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Structural and quantitative analysis of plant hormone and its metabolites with LC-MS/MS

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Introduction

Indole-3-acetic acid (IAA) is the major auxin hormone that regulates many aspects of growth and development in plants. It has been suggested that most of the IAA in plants is present in conjugated forms, since a large amount of IAA is released by hydrolysis of plant extracts. The conjugates are thought to be intermediates in the catabolic processing of IAA, but, in some cases, to play a role in storage or transportation of the hormone. Here, we developed LC-ESI-MS/MS method, based on MRM for quantitative analysis for the concentrations of various metabolites of IAA in plant and based on precursor ion and neutral loss scanning for new metabolites screening.

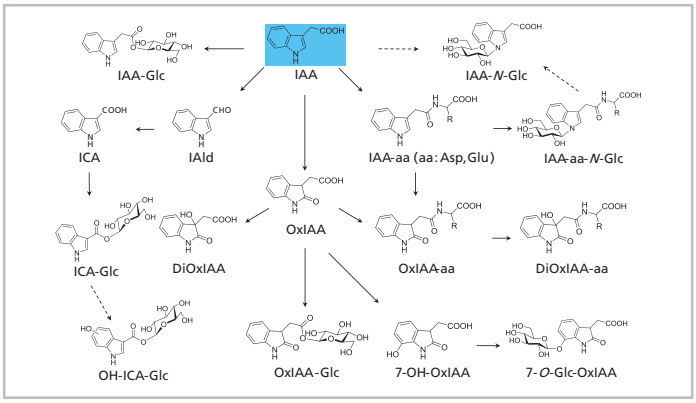


Fig. 1
IAA metabolism skim in plant
The main metabolic reactions of IAA are oxidation, decarboxylation and amino acid conjugation and sugar conjugation.

Methods

Two-week-old rice seedlings (Oryza sativa cv. Nipponbare) grown with either water or 10 uM IAA solution were used. Rice plants were separated into aerial part and root, which were individually homogenized in liquid N_2 and subsequently extracted with acetone-H_2O (4:1) containing 2.5 mM diethyldithiocarbamic acid. After the extraction procedure was repeated, the combined extract was concentrated under reduced pressure. The concentrate was applied to ODS cartridge column to be partially purified. Eluate by 70 % acetonitrile was collected and concentrated. The prepared sample was diluted to water (aerial part; 0.1 g-plant-weight/mL, root; 0.067 g-plant-weight/mL) and then analyzed using a LC-ESI-MS/MS system consisting of a binary gradient system HPLC coupled to a triple quadrupole mass spectrometer (LCMS-8030, Shimadzu Corporation, Japan).

IAA metabolites provide rich fragment information in both positive and negative ion modes. For example, metabolites sharing the common indole-3-acetly moiety sub-structure produce a quinolinium ion at *m/z* 130 in positive ion mode, while an ion at *m/z* 146 is characteristic of amide conjugates of oxidized IAA. With such characteristic fragment ions it was also possible to use neutral loss scan of *m/z* 161 in positive ion mode for Asp conjugates, and *m/z* 162 in negative ion mode for Glc conjugates.

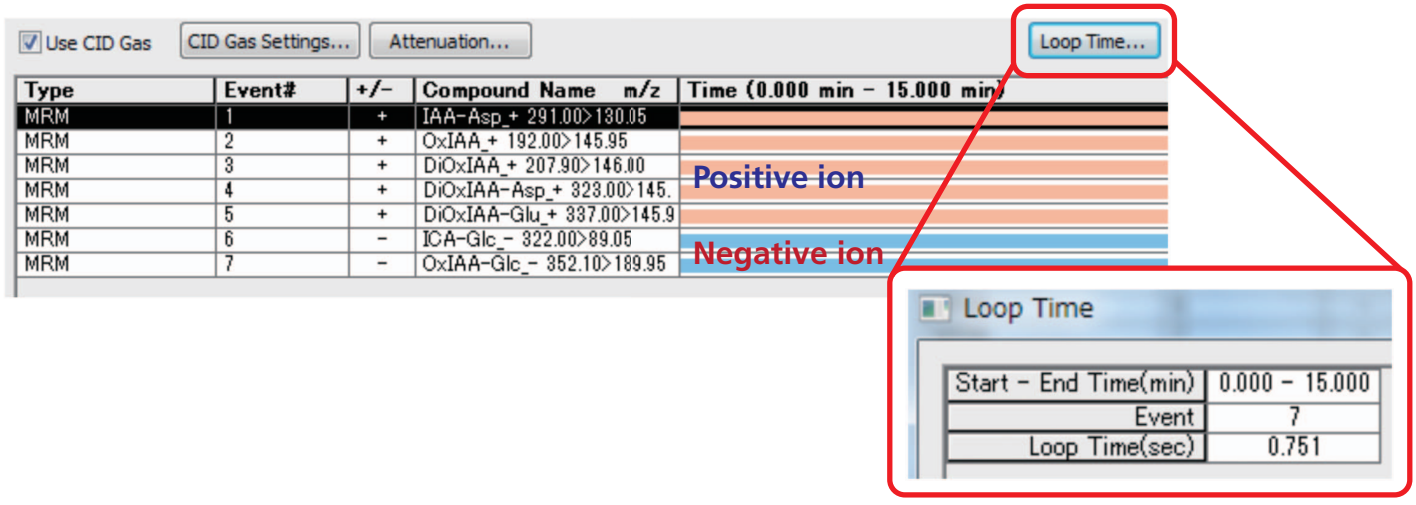
To detect and quantify IAA metabolites, the MRM transition and collision energy was optimized for IAA-Asp, 2-oxo-IAA (OxIAA), 3-hydroxy-2-oxo-IAA (DiOxIAA), DiOxIAA-Asp, DiOxIAA-Glu and OxIAA-Glc. DiOxIAA-Glu and OxIAA-Glc for negative ion detection; IAA-Asp, OxIAA, DiOxIAA, DiOxIAA-Asp, DiOxIAA-Glu were optimized in positive ion mode.
In positive ion mode, the fragment ion at *m/z* 146 was common to all the metabolites examined, with the exception of IAA-Asp

Table 1 Analytical conditions for quantitation of IAA and IAA metabolites

HPLC: Nexera UHPLC system (SHIMADZU CORPORATION)	
Column	Cadenza CD-C18 (3 um, 75 mm x 2 mmI.D.) Imtakt Corporation, Japan
Flow rate	0.2 mL/min
Injection volume	3 uL
Mobile phase	A: 0.05 % Acetic acid B: Methanol
Gradient program	10 % B – 90 % B (15 min)
Column temperature	30 °C
MS: LCMS-8030 Triple quadrupole LC-MS/MS (SHIMADZU CORPORATION)	
Ionization	ESI (Positive or Negative)
Mode	Multiple reaction monitoring (MRM)

Fig. 2
LCMS-8030 triple quadrupole mass spectrometer

Results



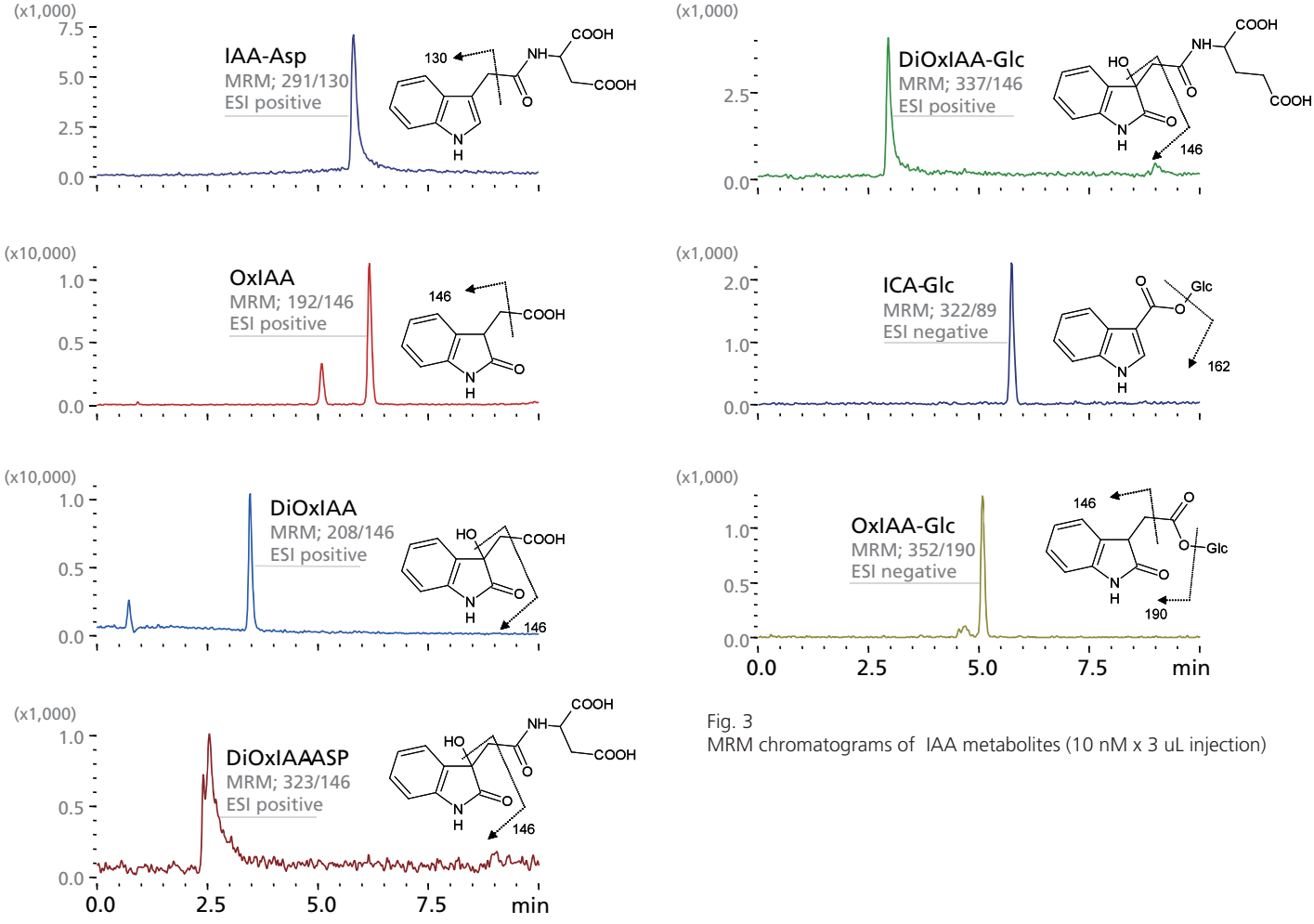


Fig. 3
MRM chromatograms of IAA metabolites (10 nM x 3 uL injection)

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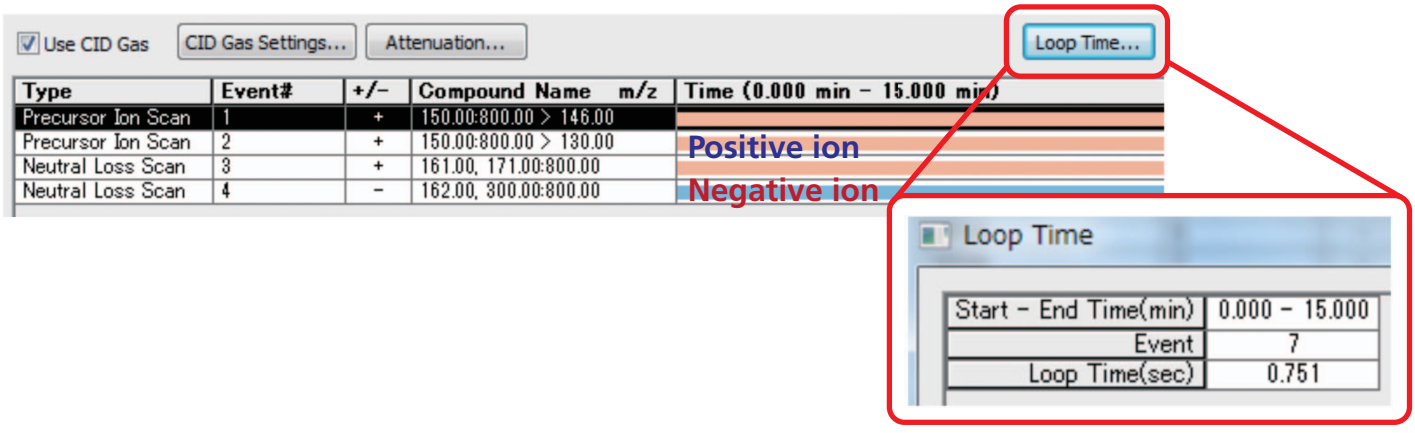
Achieving a high sampling rate across a peak with multiple MS events (MRM, polarity switching and neutral loss) required a high speed data acquisition system (15,000 u/sec), fast polarity switching (15 msecs) and minimal dwell times (1 msec) and pause times (1 msec).
IAA metabolites were determined by fast scanning LC-MS/MS analysis in rice seedlings at 14 days after germination. All metabolites were found in samples grown with 10 uM IAA solution at a higher concentration compared to samples grown in water; metabolite levels in root were higher than in aerial part.
All compounds were detected over a calibration range from 1 nM to 1000 nM (r> 0.999).

Table 2
Calculated concentration of metabolites in aerial part and root; control and IAA spiked (n=3 average)

Compound	Aerial part (nM)		Root (nM)	
	Control	IAA spiked	Control	IAA spiked
IAA-Asp	<1	**8.48**	1.67	**781**
OxIAA	<1	**5.17**	<1	**5.31**
DiOxIAA	2.24	**3.74**	1.12	**17.1**
DiOxIAA-Asp	<1	<1	6.59	**156**
DiOxIAA-Glu	<1	<1	1.16	**11.9**
ICA-Glc	<1	<1	2.49	**16.4**
OxIAA-Glc	<1	<1	<1	**1.60**

Root part extraction sample where IAA was spiked was analyzed to discover new IAA metabolite candidates using precursor ion scans of *m/z* 146(+), 130(+) and neutral loss scan; loss of *m/z* 161(+) and 162(-) suggested from the fragmentation patterns of the known IAA metabolites.

The fast polarity switching (15msecs) and fast scan speed (max. 15000 u/sec) capacity can set the loop time within one second even if there are 4 full scans with both positive and negative modes.



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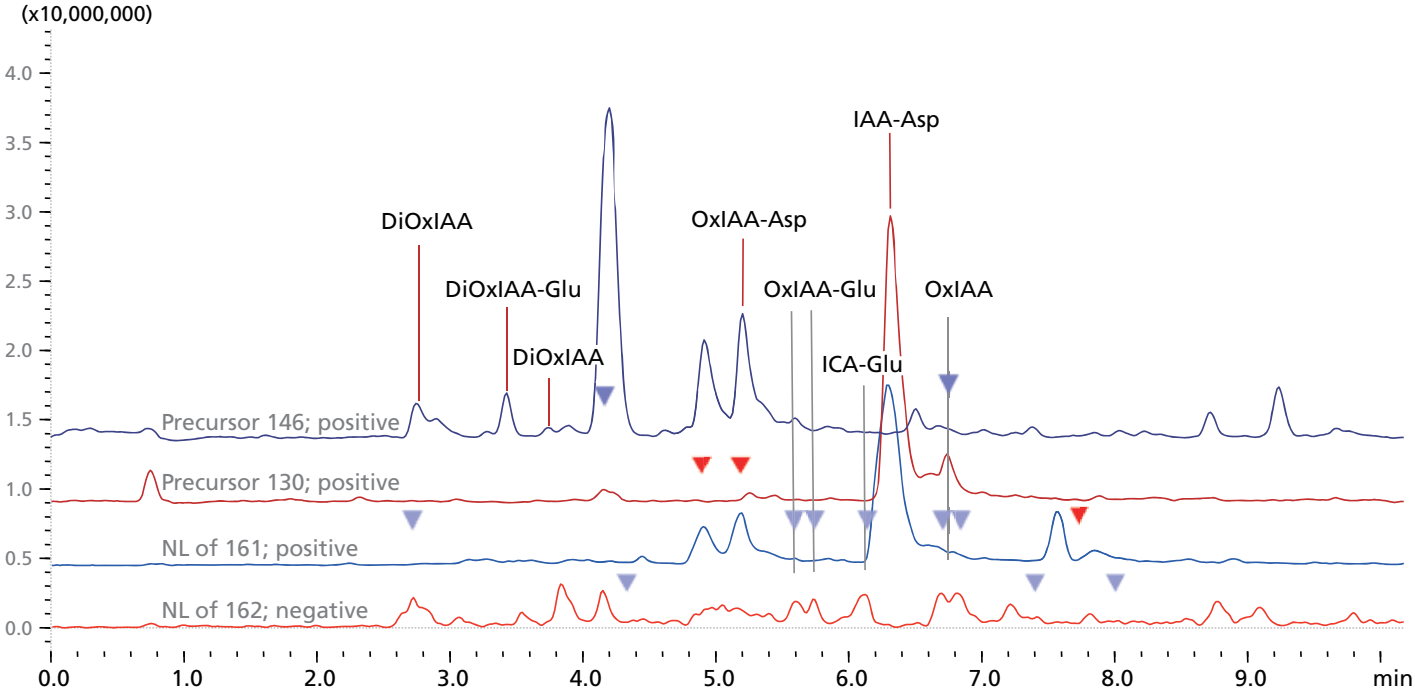

Fig. 4
MS/MS scanning functions to detect IAA metabolite candidates

In this case 4 full scan events were acquired at the same time. However, as the MS data acquisition speed is high the number of data points across a peak results in well defined peak shape. Six of the seven compounds in Table 1 were detected. However, using multiple precursor ion scan and neutral loss experiments (including polarity switching) in a single data acquisition also helps to identify IAA metabolite candidates. This approach confirmed published metabolite data and identified new metabolite candidates.

Conclusions

IAA and its metabolites were determined using a new high speed data acquisition system using multi-polar MRMs. Simultaneous multi precursor ion scanning and the neutral loss scanning helped to identify and quantitate plant hormone and its metabolites using a single analytical run.

References

K. Kai et. al., Phytochemistry., (68) 2007,2512–2522
K. Kai et. al., Phytochemistry., (68) 2007,1651–1663

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