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RESEARCH ARTICLE

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Aromatic constituents in fresh leaves of *Lingtou Dancong* Tea induced by drought stress

Abstract The effect of different degrees of drought stress on the aromatic constituents and their relative contents in fresh leaves of *Lingtou Dancong* tea plants was studied in this paper. The results showed that drought stress could evidently increase the kinds of aromatic components in the fresh leaves. The largest number of kinds (58) of the aromatic constituents was detected when soil relative water content was 53.90% among all the designed treatments, while the lowest number was found under a soil relative water content of 99.75%. The total amount of relative contents of 17 kinds of aromatic components such as linalool etc., increased with drought stress, whereas 12 kinds of aromatic components such as tetradecanoic acid etc., decreased with drought stress. Linalool, linalool oxide, tetradecane, 10-methylnonadecane, and dodecanal showed high contents under the soil relative water content of 53.90%; Cyclohexane, 1-hexadecene, and 1-tricosanolonly were induced in the soil relative water content of 53.90% and 29.25%; while drought stress could inhibit the synthesis of constituents of 7 kinds such as nonanedioic acid monomethyl ester, etc. Different degrees of drought stress could induce various kinds of aromatic constituents, and the number of aromatic constituents induced in fresh leaves increased with the strengthening of drought stress.

Keywords drought stress, tea plant, volatile aromatic of tea, fresh tea leaves

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1 Introduction

The aromatic constituent of tea has been a hot research field in tea science both domestically and internationally (Wang et al., 2002; Wang, 1998). The large-leaved black tea cultivars planted in two different regions in China were found to have different aroma due to planting locations (Takeo et al., 1992). Tea prepared from raw leaves collected in different growing seasons had greatly different aroma constituents (Chen et al., 1985). The volatile aromatic constituent of tea planted in the plains was three times as high as those planted in high-altitude areas in Darjeeling of Indian (Mahanta et al., 1988).

The tea with high aroma in Sri Lanka is the result of the country’s specific climate of ocean monsoon and continent monsoon, which creates a distinct alternation of dryness and wetness. The reason for the high aroma content in mountain tea leaf is that, up in the mountains, the temperature at night is low, air pressure is low, and water evaporates fast (Owuor et al., 1990). The special aroma and flavor of China Qimen Black tea is the result of the special climatic conditions with moderately high temperature and temporary drought in autumn in its growing region (Wang et al., 1993).

Usually, a favorable growing environment is good for tea growth, and also good for the formation and accumulation of the effective constituents such as aroma acid and polyphenols in tea plants, which may guarantee the high quality of tea (Tong and Luo, 2000; Cao et al., 2002). But in recent years, more and more researches have proved that many plants under environmental stress would produce some secondary substances to cope with the unfavorable environment (Agrawal, 1998; Cao and Luo, 1994; Dicke, 1994; Liu et al., 2005; Pan and Wu, 1996). The special aroma of tea maybe induced under a stressed environment instead of the certainty of a favorable growing environment.

This research, taking *Lingtou Dancong* tea as the material, was carried out to analyze the change of aromatic constituents in fresh leaves under different drought stress conditions, to investigate the relationship between the accumulation of aromatic constituents in fresh leaves and the water change in soil, and to try to reveal the inducing mechanism of the aromatic constituents in fresh leaves of this tea variety by environmental stress.

2 Materials and methods

2.1 Materials

Lingtou Dancong, the most widely grown tea variety in Guangdong province, was selected as the trial material.

Lingtou Dancong tea plants pot-cultured for three years were moved into a shed covered with plastic film, when the shoots grow into one bud and three or four leaves in early summer. After nine days of water stressing, the shoots were collected.

2.2 Methods

2.2.1 Water stress trial

Tea plants potted with red-soil were used for water stress. Four treatments were designed in the trial with three replicates, and the relative soil water contents were 75.75% (T1), 53.90% (T2), 29.25% (T3), respectively. Tea plants were watered everyday to create a similar field environment, with an average relative soil water content of 99.75% (CK).

2.2.2 Analytical apparatus for aromatic component and liquid conditions

(1) Extraction of aromatic-essence: 25 g of ground leaf samples were put into a 1 000-mL flask of the apparatus SDE. Then 500 mL of boiling redistilled water was added, and the water was kept at a boiling temperature using a heater with a thermostat. An extracting flask was filled with 50 mL sublimed diethyl ether, and then taken for a water bath at a water temperature of 50°C for one hour. The extracted liquid was then dried with 5 g anhydrous sodium sulfate and condensed into a volume of 0.2 mL to test the essential oil. Thereafter, the oil was placed into a test tube with a stopple for the analyses of aromatic components. (2) Analyzing apparatus and liquid conditions. Analytical apparatus: Finiga TRACE GC-MS. Spectrum conditions: The temperature was kept at 80°C for 5 min, then raised to 180°C at a rate of 3°C/min, then to 220°C at a rate of 5°C/min and maintained for half an hour. The temperature both at the entrance and the connective points was 280°C. (3) Chromatographic column: DB-5 30 m $\times$ 0.25 mm; helium 1.0 mL/min. (4) Mass spectrum conditions: EI was used as the power source, the bombardment voltage was 70 eV.

3 Results and analysis

Table 1 shows that 81 kinds of aromatic components in the fresh tea leaves under the designed water stress conditions were detected. The largest number (58 kinds) of aromatic constituents was found in the trial with relative soil water content of 53.90% among all the treatments, while the lowest number was for the 99.75% water stress treatment. With drought stress strengthening, the kinds of alcohol increased from 7 to 14, ketone increased from 4 to 6, ester decreased from 13 to 11, decane increased from 10 to 16, alkene increased from 2 to 5, and acid and aldehyde all increased by two kinds.

The results also indicated that the relative contents of the following 17 kinds of aromatic components such as linalool and its oxides, α-terpineol, tetradecane, 10-methylnonadecane, dodecanal, (apric aladehyde,2-ethyl-3-methyl-4-C2-methylpropyl)-2-cyclopenten-1-one, hexadecane, 2,6,10,14-tetramethyl, hexadecane, 1,2-benzenedicarboxylic acid, dibutyl ester, jasmone, farnesene, 6,10,14-trimethyl-2-pentadenanone, 9,12-octadecadienoic acid, and methyl ester, increased with the decreasing of soil relative water content, of which, the linalool and its oxides, α-terpineol, tetradecane, 10-methylnonadecane and dodecanal reached the maximum in T2 treatment; whereas 12 kinds of aromatic components such as tetradecanoic acid, 2,6,10-trimethyl dodecane, pentadecane, 3,7,11,15-tetramethyl-2-hoxadecen-1-ol, tetradecanoic acid, ethyl ester, hexadecanoic methyl ester, hexadecanoic acid ethyl ester, 2-(octadecyloxy)-ethanil and farneryl acetone, decreased with the strengthening of water stress. Linalool, linalool oxide, etradecane, 10-methylnonadecane, and dodecanal showed the highest content in the treatment with relative soil water content of 53.90%. Cyclohexane, 1-hexadecene, and tricosanolonly were induced in the treatments with relative soil water contents of 53.90% and 29.25%. However, drought stress could inhibit the synthesis of the following seven aromatic substances such as benzothiazole, nonanedioic acid monomethyl ester, 7-nonenoic acid methyl ester, pentadecanoic acid methyl ester, 2,6,10,15-tetramethyl heptadecane, octadecanol and eicosanoic acid methyl ester. The different degree of drought stress could induce special aromatic constituents such as tridecane, 2,6,10,14-tetramethyl pentadecane, 2-(2-naphthyl)-2-butene, hexadecanol, tetradecanal, 3-methyl petadecane, 2-methyl-1-hexadecanol, octadecane, heneicosane, 3-decanone, 9,12-octadecadienoic acid ethyl ester and octadecanoic acid ethyl ester. Eicosyl cyclohexane, 1-hexadecane and 1-tricosanol were only detected in T1 and T2 treatments. Globulol, 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol and geraniol were only found in the T2 and T3 treatments. 2,6,10,14-Tetramethyl-pentadecane, 1-tricosanol and neophytadiene reached the highest content in the T1 treatment. 1,2,3,4-Tetrahydro-5,8-dimethyl-1-octyl-naphthalene, 2-nonadecanone, pentadecanoic acid and 2-methyl-hexadecanal were detected only in the T1 treatment. 2-Mehtyl-1-hexadecanol, 1-dodecanethiol, isomethone, hexadecadienoic acid methyl ester and 7,3,4-trimethoxy quercetin were only found in the T2 treatment. 6,10-Dimethyl-5,9-undecadien-2-one, bicyclo(4.2.1)nonan-1-ol hexadecanenitrile, isohepptadecanol, 1-eicosanol,

Table 1 Aromatic constituents and relative contents in fresh leaves in different treatments

No.	Chemical constituents	CK	T1	T2	T3
1	Linalool	5.44	5.26	7.64	6.10
2	α-Terpineol	1.01	1.31	1.37	0.61
3	Benzothiazole	4.61	—	—	—
4	Farnesene	0.18	0.23	0.42	0.65
5	2,3,7-trimethyl- octane	0.84	1.12	1.77	—
6	Tridecane	—	3.24	4.08	4.02
7	1,2,3,4-tetrahydro-5, 8-dimethyl-1-octyl-naphthalene	—	3.34	—	—
8	Tetradecane	3.35	3.92	4.34	3.19
9	10-methylnonadecane	0.41	0.53	0.92	0.56
10	2,6,10,14-tetramethyl-pentadecane	—	1.97	1.78	0.69
11	2,6,10-trimethyl- dodecane	2.80	0.79	0.50	—
12	Pentadecane	16.97	5.58	3.39	2.54
13	Dodecanal	0.40	0.66	0.84	0.72
14	2-(2-naphthyl)-2-butene	—	1.34	1.42	1.83
15	6,10-dimethyl-5,9-undecadien-2-one	—	—	—	1.94
16	Capric aldehyde	0.58	1.15	1.33	1.82
17	Heptadecane	2.05	2.40	2.72	2.61
18	Bicyclo(4.2.1)nonan-1-ol	—	—	—	1.44
19	2-ethyl-3-methyl-4-(2′-methylpropyl)-2-Cyclopenten-1-one	1.29	1.64	1.84	2.64
20	Cyclohexane, eicosyl	—	0.43	0.67	—
21	Nerolidol	1.16	1.28	1.53	1.82
22	Nonanedioic acid, monomethyl ester	1.37	—	—	—
23	7-Nonenoic acid, methyl ester	1.24	—	—	—
24	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	11.82	10.41	4.86	3.66
25	1,6,10-Dodecatrien-3-ol, 3,7, 11-trimethyl- ol	—	—	1.07	2.19
26	2-Nonadecanone	—	0.73	—	—
27	Globulol	—	—	0.50	1.55
28	Linalool oxide	3.84	4.21	4.60	4.01
29	Hexadecane	2.39	2.60	3.90	4.13
30	1-Hexadecanol	—	0.34	0.52	0.91
31	Hexadecene	—	0.49	0.61	—
32	Hexadecanenitrile	—	—	—	1.34
33	Tetradecanoic acid	1.40	0.75	0.75	0.47
34	Tetradecanoic acid, ethyl ester	0.5	0.29	0.25	—
35	Tetradecanal	—	0.22	0.86	0.78
36	3-methyl-pentadecane	—	0.49	0.25	0.79
37	2-methyl-1-hexadecanol	—	0.30	0.78	1.65
38	2-methyl- hexadecene	—	—	0.43	—
39	Isoheptadecanol	—	—	—	0.84
40	1-Eicosanol	—	—	—	0.73
41	2,6,10,14-tetramethyl-hexadecane	0.63	0.77	0.93	1.04
42	Pentadecanoic acid, methyl ester	0.77	—	—	—
43	Isopropyl Myristate	1.12	1.40	1.76	1.67
44	Neophytadiene	5.91	6.22	4.25	3.39
45	6,10,14-trimethyl-2-pentadecanone	1.16	1.61	1.60	1.82
46	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.79	1.03	1.11	1.22
47	Pentadecanoic acid	—	0.67	—	—
48	Hexadecanoic acid	7.75	6.97	4.90	3.48
49	Hexadecanoic acid, methyl ester	2.85	0.90	0.81	0.44
50	Hexadecanoic acid, ethyl ester	2.38	2.65	2.15	0.91
51	Hexadecanoic acid, 2,3-dihydroxypropyl ester	—	—	—	0.12
52	Octadecane	—	1.43	1.46	2.59
53	1-Nonadecene	—	—	—	0.32
54	Heneicosane	—	2.70	3.83	2.69
55	Geraniol	—	—	0.37	1.87
56	Cis -Jasmone	—	1.47	3.41	3.73
57	3-Decanone	—	0.15	0.87	1.54
58	9,12-Octadecadienoic acid, methyl ester	0.50	0.79	0.91	1.92
59	2-(octadecyloxy)-ethanol	0.15	0.26	0.33	—
60	Farnesyl acetone	0.60	0.31	0.22	—
61	2-methyl-hexadecanal	—	0.63	—	—
62	1-Tricosanol	—	5.86	3.30	—
63	Isophytol	0.68	0.34	0.31	—
64	1,2-Benzenedicarboxylic acid, dibutyl ester	0.70	0.83	0.89	1.47
65	Octadecanol	1.65	—	—	—
66	9,12-Octadecadienoic acid, ethyl ester	—	0.81	1.02	1.30
67	Octadecanoic acid, ethyl ester	—	0.40	0.75	0.68
68	9,12,15-Octadecatrienoic acid, methyl ester,	0.76	1.32	1.62	2.13
69	2,6,10,15-tetramethyl-heptadecane	1.54	—	—	—
70	Eicosanoic acid, methyl ester	1.86	—	—	—
71	9-Octadecenoic acid	2.54	1.63	1.26	1.03
72	Octadecanoic acid	0.26	0.36	0.97	1.49
73	Pentatriacontane	—	—	—	3.77
74	1-Dodecanethiol	—	—	0.68	—
75	Hexadecadienoic acid, methyl ester	—	—	0.74	—
76	Quercetin 7,3’,4’-trimethoxy	—	—	0.68	—
77	Isomenthone	—	—	0.55	—
78	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	—	—	—	0.63
79	Cyclohexane, (1-hexadecylheptadecyl)	—	—	—	0.53
80	Triacontane	—	—	—	0.86
81	1-Heptatriacotanol	—	—	—	0.69
	Total	98.45	98.53	98.42	98.62

Notes: T1, T2 and T3 represent soil relative water contents of 75.75%, 53.90% and 29.25%, respectively; CK is for relative water content of 99.75%; the relative contents in the table are the ratios of peak area of each constituent to the total peak area.

hexadecanoic acid-2,3-dihydroxypropyl ester, 1-nonadecene, pentatriacontane, 2,2,4-trimethyl-1,3-pentanediol diisobutyrate, (1-hexadecyl-heptadecyl) cyclohexane, triacontane and 1-heptatriacotanol were only discovered in the T3 treatment. It is concluded that the number of aromatic constituents in fresh leaves was increased under drought stress.

4 Discussion

Tea aroma formation is related to the environmental factors, especially season of collection. Oolong tea produced in winter and autumn has a strong aroma but weak in summer tea. The seasonal difference is created mainly by the meteorological factors including light, temperature and humidity. The quality of various teas in different seasons maybe affected by a single factor or by the interactions of many factors. This trial confirmed that water is an important factor that affected the aromatic component variation in fresh leaves, under the condition of drought stress, indicating that many aromatic constituents and their relative contents in fresh leaves changed along with the change in the soil water content, and could induce some specific aromatic constituents.

Tea aroma depends on the proportion and content of different kinds of aromatic components (Wan, 2003), so the changes of the kinds or relative contents of aromatic constituents, whether increased or reduced, would greatly affect tea aroma. Although sometimes the change in kinds is not obvious, certain kinds of components still have a great proffer on aroma, whose minimum change can change the aroma style sufficiently and take a pivotal part in the formation of aroma, especially for certain styles. In the condition of moderate drought stress, many aromatic constituents and their relative contents in fresh leaves may be changed, and the tea aroma could be more balanced. Therefore, water stress is an important factor for the expression of strong tea aroma produced in winter and autumn.

The leaf aromatic constituents in the T2 and T3 treatments were induced in the condition of moderate drought stress, making it possible to form the distinctive aroma. When the soil relative water content was reduced to a certain level, some aromatic constituents were reduced to the lowest content or maybe disappeared, which is not certainly harmful to the aroma. But when the drought stress is too serious or its duration is too long, it would affect the normal physiological function of the tea plants, and even make their metabolism disordered, resulting in the reduction of effective substances in leaves and low tea quality. Therefore, moderate drought stress and duration can induce the formation of effective aromatic constituents in tea leaves and can improve their distinctive aroma.

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