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Effect of soil factors on flavonoid metabolites in *Striga asiatica* using LC–MS based on untargeted metabolomics

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Abstract

Background *Striga asiatica* (L.) O. Kuntze is a traditional medicinal plant rich in flavonoids, which has various pharmacological effects such as anti-hepatitis and antioxidant activities. However, there is a scarcity of resources, and artificial cultivation has not yet been achieved. This study explored the association between flavonoid metabolites and soil physicochemical properties and trace elements in different habitats, with the aim of offering theoretical guidance for the high-quality artificial cultivation of *S. asiatica*.

Results The results showed that *S. asiatica* has low requirements for soil fertility and prefers to grow in acidic soil with high contents of potassium and available potassium, while low contents of phosphorus, nitrogen and alkali hydrolyzed nitrogen. Additionally, 1592 kinds of metabolites were identified from *S. asiatica*, including 78 flavonoids.

Conclusions The flavonoid metabolites were strongly related to soil factors. Reasonable application of nitrogen and potassium fertilizers as well as controlling the contents of sodium, manganese and boron in the soil, can promote the synthesis of flavonoid metabolites in the plant. Moreover, kaempferide, glycitein, luteolin, apigenin and genistein may be the metabolic markers for identifying different regions.

Keywords *Striga asiatica* (L.) O. Kuntze, Soil physical and chemical properties, Metabolic pathways, KEGG, Habitat
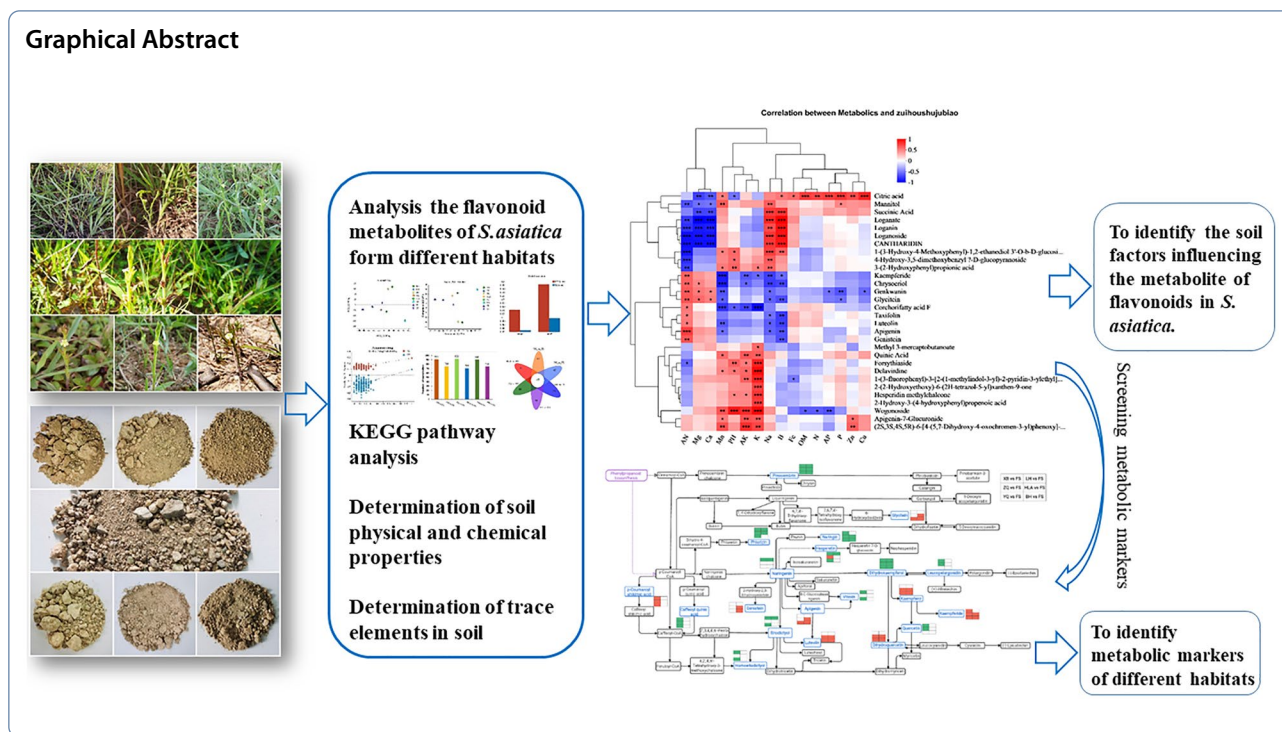
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Graphical Abstract

Introduction

Striga asiatica (L.) O. Kuntze belonging to the Scrophulariaceae family, is an annual semi-parasitic herbaceous plant that often parasitizes the roots of Gramineae plants such as *Eleusine indica* (L.) Gaertn., *Digitaria sanguinalis* (L.) Scop., and *Kummerowia striata* (Thunb.) Schindl. It is mainly found in barren mountains, valleys, grasslands, and fields, usually distributed in China, India, Myanmar, Nepal, Thailand, Vietnam, Bangladesh, Cambodia and Africa region [1]. In the southern folk of China, *S. asiatica* is a traditional medicinal plant that was commonly used to promote digestion and absorption, alleviate symptoms such as infantile malnutrition, parasitic diseases, infantile anorexia, icterohepatitis, and indigestion [2]. The classics "Dictionary of Chinese Medicine" recorded that taking 9–15 g of dried *S. asiatica* and boiling it in water can alleviate pediatric anorexia. Additionally, consuming 15–30 g of dried *S. asiatica* in boiling water with pig liver can effectively treat night blindness [3]. The traditional Chinese patent drug, "Xiao er gan ji tang", contains ingredients such as *S. asiatica* (234 g), *Tadehagi triquetrum* (L.) Ohashi (781 g), *Areca catechu* L. (469 g) and *Melia azedarach* L. (156 g), which is used to address infantile anorexia, emaciation, restlessness at night, abdominal distension, and vomiting [1]. It is found that the decoction of the combination of *S. asiatica* with *Codonopsis pilosula* (Franch.) Nannf., *Atractylodes macrocephala* Koidz. and *Poria cocos* (Schw.)

Wolf. had a good effect on appetite loss in patients with malignant tumors after chemotherapy [4]. Due to its excellent effects, it was widely used in the Guangdong and Guangxi provinces of China, making it highly attractive in the market. According to our on-site investigation, the price of dried *S. asiatica* is as high as \$270–350 per kilogram in Guangdong Province in 2023. Moreover, it is utilized as a folk prescription to treat loss of appetite and hypertension in India and Malaysia. Modern researches have shown that one of the main chemical composition groups of *S. asiatica* is flavonoids, which possess a wide range of biological activities, such as antioxidant, anti-inflammatory, antibacterial, anticancer, antiviral and anti-parasitic diseases [5–7]. In addition, Zou [8] found that the ethyl acetate extract and two kinds of flavonoids (apigenin and luteolin) from *S. asiatica* have good anti-malaria and anti-cytotoxic activities.

However, due to the unique semi-parasitic characteristics of *S. asiatica* and the deep dormancy characteristics of the seeds, specific environmental and host conditions were required to induce their germination [9], which resulted in its community competitiveness was weak and natural regeneration was slow. In recent years, there has been an increasing market demand for *S. asiatica*, but artificial cultivation has not yet been attained. Unfortunately, overharvesting and environmental damage have led to the endangerment of wild resources of *S. asiatica*. There is an urgent

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need to accomplish artificial cultivation to meet market demands and protect the species. Soil factors play a vital role in planting growth, which can impact on root nutrition and physiological metabolic activities. What's more, they are the influence factors that significantly impact the formation and accumulation of plant metabolites [10, 11]. It is necessary to conduct more in-depth research regarding the effects of soil factors on the growth of *S. asiatica*. On one hand, it aids in comprehending how soil factors influence the growth and enable the implementation of effective measures to enhance the feasibility of artificial cultivation. On the other hand, it facilitates better production guidance, leading to the cultivation of *S. asiatica* with robust growth and high-quality active ingredients. This will not only meet market demands, but also enhance the resource utilization efficiency of *S. asiatica.*

Metabolomics techniques enable the simultaneous assessment of various plant metabolites, offering opportunities to investigate the multitude of plant metabolites. Metabolomics provides a comprehensive view of the advantages of various biological systems and physiological states [12, 13]. Currently, metabolomics techniques have made significant progress in the identification of phytochemical components, differentiation of growth stages, studies of plant metabolic pathways, authentication and quality assessment of genuine medicinal materials, etc. Yang et al. investigated the metabolic changes in four developmental stages of *Lycium barbarum* fruits using metabolomics, preliminarily identifying 49 compounds including anthocyanins and alkaloids, and distinguishing the four stages of fruit development through 15 potential biomarkers [14]. Li used extensively targeted metabolomics technology to identify a total of 161 secondary metabolites in *Codonopsis pilosula* (Franch.) Nannf. from both Shanxi and Gansu provinces of China. Through a metabolic pathway enrichment analysis, significant differences in phenolic acids and flavonoids secondary metabolites were observed between the two regions [15]. Lau utilized metabonomics and transcriptomics techniques to improve and perfect the biosynthesis pathway of podophyllotoxin in *Sinopodophyllum hexandrum* (Royle) T. S. Ying [16].

This study collected *S. asiatica* samples and their respective habitat soils from seven locations. Through metabolomics analysis, we investigated the response patterns of flavonoid metabolites in *S. asiatica* to soil factors. The objective of this study was to identify the critical soil factors that influence flavonoid metabolites and offer theoretical guidance for the artificial cultivation of *S. asiatica*, thereby expediting its cultivation progress.

Materials and methods
Sample collection
The whole plant of *S. asiatica* and soil were collected from seven different habitats. Soil samples were collected by randomly selecting three points in each plot, and then mixing the three soil samples to obtain a composite soil sample for that plot. The soil collection method involved using a soil auger to collect the surface soil (0–10 cm) from the area 0–30 cm away from the roots of *S. asiatica*, with the removal of surface litter and fallen leaves. The habitat information was shown as follows: XB (Daling Town, Huizhou City, Guangdong Province); BH (Baihua Town, Huizhou City, Guangdong Province); HLA (Hengli Town, Huizhou City, Guangdong Province); LH (Lianghua Town, Huizhou City, Guangdong Province); ZQ (Guanxu Town, Zaoqing City, Guangdong Province); YQ (Yanqian Town, Longyan City, Fujian Province); FS (Shashi Town, Ganzhou City, Jiangxi Province). The photos of plants and soil in each habitat are shown in Fig. 1.

Determination of soil physicochemical properties and trace element content
The indices of measuring soil physical and chemical properties include pH value, organic matter (OM), available potassium (AK), available phosphorus (AP), alkali hydrolyzed nitrogen (AN), and potassium (K), phosphorus (P), nitrogen (N). Among them, AN and AK were determined according to the method described in the textbook [17]. OM, P, K, and AP were determined in accordance with the National Standard of the People's Republic of China [18–21], and the pH value was measured using the glass electrode method. The levels of sodium (Na), magnesium (Mg), zinc (Zn), copper (Cu), calcium (Ca), manganese (Mn), boron (B), and iron (Fe) were assessed by means of ICP-OES/MS methodology, respectively, using nitric acid, hydrogen peroxide, hydrofluoric acid, and multiacid digestion [22].

Metabolite extraction
Plant sample preparation
In a 2-mL centrifuge tube, 50 mg of dried *S. asiatica* sample was combined with a 6-mm diameter grinding bead, and then 400 μL of an extraction solution composed of methanol and water (4:1, v/v) was added. This solution also contained 0.02 mg/mL of L-2-chlorophenylalanine as an internal standard. The samples underwent grinding in a cryogenic tissue grinder at − 10 °C for 6 min with a frequency of 50 Hz, followed by sonication extraction at 5 °C for 30 min at 40 kHz. Post-extraction, the samples were cooled to − 20 °C for 30 min, then centrifuged at 13,000 rpm and 4 °C for 15 min. The resulting

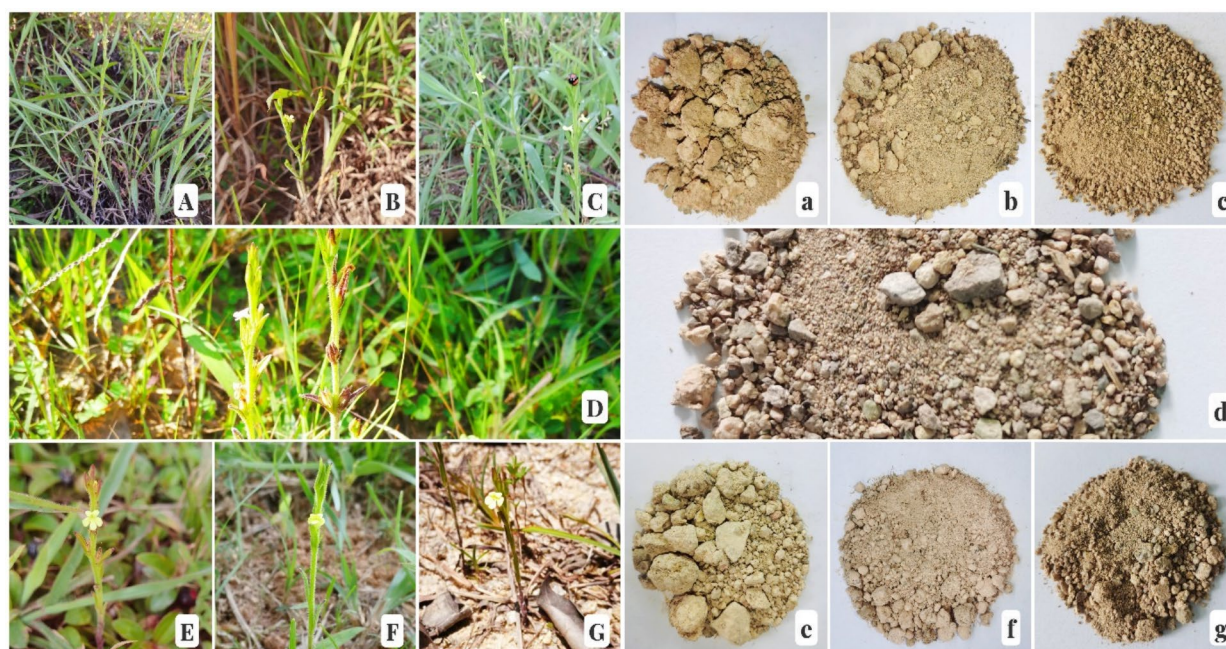Tang *et al. Chem. Biol. Technol. Agric.*   (2024) 11:89

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Fig. 1 Plant of *S. asiatica* and soil from seven habitats. **A** represents the plant of *S. asiatica* in BH, **a** represents BH's soil; **B** represents the plant of *S. asiatica* in LH, **b** represents LH's soil; **C** represents the plant of *S. asiatica* in ZQ, **c** represents ZQ's soil; **D** represents the plant of *S. asiatica* in HLA, **d** represents HLA's soil; **E** represents the plant of *S. asiatica* in YQ, **e** represents YQ's soil; **F** represents the plant of *S. asiatica* in XB, **f** represents XB's soil; **G** represents the plant of *S. asiatica* in FS, **g** represents FS's soil

supernatant was transferred to an injection vial equipped with an inserted tube for subsequent UPLC–MS/MS analysis [23].

QC sample preparation

All samples were metabolite-mixed to prepare quality control (QC) samples with equal volumes. During instrumental analysis, a QC sample was inserted every 6 samples to assess the reproducibility of the entire analysis process.

UPLC–MS/MS analysis

The sample was analyzed by using Thermo UPLC-Q Exactive HF-X system equipped with an ACQUITY HSS T3 column (100 mm×2.1 mm i.d., 1.8 μm; Waters, USA) via LC–MS/MS method. The UPLC–MS/MS conditions were documented in our prior studies [24].

Metabolite data analysis

Preprocessing of LC/MS raw data was conducted using Progenesis QI software (Waters Corporation, Milford, USA), which exported a three-dimensional data matrix in CSV format. The three-dimensional matrix contained sample information, metabolite names, and mass spectral response intensities. Internal standard peaks, along with any identified false positive peaks (such as noise, column bleed, and derivatized reagent peaks), were eliminated

from the data matrix, ensuring redundancy removal and peak pooling. Metabolites were simultaneously identified through database searches, primarily utilizing the HMDB (http://www.hmdb.ca/), Metlin (https://metlin.scripps.edu/), and Majorbio Database.

The database-searched data matrix was uploaded to the Majorbio cloud platform (https://cloud.majorbio.com) for subsequent data analysis. Initially, the data matrix underwent preprocessing, which involved retaining a minimum of 80% of metabolic features detected across any sample set. Subsequently, for samples exhibiting metabolite levels below the lower limit of quantification post-filtering, the minimum metabolite value was estimated, and normalization to the sum was performed for each metabolic signature. In order to mitigate errors stemming from sample preparation and instrument instability, the response intensities of mass spectrometry peaks in the samples were normalized to yield the data matrix. Simultaneously, variables from QC samples exhibiting a relative standard deviation (RSD) > 30% were excluded and logarithmically transformed using base 10 (log10) to derive the final data matrix for subsequent analysis.

Then, the R package "ropls" (Version 1.6.2) was used to perform partial least squares discriminant analysis (PLS-DA) and principal component analysis (PCA) on the preprocessed data matrix, and 7 rounds of cyclic

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interaction validation were used to evaluate the stability of the model [24]. Based on the partial least squares discriminant analysis model, the metabolites with variable importance in the projection (VIP) values > 1 and *P* values < 0.05 were identified as significantly different metabolites, which taking into account of the VIP and *P* values generated by the Student's t test. Differential metabolites were annotated through the KEGG database to obtain the pathways involved in differential metabolites (https://www.kegg.jp/kegg/pathway.html). Enrichment analysis, aiming to identify the most pertinent biological pathways for experimental treatments, was conducted utilizing the Python package "scipy. stats" (https://docs.scipy.org/doc/scipy/) [25, 26].

Statistical analysis

Excel 2016 was employed to organize and visualize the physiological and biochemical data. The pathways involved in the differential metabolites were obtained through metabolic pathway annotation using the KEGG database (https://www.kegg.jp/kegg/pathway.html). All of the metabolites data were analyzed through the free online platform of majorbio cloud platform (cloud. majorbio.com). The SPSS 18.0 software was operated in the correlation and significance analysis (Tukey's test).

Results

Multivariate statistical analysis

We performed untargeted metabolomics analysis on *S. asiatica* to characterize the variations in metabolites across different habitats (Fig. 2A–D). By conducting a principal component analysis of samples (including quality control samples), one can gain a preliminary understanding of the overall metabolic differences among sample groups and the variability in each sample group (Fig. 2A, B). In the PCA and PLS-DA charts, it is observed that three samples from the same habitat were clustered together and situated at a distance for samples from different habitats, which indicated that samples from the same environment have similar chemical compositions and relatively stable quality. However, samples from different habitats displayed substantial variations in their chemical compositions and varying quality.

In the PCA score chart, the first principal component contributes 32.2%, the second principal component contributes 22.5%, and the combined contribution rate of the two principal components is 54.7%. This suggests that they effectively reflect the main characteristic information of the *S. asiatica* samples (Fig. 2A). Upon conducting additional PLS-DA analysis on the data, the first main component (Component 1) accounts for an interpretation of 32%, while the second main component (Component 2) accounts for an interpretation of 23.6% (Fig. 2B).
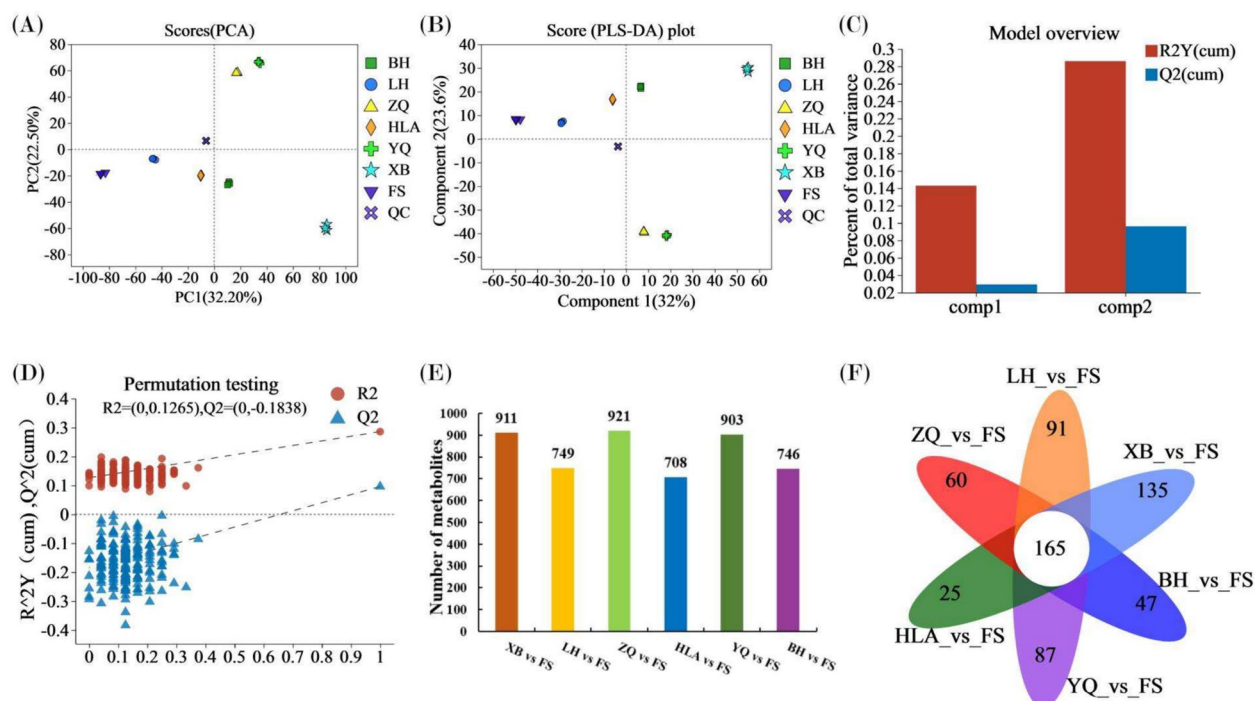

Fig. 2 Overview of *S. asiatica* metabolites information. **A** PCA score; **B** PLS-DA score; **C** PLS-DA model overview; **D** PLS-DA permutation testing; **E** comparison group metabolites; **F** comparison group Venn diagram

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R2Y and Q2 are employed to evaluate the modeling and predict capabilities of the PLS-DA model, respectively. A higher cumulative value of R2Y and Q2 indicates a more stable and reliable model (Fig. 2C). The decrease in R2 and Q2, coupled with an upward trend in the regression line, suggests that the permutation test was successful and the model did not exhibit overfitting (Fig. 2D).

Differential metabolite (DM) analysis

In total, 1592 kinds of metabolites were identified in *S. asiatica* from seven habitats (Table S1). Among them, carboxylic acids and their derivatives, organooxygen compounds, fatty acyls, prenol lipids, flavonoids, benzene and substituted derivatives, and glycerophospholipids accounted for 14.26%, 12.94%, 10.93%, 7.85%, 4.90%, 4.52%, and 2.70%, respectively (Fig. S1A).

The study detected a total of 911 metabolites between XB and FS, with flavonoids representing 6.52% (Fig. 2E; Table S2; Fig. S1B). Between LH and FS, a total of 749 metabolites were identified, with flavonoids comprising 5.43% of the total (Fig. 2E; Table S3; Fig. S1C). Similarly, between ZQ and FS, a total of 921 metabolites were found, with flavonoids accounting for 5.33% (Fig. 2E; Table S4; Fig. S1D). Also, between HLA and FS, a total of 708 metabolites were detected, with flavonoids comprising 4.78% of the total (Fig. 2E; Table S5; Fig. S1E). Between YQ and FS, a total of 903 metabolites were identified, with flavonoids accounting for 5.20% (Fig. 2E; Table S6; Fig. S1F). Besides, between BH and FS, a total of 746 metabolites were detected, with flavonoids accounting for 4.69% (Fig. 2E; Table S7; Fig. S1G).

There were 165 metabolites shared among all comparison groups. 135 differential metabolites (DMs) between the XB and FS comparison groups, 91 DMs between the LH and FS comparison groups, 60 DMs between the ZQ and FS comparison groups, 25 DMs between the HLA and FS comparison groups, 87 DMs between the YQ and FS comparison groups, and 47 DMs between the BH and FS comparison groups (Fig. 2F).

These results indicated that there are distinct differences in the metabolic compositions between the different comparison groups. Additionally, we visually represented the different metabolites in different comparison groups using volcano maps (Fig. 3).

Determination of soil physical and chemical properties

The physical and chemical properties of soil samples from seven habitats were measured (Table 1). The soil texture of FS and HLA plots is sandy, whereas the remaining plots exhibit a clay texture. Overall, the pH levels of the soil range from 4.85 to 5.76, indicating that the soil were acidic. Referring to the "National Soil Nutrient Content Grading Standard" [27], the soil nutrient content levels of seven habitats can be determined. The content of P ranges from 136.29 to 471.79 mg kg^{-1}, which belongs to extremely low levels. The content of AP ranges from 4.82 to 14.14 mg kg^{-1}, with FS at a low level, YQ/XB/BH/HLA at a moderate level, and ZQ/LH at an upper-middle level. The K content ranges from 16,018.44 to 39,380.17 mg kg^{-1}, with ZQ/YQ/XB at a high level and LH/BH/FS/HLA at an extremely high level. The content of AK ranges from 141.95 to 306.59 mg kg^{-1}, with XB/LH/HLA in an upper-middle level, ZQ/YQ at a high level, and BH/FS at an extremely high level. The N content ranges from 589.67 to 2387.67 mg kg^{-1}, with LH at a moderate level and ZQ at a very high level, while the other habitats are at low levels. The AN content ranges from 23.46 to 73.71 mg kg^{-1}, with LH at an extremely low level, YQ/XB/FS/HLA at a low level, and ZQ/BH at a moderate level. The OM content ranges from 8240.09 to 42,868.27 mg kg^{-1}, with YQ/HLA at a low level, XB/LH/BH/FS at a moderate level, and ZQ at an extremely high level. In summary, the contents of K and AK are generally higher, the content of AP is at a moderate level, while the content of P, N, AN and OM was relatively lower overall.

Determination of trace elements in soil

According to Table 1, the Na content in the soil varies from 120.5 to 5873.56 mg kg^{-1}, showing significant differences among various habitats. In addition, the contents of Zn (13.67–82.37 mg kg^{-1}), Mn (9.7–897.35 mg kg^{-1}), B (6.01–3828.69 mg kg^{-1}), and Fe (82.88–37,271.38 mg kg^{-1}) present significant differences among most habitats. The results demonstrated that *S. asiatica* is non-selective regarding aforementioned elements in soil, and these indicators are non-restrictive factors for its growth. However, the levels of Ca (46.65–510.08 mg kg^{-1}), Cu (0.21–11.14 mg kg^{-1}), and Mg (29.61–683.77 mg kg^{-1}) in the soil did not show significant differences among most habitats, which indicated that the growth of *S. asiatica* may have certain selectivity towards these indicators. Hence, it is necessary to conduct further exploration on how the content of soil indicators affected the growth of *S. asiatica*.

Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis

KEGG analysis was carried out on the biosynthetic pathways of *S. asiatica* in various habitats (Table 2). Among the six comparison groups, DMs in the pathway of flavone and flavonol biosynthesis, flavonoid biosynthesis, isoflavonoid biosynthesis, and phenylpropanoid biosynthesis all showed obvious enrichment. Significant enrichment of accumulated DMs was observed in both the pathway of flavone and flavonol biosynthesis as well as phenylpropanoid biosynthesis, across all comparison

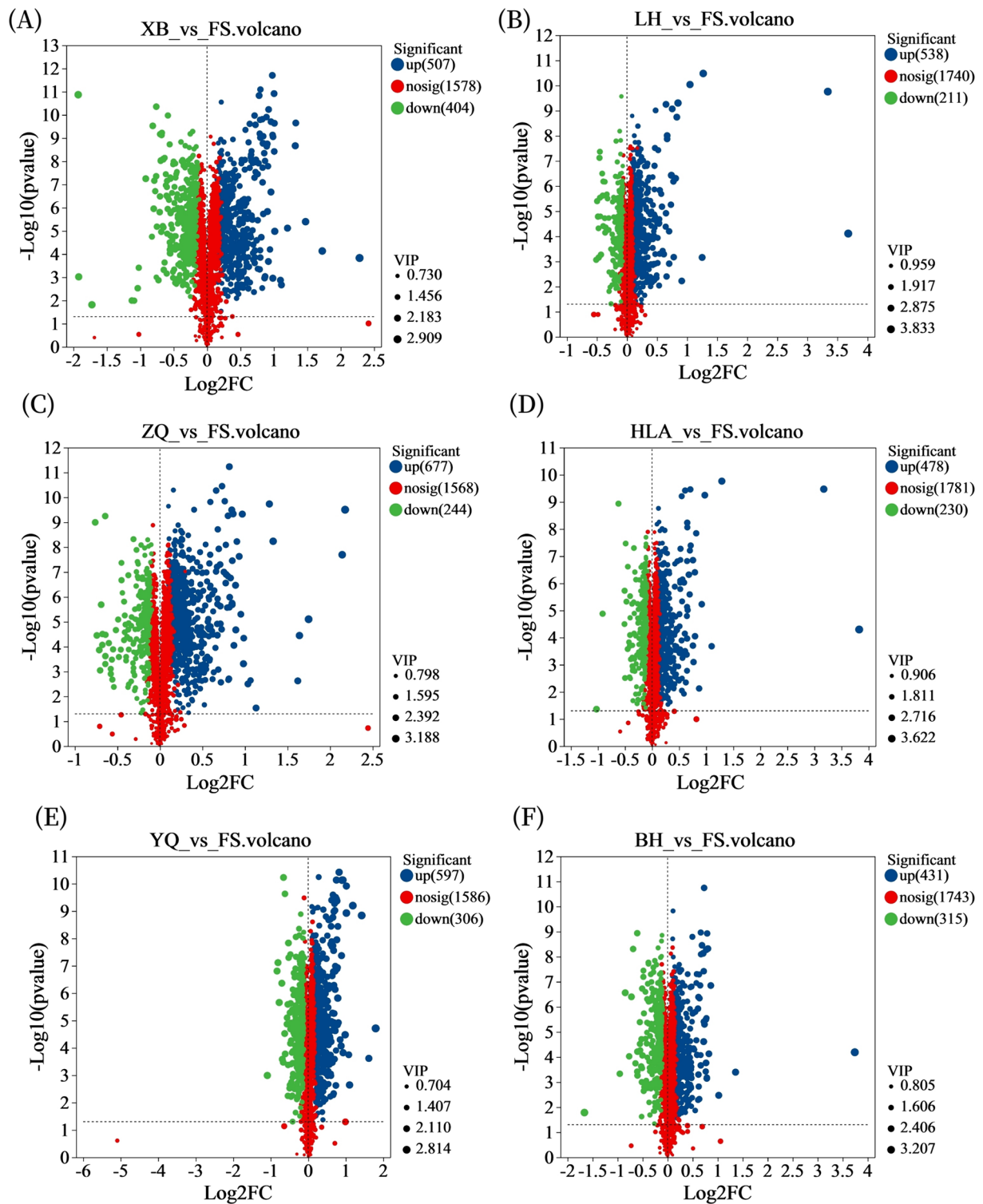**Fig. 3** DM volcano map. Each dot represents a distinct metabolite, and its size indicates the VIP value. Differentially downregulated metabolites are located on the left, whereas differentially upregulated metabolites are situated on the right. The further to the left and right they are, the higher the significance of the point above. **A** XB vs FS, **B** LH vs FS, **C** ZQ vs FS, **D** HLA vs FS, **E** YQ vs FS, **F** BH vs FS

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Table 1 Soil physicochemical properties and trace element analysis of *S. asiatica* from different habitats ($\bar{x} \pm$ s, $n = 3$)

Sample	ZQ	YQ	XB	LH	BH	FS	HLA
Soil parameters (mg/kg)							
AK	182.84 ± 7.00c	152.92 ± 1.84d	145.39 ± 2.54e	142.53 ± 1.97e	235.44 ± 3.05b	306.59 ± 3.04a	141.95 ± 2.89e
AP	14.14 ± 0.71a	5.34 ± 0.08de	5.01 ± 0.08de	10.95 ± 0.14b	5.96 ± 0.14c	4.82 ± 0.45e	5.48 ± 0.19 cd
AN	61.72 ± 1.69b	52.56 ± 1.27c	59.01 ± 1.01b	23.46 ± 2.94f	73.71 ± 0.99a	43.48 ± 1.11d	31.78 ± 0.97e
K	17,307.03 ± 271.95e	16,018.44 ± 128.75f	13,030.88 ± 91.57 g	20,294.89 ± 264.92d	34,589.97 ± 1246.11b	39,380.17 ± 429.29a	30,033.99 ± 281.79c
P	471.79 ± 22.28a	145.54 ± 6.54e	136.29 ± 2.79e	298.63 ± 9.22b	185.2 ± 7.53d	231.52 ± 9.31c	241.3 ± 2.54c
N	2387.67 ± 103.74a	596.33 ± 19.09e	657.67 ± 30.6de	970.67 ± 32.32b	746.33 ± 4.62c	589.67 ± 18.04e	707.33 ± 10.69 cd
OM	42,868.27 ± 1229.93a	8240.09 ± 180.8e	13,238.07 ± 87.16c	14,665.5 ± 481.06b	12,347.38 ± 174.29c	11,067.85 ± 494.63d	8410.21 ± 21.78e
pH	4.85 ± 0.01f	5.58 ± 0.04b	5.32 ± 0.03e	5.44 ± 0.01d	5.53 ± 0.03c	5.76 ± 0.01a	5.49 ± 0.02c
Na	1871.51 ± 31.74f	4083.21 ± 24.07b	3036.24 ± 33.49c	2814.31 ± 48.74d	120.5 ± 0.71 g	5873.56 ± 68.08a	2354.77 ± 81.3e
Mg	29.61 ± 0.65e	137.53 ± 4.89b	114.05 ± 1.03c	108.85 ± 7.56c	683.77 ± 9.4a	64.31 ± 1.11d	60.05 ± 1.9d
Ca	49.28 ± 0.16c	81.48 ± 53.63bc	114.82 ± 43.75b	76.33 ± 1.30bc	510.08 ± 14.13a	115.49 ± 4.22b	46.65 ± 1.45c
Zn	82.37 ± 5.02a	50.32 ± 0.37b	43.21 ± 0.90c	13.67 ± 1.29f	24.02 ± 0.09e	79.08 ± 1.88a	38.02 ± 1.04d
Mn	217.03 ± 8.74b	57.32 ± 3.37e	86.5 ± 1.25d	28.33 ± 1.97f	9.78 ± 1.05f	897.35 ± 26.99a	122.79 ± 3.29c
Cu	11.14 ± 0.42a	0.21 ± 0.04e	0.47 ± 0.04de	0.69 ± 0.05d	1.53 ± 0.23c	6.84 ± 0.11b	0.4 ± 0.08de
B	1459.39 ± 23.53d	2792.84 ± 80.25c	3139.83 ± 39.13b	2718.09 ± 37.77c	6.01 ± 0.80f	3828.69 ± 83.07a	1309.77 ± 85.57e
Fe	37,271.38 ± 212.8a	21,734.33 ± 588.01c	20,727.14 ± 631.77b	13,064.12 ± 1281.45e	82.88 ± 10.09f	17,330.96 ± 590.92d	27,734.62 ± 314.66b

Different lowercase letters in the same line indicated significant differences in $P < 0.05$

groups ($P < 0.05$, Table 2). The results above indicated that various growth environments and soil factors significantly affected the flavonoid metabolites of *S. asiatica*.

Correlation analysis between soil factors and metabolites of *S. asiatica*

Figure 4 illustrates the correlation analysis between soil factors and metabolites. We have chosen the top 30 metabolites based on their abundance in *S. asiatica*, which included 11 flavonoid metabolites such as kaempferide, chrysoeriol, genkwanin, glycitein, taxifolin, luteolin, apigenin, genistein, hesperidin methylchalcone, wogonoside, and apigenin-7-glucuronide (Fig. 5). Kaempferide had a significant positive correlation with AN ($P < 0.01$), while having a significant negative correlation with Mn ($P < 0.001$), AK/Na ($P < 0.01$), and B/K ($P < 0.05$). Chrysoeriol had a significant positive correlation with AN ($P < 0.01$) and Mg ($P < 0.05$), while showing a significant negative correlation with Mn ($P < 0.001$), B/Na ($P < 0.01$) and AK ($P < 0.05$). Genkwanin had a significant positive correlation with AN ($P < 0.01$) and Ca/Mg ($P < 0.05$), while displaying a significant negative correlation with Mn ($P < 0.01$) and Na ($P < 0.05$). Glycitein was significantly and positively correlated with AN ($P < 0.01$) and Ca/Mg ($P < 0.05$), while dramatically and negatively correlated with B ($P < 0.01$) and Mn/Na ($P < 0.05$). Taxifolin was significantly and positively correlated with AN ($P < 0.05$), and prominently and negatively correlated with B ($P < 0.01$) and Na ($P < 0.05$). Luteolin were markedly positively correlated with AN ($P < 0.05$), yet significantly

negatively correlated with Mn/B/Na ($P < 0.05$). Apigenin was memorably positively correlated with AN ($P < 0.001$), yet significantly negatively correlated with B/Mn/Na ($P < 0.05$). Genistein was significantly positively correlated with AN ($P < 0.01$), while significantly negatively correlated with B ($P < 0.01$). Hesperidin methylchalcone was substantially positively correlated with K ($P < 0.001$) and AK ($P < 0.05$). Wogonoside was significantly positively correlated with AK/K ($P < 0.001$) and Mn ($P < 0.01$). Apigenin-7-glucuronide was obviously positively correlated with AK/K ($P < 0.01$) and Mn ($P < 0.05$). These evidences suggested that soil factors have a significant impact on the flavonoid metabolites of *S. asiatica*.

KEGG metabolic pathway

We have drawn a diagram for illustrating the synthesis of flavonoid metabolites (Fig. 6). Phenylpropane biosynthesis served as the initial source of flavonoid metabolites, and the metabolites depicted in the blue box of the figure exhibit significant downregulation or upregulation in each comparison group, suggesting their crucial role as intermediates in the flavonoid biosynthesis pathway. Among them, kaempferide displayed a noteworthy upregulation in five comparative groups, namely XB vs FS, ZQ vs FS, YQ vs FS, HLA vs FS, and BH vs FS. Glycitein revealed a remarkable positive regulation in three comparative groups, namely YQ vs FS, HLA vs FS, and BH vs FS. Meanwhile, luteolin demonstrated a prominent upregulation among four comparative groups, including ZQ vs FS, YQ vs FS, HLA vs FS and BH vs FS. Genistein

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Table 2 KEGG pathways associated with differential metabolites (DMs)

[a]Pathway description	[b]Pathway_ID	[c]Ratio_in_study	[d]Metabolites	[e]Ratio_in_pop	[f]P value_corrected
KEGG pathways enriched in DMs identified between XB and FS					
Flavone and flavonol biosynthesis	map00944	9/186	C01460;C00389;C10098;C12638; C12629;C01265;C05623;C12626; C12249	51/5045	0.001086746
Flavonoid biosynthesis	map00941	13/186	C01709;C00389;C00509;C05631; C09789;C09756;C03648;C01460; C02947;C09827;C01604;C00852; C00974	74/5045	0.000087839
Isoflavonoid biosynthesis	map00943	5/186	C10516;C00509;C16192;C15511; C10522	64/5045	0.423604795
Phenylpropanoid biosynthesis	map00940	12/186	C00079;C02947;C00590;C02666; C01494;C01175;C01533;C10452; C00761;C01197;C00852;C12205	58/5045	0.000070500
KEGG pathways enriched in DMs identified between LH and FS					
Flavone and flavonol biosynthesis	map00944	7/141	C04608;C12629;C01265;C05623; C01470;C12626;C12249	51/5045	0.006741912
Flavonoid biosynthesis	map00941	6/141	C00509;C01709;C05631;C09827; C01604;C00974	74/5045	0.107878873
Isoflavonoid biosynthesis	map00943	6/141	C16223;C00509;C00786;C15511; C00858;C16192	64/5045	0.06692146
Phenylpropanoid biosynthesis	map00940	9/141	C01533;C00590;C20465;C20225; C02666;C00079;C01494;C02325; C12205	58/5045	0.000949847
KEGG pathways enriched in DMs identified between ZQ and FS					
Flavone and flavonol biosynthesis	map00944	10/192	C00389;C04293;C10098;C12638; C12629;C01514;C05903;C01265; C05623;C12626	51/5045	0.001254791
Flavonoid biosynthesis	map00941	12/192	C00389;C09789;C05903;C01514; C03648;C01709;C02947;C09827; C01604;C01617;C00852;C00974	74/5045	0.000686971
Isoflavonoid biosynthesis	map00943	5/192	C00858;C10522;C16223;C16192; C00786	64/5045	0.3287625
Phenylpropanoid biosynthesis	map0094	10/192	C00079;C01533;C00590;C02947; C01175;C02666;C01494;C02325; C00852;C12205	58/5045	0.001353781
KEGG pathways enriched in DMs identified between HLA and FS					
Flavone and flavonol biosynthesis	map00944	7/161	C04293;C10098;C01514;C01265; C05623;C01477;C05903	51/5045	0.017003907
Flavonoid biosynthesis	map00941	8/161	C01617;C09789;C01514;C09827; C01604;C01477;C05903;C00974	74/5045	0.020449988
Isoflavonoid biosynthesis	map00943	7/161	C14536;C06563;C16223;C15511; C00858;C01477;C10522	64/5045	0.032255791
Phenylpropanoid biosynthesis	map00940	7/161	C00079;C00590;C01175;C20225; C02666;C01494;C12205	58/5045	0.026166315
KEGG pathways enriched in DMs identified between YQ and FS					
Flavone and flavonol biosynthesis	map00944	9/190	C04293;C10098;C05903;C12629; C01514;C01265;C05623;C01477; C01460	51/5045	0.002068682
Flavonoid biosynthesis	map00941	14/190	C01709;C05631;C09789;C01477; C01514;C09756;C05903;C01460; C02947;C09827;C01604;C01617; C00852;C00974	74/5045	0.000038023
Isoflavonoid biosynthesis	map00943	8/190	C14536;C00786;C15511;C00858; C16231;C01477;C10522;C16192	64/5045	0.021505773
Phenylpropanoid biosynthesis	map00940	10/190	C00079;C00590;C02947;C01175; C02666;C01494;C10469;C00761; C00852;C12205	58/5045	0.002076615

Table 2 (continued)

ªPathway description	ᵇPathway_ID	ᶜRatio_in_study	ᵈMetabolites	ᵉRatio_in_pop	ᶠP value_corrected
KEGG pathways enriched in DMs identified between BH and FS					
Flavone and flavonol biosynthesis	map00944	7/159	C04293;C10098;C01514;C01265; C05623;C01477;C05903	51/5045	0.015794804
Flavonoid biosynthesis	map00941	7/159	C09789;C01514;C09827;C01617; C01477;C05903;C00974	74/5045	0.083861778
Isoflavonoid biosynthesis	map00943	7/159	C14536;C06563;C16223;C00786; C15511;C01477;C10522	64/5045	0.042993096
Phenylpropanoid biosynthesis	map00940	8/159	C00079;C00590;C01175;C20225; C02666;C01494;C16930;C12205	58/5045	0.016571475

ª Pathway description: The description for the name of KEGG pathway

ᵇ Pathway_ID: KEGG pathway ID

ᶜ Ratio_in_study: The fraction of metabolites assigned to the target pathway within the target metabolites, the count of target metabolic sets annotated to the left side of the diagonal, and the total number of KEGG compound identifiers for the target metabolic sets annotated across all pathways

ᵈ Metabolites: Metabolites engaged in metabolic pathways

ᵉ Ratio_in_pop: The fraction of metabolites assigned to the background pathway among the background metabolites, the count of background metabolic sets labeled to the left of the diagonal, and the total number of KEGG compound identifiers for the background metabolic sets annotated across all pathways

ᶠ P value_corrected: With a P-value less than 0.05, this function was deemed as a term indicating significant enrichment
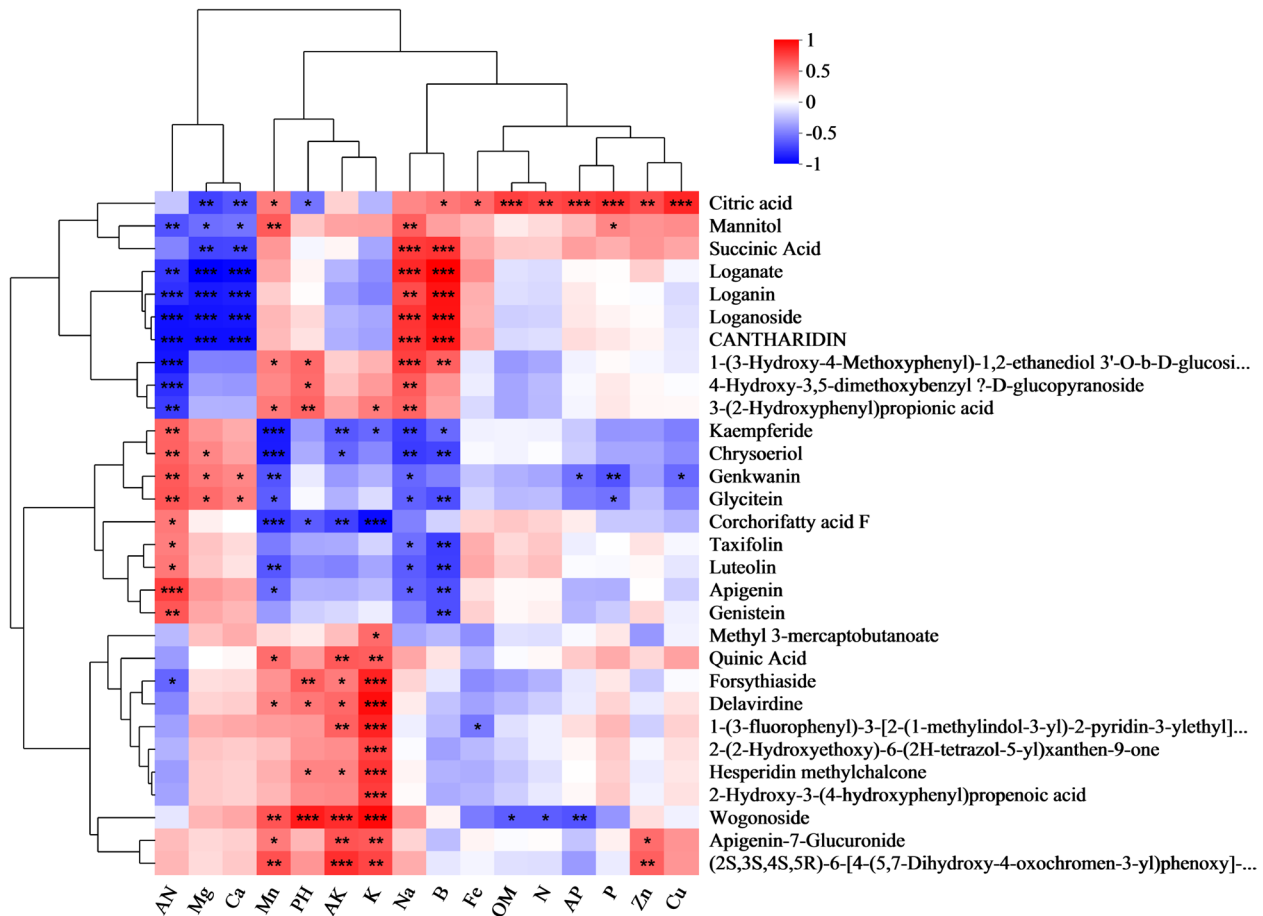

Fig. 4 Correlation analysis of the AK, AP, AN, K, P, N, OM, pH, Na, Mg, Ca, Zn, Mn, Cu, B, Fe with DMs among seven habitats. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$
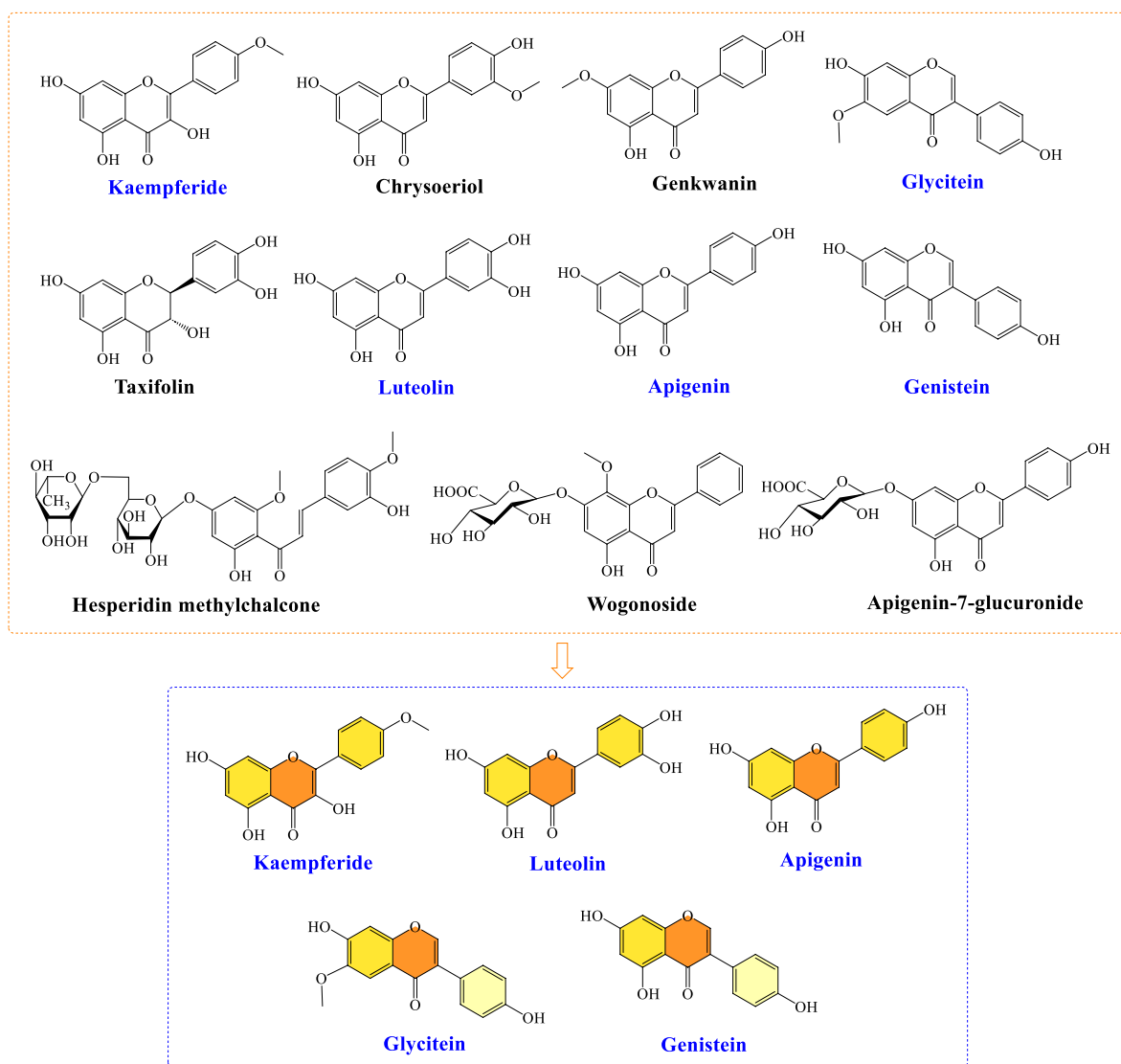
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Fig. 5 Chemical structures of 11 major flavonoid metabolites and five flavonoid metabolic markers

showed significant positive regulation between HLA vs FS and BH vs FS. Furthermore, apigenin exhibited dominant upregulation among three comparative groups, including YQ vs FS, HLA vs FS and BH vs FS (Fig. 6). The detailed schematic diagram of the biosynthetic pathways of aforementioned five metabolites is illustrated in Fig. 7.

Taken together, based on the correlation analysis of soil factors and metabolites mentioned above, it can be found that flavonoids (kaempferide, chrysoeriol, genkwanin, glycitein, taxifolin, luteolin, apigenin, genistein, hesperidin methylchalcone, wogonoside, and apigenin-7-glucuronide) are closely related to soil factors, which can be considered as potential metabolic biomarkers. What's more, kaempferide, glycitein, luteolin, apigenin, and

genistein are the key metabolites in the flavonoid biosynthesis pathway (Figs. 6, 7). According to these findings, we preliminarily speculate that kaempferide, glycitein, luteolin, apigenin, and genistein may serve as metabolic markers for distinguishing different habitats.

Receiver operating characteristic (ROC) analysis

This study utilized ROC analysis to examine the similarity of flavonoid metabolites in various comparison groups, aiming to assess the significance of differences between the groups. As depicted in Fig. 8, the AUC ratio of apigenin and glycitein was 1 in the comparisons of HLA vs FS, YQ vs FS, and BH vs FS. At the same time, the AUC ratio of genistein was also 1 in the comparisons of HLA
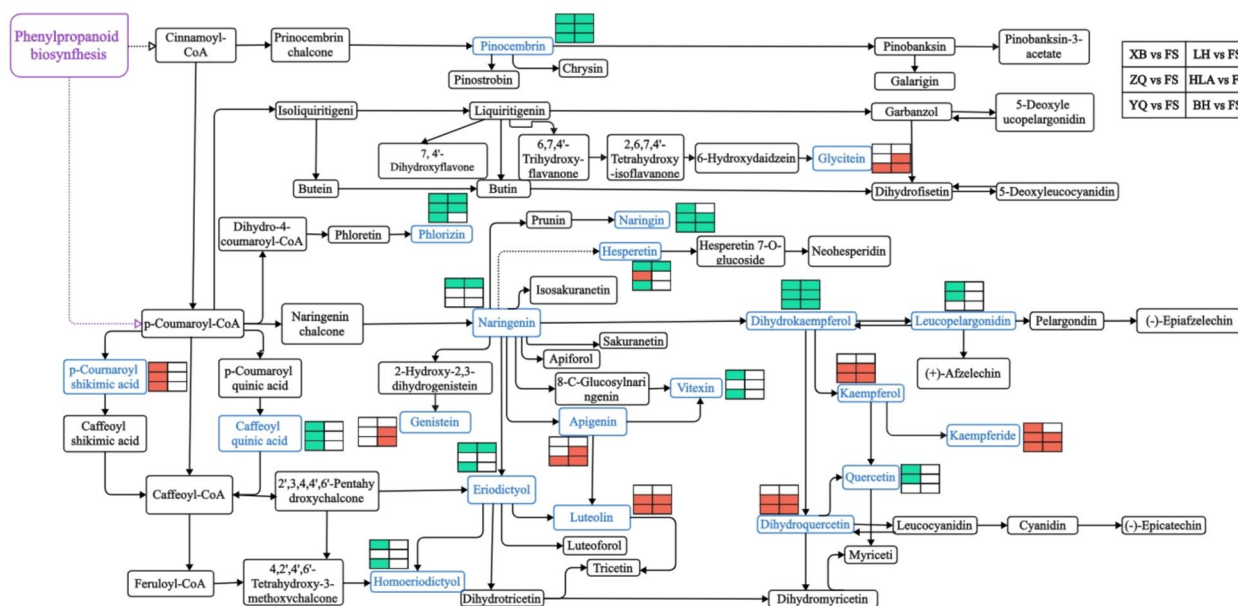
Fig. 6 Overview of the possible regulation of some key metabolites in metabolic pathways in pairwise comparisons of seven samples. The key metabolites are shown in the blue rectangle. Small red rectangles indicate significant upregulation of the metabolite content; small green rectangles indicate significant downregulation of the metabolite content; and small white rectangles indicate no significant difference in the metabolite content

vs FS and BH vs FS. Similarly, the AUC ratio of luteolin was 1 in the comparisons of ZQ vs FS, HLA vs FS, YQ vs FS, and BH vs FS. Likewise, the AUC ratio of kaempferide was 1 in the comparisons of XB vs FS, ZQ vs FS, HLA vs FS, YQ vs FS, and BH vs FS. These findings suggested that there are significant variations in flavonoid metabolites across different habitats, which further validated the potential of these five metabolites as crucial markers for distinguishing the various habitats of *S. asiatica.*

Discussion

In the natural environment, various factors influence the secondary metabolites of plants, with soil factors exerting a significant impact on plant metabolites [28]. This study focused on seven habitats of *S. asiatica* and soil (Fig. 1) to analyze the impact of soil factors on metabolites, especially on their flavonoid metabolites, and explore the key soil factors that affect the variation of metabolites.

Numerous researches have shown that soil physicochemical properties, pH value, and trace elements could affect the accumulation of plant secondary metabolites [29, 30]. Soil acidity and alkalinity provided appropriate environmental conditions for various chemical reactions, affecting the synthesis and decomposition of substances, as well as the absorption of anions and cations by plant roots. They also had a significant impact on soil fertility and the transformation and transportation of nutrients [31]. This experiment conducted a significant analysis

of the physical and chemical properties and trace elements in soil of seven habitats (Table 1). It was found that *S. asiatica* has small soil fertility requirements and prefers to grow in acidic soil with high content of K and AK, while exhibits a low demand for P, N, AN, and OM. The trace elements of Ca, Cu, and Mg in the soil existed a significant impact on the growth of *S. asiatica.* However, further investigation is required to establish the exact correlation between their levels and the growth of *S. asiatica.*

This results were closely linked to the semi-parasitic characteristics of *S. asiatica.* Not only did the germination of *S. asiatica* depend on the suitable light, temperature, and water vapor in the environment and soil, but also required stimulants from the host to break its dormancy. The seeds cannot germinate without effective stimulants. Recent research has shown that the environments rich in K, but deficient in P and N can induce a significant amount of germination stimulants for *Orobanche coerulescens* in tobacco plants, while the deficiency of N and P can promote the accumulation of these stimulants in the roots of tobacco [32]. Raju demonstrated that as N application increasing, the activity of sorghum infected by *S. asiatica* showed a declining trend [33]. The application of high-content (150 mg kg^{-1}) N to the soil was unable to trigger the germination of *S. asiatica*, even when sorghum produced stimulants. Therefore, the soil with high content of K and low content of P and N is
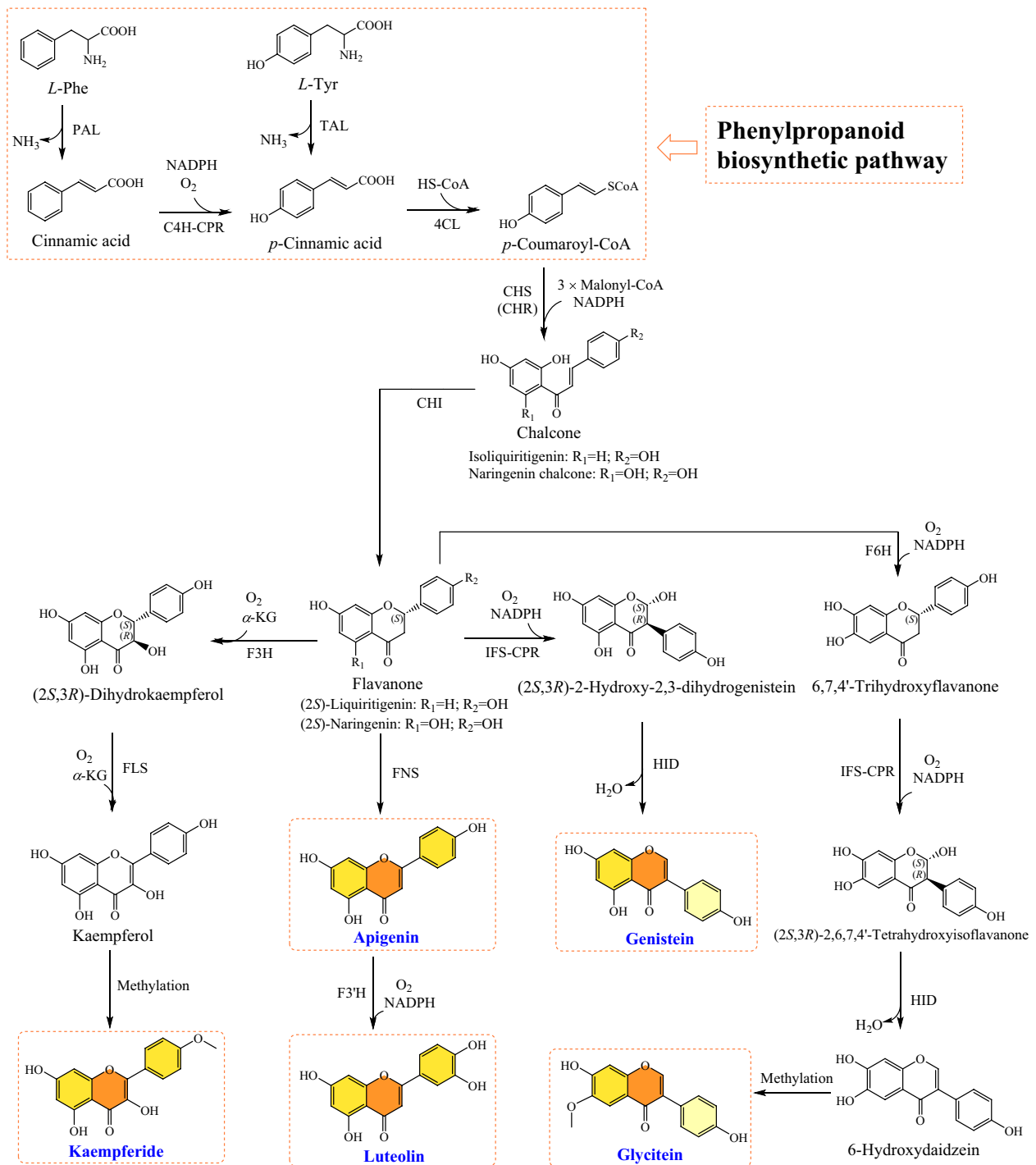
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Fig. 7 Biosynthetic pathways of five critical flavonoid metabolic markers. *PAL* phenylalanine ammonia-lyase, *C4H* cinnamate-4-hydroxylase, *TAL* tyrosine ammonia-lyase, *CPR* cytochrome P450 reductase, *4CL* 4-Coumarate: coenzyme A ligase, *CHR* chalcone reductase, *CHS* chalcone synthase, *CHI* chalcone isomerase, *F3H* flavanone-3-hydroxylase, *IFS* isoflavone synthase, *CPR* cytochrome P450 reductase, *F6H* flavanone-6-hydroxylase, *FLS* flavonol synthase, *α-KG* α-ketoglutaric acid, *FNS* flavone synthase, *HID* 2-hydroxyisoflavanone dehydratase, *NADPH* nicotinamide adenine dinucleotide phosphate, *F3'H* flavanone-3'-hydroxylase
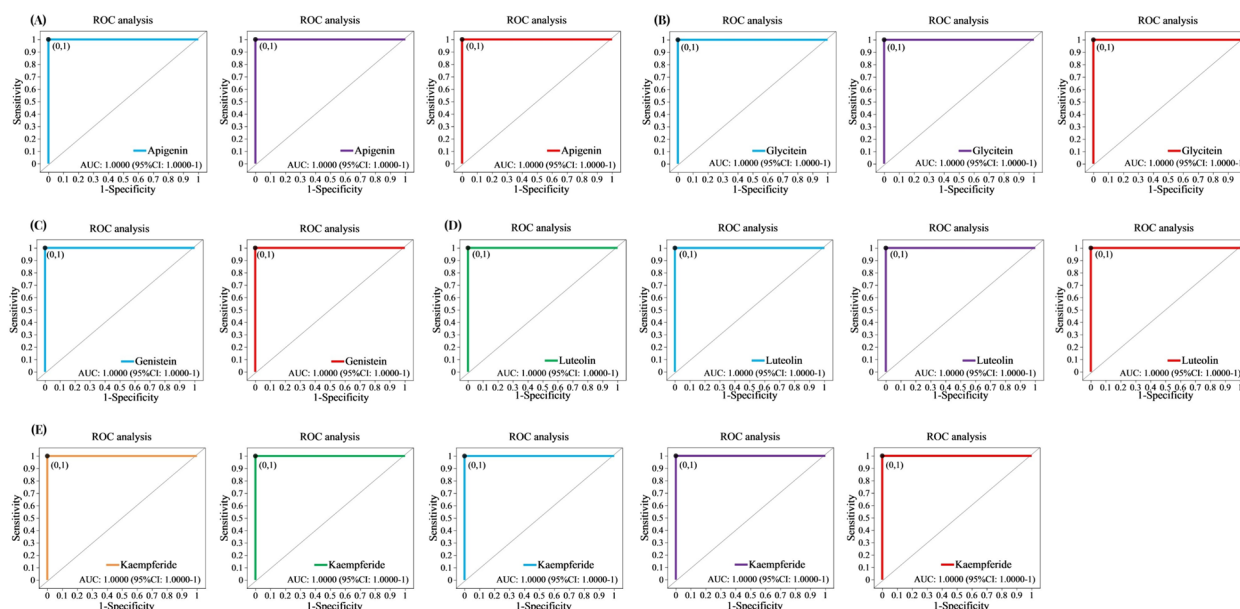
Fig. 8 ROC analysis. Blue represents HLA vs FS, purple represents YQ vs FS, red represents BH vs FS, green represents ZQ vs FS, orange represents XB vs FS. **A** ROC analysis of apigenin in different habitats; **B** ROC analysis of glycitein in different habitats; **C** ROC analysis of genistein in different habitats; **D** ROC analysis of luteolin in different habitats; **E** ROC analysis of kaempferide in different habitats. The *x*-axis is specificity, and the coordinate axis is 1–0. The *y*-axis is sensitivity, and the coordinate axis is 0–1. The AUC is the area under the corresponding curve. CI represents the 95% confidence interval of the AUC calculated based on the nonparametric resampling method. The point on the curve refers to the best threshold to distinguish the two groups based on the ROC curve

more suitable for the host to secrete effective germination stimulants, which promotes the growth and development of *S. asiatica*. As far as we know, organic matter can reflect soil fertility, yet serves as an indicator for evaluating soil fertility, fertility level, fertility retention capacity and buffering capacity [34]. Cecile found that the secretions of the plant rhizosphere were increased under the conditions of nutritional stress. As a result, weak soil nutrient conditions and low fertility levels were beneficial to stimulate the production of rhizosphere secretions by host plants, which in turn induced the germination of *S. asiatica* [35].

Additionally, *S. asiatica* is a semi-parasitic plant that exhibits degraded chloroplasts and an underdeveloped photosynthetic system. A suitable soil environment is required for optimal photosynthesis to meet its daily requirements. A document has shown that Ca, Cu and Mg possessed regulatory effects on plant photosynthesis. According to the research of Chen et al., Ca could regulate plant photosynthesis and enhance the content of soluble sugar, which further amplified the plant's resistance to factors such as low temperature, high temperature, drought, high salt, and diseases [36]. Yuan conducted a study on the impact of Mg on the growth and physiological characteristics of *Medicago sativa* L., and discovered that Mg had the ability to safeguard the chlorophyll structure, enhance the absorption and utilization of light

energy by plants, maintain the energy transfer process of the photosynthetic system, and supply enough energy for photosynthesis [37]. Consequently, *S. asiatica* displays a strong selectivity for Ca, Cu, and Mg in soil. Nevertheless, additional exploration is necessary to examine the relationship between the levels of these trace elements and *S. asiatica* growth.

Both the physicochemical properties of soil and trace elements can affect the content of flavonoids in plants. For instance, drought stress inhibits the accumulation of flavonoid content in the leaves of *Bupleurum chinense* [38]. The primary soil factors that influence the flavonoid content in *Glycyrrhiza uralensis* Fisch. are Ca and K [39]. Meanwhile, flavonoid metabolites are important components of *S. asiatica*, with a total of 78 ones detected in samples from seven habitats, accounting for 4.9% of all identified components of *S. asiatica*. Moreover, flavonoid secondary metabolites have diverse pharmacological properties such as anti-inflammatory, anti-neuroinflammatory, neuroprotective, anti-tumor, anti-bacterial, anti-hepatitis, hepatoprotective, and gastric ulcer treatments and so on, all of which have positive impacts on human health [40–42].

The correlation analysis between metabolites and soil factors revealed a significant positive correlation between AN and kaempferide, chrysoeriol, genkwanin, glycitein, taxifolin, luteolin, apigenin, and genistein (Fig. 4). This

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indicated that, within a certain range, a higher content of AN in the soil is conducive to the synthesis of the aforementioned flavonoid metabolites. On the contrary, an evidently negative correlation was observed between kaempferide, chrysoeriol, glycitein, luteolin, and apigenin with Mn, B, and Na. This relevance suggested that, within a certain range, higher levels of Mn, B, and Na in the soil may hinder the synthesis of the aforementioned flavonoid metabolites. Hesperidin methylchalcone, wogonoside, and apigenin-7-glucuronide demonstrated a noticeable positive correlation with AK and K, suggesting that AK and K contributed to the biosynthesis of these compounds for *S. asiatica*.

The KEGG is a comprehensive biological information database that visualizes pathways based on gene-related information. By aligning metabolites to KEGG compound IDs, information on the metabolic pathways in which the metabolites are involved can be obtained, allowing for the assessment of their impact on biological metabolic processes [43, 44]. Yuan et al. provided new insights into the biosynthesis of flavonoids in *Dendrobium huoshanense* by integrating transcriptome and metabolome approaches. This study identified 428 differentially accumulated metabolites and 1802 differentially expressed genes in *D. huoshanense*. KEGG enrichment analysis of these genes and metabolites unveiled significant variances in pathways such as flavonoids biosynthesis and phenylpropanoid biosynthesis [45]. The research indicated remarkable differences in the contents of total saponins, total flavonoids, and total polysaccharides in *Astragalus membranaceus* (Fisch.) Bunge from different regions, which could serve as predictive analysis indicators for the quality assessment of *A. membranaceus* [46]. In this study, KEGG pathway analysis revealed a significant enrichment of accumulated metabolites in the pathways of flavone and flavonol biosynthesis, as well as phenylpropanoid biosynthesis, among the comparison groups ($P < 0.05$, Table 2). Combining the expression of key flavonoid metabolites in the KEGG metabolic pathway, kaempferide, glycitein, luteolin, apigenin, and genistein were prominent upregulation among the comparison groups, which demonstrated that they could be the key metabolites of the flavonoid metabolic pathway. Besides, recent pharmacological studies have shown that the aforementioned flavonoid metabolites possess anti-oxidant, anti-inflammatory, anti-hepatitis, and anti-tumor properties [47–49]. The pharmacological activities of these five metabolites are similar to the efficacy of *S. asiatica*, thus they are considered as one of the main groups of active ingredients in *S. asiatica*. Based on the above results, it was speculated that five metabolites may be used as metabolic biomarkers to distinguish different habitats of *S. asiatica*. This study further analyzed

the ROC curves of kaempferide, glycitein, luteolin, apigenin and genistein to verify whether these five metabolites are metabolic biomarkers for identifying different habitats (Fig. 8). The results showed that the AUC ratio of each metabolite was 1, indicating significant differences among the comparison groups for these five metabolites, which can be used as key metabolic markers to identify different habitats. Besides, the research results offered some guidance to improve the content of flavonoid metabolites in *S. asiatica*.

Conclusion

In summary, *S. asiatica* has low requirements for soil fertility and prefers to grow in acidic soil with high content of K and AK, while low content of P, N and AN. During the cultivation process, attention should be paid to regulating the physical and chemical properties of the soil and trace elements, which can help cultivate high-yield, high-quality and high-content flavonoid metabolites of *S. asiatica*. Soil factors are closely related to flavonoid metabolites in *S. asiatica*. Kaempferide, glycitein, luteolin, apigenin, and genistein can be served as crucial metabolic biomarkers to identify different habitats. Reasonable application of N and K fertilizers, as well as control the contents of Na, Mn, and B in the soil, can promote the biosynthesis of flavonoid metabolites aforesaid, so as to enhance its anti-inflammatory, anti-hepatitis and antioxidant effects. The results of this investigation contribute to accelerating the process of artificial cultivation of *S. asiatica* and provide theoretical guidance for cultivating the *S. asiatica* with high content of flavonoid metabolites. Furthermore, based on the findings of this study, we propose a preliminary method for cultivating *S. asiatica*, wherein acidic, low-phosphorus, and low-nitrogen growth medium were prioritized. Following the planting of suitable host plants, *S. asiatica* seeds are sown, potassium fertilizer is applied at appropriate times, and rational irrigation and fertilization management are carried out.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00614-9.

Supplementary Material 1. Fig S1. Statistical map of compounds. Table S1. Metabolite information of *S. asiatica*. Table S2. Metabolite information comparing XB and FS. Table S3. Metabolite information comparing LH and FS. Table S4. Metabolite information comparing ZQ and FS. Table S5. Metabolite information comparing HLA and FS. Table S6. Metabolite information comparing YQ and FS. Table S7. Metabolite information comparing BH and FS.

Author contributions
Siqi Tang: field investigation, sampling, writing—original draft. Kaixin Wei, Xinghua Li, Yuxin Min, Jiayi Tai: field investigation, sampling, data curation. Hao

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Huang: investigation, formal analysis. Yi Xu, Lei Chen: resources, validation. Shimeng Yan: Software. Qiangqiang Xiong, Xiaojun Li: conceptualization, project administration, supervision, writing—review and editing.

Data availability
Data are provided within the manuscript or supplementary information files.

Declarations

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could potentially have influenced the work reported in this paper.

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