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Research article

Mineral and metabolic profiles in tea leaves and flowers during flower development

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ABSTRACT

Tea [*Camellia sinensis* (L.) O. Kuntze] is one of the most popular non-alcoholic beverage crops in the world, and the physiological processes and gene regulations involved in development in tea plants have been well characterized. However, relatively little is known about the metabolic changes combined with mineral distributions that occur during flower development. Here we detected the contents of 11 elements in tea leaves and flowers and found that, some of them, especially phosphorus, sulfur and copper, showed significant changes during tea flowering. We also detected 122 metabolites in tea leaves and flowers and found that, 72 of them showed significant differences between flowers and leaves, of which sugars, organic acids, and flavonoids dominated. The sugars, such as trehalose and galactose, all accumulated in tea flowers, and the organic acids, such as malic acid, citric acid and fumaric acid involved in TCA cycle. The flavonoids, like epicatechin, catechin gallate and epigallocatechin, were more abundant in leaves. Furthermore, we found that the contents of 33 metabolites changed during the development of flowers. Especially, citric acid, phenylalanine and most flavonoids decreased while fructose and galactose increased during flowering stages in flowers. We also analyzed the correlations between the ions and metabolites and found that, some mineral nutrients including phosphorus, sulfur, manganese and zinc had close relations to organic acids, flavonoids, sugars and several amino acids during flowering. We mapped the metabolic pathway according to the KEGG database. This work will serve as the foundation for a systems biology approach to the understanding of mineral metabolism.

1. Introduction

Tea [*Camellia sinensis* (L.) O. Kuntze] is an evergreen woody perennial and bears flowers after 4–5 years being planted. Flowering, consuming large amounts of nutrients, restrains vegetative growth and severely influences the yield and quality of tea. Thus, in order to increase the yield and quality of tea, we should take some technologies to reduce quantities of flowers, which could avoid the nutrient consumption in tea plants.

In recent years, many researchers devoted to study the distribution of mineral nutrients and some metabolites in other plants. It was demonstrated that some mineral nutrients (such as N, P and Ca), as well as metabolites (sugars) changed significantly during flowering in *Hibiscus rosa-sinensis* (Trivellini et al., 2011). It was also described that the flowers had a higher concentration of N, P, and K, but a lower Mn concentration than in leaves in *Ceratonia silique* (Custodio et al., 2007). And it was reported that the increase in accumulation of N, P, K, Ca, and Mg preceded the dry matter accumulation of *Tulipa gesneriana* (Niedelzia et al., 2015). However, in tea plants, most studies focused on the molecular mechanisms and some biochemical regularities during flowering (Joshi et al., 2011; Liu and Han, 2010; Yang et al., 2012; Zhang et al., 2015). The systematic mineral distributions and metabolite changes in tea leaves and flowers during tea flower development are largely unexplored.

In order to investigate the characteristic of mineral and metabolic changes involved in tea flowering, we conducted metabolomic and ionomic studies to capture the relevant profiles from tea

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leaves and flowers. We hope that this work would provide the framework for a better understanding of the flowering molecular mechanism of tea plants, and the resulting information would facilitate the effective management of tea plantation and consequently reducing the nutrient consumption during flowering.

2. Materials and methods

2.1. Plant materials

The tea plants, *C. sinensis* cv. QN06, were planted in the plantation of Tea Research Institute, Qingdao Agricultural University in Shandong province of China (36°19′N, 120°23′ E, 54.88 m above sea level). The soil samples were obtained from the tea plantation by soil auger and detected by ICP-OES (Inductively coupled plasma-optical emission spectrometry, PE companies in the United States). The nutrient status of the soil is shown in Appendix A-Table S1.

To guarantee comparability of data, all samples were produced from the same growth status. Flowers and leaves of tea plants were harvested on October 13, 2014 at three opening stages of flower development: alabastrum (F1), half opened flower (F2) and full bloom (F3). The leaves locating at the positions corresponding to the different stages of flowers were named L1, L2 and L3, respectively (Fig. 1). We harvested all samples on a single day to avoid the influences caused by the different temperature, water, light and so on (Yang and Wei, 2015). Materials were divided into two parts, one part was collected and dried for element determinations (each developmental stage in four replicates, 24 samples in total); the other part was immediately frozen in liquid nitrogen, and then stored at −80 °C for metabolome analysis (each developmental stage in four replicates, 24 samples in total).

2.2. Pre-processing for metabolome

The frozen tea leaves and flowers were grinded into fine powders under liquid nitrogen by a chilled pestle and mortar. Metabolite extraction for gas chromatography-mass spectrometer (GC-MS) was carried out as described in previous research with little modification (Shen et al., 2015).

The frozen samples for the method, ultra high performance liquid chromatography time of flight tandem mass spectrometry (UHPLC-TOF-MS/MS), were also homogenized to fine powders and a subsample was transferred to a 15 mL plastic eppendorf tube with the same method of GC-MS. A 1000 μL aliquot of chilled methanol (−20 °C) was added. This was followed by vortex-mixing for 30 s and transferred it to ultrasonic cleaner (70 °C) for 15 min. Then 500 μL chloroform and 1000 μL ddH_2O were added. The mixture was vortexed for 1 min and centrifuged for 15 min (4000 rpm). The supernatant was transferred to a new plastic eppendorf tube and passed through a 0.2 μm filter membrane.

We introduced the quality control (QC) samples, to ensure the stability of the system. In order to avoid system error, all the samples were injected into apparatus randomly.

2.3. Detection procedure by GC-MS and UHPLC-TOF-MS/MS

Agilent 7890A/5975C GC-MS system (Agilent, USA) was used for GC-MS and operated as previous research (Shen et al., 2015).

Acquity Ultra Performance LC system (Waters, USA) was used in this study and operated as previous research with little modification (Degu et al., 2014). Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm, Waters, USA) maintained at 40 °C. Sample volume of 4 μL with the partial loop injection mode was used in all experiments. Autosampler temperature was kept at 4 °C. The mobile phase consisted of 0.1% aqueous formic acid (A) and 0.1% formic acid in acetonitrile (B) with flow 0.3 mL min^{-1}. The following linear gradient elution was carried out at 0.5 min eluent B 2%; 0.5–9 min eluent B 2–50%; 9–12 min eluent B 50–98%; 12–13 min eluent B 98%; 13–14 min eluent B 98-2%. Balance the chromatographic column 1 min and take the next sample. Keep the sample size at 2 μL every time, column temperature at 40 °C, and automatic injector temperature at 4 °C. The leucine enkephalin was used as the lock and spray (0.4 ng L^{-1}, 0.1% formic acid, CAN/H_2O 50/50).

The mass spectrometer was equipped with an electrospray ionization (ESI) source. In the ESI, parameter settings used for the measurement were as follows: capillary voltage in positive ESI (+3000 V), capillary voltage in negative ESI (−2800 V), sampling cone voltage (27 eV), extraction cone voltage (4 eV), collision energy voltage (6 eV), source temperature (120 °C), desolvation temperature (300 °C). Nitrogen was used as desolvation and cone gas at flow 650 L h^{-1} and 50 L h^{-1}. Mass spectra were acquired using full scan monitoring mode with a mass scan range of 50–1500 $m \cdot z^{-1}$.

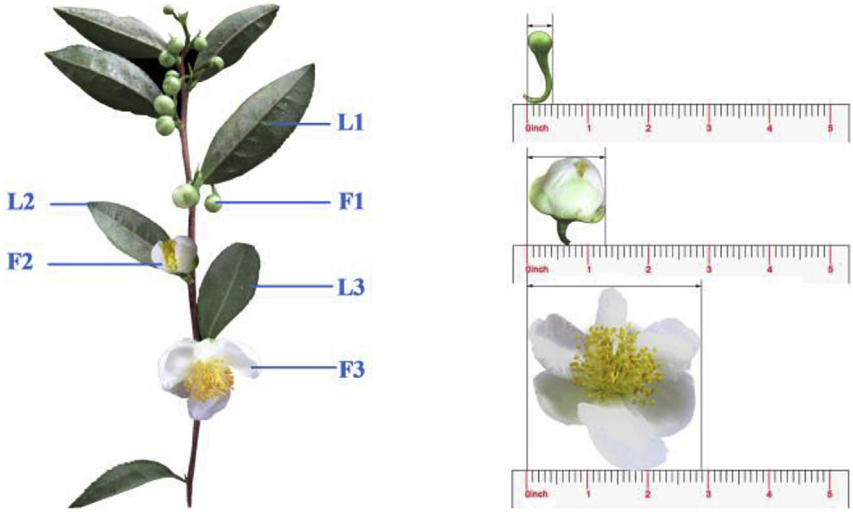

Fig. 1. The morphological characteristics of three development stages in this study. F1: sepals separated and their ends just started to go away from each other; F2: petals split and half opened; F3: full bloom. L1: the leaf locating at the same leaf position with F1; L2: the leaf locating at the same leaf position with F2; L3: the leaf locating at the same leaf position with F3.