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Responses of aroma related metabolic attributes of Opisthopappus longilobus flowers to environmental changes

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Abstract

Various metabolic processes are activated in response to environmental changes. *Opisthopappus longilobus* (*Opisthopappus*) and its descendant species, *Opisthopappus taihangensis* commonly thrive on the Taihang Mountains of China. Being typical cliff plants, both *O. longilobus* and *O. taihangensis* release unique aromatics. We investigated whether these aromatics might be differentiated and inherently adaptive attributes. To determine the potential differentiation and environmental response patterns between *O. longilobus* and *O. taihangensis*, comparative metabolic analysis was performed on *O. longilobus* wild flower (CLW), *O. longilobus* reciprocal transplant flower (CLT), and *O. taihangensis* wild flower (TH) groups. Significant differences in the metabolic profiles between *O. longilobus* and *O. taihangensis* flowers were observed, and 29 substances related to their scents were obtained (two alkenes, two aldehydes, three esters, eight phenols, three acids, three ketones, three alcohols, and five flavonoids) of which eugenol and chlorogenic were the primary aromatictic molecules. The chlorogenic content of TH was significantly higher than that of CLW and CLT, while the eugenol content was highest in CLT followed by TH, and both metabolites were enriched in the phenylpropane pathway. In addition, the aromatic related compounds were significantly correlated with the lowest temperatures in October and in December. The results above indicated that phenylpropane, particularly eugenol and chlorogenic, played important roles in the responses of *O. longilobus* species to environmental changes.

Introduction

The metabolic processes of organisms are dynamic and complex systems that are regulated by multiple factors [1]. From the most upstream genomic DNA, mRNA, and from proteins to traits, organisms continually adjust their metabolic responses and metabolites to maintain a dynamic balance between their internal and external environments [2]. All reactions, from genes to traits are the final comprehensive result of the co-regulation of genes and the external environment [3]. Thus, changes in the metabolites of organisms, the relationships between them, and their external manifestations are essential for elucidating their adaptations and evolutionary processes [4]. Conversely, metabolic data can more accurately reflect the physiological status and phenotypic presence of plants, while clearly identifying minor changes in genetic and protein expression [2].

Differences in the metabolic activities of plants at different growth stages can facilitate the exploration and tracking of changing metabolite distribution patterns [5]. Based on the differences and interactions between metabolites, environmental influences on the metabolites of plants, as well as their adaptive pathways and metabolic networks may be inferred [6].

Continuous and dynamic changes in the environment typically result in adaptions by plants [7]. These adaptations involve the production and accumulation of a diverse set of metabolites, ranging from signaling hormones, primary metabolites, to a wide array of specialized multifunctional metabolites [8, 9]. When plants are subject to environmental stress, specialized metabolites can improve their protection and competitiveness for survival [10].

Different models have been proposed to describe the relationships between plant metabolism and the environment. Researchers have suggested that some plants synthesize and accumulate metabolites regardless of their environment. However, for most plants, the synthesis and accumulation of metabolites are altered with changes in the environment [11]. Plants typically allocate various types and quantities of metabolites in response to specific environmental changes. They either synthesize particular metabolites, or significantly increase their concentrations under certain environmental conditions [12]. For example, the volatile secondary metabolites of insect repellents in cotton are synthesized after being stimulated by insects, rather than prior to being synthesized and stored [13].

During the growth and development of plants, myriad environmental influences can impact the synthesis and accumulation of metabolites, including biological and abiotic factors [13, 14]. Further, the effects of environmental factors on plant metabolism are very complex. The synthesis and accumulation of one type of metabolite can be induced by several environmental factors, whereas one type of environmental factor can induce the synthesis and accumulation of multiple metabolites [15]. Identical environmental factors may also have distinct effects on different plants [16]. Although any environmental factor may affect the metabolism of plants for various species under different natural conditions, the degree of influence can vary [16, 17].

Opisthopappus longilobus, belongs to the genus *Opisthopappus*, which is mainly distributed in the Hebei Provinces of China [18]. This species, which grows on the slopes or cliffs of the Taihang Mountains is a typical cliff plant [19] that has good cold and drought resistance [20]. *O. longilobus* is rich in aromatictic oils with a strong and persistent fragrance, and has high ornamental and medicinal value [21, 22].

Within the *Opisthopappus* genus, *Opisthopappus taihangensis* is another cliff residing species that is primarily located in Henan Province, China. Based on our previous study, *O. taihangensis* is regarded as a descendant of *O. longilobus* [23]. They diverged during the early Miocene under differing precipitation regimes due to the intensification of the Asian monsoon [23]. Though the two species present a significant genetic differentiation [18], they exhibit morphological similarities under different habitats [23]. The morphological differentiation occurred only in the leaf segmentation pattern with or without bracts [23, 24]. Through field investigations we observed that the flowers of the two species evolved some morphological differentiation. The flower size and ligulate flower length of *O. longilobus* was smaller than that of *O. taihangensis* (Fig. 1), and the petal colors of the two species changed with the flowering cycle (Fig. 1). The petal color of *O. taihangensis* primarily appeared pink and then changed to white, while that of *O. longilobus* occasionally presented as pink at the end of the flowering cycle.

Apart from this, the flowers of both *O. longilobus* and *O. taihangensis* can release unique and potent aromatic. The aromatictic elements of plants generally consist of secondary metabolites. We investigated whether the metabolites and metabolic pathways of the *O. longilobus* ancestor plant were identical to those of *O. taihangensis* flowers. Further, if there was any differentiation what were the key aromatic metabolites, involved pathways, and associated genes? Under these differentiations what were

the potential mechanisms, and were they adaptive responses to the disruptions caused by different Taihang Mountain habitats?

To answer the above questions, for this study we initially identified the types of aromatic metabolites involved in the flowers of the two species. Subsequently, the different aromatic metabolites and metabolic pathways were comparatively analyzed between the *O. longilobus* ancestor and *O. taihangensis* descendant. Finally, the potential mechanisms of the two species in response to different habitats were elucidated.

Methods

Sample sites and materials

The Shennong Mountains inhabit Henan Province, while the Xiangtang Mountains reside in Hebei Province. Both of these mountains occupy branches of the Taihang Mountain Range. Despite the two areas being in the center of the Taihang Mountains, the Xiangtang Mountains are 330 kilometers away from the Shennong Mountains, and the environmental conditions are unique for each. The Shennong Mountains have a warm temperate continental climate with four distinct seasons. In contrast, the Xiangtang Mountains are home to a warm temperate semi - humid semi - arid continental monsoon climate where *O. longilobus* is found, while *O. taihangensis* are distributed among the Shennong Mountains.

In 2016, we established a reciprocal transplant garden in Xiangtang Mountains, where *O. longilobus* flowers were collected from July to October of 2020. Concurrently, *O. taihangensis* wild flowers were collected as comparative samples from the Shennong Mountains (Table 1).

Table 1
CLT, CLW, and TH samples grouping and location Information.

sample	location	batch	class	order
QC1	—	1	QC	1
CLW1	Xiangtang Moutains, Hebei	1	CL	2
THW1	Shennong Moutains, Henan	1	TH	3
CLW2	Xiangtang Moutains, Hebei	1	CL	4
THW2	Shennong Moutains, Henan	1	TH	5
CLW3	Xiangtang Moutains, Hebei	1	CL	6
THW3	Shennong Moutains, Henan	1	TH	7
QC2	—	1	QC	8
CLW4	Xiangtang Moutains, Hebei	1	CL	9
THW4	Shennong Moutains, Henan	1	TH	10
CLT5	Xiangtang Moutains, Hebei	1	CL	11
THW5	Shennong Moutains, Henan	1	TH	12
CLT6	Xiangtang Moutains, Hebei	1	CL	13
THW6	Shennong Moutains, Henan	1	TH	14
QC3	—	1	QC	15

For sampling, fresh and healthy whole flowers in the full bloom stage were collected and placed into a Ziploc bags (one bag for each group), and quickly put into a dry ice box. All flower samples were processed as three groups (*O. longilobus* wild flowers (CLW), *O. longilobus* reciprocal transplant flowers (CLT), and *O. taihangensis* wild flowers (TH). CLW, CLT and TH, the plants were sampled from Shanxi Province where the two have adjacent distribution as closely as possible (Study protocol comply with relevant institutional, national, and international guidelines and legislation, and collection of CLW, CLT and TH have obtained the permission from the College of Life Sciences of Shanxi Normal University). Shi Tao and Jin Shuying identified *O. longilobus* the on May 21, 1989, and the specimen is in the Herbarium of the Institute of Botany, Chinese Academy of Sciences (PE), No. 01710937.

Total metabolite extraction and analysis

Total metabolite extraction

The collected samples were thawed on ice, and the metabolites were extracted from 20 μL of each sample using 120 μL of a precooled 50% methanol buffer. Subsequently, the metabolite mixture was vortexed for 1 min and incubated for 10 min at room temperature and stored at -20°C overnight. The

mixture was then centrifuged at 4,000 g for 20 min, and the supernatant was transferred to 96-well plates and stored at -80°C pending LC-MS analysis. Pooled quality control (QC) samples were also prepared by combining 10 μL of each extraction mixture.

All samples were analyzed using a TripleTOF 5600 Plus highresolution tandem mass spectrometer (SCIEX, Warrington, UK) with both positive and negative ion modes. Chromatographic separation was performed using an ultra performance liquid chromatography (UPLC) system (SCIEX, UK). An ACQUITY UPLC T3 column (100mm*2.1mm, 1.8μm, Waters, UK) was used for the reversed-phase separation. It was introduced for the separation of metabolites that the mobile phase consisted of solvent A (water, 0.1% formic acid) and solvent B (Acetonitrile, 0.1% formic acid). The gradient elution conditions were as follows with a flow rate of 0.4 ml/min: 5% solvent B for 0-0.5 min; 5-100% solvent B for 0.5-7 min; 100% solvent B for 7–8 min; 100-5% solvent B for 8-8.1min; and 5% solvent B for 8.1-10min. The column temperature was maintained at 35℃. The TripleTOF 5600 Plus system was used to detect metabolites eluted from the column. The curtain gas pressure was set at 30 PSI, the ion source gas1 and gas2 pressure was set at 60 PSI. The interface heater temperature was 650℃.For the positive-ion mode, the ion spray floating voltage was set at 5kV, and For the negative-ion mode, it was set at -4.5kV. The MS data were acquired in the IDA mode. The TOF mass range was 60-1200Da. Survey scans were acquired every 150ms, and as many as 12 product ion scans were collected if the threshold of 100 counts/s was exceeded with a 1 + charge state. The total cycle time was fixed at 0.56s. Four-time bins were summed for each scan at a pulse frequency of 11 kHz by monitoring the 40 GHz multichannel TDC detector with four-anode/channel detection. Dynamic exclusion was set for 4s.

Determination of differential metabolites

The pretreatment of acquired LC-MS data was performed using XCMS software. Raw data files were converted to mzXML format and then processed using the XCMS, CAMERA and metaX toolbox included in R software. The data was then matched to in-house and public databases. The peak intensity data was further preprocessed using metaX, and metabolic substances with standard deviations of > 30% were removed. The dataset groups were normalized prior to analysis using the probabilistic quotient normalization algorithm. The P value analyzed by student t-tests was adjusted for multiple tests using an FDR (Benjamini–Hochberg) and then used for the selection of various metabolites. In conjunction with multivariate statistical analysis, the VIP value obtained by PLS-DA was analyzed to screen differential metabolic ions. The different ions met the following requirements: 1. Ratio ≥ 2 or Ratio ≤ 1/2; 2. Q value ≤ 0.05; 3. VIP ≥ 1 for the screening of differential metabolites. Assessment of nutritional value and quantitative analysis of bioactive phytochemicals through targeted LC-MS/MS method.

KEGG, Venn diagram, and relationship network diagram analysis of differential metabolites

The metabolomic database was annotated using KEGG to retrieve all the pathways mapped by the differential metabolites. The pathways of the differential metabolites were then further screened to

identify key pathways that had the highest correlations with the different metabolites. Fold-change analysis and the T. test statistical test were employed to conduct BH corrections to obtain the Q-value.

Differential metabolic enrichment analysis was performed between the CLW, CLT, and TH groups and those with different expressions were screened by Origin to develop a Venn diagram. The developed metabolic network was displayed using Cytoscape 3.7.0.

Aromatic substances of flowers

Determination of aromatic substances

Plant flowers are critical organs that emit odoris compounds to attract pollinators and defend against florivores. To evaluate the biochemical differences between *O. longilobus* and *O. taihangensis* flowers under various conditions, the metabolic scent profiles were compared.

According to the original data the differential metabolites of $P < 0.05$ and $VIP > 1$ were screened, and the similarity > 700 was also screened. The aromatic-related metabolites were then identified and classified, and a metabolic pathway map was illustrated using Visio software (Office Visio 2013, Microsoft USA).

Variation coefficient of identified aromatic compounds

Variation coefficients were statistic indices used to quantify the degree of variation for each metabolite. For this study, the variation coefficient was calculated using the formula: $CV = \sigma/\mu$, where σ is the standard deviation and μ is the average value. The variation coefficients of different metabolic aromatic compounds were subsequently plotted using SPSS (IBM SPSS Statistics 26) software.

PCA and heat map of different aromatic compounds

A PCA diagram of different aromatic metabolites was plotted using Origin (Origin 2018) software. The relative content maps of differential metabolites were plotted with SigmaPlot version 10.0 (Systat Software Inc., USA) software. The metabolic pathway map was depicted using Visio software (Office Visio 2013, Microsoft USA)

Correlations between floral metabolites and environment factors

Climate data for the sampled sites was extracted using R software, through which a total of 103 climatic factors were obtained according to their longitude and latitude. According to the VIF and PCA of Origin software significant environmental factors were obtained. Pearson correlations between significant environmental factors and different aromatic substances were then calculated and plotted using Origin (Origin 2018) software [25]. It was generally believed that there was a significant relationship when the P value was < 0.05.

Results

Non-targeted metabolism

In the flower metabolites, a total of 95,655 features were detected in the positive ion mode and 36,478 features were detected in the negative ion mode. A total of 5439 metabolites were obtained in the positive and negative ion mode, which accounted for 97.6% of those of the flowers. Based on quantitative analysis, 14262 and 10004 high-quality substances were obtained in the positive and negative ion mode, respectively.

Within the screened Venn diagram (Fig. 2), there were 14713 substances shared between the three groups (1954 metabolites between CLW and CLT, 1245 between CLW and TH, and 695 between CLT and TH).

It was found that 638 metabolites were unique to the CLW group, 748 to the CLT group, and 2248 to the TH group. Thus, significantly distinct metabolic expressions occurred between the CLW, CLT, and TH groups. For the *O. longilobus* wildflowers and *O. longilobus* reciprocal transplant flowers (CLW/CLT), the differential metabolites were upregulated by 919 and decreased by 1189. The differential metabolites of wildflowers of *O. taihangensis* and those of *O. longilobus* (TH/CLW) were upregulated by 4020 and downregulated by 3722. Between TH/CLT the differential metabolites were upregulated by 2560 and downregulated by 3135.

KEGG enrichment analysis revealed that the differential metabolites were enriched into 47 pathways in the CLW/CLT groups, 59 metabolic pathways in the TH/CLW groups, and 61 pathways in the TH/CLT groups. The significantly different metabolic pathways were all involved in secondary plant metabolite biosynthesis, phenylpropanoid biosynthesis, and arginine biosynthesis between the different groups, namely, TH/CLT, TH/CLW, and CLW/CLT.

A correlation metabolic network was developed for the metabolites (Fig. 5). Closely linked metabolites were likely involved in the same or related metabolic pathways. Further investigations into this network indicated that most of the metabolites were relatively dispersed, which implied that they were fundamental for growth and involved in various metabolic pathways.

Detection of aromatic compounds

Through the screening of secondary metabolic data, a total of 29 compounds related to aromatic compounds were identified in the *O. longilobus* wildflowers (CLW), *O. longilobus* reciprocal transplant flowers (CLT), and *O. taihangensis* wild flowers (TH), including two alkenes, two aldehydes, three esters, eight phenols, three acids, three ketones, three alcohols, and five flavonoids. These compounds were chlorogenate, caffeate, carvacrol, thymol, eugenol, cinnamaldehyde, scopoletin, coniferyl aldehyde, trans-2-hydroxycinnamate, 4-hydroxycinnamyl aldehyde, 4-hydroxystyrene, quercetin, vitexin, luteolin 7-O-beta-D-glucoside, chrysoeriol, rutin, syringetin, taxifolin, delphinidin, naringenin chalcone, 4-hydroxystyrene, dioctyl phthalate, p-cresol, 3-hydroxybenzaldehyde, phenyl acetate, 3-nitrophenol, trans-2-hydroxycinnamic acid, and hesperetin, respectively. The CLT, CLW, and TH possessed the same compounds, albeit different compound expressions.

The most highly expressed metabolite for CLT was eugenol, with a relative content of 450751.55 (Fig. 3, Table 2), followed by caffeate, carvacrol, thymol, and chlorogenate, with relative contents of 166712.44, 123258.45, 105479.21, and 92435.68, respectively. The least expressed metabolite was hesperetin, with a relative content of 1242.84.

Table 2
Aromatic compound expression in CLT, CLW, and TH.

substance	CLT	CLW	TH
Chlorogenate	105479.21	19921.39	274799.37
Caffeate	10786.84	26437.30	169303.97
Carvacrol	45265.46	5310.48	13994.04
thymol	92435.68	16687.11	40157.56
eugenol	450751.55	11388.27	3814.11
cinnamaldehyde	12952.79	5892.94	4449.50
Scopoletin	59001.51	10522.40	132320.74
Coniferyl aldehyde	2351.69	2560.67	4449.50
trans-2-Hydroxycinnamate	1242.84	1394.90	16694.42
4-Hydroxycinnamyl aldehyde	3320.20	1792.73	7750.14
4-Hydroxystyrene	9294.67	7470.99	2751.54
Quercetin	2609.50	3928.13	9140.53
Vitexin	2274.42	9813.69	3345.64
Luteolin 7-O-beta-D-glucoside	6273.09	7178.23	16711.09
Chrysoeriol	78813.62	120830.08	21661.31
Rutin	4227.24	5222.34	20086.61
Syringetin	45869.14	12767.33	41409.86
Taxifolin	15370.62	6753.96	8319.35
Delphinidin	2436.45	18204.98	4483.08
Naringenin chalcone	8136.07	16785.24	10369.81
Dioctyl phthalate	123258.45	39622.58	47210.77
Phenol	3296.28	3956.37	2215.23
p-Cresol	1951.54	514.12	327.25
3-Hydroxybenzaldehyde	8049.15	7936.81	1037.18
Phenyl acetate	166712.44	148542.13	13535.54
3-Nitrophenol	8723.93	14561.86	5941.29
trans-2-Hydroxycinnamic acid	25270.25	4991.71	1565.01

substance	CLT	CLW	TH
Hesperetin	88158.46	56960.99	39691.62

For CLW, the most highly expressed metabolite was phenyl acetate, at 148542.13, followed by chrysoeriol, hesperetin, dioctyl phthalate, caffeate, with relative contents of 120830.08, 56960.99, 39622.58, and 26437.30, respectively. The least expressed metabolite was p-Cresol with a relative content of 514.12.

For TH, chlorogenate was the most highly expressed metabolite with a content pf 274799.37, followed by caffeate, scopoletin, dioctyl phthalate, and syringetin, with relative contents of 169303.97, 132320.74, 47210.76, 47210.77, and 41409.86, respectively. The least expressed metabolite was p-Cresol, with a relative content of 327.25.

The PCA of aromatic substances (Fig. 4) revealed that the three groups could be distinguished from each other. The overall differences in aromatic substances were obvious between the different groups, which indicated that the aromatic compounds in CLT, CLW, TH were significantly different.

Coefficient of variation and relationship network diagram

Based on the identified aromatic substances the coefficients of variation (CV) (Table 3) of fragrance metabolism were calculated. For the CLT, the CV ranged from 0.1–48.72%, whereas for the CLW, it ranged from 0.58% – 55.25%, and for TH it ranged from 2.17% -80.90%.

Table 3
Coefficient of variation (CV) for CLT, CLW, and TH.

substance	CLT- CV (%)	CLW- CV (%)	TH- CV (%)
Chlorogenate	4.36	2.59	8.80
Caffeate	3.97	3.03	4.60
Carvacrol	11.79	20.25	6.01
thymol	7.58	6.66	3.03
eugenol	6.54	5.70	4.05
cinnamaldehyd	4.25	24.60	6.88
Scopoletin	3.61	8.01	4.96
Coniferyl aldehyde	11.83	55.25	6.88
trans-2-Hydroxycinnamate	48.72	29.11	8.26
4-Hydroxycinnamyl aldehyde	3.16	23.66	29.63
4-Hydroxystyrene	8.34	34.21	2.17
Quercetin	47.03	33.96	20.87
Vitexin	17.04	6.76	9.71
Luteolin	1.46	53.42	31.48
Chrysoeriol	5.49	4.31	14.87
Rutin	10.91	7.93	5.42
Syringetin	4.28	4.80	10.89
Taxifolin	7.36	9.03	10.61
Delphinidin	13.97	10.22	38.06
Naringenin chalcone	3.00	6.24	36.66
4-Hydroxystyrene	8.34	34.21	2.17
Dioctyl phthalate	21.35	39.00	38.26
Phenol	35.77	44.32	10.05
p-Cresol	18.11	5.94	33.29
3-Hydroxybenzaldehyde	6.76	9.14	27.61
Phenyl acetate	7.71	6.63	10.97
3-Nitrophenol	1.65	9.08	10.75

substance	CLT- CV (%)	CLW- CV (%)	TH- CV (%)
trans-2-Hydroxycinnamic acid	8.11	4.38	14.84
Hesperetin	3.78	7.47	11.98
range	0.1%-48.72%	0.58%-55.25%	2.17%-38.06%

Interesting, for the relatively higher content of some aromatic compounds (e.g., chlorogenic and eugenol) among the identified fragrance metabolites, their CV were at a medium level between the three groups.

Enrichment pathway of aromatic substances

The results of enrichment analysis indicated that 29 aromatic compounds were engaged in phenylpropanoid biosynthesis, flavone and flavonol biosynthesis, chemical carcinogenesis-receptor activation, bisphenol degradation, toluene degradation, phenylalanine metabolism, and aminobenzoate degradation.

The same, albeit significantly different, aromatic metabolic pathways in the TH/CLT, TH/CLW, and CLW/CLT involved phenylpropanoid biosynthesis, as well as flavone and flavonol biosynthesis.

Among the 29 aromatic metabolites, eugenol was highest in CLT, followed by that in TH, and was involved in the phenylpropanoid biosynthesis pathway. Chlorogenate was the most prevalent aromatic compound in TH, followed by that in CLW, which also involved in phenylpropanoid biosynthesis (Fig. 6).

Environmental correlation of aromatic substances

Using VIF and PCA (Tables 4, 5) it was found that two primary environmental factors, namely the lowest temperatures in October and December, were the most important for the sampled sites (Table 4). According to correlation analysis, chlorogenic was significantly correlated with the minimum temperature in December, and the Pearson's correlation coefficient was 0.72105. Further, eugenol was significantly correlated with the minimum temperatures in October and December, and the Pearson's correlation coefficients were 0.88483 and 0.82039, respectively.

Table 4
VIF values of major environmental factors.

VIF Variables	
Lowest temperature October	1.460
Lowest temperature December	1.502

Table 5
Correlations between aromatic substances and two environmental factors

	Lowest temperature in October	Lowest temperature in October	Lowest temperature in December	Lowest temperature in December
	Pearson correlation	p-value	Pearson correlation	p-value
Chlorogenate	-0.2601	0.41424	0.72105*	0.00814
Caffeate	-0.54904	0.06449	0.47613	0.11764
Carvacrol	0.93519*	8.07E-06	0.73249*	0.00674
thymol	0.88483*	1.31E-04	0.82039*	0.00108
eugenol	0.88483*	1.31E-04	0.82039*	0.00108
cinnamaldehyd	0.95583*	1.23E-06	0.3087	0.32893
Scopoletin	-0.21318	0.50589	0.75863*	0.00423
Coniferyl aldehyde	-0.44013	0.15218	0.38082	0.22198
trans-2-Hydroxycinnamate	-0.49241	0.10389	0.52838	0.0774
4-Hydroxycinnamyl aldehyde	-0.28309	0.37261	0.60134*	0.03861
4-Hydroxystyrene	0.60689*	0.03639	-0.30418	0.33643
Quercetin	-0.55199	0.06278	0.35871	0.2522
Vitexin	-0.37871	0.22477	-0.98711	2.75E-09
Luteolin 7-O-beta-D-glucoside	-0.42553	0.16785	0.38042*	0.22251
Chrysoeriol	0.19279	0.54829	-0.77187*	0.00327
Rutin	-0.5253	0.07946	0.49815	0.0993
Syringetin	0.36739	0.24007	0.97295*	1.09E-07
Taxifolin	0.9309*	1.10E-05	0.68244*	0.01447
Delphinidin	-0.36309	0.24603	-0.9719*	1.32E-07
Naringenin chalcone	-0.39108	0.20873	—0.80275*	0.00167
4-Hydroxystyrene	0.60689*	0.03639	-0.30418	0.33643
Dioctyl phthalate	0.86946*	2.39E-04	0.53605	0.07241
Phenol	0.14631	0.65002	-0.48033	0.11398

	Lowest temperature in October	Lowest temperature in October	Lowest temperature in December	Lowest temperature in December
p-Cresol	0.97346*	9.92E-08	0.37682	0.22728
3-Hydroxybenzaldehyde	0.49574	0.10121	-0.52455	0.07997
Phenyl acetate	0.56086	0.05782	-0.46192	0.13059
3-Nitrophenol	-0.0152	0.9626	-0.87167*	2.20E-04
trans-2-Hydroxycinnamic acid	0.98848*	1.57E-09	0.34875	0.26656
Hesperetin	0.89367*	8.93E-05	0.07483	0.81721

Discussion

Different metabolites response to the environment

Generally, the regulation of primary metabolite allocation affects fundamental processes such as growth and development. Other processes aimed at increasing plant fitness in specific environments are more associated with specialized metabolic processes, such as those involved with protection against herbivores and pathogens, the reduction of oxidative stress, or competition with other plants [26].

Following reciprocal transplantation, it was found that there were 1–5 days difference between the *O. longilobus* and *O. taihangensis* flowering phases. The data revealed that arginine promoted root growth and improved the salt tolerance of plants. The significantly enriched arginine biosynthesis pathway might have affected the florescence of the *O. longilobus* ancestor and *O. taihangensis* descendant.

During various developmental processes plants need to continuously adjust the distribution of metabolites to maintain growth and survival under changing environments [27]. The differential metabolites were engaged in both TH/CLW and TH/CLT (Table 2). *O. taihangensis* flourishes in a different habitat than its ancestor *O. longilobus* [23]. To respond to different environments while maintaining its reproductive capacity, *O. taihangensis* regulated its phenylpropane metabolism at different stages during the whole flower phase.

Within the *O. longilobus* species there were also obviously different metabolites between CLW and CLT. When *O. longilobus* was transplanted from the wild site to the reciprocal transplant garden, it adjusted its phenylpropane biosynthesis to respond the changed environment to ensure that its flowers to bloomed normally.

Environmental influences on aromatic substances

Under different environments, the growth and development of plants can be modified by changing environmental factors [28]. Plants can regulate different signaling pathways by controlling the expression of genes; thus, impacting metabolic levels and responding to habitat changes [29]. The synthesis and accumulation of secondary metabolites involve very complex processes that are affected by genetic, developmental, and environmental factors [30]. The secondary metabolism of plant flowers in different habitats were significantly different due to the influences of habitat heterogeneity [31]. Consequently, plants generate many specialized metabolites that contribute to their health and survival and play roles in their capacities to adapt to localized environmental conditions. The synthesis and accumulation dynamics of aromatic compounds are regulated at different development stages by circadian rhythms, as well as biological and abiotic factors [10]. The emission of fragrances from flowers is regulated by metabolic processes [32]. The biosynthesis and emission of floral fragrances are modulated not only by the stages of flower maturity and circadian rhythms, but also by other environmental factors such as temperature [33, 34]. Environmental conditions not only influence the vaporization of volatile compounds from flowers, but also their biosynthesis, particularly under different ambient air temperature regimes (Cheng, Fu et al. 2016), which are known to play major roles in the biosynthesis and release of floral fragrances [34, 35]. Plants produce a diversity of secondary metabolites, while generating and storing an extensive range of volatile organic compounds in their flowers. Typically, aromatic substances are important metabolites in plants for attracting insects, resisting diseases and pests, and adapting to altered habitats [36]. Fragrances can often be efficient attractants to lure insects for food or "copulation" and thus pollination [37]. Further, plant scents attract and repel various pollinators for different pollination processes [38]. Plants can also establish chemical and physical defenses to defend against herbivores [39].

The floral scents of CLT, CLW, TH were mainly composed of phenylpropanes, such as eugenol and chlorogenic, which was consistent with the general pattern of the chemical composition of floral scents [40]. Being the main aromatic compound, it was indicated that chlorogenic might play an important role in the attraction of pollinators and against herbivores and pathogens for CLT, CLW and TH. Further, chlorogenic is a phenylpropanoid compound produced via the shikimic acid pathway in plants during aerobic respiration, which exhibits anti-inflammatory, antibacterial, antiviral, and antidepressant pharmacological effects in vitro [41]. As the descendant of *O. longilobus*, additional chlorogenic might induce *O. taihangensis* to not only attract more and different pollinators to facilitate its reproduction, but also to respond to different environments compared with its ancestor to better survive and develop. This may be responsible for the enhanced flower color and relatively different flower size of *O. taihangensis* in contrast to *O. longilobus.*

Eugenol is an important type of phenylpropane volatile that is commonly found in plant flowers and mature fruits, which possesses a variety of biological and therapeutic effects [30]. Eugenol can attract biological pollinators, facilitate seed transmission, protect against herbivores, and be used as a protective agent against pathogenic parasites [42]. Eugenol also functions as an antioxidant, antibacterial, in insect resistance, and food seasoning. Eugenol had the highest content in CLT, followed by CLW and TH (Table 2). When *O. longilobus* was transplanted into the reciprocal transplant garden from the wild

growth site, eugenol would accumulate to enhance survival under changing environmental conditions. Further, eugenol is a semi-volatile phenolic compound derived from plants that has a strong carnation musk odor [43], that creates the different fragrances for *O. longilobus* and *O. taihangensis*.

In this study, there was a significant correlation between chlorogenic and the minimum temperature in October, and between eugenol and the minimum temperature in October and December. It is possible that these two environmental factors directly affect the expression of chlorogenic and eugenol, or indirectly affect the expression of chlorogenic and eugenol synthase. It was reported that low temperatures can induce the expression of PAL and other phenylpropane metabolism genes; however, not all genes in phenylpropane metabolism pathway can be induced [44]. Flower buds could only be formed after low temperature treatment, which may be reasonable for these two species.

Aromatic compounds and differences in enrichment pathway

In this study, the pathways related to flower fragrances were primarily phenylpropanoid, plavone, and flavonol biosynthesis in CLW, CLT, and TH. Metabolism occurs between phenylpropane metabolism and other secondary metabolic pathways, which maintains a dynamic balance between phenylpropane metabolism and resisting environmental degradation [45]. When downregulated, chlorogenic (a compound in the phenylpropanoid biosynthesis pathway) based metabolites can be metabolized to storage forms such as glycosyl based derivatives, or decomposed to their corresponding forming units (quinic acid and caffeic acid) and further metabolized to more complex molecules such as lignin [46]. It contributes to plant development and plant-environment interactions, and phenylpropanoid-based polymers such as lignin are required for mechanical support to facilitate plant growth and the long-distance transport of water and nutrients [47]. Phenylpropanoid homeostasis between the different branches of phenylpropanoid metabolism, exhibits extraordinary complexity and a high-level of plasticity during successive developmental stages in response to environmental stimuli and changes [47, 48]. Lignin encapsulates carbohydrates comprised of cellulose and hemicellulose to form a composite wood fiber barrier to resist the attack and destruction of plant tissues by microorganisms and the surrounding environment [49].

During their synthesis, both chlorogenic and eugenol compounds are involved in the phenylpropane pathway (Fig. 6), where chlorogenic is upstream and eugenol is downstream, synthetic chlorogenic and eugenol prerequisite substances are the same, and the substance of synthetic chlorogenic is highly expressed in *O. longilobus* and *O. taihangensis*, and the substance behind synthetic eugenol is probably due to low expression not detected. The first step of the phenylpropane chlorogenic metabolic pathway involves the dissociation of ammonia from l-phenylalanine via phenylalanine ammonia lyase and the production of trans-cinnamic acid, which is a key enzyme [50–52]. This enzyme belongs to the aromatic amino acid lyase family, is the first key enzyme in the phenylpropane pathway, and is related to most bioactive metabolites, such as flavonoids and anthocyanins. Caffeoyl-CoA is the final step in the synthesis of chlorogenic, where 4-coumaryl-CoA ligase is one of the key enzymes in the phenylpropane

metabolic pathway toward the metabolism of other substances, which can catalyze cinnamic acid and its hydroxyl groups. Thus, 4-coumaryl-CoA ligase is of great value in the biosynthesis of phenylpropane compounds such as chlorogenic and flavonoids [53]. Intermediates of the phenylpropane metabolic pathway and their secondary metabolites cannot only improve the disease resistance of plants, but also regulate and promote plant resistance to abiotic stresses such as low temperatures, high temperatures, and ultraviolet radiation. These might be an explanation for two groups (CLW and CLT) of *O. longilobus* under different surroundings.

Conclusion

Through LC-MS/MS methods, the expressions of metabolic compounds were shown to be significantly different between *O. longilobus* wildflowers (CLW), *O. longilobus* reciprocal transplant flowers (CLT), and *O. taihangensis* wild flowers (TH). Two types of phenylpropane metabolites (eugenol and chlorogenic) were responsible for the differences in aromatic components between the CLT, CLW, and TH groups. Following reciprocal transplantation, *O. longilobus* exhibited a positive response toward adapting to biological and abiotic stresses via the phenylpropanoid biosynthesis pathway. The results revealed the potential response mechanisms of *O. longilobus* to different habitats, while providing a scientific basis for the synthesis and regulation of aromatic substances in *O. longilobus* and *O. taihangensis.*

Declarations

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Author contributions

Liu Zhixia, Lan Yafei and Zhang Hao wrote the main manuscript text; Hao Weili and He Shan prepared figures 1-6, Feng XL and Qie QY prepared table1-5; Liu li and Chai Min completed analyzed data; Sun GL completed software-Equal; Wang Yiling completed writing-review and editing-Lead. All authors reviewed the manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article

Ethics approval and consent to participate

All the experimental research and field studies on plants, including the collection of plant material, were carried out in accordance with local institutional, national, and international guidelines and legislation of China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Figures



Figure 1

O. longilobus (left) flowers and *O. taihangensis* flowers (right) in blooming period. The state is ligule in full bloom.

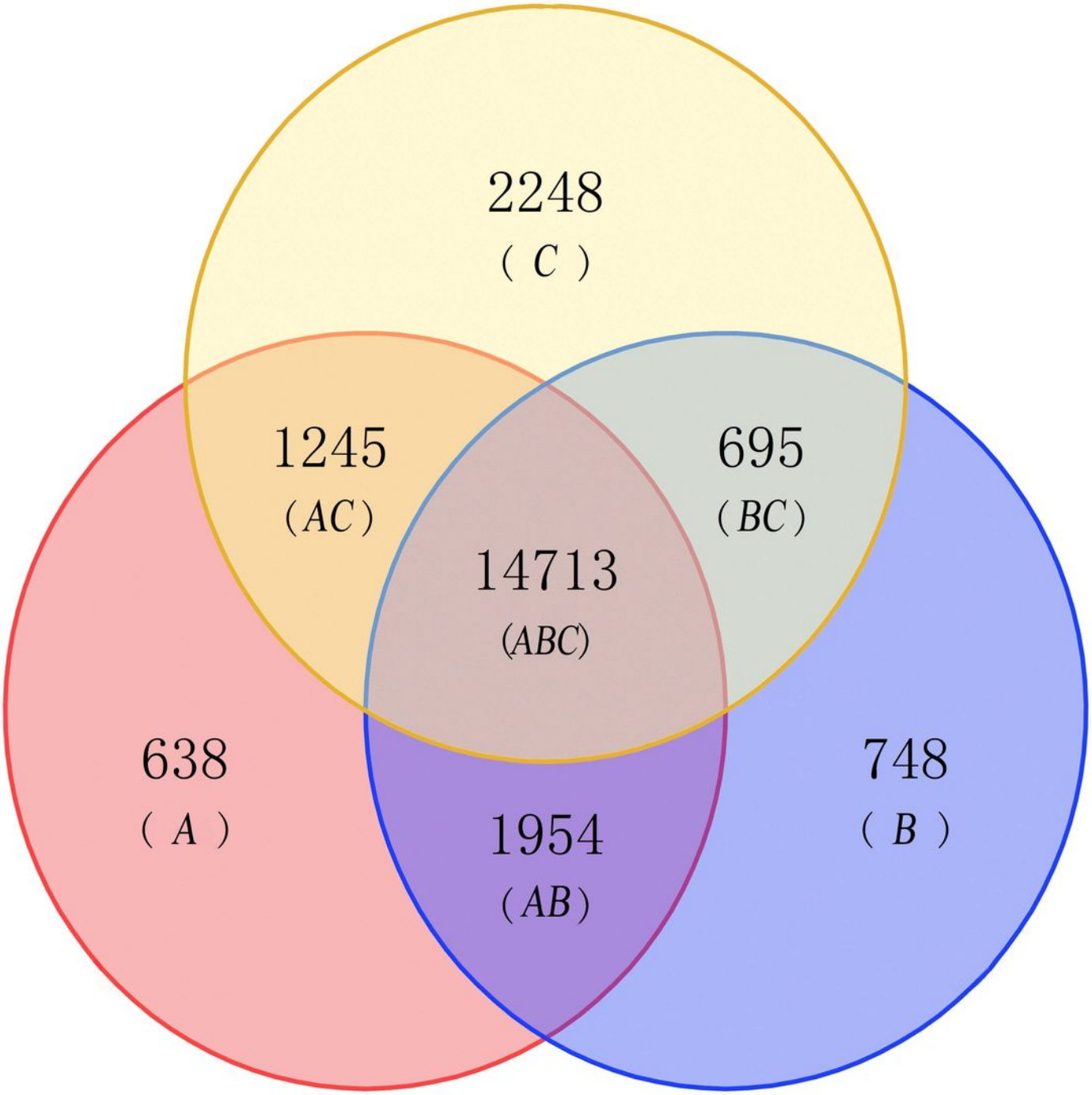


Figure 2

Venn diagrams for flowers metabolites, showing CLW, CLT, TH Intersection. A.CLW; B.CLT; C.TH.

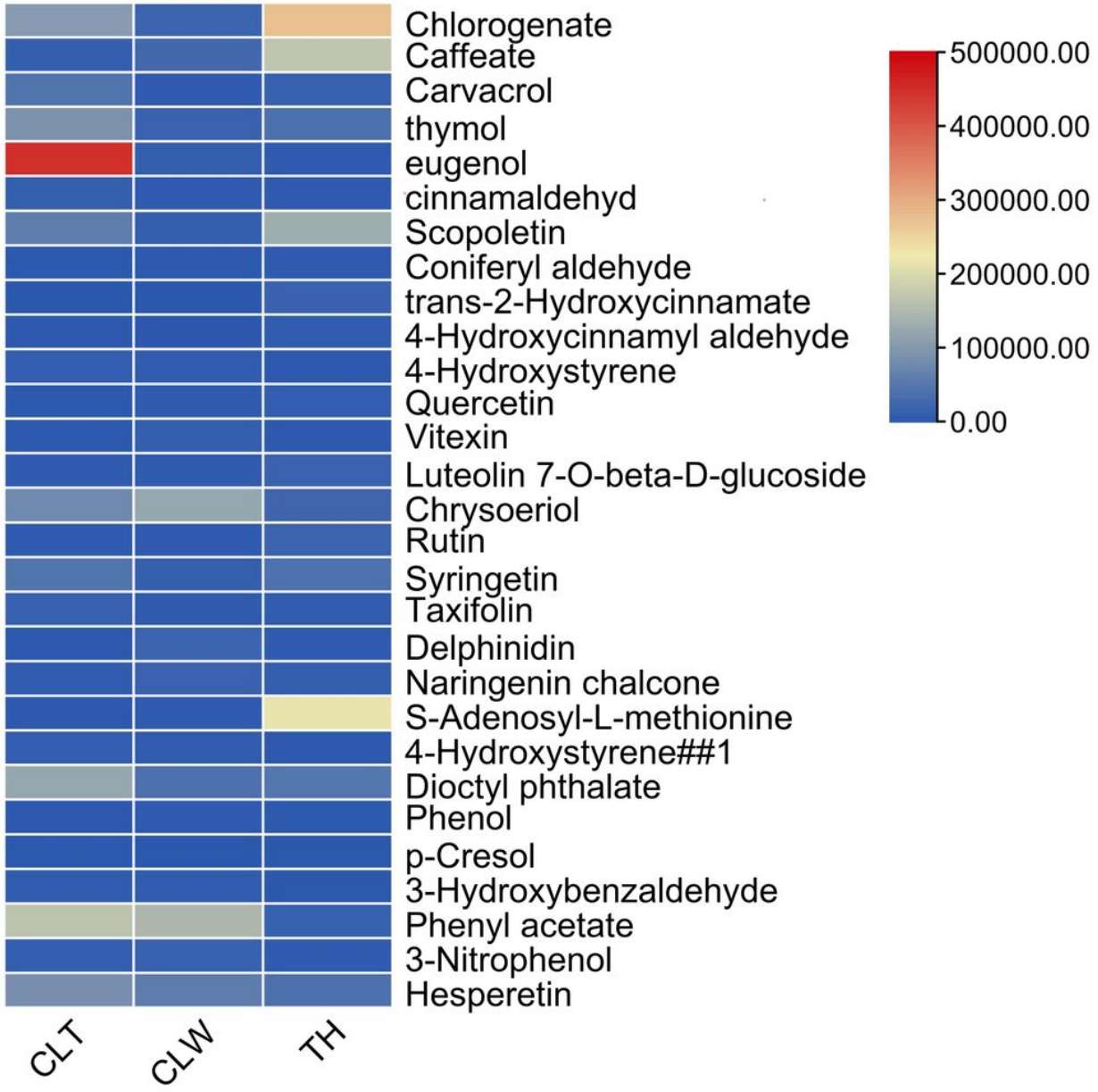


Figure 3

CLT, CLW, and TH group aroma substances expression heatmap. The top right corner is the highest to lowest expression, red is the highest expression color.

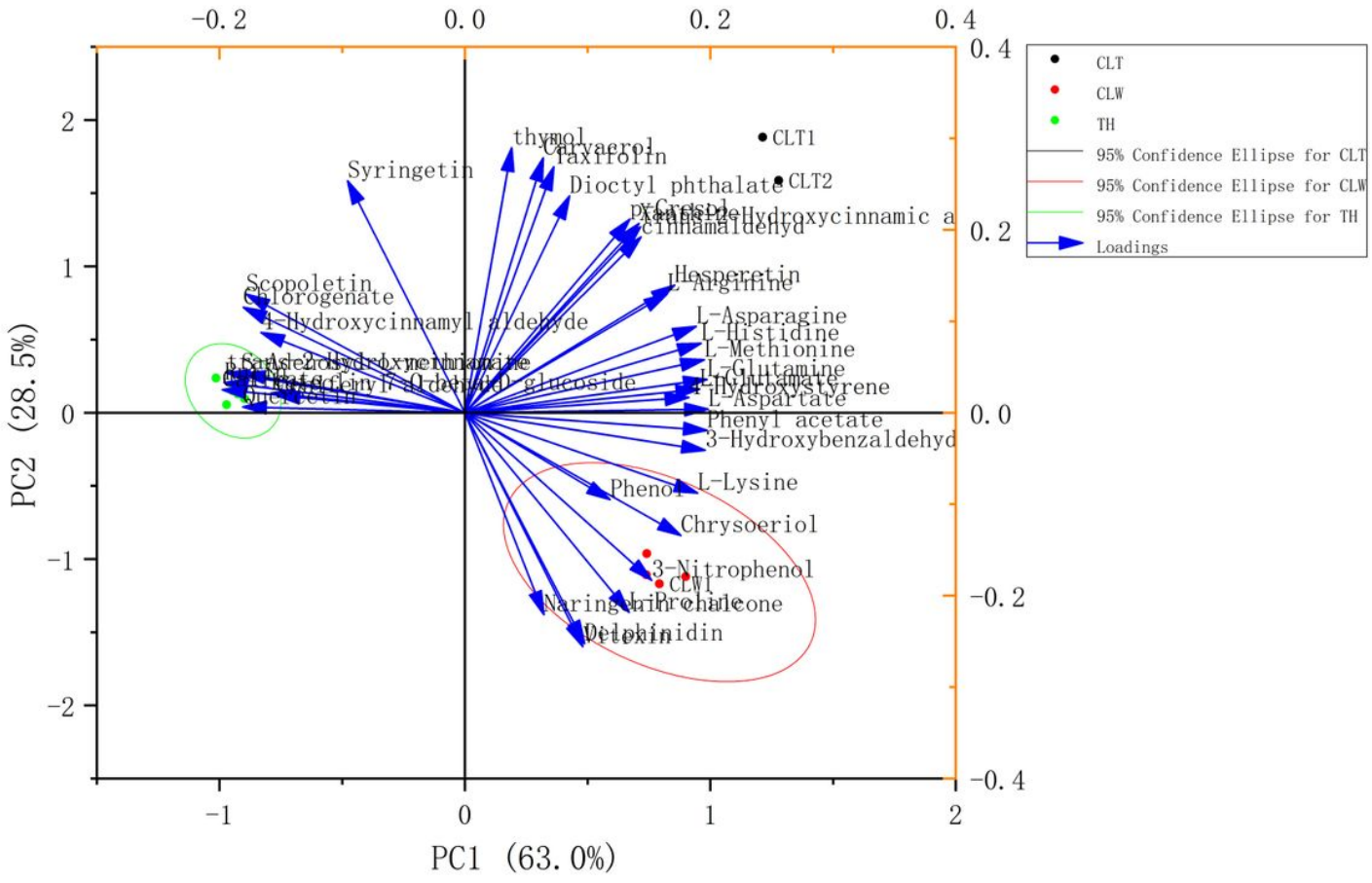


Figure 4

PCA diagram of significant aromatic substances of CLT, CLW, TH group.

Green points are for TH, red points are for CLW, black points are for CLW.

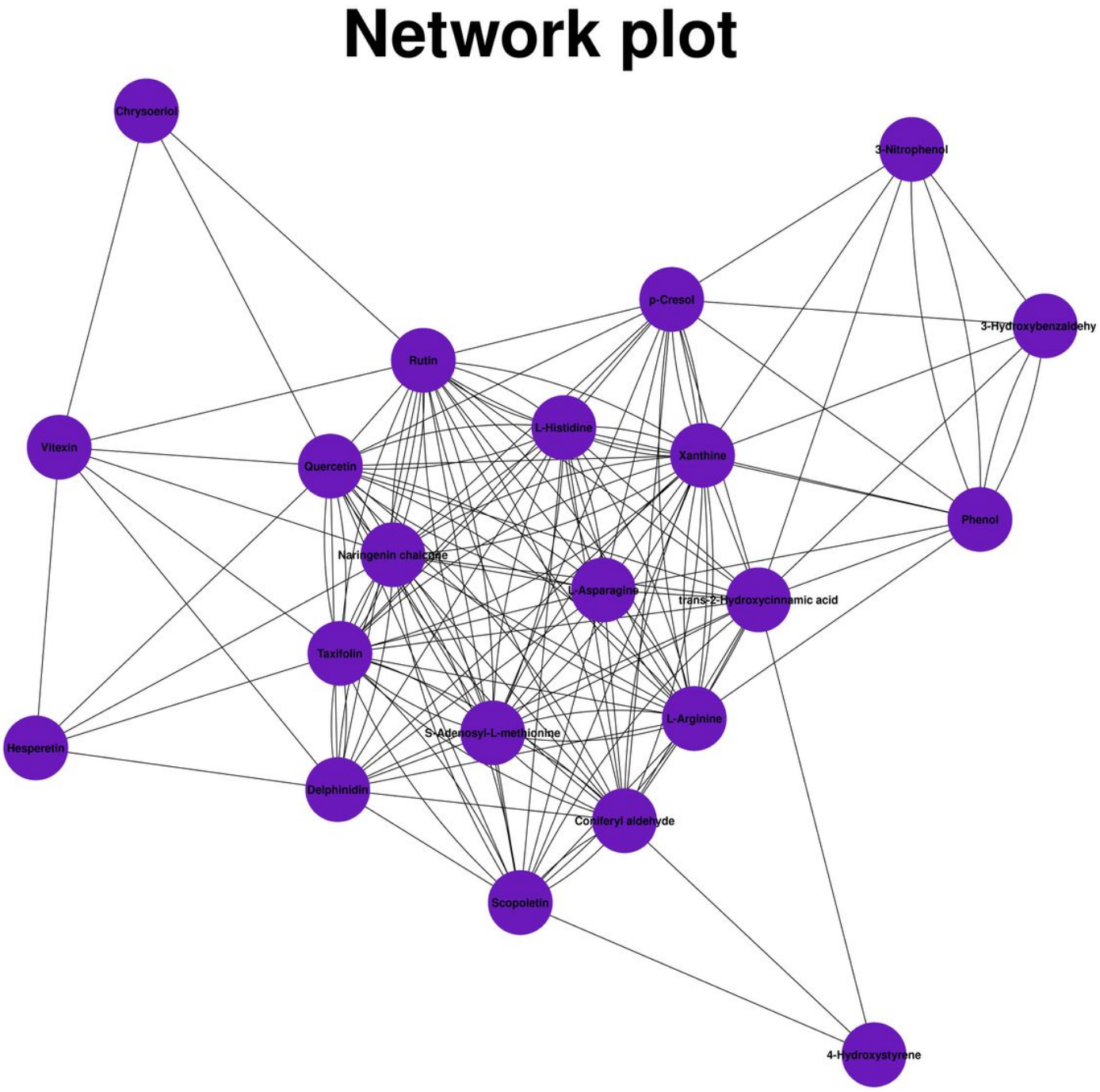


Figure 5

Metabolite network relationship diagram of aroma substances. Each node represents a metabolite.

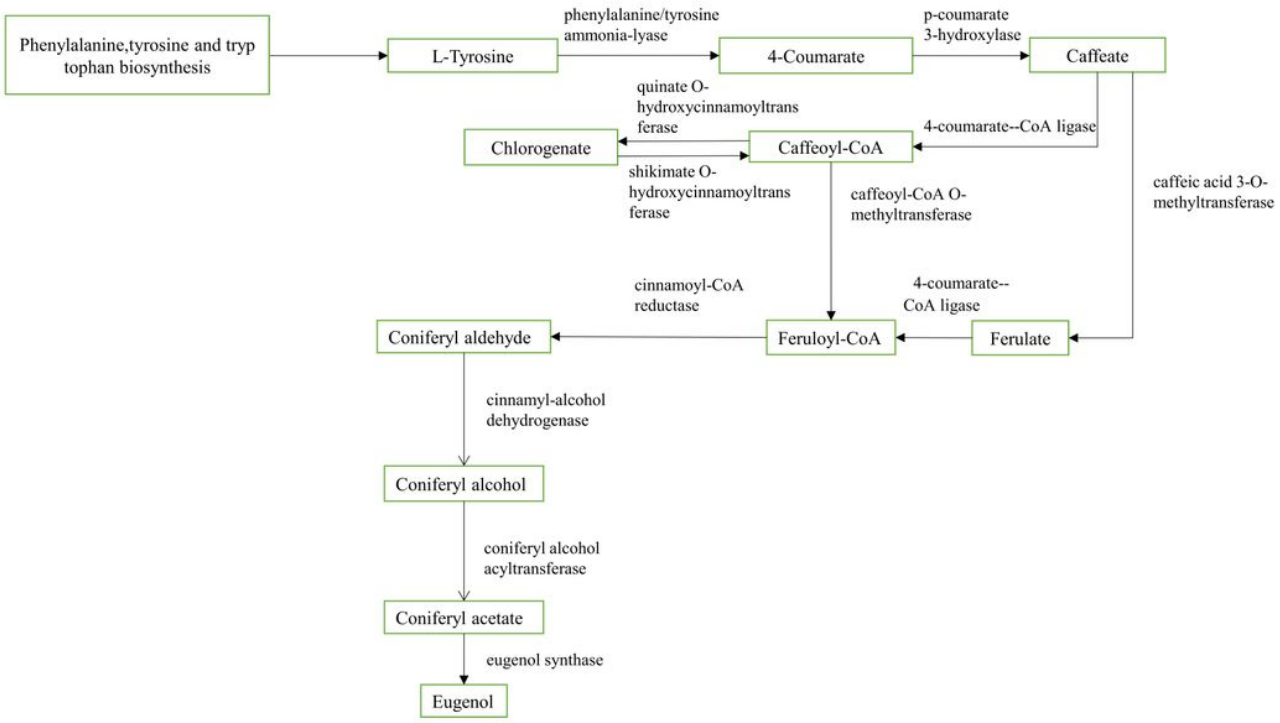


Figure 6

Chlorogenic and eugenol synthetic related pathway in phenylpropanoid biosynthesis pathway.