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Application News

Imaging Mass Spectrometry Microscope, iMScope™ QT

A Study of the Spatial Distribution of Gossypol and Other Terpenoids in Cotton Leaves and Ovules

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User Benefits

- High spatial resolution(5 μm) of the integrated optical microscopy and MS imaging technique that enables observation of the spatial distribution of endogenous metabolites in tiny areas (e.g., in glands)
- Uniform and fine matrix crystal (sub-microscale in size) coating by matrix sublimation that further supports the high spatial resolution
- Highly-sensitive MS imaging that yields high definition and tissue-specific distribution images of gossypol and hemigossypolone in the glands in cotton ovule despite the MS signal intensity differs by a factor of more than 1000 between the two chemicals

■ Introduction

Terpenoids are a diverse group of metabolites. Plants synthesize different terpenoids in response to different environments, which can attract pollinators and seed dispersers, defend against pathogens and herbivores. Terpenoids are synthesized in specific organs of different cell types. Cotton plants possess lysigenous glands in their leaves, stems, and seeds, which accumulate various non-volatile terpenoids such as gossypol and hemigossypolone. But direct visual evidence is lacking.

iMScope QT mainly consists of a high-resolution optical microscope, an atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI) ion source and an quadruple-time-of-flight (Q-TOF) analyzer. We can not only use iMScope QT to observe the morphology of the sample in detail, but also to obtain the distribution and content information of compounds in specific parts of the sample. The spatial distribution of gossypol and other terpenoids (hemigossypol and hemigossypolone) in cotton leaves and cotton ovules was studied using iMScope QT. It was found that gossypol, hemigossypol and hemigossypolone were mainly distributed in the glands, and the content of gossypol in cotton ovule was much higher than that in leaves, which was consistent with literature reports. In addition, the study also revealed that the distribution level of hemigossypolone is high in leaf but low in cotton ovule. The above findings provide a reference for the study of the synthesis and transformation mechanism of gossypol and other terpenoids, and also indicate that iMScope QT is a reliable method for both microscopic observation and MS imaging studies of various compounds.

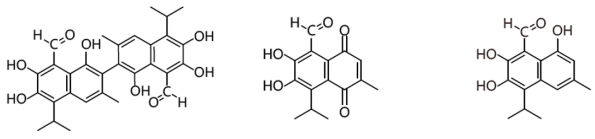

Figure 1. Structural formula of gossypol and other terpenoids

■ Sample pretreatment and instrumental analysis conditions

In this study, the samples were leaves and ovules from fresh cotton plant, and the matrix was CHCA (α-cyano-4-hydroxy cinnamic acid). For detailed information of gossypol, hemigossypol, and hemigossypolone reference standards, refer to Table 1.

Table 1. Information of reference standards

Name	Molecular Formula	Molecular weight$_{mono}$(Da)
Gossypol	$C_{30}H_{30}O_8$	518.1941
Hemigossypol	$C_{15}H_{16}O_4$	260.1049
Hemigossypolone	$C_{15}H_{14}O_5$	274.0841

Preparation of slides: The cotton ovules were embedded in 10% gelatin (wt/vol) solutions and rapidly frozen using liquid nitrogen for 2 min to obtain frozen gelatin blocks suitable for sectioning. Subsequently, sections with a thickness of 10 μm were obtained using a freezing microtome equipped with a retraction system (Leica Biosystems CM1950, Wetzlar, Germany) at a temperature of -20 ˚C . The resultant sections were directly placed on indium-tin-oxide coated glass slides, which were pre-dried in a 50-mL centrifuge tube containing desiccant for 20 min prior to matrix application. As for cotton leaves, they were directly affixed onto indium tin oxide conductive glass using conductive double-sided adhesive tape.

Matrix coating: The slides were coated with CHCA (a-cyano-4-hydroxycinnamic acid) matrix using the iMLayer™ matrix sublimation apparatus with a thickness of 0.7 μm. Following matrix deposition, the slide was placed inside a sealed container containing methanol vapor to facilitate matrix recrystallization, with a recrystallization time of 5 min.

Figure 2. Cotton plant

Table 2 Analytical conditions

Mode of analysis	:	Negative
Pixel pitch	:	5×5 μm
Laser diameter setting	:	0 (5 μm)
Laser intensity	:	45
Number of laser shots	:	700 shots
Laser repetition rate	:	2000 Hz
Detector voltage	:	2.2+0.2 kV
Scan range:	:	*m/z* 150-650

■ Test results of reference standards

The reference standards of gossypol, hemigossypol and hemigossypolone were tested in positive and negative ion modes respectively using iMScope QT, with CHCA as matrix. Significant ion peaks were detected in all of them. The background interferences of matrix were fewer in negative mode. Therefore, negative ion mode was selected for subsequent MS acquisition of samples.

■ MS imaging of cotton leaf and cotton ovule

Optical images of cotton leaves and ovules were taken under 5X objective lens and mass spectrum acquisition was performed using iMScope QT. The acquired data were analyzed using the data processing software IMAGEREVEAL MS. Five glands from each leaf and ovule were selected as regions of interest (ROI) for analysis. The signal intensity of gossypol, hemigossypol, and hemigossypolone was shown in Table 3. The spatial distribution images are shown in Figure 3 and Figure 4.

The results showed that gossypol, hemigossypol, and hemigossypolone are mainly distributed in the glands in cotton leaf and ovule. It was found that Gossypol and Hemigossypol were more abundant in the ovule than in the leaf gland, while Hemigossypolone was much more abundant in the leaf than in the ovule gland (Figure 3 and Figure 4, Table 3). The leaf glands are circular in shape, but their sizes vary somewhat. Glands with larger dimensions contain higher levels of the three compounds (e.g., ROI-3 > ROI-5), while glands with similar sizes show relatively comparable amounts of these compounds. The ovule gland reveals irregular shapes and varying sizes, with some glands showing cavities, possibly due to the sectioning position.

Additionally, we can observe each gland in detail, taking ROI-3 as an example (Figure 3 and Figure 4). The magnified MS image of both glands show that the localization of the three components in the ovule gland is relatively uniform, whereas the localization of Gossypol and Hemigossypol (and Hemigossypolone) in the leaf glands is heterogeneous and higher distribution area is limited to the crescent-shaped region on the left. Such findings can only be obtained by measuring at a high spatial resolution, such as 5 µm.

Table 3. Signal intensities of gossypol, hemigossypol, and hemigossypolone in cotton leaf and ovule glands

Name	[M-H]⁻ (*m/z*)	Leaf ROI-1	Leaf ROI-2	Leaf ROI-3	Leaf ROI-4	Leaf ROI-5	Average Leaf	Ovule ROI-1	Ovule ROI-2	Ovule ROI-3	Ovule ROI-4	Ovule ROI-5	Average Ovule
Gossypol	517.19	25625	28582	25377	23592	15094	23654	106175	221420	151898	101462	163324	148856
Hemigossypol	259.10	419	361	373	182	74	282	1505	1339	1776	844	3106	1714
Hemigossypolone	273.08	17767	17378	16420	11280	4512	13471	138	65	172	77	271	145

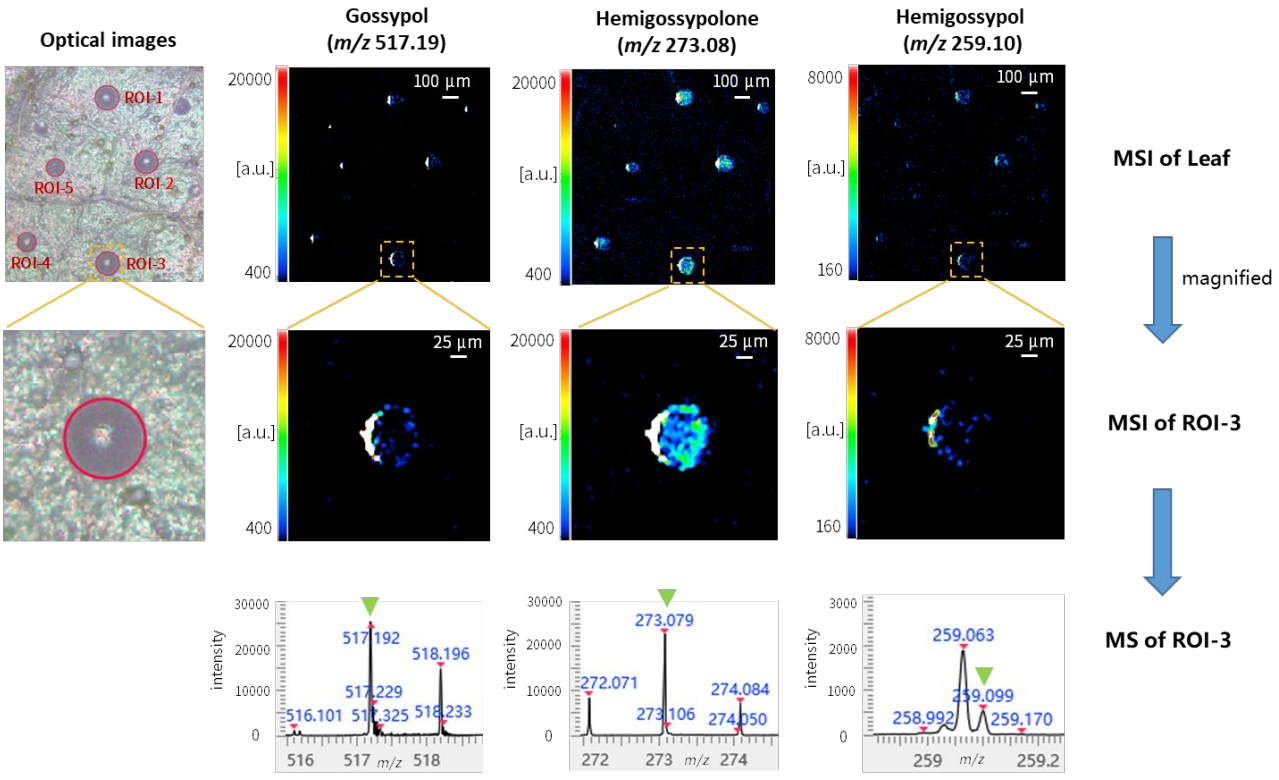

Figure 3. Optical and MS images of cotton leaf and the magnified view of ROI-3 (upper) and the mass spectra of ROI-3 (lower)

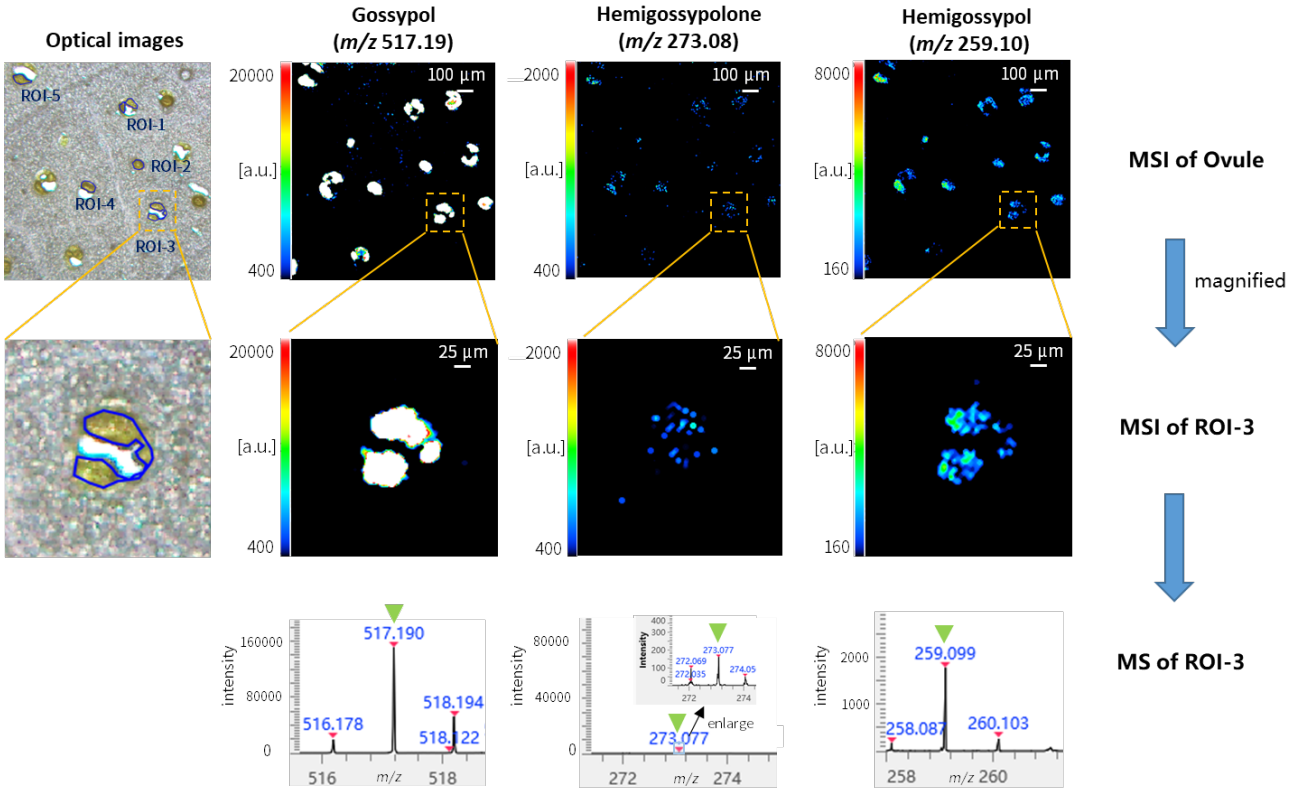

Figure 4. Optical and MS images of cotton ovule and the magnified view of ROI-3 (upper), and the mass spectra of ROI-3 (lower)

■ Conclusions

In this paper, the spatial distribution of gossyphenol, hemigossyphenol and hemigossyphenone in cotton leaves and ovule was analyzed using Shimadzu imaging mass spectrometry microscope iMScope QT. The spatial distribution information of compounds with high spatial resolution of 5 µm was obtained, and the distribution of compounds in tissues could be directly observed at the glandular level through the In situ visual method. It provides clues for the synthesis and transformation mechanism of the terpenoids such as gossyfol. This study provides a reference for the spatial distribution analysis of various endogenous metabolites in plants, and provides a new research method and technical tool for the visual study of endogenous metabolites and the exploration of their physiological functions.

■ Acknowledgment

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