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Visualization of Abscisic Acid Distribution in Brown Rice Using Mass Spectrometry Imaging

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■ Abstract

Phytohormones function as biochemical messengers that allow plants to adapt to external environmental conditions and regulate growth and development. These hormones are very potent in trace amounts and affect many physiological processes. However, direct visualization of these hormones is difficult, and mass spectrometry imaging is considered to be useful to solve this problem. This article describes the application of iMScope™ QT, which enables mass spectrometry imaging with high sensitivity and resolution, to visualize the distribution of abscisic acid, a plant hormone.

1. Introduction

Plant hormones are chemicals involved in plant growth, development, and responses. These hormones are synthesized in trace amounts in the body and affect distant parts, thus regulating plant physiological processes. Generally, plant hormones are divided into the following major categories:

1. Abscisic Acid: Abscisic acid regulates seed dormancy under stress conditions, reduces transpiration, and controls responses to water stress.
2. Auxins: Auxins play an important role in controlling plant elongation, growth, and orientation. They can reorient plants in response to light and gravity, promoting the formation of new tissues and the elongation of cells.
3. Gibberellins: Gibberellins are involved in plant growth and development, including seed germination, stem elongation, flower formation, and fruit ripening.
4. Cytokinins: Cytokinins promote cell division and affect the formation of new tissues, bud growth, and delayed leaf senescence.
5. Ethylene: Ethylene is involved in fruit ripening, inhibition of footfall, defoliation, and sometimes stress responses.

Plant hormones regulate a variety of physiological processes, depending on the plant's life cycle and environmental conditions. The balance and regulation of these hormones are very important in plant growth, reproduction, and stress response.

Abscisic acid (ABA) plays a particularly important role in stress response. During seed development, ABA content peaks at the completion of seed morphology. In this study, we performed mass spectrometry imaging (MSI) of ABA in rice seeds at the maturity stage near harvest.

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2. Experiments

2-1 reagents

Abscisic acid (ABA), Girard's reagent T (GirT), Girard's reagent P (GirP), 2, 5-dihydroxybenzoic acid (DHB), 1, 5-diaminonaphthalene (1,5-DAN), and porcine gelatin were purchased from Merck (Darmstadt, Germany). Methanol (MeOH, LCMS grade), acetonitrile (ACN, LCMS Grade), and trifluoroacetic acid (TFA) were also purchased from Fujifilm Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2-2 Sample Information

The hulls of Hinohikari (*Oryza sativa L. cv. Hinohikari*) grown in Okayama in FY 2023 were peeled off from the unhulled rice with tweezers to make a sample (brown rice).

2-3 MSI Measurement Sample Preparation

The prepared brown rice samples were embedded in 10% gelatin and quickly frozen at -80 ˚C. The embedded samples were placed in a cryostat (CM1950, Leica), and sections with a thickness of 15 µm were prepared. The prepared sections were collected on a cryofilm (Cryofilm type2C (9), SECTION-LAB) and dried in a silica gel tube at room temperature. Conductive tape (3M) was applied to the conductive surface of ITO-coated slide glass (MATSUNAMI) and used for MSI measurement.

Fig. 1 iMScope™ QT Imaging Mass Microscope

2-4 Derivatization and Matrix Supply

An equal amount of ABA standard solution and GirT solution or GirP solution (5 mg/mL, MeOH/H2O/TFA: 80/20/2, v/v/v) were mixed and allowed to react at room temperature for 30 minutes. Furthermore, an equal amount of the mixture with DHB solution (30 mg/ml, 70% MeOH) was used as GirT-derivatized ABA (GirT-ABA). For the MSI test sample, GirT solution was uniformly sprayed onto the sample surface with an airbrush (GSI Creos) (200 µL/slide) and allowed to react in a room kept at 25 ˚C for 30 minutes. After drying in a desiccator under reduced pressure for 10 minutes, DHB solution (30 mg/ml, 70% MeOH) was supplied with an airbrush (300 µL/slide).

2-5 Mass Spectrometry

Measurements were performed with iMScope QT (Fig. 1). Mass calibration was performed using DHB as an external standard. GirT-ABA is detected as $[M]^+$ at m/z 378.24. Therefore, mass spectra of the standard were obtained in the mass range of m/z 350-400 in the positive ion mode. MS/MS spectra were obtained after dissociation with argon gas at m/z 378.24 as precursor ion. As a control experiment, equal amounts of ABA standard solution and DAN solution (10 mg/mL, 70% ACN) were mixed and analyzed in the negative ion mode. At this time, underivatized ABA was detected as [M-H]- at m/z 263.13, and mass spectra were obtained in the mass range of m/z 250-300. For MSI measurement, the pixel size was 25 µm x 25 µm, and other conditions are shown in Table 1. After measurement, analysis was performed using the analysis software IMAGEREVEAL™ MS (Shimadzu Corporation) (Fig. 2).

Table 1 MSI Analysis Parameters

MS analysis conditions	
Ion species	Positive ion mode
m/z measurement range	350-400
Number of accumulations	1
DL temperature	250 ˚C
Heat block temperature	450 ˚C
Sample voltage	4.50 kV
Detector voltage	2.10 kV (standard), 2.30 kV (brown rice)
Number of MS stages	1 and 2
CID gas	150 kPa (MS1), 230 kPa (MS2)
Laser irradiation conditions	
Number of times of irradiation	80
Repeat frequency	1000 Hz
Irradiation diameter setting value	2
Laser intensity	65

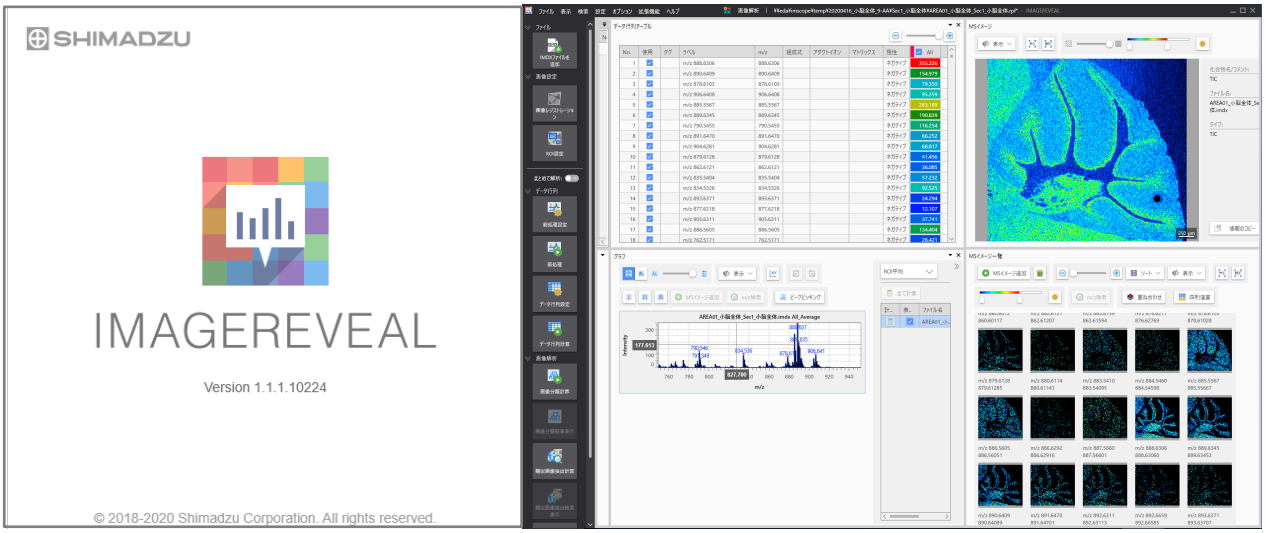

Fig. 2 IMAGEREVEAL™ MS Mass Spectrometry Imaging Data Analysis Software

3. Results

The mass spectrum of GirT-ABA in the positive ion mode is shown in Fig. 3A. It is known that ABA can be detected with high sensitivity by GirT derivatization in the positive ion mode 1). In this experiment, a clear peak derived from GirT-ABA was detected at m/z 378.24 as $[M]^+$ due to the improvement of ionization efficiency.

Using $[M]^+$ of GirT-ABA as the precursor ion, MS/MS measurements showed that m/z 319.17, which is the ion from which the trimethylamine moiety of GirT was removed, and *m/z* 291.17, which is 87 Da smaller than that of GirT-ABA, were detected as shown in Fig. 3B. As a control experiment, both GirP-derivatized ABA and underivatized ABA were analyzed in negative ion mode. As shown in Fig. 3C, the peak intensity was significantly lower than that of GirT-ABA.

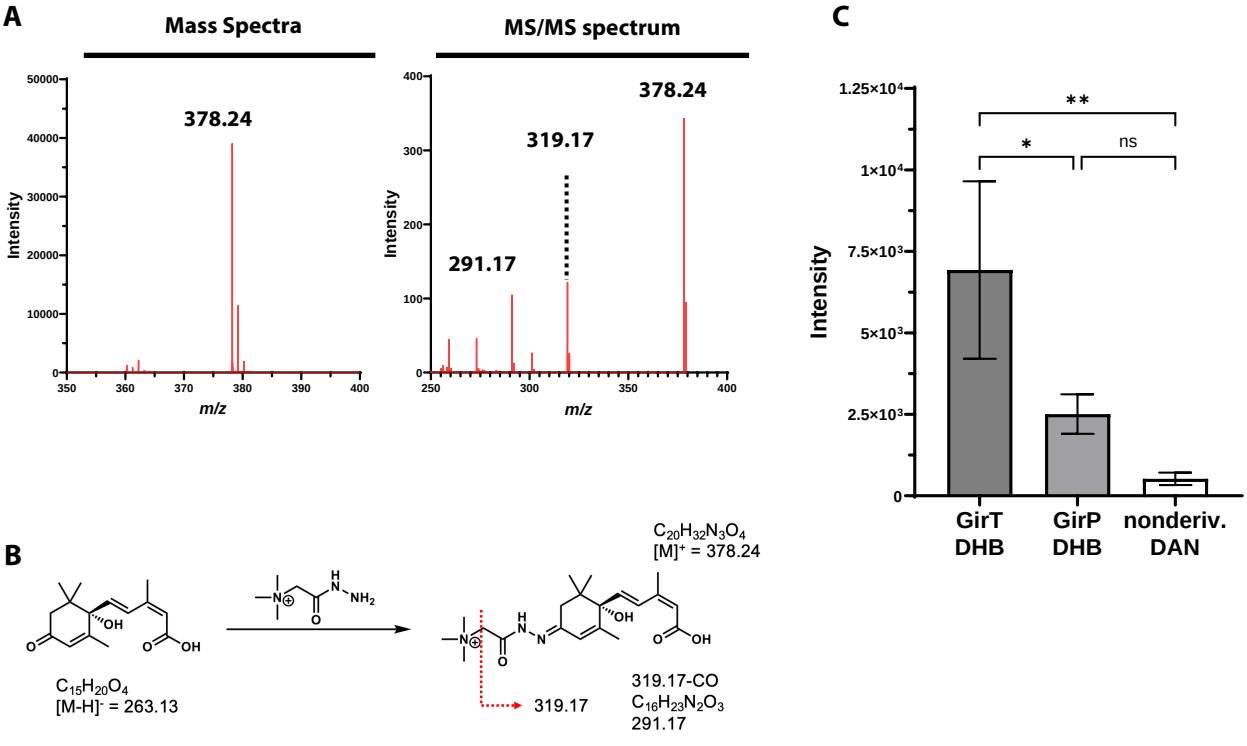

Fig. 3 Measurement with ABA Equivalent
(A)Mass and MS/MS Spectra of GirT-ABA in Positive Ion Mode. (B) Dissociation pattern in MS/MS spectra of GirT-ABA. (C) Comparison of ionic strength of GirT-ABA, GirP-ABA, and underivatized ABA (n = 3, error bars: standard deviation, t-test). GirT-ABA and GirP-ABA were measured in positive ion mode using DHB, and underivatized ABA was measured in negative ion mode using 1,5-DAN.

Based on the results of the measurement of the reference standard, 3 seeds of Hinohikari produced in Okayama in 2023 were sectioned and subjected to GirT derivatization on tissue, followed by MSI measurement. The imaging results of GirT-ABA in rice seeds are shown in Fig. 4A, and the obtained mass spectra and product ion spectra are shown in Fig. 4B. In addition, 3 seeds were sectioned and subjected to MSI measurement, and the resulting peak intensity is compared between embryo and endosperm, and a graph is shown in Fig. 4C.

From the results of the MS imaging, it was visualized that GirT-ABA accumulated mainly in the embryo, and clear peaks were obtained in the mass spectra and MS/MS spectra, which were consistent with the reference standard.

(The peaks corresponding to the fragment ions are m/z 291.17 and 319.17 shown in Fig. 3B.)

Comparison of ionic strength showed that there was a significant difference between embryo and endosperm. These results indicate that ABA accumulates in embryos in brown rice.

4. Conclusion

In this experiment, we successfully visualized abscisic acid in brown rice samples by performing derivatization with Girard reagent T and MSI measurement with iMScope QT. It is expected that various phytohormones will be visualized by combining tissue derivatization with MSI in the future.

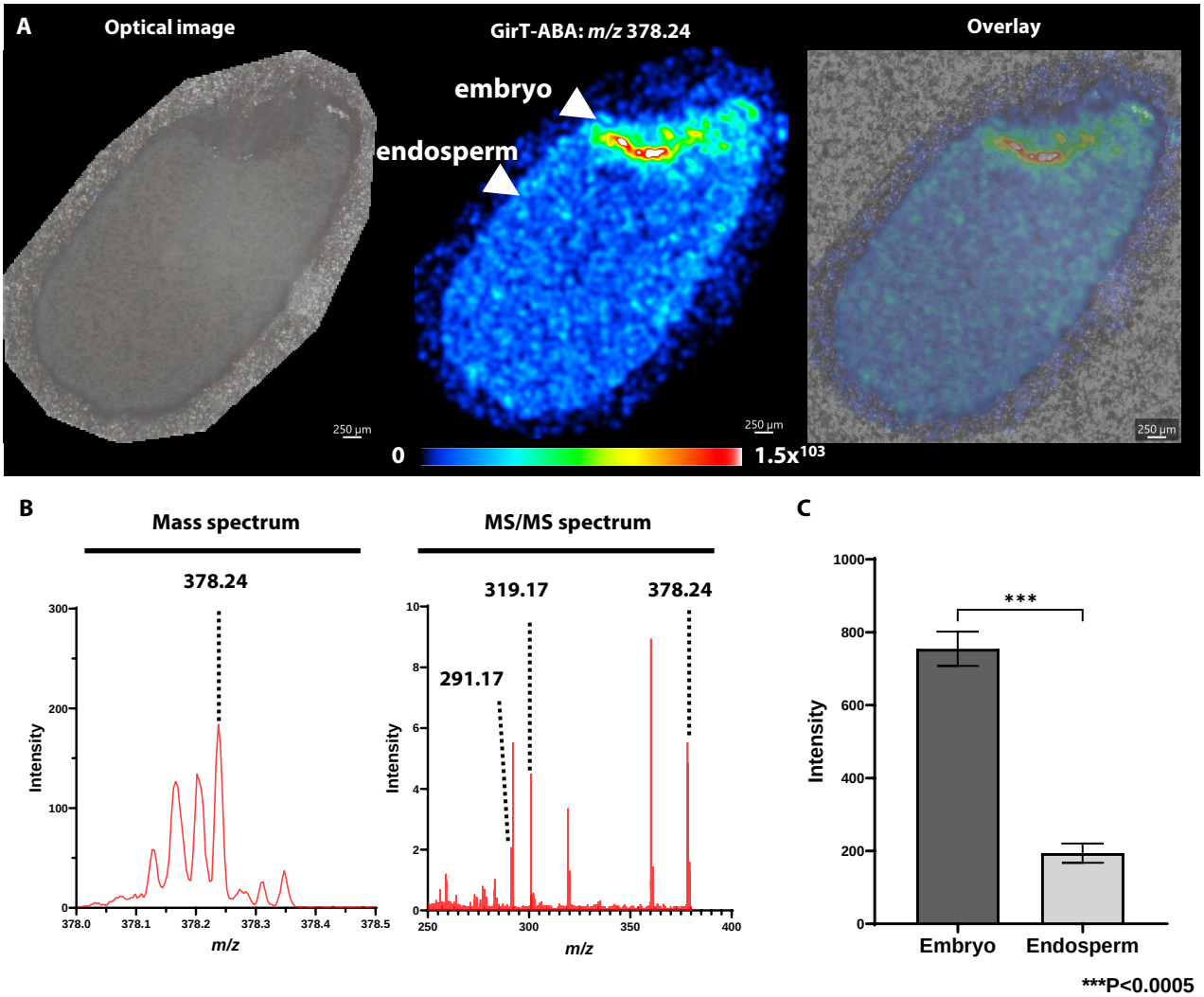

Fig. 4 MSI results of GirT-ABA in brown rice.

(A)Imaging results (From left, optical image, m/z 371.24 distribution, superposition with engineering image), scale bar: 250 µm. (B) Mass spectrum and MS/MS spectrum obtained from brown rice sections. (C) Ion intensity comparison between embryo and endosperm (n = 3, error bars: standard deviation, t-test).There was a significant difference between the embryo and endosperm.

<References>

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