Combined metabolome and transcriptome to analyze the regulatory network of key enzymes in the synthesis of Senkirkine in Emilia sonchifolia

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Abstract

Emilia sonchifolia (L.) DC. is a commonly used medicinal and edible plant in the folk, and its toxic component of Senkirkine (Sek) has hepatotoxicity, which can lead to sinusoidal obstruction syndrome if exposed in large amounts, and will cause hepatic megaloblastosis and hepatic fibrosis if ingested in small amounts over a long period of time. This study was based on the metabolomics of *Emilia sonchifolia*, which analysed and identified the alkaloid constituents of 4 parts of *Emilia sonchifolia*, namely, the root, stem, leaf and flower. A total of 91 differential metabolites were detected, belonging to 10 alkaloids, among which pyrrolizidine alkaloids accounted for 13%. The relative content of Senkirkine differed most significantly between the group of root and flower comparison, and the relative content of Senkirkine in root was significantly higher than in flower. A total of 119,886 unigenes and 32,797 differentially expressed genes were identified in the transcriptome data, and the 45 key enzymes of the biological synthesis pathway of Senkirkine were selected, which belonged to 7 categories. There are a total of 172 candidate enzyme genes in it, of which methyltransferase, alcohol dehydrogenase, acyltransferase, and cyclooxygenase genes were significantly more expressed in roots than in flowers, and the metabolome and transcriptome expression patterns were basically the same. The 5 key enzyme gene sequences of the biological synthesis pathway of Senkirkine were selected for qRT-PCR. The results showed that, using flowers as the control group, 4 key enzyme genes (*BAHD-Ats*, *CCoAOMT*, *BOMT*, *3AT*) were all highest in roots, while the relative expression of *CAD* was highest in stems, which was basically consistent with the transcriptome pattern. This study provided a reference for the functional expression analysis of the genes of *Emilia sonchifolia*, and laid a foundation for the future analysis of the Senkirkine synthesis pathway and the regulation of the key enzyme genes of the toxic components, so as to obtain the low-toxic raw materials of *Emilia sonchifolia*.

Introduction

Emilia sonchifolia (L.) DC., known as “Yi Dian Hong” or “Zi Bei Cao” in Chinese, belongs to tribe Emilia in the family Asteraceae [1]. It is an annual herb, widely distributed in Guangxi, Yunnan and other places. Its whole plant has the efficacy of clearing heat and detoxifying, anti-inflammatory and diuretic, dispersing blood stasis and reducing swelling so on, and it is often supposed to be used as our country's commonly used traditional Chinese medicine and ethnomedicine in the treatment of upper respiratory tract infection, mouth ulcers, urinary tract infections, pneumonia, mastitis, enteritis and traumatic infectionsetc in China [2–4]. It is often used to treat inflammation and mosquito bites in India and is commonly used to treat mosquito bites in Brazil [5, 6].

E. sonchifolia has a high medicinal value and is often used as a single herb or as a prescription preparation for a wide range of applications. Among the recorded prescription preparations and single-flavour remedies in the 2015 edition of the Chinese Pharmacopoeia [7], *E. sonchifolia* as the main raw material of the Chinese patent medicine preparation of huahong tablets, the main treatment for menstrual disorders, dysmenorrhoea, endometritis, adnexitis, pelvic inflammation and other gynecological inflammatory diseases [8]. *E. sonchifolia* is a famous edible and medicinal wild vegetable,

which contains a variety of nutrients, and it is unique in flavor, fragrant and refreshing. In recent years, *E. sonchifolia* has been developed into a famous wild vegetable that has been put on the table, which is favored by people and has broad application prospects [9].

It was found that the main chemical constituents of *E. sonchifolia* are flavonoids, alkaloids, phenols, and so on [10, 11]. The main alkaloids known to be contained in *E. sonchifolia* to date are Senkirkine (Sek), Senecionine N-oxide (SnNO), etc [12], all of which are pyrrolizidine alkaloids (PAs). At present, scholars at home and abroad have carried out relevant research on the PAs components in *E. sonchifolia*. For example, Ni Xiaozhi et al. used ethanol reflux extraction to obtain alkaloid extracts, which were identified as Sek [13]; Cheng et al. isolated and identified PAs polymesterine from *E. sonchifolia* [14]; Shen Shoumao isolated a little red alkali from *E. sonchifolia* [15]; Ning Yu et al. used HPLC to determine the average content of Sek in the different origins of *E. sonchifolia* was 0.40 $mg{\cdot}g^{-1}$ [1]; Zhou Wuping et al. analysed the total alkaloid content of *E. sonchifolia* from different harvesting periods in Guangxi ranged from 4.31 to 13.99 $mg{\cdot}g^{-1}$ [16]; HSIEH C H et al. used GC-MS to identify 11 PAs such as Sek, Seneciphylline (Sp), etc. from *E. sonchifolia* [17]; LUO Z M et al. used UPLC-Q-TOF-MS technology to identify 7 kinds of PAs including Sp from *E. sonchifolia*, and found that *E. sonchifolia* had antioxidant and anti-inflammatory effects [18–20].

Studies have found that PAs can cause severe liver damage [21, 22], which can lead to sinusoidal obstruction syndrome if exposed in large amounts, and will cause hepatic megaloblastosis and hepatic fibrosis if ingested in small amounts over a long period of time [23–28]. At present, the research on the toxic components of PAs has a certain foundation at home and abroad, and relevant national and international institutions have formulated strict standards for the limits of PAs and the regulations on the use of them. The World Health Organisation issued IPCS Health and Safety Guidelines No.26: Guidelines for the Health and Safety of Pyrrolizidine Alkaloids in 1989, recommending an intake limit of PAs of 0.015 $mg{\cdot}kg^{-1}$ [29]. Sek is the highest content alkaloid in *E. sonchifolia*, and this is also one of the main characteristic components in *E. sonchifolia*. Therefore, the research and analysis of the toxic components of *E. sonchifolia* to ensure that it can be used safely and reasonably is an urgent problem to be solved in the industry of *E. sonchifolia*.

With the development of multi-omics technology in recent years, the molecular mechanism of secondary metabolite biosynthesis in traditional Chinese medicine based on metabolome and transcriptome sequencing has been widely studied and applied. You Liqian et al. constructed the gene knockout vector of the effector g12593 of Fusarium oxysporum f. sp. cubense by genome-wide and transcriptome techniques, laying a foundation for the prevention and treatment of banana Fusarium wilt [30]; Xu Ming et al. used bioinformatics analysis, transcriptome and other techniques to knock out the up-regulated chitin deacetylase gene in wheat, revealing the molecular mechanism of Fusarium graminearum chitin deacetylase family pathogenesis [31]; Zhang Yihan et al. used transcriptomics to knock out 3 protein kinases in the BdGpmk1-MAPK signaling pathway of Botryosphaeria dothidea, which provided a theoretical basis for exploring the molecular mechanism of regulating the growth and development and pathogenicity of Botryosphaeria dothidea [32]. In this experiment, based on the metabolome and

transcriptome sequencing of *E. sonchifolia*, the candidate sequences of the key enzyme genes in the biosynthetic pathway of Sek were analyzed and screened, and quantitative real-time PCR (qRT-PCR) was performed to verify, which laid a foundation for analyzing the Sek and even PAs synthesis pathway, regulating key enzyme genes, cultivating new varieties of *E. sonchifolia* with detoxification or attenuation in the future, and promoted the development of the industry of *E. sonchifolia*.

Results

Metabolomic analysis of alkaloids with E. sonchifolia

The total ions current plots (TIC plots) analysed by mass spectrometry detection of different quality control samples (QC) were analysed by overlapping display. According to the TIC plots (Fig. 1a & Fig. 1b), the results show that the curves of different QC samples have a high degree of overall overlap, its signal stability is also good, and the system error is small, so the detected data can be used for subsequent analysis. The results of the Principal Component Analysis (PCA) showed that the groups of samples of roots, stems, leaves, and flowers of *E. sonchifolia* were better clustered within the groups, indicating that the variability within the groups of samples was less (Fig. 1c). In the first principal component, stems and leaves were more similar to the QC, on the contrary, there was a significant trend of separation between roots and flowers, indicating that the overall metabolite differences between roots and flowers in the first principal component were larger. In the second principal component, roots and flowers were similar to QC, on the contrary, stems and leaves showed a clear separation trend, indicating that the overall metabolites of stems and leaves in the second principal component were significantly different, while the differences between roots and flowers were small. According to the orthogonal partial least squares-discriminant analyses (OPLS-DA) score plot, there was a clear trend of separation between the comparison groups of different parts of *E. sonchifolia* in the horizontal coordinate, while the samples were more aggregated within each group in the vertical coordinate, which indicated that there were inter-group differences between the samples of different parts of *E. sonchifolia*, while the intra-group reproducibility was higher (Fig. 2).

Screening of differential metabolites

A total of 69 differentially expressed metabolites (DEMs) were detected in the metabolome, belonging to 10 kinds of alkaloids **(Supplementary Table 1)**. The number of pyrrolizidine alkaloids and indole alkaloids is higher (Fig. 3), which proportions were 13% and 13%, respectively. In order to evaluate the differences of metabolites in different parts of *E. sonchifolia*, the number of DEMs was compared two-by-two according to the conditions of VIP > 1 and |log2 FC|>= 1, there were 47 DEMs (35 down-regulated, 12 up-regulated) in the group of CBr vs CBh, 44 DEMs (20 down-regulated, 24 up-regulated) in the group of CBl vs. CBr, 33 DEMs (17 down-regulated, 16 up-regulated) in the group of CBl vs CBs, 48 DEMs (16 down-regulated, 32 up-regulated) in the group of CBs vs CBr, 37 DEMs (11 down-regulated, 26 up-regulated) in the group of CBh vs CBl, and 33 DEMs (10 down-regulated, 23 up-regulated) in the group of CBh vs CBs.

Dynamic distribution of relative content differences in PAs

The top 10 up- and down-regulated DEMs were analysed according to the value of the FC (FC), with a particular focus on PAs of which **(Supplementary Table 2 &** Fig. 4a**)**. It was found that there were 10 DEMs in the CBr vs CBh, which were the most in each comparison group. Among them, the up-regulated metabolites (Isa, SnNO, ADO, EfNO, Sek, SpNO) were more than the down-regulated metabolites (INT, HMPP, Re, Py). There were 3 DEMs in CBh vs CBs, among which the up-regulated metabolites (HMPP, Re) were more than the down-regulated metabolites (Sek). In order to further clarify the content differences of PAs in different parts of *E. sonchifolia*, the relative content of PAs in the four parts of *E. sonchifolia*, including root, stem, leaf and flower, was compared, and the cluster heat map of relative content was drawn on the Metware cloud platform **(**Fig. 4b**)**. As can be seen from Fig. 4b, the relative contents of Isa, SnNO, ADO, EfNO, Sek and SpNO in different parts were significantly different, and the relative content of root was the highest. HMPP, Re and INT were the highest in flowers. Py was the highest in leaves. It can be clearly seen from Fig. 4c that the relative content of Sek in roots was much higher than that in other parts, and its relative content decreased in the trend of roots, stems, leaves and flowers.

Functional annotation and enrichment analysis of DEMs in KEGG

By KEGG enrichment analysis, a total of 69 DEMs were annotated in 28 pathways, mainly enriched in 5 of them **(**Fig. 5**)**: ko01100 (Metabolic pathways), ko01110 (Biosynthesis of secondary metabolites), ko00999 (Biosynthesis of various plant secondary metabolites), ko00380 (Tryptophan metabolism), ko00960 (Tropane, piperidine and pyridine alkaloid biosynthesis). Among the PAs in the DEMs, only Re and SnNO were annotated to the KEGG pathway, and both were present in 2 pathways: ko01110 and ko00960.

Overall Analysis of transcriptome sequencing results for E. sonchifolia

Transcriptome sequencing was performed on different parts of *E. sonchifolia* and the library was constructed using the Illumina platform. a total of 80.21 Gb Clean Data was obtained. The Clean Data of each sample reached 6 Gb, and the percentage of Q30 bases was 93% and above. A total of 119,886 Unigenes were obtained after assembly of Clean Date, and 32,797 DEGs were screened by analyzing transcriptome data.

Screening and analysis of differentially expressed genes

In order to evaluate the difference of gene expression in different parts of *E. sonchifolia*, a two-by-two comparison of the number of differentially expressed genes (DEGs) was performed according to the conditions of |log2 FC| >= 1 and FDR < 0.05 **(**Fig. 6**)**: 22,122 DEGs (10,208 down-regulated, 11,914 up-regulated) in CBr vs CBh, 16,153 DEGs (8,089 down-regulated, 8,064 up-regulated) in CBl vs CBr, 15,650 DEGs (8,507 down-regulated, 7,143 up-regulated) in CBh vs CBl, 14,706 DEGs (7,583 down-regulated,

7,123 up-regulated) in CBh vs CBs, 9,648 DEGs (5,513 down-regulated, 4,135 up-regulated) in CBs vs CBr, 5,462 DEGs (2,042 down-regulated, 3,420 up-regulated) in CBl vs CBs.

Functional annotation and enrichment analysis of differential genes

A total of 32,797 DEGs were annotated in 146 pathways by KEGG enrichment analysis. The KEGG enrichment results of each comparison group were sorted by Qvalue, and the 15 pathways with the most significant enrichment were taken and combined for display **(**Fig. 7**)**. The results showed that KEGG of the differentially expressed genes of *E. sonchifolia* was mainly enriched in 5 pathways: ko01100 (Metabolic pathways), ko01110 (Biosynthesis of secondary metabolites), ko04626 (Plant-pathogen interaction), ko04075 (Plant hormone signal transduction), and ko04016 (MAPK signaling pathway).

Screening of key enzyme genes of Sek biosynthesis pathway in E. sonchifolia

Studies have shown that PAs form spermine by ornithine or arginine under the action of decarboxylase, followed by hydrolysis to form putrescine [33]. Putrescine reacts with spermidine under the action of High Spermine Synthetase (HSS) to form high spermine, which is then restored by NADH to form high spermidine, and forms a pyrrole dialkyl skeleton through cyclization reaction [34], on the basis of which a series of reactions such as oxidation, reduction, acylation, methylation and so on further modified to form alkaloids such as Re, SnNO and Sek, etc [35]. According to the transcriptome data, and based on the expression level of transcriptome genes and the size of |Log2 FC|, a total of 45 key enzymes (including 1 HSS, 1 diamine oxidase, 1 cyclase, 5 alcohol dehydrogenases, 11 acyltransferases, 23 methyltransferases, 3 Pyridoxal 5'-phosphate -dependent enzymes (PLP)) were screened out **(Supplementary Table 3)**, which belonged to 7 categories of key enzymes, and a total of 172 key enzyme candidate genes were screened out. The heat map of gene expression of key enzymes was drawn by Metware cloud platform **(**Fig. 9**)**. The results showed that the key enzyme genes in the synthesis pathway had significant differences in different parts of *E. sonchifolia*, and the expression levels of most genes in roots were significantly higher than those in flowers, followed by stems and leaves, and the expression levels in flowers were the lowest. The expression of PLP gene was higher in flowers. A few genes of methyltransferase, alcohol dehydrogenase, acyltransferase, cyclase and diamine oxidase were highly expressed in stems or leaves. The expression of key enzyme genes and the relative content of DEMs in the biosynthetic pathway of Sek in different parts of *E. sonchifolia* were used to draw a heat map of the biosynthetic pathway with ChemDraw 21.0.0 **(**Fig. 8**)**. The results showed that HSS, diamine oxidase, cyclooxygenase, acyltransferase all had the highest expression in roots, ADH had high expression in roots and stems, most of the methyltransferases had the highest expression in roots, while PLP had the lowest expression in roots. The relative content of SnNO was highest in roots, the expression of acyltransferase was the highest in roots, while the relative content of Retronecine (Re) was the lowest in roots. It is speculated that Re might have generated SnNO under the action of acyltransferase in the root. The relative content of Sek in roots was the highest, most of the methyltransferases were highly expressed in roots, while the expression of PLP was the lowest in roots, which may be due to the conversion of SnNO into Sek under the action of some methyltransferases.

Combined analysis of metabolome and transcriptomics of PAs in E. sonchifolia

To further understand the relationship between the relative content of PAs and the expression of key enzyme genes of the synthesis pathway of Sek in different parts of *E. sonchifolia*, the correlation between the relative content of PAs and the expression of DEGs was compared between the groups of CBr and CBh (Fig. 10). The results showed that differential metabolites were significantly correlated with differential genes. Among them, differential metabolites of Sek, SnNO, SpNO, Isa, ADO, EfNO showed significant positive correlation but INT showed significant negative correlation. The differential genes of K1, K2, K3, K4 and K5 enzymes were correlated.

qRT-PCR expression analysis of key enzyme genes

The 5 candidate gene sequences with the largest multiplicity of difference (CBr vs CBh) among the key enzymes of the Sek biosynthetic pathway of *E. sonchifolia* were selected for qRT-PCR validation. Using flowers as the control, the relative expression of all four candidate genes, *BAHD-Ats*, *CCoAOMT*, *BOMT* and *3AT*, was highest in roots, while the relative expression level of *CAD* was the highest in stems. The relative expression of *BOMT* was root, stem, leaf and flower in turn. The relative expression levels of *BAHD-Ats* and *3AT* were root, stem, flower and leaf in turn. The relative expression levels of *CAD* were stem, flower, root and leaf in turn. Compared with roots, the expression of *CCoAOMT* was not obvious in stems, leaves and flowers (Fig. 11).

Discussion

In this study, a total of 91 DEMs were detected by metabolomics and transcriptome sequencing, belonging to 10 types of alkaloids, and a total of 32,797 DEGs were screened out. Among the total DEMs, the up-regulated metabolites were more than the down-regulated metabolites, and the down-regulated genes were more than the up-regulated genes among the total DEGs. Among them, the relative contents of PAs in different parts of *E. sonchifolia* were significantly different, while Sek had the highest relative contentin roots and the lowest in flowers. The Sek and SnNO were significant up-regulated metabolites, and Re was a significant down-regulated metabolite.

Currently, the synthetic pathway of PAs is relatively complex, requiring a series of reactions such as cyclisation, oxidation, reduction, acylation, methylation, etc. Only the enzyme has been characterized, namely HSS, and other key enzymes that have not yet been characterized are yet to be explored. In order to further analyze the synthesis pathway of Sek, 45 key enzymes in the biosynthesis pathway were screened based on transcriptome data, belonging to 7 categories, with a total of 172 candidate enzyme genes. The expression of the key enzyme genes was significantly different in different parts of *E. sonchifolia*, and the expression in roots was significantly higher than that in flowers. Combined analysis of metabolic group and transcriptome data showed that there was a significant correlation between differential metabolites and differential genes. Among the differential genes, methyltransferase, alcohol dehydrogenase, acyltransferase, HSS and cyclase genes were all correlated. By qRT-PCR, using the

stable internal reference gene of *UPL* as a reference, it was verified that using flowers as a control group, 4 key enzyme genes (*BAHD-Ats*, *CCoAOMT*, *BOMT* and *3AT*) all had the highest relative expression in roots, while the relative expression of *CAD* was highest in stems, which was basically consistent with the pattern of transcriptome data. In order to reduce the hepatotoxicity of Sek in *E. sonchifolia*, it can be considered to regulate the biosynthesis synthesis of key enzyme genes of Sek in the root of *E. sonchifolia*, so as to further ensure the safety of of the medication of *E. sonchifolia*.

Conclusion

In this study, based on metabolomics and transcriptomics, 45 key enzymes in the biosynthesis pathway of Sek were screened out, belonging to 7 categories, with a total of 172 candidate enzyme genes. The expression of the first 5 candidate gene sequences with the largest difference multiple were analyzed by qRT-PCR. The relative expression levels of 4 key enzyme genes (*BAHD-Ats*, *CCoAOMT*, *BOMT*, *3AT*) in roots were the highest, and the relative expression level of *CAD* was the highest in stems, which was basically consistent with the transcriptome data. It provided a reference for the functional expression analysis of *E. sonchifolia*, and laid a foundation for the future analysis of synthesis pathway of Sek and the regulation of key enzyme genes of toxic components, so as to obtain low-toxic raw materials of *E. sonchifolia*.

Materials and Methods

Plant material collection

The sample of *E. sonchifolia* (L.) DC. was collected on August 17, 2023 in Qingxiu District, Nanning City, Guangxi Zhuang Autonomous Region. It was identified as the whole grass of *E. sonchifolia* (L.) DC. by professor Wang Liuping of Guangxi University of Traditional Chinese Medicine.

After collecting the whole plant of *E. sonchifolia*, wash, dry, divide the roots, stems, leaves and flowers, put them into 15 ml centrifuge tubes, number them, put them into liquid nitrogen for quick freezing, and store them in an ultra-low temperature refrigerator at -80 ℃ for spare parts.

Metabolome sequencing of E. sonchifolia

The 4 samples of roots, stems, leaves and flowers of *E. sonchifolia* were taken from the ultra-low temperature refrigerator, each with 3 biological replicates, and divided into two parts. One was sent to Wuhan Metware Biotechnology Inc for the analysis of UPLC-MS/MS. The conditions of UPLC analysis were as follows: column: Agilent SB-C18 (1.8 μm, 2.1 mm × 100 mm); mobile phase A (ultrapure water) with 0.1% formic acid, and mobile phase B (acetonitrile) with 0.1% formic acid; gradient elution: 0–9 min, 5%-95% B; 9–10 min, 95% B; 10-11.1 min, 95% B; and 10-11.1 min.; 10 ~ 11.1 min, 5% B; 11.1 ~ 14 min, 5% B; flow rate 0.35 mL/min; column temperature 40 ℃; injection volume of 2 μL. Mass spectrometry (MS) conditions: ESI temperature 550 ℃, mass spectrometry voltage 5500 V (positive ion mode), mass spectrometry voltage − 4500 V (negative ion mode), ion source gas I (GSI), gas II (GSII), and curtain gas

(CUR) were set to 50, 60, and 25 psi, respectively. The chromatographic peaks of the target metabolites were scanned and detected using the MRM mode of a triple quadruple pole, and the TIC plots of the mass spectrometry detection analyses of the different QC samples were analyzed by overlapping display. The metabolome data obtained from UPLC-MS/MS detection and analysis were analyzed, and the PCA was performed on all the samples to observe the inter- and intra-group differences of different parts of *E. sonchifolia*, based on the OPLS-DA, the Variable Importance in Projection (VIP) and FC values obtained from univariate analysis were used to further analyse and screen for DEMs. DEMs were further analyzed and screened by setting VIP > 1 and |log2 FC|>= 1 as the thresholds for DEMs, and the KEGG database was used for KEGG annotation and enrichment analysis of the screened DEMs.

Transcriptome sequencing of E. sonchifolia

Another copy of the same samples used for metabolome sequencing, a total of 12 samples, were extracted for total RNA, mixed in equal amounts, and sent to Wuhan Metware Biotechnology Inc for transcriptome sequencing analysis using the Illumina platform. The expression level of transcripts was calculated by fragments per kilobase of transcri pter million fragments (FPKM).

Differential analysis was performed using DESeq2, and the screening criteria forDEGs were set as |log2 FC| >= 1, FDR < 0.05. The KEGG functional annotation and enrichment analysis of the screened DEGs were carried out using the KEGG database to explore the signaling pathways that may play a role in the key enzymes of Sek biosynthesis.

The qRT-PCR verification of key enzyme genes

The first 5 candidate gene sequences with the largest |log2 FC| in the key enzymes for Sek synthesis in the CBr vs CBh group were selected. The ubiquitin-protein ligase (UPL) gene was used as the internal reference gene, and the specific primers were designed using Premier 5.0 **(Supplementary Table 4)**. The primers were synthesized by Nanning Jenice Biotechnology Co., Ltd. The total RNA was extracted from different parts of *E. sonchifolia* using the Eastep®Super Total RNA Extraction Kit, and its concentration and quality were detected and then reverse transcription reaction into cDNA was performed according to the instructions of the reverse transcription kit. The first incubation system was 16 ul, the second incubation system was 20 ul, and 3 technical replicates were set for each sample. The transcribed cDNA was diluted 350-fold with RNase-Free ddH_2O and used as the gene template. The reaction system was configured according to the instructions of the ChamQ SYBR qPCR Master Mix kit, and the reaction was performed according to 95 ℃ 3 min, 95 ℃ 10 s, 60 ℃ 30 s, 95 ℃ 10 s, 65 ℃ 6 s, 95 ℃ 50 s, 39 cycles of the amplification procedure were validated by qRT-PCR, and the relative expression of the candidate genes was calculated using the formula of $2^{-\Delta\Delta Ct}$, in which each sample was repeated 3 times. The specific expression patterns of the screened synthesis key enzyme candidate genes of the synthesis of Sek in different parts of *E. sonchifolia* were researched and jointly analyzed with transcriptomic data and metabolomic data to preliminarily validate the function of the biosynthesis key enzyme gene of Sek in *E. sonchifolia*

Declarations

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Authors’ contributions

Jinli yao: Methodology, Software, Writing - original draft. **Yanqing Qin:** Resources, Methodology, Software. **Dongping Tu:** Supervision, Conceptualization, Methodology, Writing - review & editing. **Yumei Huang:** Resources, Conceptualization. **Liuguan Liang:** Conceptualization, Resources. **Wenhui Cai:** Resources, Methodology. **Mingli Huang:** Resources, Conceptualization. **Xiaoqi Zhu:** Resources, Methodology. **Xinling Lao:** Resources, Methodology.

Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Ethics approval and consent to participate

The methods involved in this study were carried out in compliance with local and national regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Figures

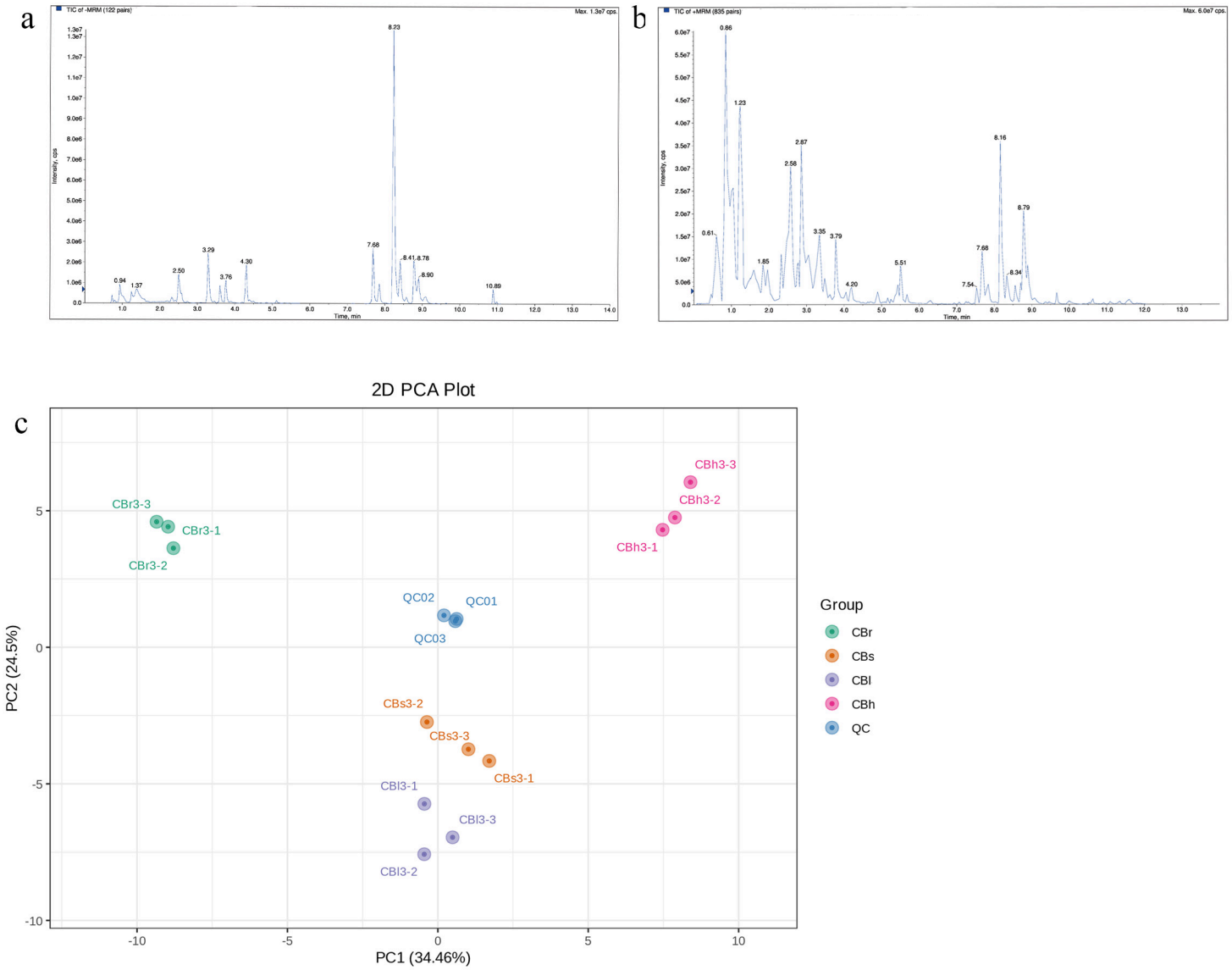

Figure 1

Qualitative and quantitative analysis of metabolome of *E. sonchifolia*. **a** Sample mass spectrometry detection TIC overlap map-N of *E. sonchifolia*. **b**Sample mass spectrometry detection TIC overlap map-P of *E. sonchifolia*. **c**PCA diagram of metabolites in different parts of *E. sonchifolia.*

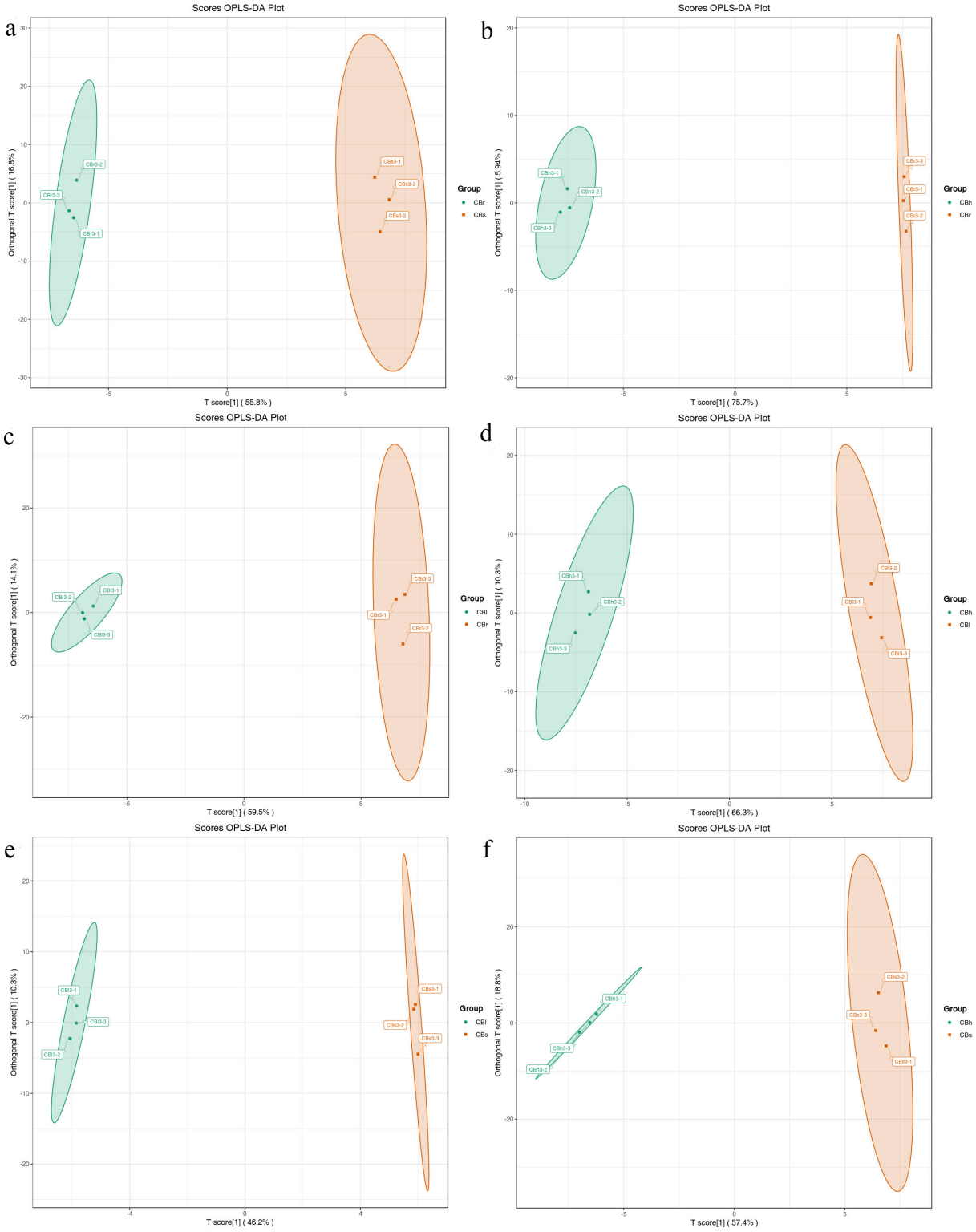

Figure 2

OPLS-DA score plots of different parts of *E. sonchifolia*. **a** OPLS-DA score plots in CBr and CBs comparison groups. **b** OPLS-DA score plots in CBh and CBr comparison groups. **c** OPLS-DA score plots in CBl and CBr comparison groups. **d** OPLS-DA score plots in CBh and CBl comparison groups. **e** OPLS-DA score plots in CBl and CBs comparison groups. **f** OPLS-DA score plots in CBh and CBs comparison groups.

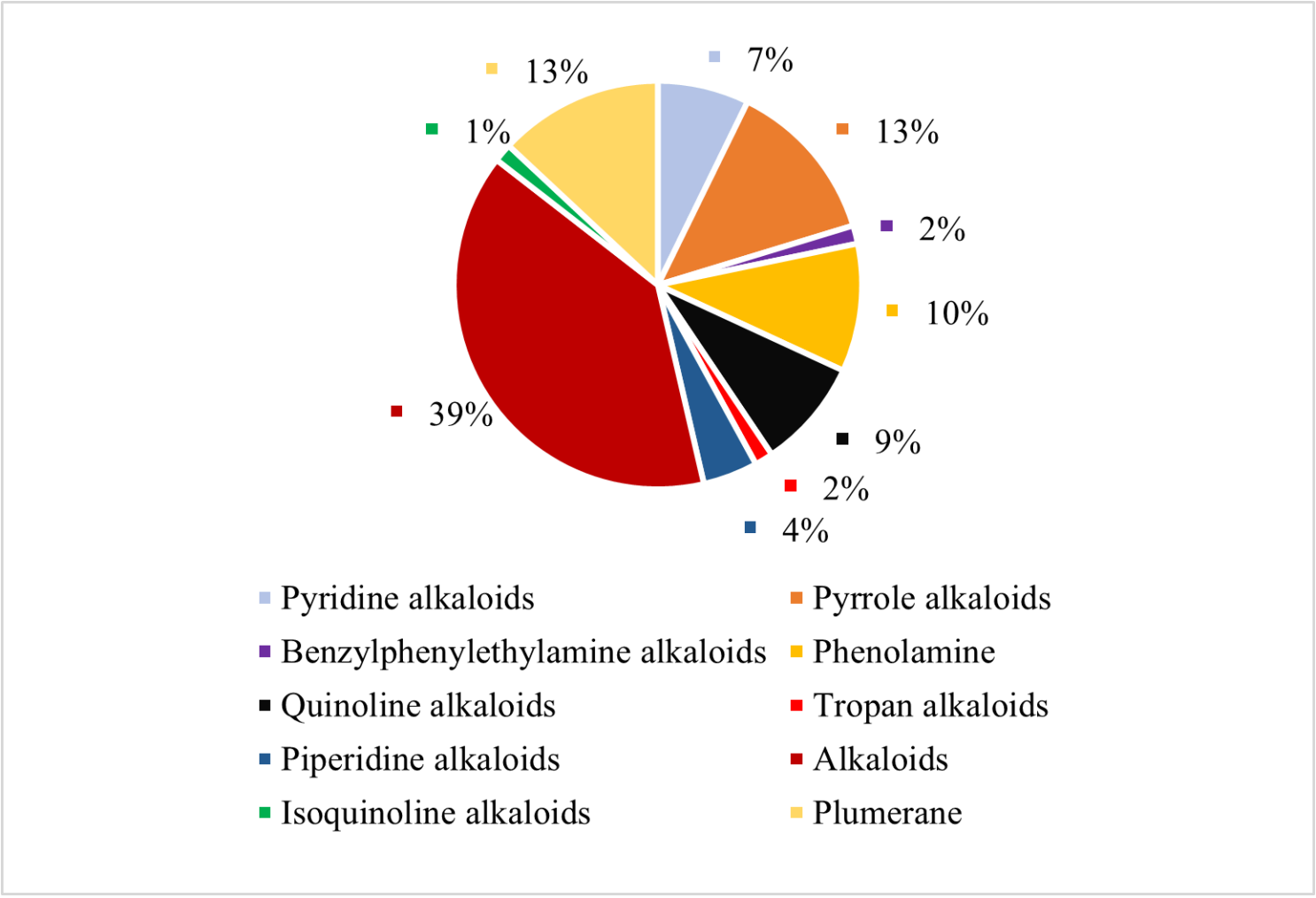

Figure 3

The overall situation of differential metabolite alkaloids

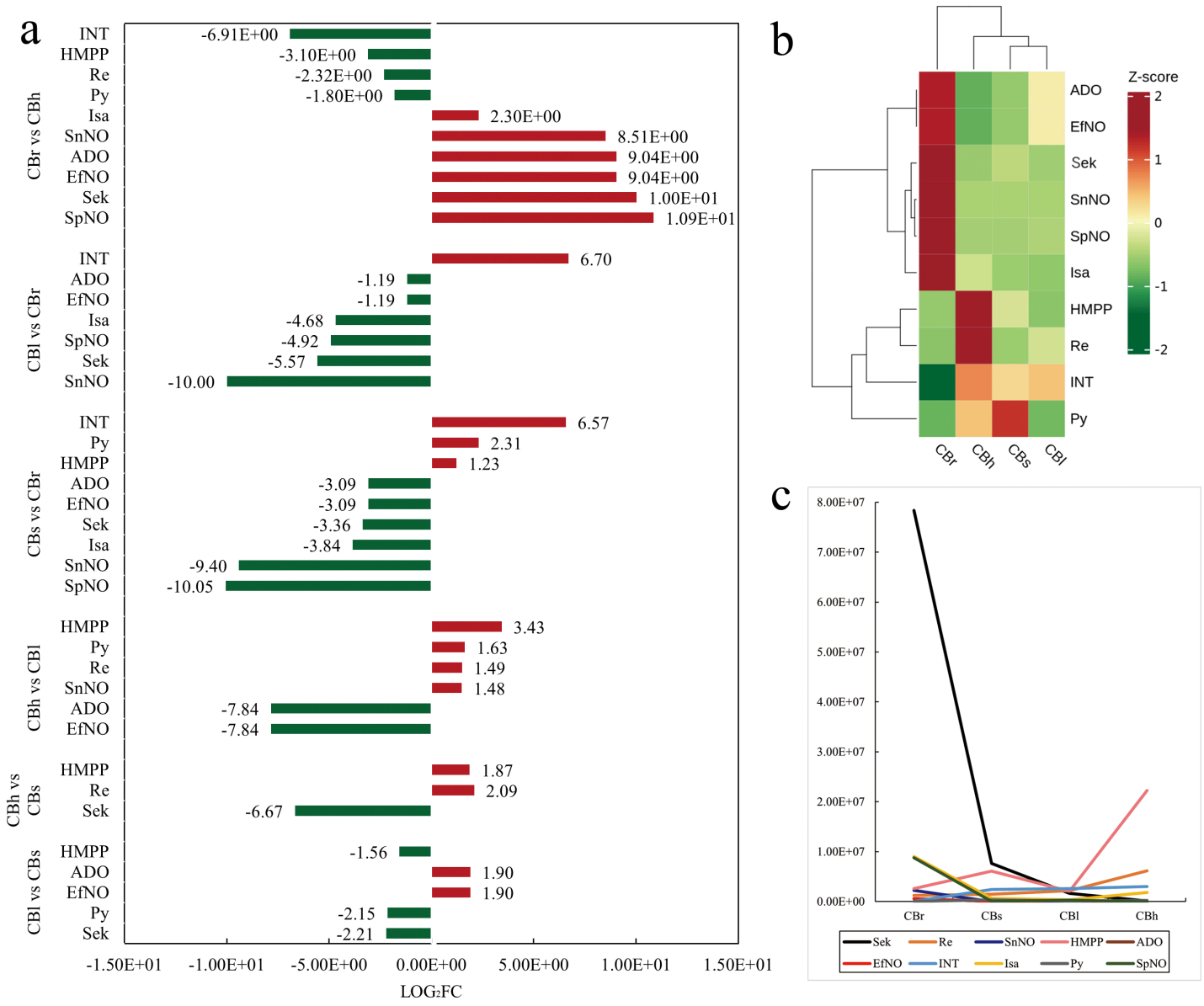

Figure 4

Dynamic distribution of relative content difference of PAs in *E. sonchifolia*. **a** The relative content of PAs in different parts of *E. sonchifolia*. **b** Cluster heat map of relative content of pyrrolizidine alkaloids. **c** The relative content of Sek, Re and SnNO in different parts of *E. sonchifolia*

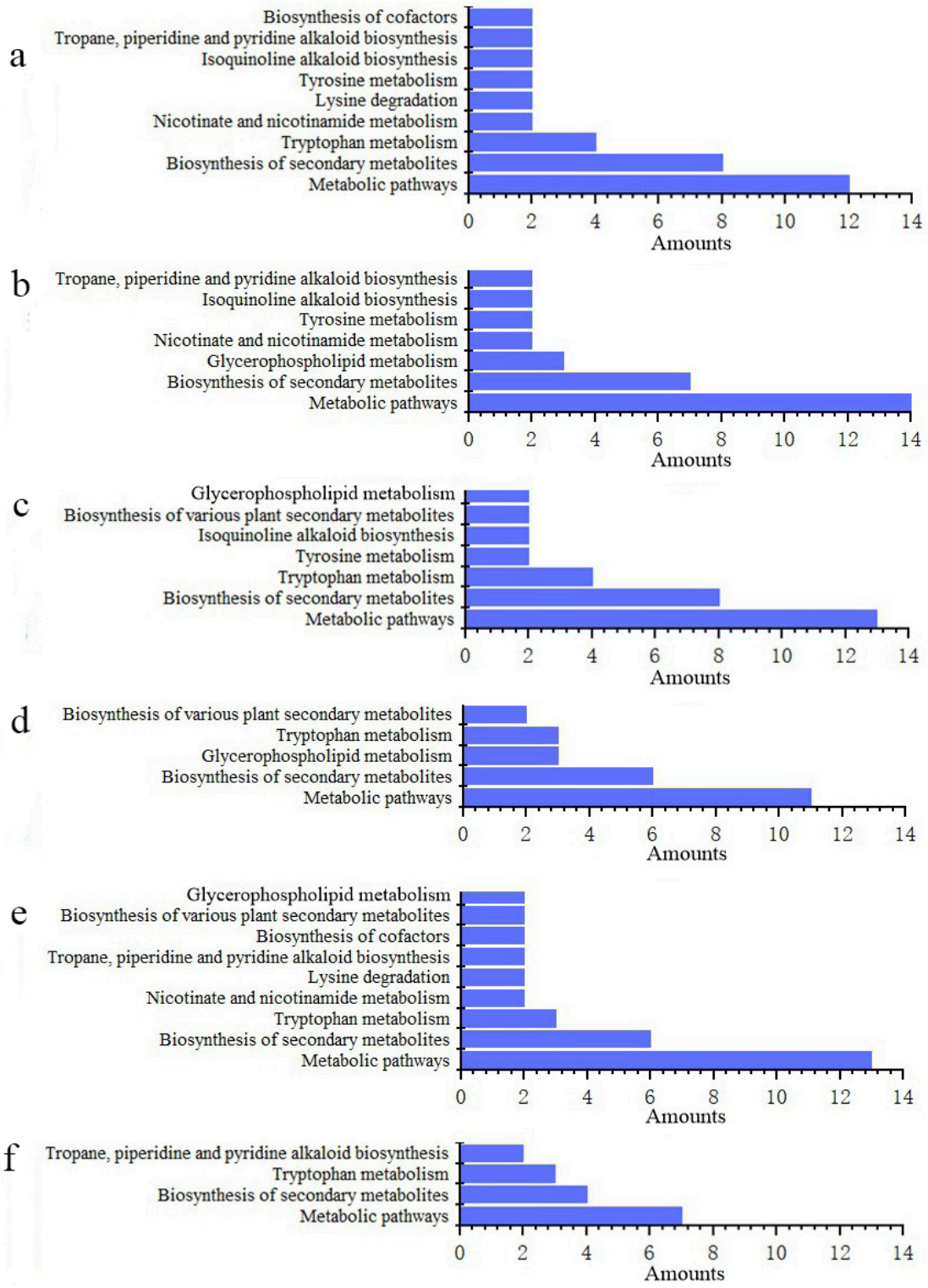

Figure 5

The histogram of differential metabolites KEGG. **a** KEGG histogram of differential metabolites in CBr and CBh comparison groups. **b** KEGG histogram of differential metabolites in CBl and CBr comparison groups. **c**KEGG histogram of differential metabolites in CBs and CBr comparison groups. **d**KEGG histogram of differential metabolites in CBh and CBl comparison groups. **e**KEGG histogram of

differential metabolites in CBh and CBs comparison groups. **f**KEGG histogram of differential metabolites in CBl and CBs comparison groups.

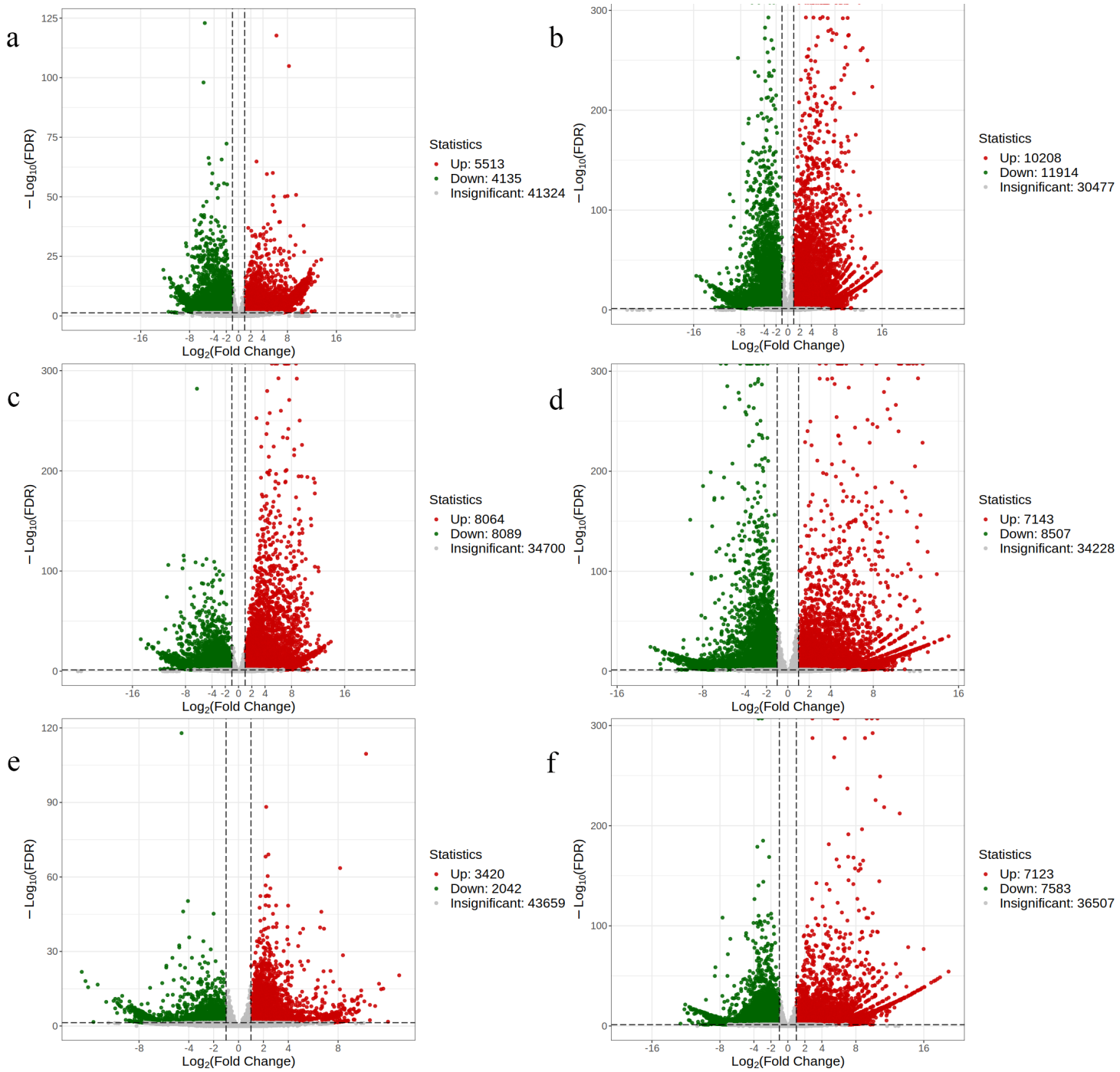

Figure 6

Volcano map of differential genes in different parts of *E. sonchifolia*. **a** Volcano maps of differential genes in CBr and CBs comparison groups. **b** Volcano maps of differential genes in CBh and CBr comparison groups. **c** Volcano maps of differential genes in CBl and CBr comparison groups. **d** Volcano maps of differential genes in CBh and CBl comparison groups. **e** Volcano maps of differential genes in CBl and CBs comparison groups. **f** Volcano maps of differential genes in CBh and CBs comparison groups.

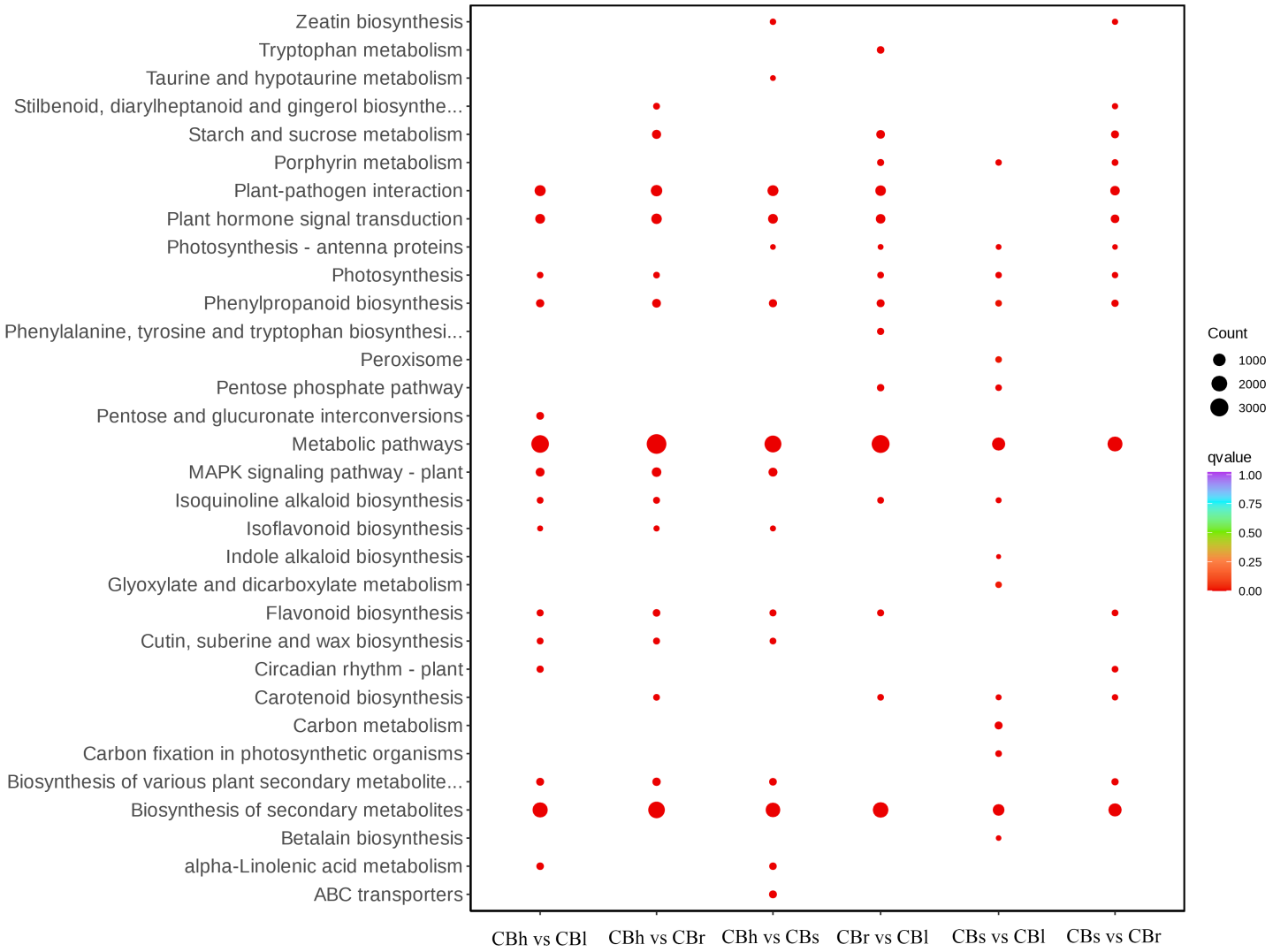

Figure 7

Bubble map enriched by differential gene in KEGG of *E. sonchifolia.*

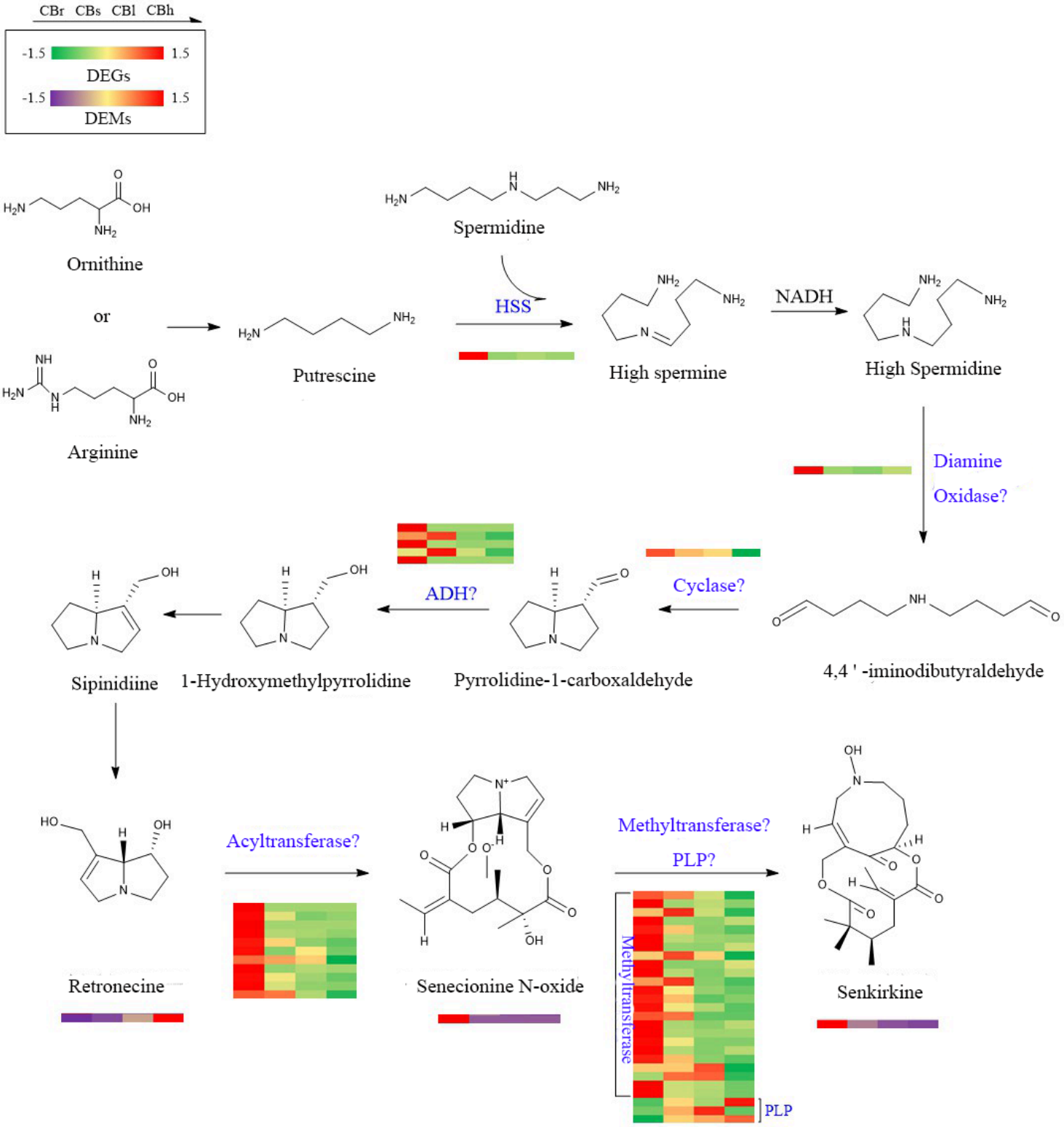

Figure 8

Pathway heat map of Sek biosynthetic pathway in *E. sonchifolia.*

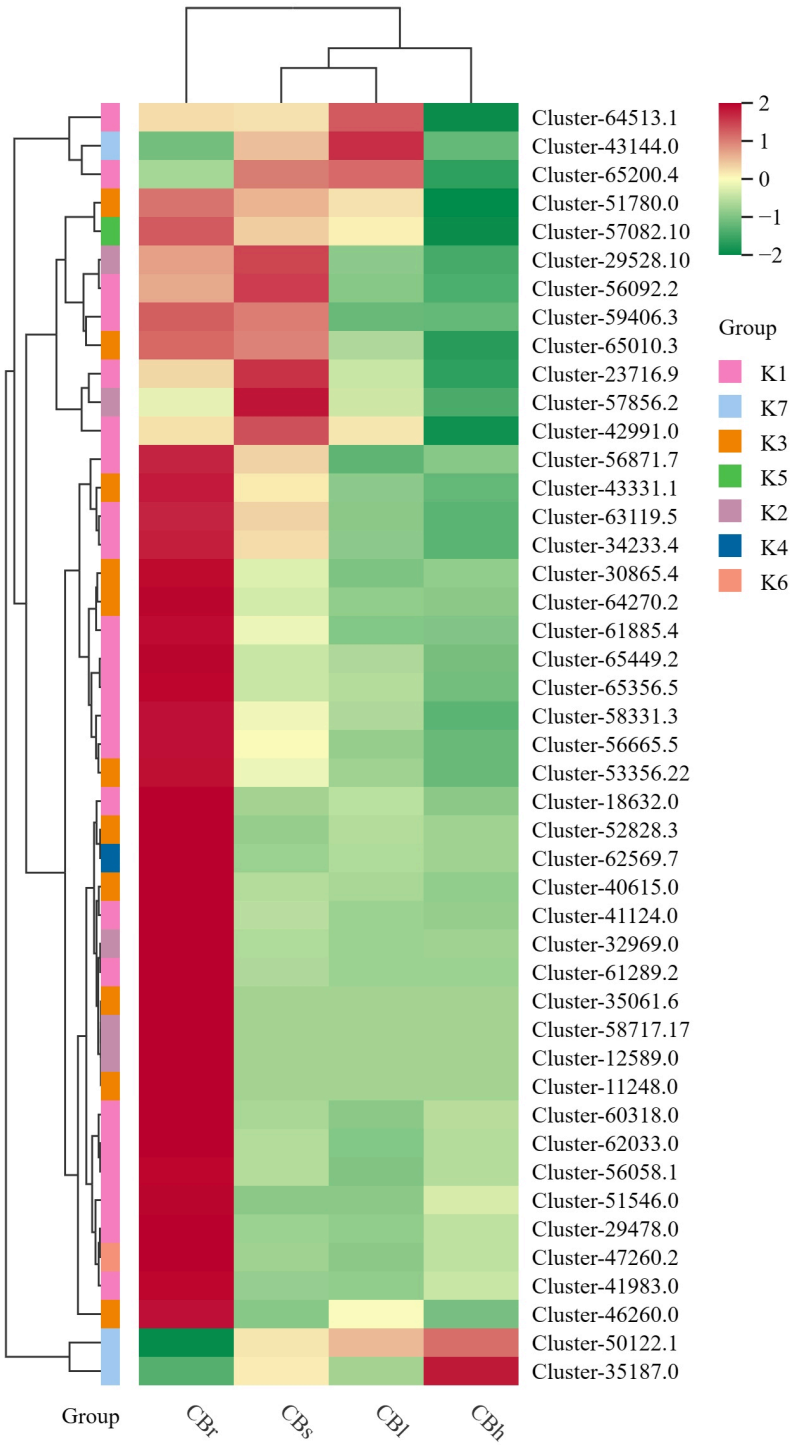

Figure 9

Differential gene expression quantity heat map of *E. sonchifolia.*

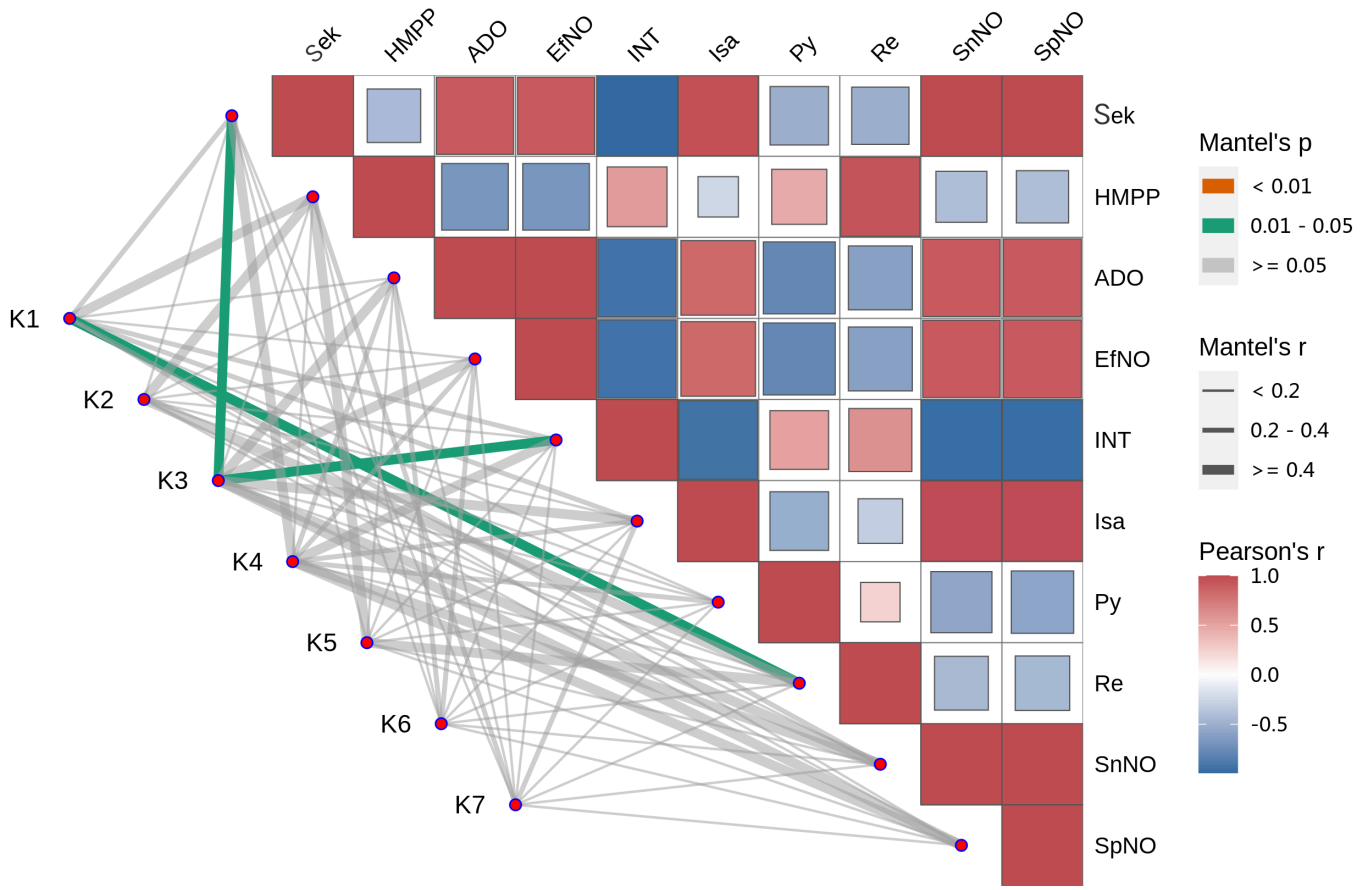

Figure 10

Correlation analysis between differential metabolites and differential genes in CBr and CBh groups.

Notes “K1” is methyltransferase, “K2” is alcohol dehydrogenase (ADH) [35], “K3” is acyltransferase [35], “K4” is HSS [34], “K5” is cyclase, “K6” is diamine oxidase [36], “K7” is 5 ' -pyridoxal phosphate-dependent bifunctional enzyme (PLP) [37].

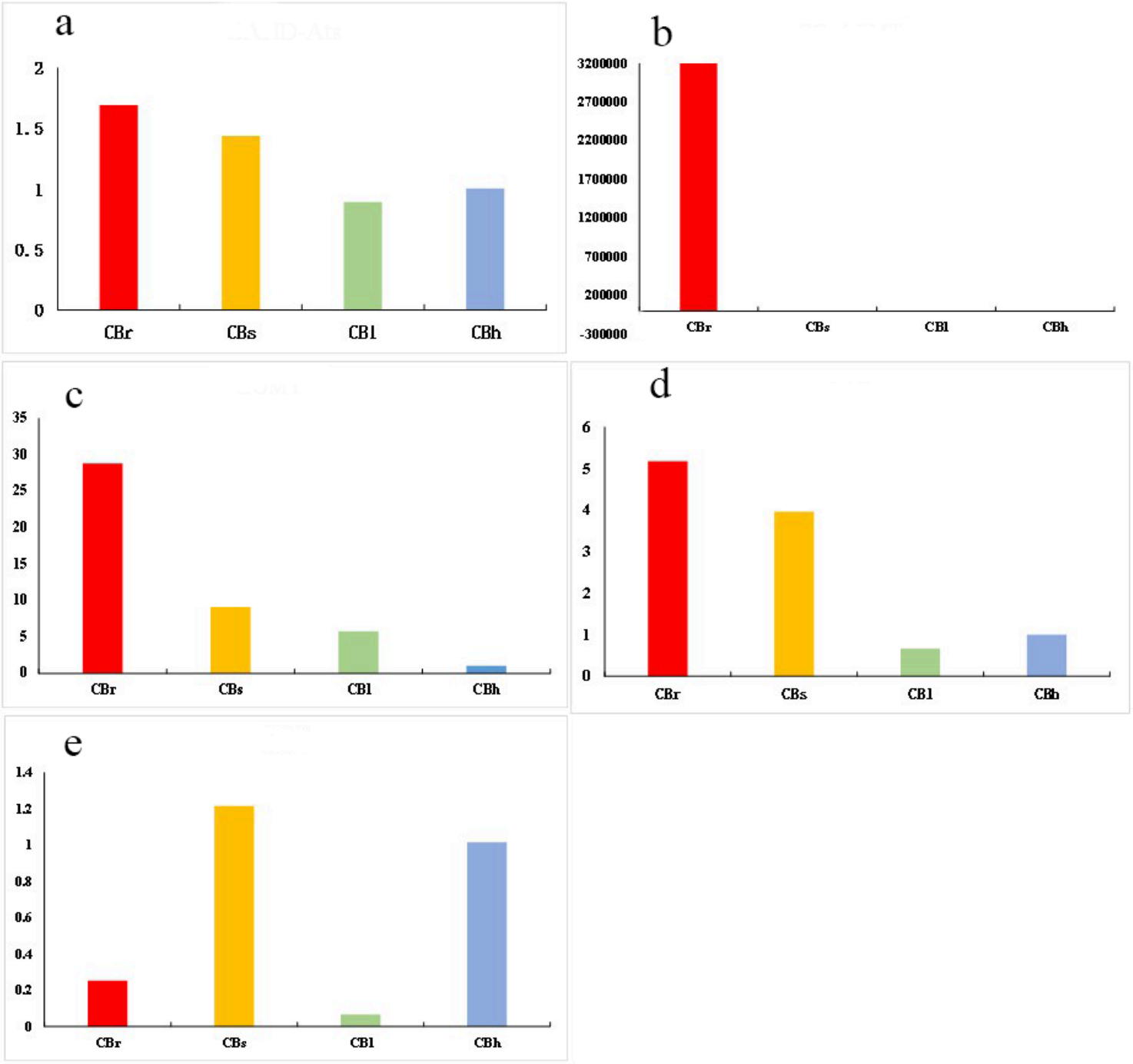

Figure 11

Relative expression of target gene. **a** Relative expression of gene "*BAHD-Ats*". **b** Relative expression of gene "*CCoAOMT*". **c** Relative expression of gene "*BOMT*". **d** Relative expression of gene "*3AT*" **e** Relative expression of gene "*CAD*".

Notes: "*BAHD-Ats*" is a BAHD acyltransferase gene, "*CCoAOMT*" is a caffeoyl-CoA O-methyltransferase gene, "*BOMT*" is a benzoate O-methyltransferase gene, "*3AT*" is an anthocyanidin 3-O-glucoside 6"-O-acyltransferase gene, and "*CAD*" is a cinnamyl-alcohol dehydrogenase gene.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.xlsx