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Effects of tea garden soil on aroma components and related gene expression in tea leaves

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Summary

In order to explore the effect of soil on the synthesis of aroma components in tea leaves, tea seedlings replanted in tea rhizosphere soil of different ages were used as research materials. Tea seedlings were replanted in soils aged 0, 4, 9, and 30 years, and after one year of growth, 34, 37, 29, and 26 substances were detected in the tea leaves, respectively, using gas chromatography-mass spectrometry (GC-MS). The relative contents of terpenoids and alcohols in the tea leaves dropped from 66.40% to 44.52% and 5.21% to 2.61%, respectively, as the age of the rhizosphere soil increased. Aldehydes, esters, and nitrogen compounds increased from 3.80% to 22.36%, 1.33% to 12.02%, and 3.13% to 19.96%, respectively, as the age of the rhizosphere soil increased. Gene differential expression measured by fluorescence quantitative PCR (qRT-PCR) showed that the number of nerolidol synthetase and linalool synthase genes in tea leaves increased significantly, and the terpineol synthetase, phellandrene synthase, myrcene synthetase, ocimene synthase, limonene synthetase, germacrene synthase, and farnesene synthase genes declined significantly with the increase in soil age. In summary, as the number of years tea had been planted in the soil increased, the soil significantly affected the expression of terpene synthase genes in tea leaves, and then the composition and content of aroma substances in tea leaves changed. The results provide a theoretical basis for the improvement of tea quality.

Key word: Tea tree; Rhizosphere soil; Aroma components; Terpenoid synthases; Gene expression

Introduction

Tea trees are acidophilic crops with an adequate soil pH range of 4.0-6.5 and an optimal pH value of 5.0-5.5. When the soil pH is lower than 4.0, the growth of tea trees is restricted, and the yield and quality of tea leaves decline (Mehra and Baker, 2007; Mohammad et al., 2014). Wang et al. (2018a, 2018b) investigated the acidification of tea garden soil in Anxi county, China, and they found that 37.67% of the tea soil had become acidic (pH < 4.5), and 10.03% of the soils were not suitable for tea cultivation. Further, their results showed that the number of continuous planting years and rhizosphere soil pH value exhibited a significant negative correlation; whereas, the yield and quality of tea and the rhizosphere soil pH showed a significant positive correlation. Ye et al. (2016a, 2016b) found that the pH of tea soil decreased as planting years increased, the potential of soil autotoxicity increased, and the yield and quality of tea declined. They suggested that this phenomenon was related to the accumulation of acidic substances in the rhizosphere soil. All the results indicated that as the number of continuous planting years of tea increase, the soil becomes increasingly acidified, which could lead to a reduction in tea yield and quality.

Professional tea evaluators generally use sensory assessment to evaluate the quality of tea. For example, the maximum total score for oolong tea is 100 points, including 15 points for appearance, 10 points for soup color, 35 points for aroma, 30 points for taste and 10 points for leaf base (GB/T 30357.2-2013). In the evaluation index system of tea, taste and aroma account for 65% of the score, constituting the most important factor in the index. Therefore, many researchers evaluate the quality of tea primarily on aroma and taste. For example, in terms of taste, the content of polyphenols, theanine, caffeine, and amino acids in tea leaves are commonly assessed to evaluate the quality of tea (Jia et al., 2017, 2018). In terms of aroma, tea quality is evaluated by quantitative or qualitative analysis of aroma substances extracted by different methods. The aroma of tea mainly comes from volatile aroma substances. As a key factor of sensory quality of tea, aroma accounts for 30-35% of the score in the evaluation system of all kinds of tea (GB/T 30357.2-2013). Therefore, many researchers study differences in the content of aroma substances by type of tea, processing method, and harvesting time, so as to evaluate the quality of tea leaves from an objective perspective (Lv et al., 2015; Kowalsicka et al., 2014; Yi et al., 2015). However, there are few reports on whether planting soil affects the content of aroma substances in tea tree leaves after long-term planting of tea trees.

In this study, rhizosphere soil was collected from Tieguanyin tea trees planted in different years and used to replant new tea seedlings. The aroma substances in tea leaves were measured after tea trees had been replanted in order to compare aroma substances from plants grown in tea soils of different ages. In addition, fluorescent quantitative PCR was used to analyze the differential expression of the aroma substances synthetase gene. This research aims to lay a theoretical foundation for the improvement and promotion of tea quality.

Materials and methods

Sampling sites and processing

In this study, rhizosphere soil of the Tieguanyin tea cultivar was collected from the Huaxiangyuan tea garden, located in Zhuta village, Longjuan town, Anxi county, Fujian province of China (N24°57'53.89'', E117°40'8.74''). Tieguanyin tea trees that had been planted for 4, 9 and 30 years in this tea garden were selected, and 100 tea trees of each age were selected as a sample site, three replicates per sample. In each sample site, 15 tea trees were randomly selected to collect 15 kg of rhizosphere soil as a sample. Three independent soil samples were collected from tea trees of each age. The tea rhizosphere soil was collected using the method of Wang et al. (2018b) and then used to replant the tea seedlings. Briefly, for each sample, leaf litter was removed from the soil surface, tea trees were dug out, and rhizosphere soil was collected from the tea tree. The control sample was nearby soil in which no tea trees (0 year) had

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grown. The control soil was collected from a depth of 15-25 cm was collected and the collection was repeated three times. Basic physical and chemical indexes of rhizosphere soil of tea trees with different years are shown in Tab. 1.

Planting tea seedlings

The soil sample collected as described above was dried, ground, and sifted through 40 meshes. Then 8 kg of ground soil was put into a pot, and three pots were prepared for each sample. Five one-year-old tea seedlings were planted in each pot. The experiment was conducted for one year from April 2017 to April 2018. During the planting process, fertilizer was applied according to the routine management of tea trees. After one year, the tea leaves with one bud and two leaves were picked for the following assessments.

Determination of aroma substances in tea leaves

The aroma substances contained in the tea leaves obtained as described above were determined using a gas chromatography-mass spectrometer (GC-MS) with three replicates per sample. The fresh tea leaves were cut into 0.2 cm pieces; 2 g of tea leaves were placed into a sample bottle with 20 mL of headspace, and 10 mL of boiling ultrapure water was added to each bottle. Then the bottles were placed in a water bath at 90 °C for 60 min and inserted immediately thereafter into a manual sampler fitted with a 50/30 μm DVD/CAR/PDMS extraction head. The extraction head was then immediately inserted into the GC-MS (7890A-5975C). Injection port temperature was 250 °C and thermal desorption was 5 min. The chromatographic column was an elastic quartz capillary column of HP-5MS (30 m × 0.25 mm × 0.25 μm). The temperature procedure was as follows: initial column temperature was 40 °C, maintained for 5 min, and then increased to 240 °C at 5 °C/min for 60 min. The carrier gas was high purity helium, the flow rate was 1 mL/min, the ion source was EI source, the electron energy was 70 eV, the ion source temperature was 230 °C, the four-stage bar temperature was 150 °C, and the interface temperature was 280 °C. The mass scanning range was 30-500 amu, and the solvent delay was 1 min. The substance was identified using the NIST.11 library, and the relative content of substances was quantified by the peak area normalization method. The substance whose matching rate was greater than 80% was used for analysis.

Differential expression of terpene synthase genes in tea leaves

The total RNA of the tea leaves obtained as previously described was extracted using an RNAiso Plus (TaKaRa) Kit, and the residual DNA was removed by DNase; 1% agarose gel electrophoresis was used to detect RNA integrity. Next, the RNA was reversed into cDNA by using a PrimeScript RT reagent Kit (TaKaRa). The fluorescence quantitative PCR (qRT-PCR) was performed using a TransStart Top Green qPCR Mix kit (TransGen). The reaction system measured 25 μL, including 1.0 μL of template DNA, 12.5 μL of SYBR Premix Ex Taq, and 1 μL each of positive and negative primers, and the remaining was ddH_2O. The PCR reaction procedure was as follows: predenaturation at 94 °C for 1 min, 94 °C for 10s, 55 °C for 30s, 72 °C for 20s, repeated for 35 cycles, and fluorescence signals were collected in each cycle. After that the PCR reaction system extended at 72 °C for 5 min. Five technical replicates per sample were performed. The average of the five technical replicates of each sample was used as a single measurement. The standard deviation was calculated from the three independent samples of tea leaves. The primer of terpenoid synthase genes are shown in Tab. 2 (Li et al., 2014; Liu et al., 2014; Ma et al., 2014). The glyceraldehyde-3-phosphate dehydrogenase gene (CsGAPDH) was used as an internal gene. The segments of product size was 206 bp, the expression quantity of all terpenoid synthase genes were calculated by the method of $2^{-\ddot{A}\ddot{A}Ct}$.

Data Analysis

Excel was used for data classification and percentage calculation analysis. Statistical analyses for comparing the average results of the different samples were performed using a one-way analysis of vari-

Tab. 1: Physico-chemical properties of tea rhizosphere soil of different ages

Planting years	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
0	2.63±0.08	1.37±0.11	1.72±0.04	27.2±1.23	79.3±2.57	305.2±4.85
4	2.58±0.12	1.29±0.07	1.64±0.07	29.3±1.54	87.4±3.62	312.1±3.97
9	2.47±0.06	1.21±0.05	1.71±0.05	28.1±1.29	88.5±2.84	320.2±3.26
30	2.49±0.09	1.24±0.13	1.69±0.08	28.7±1.38	89.2±2.16	324.5±2.53

Tab. 2: The *q*RT-PCR primers of terpenoid synthase genes in tea tree leaves

Gene name	Primer sequence		Product size (bp)
3-Hydroxy-3-methylglutaryl coenzyme A reductase	5-ctcttcctcctcctcctcct-3	5-cctttgtgcccttggatagt-3	200
Farnesene synthase	5-taatgctctattctttaggctcc-3	5-tgaattggtgatattcttcagaat-3	226
Germacrene synthase	5-agtgagaaagatgttagtggcag-3	5-tccttgttgtctaagtaaccgaa-3	217
Limonene synthase	5-aacactacctttggtgctctg-3	5-aggcatgtccttatattgtgtg-3	211
Ocimene synthase	5-cttctgttcagttggaatggtc-3	5-gaagcatagtttcaggcagtct-3	205
Myrcene synthase	5-acgatgggaatttcaaacc-3	5-agacctagtcatttgccattgt-3	233
Phellandrene synthase	5-ctaaaaatctcaaagaaaacctca-3	5-ttcagatcttcttggtaagttgtt-3	220
Terpineol synthase	5-atctgcgaactaccaacctcc-3	5-ccttgaagtgatagaacactcca-3	209
Linalool synthase	5-cagcacaaacgaaatttcct-3	5-cattccatgacccaagagaa-3	226
Nerolidol synthase	5-attcttaaaatggacgggct-3	5-tgaggacatcttcgaacaag-3	226
CsGAPDH (internal gene)*	5-ttggcatcgttgagggtct-3	5-cagtgggaacacggaaagc-3	206

* CsGAPDH – glyceraldehyde-3-phosphate dehydrogenase gene as internal gene.

ance (ANOVA) followed by Scheffe's test for multiple comparisons. Significance and correlation analysis were performed using SPSS software package 11.0, and principal component analysis was performed using DPS software package 7.05

Results and discussion

Analysis of aroma components in tea leaves

The aroma substance analysis detected 34, 37, 29, and 26 substances in tea leaves from tea seedlings replanted in soils aged 0, 4, 9, and 30 years, respectively (Tab. 3). The substances detected can be divided into the following six types: terpenoids, alcohols, aldehydes, ketones, esters, and nitrogen compounds. The number of terpenoids and ketones exceeded the number of other types of aroma substances. In leaves of tea seedlings replanted in 0-, 4-, 9-, and 30-year-old soils, the proportions of terpenoids were 52.94%, 56.76%, 58.62%, and 53.85%, respectively, and the proportions of ketones were 17.65%, 13.51%, 10.34%, and 15.38%, respectively (Fig. 1).

Aroma substances are one of the key indicators in tea quality evaluation, and they come from secondary metabolites of tea trees, which have attracted the attention of many researchers in recent years. Wan and Xia (2015) found that the main aroma substances of tea leaves were terpenes, alcohols, phenols, ketones, acids, esters and heterocyclic. Ma et al. (2014) used HS-SPME (Headspace solid-phase microextraction) combined with GC-MS to analyze and compare the aroma components between conventional treatment and freezing treatment of Dangui varieties of Wuyi rock tea. They established the HS-SPME–GC-FID detection method with nerolidol as an evaluation index and then analyzed the changes of nerolidol content in three grades of oolong tea during processing. Kowalsicka et al. (2014) studied the aroma substances of 201 spring tea samples and 196 post-season tea samples, finding that 59 aroma substances were unique substances, which were mainly composed of oxygenated monoterpene. Our results indicate that the relative contents of terpenoids and alcohols in tea leaves decreases with increasing age of tea rhizosphere soil (Tab. 3). When the soil age was 0 years, the relative contents of terpenoids and alcohols were the greatest, measuring 66.40% and 13.09%, respectively. In contrast, the relative contents of aldehydes, esters and nitrogen compounds increased with increasing age of the soil. When the soil age was 30 years, these compounds had the largest presence, measuring 22.36%, 12.02% and 16.96%, respectively. The results indicate that tea soil age could significantly affect the composition and content of aroma substances in tea leaves, especially terpenoids.

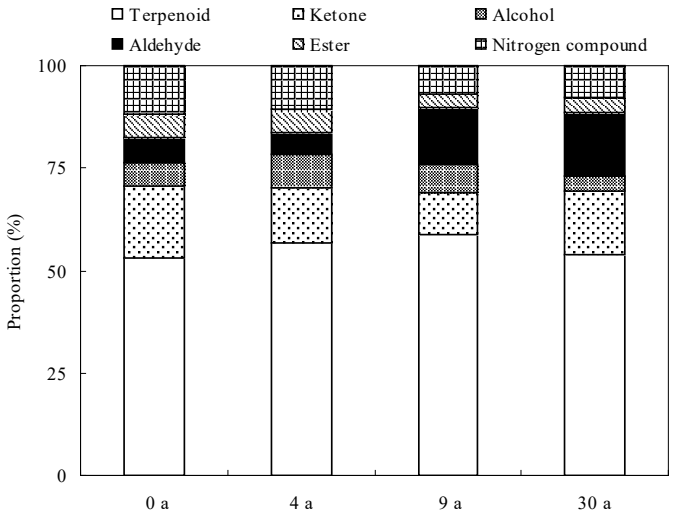

Fig. 1: Proportion of each type number to total number of detected substances in hot-water extract of tea leaves.

Principal component analysis of aroma substances in tea leaves

The results of the principal component analysis (PCA) showed that the aroma substances in tea leaves could be divided into three major components (PC1, PC2, and PC3), and the contribution rates of the three principal components were 80.44%, 12.39%, and 7.17%, respectively (Fig. 2). The tea leaves sampled from the 0-, 4-, 9-, and 30-year-old soils could be effectively divided into different areas.

The correlation analysis results showed that 29 substances were significantly correlated with PC1 (with 80.44% contribution rate; Tab. 4), 16 of the 29 substances were significantly positively correlated, and 13 of the 16 substances were terpenoids. The results showed that the proportion of positively correlated terpenoids accounting for 81.25%. Furthermore, 13 of the 29 substances were significantly negatively correlated, and 6 of the 13 substances were terpenoids, accounting for 46.15%. The results suggest that aroma substances could effectively be distinguished from the four different samples, and terpenoids play a major role in the process of differentiation.

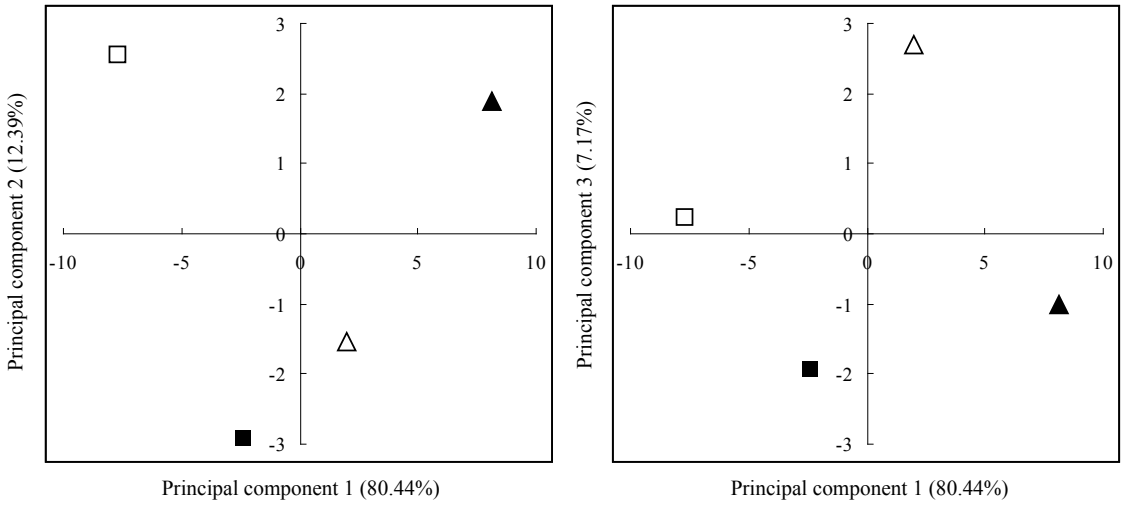

Fig. 2: Principal component analysis of aroma substances in tea leaves.
▲: 0 a, △: 4 a, ■: 9 a, □: 30 a.

Tab. 3: Analysis of aromatic substances in tea leaves by GC-MS

Classification	Retention time (min)	Compound	Molecular formula	Relative content of aromatic substances in tea leaves (*P*/%) 0 a	4 a	9 a	30 a
Terpenoids	11.395	2,6-Dimethyl-2-trans-6-octadiene	$C_{10}H_{18}$	2.443±0.105	0.661±0.033	ND	ND
	14.456	α-Terpinene	$C_{10}H_{16}$	1.386±0.028	0.996±0.042	ND	0.214±0.035
	14.463	β-Myrcene	$C_{10}H_{16}$	ND	1.125±0.038	ND	0.394±0.037
	15.162	D-Limonene	$C_{10}H_{16}$	2.963±0.125	1.633±0.026	1.486±0.034	0.398±0.026
	18.809	o-Cymene	$C_{10}H_{14}$	2.747±0.131	1.242±0.084	0.708±0.048	ND
	19.121	α-Ocimene	$C_{10}H_{16}$	1.950±0.087	1.106±0.045	0.616±0.028	ND
	23.928	2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	$C_{10}H_{16}$	2.298±0.142	0.451±0.082	ND	ND
	27.144	1,3-Cyclohexadiene, 1,5,5,6-tetramethyl-	$C_{10}H_{16}$	0.383±0.035	0.332±0.042	ND	ND
	29.239	Isodurene	$C_{20}H_{20}$	0.749±0.021	0.682±0.054	0.504±0.047	0.434±0.039
	30.844	4-Carene	$C_{10}H_{16}$	0.011±0.009	0.694±0.025	0.782±0.028	1.582±0.023
	46.132	β-Ocimene	$C_{10}H_{16}$	0.598±0.054	1.384±0.047	1.635±0.083	2.550±0.052
	19.136	Terpinen-4-ol	$C_{10}H_{18}O$	1.143±0.058	1.102±0.032	1.007±0.041	ND
	32.314	Linalool	$C_{10}H_{18}O$	ND	0.827±0.024	0.969±0.032	1.773±0.042
	35.048	β-Cyclocitral	$C_{10}H_{16}O$	0.986±0.037	1.193±0.081	1.326±0.067	1.528±0.057
	50.336	Carvenone	$C_{10}H_{16}O$	1.849±0.036	0.717±0.027	0.673±0.032	ND
	52.483	L-Menthol	$C_{10}H_{20}O$	2.037±0.128	1.296±0.018	0.408±0.032	0.317±0.042
	31.141	α-Ionone	$C_{13}H_{20}O$	0.559±0.038	0.606±0.042	1.107±0.038	2.909±0.037
	37.864	2-Undecanone, 6,10-dimethyl-	$C_{13}H_{26}O$	1.897±0.107	1.069±0.076	0.866±0.046	0.610±0.017
	43.925	5,9-Undecadien-2-one, 6,10-dimethyl-	$C_{13}H_{22}O$	3.592±0.083	3.072±0.054	1.498±0.057	0.898±0.038
	48.063	Nerolidol	$C_{15}H_{26}O$	ND	ND	0.108±0.035	0.431±0.051
	49.415	Phytone	$C_{18}H_{36}O$	ND	0.420±0.032	0.636±0.054	1.941±0.064
	51.406	Isophytol	$C_{20}H_{40}O$	0.631±0.048	0.359±0.025	0.131±0.018	ND
Ketones	21.357	Cyclohexanone, 2,2,6-trimethyl-	$C_9H_{16}O$	0.439±0.042	0.327±0.016	0.538±0.052	ND
	22.850	5-Hepten-2-one, 6-methyl-	$C_8H_{14}O$	0.643±0.025	0.534±0.038	0.220±0.052	0.114±0.018
	34.394	6-Methyl-3,5-heptadiene-2-one	$C_8H_{12}O$	ND	0.272±0.029	ND	1.572±0.028
	36.400	Acetophenone	C_8H_8O	0.427±0.043	0.184±0.036	ND	0.054±0.004
	38.123	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	$C_9H_{12}O_2$	1.610±0.058	ND	ND	ND
	41.192	Ethanone, 1-(2-methylphenyl)-	$C_9H_{10}O$	1.683±0.104	0.890±0.053	0.806±0.041	0.794±0.049
	42.967	1-(4-tert-Butylphenyl)propan-2-one	$C_{13}H_{18}O$	0.761±0.024	ND	ND	ND
Alcohols	44.698	Benzyl alcohol	C_7H_8O	ND	0.177±0.018	0.238±0.029	0.938±0.058
	45.612	Phenylethyl alcohol	$C_8H_{10}O$	0.436±0.035	0.401±0.023	ND	ND
	49.913	1,3-Benzenediol, 5-pentyl-	$C_{11}H_{16}O_2$	1.778±0.132	0.604±0.041	0.393±0.027	ND
Aldehydes	30.012	2,4-Heptadienal, (E,E)-	$C_7H_{10}O$	0.231±0.027	0.399±0.031	0.776±0.047	4.376±0.053
	33.451	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	1.386±0.082	2.529±0.026	3.201±0.043	2.282±0.046
	44.178	2-Propenal, 3-(2-furanyl)-	$C_7H_6O_2$	ND	ND	0.308±0.038	0.401±0.042
	45.225	Benzaldehyde, 2,4,5-trimethyl-	$C_{10}H_{12}O$	ND	ND	0.379±0.045	0.968±0.058
Esters	41.080	Methyl salicylate	$C_8H_8O_3$	1.127±0.035	1.938±0.047	2.194±0.045	4.313±0.053
	50.567	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	0.202±0.023	0.209±0.028	ND	ND
Nitrogen compound	15.422	1-Ethylpyrrole	C_6H_9N	2.537±0.039	0.937±0.039	ND	ND
	35.947	Benzenamine, 4-methoxy-2-methyl-	$C_8H_{11}NO$	0.659±0.054	1.577±0.039	2.238±0.061	2.806±0.032
	53.330	Indole	C_8H_7N	0.225±0.041	0.108±0.028	ND	ND
	53.858	1H-Indole, 3-methyl-	C_9H_9N	0.138±0.021	0.186±0.032	0.274±0.048	1.296±0.053

Note: ND: Not detected. Means standard error (± SE) from three replications for each sample is shown.

Analysis of the differential expression of terpenoid synthase genes in tea leaves

The synthesis of terpenoid substances was closely related to the gene expression of key enzymes in the terpenoid metabolic pathway. According to previous reports, the isoprene pyrophosphate and its isomer come from the terpenoid precursor synthesis pathway and could be catalyzed by isoamyl alkenyl transferase and produced geranyl pyrophosphate and faranyl pyrophosphate. These products could be catalyzed by monoterpene and sesquiterpene synthases to produce volatile monoterpenes and sesquiterpenes (DEGENHARDT et al., 2009; AHARONI et al., 2003; XIANG et al., 2013). The results of this study (Fig. 3) show that the expression of nerolidol synthase and linalool synthase increased with increasing soil age. With comparison to the internal reference gene, the two genes of tea leaves corresponding to 0- and 30-year-old soils showed that nerolidol synthase gene was up-regulated 3.86 and 6.21 times, and linalool synthase was up-regulated 2.02 and 5.18 times, respectively. The expression of terpineol synthase, phellandrene synthase, myrcene synthase, ocimene synthase, limonene synthase, germacrene synthase and farnesene synthase decreased with the increase in soil planting years. Corresponding to

Tab. 4: Correlation analysis of different compounds and principal component 1

Compound	r	Compound	r
2,6-Dimethyl-2-trans-6-octadiene	0.91*	2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	0.89*
1,3-Cyclohexadiene, 1,5,5,6-tetramethyl-	0.90*	5-Hepten-2-one, 6-methyl-	0.96**
2-Undecanone, 6,10-dimethyl-	0.96**	5,9-Undecadien-2-one, 6,10-dimethyl-	0.97**
Acetophenone	0.94*	Phenylethyl alcohol	0.89*
D-Limonene	0.98**	α-Ocimene	1.00**
Pinolene	0.88*	Isodurene	0.97**
Carvenone	0.96**	L-menthol	0.96**
1,3-Benzenediol, 5-pentyl-	0.96*	Isophytol	0.99**
Phytone	-0.93*	2-Propenal, 3-(2-furanyl)-	-0.91*
Benzaldehyde, 2,4,5-trimethyl-	-0.91*	Linalool	-0.98**
Benzenamine, 4-methoxy-2-methyl-	-1.00**	β-Ocimene	-0.99**
4-Carene	-0.97**	1-Ethylpyrrole	0.93*
o-Cymene	0.98**	Methyl salicylate	-0.93*
Indole	0.95*	Benzyl alcohol	-0.89*
β-Cyclocitral	-1.00**		

*: Significant correlation at the 0.05 level; **: Significant correlation at the 0.01 level.

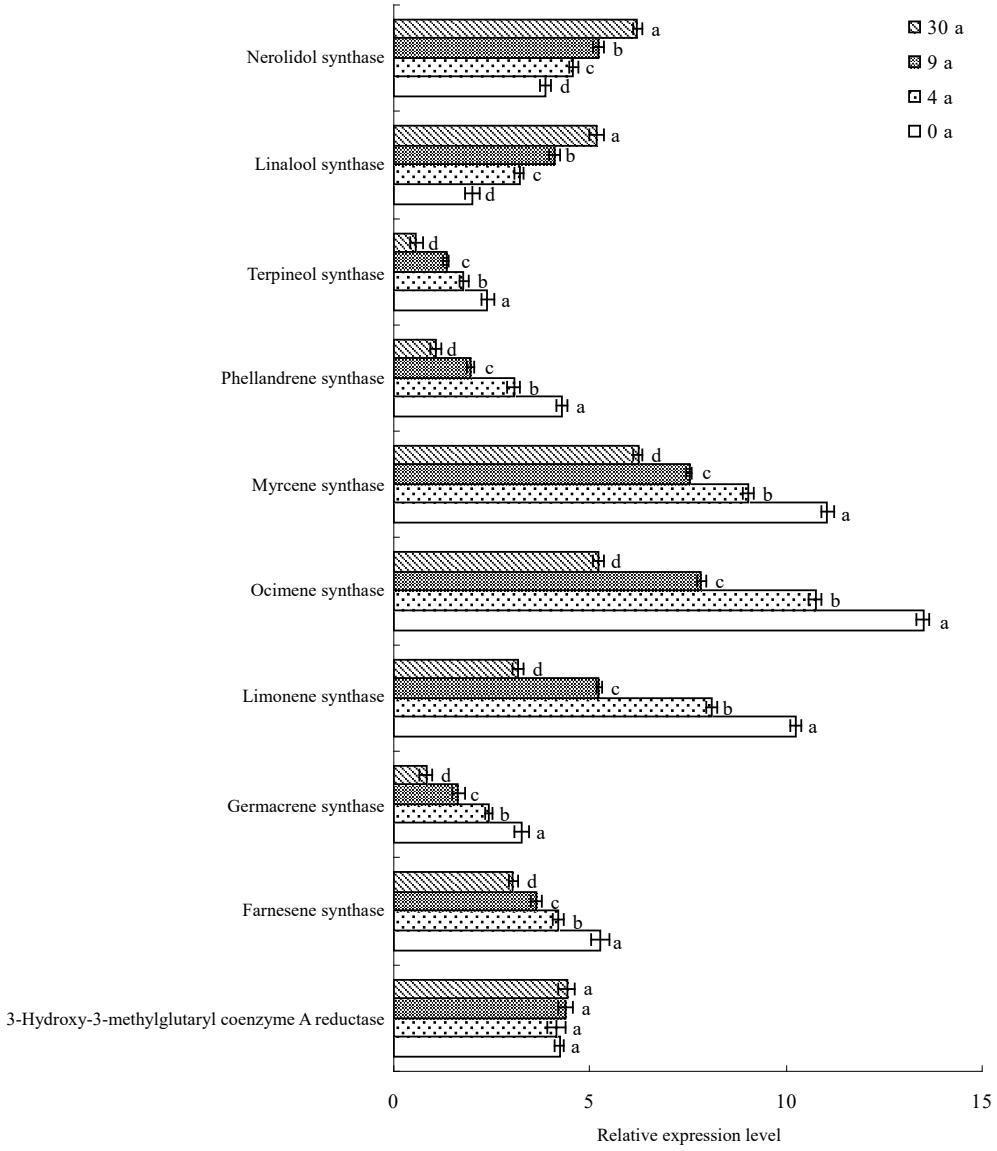

Fig. 3: Analysis of differential expression of terpene synthase genes in tea leaves. The bars represent standard errors of the mean (n = 3). Different letters indicate significant differences at $p < 0.05$ among soils of different ages.

0- and 30-year-old soils, they were showed that terpineol synthase gene was up-regulated 2.39 and 0.58 times, phellandrene synthase gene was up-regulated 4.27 and 1.05 times, myrcene synthase gene was up-regulated 11.05 and 6.23 times, ocimene synthase gene was up-regulated 13.49 and 5.23 times, limonene synthase gene was up-regulated 10.26 and 3.18 times, germacrene synthase gene was up-regulated 3.25 and 0.83 times, and farnesene synthase gene was up-regulated 5.27 and 3.04 times, respectively, compared to the internal reference gene. No significant difference in expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase (Fig. 3) was observed. The expression of terpene metabolic genes in tea leaves showed significant differences among tea seedlings transplanted into soil of different ages. Among 10 terpene synthase genes, 2 genes showed significant upward trends as soil age increased, 7 genes showed significant downward trends, and only 1 gene showed no significant difference in expression. The amount of terpenoids produced was related to the expression intensity of terpenoid metabolic pathway genes. The higher the expression of terpenoid metabolic genes, the more terpenoids were synthesized, and the opposite was also observed (KEELING and BOHLMANN, 2006). YAHYAA et al. (2015) found that increased expression levels of terpene synthase, sesquiterpene synthase, and monoterpene synthase genes in carrot tissues could promote increased terpenoid content in carrot tissue. In our study, it was observed that with increased tea tree planting years, soil could significantly affect expression of terpene synthase genes in tea leaves. The expression of most terpene synthase genes decreased significantly as the age of the soil increased, leading to the reduction of terpene content in tea leaves.

Conclusion

Volatile terpenoids play an important role in the formation of tea aroma quality and are often used to evaluate the quality of tea (OWUOR, 1992; ZHU et al., 2017). RAVICHANDRAN (2002) used terpenoid content to evaluate the aroma quality of tea and found that the higher the terpenoid content, the better the aroma quality of tea. In this study, we found that as soil age increased, the expression of terpene synthase genes in tea leaves was down-regulated, and the content of terpene aroma substances in tea leaves decreased, which led to a decline in tea quality. In addition, some non-terpene aroma substances have been identified, and the effects of these substances on tea quality and their contributions needs to be further studied. These results provide a basis for further improvement of tea quality.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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