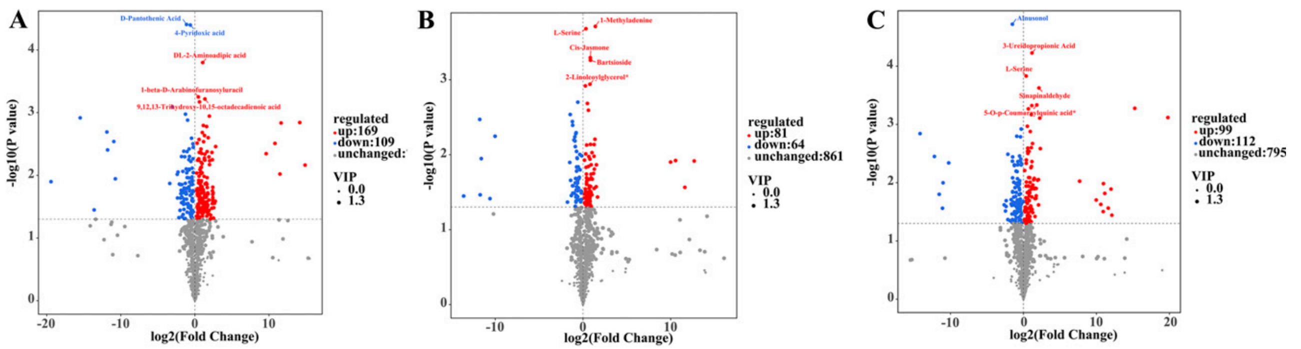


Supplemental Fig. 1. Orthogonal partial least-squares discriminant analysis (OPLS-DA) model arrangement verification diagram. (**A**) CK vs. T1; (**B**) CK vs. T2; and (**C**) T1 vs.T2. Q2Y indicates the predictive ability of the model; R2Y represents the interpretation rate to the Y matrices.



Supplemental Fig. 2. Volcanic maps of each tomato samples pairwise comparisons. (**A**) CK vs. T1; (**B**) CK vs. T2; and (**C**) T1 vs.T2.

Supplemental Table 1. A total of 126 overlapping differential metabolites were considered as key metabolites among T1 vs. T2. KEGG = Kyoto Encyclopedia of Genes and Genomes.

Class	Compounds	KEGG ID
Others	Bartsioside	
	dihydro-4-hydroxy-5-hydroxymethyl-2(3H)-furanone	
	4-O-acetyl-3-O-caffeoyl-2-C-methyl-D-erythronate	
	2-Dehydro-3-deoxy-L-arabinonate	
	Glucarate O-Phosphoric acid	
	D-Sedoheptuiose 7-phosphate	
	Pyridoxine	
	Lactobiose	
	2,6-Dimethyl-7-octene-2,3,6-triol	
	L-Xylose	
	D-Maltose	
Phenolic acids	Sinapylalcohol	C02325
	1,6-Di-O-caffeoyl-β-D-glucose*	
	Alnusonol	
	Cryptochlorogenic acid (4-O-Caffeoylquinic acid)*	
	Anisic acid-O-feruloyl glucoside	
	1-O-p-Coumaroyl-β-D-glucose*	
	1-O-Vanilloyl-D-Glucose	
	Dimethyl Phthalate	
	Chlorogenic acid (3-O-Caffeoylquinic acid)*	
	8-O-p-Coumaroylquinic acid*	
	Phenolic acids	
	6-O-Caffeoyl-D-glucose	
	3-(3-Hydroxyphenyl)-3-hydroxypropanoic acid	
	Isochlorogenic acid C*	
	Benzyl-β-gentiobioside*	
	Coniferyl alcohol	
	1,3-O-Dicaffeoylquinic Acid (Cynarin)*	
	Orcinol glucoside	
	Ferulic acid*	
	3-O-Feruloylquinic acid	
	3,5-Dicaffeoylquinic acid	
	3,4-Di-O-caffeoylquinic acid methyl ester	
	Dicaffeoylquinic acid-O-glucoside	
	4-O-p-Coumaroylquinic acid*	
	4,5-O-Dicaffeoylquinic Acid Methyl Ester	
	3,6-Di-O-caffeoyl glucose*	
	(1'R,3R,5R,8'S)-Dihydrophaseic acid-O-β-D-glucoside	
	Sinapinaldehyde	
	6'-O-Feruloyl-D-sucrose	
	Eugenol	
	p-Coumaric acid	
Lipids	13S-Hydroxy-9Z,11E,15Z-octadecatrienoic acid	
	9,12,13-Trihydroxy-10,15-octadecadienoic acid	
	1-α-Linolenoyl-glycerol*	
	9,10,13-Trihydroxy-11-Octadecenoic Acid	
	LysoPE 16:1(2n isomer)*	
	9-Hydroperoxy-10E,12,15Z-octadecatrienoic acid	
	9,10,11-Trihydroxy-12-octadecenoic acid	
	LysoPC 20:2*	
	Punicic acid (9Z,11E,13Z-octadecatrienoic acid)	
	LysoPC 19:1	
	LysoPE 16:1*	
	2-Linoleoylglycerol*	
	9-Hydroxy-13-oxo-10-octadecenoic Acid	
	LysoPC 16:1(2n isomer)*	
	1-(9Z-Octadecenoyl)-2-(9-oxo-nonanoyl)-sn-glycero-3-phosphocholine	
	LysoPE 20:4(2n isomer)*	
	LysoPE 20:4*	
Alkaloids	Dihydrocaffeoylputrescine	
	Coumaroyl amide derivative	
	N-benzoyl-2-aminoethyl-β-D-glucopyranoside	
	Tryptamine	C00398
	Betanin (Betanidin-5-O-glucoside)	
	N-(4-Aminobutyl)benzamide	
	2-Ethyl-2,6,6-trimethylpiperidin-4-one	
	DL-2-Aminoadipic acid	C00956
	Sinapoylputrescine	
	Agmatine	
	Esculeogenin B-O-sophoroside	C00179
	2,5-Dimethyl pyrazine	
Flavonoids	Naringenin chalcone; 2',4,4',6'-Tetrahydroxychalcone	

(Continued on next page)

Supplemental Table 1. (*Continued*)

Class	Compounds	KEGG ID
	Kaempferol-3-O-(6”-malonyl)galactoside*	
	Luteolin-7-O-(6”-malonyl)glucoside	
	Eriodictyol-8-C-glucoside	
	Naringenin (5,7,4’-Trihydroxyflavanone)*	
	Aromadendrin-7-O-glucoside	
	3,5,4’-Trihydroxy-7-methoxyflavone (Rhamnocitrin)*	
	6,7,8-Tetrahydroxy-5-methoxyflavone*	
	Kaempferol-3-O-(6”-malonyl)glucoside*	
	Chrysin-5-O-glucoside (Toringin)	
	Kaempferol-3-O-neohesperidoside-7-O-glucoside	
	8-Methoxykaempferol-7-O-rhamnoside	
	Quercetin-7-O-(6''-malonyl)glucoside	
	Hispidulin (5,7,4'-Trihydroxy-6-methoxyflavone)*	
	Diosmetin (5,7,3′-Trihydroxy-4'-methoxyflavone)*	
	Pratensein*	
Nucleotides and derivatives	Vidarabine*	
	2'-Deoxyguanosine	C00330
	2-beta-D-Arabinofuranosyluracil	
	Cytarabine	
	L-Methionine	C01733
	Uridine 5′-monophosphate	
	8-Methylcytosine	C02376
	9-Methylmercaptopurine	C16614
	Adenosine*	
Amino acids and derivatives	γ-Glutamylphenylalanine	
	L-Glutamic acid	
	2-Aminopurine	
	γ-Glutamyltyrosine	
	Guanosine	C00144
	L-Aspartic Acid	
	S-Allyl-L-cysteine	
	5-Hydroxy-L-tryptophan	C00643
	L-Proline	C16435
	L-Arginine	
	S-(2-Carboxypropyl)cysteine	
	L-Homocysteine	
	O-Phospho-L-serine	
Organic acids	Iminodiacetic acid	C19911
	Mevalonic acid	C00418
	Methylmalonic acid*	
	Succinic acid*	
	L-Citramalic acid	
	Muconic acid	C02480
	3-Guanidinopropionic acid	C03065
	2-Hydroxyglutaric Acid	
Lignans and Coumarins	Umbelliferone	
	Aesculetine	
	isoscopoletin-glucoside	C09315
	Skimmin (7-Hydroxycoumarin-7-O-glucoside)	
Quinones	1,4,8-Trihydroxynaphthalene-1-O-glucoside	
Terpenoids	11,12-O-Isopropyfidenesolajiangxin F(ISO4)	
	6-O-Trans-Caffeoyl Ajugol	
	Asperulosidic acid	
Steroids	Tomatoside B	