Beverage Plant Research

ARTICLE

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https://doi.org/10.48130/BPR-2023-0017

Beverage Plant Research **2023**, 3:17

Characteristic volatile components and transcriptional regulation of seven major tea cultivars (*Camellia sinensis*) in China

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Abstract

The volatiles in the young shoots of tea cultivars are the important material basis for the formation of tea aroma, but the cultivar-specific aroma and its molecular regulation are still lacking in research. In this study, the characteristic volatiles of seven tea cultivars in China were detected, and the results showed that the green tea cultivars 'Fuding Dabaicha' (FDDB), 'Longjing43' (LJ43), 'Shuchazao' (SCZ), and 'Baihaozao' (BHZ) were rich in (*E*)-3-hexenol, phenylethyl alcohol, phenylacetaldehyde, and β-ionone. For oolong tea cultivars, the characteristic volatiles of 'Tieguanyin' (TGY) were heptanal and eugenol, while the contents of (*E*)-β-ocimene, geraniol, and methyl salicylate were significantly increased in 'Jinxuan' (JX). In addition, 'Fujian Shuixian' (FJSX) has the highest content of esters, mainly jasmonolactone and dihydrojasmonolactone. Transcriptomic analysis showed that the different tea cultivars were significantly enriched in different levels of gene transcription in the three pathways related to aroma biosynthesis. Potential regulatory modules and genes of several characteristic volatiles were identified by WGCNA, among which *CsbHLH* (CsTGY12G0001520) may regulate the expression of *CsTPS* (CsTGY05G0001285) to directly affect the accumulation of β-caryophyllene in young shoots, while *CsMYB* (CsTGY01G0001203, CsTGY04G0001918, CsTGY06G0002545) may affect the synthesis of (*Z*)-3-hexenol and (*E*)-3-hexen-1-ol acetate by regulating the *CsADH* (CsTGY09G0001879). In addition, the transcription factors bHLH, WRKY, ERF, and MYB may be involved in the biosynthesis of linalool by regulating the expression of *CsLIS/NES* (CsTGY08G0001704, CsTGY08G0000359) genes individually or through interaction. These results revealed the characteristic volatiles and their key regulatory genes of seven tea cultivars, which will provide a theoretical basis for breeding and suitability research of tea cultivars.

Citation: Gao T, Shao S, Hou B, Hong Y, Ren W, et al. 2023. Characteristic volatile components and transcriptional regulation of seven major tea cultivars (*Camellia sinensis*) in China. *Beverage Plant Research* 3:17 https://doi.org/10.48130/BPR-2023-0017

Introduction

Tea aroma is affected by the tea cultivars, growing environment, and manufacturing process, and is an essential factor in determining the quality of tea[1]. As a high-weight trait in tea plant breeding, the volatile content of tea cultivars with different adaptability is usually different[2,3]. Studies have shown that the contents and ratios of linalool and geraniol are genetically specific and stable in tea cultivars[4]. The aroma quality of oolong tea is related to the release of aroma glycosides in the leaves of cultivars during the shaking process[5]. The major volatiles in tea are derived from either the terpenoid and shikimate pathways or by the oxidation of fatty acids and carotenoids[6]. It was found that aroma components can be synthesized by a single gene or multiple gene interactions[7]. Moreover, many transcription factor (TFs) can also participate in the formation of volatiles by regulating the expression of aroma synthesis pathway genes[8]. In summary, the formation and regulation of tea aroma is a complex process.

Oolong tea, which is a semifermented tea, possesses an elegant floral odour and is gaining popularity in China due to its distinct and characteristic aromas. Furthermore, the aroma quality of oolong tea can vary greatly because of cultivars, tea manufacturing process, regions, climate conditions, season of harvest, and quality of fresh tea leaves, with the cultivar being the most important factor[2,3]. 'Tieguanyin' (TGY, Registration No. GS13007-1985) tea is a typical cultivar of Chinese oolong tea, which is famous for its unique rich flavour and orchid-like aroma[2,9,10]. 'Jinxuan' (JX, Registration No. MS2011002) is one of the main cultivated tea cultivars in Fujian Province, China. Oolong tea processed from JX is popular among tea drinkers due to its unique floral and creamy aroma[11]. 'Fujian Shuixian' (FJSX, Registration No. GS13009-1985) is considered to be one of the most suitable cultivars for producing oolong tea[12]. Oolong tea processed from 'Shuixian' is popular among tea drinkers due to its sharp and typical floral odour[13]. White tea is a lightly fermented tea popular for its sweetness, clear fragrance, mellow aroma and outstanding health benefits, and it is rich in volatile compounds inherent to fresh leaves, such as aldehydes and alcohols[14,15]. 'Fuding Dabaicha' (FDDB, Registration No. GS13001-1985) is a major cultivar suitable for making white tea and an important parent for breeding green and black tea cultivars, which played important roles in the Chinese tea breeding history[16]. Chinese green tea, the most popular tea in China, presents different characteristic aroma types according to its sensory quality, such as floral, green, and

delicate aromas[17]. For instance, the representative green tea cultivars 'Longjing43' (LJ43, Registration No. GS 13037-1987), 'Baihaozao' (BHZ, Registration No. GS13017-1994), and 'Shuchazao' (SCZ, Registration No GS2002008), which come from Fujian, Zhejiang, Hunan, and Anhui Provinces, respectively, were identified as Chinese national improved cultivars.

In our previous study[18], we have analyzed the characteristic metabolites of seven tea cultivars using targeted metabolomics and widely targeted metabolomics, and combined transcriptome data to construct transcriptional regulatory networks for the characteristic metabolites of different cultivars. In addition to non-volatile metabolites, tea cultivars also affect the content of aromatic substances in its fresh leaves, which are the material basis for the formation of tea aroma. Although there have been many studies on the volatile components of tea, most of them have focused on tea processing and finished tea[19–21], and the influence of tea cultivars on aroma formation has received little attention. In this study, volatile metabolomics and transcriptomics were used to analyze the characteristic aroma components and differential genes of seven tea cultivars. Then the transcriptome data and aroma components were correlated by weighted gene co-expression network analysis (WGCNA), and co-expressed gene modules were screened to construct transcriptional regulatory networks of characteristic aroma components. These data and results will provide a theoretical basis for the production adaptability of tea cultivars at the aroma component and molecular level.

Materials and methods

Tea plant materials

In April 2021, the young shoots (one bud and two leaves) of the tea plants of *Camellia sinensis* (L.) O. Kuntze 'Tieguanyin' (TGY), 'Jinxuan' (JX), 'Fujian Shuixian' (FJSX), 'Fuding Dabaicha' (FDDB), 'Baihaozao' (BHZ), 'Longjing 43' (LJ43), and 'Shuchazao' (SCZ) were collected from the tea germplasm plantation of Wuyi University (Wuyishan City, Fujian, China; 27°73′17″ N, 118°00′18″ E) for detection of released volatiles and transcriptome analysis. Indeed, all tea plants were grown under the same cultivation practices. Three independent biological replicates were set up. The collected samples were immediately frozen with liquid nitrogen and stored in a freezer at −80 °C.

Analysis of volatile metabolites

The method for determining and analysing volatile metabolites was consistent with our previous report[2]. In brief, the samples were ground into powder in liquid nitrogen, and then 1 g of the powder was immediately transferred to a 20 mL Agilent headspace vial (CA, USA) containing saturated NaCl and 10 μL (50 μg/mL) [2H3]-β-ionone internal standard solution. After 5 min of constant temperature at 100 °C, 120 μm DVB/CAR/PDMS extraction head was inserted into the headspace bottle, and the headspace extraction was carried out for 15 min, and the sample was analyzed at 250 °C for 5 min. The volatile metabolites were detected using an Agilent Model 8890 GC and a 5977B mass spectrometer (Agilent). The analytical conditions were set as follows: desorption of the volatiles from the fibre coating at 250 °C for 5 min in the splitless mode. The carrier gas was helium, and the linear velocity was 1.0 mL/min. The temperature of the injector was kept at 250 °C, and the temperature of the detector was kept at 280 °C. Mass spectra were recorded in electron impact ionization mode at 70 eV. The quadrupole mass detector, ion source, and transfer line temperatures were set at 150, 230, and 280 °C, respectively. Mass spectra were scanned in the range m/z 50−500 amu at 1 s intervals.

Detection of volatile compounds

Volatile metabolites were identified by comparing the mass spectra with the data system library (MWGC or NIST) and the linear retention index. Each sample was repeated three times, and the data are expressed as the mean ± standard deviation. The concentrations of volatile compounds in tea plants were quantified based on their peak areas and the peak area of the internal standard compound. The bar charts were made by Excel, and the line charts were made by GraphPad Prism 9.0. Analysis of variance and significant difference analysis were performed by SPSS 26.0. Principal component analysis (PCA) of the identified metabolites was performed using the R package (www.r-project.org). Based on the variable importance in project (VIP) score obtained by the OPLS-DA model, metabolites with VIP ≥ 1.0 and fold change (FC) ≥ 1.5 or FC ≤ 0.67 were defined as significantly changed metabolites (SCMs). The calculation method for the odour activity values (OAVs) was the same as that used in a previous study. OAV = C/OT, where C is the concentration of the volatile compound and OT is its odour threshold[22]. Compounds with OAV ≥ 1 were considered potential contributors to the tea aroma profile.

Transcriptome-based analysis of aroma component-related pathways and differentially expressed genes

The transcriptome data was based on contemporaneous data that we previously published[18]. All RNA-seq data are publicly available in the BIG Data Center (https://bigd.big.ac.cn) under project number PRJCA009753. Differential expression analysis with DESeq2 software. Genes with $|\log_2 FC| \geq 1$ and p-value < 0.05 were considered to be differentially expressed genes (DEGs) by the DESeq2 R package[23]. All the genes assembled by the transcriptome were compared with six databases (NR, Swiss-Prot, Pfam, EggNOG, GO, and KEGG) to obtain the functional information of genes, and the annotation of each database was statistically analysed. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using the ClusterProfiler v4.0.0 R package[24] and BLAST was set to e-value ≤ 10^{-5}. For *TPS* gene, the hidden Markov models of PF01397 and PF03936 were downloaded from Pfam (http://pfam.xfam.org/) database, and HMMER software was used to search the *TPS* gene family sequence. The BLASTP of NCBI and Swiss-Prot was used to predict the possible function of *CsTPS* gene, and the threshold was set as *E*-value < 10^{-5} and identity > 90%. For transcription factors (TFs), all the genes were annotated in the Plant Transcription Factor Database (PlantTFDB v5.0) to determine whether they are TFs. TBtools software was used to make a heatmap for visualization of DEGs.

Gene coexpression analysis

Weighted gene coexpression network analysis (WGCNA) was performed using the WGCNA R package. Genes with TPM > 1 and coefficient of variation (cv) > 0.1 were used to construct the coexpression network. After filtering, the abundance of 11,222 genes and 20 metabolites was used to build a signed coexpression network by calculating Pearson correlations. The soft-thresholding power of the correlation network was set to 14,

the minimum module size was equal to 30, and the minimum height for merging modules was set to 0.5. The module networks were visualized using Gephi software.

Quantitative real-time PCR verification

cDNA synthesis and qRT–PCR tests were performed to verify the reliability of the RNA-Seq data according to previous methods[25]. *CsGAPDH* (GE651107) was used as a reference control, and the primers of validated genes were designed using Primer3Plus (www.primer3plus.com). The primer information is listed in Supplemental Table S1. All samples were analysed in three biological replicates. The relative expression level was calculated using the $2^{-\Delta\Delta C_T}$ method[26].

Results

The volatile components of seven tea cultivars

In total, 88 volatiles were identified by GC–MS in the seven tea cultivars (Table 1), including alcohols (22), phenols (3), aldehydes (12), acids (5), terpenoids (13), ketones (7), hydrocarbons (3), heterocyclic compounds (3) and esters (20). Alcohols and esters accounted for 47.72% of the total aroma content, among which geraniol, linalool, methyl salicylate and (*E*)-3-hexen-1-ol acetate had higher contents. There were 19 volatiles with relative contents greater than 100 μg/kg. Geraniol and linalool contain more than 1,000 μg/kg, accounting for 1%–5% of the total content. The total contents of volatiles in the seven tea cultivars were in the following order: JX > FDDB > SCZ > FJSX > LJ43 > TGY > BHZ.

The phenotypes of seven tea cultivars are shown in Fig. 1a. The PCA score plot of the main chemical components indicated that the first two principal components explained 27.5% and 21.3% of the total variance, respectively, and the cumulative variance contribution reached 49.8% (Fig. 1b), which indicated that PC1 and PC2 were selected to analyze the samples with good reliability. The seven tea cultivars showed different distribution characteristics in the PCA score plot: JX, BHZ, and TGY were far away from other cultivars; FDDB and FJSX were relatively close; and LJ43 and SCZ were relatively close.

The comparative analysis identified differences in the relative content of volatiles in the seven tea cultivars (Fig. 1c, d). The volatile aroma substances with the highest contents in TGY were mainly phenols. In particular, eugenol had the highest levels of contents in TGY as compared to other cultivars. Among these compounds, alcohols and esters were present in the greatest numbers, indicating major contributions to aroma. The content of alcohols and esters in JX were higher than in other cultivar aroma types, nerol, geraniol, myrcene, (*E*)-*β*-ocimene, *δ*-cadinene and *d*-limonene had higher concentrations in JX than other cultivars. Compared with other cultivars, these aroma categories varied in FJSX was not abundant. Interestingly, Jasmine lactone had the highest concentrations in FJSX. The representative green tea cultivars showed higher contents of alcohol and aldehyde. In the alcohol group, linalool had the highest concentrations in FDDB than other cultivars, (*E*)-nerolidol and (*Z*)-3-hexenol had the highest concentrations in SCZ than other cultivars. The content of aldehydes in FDDB and SCZ than other cultivars, for example, phenylacetaldehyde had higher concentrations than other cultivars. In the aldehyde group, hexanal and butanal had the highest concentrations in LJ43. It is worth noting that indole had the highest concentrations in BHZ than other cultivars.

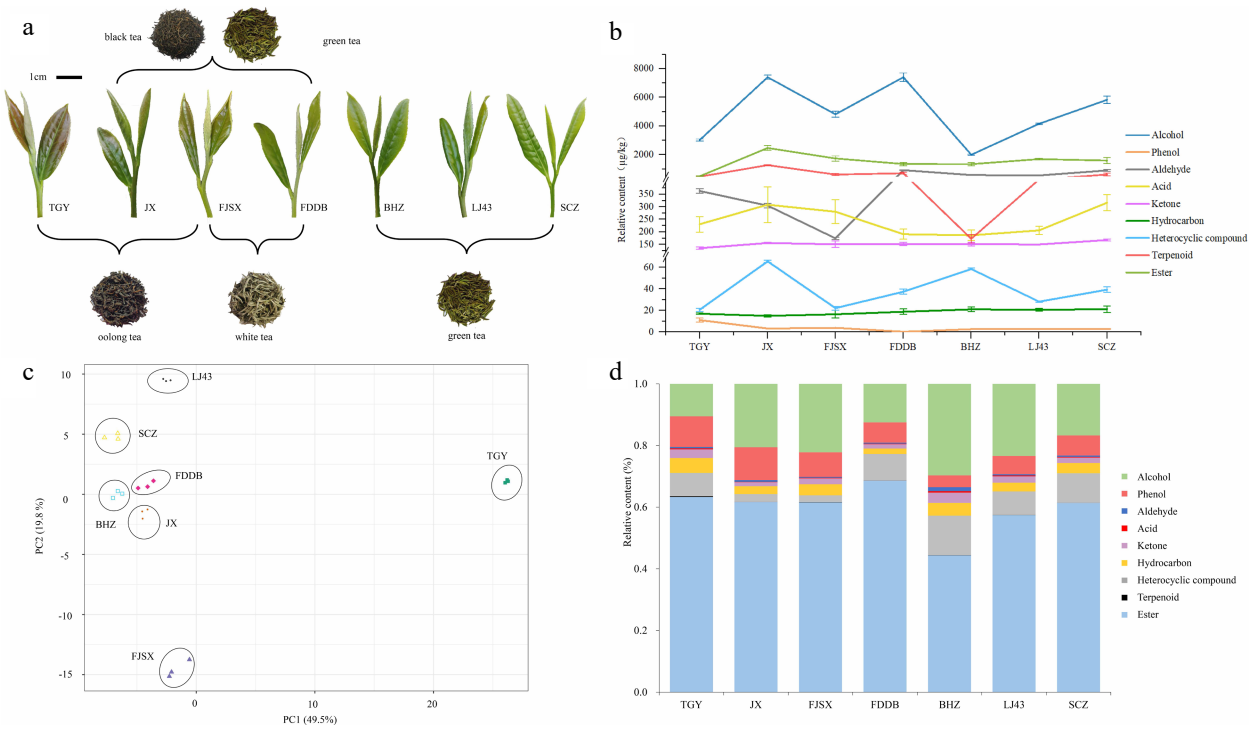

Fig. 1 Multivariate statistical analysis of volatile components of seven tea cultivars. (a) Phenotypes of one bud and two leaves and their suitability. (b) PCA principal component analysis. (c) Types and relative contents of volatile components. (d) Proportions of volatile components.

Table 1. The volatile components of seven tea cultivars.

ID	CAS	RT /min	Compounds	Relative contents (μg/kg)						
				TGY	JX	FJSX	FDDB	BHZ	LJ43	SCZ
1	106-24-1	14.27	Geraniol	1,358.90 ± 49.69^{d}	5103.36 ± 85.9^{a}	3,017.74 ± 80.37^{b}	2,573.91 ± 69.41^{c}	239.72 ± 10.4^{e}	1,321.78 ± 27.49^{d}	2,510.78 ± 62.78^{c}
2	78-70-6	11.46	Linalool	1,012.66 ± 10.81^{d}	1,258.39 ± 74.46^{bc}	1,106.32 ± 71.3^{cd}	2,465.09 ± 167.61^{a}	478.07 ± 44.92^{e}	1,212.96 ± 18.69^{bcd}	1,408.27 ± 132.44^{b}
3	40716-66-3	19.76	(*E*)-Nerolidol	54.73 ± 2.25^{c}	54.71 ± 0.86^{c}	72.67 ± 5.77^{b}	34.06 ± 1.59^{d}	107.39 ± 4.41^{a}	67.08 ± 3.28^{b}	115.07 ± 5.96^{a}
4	106-25-2	13.75	Nerol	22.42 ± 0.62^{e}	72.25 ± 1.44^{a}	49.45 ± 4.07^{cd}	60.73 ± 1.75^{b}	11.29 ± 0.31^{f}	35.87 ± 0.79^{d}	60.29 ± 3.10^{b}
5	928-96-1	6.67	(*Z*)-3-Hexenol	34.90 ± 0.64^{d}	33.82 ± 1.24^{d}	79.71 ± 11.02^{c}	177.94 ± 10.84^{b}	73.79 ± 9.13^{c}	170.01 ± 4.20^{b}	229.24 ± 26.97^{a}
6	100-51-6	12.50	Benzyl alcohol	0.28 ± 0.02^{a}	0.16 ± 0.03^{cd}	0.16 ± 0.03^{bcd}	0.22 ± 0.03^{ab}	0.12 ± 0.01^{d}	0.21 ± 0.00^{bc}	0.17 ± 0.02^{bcd}
7	60-12-8	11.69	Phenylethyl alcohol	95.04 ± 6.81^{e}	114.96 ± 1.21^{e}	15.49 ± 0.09^{f}	739.79 ± 17.52^{a}	414.46 ± 7.66^{c}	338.25 ± 5.13^{d}	689.34 ± 36.16^{b}
8	505-32-8	23.78	Isophytol	10.36 ± 2.57^{b}	9.28 ± 1.52^{b}	12.55 ± 2.90^{b}	7.46 ± 2.43^{b}	24.32 ± 8.12^{a}	8.23 ± 3.84^{b}	14.38 ± 3.96^{ab}
9	150-86-7	24.63	Phytol	11.05 ± 3.74^{ab}	9.64 ± 1.83^{ab}	18.00 ± 6.78^{ab}	7.29 ± 3.37^{b}	30.89 ± 18.38^{a}	6.36 ± 4.45^{b}	15.20 ± 9.07^{ab}
10	29171-23-1	24.77	Dehydroisophytol	2.73 ± 0.71^{b}	1.83 ± 0.39^{b}	3.36 ± 1.03^{ab}	1.71 ± 0.63^{b}	5.53 ± 1.83^{a}	1.73 ± 0.93^{b}	2.86 ± 0.96^{b}
11	143-08-8	12.74	1-Nonanol	10.40 ± 0.33^{c}	8.33 ± 0.59^{cd}	6.59 ± 0.56^{d}	29.29 ± 0.77^{a}	21.15 ± 1.31^{b}	31.58 ± 1.08^{a}	31.27 ± 2.04^{a}
12	111-70-6	9.03	1-Heptanol	6.53 ± 0.27^{d}	5.88 ± 0.27^{d}	3.77 ± 0.51^{d}	19.03 ± 1.98^{a}	11.75 ± 1.10^{bc}	10.68 ± 0.49^{c}	13.75 ± 2.12^{b}
13	10482-56-1	13.24	α-Terpineol	26.66 ± 0.24^{a}	26.81 ± 1.26^{a}	19.35 ± 1.44^{b}	24.79 ± 1.22^{a}	6.21 ± 0.53^{e}	10.88 ± 0.71^{d}	14.51 ± 1.87^{c}
14	10340-23-5	12.43	(*Z*)-3-Nonen-1-ol	0.97 ± 0.13^{d}	1.44 ± 0.12^{bc}	1.22 ± 0.16^{cd}	2.26 ± 0.09^{a}	1.27 ± 0.05^{cd}	2.34 ± 0.17^{a}	1.75 ± 0.21^{b}
15	2425-77-6	22.13	2-Hexyl-1-decanol	2.22 ± 0.05^{a}	1.22 ± 0.11^{d}	1.85 ± 0.35^{bc}	1.63 ± 0.08^{c}	2.05 ± 0.07^{ab}	1.64 ± 0.02^{c}	1.91 ± 0.02^{bc}
16	481-34-5	21.18	α-Cadinol	3.08 ± 0.08^{d}	11.32 ± 0.47^{a}	4.30 ± 0.28^{c}	1.89 ± 0.13^{e}	5.97 ± 0.16^{b}	2.18 ± 0.09^{e}	1.67 ± 0.14^{e}
17	5944-20-7	13.83	Isogeraniol	5.75 ± 0.21^{e}	7.19 ± 0.26^{e}	23.45 ± 2.80^{b}	16.03 ± 1.19^{c}	11.98 ± 0.87^{d}	30.30 ± 0.99^{a}	14.74 ± 1.49^{cd}
18	498-00-0	14.09	Vanillyl alcohol	246.47 ± 2.27^{b}	242.40 ± 4.17^{b}	251.82 ± 8.47^{ab}	257.91 ± 7.73^{ab}	243.94 ± 6.00^{b}	265.31 ± 4.52^{a}	244.78 ± 11.91^{b}
19	15051-81-7	19.43	epi-g-Eudesmol	1.09 ± 0.10^{d}	0.90 ± 0.09^{de}	1.13 ± 0.10^{cd}	0.66 ± 0.06^{e}	1.76 ± 0.14^{ab}	1.43 ± 0.13^{bc}	1.79 ± 0.29^{a}
20	18675-33-7	15.29	(+)-Neodihydrocarveol	2.54 ± 0.12^{a}	1.61 ± 0.06^{b}	0.79 ± 0.04^{d}	0.49 ± 0.02^{e}	1.02 ± 0.11^{c}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}
21	5989-33-3	10.90	(*Z*)-Linalool oxide (furanoid)	26.59 ± 1.08^{e}	97.88 ± 5.72^{d}	43.45 ± 4.25^{e}	255.98 ± 13.68^{a}	84.79 ± 6.13^{d}	202.69 ± 13.96^{b}	130.23 ± 11.66^{c}
22	34995-77-2	11.20	(*E*)-Linalool oxide (furanoid)	81.38 ± 4.35^{e}	314.5 ± 19.75^{c}	81.20 ± 5.77^{e}	666.69 ± 29.84^{a}	189.5 ± 12.55^{d}	408.19 ± 31.09^{b}	299.17 ± 27.17^{c}
23	39028-58-5	12.84	(*E*)-Linalool oxide (pyranoid)	15.78 ± 0.36^{d}	40.13 ± 2.58^{b}	12.91 ± 0.95^{d}	69.64 ± 0.99^{a}	39.31 ± 2.55^{b}	34.67 ± 1.30^{c}	36.72 ± 1.82^{bc}
		Alcohols (23)		3,032.53 ± 74.37^{e}	7,418.01 ± 149.51^{a}	4,827.26 ± 203.54^{c}	7,414.48 ± 291.74^{a}	2,006.27 ± 48.02^{f}	4,164.36 ± 50.25^{d}	5,837.9 ± 253.16^{b}
24	97-53-0	16.12	Eugenol	3.17 ± 0.58^{a}	0.73 ± 0.01^{c}	1.82 ± 0.44^{b}	1.67 ± 0.13^{b}	0.95 ± 0.05^{c}	1.94 ± 0.04^{b}	2.01 ± 0.14^{b}
25	3228-02-2	15.13	3-Methyl-4-isopropylphenol	5.46 ± 1.38^{a}	1.03 ± 0.11^{c}	3.14 ± 0.68^{b}	1.82 ± 0.07^{bc}	0.76 ± 0.12^{c}	0.99 ± 0.15^{c}	0.87 ± 0.18^{c}
		Phenols (2)		8.63 ± 1.85^{a}	1.76 ± 0.12^{c}	4.96 ± 1.11^{b}	3.49 ± 0.09b^{c}	1.71 ± 0.14^{c}	2.93 ± 0.14^{c}	2.88 ± 0.29^{c}
26	141-27-5	14.55	Neral	25.34 ± 1.36^{c}	93.8 ± 3.25^{a}	60.48 ± 7.92^{b}	65.70 ± 7.92^{b}	5.30 ± 0.95^{d}	37.78 ± 1.06^{c}	59.69 ± 6.84^{b}
27	121-33-5	13.69	Vanillin	3.25 ± 0.28^{d}	0.74 ± 0.11^{e}	10.16 ± 2.30^{a}	5.28 ± 0.45^{bcd}	3.90 ± 0.21^{cd}	6.27 ± 0.86^{b}	6.15 ± 0.11^{bc}
28	34246-54-3	12.67	3-Ethylbenzaldehyde	0.65 ± 0.01^{cd}	0.62 ± 0.02^{de}	0.42 ± 0.05^{e}	0.70 ± 0.07^{cd}	0.86 ± 0.12^{bc}	1.01 ± 0.02^{ab}	1.10 ± 0.11^{a}
29	6728-26-3	14.46	(*E*)-2-Hexenal	3.25 ± 0.48^{cd}	5.99 ± 0.58^{a}	4.38 ± 0.19^{b}	4.10 ± 0.31^{bc}	1.93 ± 0.12^{e}	3.16 ± 0.37^{d}	4.01 ± 0.36^{bcd}
30	100-52-7	8.87	Benzaldehyde	9.82 ± 0.70^{ab}	8.20 ± 0.37^{bc}	6.17 ± 1.08^{c}	8.72 ± 1.21^{bc}	9.00 ± 1.21^{bc}	11.07 ± 0.73^{ab}	12.86 ± 2.30^{a}
31	122-78-1	11.69	Phenylacetaldehyde	99.41 ± 7.28^{e}	121.13 ± 1.40^{e}	18.87 ± 0.40^{f}	746.69 ± 12.32^{a}	435.00 ± 6.55^{c}	356.40 ± 14.09^{d}	700.08 ± 30.99^{b}
32	112-31-2	13.40	Decanal	0.59 ± 0.07^{b}	0.64 ± 0.15^{b}	0.69 ± 0.07^{b}	0.82 ± 0.17^{b}	0.86 ± 0.14^{b}	1.19 ± 0.12^{a}	0.81 ± 0.04^{b}
33	111-71-7	7.67	Heptanal	143.12 ± 4.70^{a}	2.18 ± 0.24^{b}	2.16 ± 0.41^{b}	1.60 ± 0.24^{b}	1.15 ± 0.19^{b}	1.09 ± 0.11^{b}	0.90 ± 0.11^{b}
34	66-25-1	5.45	Hexanal	7.83 ± 1.19^{d}	15.44 ± 3.43^{bcd}	12.48 ± 1.33^{cd}	18.63 ± 2.71^{bc}	27.58 ± 6.03^{a}	29.47 ± 3.54^{a}	22.46 ± 1.58^{ab}

(to be continued)

Table 1. (continued)

ID	CAS	RT /min	Compounds	Relative contents (μg/kg)						
				TGY	JX	FJSX	FDDB	BHZ	LJ43	SCZ
35	123-72-8	5.45	Butanal	6.59 ± 1.30^{d}	14.07 ± 3.66^{bcd}	11.59 ± 1.53^{cd}	16.53 ± 1.67^{bc}	26.64 ± 5.32^{a}	28.38 ± 3.38^{a}	21.63 ± 2.66^{ab}
36	110-62-3	4.83	Valeraldehyde	14.41 ± 1.13^{de}	20.84 ± 1.88^{cd}	13.41 ± 2.76^{e}	24.21 ± 1.66^{c}	33.52 ± 2.5^{ab}	31.13 ± 1.22^{b}	40.15 ± 5.75^{a}
37	590-86-3	5.03	Isovaleraldehyde	48.88 ± 1.45^{a}	21.11 ± 1.69^{b}	33.19 ± 5.46^{ab}	37.00 ± 5.6^{ab}	34.38 ± 8.89^{ab}	45.65 ± 1.09^{a}	39.15 ± 11.95^{a}
			Aldehydes (12)	363.15 ± 9.42^{c}	304.75 ± 8.93^{c}	174.01 ± 3.96^{d}	929.96 ± 12.47^{a}	580.11 ± 28.65^{b}	552.59 ± 8.81^{b}	908.99 ± 56.08^{a}
38	459-80-3	16.02	Geranic acid	37.94 ± 9.28^{bc}	115.49 ± 33.01^{a}	62.74 ± 23.82^{b}	20.81 ± 3.51^{bc}	8.82 ± 2.85^{c}	19.69 ± 2.49^{c}	131.88 ± 17.95^{a}
39	112-05-0	14.48	Nonanoic acid	2.58 ± 0.66^{c}	4.45 ± 1.22^{ab}	4.80 ± 0.65^{ab}	4.39 ± 0.67^{ab}	5.51 ± 0.47^{a}	3.51 ± 0.44^{bc}	4.64 ± 0.40^{ab}
40	111-14-8	12.69	Heptanoic acid	119.89 ± 0.98^{a}	101.69 ± 5.30^{cd}	115.81 ± 5.88^{ab}	107.27 ± 3.55^{bcd}	98.31 ± 5.48^{d}	111.4 ± 2.49^{abc}	104.84 ± 6.82^{bcd}
41	109-52-4	14.48	Pentanoic acid	4.92 ± 0.63^{b}	7.14 ± 1.45^{ab}	8.07 ± 1.02^{a}	8.00 ± 1.74^{a}	8.12 ± 1.10^{a}	6.55 ± 0.54^{ab}	7.57 ± 0.45^{ab}
42	79-09-4	12.05	Propanoic acid	65.13 ± 26.72^{a}	79.82 ± 37.7^{a}	89.19 ± 28.57^{a}	50.37 ± 21.63^{a}	65.56 ± 23.49^{a}	64.99 ± 18.33^{a}	67.71 ± 24.17^{a}
			Acids (5)	230.47 ± 31.08^{ab}	308.59 ± 71.53^{a}	280.6 ± 47.71^{ab}	190.84 ± 20.11^{b}	186.32 ± 20.63^{b}	206.13 ± 16.59^{b}	316.64 ± 32.43^{a}
43	127-41-3	9.92	α-Ionone	12.05 ± 0.88^{cd}	23.28 ± 0.54^{a}	14.7 ± 1.06^{bc}	17.81 ± 1.66^{b}	2.60 ± 0.43^{e}	10.34 ± 1.64^{d}	14.11 ± 2.26^{c}
44	79-77-6	18.39	β-Ionone	87.2 ± 1.64^{c}	90.97 ± 0.81^{c}	92.93 ± 7.34^{c}	103.48 ± 5.56^{b}	111.25 ± 4.28^{ab}	108.43 ± 2.05^{ab}	114.18 ± 2.52^{a}
45	689-67-8	17.79	Geranylacetone	10.67 ± 0.30^{bc}	12.01 ± 0.49^{a}	11.15 ± 1.07^{abc}	10.07 ± 0.57^{c}	11.79 ± 0.19^{ab}	10.58 ± 0.05^{bc}	11.43 ± 0.29^{ab}
46	502-69-2	23.10	Fitone	6.01 ± 0.21^{bc}	4.82 ± 0.37^{de}	6.70 ± 0.61^{b}	7.05 ± 0.24^{b}	9.15 ± 0.70^{a}	4.17 ± 0.21^{e}	5.51 ± 0.61^{cd}
47	471-15-8	11.62	3-Thujone	11.55 ± 3.73^{a}	1.49 ± 1.68^{b}	6.56 ± 2.92^{ab}	0.84 ± 0.34^{b}	4.92 ± 4.11^{b}	2.18 ± 1.52^{b}	0.65 ± 0.44^{b}
48	488-10-8	16.84	Jasmone	6.64 ± 0.35^{d}	4.79 ± 0.61^{e}	4.24 ± 0.28^{e}	5.18 ± 0.26^{e}	9.53 ± 0.41^{b}	7.84 ± 0.53^{c}	12.43 ± 0.39^{a}
49	23726-93-4	16.53	β-Damascenone	1.01 ± 0.15^{e}	18.05 ± 0.75^{a}	14.26 ± 1.60^{b}	7.58 ± 0.13^{cd}	1.82 ± 0.24^{e}	6.11 ± 0.14^{d}	9.02 ± 0.31^{c}
			Ketones (7)	135.12 ± 4.62^{c}	155.41 ± 3.29^{ab}	150.53 ± 12.41^{bc}	152.02 ± 6.26^{ab}	151.07 ± 7.13^{bc}	149.65 ± 0.72^{bc}	167.33 ± 4.26^{a}
50	544-76-3	20.41	Hexadecane	3.25 ± 0.34^{a}	4.04 ± 0.38^{a}	3.41 ± 0.90^{a}	4.73 ± 1.33^{a}	4.82 ± 0.68^{a}	4.11 ± 0.54^{a}	4.26 ± 0.56^{a}
51	629-59-4	16.97	Tetradecane	7.30 ± 00.43^{ab}	5.87 ± 0.54^{b}	6.21 ± 0.46^{b}	7.82 ± 1.40^{ab}	7.82 ± 0.95^{ab}	8.41 ± 0.70^{a}	7.27 ± 0.84^{ab}
52	3891-99-4	18.03	2,6,10-Trimethyltridecane	6.51 ± 0.12^{bc}	5.13 ± 0.72^{c}	6.91 ± 2.37^{abc}	6.36 ± 0.50^{bc}	8.50 ± 0.66^{ab}	8.21 ± 0.16^{ab}	9.67 ± 1.60^{a}
			Hydrocarbons (3)	17.07 ± 0.59^{ab}	15.04 ± 1.09^{b}	16.54 ± 3.61^{ab}	18.9 ± 2.56^{ab}	21.14 ± 2.03^{a}	20.72 ± 1.21^{ab}	21.2 ± 2.99^{a}
53	120-72-9	15.05	Indole	0.00 ± 0.00^{f}	36.36 ± 1.23^{b}	0.00 ± 0.00^{f}	11.8 ± 0.78^{d}	42.41 ± 1.36^{a}	7.78 ± 0.62^{e}	24.75 ± 2.46^{c}
54	91-64-5	17.72	Coumarin	16.81 ± 1.45^{a}	18.38 ± 0.34^{a}	17.67 ± 1.54^{a}	11.5 ± 0.53^{b}	10.28 ± 0.58^{b}	11.43 ± 0.48^{b}	10.50 ± 0.61^{b}
55	36431-72-8	15.22	Theaspirane	3.92 ± 0.05^{c}	10.98 ± 0.92^{b}	4.57 ± 0.49^{c}	14.31 ± 1.95^{a}	5.88 ± 0.24^{c}	9.10 ± 0.19^{b}	4.23 ± 0.44^{c}
			Heterocyclic compounds (3)	20.73 ± 1.41^{f}	65.72 ± 0.98^{a}	22.24 ± 1.79^{f}	37.61 ± 2.32^{d}	58.57 ± 0.92^{b}	28.31 ± 0.70^{e}	39.48 ± 2.71^{c}
56	6753-98-6	18.06	α-Humulene	6.58 ± 0.40^{a}	4.63 ± 0.11^{b}	1.16 ± 0.04^{e}	1.19 ± 0.07^{e}	3.06 ± 0.21^{d}	0.86 ± 0.04^{e}	3.68 ± 0.42^{c}
57	87-44-5	17.43	β-Caryophyllene	14.30 ± 0.46^{a}	14.97 ± 0.30^{a}	2.04 ± 0.28^{d}	4.54 ± 0.73^{c}	8.49 ± 0.48^{b}	0.50 ± 0.19^{e}	1.62 ± 0.26^{d}
58	502-61-4	18.79	α-Farnesene	28.28 ± 0.62^{c}	34.79 ± 0.54^{b}	12.15 ± 1.56^{e}	14.98 ± 1.35^{e}	43.86 ± 1.15^{a}	12.50 ± 0.69^{e}	24.02 ± 1.91^{d}
59	123-35-3	9.39	Myrcene	241.44 ± 15.17^{c}	669.11 ± 7.54^{a}	374.82 ± 41.18^{b}	426.14 ± 44.52^{b}	44.16 ± 6.77^{d}	230.59 ± 33.5^{c}	363.94 ± 50.74^{b}
60	3779-61-1	10.44	(*E*)-β-Ocimene	107.36 ± 5.92^{c}	263.91 ± 3.58^{a}	147.98 ± 15.26^{b}	174.1 ± 17.53^{b}	23.92 ± 4.42^{d}	99.25 ± 13.74^{c}	155.4 ± 25.28^{b}
61	7216-56-0	11.95	Allo-ocimene	14.16 ± 1.00^{c}	37.51 ± 0.65^{a}	21.17 ± 2.50^{b}	23.85 ± 2.91^{b}	2.43 ± 0.34^{d}	12.92 ± 2.22^{c}	20.17 ± 2.98^{b}
62	99-85-4	10.68	γ-Terpinene	11.22 ± 1.12^{b}	17.20 ± 0.51^{a}	12.16 ± 0.68^{b}	13.79 ± 1.33^{b}	2.42 ± 0.36^{d}	8.25 ± 1.30^{c}	11.29 ± 2.04^{b}
63	5208-59-3	16.78	β-Bourbonene	4.85 ± 0.11^{d}	4.44 ± 0.06^{d}	12.08 ± 2.07^{b}	8.51 ± 1.21^{c}	17.81 ± 1.93^{a}	5.83 ± 0.16^{cd}	3.54 ± 0.47^{d}
64	29050-33-7	9.91	4-Carene	12.04 ± 0.92^{cd}	23.33 ± 0.51^{a}	14.74 ± 1.06^{bc}	17.81 ± 1.66^{b}	2.66 ± 0.40^{e}	10.34 ± 1.64^{d}	14.13 ± 2.31^{c}
65	483-76-1	19.10	δ-Cadinene	12.99 ± 0.13^{c}	93.21 ± 2.13^{a}	7.78 ± 0.97^{de}	6.12 ± 0.41^{e}	9.49 ± 0.47^{d}	17.99 ± 0.70^{b}	6.78 ± 0.55^{e}
66	21391-99-1	19.51	α-Calacorene	10.27 ± 0.10^{b}	42.73 ± 0.64^{a}	6.58 ± 0.53^{d}	4.65 ± 0.21^{e}	8.12 ± 0.70^{c}	10.1 ± 0.44^{b}	5.53 ± 0.59^{de}
67	483-77-2	19.16	Calamenene	6.57 ± 0.15^{c}	61.3 ± 1.03^{a}	4.16 ± 0.49^{de}	4.66 ± 0.12^{de}	5.46 ± 0.70^{cd}	16.02 ± 0.98^{b}	3.38 ± 0.62^{e}
68	5989-27-5	12.19	*d*-Limonene	5.38 ± 0.38^{cd}	14.19 ± 0.42^{a}	8.26 ± 0.99^{b}	8.95 ± 1.46^{b}	0.94 ± 0.23^{e}	4.99 ± 0.79^{d}	7.49 ± 1.14^{bc}

(to be continued)

Table 1. (continued)

ID	CAS	RT /min	Compounds	Relative contents (μg/kg)						
				TGY	JX	FJSX	FDDB	BHZ	LJ43	SCZ
		Terpenoids (13)		475.44 ± 24.58^{c}	1281.32 ± 15.03^{a}	625.07 ± 57.13^{b}	709.29 ± 67.59^{b}	172.83 ± 17.3^{d}	430.14 ± 56.04^{c}	620.98 ± 89.21^{b}
69	3681-82-1	9.64	(*E*)-3-Hexen-1-ol acetate	125.08 ± 1.45^{d}	223.82 ± 15.90^{cd}	1030.93 ± 119^{a}	690.15 ± 92.67^{b}	398.2 ± 79.2^{c}	981.62 ± 42.12^{a}	871.21 ± 172.5^{ab}
70	61444-38-0	16.64	(Z)-3-Hexenyl (*Z*)-3-hexenoate	10.90 ± 0.08^{d}	10.16 ± 0.50^{d}	46.93 ± 2.48^{b}	48.58 ± 3.24^{b}	7.03 ± 0.63^{d}	23.17 ± 1.03^{c}	54.08 ± 3.55^{a}
71	31501-11-8	16.57	(*E*)-hex-3-enyl hexanoate	5.27 ± 0.10^{e}	23.15 ± 0.86^{d}	76.15 ± 6.66^{a}	26.00 ± 2.08^{d}	22.04 ± 1.86^{d}	43.61 ± 1.26^{b}	36.01 ± 2.91^{c}
72	2497-18-9	9.82	(*E*)-2-Hexenyl acetate	0.00 ± 0.00^{c}	1.48 ± 0.15^{bc}	8.98 ± 1.42^{a}	2.34 ± 0.40^{bc}	7.07 ± 1.83^{a}	0.00 ± 0.00^{c}	3.23 ± 0.91^{b}
73	65405-77-8	22.84	(*Z*)-3-Hexenyl salicylate	0.12 ± 0.01^{d}	4.60 ± 0.07^{a}	4.55 ± 0.53^{a}	2.40 ± 0.32^{c}	4.28 ± 0.37^{b}	3.78 ± 0.16^{b}	2.22 ± 0.21^{d}
74	41519-23-7	12.99	(*Z*)-3-Hexenyl isobutyrate	9.20 ± 0.41^{d}	20.40 ± 1.44^{c}	83.98 ± 8.54^{a}	12.03 ± 1.63^{cd}	12.81 ± 1.82^{cd}	39.75 ± 1.61^{b}	34.51 ± 4.53^{b}
75	53398-85-9	14.82	(*Z*)-3-Hexenyl 2-methylbutyrate	0.00 ± 0.00^{e}	0.22 ± 0.02^{d}	1.23 ± 0.15^{a}	0.21 ± 0.02^{d}	0.28 ± 0.02^{d}	0.87 ± 0.06^{b}	0.53 ± 0.08^{c}
76	35852-46-1	13.90	(*Z*)-3-Hexenyl valerate	0.23 ± 0.05^{e}	0.61 ± 0.10^{e}	5.30 ± 0.89^{a}	1.89 ± 0.32^{cd}	3.32 ± 0.51^{b}	2.15 ± 0.05^{c}	1.05 ± 0.13^{de}
77	1189-09-9	15.51	Methyl geranate	3.73 ± 0.18^{de}	28.76 ± 1.08^{a}	23.44 ± 2.68^{b}	5.40 ± 0.07^{d}	3.02 ± 0.09^{de}	1.48 ± 0.24^{e}	10.69 ± 0.77^{c}
78	150-84-5	23.07	Citronellyl acetate	26.23 ± 4.83^{bc}	22.86 ± 3.66^{bc}	24.37 ± 1.79^{bc}	15.38 ± 4.07^{c}	50.53 ± 12.99^{a}	18.84 ± 7.95^{bc}	32.85 ± 6.84^{b}
79	2051-49-2	16.72	Hexyl hexanoate	0.00 ± 0.00^{e}	2.77 ± 0.15^{c}	9.44 ± 1.23^{a}	1.56 ± 0.19^{cd}	4.97 ± 0.39^{b}	0.97 ± 0.05^{de}	1.18 ± 0.08^{de}
80	142-92-7	9.78	Hexyl acetate	0.00 ± 0.00^{d}	1.57 ± 0.27^{cd}	8.85 ± 1.48^{a}	2.79 ± 0.49^{bc}	4.39 ± 1.06^{b}	2.59 ± 0.17^{bc}	1.92 ± 0.54^{c}
81	120-51-4	22.56	Benzyl benzoate	2.17 ± 0.12^{cd}	4.02 ± 0.53^{a}	2.95 ± 0.53^{bc}	3.00 ± 0.45^{bc}	3.85 ± 0.42^{b}	1.96 ± 0.18^{d}	2.52 ± 0.27^{cd}
82	110-27-0	22.96	Isopropyl myristate	0.06 ± 0.01^{e}	2.55 ± 0.18^{a}	1.35 ± 0.12^{bc}	1.34 ± 0.07^{bc}	1.14 ± 0.07^{cd}	1.03 ± 0.03^{d}	1.44 ± 0.06^{b}
83	606-45-1	15.77	Methyl 2-methoxybenzoate	7.30 ± 0.20^{a}	0.56 ± 0.03^{e}	3.00 ± 0.26^{b}	2.02 ± 0.08^{c}	0.42 ± 0.03^{e}	1.01 ± 0.02^{d}	0.97 ± 0.11^{d}
84	102-16-9	14.94	Benzyl phenylacetate	0.97 ± 0.07^{b}	1.64 ± 0.04^{a}	1.07 ± 0.19^{b}	0.73 ± 0.07^{c}	0.38 ± 0.02^{d}	0.49 ± 0.02^{d}	0.74 ± 0.05^{c}
85	7011-83-8	16.72	Dihydrojasmone lactone	0.00 ± 0.00^{e}	3.14 ± 0.11^{c}	11.60 ± 1.36^{a}	1.65 ± 0.18^{d}	5.14 ± 0.36^{b}	1.36 ± 0.02^{d}	1.52 ± 0.11^{d}
86	25524-95-2	16.57	Jasmine lactone	5.34 ± 0.11^{e}	23.23 ± 0.92^{d}	76.66 ± 6.57^{a}	26.21 ± 1.94^{d}	22.16 ± 1.84^{d}	43.74 ± 1.33^{b}	36.1 ± 2.91^{c}
87	1211-29-6	23.24	Methyl jasmonate	12.81 ± 2.06^{b}	13.26 ± 2.07^{b}	12.50 ± 1.11^{b}	8.73 ± 1.89^{b}	27.01 ± 4.9^{a}	10.85 ± 4.47^{b}	15.19 ± 1.67^{b}
88	119-36-8	13.19	Methyl salicylate	294.81 ± 22.12^{d}	2,085.7 ± 131.83^{a}	307.14 ± 36.23^{d}	498.63 ± 15.53^{c}	764.22 ± 42.52^{b}	516.16 ± 30.26^{c}	483.65 ± 46.37^{c}
		Esters (20)		504.25 ± 27.11^{d}	2,474.51 ± 157.38^{a}	1,740.43 ± 183.45^{b}	1,351.05 ± 114.32^{c}	1,342.27 ± 103.23^{c}	1,695.42 ± 61.37^{b}	1,591.61 ± 204.25^{bc}

All data are shown as the mean ± standard deviation (SD). Significant differences among various groups are represented by different letters ($p < 0.05$).. RT: retention time.

Analysis of differential volatile components of seven tea cultivars

A total of 54 significantly changed metabolites (SCMs) were identified in seven tea cultivars (Fig. 2). Specifically, the study found that the proportion of up-regulated terpenoids was highest in JX, while the proportion of down-regulated terpenoids was highest in BHZ. Among the esters, the proportion of up-regulated compounds was highest in FJSX, while the proportion of down-regulated compounds was highest in TGY. Additionally, the content of three phenolic compounds was significantly up-regulated in TGY, and the content of alcohol compounds was significantly up-regulated in FDDB.

Furthermore, the study conducted a more detailed analysis of the compounds with significantly increased content in each tea variety. In TGY and JX, the content of β-caryophyllene and α-caryophyllene was significantly higher than in other cultivars. The highest proportion of eight ester compounds was found in FJSX. Phenylethyl alcohol and phenylacetaldehyde levels showed significant variation in seven cultivars, with the highest levels found in FDDB. In SCZ, LJ43, and BHZ, the content of (*Z*)-3-hexenol was significantly up-regulated. In SCZ and BHZ, the levels of (*E*)-nerolidol and jasmone were significantly up-regulated. Finally, the content of indole was significantly accumulated in JX and BHZ, while the levels of α-farnesene and methyl jasmonate were significantly higher in BHZ than in other cultivars.

OAV value of differential volatile components in seven tea cultivars

The odor activity values (OAVs) of the identified volatiles are shown in Table 2. A total of 26 volatiles were determined to have OAV > 1 in tea samples, of which two volatiles had OAVs ≥ 1000. The OAVs of β-damascenone (OAV: 503.45-9026.77), β-ionone (OAV: 1245.71-1631.16), geraniol (OAV: 31.96-680.45), linalool (OAV: 79.68-410.85) and phenylacetaldehyde (OAV: 4.74-186.67) were higher than those of other compounds, indicating that they played significant roles in the aroma of the seven tea cultivars.

There were 21 volatiles with 1 ≤ OAV ≤ 100 in tea samples, of which eight volatiles (phenylethyl alcohol, heptanal, decanal, isovaleraldehyde, α-ionone, myrcene, (*E*)-3-hexen-1-ol acetate, methyl salicylate) had OAVs ≥ 10. Importantly, we found that heptanal was abundant in TGY with OAV > 1. In total, 13 volatile compounds had OAVs > 1 in tea samples, of which (*Z*)-3-hexenol (SCZ, FDDB, LJ43), (*Z*)-linalool oxide (FDDB, LJ43), and jasmone (SCZ, BHZ, LJ43) had OAVs ≥ 1. In addition, indole was determined to have OAVs > 1 in BHZ.

Differentially expressed genes (DEGs) related to three volatile synthesis pathways

Terpenoids, unsaturated aliphatic compounds and aromatic compounds are the main aroma components of tea. To explain the mechanism of up- or downregulation of SCMs in seven tea cultivars at the molecular level, we identified DEGs in relevant biosynthetic pathways.

For the terpenoid volatiles synthesis pathway, a total of 20 DEGs and 40 *TPS* genes were involved in Mevalonate pathway (MVA) and 2-methyl-D-erythritol-4-phosphate pathway (MEP), and six DEGs were involved in the carotenoid metabolic pathway (Fig. 3). Among them, *HMGR* (CsTGY05G0000313) and *HMGS* (CsTGY01G0002862) were significantly upregulated 5.55- and 5.46-fold in JX and SCZ, respectively. *CsHMGR* (3-hydroxy-3-

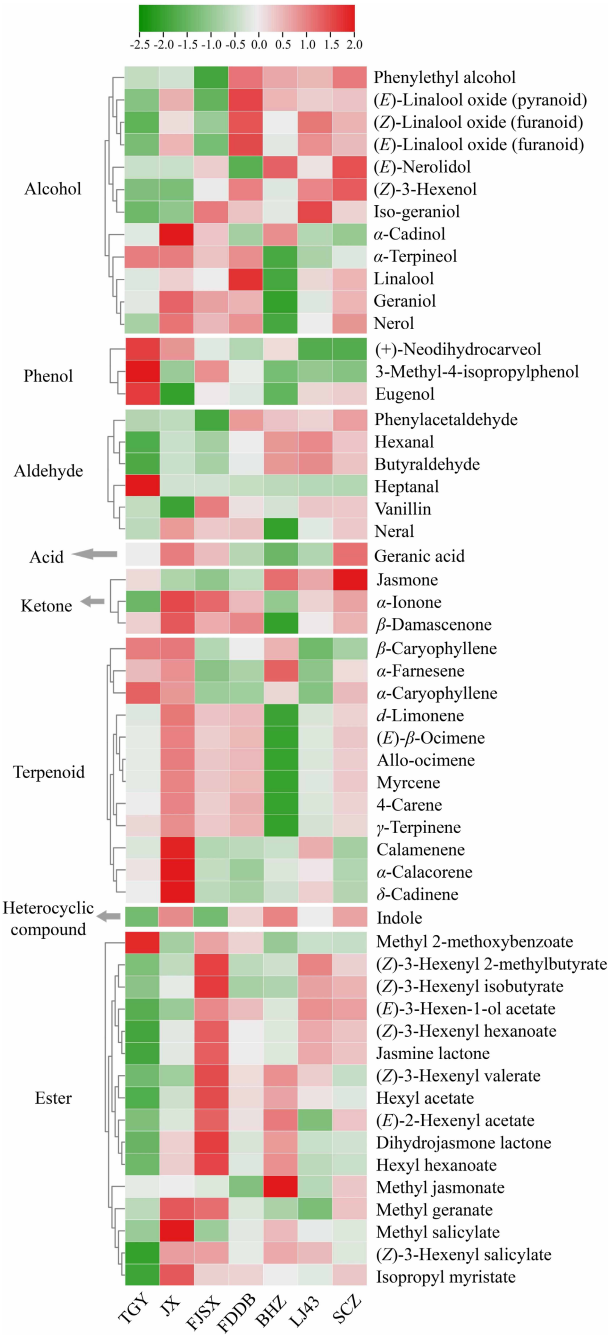

Fig. 2 Analysis of differential volatile components of seven tea cultivars.

methylglutaryl coenzyme A reductase) and *HMGS* (3-hydroxy-3-methylglutaryl-CoA synthase) are key rate-limiting enzymes in the MVA of the terpene derivative pathway[31]. *LIS/NES* (CsTGY08G0001704) and *NES/GIS* (CsTGY08G0001826) were significantly upregulated 3.09- and 4.7-fold in FDDB and BHZ, respectively. LIS/NES (linalool/nerolidol synthase) and *NES/GIS* (nerolidol/geranyl linalool synthase) are the key enzyme in the biosynthesis of linalool and nerolidol. (*E*)-nerolidol has clean and floral aromas, linalool has the fragrance of rose and fruit, and its oxidized products have woody, floral, and camphor odours, which are the main aroma components of tea[3].

For the α-linolenic acid metabolism pathway, 29 DEGs were involved in the α-linolenic acid metabolism pathway (Fig. 4),

Table 2. OAV values of differential volatile components in seven tea cultivars.

Volatile components	Aroma characteristics	Aroma thresholds[3,27–30] (μg/kg)	Relative OAV value						
			TGY	JX	FJSX	FDDB	BHZ	LJ43	SCZ
Geraniol	Rosy, sweet	7.5	181.19	680.45	402.37	343.19	31.96	176.24	334.77
Linalool	Floral, fruity	6	168.78	209.73	184.39	410.85	79.68	202.16	234.71
(*E*)-Nerolidol	Floral, citrus	15	3.65	3.65	4.84	2.27	7.16	4.47	7.67
Nerol	Rosy, orange	49	0.46	1.47	1.01	1.24	0.23	0.73	1.23
(*Z*)-3-Hexenol	Fresh, grassy	110	0.32	0.31	0.72	1.62	0.67	1.55	2.08
Phenylethyl alcohol	Floral, rosy	45	2.11	2.55	0.34	16.44	9.21	7.52	15.32
(*Z*)-Linalool oxide (furanoid)	Sweet, floral	190	0.14	0.52	0.23	1.35	0.45	1.07	0.69
(*E*)-Linalool oxide (furanoid)	Sweet, floral	190	0.43	1.66	0.43	3.51	1.00	2.15	1.57
Neral	Sweet, fruity	53	0.48	1.77	1.14	1.24	0.10	0.71	1.13
Decanal	Sweet, citrus	0.1	5.90	6.38	6.93	8.20	8.60	11.89	8.13
Heptanal	Fatty, citrus	10	14.31	0.22	0.22	0.16	0.12	0.11	0.09
Benzaldehyde	Almond, nutty	3	3.27	2.73	2.06	2.91	3.00	3.69	4.29
Phenylacetaldehyde	Woody, sweet	4	24.85	30.28	4.72	186.67	108.75	89.10	175.02
Hexanal	Fresh, fruity, fatty	4.5	1.74	3.43	2.77	4.14	6.13	6.55	4.99
Valeraldehyde	Almond, malty	12	1.20	1.74	1.12	2.02	2.79	2.59	3.35
Isovaleraldehyde	Fruity	4	12.22	5.28	8.30	9.25	8.59	11.41	9.79
α-Ionone	Violet, woody	0.4	30.13	58.20	36.75	44.53	6.50	25.85	35.27
β-Ionone	Violet, floral	0.07	1,245.71	1,299.60	1,327.56	1,478.32	1,589.21	1,549.00	1,631.16
Jasmone	Jasmine	7	0.95	0.68	0.61	0.74	1.36	1.12	1.78
β-Damascenone	Rosy	0.002	503.45	9,026.77	7,127.53	3,791.88	911.92	3,053.12	4,509.23
Indole	Floral	40	–	0.91	–	0.29	1.06	0.19	0.62
(*E*)-β-Ocimene	Floral, grassy	34	3.16	7.76	4.35	5.12	0.70	2.92	4.57
Myrcene	Fruity, balsamic	15	16.10	44.61	24.99	28.41	2.94	15.37	24.26
(*E*)-3-Hexen-1-ol acetate	Fruity, grassy	31	4.03	7.22	33.26	22.26	12.85	31.67	28.10
Methyl salicylate	Herbal, minty	40	7.37	52.14	7.68	12.47	19.11	12.90	12.09
Methyl jasmonate	Jasmine	3	4.27	4.42	4.17	2.91	9.00	3.62	5.06

'–' indicates that the OAV value cannot be calculated.

mainly including acyl-CoA oxidase (*ACX*), OPC-8:0 CoA ligase (*OPCL*), allene oxide cyclase (*AOC*), lipoxygenase (*LOX*), and alcohol dehydrogenase (*ADH*). Among these genes, *ACX* (CsTGY04G0002749) was significantly upregulated 3.76-fold in FDDB. *OPCL* (CsTGY07G0001858) was only expressed in SCZ. *AOC* (CsTGY01G0003195) was significantly downregulated 2.35-fold in FJSX, and *ADH* (CsTGY09G0001886) showed higher expression in TGY and FJSX than in the other tea cultivars. ADH (alcohol dehydrogenase) is a key enzyme responsible for the biosynthesis of the key volatile C6-compounds in green tea leaves, which are important precursors of tea aroma[32]. In particular, (*Z*)-3-hexenol has grassy odor, (*E*)-3-hexen-1-ol acetate has grassy and fruity aroma, among which the former is considered to be the main source of green tea aroma[33].

To deeply investigate the mechanisms that regulate the biosynthesis of phenylpropanoid/benzenoids in tea plants, we thoroughly studied the DEGs in the pathways (Fig. 5). In total, 11 DEGs were involved in the phenylpropanoid/benzenoid synthesis pathway, mainly including catechol-O-methyltransferase (*COMT*), caffeoyl-coenzyme A O-methyltransferase (*CCoCOMT*), alcohol dehydrogenase (*CAD*), eugenol synthase (*EGS*), and benzoic acid carboxyl methyltransferases (*BAMT*). Among these DEGs, *COMT* (CsTGY08G0002131), *CCoCOMT* (CsTGY06G0000958), and *CAD* (CsTGY04G0002121) showed the highest expression in TGY and were significantly upregulated 3.77-, 7.75-, and 4.63-fold, respectively. *COMT*, *CCoCOMT,* and *CAD* are involved in the biosynthesis of eugenol, which is the main aromatic component of cloves and orchids, with clove aroma[34]. In addition, *EGS* (CsTGY01G0002412) was significantly upregulated 3.93-fold in JX; *BAMT* (CsTGY03G0002449, CsTGY03G0002452) was significantly upregulated 3.99- and 52.52-fold in BHZ.

Coexpression network related to key aroma compound formation

To understand the gene regulation mechanism of aroma biosynthesis, we correlated 11,222 genes and 20 SCMs were used for WGCNA. After merging similar modules, 14 modules were generated, which comprised 95 to 2,503 genes. Figure 6 showed the correlation between 14 modules and 20 characteristic volatiles. Modules with larger correlation coefficients and smaller *p*-values are highly correlated phenotypes ($r \geq 0.7$, p-value < 0.05). Among these volatiles, the brown module was significantly correlated with (*Z*)-3-hexenol and (*E*)-3-hexen-1-ol acetate (r = 0.825, 0.755); the blue module was significantly correlated with β-caryophyllene (r = 0.81); the pink module was significantly correlated with linalool (r = 0.793). The results indicated that these modules play an important role in aroma biosynthesis in fresh leaves of tea plants.

To reveal the complex transcriptional regulatory network of aroma formation, genes from four modules were analysed, resulting in the identification of *CsHMGR* (CsTGY04G0000045), *CsDXS* (CsTGY02G0002953), and *CsTPS* (CsTGY05G0001285) genes in the blue module, two *CsLIS/NES* genes (CsTGY08G0001704, CsTGY08G0000359) in the pink module, and *CsMVK* (CsTGY05G0001238) and *CsADH* (CsTGY09G0001879) genes in the brown module. When r > 0.8, there may be a strong relationship between the two nodes. Therefore, transcription factors (TFs) were screened from blue,

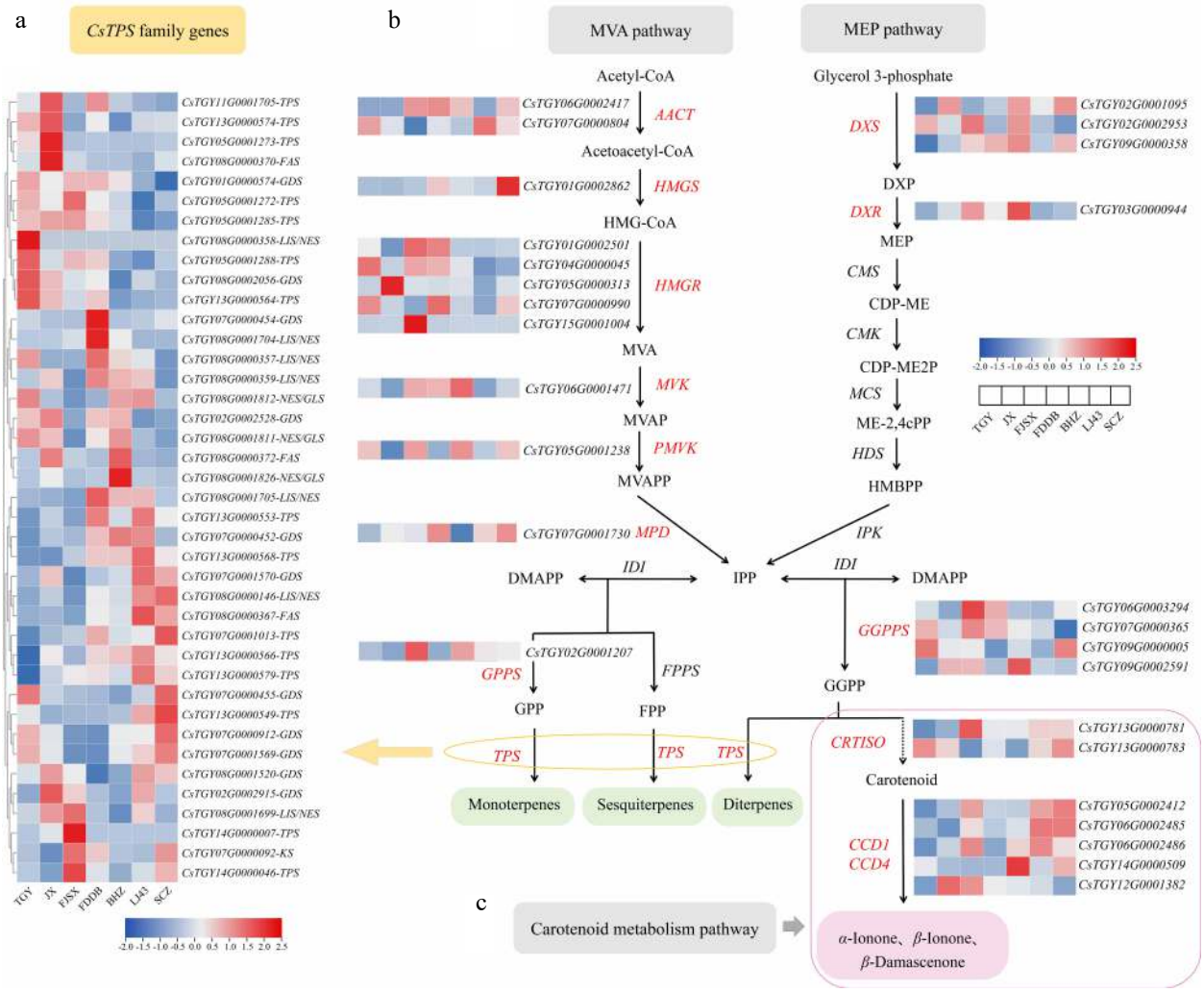

Fig. 3 Expression profiles of genes related to the terpene synthesis pathway. (a) Heatmap of the expression pattern of *CsTPS* genes. (b) Biosynthetic pathway of terpenes and expression patterns of related DEGs. (c) Metabolic pathway of carotenoids and expression patterns of related DEGs.

pink and brown modules. Transcriptional regulatory networks were constructed with the above genes (Fig. 7).

In the blue module, there were 23, 9, and 19 TFs that were significantly correlated with *CsHMGR*, *CsDXS,* and *CsTPS*, respectively. These TFs may be directly or indirectly involved in gene expression and *β*-caryophyllene biosynthesis in fresh leaves. *MYB* (CsTGY01G0002788, CsTGY02G0001042, CsTGY04G0002761, CsTGY06G0001034, CsTGY14G0000893), *bHLH* (CsTGY03G0003180), and *NAC* (CsTGY07G0002415) showed the strongest relationship to *CsHMGR* (r > 0.9); *TCP* (CsTGY04G0003490) and *HB-other* (CsTGY07G0002073) showed the strongest relationship to *CsDXS* (r > 0.9); *TCP* (CsTGY04G0003490) and *HB-other* (CsTGY07G0002073) showed the strongest relationship to *CsDXS* (r > 0.9); *NAC* (CsTGY13G0001061), *HB-other* (CsTGY01G0003094), and *bHLH* (CsTGY12G0001520) showed the strongest relationship to *CsTPS* (r > 0.9).

In the pink module, three and eight TFs were significantly correlated with *CsLIS/NES*, respectively. Among them, *bHLH* (CsTGY06G0000306) and *WRKY* (CsTGY10G0000752) showed the strongest relationship to *CsLIS/NES1* (r > 0.9); MYB (CsTGY15G0001797), *ERF* (CsTGY12G0001243), *bHLH* (CsTGY12G0001940) and *bZIP* (CsTGY09G0000052) showed a significant positive correlation with *CsLIS/NES2* (r > 0.8).

The brown module identified seven TFs, and these TFs may be involved in the regulation of *CsMVK* and *CsADH* genes. *B3* (CsTGY07G0001850) and *NAC* (CsTGY07G0000158) showed a significant positive correlation with *CsMVK* (r > 0.8); *MYB* (CsTGY01G0001203, CsTGY04G0001918, CsTGY06G0002545), *C3H* (CsTGY15G0000151) and *GRAS* (CsTGY14G0002018) showed a significant correlation with *CsADH* (r > 0.8). These results suggested that the TFs above may be involved in regulating the characteristic volatile components and their key genes in fresh leaves of tea cultivars.

Analysis of DEG expression level by PCR

To verify the accuracy of the transcriptome data, the transcript abundances of eight selected DEGs were analysed by qRT-PCR. In total, four DEGs in the terpene synthesis pathway, two DEGs, and two TFs in the LOX pathway were identified (Fig. 8). The relative expression of qRT-PCR was consistent with the trend of RNA-Seq, indicating that the transcriptome sequencing results could be reliable.

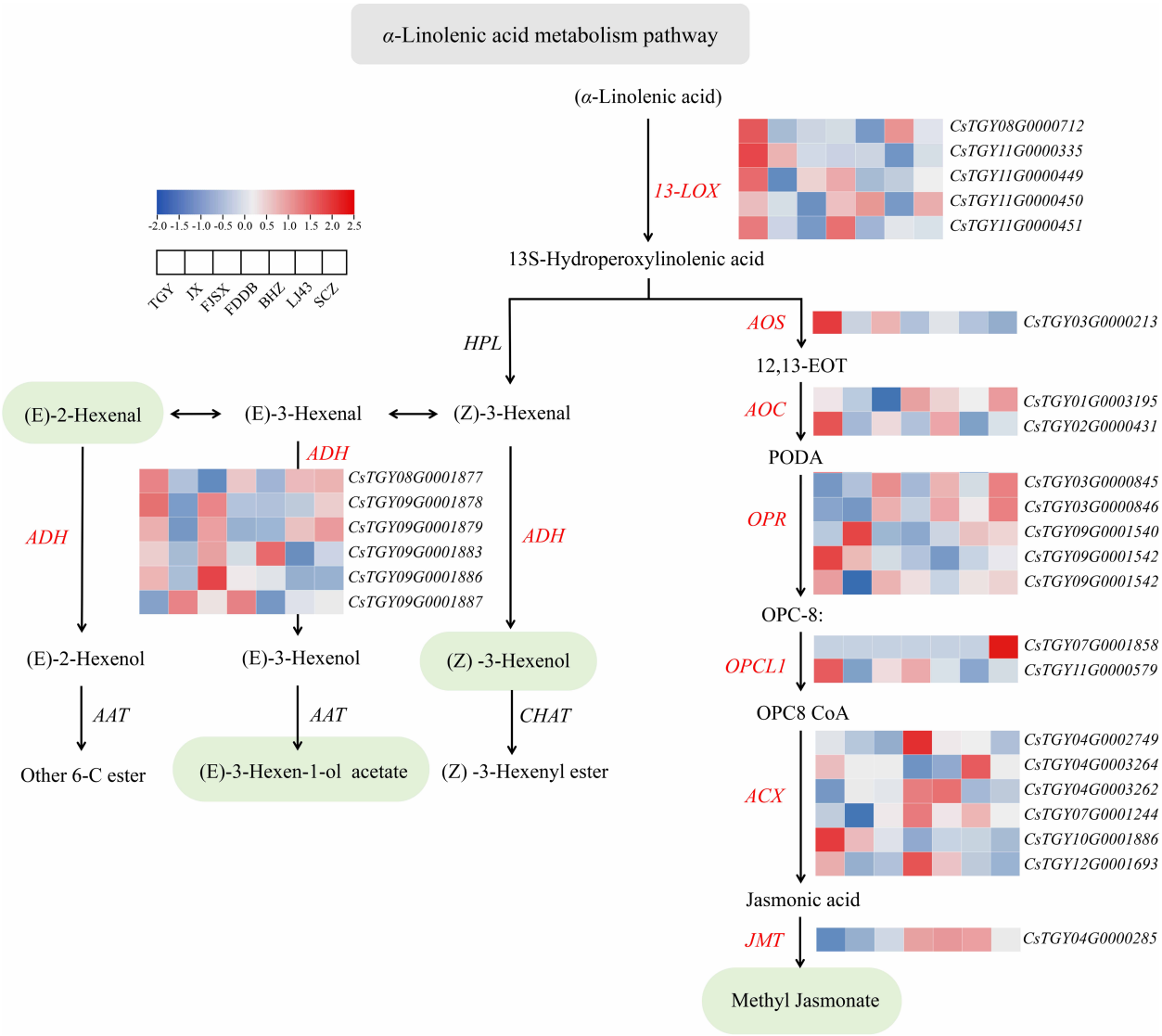

Fig. 4 Expression profiles of genes related to the α-linolenic acid metabolism pathway.

Discussion

Tea plant cultivars possess distinct genetic and biochemical characteristics, which largely determine their suitable tea species and quality[35]. In previous studies[18], we have found that the contents of catechin and purine alkaloids in TGY, JX, and FJSX were higher, the contents of sweet amino acids and sugars in FDDB were higher, and the contents of free amino acids and nucleotides in suitable green tea cultivars were higher. In addition to non-volatile metabolites, volatiles are also particularly important for the formation of tea quality. Aromatic substances in fresh leaves are the material basis for the formation of tea aroma[36]. Tea cultivars play an important role in the chemical compositions of fresh tea leaves and the enzymatic activities of aroma volatile-related enzymes[37]. Therefore, we further analyzed the volatile components of these seven tea cultivars to explore the reasons for their unique flavor from the raw materials of fresh leaves.

Each tea cultivar possesses its own phenotype and characteristic metabolites, some suitable for processing green tea, white, black, or oolong tea. The abundance of terpenes in fresh leaves plays an important role in the aroma quality of tea, which usually has an attractive floral and fruity aroma[38]. High ratio of terpenoid volatiles to green leaf volatiles could be regarded as a good indicator in screening cultivar for suitably producing high quality oolong tea[5]. The terpenoids in TGY and JX accounted for about 72% of the total contents, we speculated that this may be one of the reasons for their suitable preparation of oolong tea. Furthermore, the eugenol[34] and heptanal[28] may contribute to the fruity and flower characteristics of TGY, and methyl salicylate[3], (*E*)-*β*-ocimene[3], and geraniol[27] may be beneficial to JX tea aroma. During processing, Zeng et al.[39] found the GLVs and monoterpenes have relatively large changes in JX, the content of homoterpenes changed sharply in TGY. The differences in in gene expression regulation may all affect the production and concentration of terpenes in plants[40]. The content of esters in the fresh leaves of tea plants was only lower than that of alcohols, among which jasmine lactone is the key volatile component that gives oolong tea its fatty, dairy and floral characteristics[3], which may play an important role in the aroma formation of FJSX. Tea aroma intensity was significantly related to the contents of

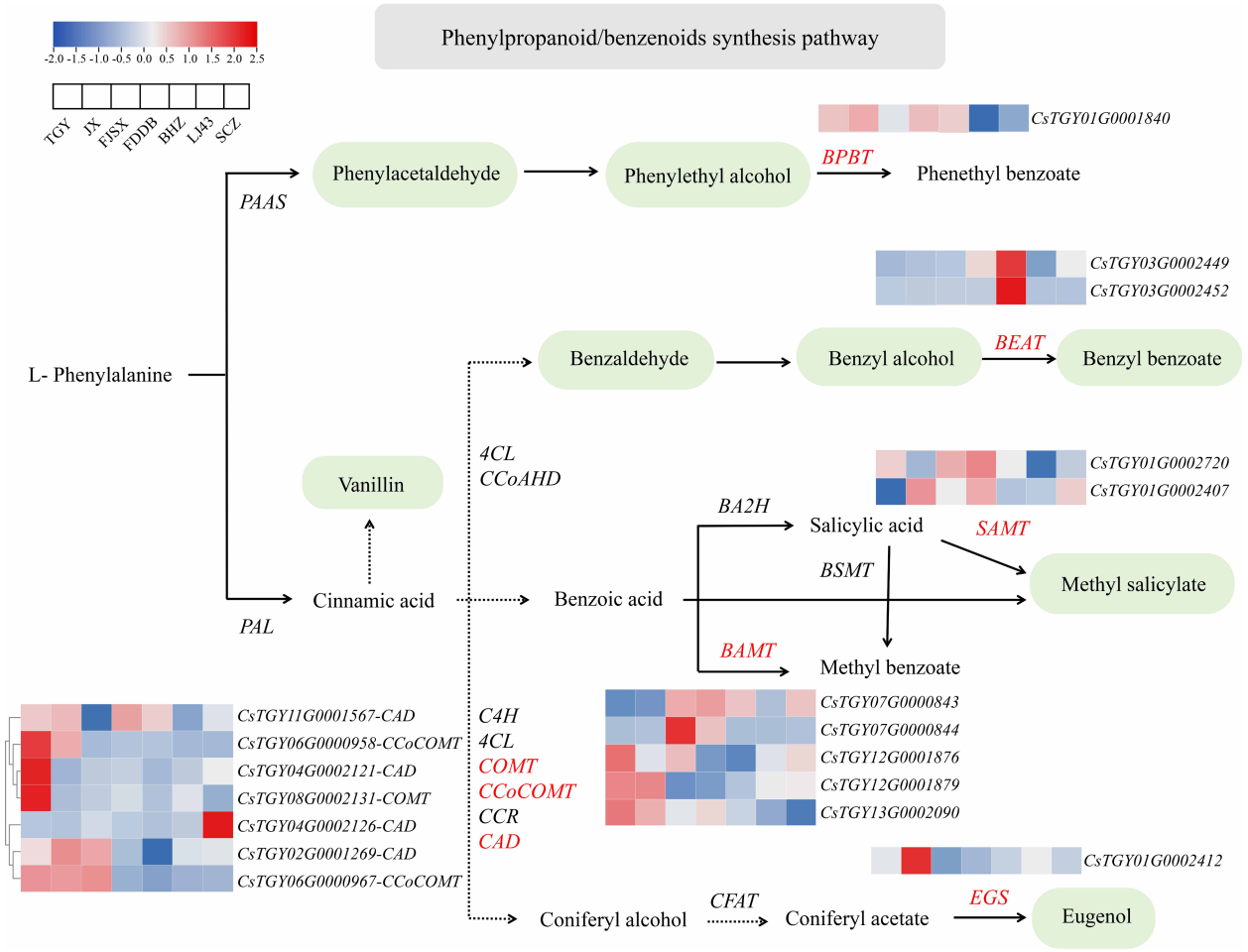

Fig. 5 Expression profiles of genes related to the phenylpropanoid/benzenoid synthesis pathway.

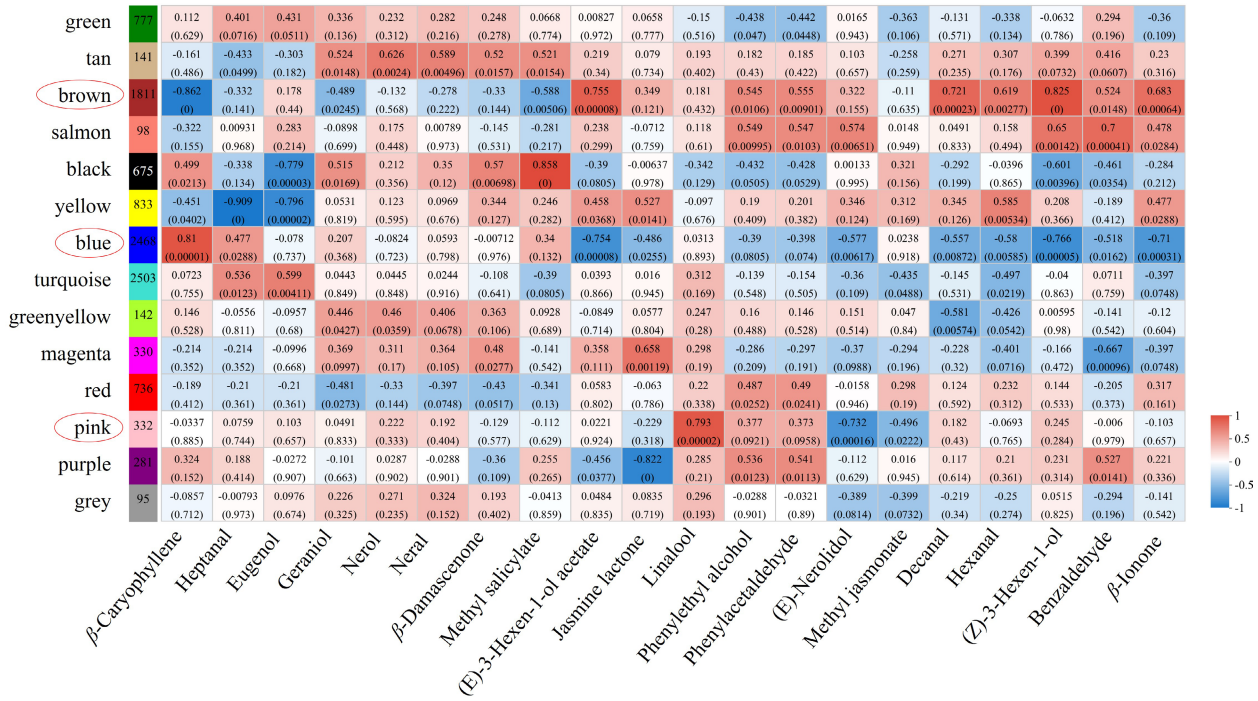

Fig. 6 Coexpression network related to key aroma compound formation. Matrix of module-metabolite associations. The abscissa represents different phenotypes, and the ordinate represents different modules. The number of genes per module is shown in the left box. Correlation coefficients and *p*-values between modules and metabolites are shown at the row-column intersection. Red means the module has a greater correlation with the phenotype, and blue means the module has a lower correlation with the phenotype.

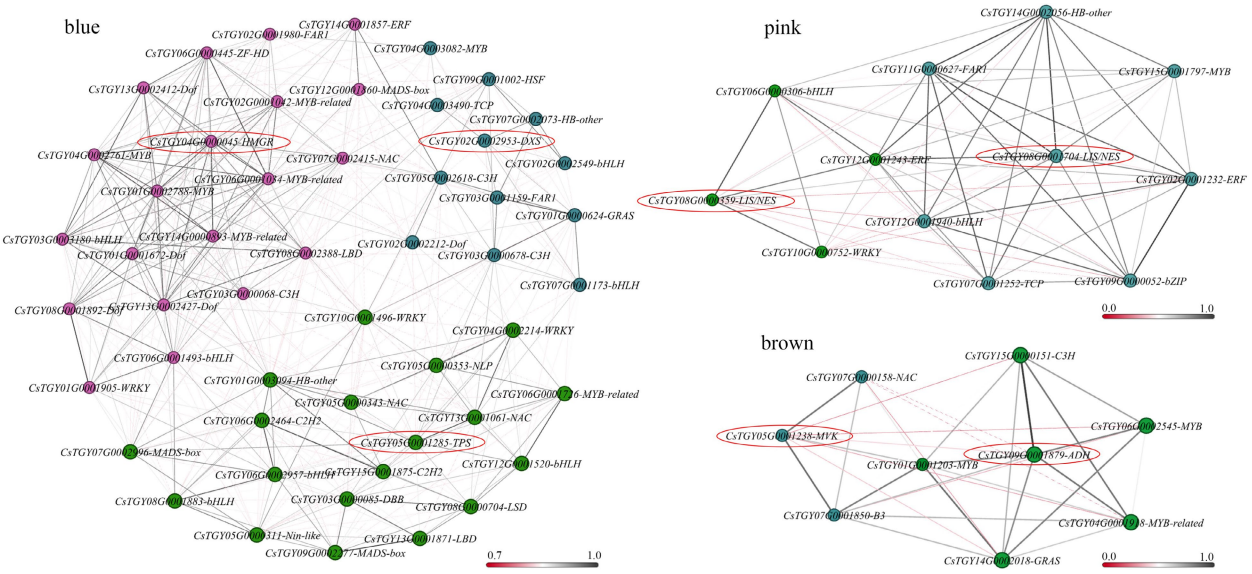

Fig. 7 Coexpression network diagram of candidate genes. When the correlation coefficient (r) is greater than 0.8, we believe that there is a regulatory relationship between the candidate genes and TFs. Nodes with the same colour indicate that the correlation coefficient between candidate genes and TFs is greater than 0.8, and the line colours between nodes indicate the strength of the correlation.

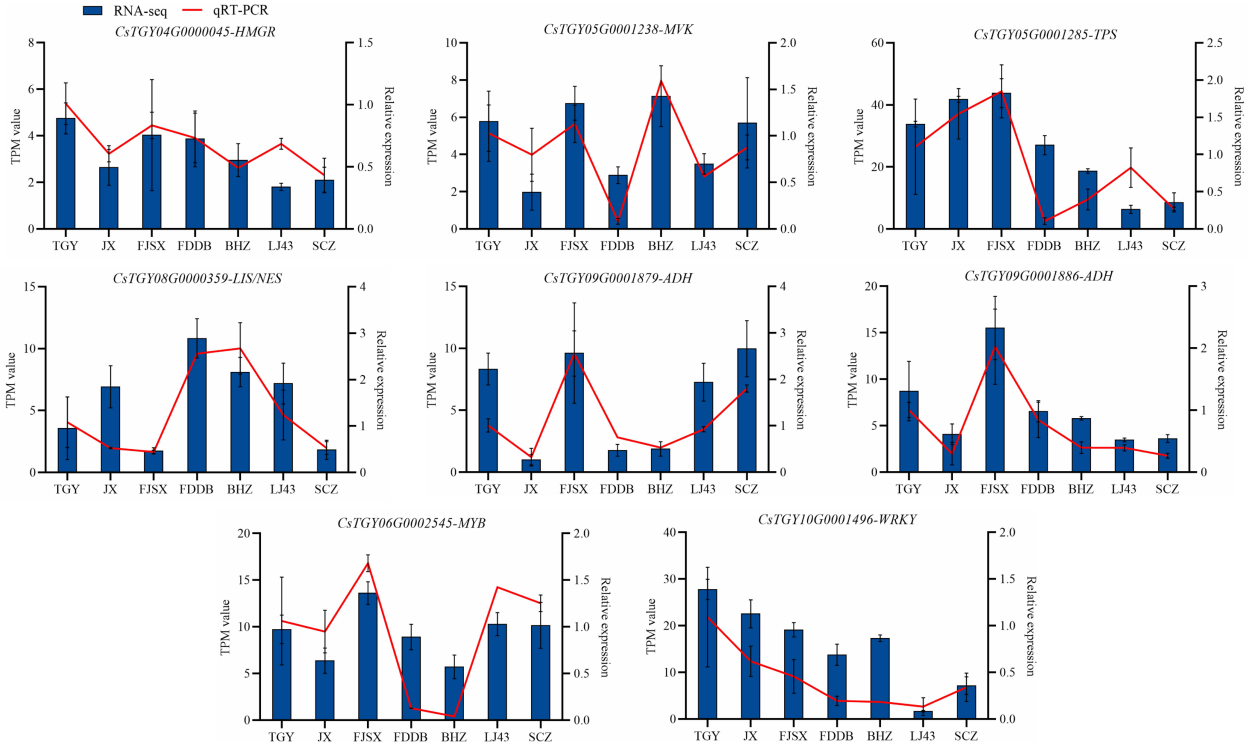

Fig. 8 Verification of the expression levels of eight differentially expressed genes.

esters in tea leaves, and the higher the content, the better the quality. The triploid tea plants increase the gene dose due to the doubling of chromosomes, and the amount of transcription and expression products will inevitably change accordingly[41]. The doubling of chromosomes first leads to changes in the genomic structure, resulting in the re-regulation of gene expression and changes in gene expression levels. The results may be related to the fact that FJSX is the triploid tea resource.

Phenylethyl alcohol, phenylacetaldehyde, linalool, and its oxidized products were the main volatiles of white tea[42], we speculated that the significant accumulation of linalool and linalool oxide content in FDDB may contribute to the clear and fresh characteristics of the white tea. Aromatic alcohols with floral and fruit aromas were not abundant in the fresh leaves of tea plants[1], among which phenylethyl alcohol and phenylacetaldehyde may be the main material basis for white tea and green tea to have mellow aroma quality[6]. Green tea has a variety of flavour characteristics, such as scent types of floral, fruity, nutty, chestnut-like fragrances, and so on[43]. (*Z*)-3-hexenol could play a determining role in the 'raw grass' odour

of finished green tea due to its overly strong and sharp green aroma[44], and (*E*)-3-Hexen-1-ol acetate has a grassy and fruity flavor[30]. The contents of these aroma components were higher in cultivars suitable for green tea, which may be closely related to the aroma formation.

The biosynthesis of terpenes with floral and fruit aromas is catalyzed by TPS enzymes via either the MVA or MEP pathway[45]. Our previous study[9,30] showed that *CsTPS* have different functions in the synthesis of terpenoids. Studies have found that *CsLIS* might be involved in the regulation of linalool accumulation in the tea manufacturing process[30], and *CsNES* were highly expressed in oolong tea during tea turning (*E*)-nerolidol accumulation[46]. In this study, *CsLIS/NES* (CsTGY08G0001704) might be the key gene affecting the accumulation of linalool in FDDB fresh leaves, and *CsNES/GIS* (CsTGY08G0001826) were significantly upregulated in BHZ which might be related to the higher content of (*E*)-nerolidol. Volatile aliphatic components were biosynthesized from linoleic acid and linolenic acid via the lipoxygenase (LOX) pathway[37]. Studies have confirmed that *COMT*, *CCoCOMT, CCR,* and *CAD* are involved in the biosynthesis of eugenol[47–50]. Our results showed that the expression levels of *CsCOMT* (CsTGY08G0002131), *CsCCoCOMT* (CsTGY06G0000958), and *CsCAD* (CsTGY04G0002121) may be closely related to the biosynthesis of eugenol in TGY.

AP2/ERF, bHLH, WRKY, MYB, NAC, and bZIP were the common TF families that regulate terpenoid synthesis[51]. Based on WGCNA study, we speculated that bHLH, MYB, WRKY, and NAC TFs may play an important role in inducing the synthesis of β-caryophyllene by regulating *CsHMGR* (CsTGY04G0000045), *CsDXS* (CsTGY02G0002953), and *CsTPS* (CsTGY05G0001285). In *Arabidopsis thaliana*, the induction of *TPS21* and *TPS11* results in increased emission of sesquiterpenes, especially (*E*)-β-caryophyllene[52]. Overexpression of *CpMYC2* and *CpbHLH13* in *Arabidopsis thaliana* and tobacco can promote the synthesis of linalool and β-caryophyllene[53,54]. Our study also identified that *CsbHLH* (CsTGY12G0001520) was annotated as *MYC2*, which may related to β-caryophyllene accumulation in fresh leaves by regulating the expression of the *CsTPS* (CsTGY05G0001285). In grape berries, VtNAC, VtC2C2-GATA, and VtbHLH were involved in the synthesis of linalool by regulating *TPS* genes[7]. TFs such as bHLH, WRKY, NAC, and ERF were directly involved in the regulation of linalool synthesis by binding with promoters of *CsLIN*[30]. Transcription factors play an essential regulatory role in the growth and development of tea plants and complex with other transcription factors to regulate plant secondary metabolism[55]. In *Freesia hybrida* and *Arabidopsis thaliana*, *AtMYB21* and *AtMYC2* were confirmed to participate in linalool synthesis by interacting with each other to form MYB-bHLH complex to control the expression of linalool synthase genes[8]. In this study, bHLH, WRKY, ERF, and MYB were involved in the biosynthesis of linalool in fresh leaves by regulating *CsLIS/NES1* (CsTGY08G0000359) and *CsLIS/NES2* (CsTGY08G0001704), especially *ERF* (CsTGY02G0001232) may be the key transcription factor affecting linalool synthesis. In tea plant, *CsMYB* is a key gene in lipid metabolism, and it mainly affects lipid metabolism by regulating *CsADH* to participate in aroma biosynthesis[25]. And *CsMYB* is involved in the biosynthesis of fatty acid derivatives by regulating the LOX pathway in green tea[56]. In the present study, we speculated that *CsMYB* (CsTGY01G0001203, CsTGY04G0001918, CsTGY06G0002545) may play an important role in regulating the expression of *CsADH* (CsTGY09G0001879) and the accumulation of (*Z*)-3-hexenol and (*E*)-3-hexene-1-ol acetate in fresh leaves.

Conclusions

In this study, we conducted a comprehensive metabolomic and transcriptomic analysis of seven tea plant cultivars to investigate the cultivar characteristic volatile components of different tea plants and their possible molecular mechanisms leading to volatile component accumulation. Overall, terpenes accounted for a large proportion of the fresh leaves of oolong tea cultivars. The aroma compositions of white and green tea cultivars were similar, and the contents of (*Z*)-3-hexenol, phenylethyl alcohol, phenylacetaldehyde, linalool, and its oxides were higher. The accumulation of volatile components is not only controlled by the expression of structural genes but also involved in the regulation of many transcription factors. Our study revealed the characteristic volatile components and their key regulatory genes of seven tea cultivars, which will provide a theoretical basis for breeding and suitability research of tea cultivars.

Acknowledgments

This research was funded by the Major Special Project of Scientific and Technological Innovation on Anxi Tea (Grant No. AX2021001), Fujian Agriculture and Forestry University Construction Project for Technological Innovation and Service System of Tea Industry Chain (K1520005A01).

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary Information accompanies this paper at (https://www.maxapress.com/article/doi/10.48130/BPR-2023-0017)

Dates

Received 12 April 2023; Revised 14 June 2023; Accepted 19 June 2023; Published online 18 July 2023

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