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Full Length Article

Effects of Nitrogen Supply on the Biochemical Attributes of Green Tea (*Camellia sinensis*)

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Abstract

The contents and concentrations of polyphenols, volatile compounds and amino acids determine the quality of tea products. Nitrogen (N) deficiency is one of the limiting factors that affected the yield and quality of tea [*Camellia sinensis* (L.) vars. "Huangdan" and "Benshan"] seedlings were supplied with different nutrient solutions containing 0, 50, 100, 300, 1200 or 6000 μM N for 13 months out-of-door. Results indicated that N supply increased leaf and plant dry weight (DW). "Huangdan" had higher N content in leaves than "Benshan" under any given N supply. With decreasing N supply, the concentrations of water extract, polyphenols, the value of (total polyphenols)/(total free amino acids (TFAA)) and (total polyphenols)/(total catechins) increased, but the concentrations of TFAA, glutamic acid (Glu) and theanine (Thea) decreased. The contents of TFAA and polyphenols were lower in "Benshan" leaves than in "Huangdan" under any given N levels, while water extract was similar between two tea varieties. Volatile compounds in leaves analyzed by GC-MS showed that the contents of hexanal, 2,4-heptadienal, 2-decenal, 3-buten-2-one, trimethyl-2- cyclohexen and phytol in leaves increased with increasing N supply, whereas ocimene, linalool, beta-cyclocitral, tetradecane, pentadecane, beta-panasinsene, limonene, dodecane and benzene decreased with increasing N supply. Low N supply decreased the contents of TFAA, Thea, Glu and some volatile aroma compounds and increasing the contents of polyphenols and catechins might result in the inferior sensory properties of green tea. Low-N-induced the changes of these parameters mentioned above were more pronounced in "Benshan" than in "Huangdan".

Keywords: Green tea; Nitrogen deficiency; Amino acid; Aroma; Catechins
Abbreviations: N: nitrogen; EGCG: epigallocatechin gallate; EGC: epigallocatechin; Glu: glutamic acid; Thea: theanine; Pro: proline; EI: electron ionization; DW: dry weight.

Introduction

Tea plant (*Camellia sinensis* L.) is a traditionally important economic crop in China (Lin *et al.* 2012; Gu *et al.* 2019). Due to the health benefits and the improvement of living standards, the quality of tea is attracting more and more attention of customers. Traditional green tea is processed by using the fresh tea with one plump bud and two young leaves. Tea quality is determined by its ingredients, among which aroma and taste were the most significant indexes for tea quality. However, the aroma and taste are easily influenced by many factors, such as biochemical components of fresh leaves and tea processing, processing conditions and techniques.

Tea polyphenols are the general terms for all polyphenols, including flavanols, flavonoids, anthocyanins and phenolic acids. The main category of tea polyphenols is the flavanol (catechin), which accounts for 60–80% of polyphenols and is one of the principal components contributing for the formation of tea color, taste and flavor, as well as the health benefits (Ruan *et al.* 2007). The polyphenols with higher contents of catechin in green tea are the epigallocatechin gallate (EGCG, 50–60%), epigallocatechin (EGC, 15–20%), epicatechin gallate (ECG, 10–15%) and epicatechin (EC, 5–10%).

According to previous research, a total of 26 amino acids were found in tea, including protein amino acids existed in the form of free amino acids and non-protein amino acids [L-theanine, γ-aminobutyric acid (GABA), glutamyl methylamine, aspartyl-ethylamine and β-alanine] (Samanta *et al.* 2017). L-theanine, also known as γ-glutamylethylamide, is a special amino acid in tea, accounting for a unique sweet flavor. L-theanine contents vary in different tea varieties and in different parts of tea

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plants, which account for 1–2% of tea dry weight. As an important source of tea taste, L-theanine endows a unique broths or savory flavor to tea. In tea infusion, the extract rate of L-theanine is up to 80%, which showed significant effects on tea taste, and the correlation coefficient between L-theanine and taste grade of green tea is 0.787–0.876. Studies have shown that tea quality is negatively related to the contents of catechin and positively related to amino acids and aroma in tea infusions, while these components are directly or indirectly influenced by nitrogen content in leaves (Taylor *et al.* 1992; Liang *et al.* 2003; 2005). And there is also a controversial question whether nitrogen deficiency in tea leaves will reduce tea quality (Watanabe 1995; Okano *et al.* 1997; Morita and Tuji 2002; Yang *et al.* 2013b). In order to clarify these questions, the effects of different nitrogen treatments on yang tea plant were conducted in this study. The effects of nitrogen on the yield and quality of tea would theoretically and practically help us to understand the relationship between nutrient balance and the yield and quality of tea (Tsai *et al.* 2004).

In this study, we investigated the effects of N supply on polyphenols, amino acids, water soluble sugars and water extract of green tea in order to determine how N-deficiency affects the quality of green tea.

Materials and Methods

Plant culture and N treatments

This experiment was carried out in the Tea Research Institute, Fujian Academy of Agricultural Sciences from Feb 2016 to Apr 2017. Nine-months-old tea seedlings (*C. sinensis* cv. "Benshan" and "Huangdan") with uniform height and size were potted in 6 L pottery pots containing clean river sands. Each pot was planted with two seedlings and cultivated in a room temperature (Day average temperature was 22℃ and night average temperature was 20℃) and natural light (out-of-door). Seedlings were supplied with 500 mL 1/4 full strength nutrient solution referred to Lin *et al.* (2016). The treatment was applied for 13 months of different N treatments after six weeks of transplanting. Each pot was supplied three times a week with 500 mL of nutrient solution with different N concentrations by 0, 50, 100, 300, 1200 or 6000 μM NH_4NO_3 at pH = 5.0.

Determination of DW and leaf nitrogen content

At the end of the treatments, tea plants were collected from different treatments (one plant per pot). The plant samples are divided into roots, stems and leaves. These plant samples were oven-dried at 80℃ for 48 h and the DWs were then measured by using electronic balance. Total N was measured by using the titration method in a continuous flow auto-analyser (AAIII; SEAL Analytical, Germany).

Processing of green tea

At the end of the experiment, tender leaves with two leaves and one bud were harvested and processed according to the manufacturing technique of green tea. There were three replicates per treatment. The tender leaves were spread out in a sieve for four hours at room temperature (temperature: 20–24℃ and humidity: 70–80%) and enzymes in leaves were inactivated by heating to 200℃ for 60 s. Then the shoots were gently rolled for 15 min and oven-dried at 80℃ for five hours. All the tea samples were grinded and sifted through 40 mesh sieve.

Determination of water extractable compounds and total polyphenol

Three grams of tea powder was accurately weighted and extracted by 450 mL de-ionized water at 95℃ for 30 min. Tea soup was filtered (GB451.2-2002) and de-ionized water was added to a 500 mL capacity bottle. The soup was used to test the contents of water extracted compounds, total polyphenols and TFAA. The water extract compounds were measured according to the method described by GB8305-2013 (Liu *et al.* 2017). Total polyphenols was measured by using ferrous tartrate method (GB8313-2008) of Lin *et al*. (2012).

Assay of free amino acids

A total of 1 g tea powder was accurately weighted and extracted with 50 mL de-ionized water at 95℃ for 60 min. Tea soup was filtered using a 0.45 μm filter mesh (Lin *et al.* 2012). Free amino acids were determined by using the Hitachi L-8900 automatic amino acid analyzer (Tianmei, Kyoto, Japan). The determination and quantification of amino acids were conducted according to the retention time and peak area of the samples.

Assay of catechins

A total of 0.8 g tea powder was accurately weighted and transferred to 100 mL de-ionized water and placed in 70℃ water bath for 30 min. Tea soup was filtered through a 0.45 μm filter (Lin *et al.* 2012). Catechins were determined by using HPLC (Shimadzu Corporation, Kyoto, Japan). A stainless steel Purospher®STAR RP-18 column (4.6 mm i.d. × 250 mm long; 5 μm particle size; HX227027, Darmstadt, Germany) was used. The column temperature was constant at 40±0.5℃. A flow rate of 1.0 mL min^{-1} was used during separation and the injected volume was 5 μL. The mobile phase consisted of a combination of 0.1% (v/v) formic acid aqueous solution and pure HPLC-grade acetonitrile. The condition for HPLC was isocratic formic acid solution lasting for 5 min. The continuous eluent gradient was formic acid solution/acetonitrile with 90/10 in 10 min, 80/20 in 14 min, 78/22 in6 min, and 75/25 in the last 5 min. The types and content of catechins were determined by comparing the retention times and peak areas of the chromatogram (Lin *et al.* 2012; Chen *et al.* 2015).

Gas chromatograph analysis of volatile constituents

The volatile compounds in tea samples were extracted by the method of headspace solid-phase micro extraction (Lv *et al.* 2012). Two grams ground sample was immersed with 5 mL distilled water in a 20 mL sealed bottle and the temperature of the headspace vial was kept at 80°C for 60 min. The volatile constituents were absorbed by 75 µm PDMS/DVB coated fiber at 60°C for 30 min, and then desorbed at 220°C for 3 min. The running conditions for GC/MS were as follows: chromatography column, HP-INNOWAX, 30× 0.25 mm× 1.0 µm; the carrier gas, helium; the flow rate, 1 mL/min; the injector temperature, 220°C. The oven temperature was programed as follows: 60°C, 2 min; to 80°C at 3°C/min for 2 min, to 180°C at 10°C/min, kept for 2 min, to 220°C at 2°C/min for 5 min. Mass spectroscopic conditions were as follows: ion source, electron ionization (EI); electric energy, 70 eV; electric tension, 350 V; scan range, 35–335 amu (Bai *et al.* 2013).

Principal component analysis (PCA)

The abundances of all the differential parameters from "Benshan" and "Huangdan" were normalized by the means and transformed for principal component analysis PCA using *princomp* package in R language (Version 3.4.3). In order to investigate the relationships among the variables, the PCA loading plots were generated by Sigmaplot software (Version 10.0) to visualize two loadings against each other.

Statistical analysis

Experiments were carried out in a completely randomized design. There were 40 pots (two seedlings per pot) per treatment. Each component determination was repeated with 3 or 6 replicates. Comparison of different means were conducted by the least significant difference (LSD) test at $P < 0.05$ level.

Results

Plant growth and total leaf N

With a decrease in N supply, the DW of leaf and whole plant significantly decreased. There was no difference between two varieties except that leaf DW was higher in “Huangdan” than in “Benshan” under 0 and 50 μM N (Fig. 1A and C). Leaf N concentration increased with increasing N supply (Fig. 1B). Leaf N content was always higher in “Huangdan” than in “Benshan” under any given N levels. Leaf protein contents decreased with a decrease in N supply (Fig. 1D).

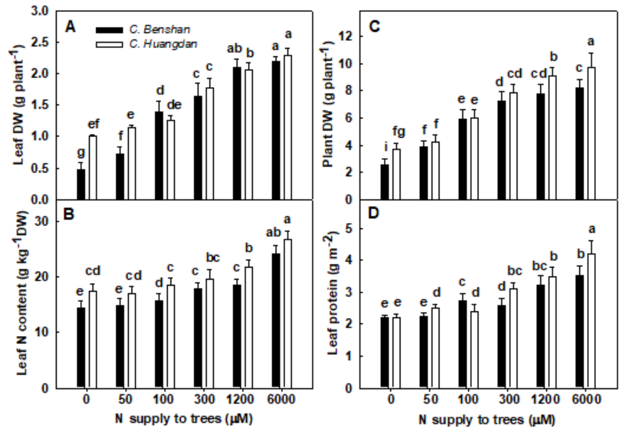

Fig. 1: Effects of N supply on leaf DW (A), leaf total N concentration (B), whole plant DW (C) and leaf protein (D) of tea plants. The data were examined using a LSD test. Each point is mean ± standard error (n = 6). Differences among twelve treatments were analyzed by two varieties × N ANOVA. Different letters above or below standard error bars indicate significant difference at $P < 0.05$

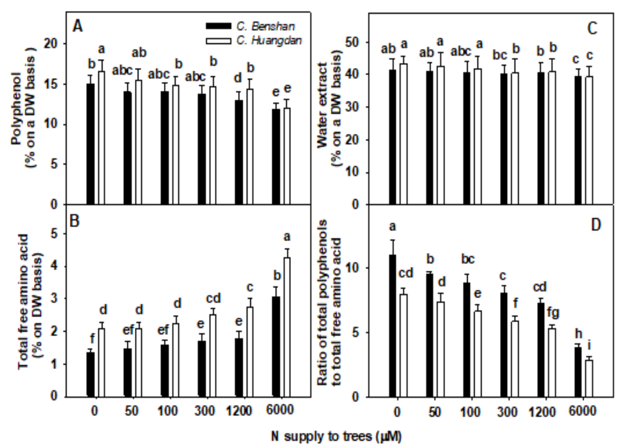

Fig. 2: Effects of N supply on the concentrations of total polyphenols (A) and total free amino acids (B), the concentrations of water extract (C) and the ratio of total polyphenols to total free amino acids (D) in green tea. The data were examined using a LSD test. Each point is mean ± standard error (n = 6). Differences among twelve treatments were analyzed by varieties × N ANOVA. Different letters above or below standard error bars indicate significant difference at $P < 0.05$

Polyphenols, TFAA, and polyphenols/TFAA ratio

The concentrations of tea polyphenols, water extracted compounds and the ratio of tea polyphenols /TFAA ratio increased with decreasing N supply (Fig. 2A, C and D), while TFAA decreased with decreasing N supply (Fig. 2B). Tea polyphenols and TFAA were higher in “Huangdan” leaves than in “Benshan” ones under any given N levels, while water extract was similar between the two tea varieties.

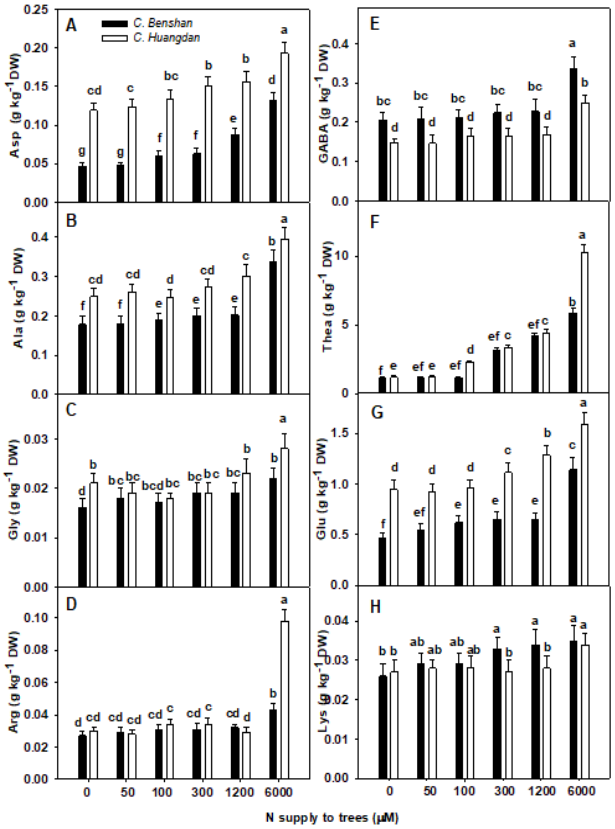

Fig. 3: Effects of N supply on the concentrations of asparagic acid (Asp, A), alanine (Ala, B), glycine (Gly, C), arginine (Arg, D), caminobutyric acid (GABA, E), theanine (Thea, F), glutamic acid (Glu, G) and Lysine (Lys, H) in green tea. The data were examined using a LSD test. Each point is mean ± standard error (n = 6). Differences among twelve treatments were analyzed by varieties × N ANOVA. Different letters above or below standard error bars indicate significant difference at $P < 0.05$

Compositions of TFAA

Aspartic acid (Asp, Fig. 3A), alanine (Ala, Fig. 3B), theanine (Thea, Fig. 3F), glutamic acid (Glu, Fig. 3G), valine (Val, Fig. 4C), leucine (Leu, Fig. 4D) and isoleucine (Ile, Fig. 4F) decreased with decreasing N supply, while the concentrations of proline (Pro, Fig. 4A) increased with decreasing N supply. The concentrations of glycine (Gly, Fig. 3C), arginine (Arg, Fig. 3D), GABA (Fig. 3E), lysine (Lys, Fig. 3H), threonine (Thr, Fig. 4E) and serine (Ser, Fig. 4B) did not change in response to different N supplies, except for increased Arg, GABA and Thr under 6000 μM N level. The concentrations of Asp (Fig. 3A), Ala (Fig. 3B), Gly (Fig. 3C), Thea (Fig. 3F), Glu (Fig. 3G), Val (Fig. 4C) and Thr (Fig. 4E) were higher in "Huangdan" leaves than in "Benshan" ones under N supply, while the concentrations of GABA (Fig. 3E), Leu (Fig. 4D) and Ile (Fig. 4F) were lower in "Huangdan" leaves than in "Benshan" ones. The concentrations of Arg (Fig. 3D), Lys (Fig. 3H) and Val (Fig. 4C) were similar between the two

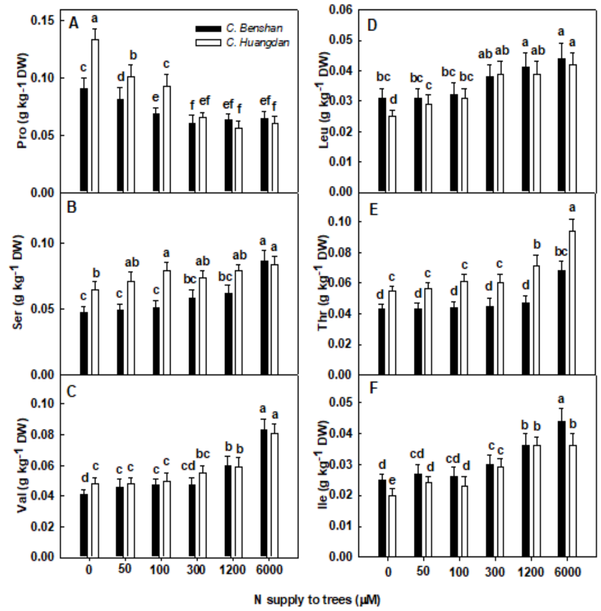

Fig. 4: Effects of N supply on the concentrations of proline (Pro, A), serine (Ser, B), valine (Val, C), leucine (Leu, D), threonine (Thr, E) and isoleucine (Ile, F) in green tea. The data were examined using a LSD test. Each point is mean ± standard error (n = 6). Differences among twelve treatments were analyzed by varieties × N ANOVA. Different letters above or below standard error bars indicate significant difference at $P < 0.05$

varieties except that Arg concentrations was higher in "Huangdan" leaves than in "Benshan" ones and Val content in "Huangdan" leaves was lower than that in "Benshan" ones.

Composition of catechins

The contents of catechin gallate (CG) in "Huangdan" remained constant from 0 μM to 1200 μM N supply (Fig. 5A). Although there was no significantly difference of CG contents among 300, 1200 and 6000 μM treatments, 6000 μM N supply significantly decreased the CG content of "Huangdan" when compared to 0, 50 and 100 μM treatments. There was no significantly difference of CG contents in "Benshan" at different N supply except for slightly decrease at 300 μM N. The contents of CG in "Huangdan" were higher than in "Benshan" under 0 to 300 μM N supply and no difference was observed under 1200 and 6000 μM N supply. Generally speaking, the L-C content of "Huangdan" was increased with decreased N supply, whereas the L-C content of "Benshan" remained constantly under different N treatments except for 0 and 6000 μM N had the highest and the lowest contents of L-C contents, respectively (Fig. 5B). The contents of L-C in "Huangdan" were lower than in "Benshan" under any given N level. The EC contents of both the two cultivars were remained constantly under different N treatments except for 0 and 6000 μM N had the highest and the lowest contents

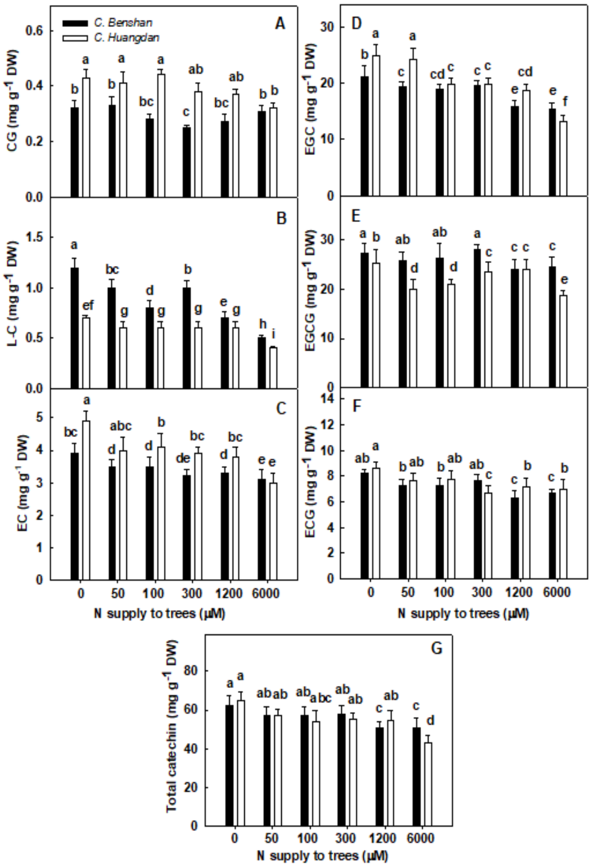

Fig. 5: Effects of N supply on the concentrations of catechin gallate (CG, A)

L-catechin (L-C, B), epicatechin (EC, C), epigallocatechin (EGC, D) epigallocatechin gallate (EGCG, E), epicatechin gallate (ECG, F) and total catechins (G) in green tea. The data were examined using a LSD test. Each point is mean ± standard error (n = 6). Differences among twelve treatments were analyzed by two (varieties)×six (N) ANOVA. Different letters above or below standard error bars indicate significant difference at $P < 0.05$

of EC, respectively (Fig. 5C). The 0 and 50 μM N-treated "Huangdan" leaves had the highest contents of ECG and 6000 μM N "Huangdan" leaves had the lowest content of ECG, respectively. "Benshan" had a similar dynamic of ECG as "Huangdan" in response to N supply (Fig. 5D). Under 0 and 50 μM N supply, "Huangdan" had a higher content of ECG than "Benshan", however under 6000 μM N level "Benshan" had a higher content of ECG than "Huangdan" (Fig. 5D). The contents of EGCG (Fig. 5E) were similar under different N supply, except for a decrease in 1200 and 6000 μM N-treated "Benshan" leaves and in 50, 100 and 6000 μM N-treated "Huangdan" leaves. The contents of ECG were higher in the two cultivars under 0, 50 and 100 μM N supply than under higher N supply (Fig. 5F). Generally, the total catechins in tea leaves were higher under low N supply (0 to 1200 μM N supply for "Huangdan" and 0 to 300 μM N supply for "Benshan") than under high N supply (Fig. 5G).

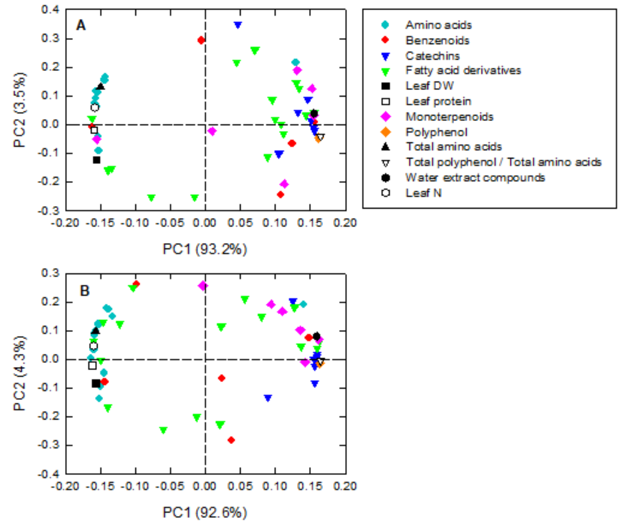

Fig. 6: Principal component analysis (PCA) loading plots of the different parameters from "Benshan" (A) and "Huangdan" (B) under different N supplies. Forty-one parameters from "Benshan" and "Huangdan" were transformed for PCA analysis. A: the first two PCs explained 96.7% of the parameter variation in response to different N levels. B: the first two PCs explained 96.9% of the parameter variation in response to different N levels

Volatile compounds

Twenty seven volatile aromatic compounds were initially identified and classified as suitable species, including 16 fatty acid derivatives, 6 terpenoids and 5 benzene compounds. Hexanal, 2,4-Heptadienal, 2-Decenal, 3-buten-2-one, trimethyl-2-cyclohexen and phytol in leaves decreased with decreasing N supply (Table 1). Ocimene, linalool, beta-cyclocitral, tetradecane, pentadecane, beta-panasinsene, limonene, dodecane and benzene in leaves increased with decreasing N supply. Total fatty acid derivatives were higher in "Huangdan" leaves than in "Benshan" ones, except for a decrease under 50 μM N. No significant difference of total monoterpenoids was observed under different N supply except for an increase in 0 μM N-treated "Huangdan" leaves. Total benzenoids decreased to a lesser extent in 0 μM N-treated "Huangdan" leaves than in "Benshan" ones, but there was no difference under other N supply. Under 0 to 300 μM N supply, two cultivars had a similar content of total volatile compounds, but under 1200 and 6000 μM N level, "Huangdan" leaves had a higher content of total volatile compounds than "Benshan" (Table 1).

PCA loading plots

PCA of the forty-one different parameters with three biological replicates from two cultivars was carried out and presented in Fig. 6. Results indicated that the first two PCs explained 96.8% of the variation in response to different N

Table 1: Relative abundance (%) of volatile compounds in leaves under different N supplies

No	Volatile compounds	RI	Cultivars	Nitrogen supply (μM)					
				0	50	100	300	1200	6000
Fatty acid derivatives									
1	Hexanal	799	*Huangdan*	0.2d	0.30c	0.11e	0.25cd	0.24cd	0.43b
			Benshan	0.1e	0.18d	0.26cd	0.63a	0.64a	0.63a
2	2,4-Heptadienal	909	*Huangdan*	16.15c	19.64b	18.11b	20.37ab	20.36ab	27.57a
			Benshan	9.58f	12.25de	13.43d	16.07c	16.27c	22.68a
3	Ocimene	1048	*Huangdan*	0.42a	0.29b	0.24b	0.20b	0.13c	0.02d
			Benshan	0.36a	0.11c	0.24b	0.16bc	0.17bc	0.1c
4	Linalool	1103	*Huangdan*	14.83b	10.67d	13.0c	9.55f	11.84d	8.14f
			Benshan	16.92a	17.94a	12de	14.26b	8.49f	10.74d
5	Decanal	1206	*Huangdan*	0.94b	0.78c	1.06ab	0.97b	1.18a	0.8b
			Benshan	0.85b	1.13a	0.85b	0.82b	0.82b	0.88b
6	Nerolidol	1569	*Huangdan*	1.27g	1.95c	2.53b	2.17bc	3.01a	1.97c
			Benshan	1.01g	1.53de	1.69d	1.56de	1.66d	1.17g
7	Beta-Cyclocitral	1216	*Huangdan*	2.01b	1.81c	1.81c	1.78c	1.6cd	1.49d
			Benshan	2.58a	1.77c	2.01b	2.09b	1.78c	1.81c
8	2-Decenal	1263	*Huangdan*	0.18c	0.2c	0.29b	0.36ab	0.34ab	0.37ab
			Benshan	0.15c	0.13c	0.19c	0.43a	0.49a	0.45a
9	Tridecane	1300	*Huangdan*	0.6d	0.66d	0.39e	0.44e	0.46e	1.08b
			Benshan	1.67a	1.19c	0.66d	0.9bc	0.81c	0.66d
10	Tetradecane	1403	*Huangdan*	3.42b	2.23de	1.88e	1.47f	1.79e	1.49f
			Benshan	5.07a	3.46b	2.96c	3.04c	2.56d	1.76e
11	3-buten-2-one, trimethyl-2-cyclohexen	1432	*Huangdan*	0.45c	0.5c	0.64b	0.52c	0.57bc	0.98a
			Benshan	0.58bc	0.57bc	0.71b	0.69b	0.65b	0.65b
12	Geranylacetone	1454	*Huangdan*	1.23c	1.65a	1.38b	1.04cd	1.36b	1.3b
			Benshan	1.43b	0.96d	1.14c	1.18c	1.2c	0.99d
13	Beta-Ionone	1486	*Huangdan*	0.69e	0.97b	0.94b	0.93b	0.97b	1.17a
			Benshan	0.9b	0.85bc	0.81bc	0.79bcd	0.87bc	0.8bcd
14	Tridecane,3-methyl	1374	*Huangdan*	0.92c	0.75d	0.81c	0.74d	0.8c	0.81c
			Benshan	1.27b	1.53a	0.87c	1.08b	0.72d	1.14b
15	4-tetradecene	1391	*Huangdan*	0.35bc	0.41b	0.41b	0.66a	0.34bc	0.4b
			Benshan	0.39b	0.57a	0.33bc	0.51ab	0.24d	0.26d
16	Pentadecane	1500	*Huangdan*	1.03bc	1.2b	1.25b	0.75e	0.9d	0.91d
			Benshan	1.77a	1.13b	1.09b	1.05bc	1.05bc	0.75e
Total			*Huangdan*	44.69	44.01	44.85	41.45	45.89	48.93
			Benshan	44.63	45.3	39.24	44.21	38.42	44.67
Monoterpenoids									
17	Benzeneacetaldehyde	1040	*Huangdan*	0.80a	0.86a	0.74ab	0.78a	0.59c	0.54cd
			Benshan	0.35d	0.4d	0.65c	0.62c	0.62c	0.84a
18	Cedrene	1410	*Huangdan*	0.39c	0.44c	0.46c	0.32d	0.31d	0.45c
			Benshan	0.28d	0.69a	0.53b	0.53b	0.42c	0.41c
19	Beta-panasinsene	1422	*Huangdan*	0.46ab	0.50a	0.43ab	0.34c	0.16d	0.3c
			Benshan	0.49a	0.53a	0.41abc	0.4abc	0.34c	0.37c
20	Geraniol	1256	*Huangdan*	3.27a	2.5c	3.04ab	2.52c	2.26d	2.61c
			Benshan	2.93ab	2.65c	2.57c	3.1a	2.67c	2.19d
21	Limonene	1027	*Huangdan*	0.82a	0.69b	0.60b	0.41c	0.27d	0.08e
			Benshan	0.62b	0.64b	0.39c	0.25d	0.22d	0.11e
22	Dodecane	1200	*Huangdan*	2.5a	1.24c	1.35c	1.1d	1.26c	0.73e
			Benshan	1.81b	1.73b	1.04d	1.4bc	1d	0.61f
Total			*Huangdan*	8.24	6.23	6.62	5.47	4.85	4.71
			Benshan	6.48	6.64	5.59	6.3	5.27	4.53
Benzenoids									
23	Butylated hydroxytoluene	1514	*Huangdan*	0.95d	0.66ef	0.73e	0.36h	0.83e	1.54c
			Benshan	2.59a	1.48c	0.86e	2.29ab	1.07d	0.5f
24	Benzyl Alcohol	1039	*Huangdan*	1.26e	1.82ab	2.09a	1.36d	1.69b	1.40d
			Benshan	1.55bc	1.42d	1.55bc	1.53bc	1.71b	0.73f
25	Benaldehyde	1528	*Huangdan*	2.04d	2.26c	2.23c	2.71a	2.17c	1.92d
			Benshan	2.73a	2.25c	2.48b	1.41e	2d	2.8a
26	Benzene	1290	*Huangdan*	0.53a	0.49a	0.41ab	0.32b	0.02e	0.03e
			Benshan	0.39ab	0.38ab	0.37ab	0.25c	0.22c	0.11d
27	Phytol	1840	*Huangdan*	0.57h	1.2de	1.78c	1.49cd	1.76c	2.14a
			Benshan	0.83g	0.94fg	1.07f	1.33d	1.58cd	1.94b
Total			*Huangdan*	5.35	6.43	7.24	6.24	6.47	7.03
			Benshan	8.09	6.47	6.33	6.81	6.58	6.08
Total volatile compounds			*Huangdan*	58.28	56.67	58.71	53.16	57.21	60.67
			Benshan	59.2	58.41	51.16	57.32	50.27	55.28

Note: Values are represented as the ratio of peak area to that of internal standard. Data are expressed as average value (n = 3). Retention indices (RI) were estimated in this work using a homologous series of n-alkanes. Differences among twelve treatments were analyzed by varieties × N ANOVA. Different letters above or below standard error bars indicate significant difference at $P < 0.05$

supplies with PC1 accounting for 93.2% and PC2 accounting for 3.5% in "Bensan" (Fig. 6A). Meanwhile, the first two PCs explained 92.6% of the variation in response to different N supplies with PC1 accounting for 92.6% and PC2 accounting for 4.3% in "Huangdan" (Fig. 6B). PCA show that leaf N, leaf DW, leaf protein and TFAA were highly clustered in both cultivars, whereas catechins, total polyphenols, polyphenol, water extract compounds and the value of (total polyphenols)/(total amino acids) were highly clustered. Interestingly, the monoterpenoids derived volatile compounds were more clustered in "Huangdan" than in "Benshan".

Discussion

Nitrogen fertilizer is indispensable for increasing crop yield and developing agricultural production. As one of the most important nutrients of tea plants, nitrogen exhibits a significant effect on its growth and quality (Bai *et al.* 2013; Lin *et al.* 2016; Liu *et al.* 2017). Tea quality is evaluated by several factors, such as color, aroma, taste, product shape and infused leaf, whereas taste and aroma are the core factors to evaluate tea quality. The taste of tea is composed of astringency, fresh, brisk, bitterness, sweetness and sourness, and the main chemical ingredients for taste include catechin (astringency and bitterness) and amino acids (fresh). The intensity and quality of taste depend on the proportion of the above ingredients. Whether adequate N fertilizer can increase the quality of tea is still debatable. A higher content of polyphenols in green tea is generally considered as poor quality of tea products (Morita and Tuji 2002; Yang *et al.* 2013b). Although Yang *et al.* (2013a) reported that nitrogen deficiency reduced the content of polyphenols in tea. In this study, we found that decreased N supply could increase polyphenols content in both of "Huangdan" and "Benshan" leaves. Deng *et al*. (2012) and Chen *et al*. (2015) reported that application amount of nitrogen fertilizer was reduced, but the content of catechin in leaves was increased. These results were similar to ours.

Amino acids, as principle components in tea, are organic compound with amino and carboxyl groups (Juneja *et al.* 1990; Thippeswamy *et al.* 2006). The composition and content of amino acids in tea, as well as their degradation products and transformation products, present a direct influence on the taste of tea infusions and quality of tea leaves. In particular, some amino acids are important ingredients in tea infusions that affect tea quality and taste. It was reported that the nitrogen contents in fresh leaves of tea plants showed highly positive correlation with the contents of amino acids in fresh leaves and produced tea (Schuh and Schieberle 2006). In this study, decreasing N supply significantly reduced the total amount and changed the composition of free amino acids in tea leaves (Fig. 3–4 and 6A), which is consistent with the results of previous studies (Schuh and Schieberle 2006). L-theanine is a unique free amino acid with the taste of sweet and fresh, which is an important indicator for the quality of green tea. L-theanine only accounts for 1–2% of dry tea weight, but it occupies 50–60% of TFAA in tea infusions (Mejia *et al.* 2009; Sharma *et al.* 2011). The synthesis of L-theanine needs to be supplied by protein, while the condensation of amino acid and ethylamine requires energy from ATP (Lin *et al.* 2016). Under nitrogen deficiency, protein content decreased significantly, as well as photosynthetic rate, and the short supply of raw materials and energy could not satisfy the needs of L-theanine synthesis, resulting in a significant decrease in its content (Syu *et al.* 2008; Samanta *et al.* 2015). This study showed that the content of L-theanine significantly decreased with a decrease of nitrogen supply (Fig. 3F), which indicated that the biochemical quality of green tea declined as a result of low nitrogen supply (Morita and Tuji 2002; Yang *et al.* 2013a). The accumulation of proline is commonly regarded as the adaptation of plants to adverse condition (Szabados and Savouré 2009).

The taste of tea is mainly determined by catechins. With the particular flavor of astringency, catechins become the main cause for the strong taste of tea infusions (Yao *et al.* 2006; Yan *et al.* 2014). Catechins are composed of eight effective monomers, including catechin, epicatechin (EC), (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG), (-)-catechin gallate (CG) and (-)-gallocatechin gallate (GCG). EGCG presents the highest part of catachins in tea plants. Our study found that the total catechins in tea leaves were higher under low N supply (0 to 1200 μM N supply for "Huangdan" and 0 to 300 μM N supply for "Benshan") than under high N supply (Fig. 5G), leading to the increase of astringent taste and the decline in green tea quality, which is in accordance with the results of previous studies (Deng *et al.* 2012; Chen *et al.* 2015).

Polyphenols and amino acids are two important substances for tea taste, and the ratio of tea polyphenols to amino acids is an essential index for the evaluation of tea products (Ruan *et al.* 1990; 2010; Hu *et al.* 2011). Tea infusions taste more fresh and sweet when the content of free amino acids is higher in tea leaves, while it tastes more bitter and astringent when tea polyphenols content is higher. When both of the ratio and the contents of tea polyphenols and amino acids are high, tea infusions taste more fragrant and astringent (Yang *et al.* 2013b). This study demonstrated that with decrease of N supply, the content of tea polyphenols, the value of tea polyphenols/amino acids slightly increased, and amino acids significantly decreased, which indicated that tea infusions had a strong taste of bitter and astringent with less flavor under low nitrogen condition (Fig. 2A, B and D). The content of tea infusions is also one of the important factors to evaluate tea quality. The results suggested that the inclusion of green tea infusions revealed by water extracts increased when N supply was decreased (Fig. 2C), which may be due to the increase of polyphenol content.

The aroma of tea was mainly composed of volatile compounds, accounting for only 0.01–0.02% of total dry weight. At present, about 600 volatile compounds have been identified, among which 41 are determined as the main sources of tea aroma (Table 1). Volatile compounds such as terpenoids and benzene compounds are synthesized by various pathways like the oxidation of fatty acids (Samanta *et al.* 2017). Several volatile compounds such as alkanes and terpenoids are mainly contributed to the green tea aroma, which improve the quality of tea. Our results indicated that with a decrease in N supply, the content of terpenoids increased significantly, especially in "Huangdan" leaves, as well as the contents of geraniol, limonene and dodecane (Table 1). The total volatile contents of these two varieties were high, especially in the "Benshan", which probably was due to increased content of polyphenols that were further converted into many volatile substances under nitrogen deficiency.

Conclusion

Low N supply increased the total polyphenols, total catechins, water extract compounds, the value of (total polyphenols)/(TFAA) and decreased TFAA. Furthermore, lower N supply could degrade the sensory and biochemical qualities of green tea.

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