

Postprint: Quantitative Analysis Methods for Odor Compounds in *Siraitia grosvenorii* Flowers

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Abstract

Siraitia grosvenorii (monk fruit) is a renowned medicinal and edible plant in the Cucurbitaceae family, widely cultivated in the Guilin region of Guangxi. The phenomenon of poor pollination following flowering urgently requires research and resolution. This study utilized male flowers of *S. grosvenorii* as experimental material to investigate quantitative analysis methods for floral scent compounds, aiming to establish a foundation for elucidating the relationship between floral scent substances and pollinator visitation frequency, ultimately identifying the causes of poor pollination. The experiment employed dynamic headspace adsorption to collect scent compounds from fresh flowers; through procedures including elution, nitrogen purging of the eluate, and GC-MS analysis, the collection, concentration, separation, and identification of floral scent substances were sequentially accomplished. Finally, the relative content of each chemical component was calculated using peak area normalization. Results indicated that the tested flowers contained volatile components including five terpenoid compounds, and one each of aromatic hydrocarbons, alkanes, and esters. Among these, the relative content of terpenoids reached 71.07%, constituting the predominant volatile compounds in the tested flowers. These results are highly consistent with the chemical composition characteristics of floral scents in Cucurbitaceae plants and demonstrate good experimental reproducibility, indicating that this experimental system represents an ideal method for collecting and identifying floral scent components of *S. grosvenorii*, and has laid an important foundation for subsequent research on monk fruit floral scent substances. Simultaneously, through comparison with multiple Cucurbitaceae species, it was discovered that floral scent compounds in *S. grosvenorii* may exhibit sexual dimorphism.

Full Text

A New Method for Quantitative Analysis of Flower Scent of *Siraitia grosvenorii*

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Abstract

Siraitia grosvenorii is a renowned edible and medicinal plant in the Cucurbitaceae family, widely cultivated in the Guilin region of Guangxi. Poor pollination following anthesis represents an urgent problem requiring investigation and resolution. This study examined quantitative analysis methods for floral scent compounds in *S. grosvenorii* using male flowers as experimental material, aiming to establish a foundation for investigating the relationship between floral scent composition and pollinator visitation frequency, ultimately clarifying the causes of pollination failure. Floral volatiles were collected from fresh flowers using dynamic headspace adsorption, followed by elution, nitrogen blowing of the eluent, and GC-MS analysis. This sequence of procedures enabled collection, concentration, separation, and identification of floral scent compounds, with relative contents of each chemical component calculated using the peak area normalization method. Results revealed that the tested flowers contained five terpenoid compounds, along with one aromatic hydrocarbon, one alkane, and one ester. Terpenoids constituted the dominant volatile compounds with a relative content of 71.07%. These findings align closely with the chemical composition characteristics of Cucurbitaceae floral scents and demonstrate good experimental repeatability, indicating that this experimental system represents an ideal method for collecting and identifying floral scent components in *S. grosvenorii*, thereby laying an important foundation for subsequent research. Furthermore, comparison with multiple Cucurbitaceae species suggests that *S. grosvenorii* floral scent may exhibit sexual dimorphism.

Keywords: *Siraitia grosvenorii*, floral scent, dynamic headspace adsorption, GC-MS analysis, Cucurbitaceae

Introduction

Among the traits plants use to attract animal pollinators, those eliciting visual and olfactory recognition responses play crucial roles (Delle-Vedove et al, 2011). However, existing research has primarily focused on visually reactive floral traits such as color (Peter & Johnson, 2008; Sara et al, 2016), corolla size, and shape (Mason & Anne, 2012), revealing important pollination and breeding mechanisms (Brodmann et al, 2009; Shuttleworth et al, 2010). In con-

trast, olfactory traits have received comparatively less attention (Raguso, 2008). The advent of highly sensitive analytical methods such as gas chromatography-mass spectrometry (GC-MS) has dramatically improved detection capabilities for trace components of floral volatile organic compounds (VOCs), enabling in-depth quantitative studies of floral scent (Delle-Vedove et al, 2017). To date, floral scent compounds have been detected in over one thousand plant species (Knudsen et al, 2006), with some compounds demonstrated to guide pollinators (Schlumpberger & Raguso, 2008; Shuttleworth & Johnson, 2009).

While GC-MS has solved the problem of precisely detecting scent compound chemistry, methods for collecting floral volatiles require further development and refinement. Traditional approaches such as solvent extraction, steam distillation, and supercritical fluid extraction require drying and pulverizing flowers before solvent immersion or high-temperature collection (丁嘉文等, 2015; 赵彦贵和张慧娟, 2018). Because these methods destroy the natural state of flowers, the identified volatile compounds differ significantly from those released under natural conditions, making them generally unsuitable for pollination biology research. Solid-phase microextraction, a later development, can collect scent compounds while maintaining flowers in their natural state, but its limitation is that it can only be performed under laboratory conditions and cannot be used for field collection of floral scents (宋述芹等, 2017). To address these shortcomings, Jennings et al (1972) proposed dynamic headspace adsorption to enable field collection of fresh floral volatiles. This method operates by placing fresh flowers in a semi-closed container and pumping air through the sealed circuit, carrying released scent components out of the container to an adsorption column where they are captured by adsorbents. After collection, adsorption columns can be stored in the field for extended periods before being transported to the laboratory for elution and analysis. Chinese scholars Li Qingliang et al (2012) subsequently improved this method, making it more convenient and applicable for field experiments.

Siraitia grosvenorii is a perennial vine in the Cucurbitaceae family whose fruit serves both edible and medicinal purposes. As a famous specialty of Guangxi, it constitutes an important component of the region's agricultural economy (李典鹏和张厚瑞, 2000). However, *S. grosvenorii* exhibits severe natural pollination failure after flowering and requires artificial pollination to set fruit, resulting in high cultivation costs that represent a major limiting factor for industry development. Given the important role of floral scent in attracting pollinators, pollination failure in *S. grosvenorii* may be related to abnormal floral scent compounds. Therefore, collecting and accurately detecting the chemical composition of *S. grosvenorii* floral scent, and comparing it with closely related Cucurbitaceae species, will help identify the causes of pollination failure and is of great significance for promoting the development of *S. grosvenorii* cultivation. Considering the advantages and disadvantages of different scent collection methods, dynamic headspace adsorption is more suitable for collecting and analyzing fresh floral volatiles in *S. grosvenorii*, though this method has not yet been successfully applied to this species. This study explored the use of dynamic

headspace adsorption to collect *S. grosvenorii* floral volatiles and GC-MS to analyze their composition and content, aiming to develop a technical system for precise analysis of *S. grosvenorii* floral scent and lay an important foundation for in-depth research on this topic.

1. Materials and Methods

1.1 Materials

To test the accuracy and stability of the experimental system, replicate experiments were conducted using flowers from a single plant. Under natural conditions, simultaneously opened flowers from one plant typically have identical scent compounds, which facilitates testing system stability across replicates. *Siraitia grosvenorii* is a dioecious plant, and since individual male plants produce far more flowers than female plants, sufficient flowers could be obtained for replicate experiments; therefore, male flowers were used as experimental material. In late September 2016, 30 fresh male flowers were quickly cut from a male *S. grosvenorii* plant at peak bloom in the experimental nursery of Guangxi Institute of Botany. The flowers were randomly divided into three groups of 10 flowers each for testing.

1.2 Instruments and Reagents

The following equipment and materials were used: HP7890A (GC)-5975C (MS) gas chromatography-mass spectrometer (Agilent, USA); Tedlar polyvinyl fluoride (PVF) collection bags (8 L, Dupont, USA); low-flow gas sampling pump (Libra-4, Beijing Saifulaibo Technology Co., Ltd.); activated carbon tubes (outer diameter 6 mm, length 75 mm); adsorption columns (100 mg/50 mg, 60 mesh/80 mesh, outer diameter 6 mm, length 75 mm, Sigma-Aldrich, USA); odorless Teflon tubes (PTFE, Dupont, USA); brown sample vials (2 mL, Agilent, USA); adsorbent (PoraPak Q 80-100, Waters, Ireland); n-hexane (chromatographic grade, Fisher, USA); ear wash bulbs; needle filters; custom cylindrical glass tubes (height 15 cm, inner diameter 5 cm, Tianjin Linghang Experimental Equipment Co., Ltd.).

1.3 Scent Collection System

Dynamic headspace adsorption (黄代红等, 2015) was used to collect *S. grosvenorii* floral volatiles. Freshly collected flowers were quickly placed inside a custom cylindrical glass tube, which was then enclosed in a Tedlar PVF collection bag sealed at both ends with clamps. Teflon tubes connected the sampling bag to an activated carbon tube, adsorption column, and gas sampling pump in series [Figure 1: see original paper]. When the gas sampling pump was activated, air was purified through the activated carbon tube before entering the sampling bag. After passing over the *S. grosvenorii* flowers in the glass tube, the air entered an adsorption column packed with PoraPak Q adsorbent, where floral volatiles were captured [Figure 1: see original paper]. The air flow rate was set

at $400 \text{ mL} \cdot \text{min}^{-1}$ and collection lasted 4 hours. Three identical systems were used simultaneously to collect volatiles from the three flower groups.

After collection, three clean brown sample vials were each filled with 500 μL of n-hexane. The three adsorption columns were vertically inserted into separate vials, with the n-hexane level just covering the lower opening of each column. An ear wash bulb was attached to the upper opening of each column, and n-hexane was repeatedly drawn up and down 30 times to elute the adsorbent. This yielded three eluent samples, which were then concentrated under nitrogen flow to approximately 50 μL for subsequent GC-MS analysis.

1.4 Gas Chromatography Analysis

Based on the method of 黄代红等 (2015) with appropriate optimization, the following GC conditions were used: HP-5MS quartz capillary column ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$, Agilent, USA); temperature program: hold at $40 \text{ }^\circ\text{C}$ for 5 min, ramp at $3 \text{ }^\circ\text{C} \cdot \text{min}^{-1}$ to $100 \text{ }^\circ\text{C}$, then ramp at $5 \text{ }^\circ\text{C} \cdot \text{min}^{-1}$ to $200 \text{ }^\circ\text{C}$ and hold for 5 min; injection volume 2 μL ; split ratio 4:1; injector temperature $250 \text{ }^\circ\text{C}$; carrier gas high-purity He at flow rate $1.0 \text{ mL} \cdot \text{min}^{-1}$.

1.5 Mass Spectrometry Analysis

MS conditions: electron ionization (EI) mode; mass scan range m/z 35-450; electron energy 70 eV; interface temperature $280 \text{ }^\circ\text{C}$; ion source temperature $230 \text{ }^\circ\text{C}$; quadrupole temperature $150 \text{ }^\circ\text{C}$. Compounds detected by GC were identified by searching the NIST 05 standard spectral library, and relative percentages of each scent component were calculated using the peak area normalization method.

2. Results

2.1 GC-MS Analysis of *S. grosvenorii* Floral Volatiles

Eluent samples were analyzed by GC-MS to obtain total ion chromatograms. The three replicate samples produced very similar total ion chromatograms, showing 13 component peaks between 7 and 41 minutes [FIGURE:2, showing only the chromatogram from replicate 1]. Library searching identified 5 impurity peaks and 8 volatile organic compound peaks.

2.2 Composition of *S. grosvenorii* Floral Scent

The three eluent samples yielded identical scent compound compositions, each consisting of 8 compounds: 5 terpenes—(S)- α -pinene, β -pinene, camphene, γ -myrcene, and limonene; 1 alkane—acetic acid, [bis[(trimethylsilyl)oxy]phosphinyl]-trimethylsilyl ester; 1 aromatic hydrocarbon—1,3-diethylbenzene; and 1 ester—2,6-bis(trimethylsilyloxy)benzoic acid trimethylsilyl ester. Not only were the chemical compositions identical across the three replicates, but the relative contents of each component were also very similar. In all three samples,

terpenes were the most abundant class, with a cumulative relative content of 71.07%. Limonene was the single most abundant component at 37.58%. Among the remaining components, aromatic hydrocarbons accounted for 4.25%, alkanes for 1.78%, and esters for 0.52% .

Discussion

The method of collecting plant floral volatiles using dynamic headspace adsorption followed by precise component analysis via GC-MS offers high analytical accuracy and quantification capability. Additional advantages include the lightweight, portable collection system and the ability to store adsorption columns for extended periods, making it particularly suitable for field collection of floral volatiles in plant pollination and evolutionary research. For example, Chinese scholar 黄代红等 (2016) used this method to identify chemical components in female and male flowers of *Phyllanthus microcarpus*, discovering sexual dimorphism in floral scent and speculating that this represents an adaptation to pollinators with highly specific pollination behaviors. Similarly, 张振国等 (2016) used this method to collect volatiles from female and male flowers of *Epicephala ancylopa*, identifying 24 volatile compounds with monoterpenes and sesquiterpenes as the main components, which were hypothesized to be the primary scent compounds attracting the pollinator *Epicephala ancylopa*.

In this study, we optimized the dynamic headspace adsorption combined with GC-MS analysis method according to the characteristics of *S. grosvenorii* flowers, establishing an experimental system for quantitative analysis of *S. grosvenorii* floral scent. The experiment detected 8 volatile components including terpenes, alkanes, aromatic hydrocarbons, and esters. Previous studies have shown that these volatile components are small, easily volatilized molecules synthesized through plant secondary metabolism and constitute the primary scent compounds of floral organs (Dudareva et al., 2013; 蒋冬月等, 2011). The relatively complete detection of these components in our experiment indicates successful collection of floral scent compounds. Comparison with floral scents of closely related Cucurbitaceae species confirms that these volatile components originated from *S. grosvenorii* flowers. In the floral scent analysis of *Luffa acutangula* and *Momordica charantia* by Fernando & Grün (2001), the main components were terpenes, aromatic hydrocarbons, and esters, showing high similarity to our results. Specifically, among terpenoid compounds, several shared compounds including -pinene, -pinene, and -myrcene were detected in both their study and ours (see TABLE 1). *Cucurbita moschata* flowers provide an even better reference: the scent of male *C. moschata* flowers consists primarily of terpenes, alkanes, aromatic hydrocarbons, and esters, with terpenes showing the highest relative content at approximately 49.59% (李昌勤等, 2012), which is almost identical to our results except that terpene content was higher in our study at 71.07%. Of course, some Cucurbitaceae species show different scent profiles; for example, *Trichosanthes kirilowii* flowers contain only benzaldehyde, phenylacetaldehyde, and aromatic alcohols (Miyasi et al.,

1998), which differs markedly from our results for *S. grosvenorii* and from those for bitter gourd, sponge gourd, and pumpkin.

Overall, our experimental results show high similarity to floral scent compounds of multiple *S. grosvenorii* relatives, confirming that these volatile components represent the scent of male *S. grosvenorii* flowers. Moreover, the high degree of similarity in chemical components across the three experimental replicates demonstrates good stability of the experimental system. In summary, the experimental system designed in this study for quantitative analysis of *S. grosvenorii* floral scent is feasible and effective, laying an important foundation for systematic research on *S. grosvenorii* floral scent. Using male *S. grosvenorii* flowers, we found scent compounds highly similar to those of sponge gourd, bitter gourd, and pumpkin. Considering that these three species do not experience pollination failure, the pollination deficiency in *S. grosvenorii* suggests possible sexual dimorphism in floral scent, where female and male flowers may have lost their ability to equally attract pollinators. This hypothesis warrants further investigation and verification.

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