Plant Science 264 (2017) 29–36

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

α-Farnesene and ocimene induce metabolite changes by volatile signaling in neighboring tea (*Camellia sinensis*) plants

Lanting Zeng[a,b,1], Yinyin Liao[a,b,1], Jianlong Li[c], Ying Zhou[a], Jinchi Tang[c], Fang Dong[d], Ziyin Yang[a,b,*]

[a] *Guangdong Provincial Key Laboratory of Applied Botany & Key Laboratory of South China Agricultural Plant Molecular Analysis and Genetic Improvement, South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Tianhe District, Guangzhou 510650, China*
[b] *University of Chinese Academy of Sciences, No. 19A Yuquan Road, Beijing 100049, China*
[c] *Tea Research Institute, Guangdong Academy of Agricultural Sciences & Guangdong Provincial Key Laboratory of Tea Plant Resources Innovation and Utilization, Dafeng Road 6, Tianhe District, Guangzhou 510640, China*
[d] *Guangdong Food and Drug Vocational College, Longdongbei Road 321, Tianhe District, Guangzhou 510520, China*

ARTICLE INFO

Keywords:
Aroma
Camellia sinensis
Metabolite
Signaling
Tea
Volatile

ABSTRACT

Herbivore-induced plant volatiles (HIPVs) act as direct defenses against herbivores and as indirect defenses by attracting herbivore enemies. However, the involvement of HIPVs in within-plant or plant-to-plant signaling is not fully clarified. Furthermore, in contrast to model plants, HIPV signaling roles in crops have hardly been reported. Here, we investigated HIPVs emitted from tea (*Camellia sinensis*) plants, an important crop used for beverages, and their involvement in tea plant-to-plant signaling. To ensure uniform and sufficient exposure to HIPVs, jasmonic acid combined with mechanical damage (JAMD) was used to simulate herbivore attacks. Metabonomics techniques based on ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry and gas chromatography-mass spectrometry were employed to determine metabolite changes in undamaged tea plants exposed to JAMD-stimulated volatiles. JAMD-stimulated volatiles mainly enhanced the amounts of 1-*O*-galloyl-6-*O*-luteoyl-α-D-glucose, assamicain C, 2,3,4,5-tetrahydroxy-6-oxohexyl gallate, quercetagitrin, 2-(2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-*4H*-chromen-8-yl)-4,5-dihydroxy-6-(hydroxymethyl)-tetrahydro-*2H*-pyran-3-yl, 3,4-dimethoxybenzoate, 1,3,4,5,6,7-hexahydroxyheptan-2-one, and methyl gallate in neighboring undamaged tea leaves. Furthermore, α-farnesene and β-ocimene, which were produced after JAMD treatments, were identified as two main JAMD-stimulated volatiles altering metabolite profiles of the neighboring undamaged tea leaves. This research advances our understanding of the ecological functions of HIPVs and can be used to develop crop biological control agents against pest insects in the future.

1. Introduction

In response to herbivore attacks, plants, especially the vegetative parts, synthesize and emit volatile compounds, which have direct and/or indirect defensive functions against herbivores [1,2]. The events occurring between herbivore attacks and volatile emissions include plasma transmembrane potential and intracellular cytosolic Ca^{2+} concentration changes, H_2O_2 formation, activation of kinases, and jasmonic acid (JA) and salicylic acid (SA) signaling [3]. The genes involved in the formation of plant volatiles can be activated by phytohormones, especially JA, which leads to the increased emission of herbivore-induced plant volatiles (HIPVs) [4]. Though emitted HIPVs are known to be involved in direct defenses against herbivores and indirect defenses by attracting herbivore enemies [2], they also play important but relatively unappreciated roles in within-plant or plant-to-plant signaling. HIPVs signaling that improves the defensive capabilities of neighboring tissues or plants have been validated in lima bean [5] and hybrid poplar [6]. Recent studies indicate that volatiles can affect neighboring tissues or plants at gene or metabolite levels, which were validated in *Arabidopsis*, *Nicotiana tabacum*, and tomato plants [7,8]. In contrast to model plants, there are only limited reports of HIPVs signaling in crops.

Tea (*Camellia sinensis*) is an important crop in almost 30 countries, including China, Japan, India, and Kenya. In tea plants, volatile compounds are a main characteristic metabolite, affecting the quality of tea

---

* Corresponding author at: Guangdong Provincial Key Laboratory of Applied Botany & Key Laboratory of South China Agricultural Plant Molecular Analysis and Genetic Improvement, South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Tianhe District, Guangzhou 510650, China.
E-mail address: zyyang@scbg.ac.cn (Z. Yang).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.plantsci.2017.08.005
Received 1 May 2017; Received in revised form 10 August 2017; Accepted 11 August 2017

product. Tea volatiles are not only responsible for the aromatic qualities of tea leaves [9], but may also be involved in the defense responses of tea plants against herbivore attack [10]. When exposed to attacks by main pest insects of tea crops, such as the smaller tea tortrix or Kanzawa spider mite, tea leaves emit numerous HIPVs, such as (*Z*)-3-hexen-1-ol, linalool, α-farnesene, benzyl nitrile, indole, nerolidol, and ocimenes, at high concentrations, which are similar to those induced by exogenous JA applications [10]. Furthermore, a principal component analysis (PCA) of metabolites (*m/z* 70–1000) in undamaged tea (*C. sinensis*) leaves with exposure (treatment group) or without exposure (control group) to HIPVs revealed that external signaling through these volatiles may lead to more drastic changes in the metabolite spectrum of tea leaves than internal signaling through vascular connections [10]. However, the following key questions remain to be answered: (1) What are the changed metabolites induced by volatile signals? and (2) What are the main volatile signals that could induce changes in metabolites? To answer these questions, metabonomics techniques based on ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC–QTOF–MS) and gas chromatography-mass spectrometry (GC–MS) were employed to determine the metabolites that underwent changes in undamaged tea plants after exposure to JA combined with mechanical damage (JAMD)-stimulated volatiles. Moreover, the main JAMD-stimulated volatiles that could induce changes in metabolites were determined. The results will provide evidence for the involvement of HIPVs in plant-to-plant signaling by altering metabolite profiles and advance our understanding of the ecological functions of HIPVs. In addition, the information should be helpful in helping to develop crop biological control agents against pest insects.

2. Materials and methods

2.1. Plant materials and treatments

The branches of *C. sinensis* var. Yinghong No. 9, which is the most popular tea cultivar in South China, were plucked from plants at the Tea Research Institute, Guangdong Academy of Agricultural Sciences (Yingde, China) in January 2017. First experimental design was used to investigate plant-to-plant communications in *C. sinensis*. The purpose of this study was mostly focused on the neighboring plants with exposure to some volatiles or without these volatiles, and to investigate effects of these volatiles on metabolic profiles of the neighboring plants. Here, insect treatment was replaced by JA treatment (combined with mechanical damage). The treatment groups were JA treated tea plants and the neighboring plants exposed to the JA treated tea plants. The control groups were undamaged tea plants and the neighboring plants exposed to the undamaged tea plants (if mechanical damaged tea plants were used as control, mechanical damage induced-volatiles may have effects on the neighboring plants). JA was dissolved in H_2O containing 1% dimethyl sulphoxide (DMSO). The amount of JA was determined according to the previous studies [4,10,11]. In these references, 0.3–6 μmol of JA were used to treat each plant. In the present study, a total of 0.6 μmol JA (2.5 mM, 240 μL/4 leaves/branch) was used to treat each tea branch. Tender leaves from these branches were exposed to the following treatments (Fig. 1): (1) daubed with H_2O containing 1% DMSO (60 μL/leaf) (CK); (2) nontreated and next to CK (CK-neighboring); (3) JA (2.5 mM, 60 μL/six holes/leaf) treatment after a single mechanical damage by needle (JAMD); (4) exposed to JAMD-stimulated volatiles (JAMD-neighboring). Then, after treatment for 2 and 4 d, the emitted volatile metabolites of each group were subsequently collected by a headspace volatile sampling system. The samples were immediately frozen in liquid nitrogen and stored at −80 °C until further analysis.

The branches of *C. sinensis* var. Yinghong No. 9 were plucked in April 2017. Second experimental design was used to investigate α-farnesene and ocimene regulation of plant-to-plant communications in *C. sinensis*. Tender leaves from these branches were exposed to the following treatments: (1) exposed to H_2O (adding 250 μL/day, total 1000 μL) (CK); (2) exposed to an α-farnesene standard (adding 250 μg/day, total 1000 μg) (Farnesene); (3) exposed to an ocimene standard (adding 250 μg/day, total 1000 μg) (Ocimene). The standards (liquid form) and H_2O were added to cotton. Cai et al. (2014) found that the contents of emitted α-farnesene and β-ocimene induced by herbivors were 268–3500 ng/h·tea plant and 376–1533 ng/h·tea plant, respectively [12]. In the first experimental design (Fig. 1), ten branches of *C. sinensis* were used to JAMD treatment in each treatment device. The content of emitted JAMD-stimulated volatiles from each branch was assumed the same as that from each plant. Therefore, 1000 μg of each authentic standard were used for the treatments. After treatment for 4 d, the samples were immediately frozen in liquid nitrogen and stored at −80 °C until further analysis.

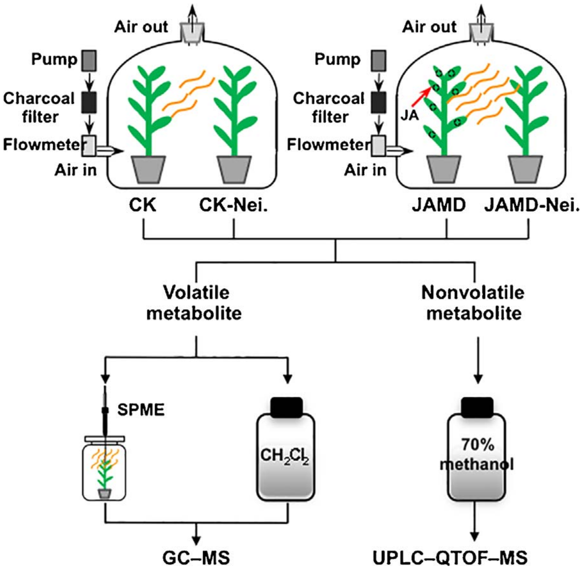

Fig. 1. Experimental design for investigating plant-to-plant communications in *Camellia sinensis*.

The black arrow indicates the airflow direction. The air was circulated using a pump. The intake air was filtered through a short plug of charcoal, and airflow was controlled using a flowmeter. The samples were collected at 2 or 4 d after treatment. GC–MS, gas chromatography–mass spectrometry; JAMD, jasmonic acid combined with mechanical damage; SPME, solid-phase microextraction; Nei., neighboring; UPLC–QTOF–MS, ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry; CK, daubed with H_2O containing 1% DMSO (60 μL/leaf); CK-neighboring, nontreated and next to CK; JAMD, JA (2.5 mM, 60 μL/six holes/leaf) treatment after a single mechanical damage by needle; JAMD-neighboring, exposed to JAMD-stimulated volatiles.

2.2. Extraction of emitted volatile metabolites by SPME

A solid-phase microextraction (SPME) fibers (2 cm-50/30 μm DVB/CarboxenTM/PDMS Stable FlexTM, Supelco Inc., Bellefonte, PA, USA) was used to collect the emitted volatile metabolites from samples according to the previous method with a slight modification [13]. The branches of each group were put into a sealed container and the volatile metabolites were collected under controlled conditions (25 ± 2 °C, 70–80% humidity) by SPME fiber for 30 min. There were four groups including CK, CK-neighboring, JAMD, and JAMD-neighboring in the present study. Five independent replicates of each group were performed. After collection, the SPME fiber with absorbing volatile metabolites was subjected to GC–MS analysis. The content of each metabolite from samples was calculated based on its peak area.

2.3. Extraction of endogenous volatile metabolites by dichloromethane

The method used to extract and analyze the endogenous volatile