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Visualizing the spatial distribution of flavonoids and phenolic acids in the tuber root of Tetrastigma hemsleyanum using AP-MALDI-MSI

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1. Overview

In this paper, we present a case of simultaneous visualization analysis of spatial distribution of multiple flavonoids and phenolic acids in the tuber root of Tetrastigma hemsleyanum using an imaging mass microscope.

2. Introduction

Tetrastigma hemsleyanum Diels et Gilg (T. hemsleyanum), as an extensively used folk Chinese herbal medicine, has the effect of clearing heat and detoxication, anti-tumor, anti-oxidant and anti-inflammotory, while flavonoids and phenolic acids are considered to be the main active ingredient. In this study, atmospheric-pressure matrix-assisted laser desorption/ ionization mass spectrometry imaging (AP-MALDI-MSI) was performed to visualize the spatial distribution of flavonoids and phenolic acids in the tuber root of T. hemsleyanum using an imaging mass microscope. The distribution characteristics and trends of these compounds can be directly observed in the plant tissue, providing a reference for the quality evaluation of T. hemsleyanum and the study of endogenous secondary metabolites.

3. Methods

Sample preparation and matrix deposition: The tuber root was embedded with 10% gelatin aqueous solution and frozen with liquid nitrogen. Frozen root sample was cut into 40 μm thickness sections using a CM1950 microtome (Leica, Wetzlar, Germany) at -20 ℃ and transported to an ITO-coated glass slides. CHCA was vapor-deposited on the sample surface using a Shimadzu iMLayer™ matrix vapor deposition system with a thickness of 0.7 μm, followed by spraying of the CHCA solution. 1,5-DAN solution was sprayed on the surface of another tissue section for using an airbrush with a 0.22-mm nozzle (PS-270, GSI Creos, Tokyo, Japan).

MALDI data acquisiton and analysis: All MALDI-MSI data were acquired using an imaging mass spectrometer (iMScope QT, Shimadzu) equipped with an built-in optical microscope, an atmospheric pressure MALDI source, and an quadruple-time-of-flight (Q-TOF) analyzer. Ions between m/z 100-800 were measured in both the positive and negative ion mode with detector voltage of 2.5 kV. The laser diameter was set to 10 μm with a 20 μm step size. IMAGEREVEAL MS software (Shimadzu) were used in data analysis and construction of the MS images.

Figure 1 Imaging Mass Microscope iMScope QT

Main Features:

- ◆ Integrated microscopic and widefield camera
- ◆ up to 5 μm spatial resolution
- ◆ up to 50 pixels/second scan speed
- ◆ Quantification and Distribution: LC-MS analysis can be performed in addition to MSI analysis

4. Result

4.1 Selection and optimization of matrix

Different kinds of matrix solutions (CHCA, DHB, 9-AA, 1,5-DAN) were added to different areas of the tissue sections. The MS data acquired were searched against the databases of flavonoids and phenolic acids and CHCA was selected for positive ion scanning and 1,5-DAN for negative ion scanning (analysis of salicylic acid) according to the response of the compounds.

4.2 Optical images of samples, ROI Settings and database search results

When using IMAGEREVEAL MS for data analysis, the software will automatically obtain optical images taken by the built-in microscope of the instrument. ROI (Region of Interest) setting was performed on optical images (Fig.2), and the signal intensity values of compounds could be obtained in each region after database searching, which can be used as quantitative analysis data (table 1-2).

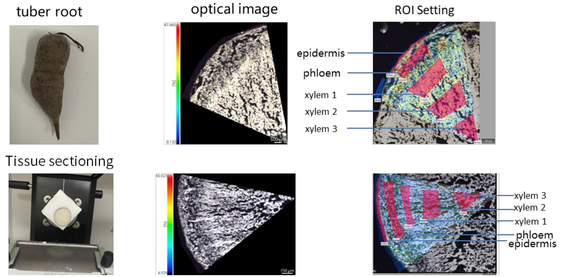

Figure 2 sample photo, tissue sectioning, optical image and ROI setting

4.3 Spatial distribution of flavonoids

Flavonoids have various pharmacological effects, such as antioxidant, anti-tumor, antibacterial, and anti-inflammatory, and are widely present in Chinese herbal medicine. They are one of the main active ingredients of T. hemsleyanum. The main flavonoids in T. hemsleyanum include quercetin, kaempferide, catechin, rutin, kaempferol, procyanidin dimmer, hyperoside, apigenin, etc.

In situ visualization analysis of flavonoids in tuber root using iMScope QT showed that the content of most flavonoids in the epidermis was higher, while the content in the phloem decreased compared to the epidermis. The area near the phloem in the xylem (xylem 1) has a higher content, while the content of flavonoids in the xylem decreases from the outside to the inside (xylem 1 to xylem 3). The high content of flavonoids in the tuber epidermis of T. hemsleyanum may be related to its protection mechanism against various biological and abiotic stress, such as the invasion of pests and diseases. Examples of the spatial distribution images of flavonoids are shown in Figure 3.

Table 1 IMAGEREVEAL MS analysis result of flavonoids

Compounds	m/z	Formula	Adduct Ion	epidermis	phloem	Xylem 1	Xylem 2	Xylem 3
quercetin	341.0064	C15H10O7	M+K	2107	1277	1672	1163	477
kaempferide	301.0707	C16H12O6	M+H	2960	2204	2765	2135	869
Catethin	329.0428	C15H14O6	M+K	1509	1019	2087	1168	389
rutin	633.1426	C27H30O16	M+Na	964	515	1006	581	241
kaempferol	287.055	C15H10O6	M+H	4086	5101	6207	5034	2227
hyperoside	503.0592	C21H20O12	M+K	1926	1245	1935	1206	533
procyanidin dimmer	617.1061	C30H26O12	M+K	1000	362	822	430	143
apigenin	271.0601	C15H10O5	M+H	1606	1331	1770	1324	489
daidzein	255.0652	C15H10O4	M+H	2640	1333	1719	1341	525
biochanin A	307.0577	C16H12O5	M+Na	1285	1056	1331	1019	409
protocatechuic acid hexoside	355.0431	C13H16O9	M+K	1968	1610	2135	2182	1137
apiosylglucosyl-4-hydroxybenzoate	455.116	C18H24O12	M+Na	1810	1708	3470	2066	1055
kaempferol-3-O-glucopyranoside	487.0643	C21H20O11	M+K	2033	1104	1732	1086	431
(Epi) catechin glucopyranoside isomer	475.1211	C21H24O11	M+Na	1686	2263	2413	2097	1355
apigenin-7-rhamnoside	485.1054	C22H22O11	M+Na	1734	1338	2080	1493	749
kaempferol-3-sambubioside	619.1065	C26H28O15	M+K	1074	527	999	551	228
isorhamnetin-3-rutinoside	647.1583	C28H32O16	M+Na	809	602	1035	670	348
robinin	779.1801	C33H40O19	M+K	391	105	263	112	42

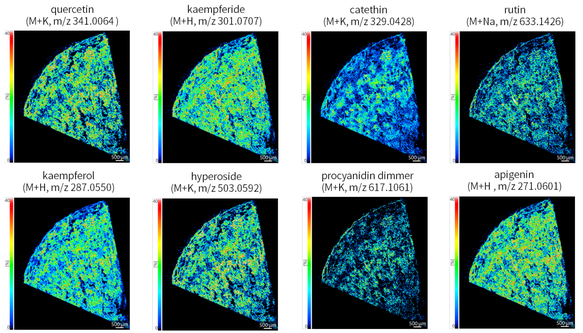

Figure 3 Examples of Spatial distribution of Flavonoids in the tuber root of T. hemsleyanum

4.4 Spatial distribution of Phenolic acids

Phenolic acids are a class of organic acids containing phenolic rings, which have antioxidant and bactericidal effects. They are also important indicator components for the quality evaluation of T. hemsleyanum. Using an Imaging Mass Microscope, in situ imaging analysis was performed on four phenolic acids, including salicylic acid, protocatechuic acid, quinic acid, and 5-p-cinnamoylquinic acid (Fig.4). It was found that the four compounds had the highest content in the epidermis, possibly related to their protective effects on plants. Except for the epidermis, salicylic acid has a higher content in the phloem, while the other three compounds have relatively higher content in the area near the phloem in the xylem. The content of phenolic acids in the xylem decreases sequentially from the outside to the inside (xylem 1 to xylem 3).

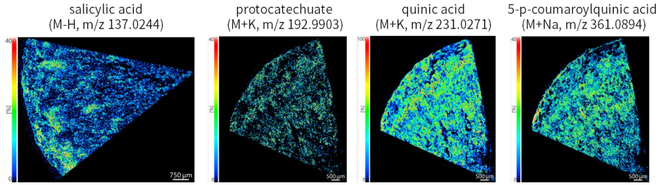

Figure 4 Spatial distribution of Phenolic acids in the tuber root of T. hemsleyanum

Table 2 IMAGEREVEAL MS analysis result of Phenolic acids

Compounds	m/z	Formula	Adduct Ion	epidermis	phloem	Xylem 1	Xylem 2	Xylem 3
salicylic acid	137.0244	C7H6O3	M-H	237	276	172	107	83
protocatechuate	192.9903	C7H6O4	M+K	207	79	101	77	18
quinic acid	231.0271	C7H12O6	M+K	4795	2802	3167	2553	826
5-p-coumaroylquinic acid	361.0894	C16H18O8	M+Na	2362	1430	2009	1448	626

5. Conclusions

In this paper, an Imaging Mass Microscope iMScope QT was applied to analyze the spatial distribution of various flavonoids and phenolic acids in the tuber root of Tetrastigma hemsleyanum. We found that most of the compounds had high content in the epidermis and xylem, providing a reference for the quality evaluation of of T. hemsleyanum medicinal herb and the study of secondary metabolites. IMScope QT integrates high-resolution optical microscopy observation of tissue sections and mass spectrometry imaging analysis of compounds, eliminating the need for complex pre-treatment steps for compound extraction. It can directly perform in situ visualization analysis of endogenous metabolites and various active molecules in plants, providing new research directions and technical means for visualizing endogenous metabolites and exploring their physiological functions.