

Metabolomic analysis of bioactive compounds in mature rhizomes and daughter rhizomes in ginger (*Zingiber officinale*)

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Research article

Keywords: ginger, rhizome, daughter rhizome, bioactive compounds, metabolomic analysis

Posted Date: November 19th, 2019

DOI: <https://doi.org/10.21203/rs.2.17463/v1>

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Abstract

Background The bioactive compounds of ginger (*Zingiber officinale*), such as gingerols, diarylheptanoids, and flavonoids, are of importance to human health because of their anticancer, antioxidant, and anti-inflammatory properties. The daughter rhizomes arise from mature rhizomes, but the pungency and pharmacological quality of the two rhizome types differ substantially.

Results In this study, a metabolomic analysis was performed to investigate the biosynthesis of major bioactive compounds in the mature rhizome (R) and daughter rhizome (DR) in ginger. A total of 1212 differentially accumulated metabolites with $p < 0.05$ and $|\text{fold change}| \geq 1$ were identified, of which 399 metabolites were more highly accumulated in R samples than in DR samples, whereas 211 metabolites were most highly accumulated in DR samples. Metabolic pathways associated with the bioactive compounds that determine the pharmacological activity of ginger, including the "terpenoid backbone biosynthesis", "stilbenoid, diarylheptanoid, and gingerol biosynthesis", and "flavonoid biosynthesis" pathways, were enriched in R samples, which indicated that bioactive compounds mainly accumulated in mature rhizomes of ginger.

Conclusion Differences in the key bioactive chemical constituents between the rhizome and daughter rhizome were analyzed. These results provide a theoretical basis for comprehensive development and utilization of ginger resources.

Background

Ginger (*Zingiber officinale* Roscoe) is a plant utilized for both medicine and food that is widely cultivated in southwestern, central, and southeastern areas of China. The pharmacological activities of ginger are utilized for the treatment of arthritis, rheumatism, fever, asthma, degenerative disorders, indigestion, cardiovascular disorders, vomiting, diabetes mellitus, and cancer [1–5]. These pharmacological activities are attributable to a complement of bioactive compounds in the rhizome.

About 2100 nonvolatile compounds have been detected in ginger by liquid chromatography–mass spectrometry (LC-MS) analysis. The bioactive components of ginger can be classified into three categories: essential oils, gingerols, and diarylheptanoids (or diphenylheptanoids) [6–8]. The aroma of ginger is mainly generated by essential oils, of which the key constituents are mainly terpenoid compounds, such as the monoterpenes α -pinene and β -phellandrene, and the sesquiterpenes α -gingene and β -erythrophylline [9]. Gingerols, including shogaols, paradols, and gingerone, are the main flavoring substances and core medicinal ingredients of ginger [10]. Diarylheptanoids are a class of derivatives that consist of a 1,7-diarylheptane skeleton and include curcuminoids [11]. In addition to these compounds, flavonoids are reported to show antioxidant, anticancer, and other pharmacological activities [12,13]. Compounds that contain a phenylpropane structure are produced directly or indirectly through the phenylpropane metabolic pathway. Some intermediates produced in the phenylpropane metabolic pathway, such as coumaric acid, *trans*-cinnamic acid, ferulic acid, and sinapic acid, can be further

converted into coumarin, chlorogenic acid, and CoA lipids to synthesize flavonoids, lignin, and other substances [14,15]. Previous studies have revealed that high contents of lignin, flavonoids, lignans, and products of other phenylpropionic acid-related synthetic pathways are not accumulated in the rhizome of ginger. The main biochemical process in ginger is conversion of sucrose into gingerols [16]. However, the developmental stage at which gingerols are accumulated in the rhizome is unknown.

The daughter rhizome develops from the rhizome. The pungency and pharmacological quality of the two rhizome types differ substantially. Previous studies have mainly focused on chemical and pharmacological analyses by transcriptomic sequencing or target extraction in ginger [4]. In the present study, the ginger cultivar 'Yujiang 1' was used as the study material and metabolomic analysis was performed by high-performance LC-MS. Differences in the key bioactive chemical constituents between the rhizome and daughter rhizome were analyzed. The results provide a theoretical basis for comprehensive development and utilization of ginger resources.

Results

Metabolomics analysis

The rhizome and daughter rhizome samples of Yujiang 1 were selected for metabolomic analysis (Fig. 1A). The LC-MS-based metabolomic analysis was performed to identify differentially accumulated metabolites between the rhizome and daughter rhizome samples. A PCA scores plot was generated to visualize the metabolic differences between the two rhizome types. The present data showed distinct discrimination of the metabolome of the rhizome and daughter rhizome samples (Fig. 1B).

Identification of differentially accumulated metabolites in ginger samples

A total of 1212 differentially accumulated metabolites with $p < 0.05$ and $|\text{fold change}| \geq 2$ were identified, of which 399 metabolites were more highly accumulated in R samples than in DR samples, whereas 211 metabolites were more highly accumulated in DR samples (Fig. 2). Among the differentially accumulated metabolites, pathway analysis of key bioactive compounds (Table 1), including volatile oils, gingerols, diarylheptanoids, and flavonoids, is shown in Table 2 together with a pathway summary (Fig. 3). The pathways "flavonoid biosynthesis" and "stilbenoid, diarylheptanoid, and gingerol biosynthesis" are branches of the general "phenylpropanoid biosynthesis" pathway.

Key bioactive compounds in ginger

The KEGG metabolic pathways associated with volatile oils, gingerols, and diarylheptanoids, such as the "terpenoid backbone biosynthesis" and "stilbenoid, diarylheptanoid, and gingerol biosynthesis" pathways, were enriched between the two samples (Fig. 4). The terpenoid backbone biosynthesis pathway includes two biosynthetic pathways: the mevalonate pathway and the non-mevalonate pathway (MEP/DOXP). In the present study, pyruvate in MEP/DOXP, mevalonate in the mevalonate pathway, and (*E,E*)-farnesyl pyrophosphate were more highly accumulated in R samples compared with DR samples. In the

“stilbenoid, diarylheptanoid, and gingerol biosynthesis” pathway, gingerols (especially 6-gingerol) showed significantly higher contents in R samples than those in DR samples; additional compounds in this pathway, such as caffeoylquinic acid and curcumin, shown similar accumulation patterns (Fig. 5).

Flavonoids show broad pharmacological activities, including antimicrobial, antioxidant, cytotoxic, chemoprevention, and anticancer activities. Kaempferol and quercetin are two intermediate products in the “flavonoid biosynthesis” pathway. In the present study, contents of flavonoid compounds, such as kaempferol and quercetin, were higher in R samples than in DR samples. Caffeoylquinic acid were also identified in the flavonoid pathway (Fig. 6).

LC-MS analysis of gingerols in R and DR samples

Gingerols are the main compounds responsible for the pungency of ginger, and are the main flavoring substances and core medicinal ingredients of ginger. To evaluate expression profiles of differentially expressed metabolites, the contents of four gingerols, namely 6-gingerol, 8-gingerol, 10-gingerol, and 6-zingiberene, were detected by LC-MS analysis. The contents of the four metabolites in R samples were 489.3 µg/g, 29.5 µg/g, 7.8 µg/g and 52.1 µg/g respectively, which were all considerably higher than those of DR samples (Fig. 7). The results were consistent with the metabolome data, which indicated that metabolomic analysis is an effective method to investigate the differences in accumulation of secondary metabolites among different tissues.

Discussion

To investigate the molecular regulatory mechanism underlying the differences in metabolite accumulation, we examined differences in metabolite accumulation between the R and DR samples. Six samples (two tissue samples, with three biological replicates for each tissue sample) were analyzed by using LC-MS methods. A total of 1212 differentially expressed metabolites were identified with $p < 0.05$ and $|\text{fold change}| \geq 2$, of which 399 metabolites were more highly accumulated in R samples than in DR samples, whereas 211 metabolites were expressed more highly in DR samples (Fig. 2). The majority of key bioactive compounds, such as volatile oils, gingerols, diarylheptanoids, and flavonoids, were accumulated in R samples rather than in DR samples.

Sesquiterpenoids and monoterpenoids produced by the “terpenoid backbone biosynthesis pathway” are major biochemical constituents of ginger volatile oils [17]. In the present study, pyruvate in MEP/DOXP, mevalonate in the mevalonate pathway, and (*E,E*)-farnesyl pyrophosphate were highly accumulated in the rhizome. Pyruvate and mevalonate are further transformed to monoterpenoids, while all three metabolites can be utilized to synthesize sesquiterpenoids (Fig. 4). Gingerols are the core pharmaceutical ingredient of ginger. On the basis of the metabolome analysis and target detection data, 6-gingerol, 8-gingerol, 10-gingerol, and 6-zingiberene were all mainly accumulated in the mature rhizome of ginger (Fig. 7). The contents of these four main gingerols, especially 6-gingerol, differed substantially between R and DR samples. Among the gingerols, the amount of 6-gingerol attains almost 75% of the total gingerol complement [18]. The present findings were consistent in that the content of 6-gingerol (489.3 µg/g) was

many times higher than that of other types of gingerols. 6-Zingiberene is an alkyl phenolic compound formed by dehydration of 6-gingerol [19]. In the present study, 52.1 µg/g and 11.28 µg/g 6-zingiberene were detected in R and DR samples, respectively, which indicated that 6-zingiberene is also an important constituent of the essential oils in ginger.

In addition to these important bioactive compounds, the ginger rhizome contains a large amount of flavonoids, which are pharmacologically active metabolites that display antioxidant, anti-aging, and anticancer properties [20,21]. Among the significantly enriched pathways detected in the ginger rhizome, the “phenylalanine metabolism” pathway (ko00360) and the “phenylpropanoid biosynthesis” pathway (ko00940) produce substrates for the “phenylpropanoid biosynthesis” pathway (ko00940), which further generates substrates for the biosynthesis of many active metabolites, such as gingerols, curcumin, and flavonoids [16]. In the current study, flavonoid compounds such as kaempferol and quercetin, intermediates of the phenylpropanoid biosynthesis pathway such as caffeoylquinic acid, and curcumin all accumulated more highly in R samples than in DR samples. Previous studies have shown that biosynthetic pathways associated with lignin, flavonoids, and phenylpropanoic acid are not up-regulated in the ginger rhizome. One of the main biochemical processes in ginger is the conversion of sucrose into gingerol [16]. The content of bioactive compounds in the rhizome of ginger differs depending on the time of harvesting. Further study of the metabolome will guide the selection, cultivation, development, and utilization of ginger cultivars.

Conclusions

Metabolomic analysis was performed to investigate the biosynthesis of major bioactive compounds in the mature rhizome and daughter rhizome in ginger. Metabolic pathways associated with the bioactive compounds that determine the pharmacological activity of ginger, including the “terpenoid backbone biosynthesis”, “stilbenoid, diarylheptanoid, and gingerol biosynthesis”, and “flavonoid biosynthesis” pathways, were enriched in R samples, which indicated that bioactive compounds mainly accumulated in mature rhizomes of ginger. The results provide a theoretical basis for comprehensive development and utilization of ginger resources.

Methods

Plant material and sample preparation

Plants of ginger ‘Yujiang 1’ were grown under local natural conditions in Chongqing, China (29°14' N, 105°52' E) from ginger planting experimental base. ‘Yujiang 1’ passed the field appraisal of experts in the Chongqing Crop Variety Approval Committee in 2014 (CVAC2015015). Pro. YL undertook the formal identification of the plant material used in our study. Rhizomes (R) and daughter rhizomes (DR) were collected, rapidly frozen in liquid nitrogen, and stored at -80 °C until LC-MS analysis (Fig. 1A).

LC-MS analysis of metabolomics

The LC-MS analysis was performed on a Waters Acquity UPLC® I-Class system equipped with a binary solvent delivery manager and a sample manager, coupled with a Waters Vion IMS QToF mass spectrometer equipped with an electrospray interface (Waters Corporation, Milford, MA, USA).

The column used was an Acquity BEH C₁₈ column (100 mm × 2.1 mm i.d., 1.7 μm; Waters). The column was maintained at 45 °C and separation was achieved using the following gradient: 5%–20% solvent B over 0–2 min, 20%–60% B over 2–8 min, 60%–100% B over 8–12 min, held at 100% B for 2 min, then 100%–5% B over 14–14.5 min, and 14.5–15.5 min hold at 5% B, at a flow rate of 0.40 mL/min, where solvent B was acetonitrile (0.1% [v/v] formic acid) and solvent A was aqueous formic acid (0.1% [v/v] formic acid). The injection volume was 3.00 μL and column temperature was 45.0 °C.

The mass spectrometric data were collected using a Waters Vion IMS QToF mass spectrometer equipped with an electrospray ionization source operating in either positive or negative ion mode. The source temperature and desolvation temperature were set at 120 °C and 500 °C, respectively, with a desolvation gas flow of 900 L/h. Centroid data were collected from 50 to 1000 *m/z* with a scan time of 0.1 s and interscan delay of 0.02 s over a 13 min analysis time.

Data analysis

The UPLC–QTOF/MS raw data were analyzed using Progenesis Q1 software (Waters). The parameters used were retention time (RT) range 0–14.0 min, mass range 50–1000 Da, and mass tolerance 0.01 Da. Isotopic peaks were excluded from the analysis, the noise elimination level was 10.00, minimum intensity was 15% of the base peak intensity, and RT tolerance was 0.01 min. The Excel file contained three-dimensional data sets including *m/z*, peak RT, and peak intensities, and RT–*m/z* pairs were used as the identifier for each ion. The resulting matrix was further reduced by removing peaks with missing values (ion intensity = 0) in more than 60% of the samples. The internal standard was used for data quality control (reproducibility). The positive and negative ion mode data were combined to generate a combined data set, which was imported into the SIMCA-P+ 14.0 software package (Umetrics, Umeå, Sweden). Principal component analysis (PCA) were carried out to visualize the metabolic differences among experimental groups, after mean centering and unit variance scaling. Pathway analysis was performed by online software http://www.genome.jp/kegg/tool/map_pathway2.html.

LC-MS determination of gingerols

For gingerols content analysis, 3 g of PEMs were collected, with each line represented by three independent biological replicates. The samples were immediately weighed (fresh weight), frozen, powdered in liquid nitrogen, and later analyzed with an LC-MS system. The instrument platform for LC-MS analysis is Ultra HPLC liquid chromatography linear ion trap mass spectrometer UHPLC-LTQ/MS from Thermo.

Declarations

Abbreviations

R: mature rhizome

DR: daughter rhizome

RT: retention time

PCA: Principal component analysis

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Competing interests

The authors declare that they have no competing interest.

Funding

The authors are grateful for the financial support provided by The Natural Science Foundation of Chongqing (cstc2019jcyj-msxmX0697 and cstc2018jcyjAX0717), The Scientific and Technological Research Program of Chongqing Municipal Education Commission (KJQN201901302) and Chongqing University of Arts and Sciences Talent Introduction Project (2017RTZ22). These funding bodies had no role in the design of the study, collection, analysis, and interpretation of data, or in writing the manuscript.

Authors' contributions

ZL conceived, designed and carried out the study and wrote the manuscript. GL provided important suggestions on the experimental design and analyses. ZC and JT offered varying degrees of help during the experimental operation. YJ, QL and YL helped to modify the manuscript. All authors read and approved the manuscript.

Acknowledgement

Not applicable.

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Tables

Tab. 1 The significantly enriched KEGG pathways for differentially expressed bioactive compounds between rhizome and daughter rhizome

Pathway Name	Match Status	P value	-log(p)
Stilbenoid, diarylheptanoid and gingerol biosynthesis	1/10	2.019E-05	4.69486
Phenylalanine, tyrosine and tryptophan biosynthesis	4/21	0.01636	1.78621
Phenylalanine metabolism	1/8	0.00834	2.07883
Phenylpropanoid biosynthesis	3/45	0.00728	2.13786
Flavone and flavonol biosynthesis	2/9	5.678E-04	3.24580
Flavonoid biosynthesis	4/43	0.00026	3.58502
Riboflavin metabolism	1/10	0.00501	2.30016
Steroid biosynthesis	2/36	0.00717	2.14448
Terpenoid backbone biosynthesis	5/25	0.00022	3.65757
Monoterpenoid biosynthesis	1/4	0.00031	3.50863
Diterpenoid biosynthesis	2/26	9.562E-05	4.01945
Ubiquinone and other terpenoid-quinone biosynthesis	1/23	0.01456	1.83683

Tab.2 Metabolites mapped to the pathways that are responsible for the biosynthesis of major bioactive compounds in ginger

Pathway Name (KO)	Metabolites	Formula	p value	Fold change (DR/R)	Change (DR/R)
backbone is	Pyruvic acid	C ₃ H ₄ O ₃	0.0185	0.11332	Down
	dimethylallyl- diphosphate	C ₅ H ₁₂ O ₇ P ₂	0.012121	0.36102	Down
	Mevalonic acid	C ₆ H ₁₂ O ₄	0.003684	0.04645	Down
	Farnesyl pyrophosphate	C ₁₅ H ₂₈ O ₇ P ₂	0.008179	6.83485	Up
	all-trans-heptaprenyl diphosphate	C ₃₅ H ₆₀ O ₇ P ₂	0.004224	5.43691	Up
diarylheptanoid and gingerol is (KO00945)	6-Gingerol	C ₁₇ H ₂₆ O ₄	0.001607	0.43341	Down
	8-Gingerol	C ₄₃ H ₃₂ O ₂₀	2.02E-05	0.19665	Down
	Curcumin	C ₂₁ H ₂₀ O ₆	0.043294	0.00000	Down
	Pinosylvin	C ₁₅ H ₁₄ O ₂	0.004511	9.00535	Up
biosynthesis (KO00941)	Quercetin	C ₁₅ H ₁₀ O ₇	0.044544	0.52164	Down
	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	0.003553	0.13237	Down
	Epicatechin	C ₁₅ H ₁₄ O ₆	0.000425	0.19846	Down
	Gallocatechin	C ₁₅ H ₁₄ O ₇	0.004082	0.08435	Down
	Kaempferol	C ₁₅ H ₁₀ O ₆	0.004614	2.40452	Up

Figures

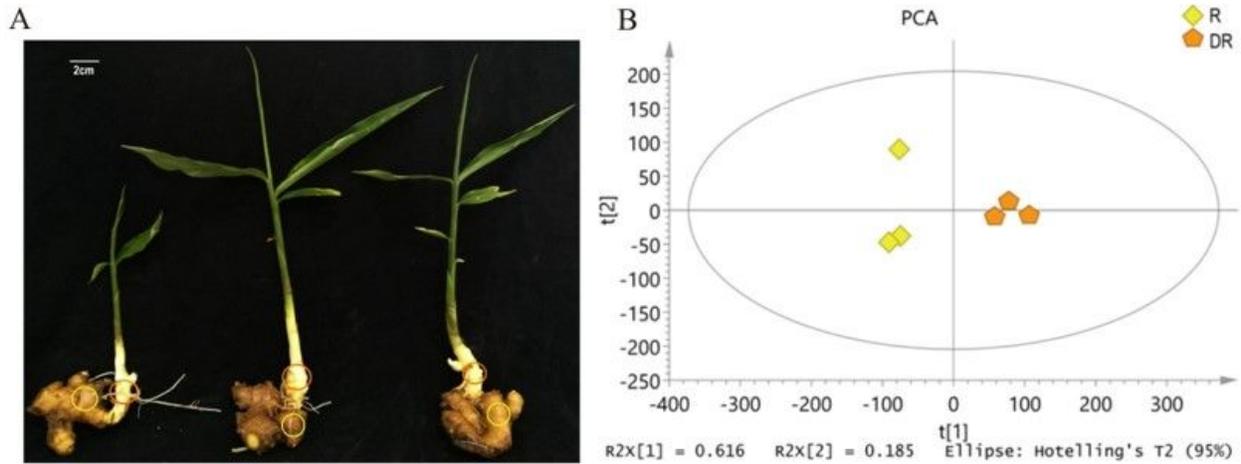


Fig.1

Figure 1

Ginger materials and Principal Component Analysis. A: Yellow circle show Rhizomes (R) and Red circle represent daughter rhizome (DR). B: Principal Component Analysis between R and DR samples. Three replicates were set for each sample.

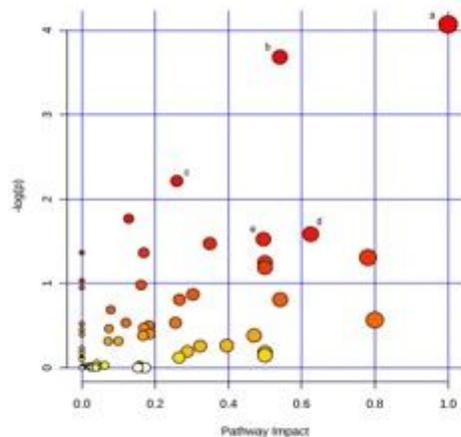


Fig.3

Figure 3

Metabolites implicating in the metabolism pathway by MetaboAnalyst 4.0. Summary of metabolism pathway analysis. (a) Terpenoid backbone biosynthesis (b) Flavonoid biosynthesis (c) Stilbenoid, diarylheptanoid and gingerol biosynthesis (d) Phenylpropanoid biosynthesis (e) Diterpenoid biosynthesis

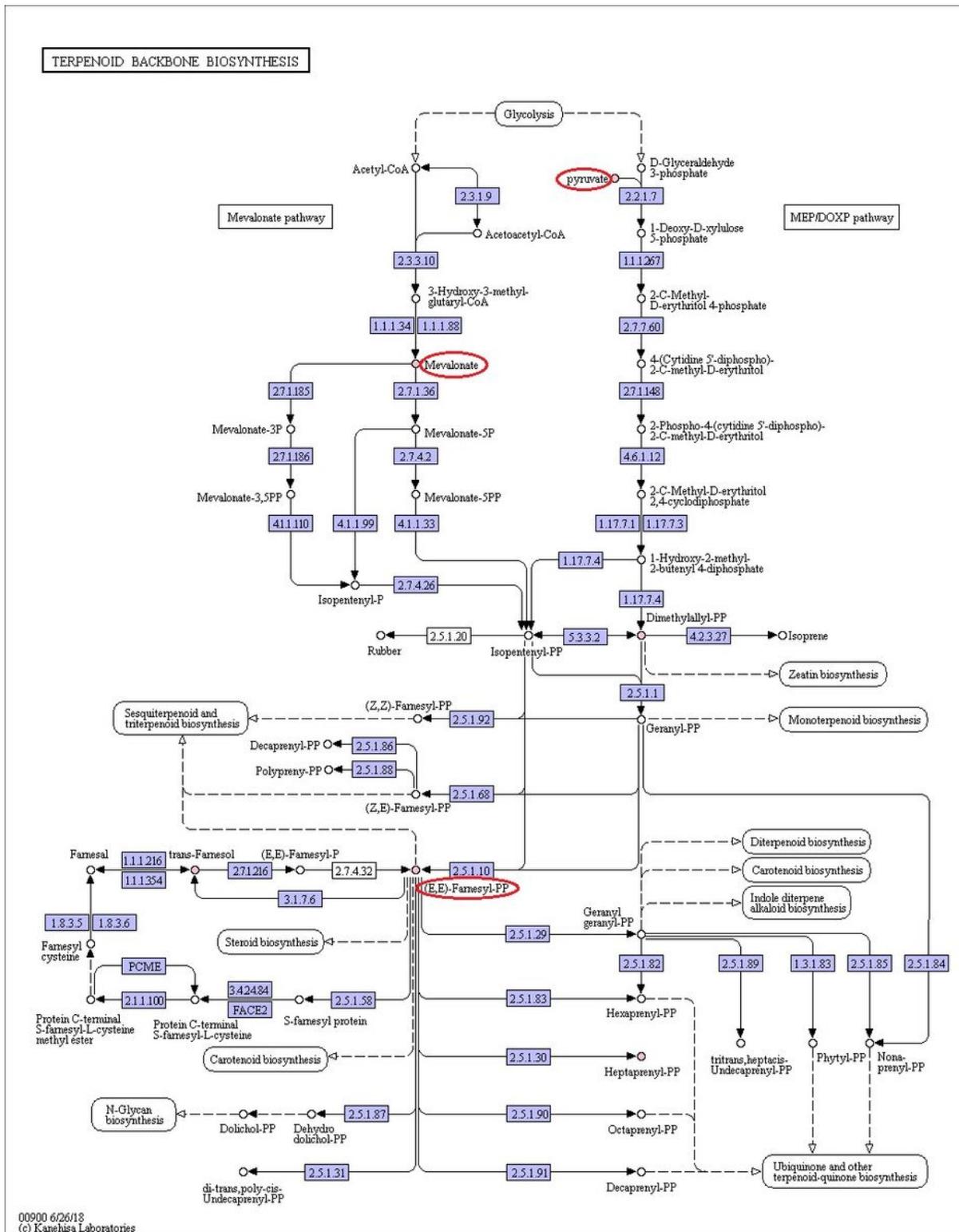


Figure 4

Diferentially accumulated metabolites involved in the "terpenoid backbone biosynthesis" pathway of ginger. The red columns indicate metabolites expressed at a significantly high level in rhizome samples. The red boxes indicate the biosynthesis of the major compounds found in ginger volatile oil. This color-coded map of DEGs corresponds to map00900 in the KEGG database (http://www.genome.jp/dbget-bin/www_bget?pathway:map00900)

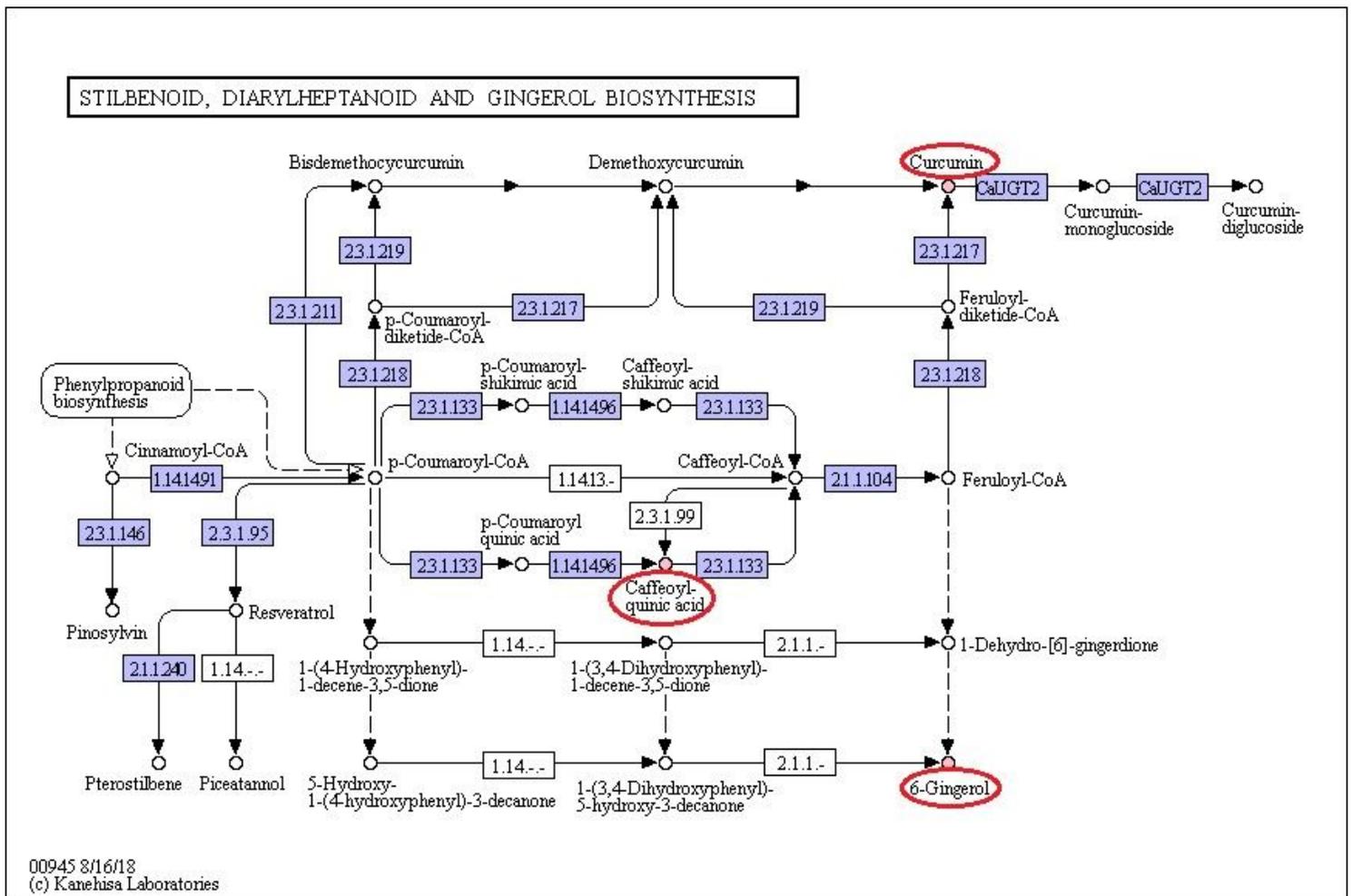


Figure 5

Diferentially accumulated metabolites involved in the "stilbenoid, diarylheptanoid and gingerol biosynthesis" pathway. The red columns indicate metabolites expressed at a significantly high level in rhizome samples. This color-coded map of DEGs corresponds to map00945 in the KEGG database (http://www.genome.jp/dbget-bin/www_bget?pathway:map00945)

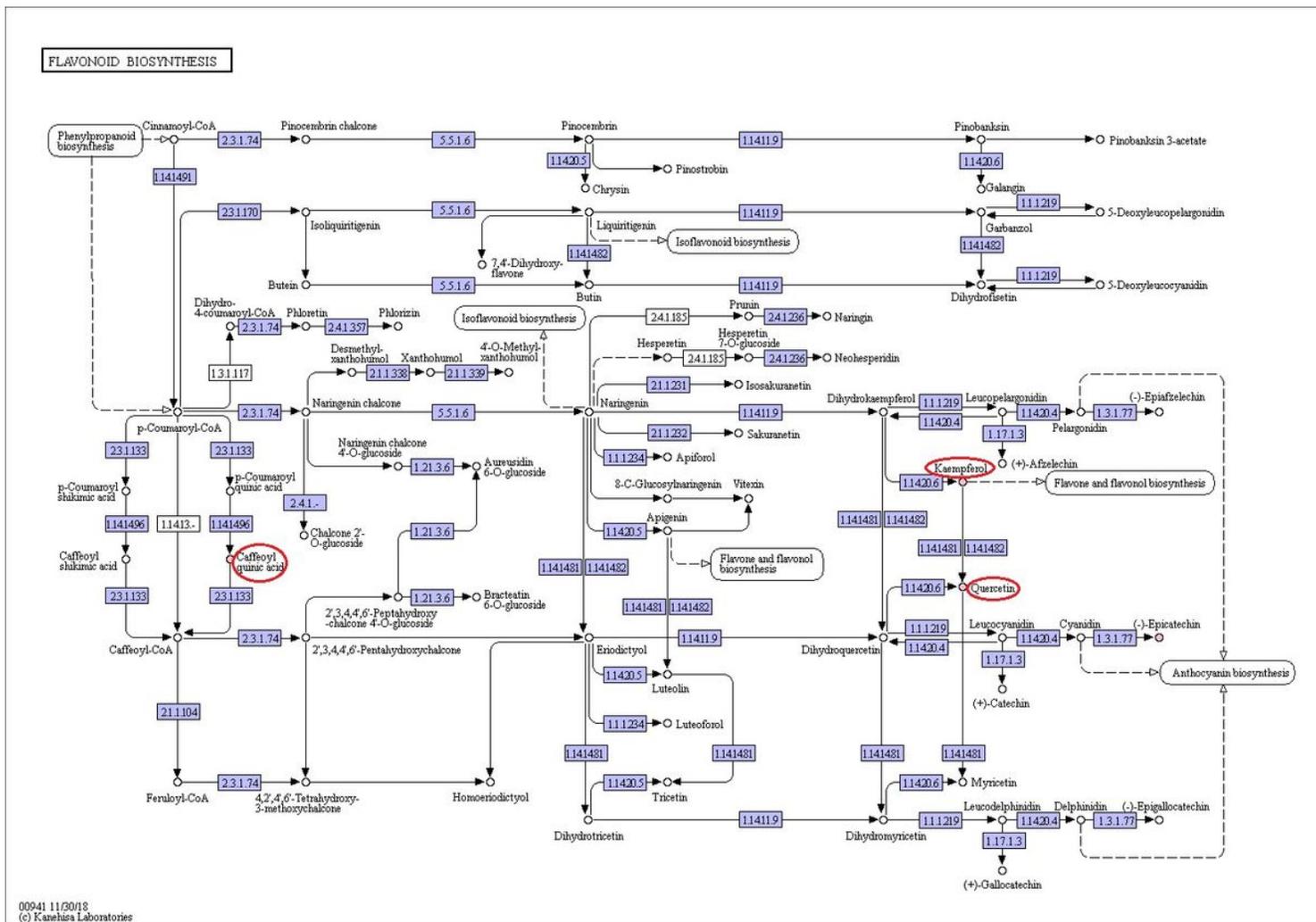


Figure 6

Diferentially accumulated metabolites involved in the "flavonoid biosynthesis" pathway. The red columns indicate metabolites expressed at a significantly high level in rhizome samples. This color-coded map of DEGs corresponds to map00941 in the KEGG database (http://www.genome.jp/dbget-bin/www_bget?pathway:map00941)

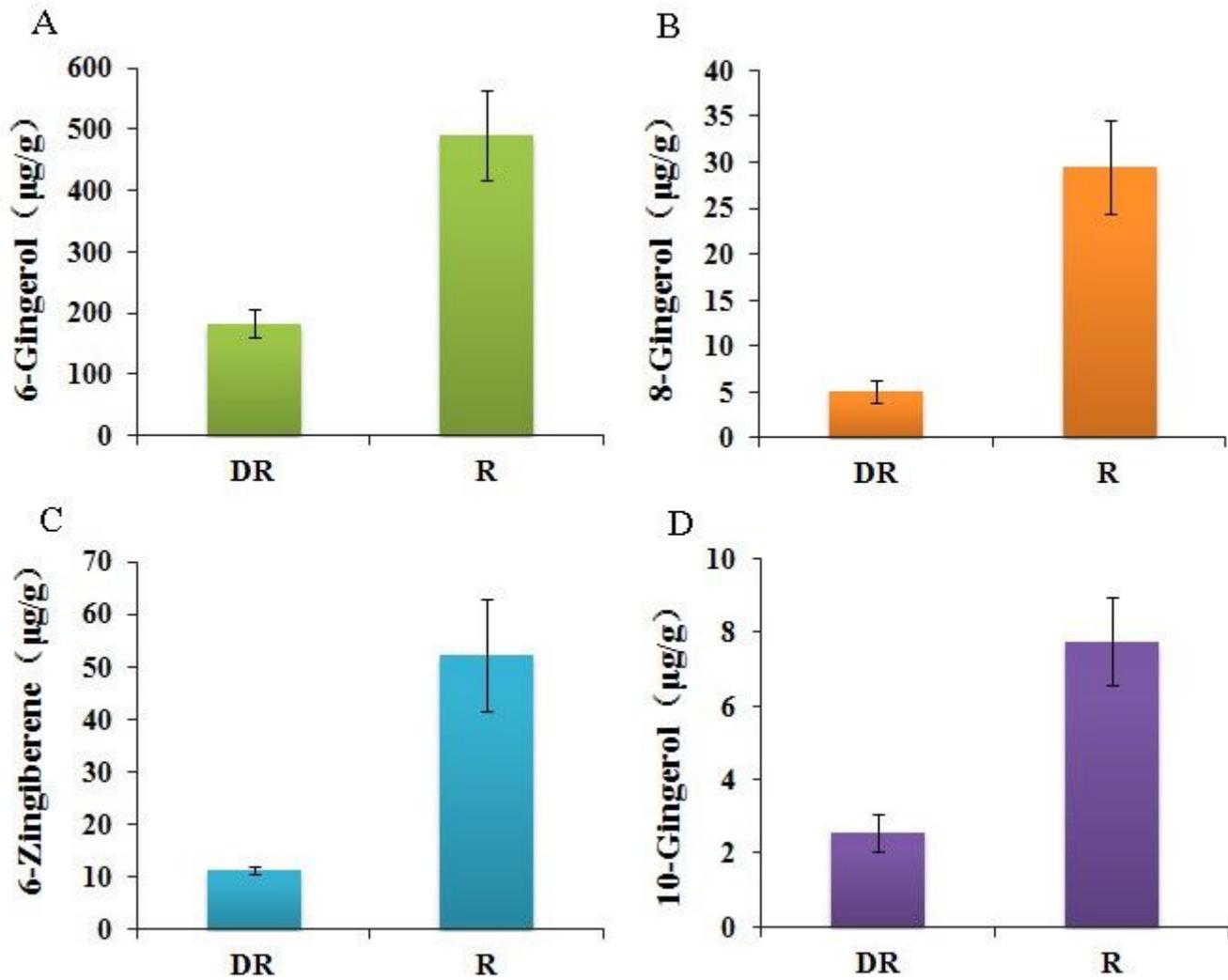


Figure 7

Gingerols contents of the two samples detected by LC-MS methods. Values are means±SE of three biological replicates.

Supplementary Files

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- [additionalfiledifferentiallyexpressedmetabolites.xlsx](#)