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Effects of magnesium on quality and aroma composition of Wuyi Rougui tea (*Camellia sinensis* L.) leaves

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ABSTRACT

In hydroponic conditions, we investigated the effects of applied magnesium (Mg) on quality of leaf and aroma of seedlings of Wuyi Rougui tea (*Camellia sinensis* L.). Different concentrations of Mg [0.4 mM (Mg^1), 0.6 mM (Mg^2) and 0.8 mM (Mg^3)] were added into the hydroponic solution. Control treatment did not contain Mg (Mg0). With the increasing availability of Mg, the polyphenols content were significantly increased. The lowest free amino acid content (2.44 %) was observed in the Mg^1 treatment. The Mg treatments significantly influenced the caffeine level, except in Mg^2 and Mg^3. Maximum total flavone content (9.30 mg/g) was in Mg^2 treatment followed by Mg^1, Mg^3 and Mg^0, respectively. The highest content of soluble sugars (9.30 %) was observed in the Mg^2 treatment followed by Mg^1, Mg^3 and Mg^0. Principal component analyses of quality indicators showed that PCoA1 and PCoA2 differed in samples treated with various Mg concentrations and the total variability was 97.73 %. Caffeine and catechin contents showed a negative correlation to increasing Mg concentrations. The last ones displayed a positive correlation (r = 0.945) with (E,E)-2,4-heptadienal levels and were inversely correlated (r = -0.967) with decanal content.

Key words: Aroma composition, *Camellia sinensis* L., correlation analysis, internal quality, magnesium concentration, tea leaves, Wuyi Rougui,

INTRODUCTION

China accounts for 45.9 % of the world's tea plantation area, it is mainly planted in the southern tropical and subtropical regions (29). The Mg content of Chinese soils gradually decreases from north to south and up to 70 % of tea plantations are Mg deficient (34). Mg-deficient tea plantations are mainly located in southern China, especially those with sandy soils or old tea plantations (20). These tea gardens are in a warm, humid and rainy climate, which, together with long-term application of nitrogen fertilizers, leads to soil acidity and serious loss of soluble Mg from tea gardens (13).

Previous studies indicate that Mg requirements of tea trees can only be overcame by adding nitrogen and potassium (22,26). At the initial stage, the Mg deficiency significantly decrease the growth thus reduce the yield of tea tree. The severe Mg deficiency causes the shoot yellowing and decreases growth of tea trees (7). Moreover, Mg content in tea tree leaves is also closely related to the quality of green tea, black tea and influence oolong tea, and the important quality parameters (theanine, catechins and caffeine) (32). Ren *et al.* (23)

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found that contents of Mg, Al, Zn, N, P, K, Cu, and B were positively correlated with the quality of tea leaves. Among them the Mg, Al, and Zn were most related to the high-quality tea, indicating that Mg plays an important role in the quality of tea tree. Li *et al*. (16) studied the effects of Mg fertilization on Yunnan big-leaf green tea and found that the contents of free amino acids, polyphenols and caffeine in leaves increased with optimum availability of Mg in the soil. The contents of amino acids and caffeine in tea leaves reached a maximum, when soil was fertilized with Mg at 229.85 kg/ha. Jayaganesh *et al*. (15) showed that the application of $MgSO_4$ increases the concentrations of amino acids, polyphenols and catechins in black tea. Soil nutrients studies found that Mg fertilization increased the leaf thickness, germination of tea buds and free amino acids : phenol-ammonia ratio in oolong tea trees however, the internode length was reduced (1,11,24). Mg content in tea leaves was positively correlated with amino acid and amino acid transferase contents. The Mg application increased theanine, proline and aspartate content, thereby, improving the freshness of oolong tea, while reducing the accumulation of astringent and bitter metabolites (15,22). Hence, study of the mechanisms of Mg affecting the quality of tea tree is important.

The regulation of Mg influence on tea quality can be attributed to some factors (availability of Mg, the N fertilization, tea growth) on the tea tree. However, there is little information about the specific factors affecting the tea tree quality. Hence present study aimed to investigate, how Mg availability affects the quality of Wuyi Rougui tea grown in hydroponic culture by determining the quality and aroma components in tea leaves.

MATERIALS AND METHODS

Hydroponics culture

One-year-old Wuyi Rougui cuttings of uniform growth were selected, washed with water to remove the root soil, and cultured in hydroponic pots (28 cm long, 25 cm wide, 14 cm high) containing 8 L of nutrients solution (0.125 mM KNO_3, 0.1875 mM $(NH_4)_2SO_4$, 0.1 mM KH_2PO_4, 0.025 mMhK_2SO_4, 0.1 mM $CaCl_2$, 0.1 mM $MgSO_4{\cdot}7H_2O$, 0.016 mM $FeSO_4{\cdot}7H_2O$) with 20 tea plants in each pot (Figure 1). After 2 months of growing, tea seedlings with uniform growth were selected and transplanted into new nutrient solution containing different concentrations of Mg. The experimental treatments were: control, 0.1 mM Mg, 0.4 mM Mg, 0.6 mM Mg and 0.8 mM Mg [i.e., Mg0, Mg1, Mg2 and Mg3]. The treatments were replicated thrice in complete Randomised Design. There was 24 h continuous aeration and the culture solution was changed once a week. The plants were applied different doses of Mg for 6 weeks. Thereafter, one bud and three leaves were collected from each tea seedling. The samples were first dried at 105 ℃ for 2 h, then oven-dried for 48 h at 80 ℃ to a constant weight. Finally, the above samples were crushed, passed through 0.25 mm sieve and used for the following analysis.

Determination of quality indexes

Standard GB/T protocols were used to estimate the leaf tea constituents. These involved the reaction with specific chromogenic with following reagents: Polyphenols with forinol (4), catechins with vanillin (33), soluble sugars with anthrone (33), total flavone with aluminum trichloride (18), caffeine with alkaline lead acetate (3), and free amino acids with ninhydrin (5). Water extract content was determined by the subtraction method (6). Each of these quality indexes were tested thrice.

Figure 1. Hydroponic model for tea seedlings

Incremental ratio is very important index and needs scientific measurement.

$$P = (\Delta m/C) \times 100\ \%$$

Where, P : Incremental ratio, Δm : A unit increment, C : Control.

Determination of aroma composition

(i). **Extraction of aroma components:** 0.5 g of tea powder samples were placed in a 20 mL headspace vial, sealed by adding 5 mL of boiling water, placed at a constant temperature of 60 ℃, stirred and equilibrated for 30 min to fully volatilize the tea aroma components; a 50/30 μm DVB/CAR/PDMS extraction head was inserted into the sample headspace vial, extracted in headspace for 30 min, resolved at 240 ℃ for 5 min. Then, GC-MS separation and determination were done.

(ii). **The GC conditions：** Agilent HP-5MS column (30 m×0.25 mm, 0.25 μm), high purity helium (purity ≥ 99.999 %) as carrier gas, constant flow rate 1.0 mL/min, inlet temperature 250 °C, no split injection, solvent delay 3.5 min. programmed ramp-up: 50 °C, hold for 2 min, ramp-up to 180 °C, hold for 5 min, 10 °C/min to 220 °C, hold for 10 min (31). 2 ℃/min to 80 ℃, hold for 4 min, 5 ℃/min to 180 ℃, hold for 5 min, 10 ℃/min to 220 ℃, hold for 10 min (31).

(iii). **MS conditions：** EI ionization mode; ionization energy 70 eV; ion source temperature 230 °C; quadrupole temperature 150 °C; emission current 34.6 μA; mass scan range 50-500 amu.

The spectrum library was searched as NIST11.L, and the final characterization was done by combining the relevant literature reports and the relative retention time of each aroma component, and the relative content of the components was expressed as the ratio of the peak area of each aroma component to the total area.

Statistical Data analysis

Data of intrinsic quality indexes were subjected to PCoA (principal component analysis) performed in R language. Mean and standard deviation were calculated with Microsoft Excel 2013, and one-way ANOVA and correlation analysis were performed using SPSS 22.0 software. Least significant difference (LSD) method was used for comparisons ($P < 0.05$).

RESULTS AND DISCUSSIONS

Intrinsic quality of fresh tea leaves

The impact of magnesium on intrinsic quality indexes of fresh leaves of Wuyi Rougui tea are shown in Figure 2. Except for 0.4 mM (Mg1) treatment (48.83 %), the water extract content of most leaf samples ranged from 45 to 47 % ($p < 0.05$). The incremental ratio of 0.6 mM was the lowest, and that of 0.04 mM was the highest. Magnesium had a significant effect on the content of free amino acids in leaves. With the increase of magnesium supply, the free amino acids increased significantly. Caffeine content showed a tendency to decrease with magnesium supply. At 0.6 mM (Mg^2 treatment), higher magnesium supply resulted in the highest incremental ratio of total flavonoids (15.53 %), while at 0.8 mM (Mg^3 treatment), the lowest incremental ratio of total flavonoids. The higher magnesium availability also increased the soluble sugars from 3.31 % (control) to 4.0 %, the incremental ratio were 24.47 % and 23.87 % (Mg^2 and Mg^3 treatments). It reduced the catechin content till it reached a constant value of 70 mg/g at 0.6 and 0.8 mM.

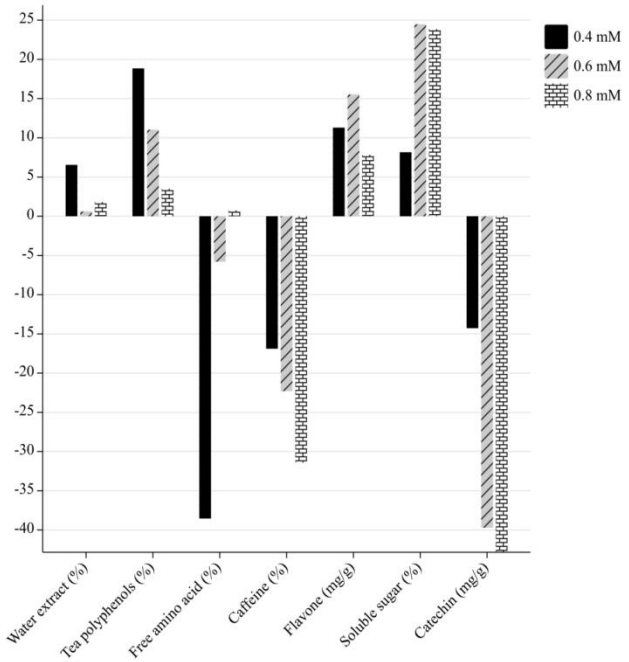

Figure 2. Effects of different Mg concentrations on the Quality indexes of Wuyi Rougui tea leaves

Hence, leaf quality indexes depended on the level of magnesium available in the hydroponic solutions. The nature of changes depended on the quality index involved. For example, magnesium improved water extract only at 0.4 mM, whereas, it slightly improved the caffeine content. At the highest Mg doses the soluble sugars were 0.4-0.6 mM. This indicated that optimum magnesium concentrations were 0.4 and 0.6 mM for best tea quality. Mg is essential for crop growth, because it is involved in most physiological and biochemical processes (22). Mg is necessary for active enzyme molecules as one of the cofactors of enzymes, such as H+-ATPase, kinase and polymerase (9). It is involved in photosynthesis of crops, including the production and distribution of photosynthetic products. Hence its deficiency decreases the photosynthetic rate and hinders the distribution of carbohydrates in plant organs, reduces the growth of storage organs and ultimately leading to low yields (14,30). Tea polyphenols, free amino acids and caffeine increased significantly in tea tree at 7 d and 15 d of Mg deficiency, while the content of catechin reached maximum at 30 d of Mg deficiency (17). In this study, the higher the Mg concentration, the lower the content of caffeine and catechin in the fresh leaves of Wuyi Rougui tea, indicating that Mg deficiency leads to accumulation of caffeine and catechin in tea trees leaves and high Mg concentration reduces the bitterness in tea leaves (28).

Principal component analysis (PCoA) of quality indexes

A PCoA relating quality indexes of tea leaves and Mg concentrations was performed (Figure 3), where components 1 and 2 contributed to 97.73 % of the total variability ($p < 0.01$). Component 1 explained 90.79 % of the differences and grouped data of treatments Mg^2 and Mg^3 in quadrants II and III of the graph, while, it located treatment Mg^1 in quadrant I and Mg^0 in quadrant IV. Samples with the same Mg concentration were grouped together and samples with different Mg concentrations were separated. This indicated that the quality indexes measured in this study can reflect the differences in quality at different Mg concentrations.

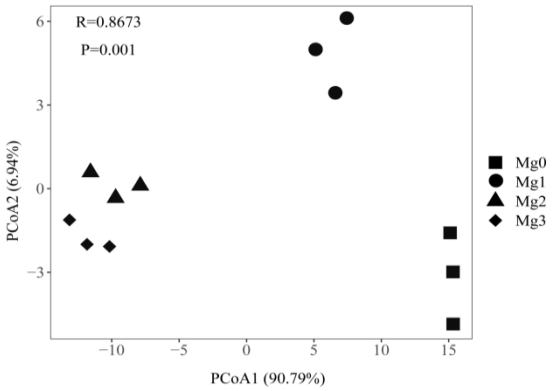

Figure 3. PCoA based on quality of tea leaves of Wuyi Rougui under different Mg concentrations

Analysis of volatile components in leaves

The volatile components provide the aroma of the fresh tea leaves. Hence, we decided to do GC-MS analysis to understand how Mg modifies them (Table 1). The analyses accounted for about 90-94 % of the compounds detected. The volatile constituents identified in the tea samples varied from 12 to 15. (E,E)-2,4-heptadienal and toluene constituted more than 60 % of the total composition of the volatiles recorded in the leaf samples. (E,E)-2,4-heptadienal content varied from 45.91 to 64.84 %, with the highest value observed in the Mg^2 treatment, while, toluene content decreased from 20.17 % (control) to 6.68 % (Mg^2 treatment) and was not detected in the Mg^3 treatment. (E,E)-2,4-heptadienal provides grassy and spicy flavours, whereas, toluene is responsible for the paint flavour. Hence, higher Mg concentrations increased grassy and spicy flavours and reduced the paint flavour.

Table 1. Analysis of main aroma components of Wuyi Rougui tea leaves regulated by Mg

No.	Main aroma components	Retention Time (min)	Relative Content (%)				Aroma Characters
			Mg^0	Mg^1	Mg^2	Mg^3	
1	Toluene	3.28	20.17	9.53	6.68	-	Paint
2	Hexanal	3.96	7.08	4.07	4.29	9.53	Grass, tallow, fat
3	2-hexenal	5.23	0.24	0.45	-	1.27	Apple, green
4	Benzeneacetaldehyde	6.42	0.33	0.44	0.52	0.81	Hyacinth
5	Benzaldehyde	9.07	1.14	5.20	5.41	4.63	Semen armeniacae amarae
6	6-methyl-5-Hepten-2-one	10.33	3.52	3.16	3.62	3.25	Fruity, fresh
7	(E,E)-2,4-heptadienal	11.45	45.91	59.87	64.84	64.69	Grass, spice flavour
8	Safranal	21.63	1.43	0.90	0.61	0.73	Potpourri
9	Decanal	22.02	1.52	1.03	0.73	0.74	Fruity
10	*β*-Cyclocitral	22.51	2.39	3.65	3.14	3.47	Fruity
11	Tridecane	25.51	4.05	-	-	-	Alkane
12	cis-3-Hexenyl caproate	28.49	0.35	0.47	0.28	0.27	Fruity
13	*α*-Cedrene	29.45	0.38	-	-	-	Cedar wood incense
14	*β*-Ionone	32.01	1.50	4.31	2.27	2.92	Violet fragrance
15	Dihydroactinidiolide	33.6	0.33	0.69	0.47	0.42	Coumarin fragrance, musk
Total			90.34	93.78	92.85	92.72	

-: Aroma component could not be detected.

Correlations between quality and main aroma components in leaves

The correlation analysis of the quality indexes obtained from leaves of Wuyi Rougui tea are shown in Table 2. There was a significant negative correlation between the catechin and soluble sugars (r = 0.990), free amino acids and water extract (r = 0.968). The concentration of Mg was negatively correlated to catechin (r = 0.959), and caffeine contents in leaves (r = 0.983), indicating that the higher the Mg concentration, the lower the content of catechin and caffeine.

The correlation analysis of aroma components of leaves from Wuyi Rougui tea trees grown under different Mg concentrations (Table 3) showed that toluene had a significant negative correlation with 2-hexenal (r = -1.000) and (E,E)-2,4-heptadienal (r = -0.999). The 2-hexenal was positively correlated with benzeneacetaldehyde (r = 1.000), whereas (E,E)-2,4-heptadienal was significantly and negatively correlated with safranal (r = -0.991) and decanal (r = -0.992). Decanal was also positively correlated with safranal (r = 0.989). Mg concentration was positively correlated to (E,E)-2,4-heptadienal content (r = 0.954) and had negative correlation with decanal content (r = -0.967).

Table 2. Correlation analysis of magnesium concentration and quality of Wuyi Rougui tea leaves

Indexes	Magnesium concentration	Water extract	Tea polyphenols content	Free amino acid content	Caffeine content	Flavone-content	Soluble sugar content
Water extract	0.132	-	-	-	-	-	-
Tea polyphenols content	0.334	0.773	-	-	-	-	-
Free amino acid content	0.011	-.968*	-0.839	-	-	-	-
Caffeine content	-.983*	-0.094	-0.197	-0.084	-	-	-
Flavone content	0.664	0.341	0.824	-0.39	-0.519	-	-
Soluble sugar content	0.926	-0.132	0.286	0.202	-0.87	0.745	-
Catechin content	-.959*	0.119	-0.221	-0.223	0.927	-0.671	-.990*

*: Significant difference, $P< 0.05$.

'Wuyi Rougi' is a highly fragrant variety of Wuyi rock tea, due to its aroma and taste, similar to the Chinese medicine cinnamon, hence, it is called "cinnamon" tea. Heptadienal is a permitted edible flavour, mainly used in the formulation of blueberry, raspberry and mixed fruit flavours. Guo *et al*. (10) described the aroma of heptadienal as fatty, green, oily and cinnamon-like. Zhan *et al*. (31) studied the effects of different roasting times on the aroma quality of Wuyi Rougui and found that heptadienal was only present in the Rougui tea after roasting, indicating that heptadienal can be transformed into other floral and fruit-like substances during the charcoal roasting process. The present study also found that heptadienal was the main aroma substance in the fresh leaves of Wuyi Rougui tea, suggesting that heptadienal plays an important role in the formation of the aroma of Wuyi Rougui variety.

Table 3. Correlation analysis of Magnesium concentration and main aroma components of Wuyi Rougui tea leaves.

Indexes	Magnesium conc	Toluene	Hexanal	2-hexenal	Benzene-acetaldehyde	Benzaldehyde	6-methyl-5-Hepten-2-one	(E,E)-2,4-heptadienal	Safranal	Decanal	β-Cyclocitral	cis-3-Hexenyl caproate	β-Ionone
Toluene	-0.991												
Hexanal	0.223	0.964											
2-hexenal	0.946	-1.000**	0.713										
Benzeneacetaldehyde	0.908	-0.973	0.608	1.000*									
Benzaldehyde	0.811	-0.988	-0.367	0.554	0.511								
6-methyl-5-Hepten-2-one	-0.276	0.113	-0.178	-0.457	-0.349	-0.315							
(E,E)-2,4-heptadienal	0.954*	-0.999*	-0.08	0.823	0.741	0.943	-0.233						
Safranal	-0.921	0.988	0.161	-0.815	-0.675	-0.94	0.111	-0.991**					
Decanal	-0.967*	0.983	0.015	-0.889	-0.774	-0.9	0.145	-0.992**	0.989*				
β-Cyclocitral	0.75	-0.816	-0.151	0.552	0.574	0.886	-0.717	0.816	-0.753	-0.745			
Cis-3-Hexenyl caproate	-0.471	0.051	-0.542	-0.673	-0.575	-0.004	-0.541	-0.31	0.344	0.424	0.231		
β-Ionone	0.387	-0.556	-0.318	0.199	0.221	0.683	-0.842	0.499	-0.427	-0.391	0.9	0.629	
Dihydroactinidiolide	0.22	-0.658	-0.64	-0.087	-0.06	0.687	-0.617	0.426	-0.399	-0.314	0.783	0.724	0.932

CONCLUSIONS

Mg is closely related to tea tree growth and leaf quality (the leaf thickness), germination of tea buds, amino acids content. Therefore, we determined the optimal magnesium concentration for better tea tree growth and leaf quality. In this study, we found that most of the intrinsic quality indexes parameters (theanine, catechins and caffeine) of Rougui tea tree leaves were significantly improved under both 0.4 mM and 0.6 mM Mg treatments. These doses also provided a distinct varietal aroma characteristics in tea leaves. Our results showed the role of Magnesium in quality improvement of Wuyi Rougui tea leaves.

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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