Plant Metabolomics

Sulfur-containing metabolite-targeted analysis using liquid chromatography-mass spectrometry

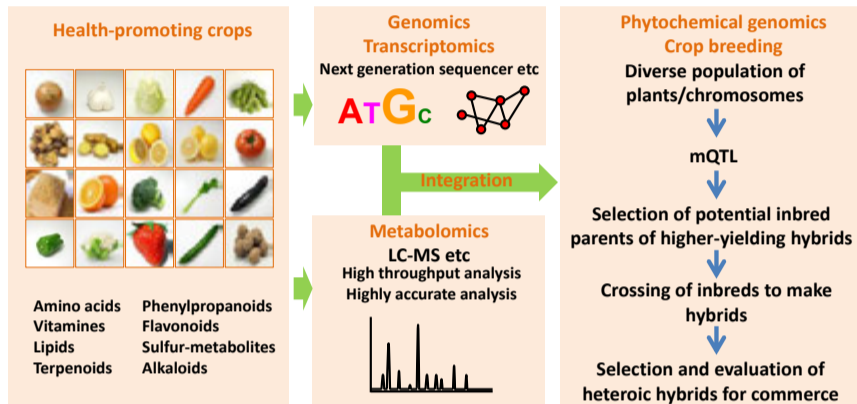## Ryo Nakabayashi[1], Yuji Sawada[1], Makoto Suzuki[1], Masami Yokota Hirai[1], Noriya Masamura[2], Masayoshi Shigyo[3] and Kazuki Saito[1,4]
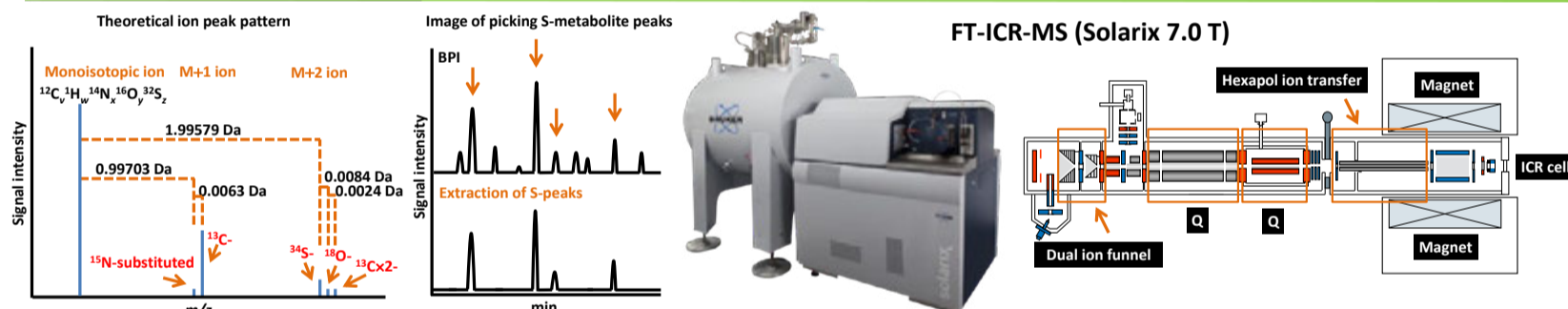
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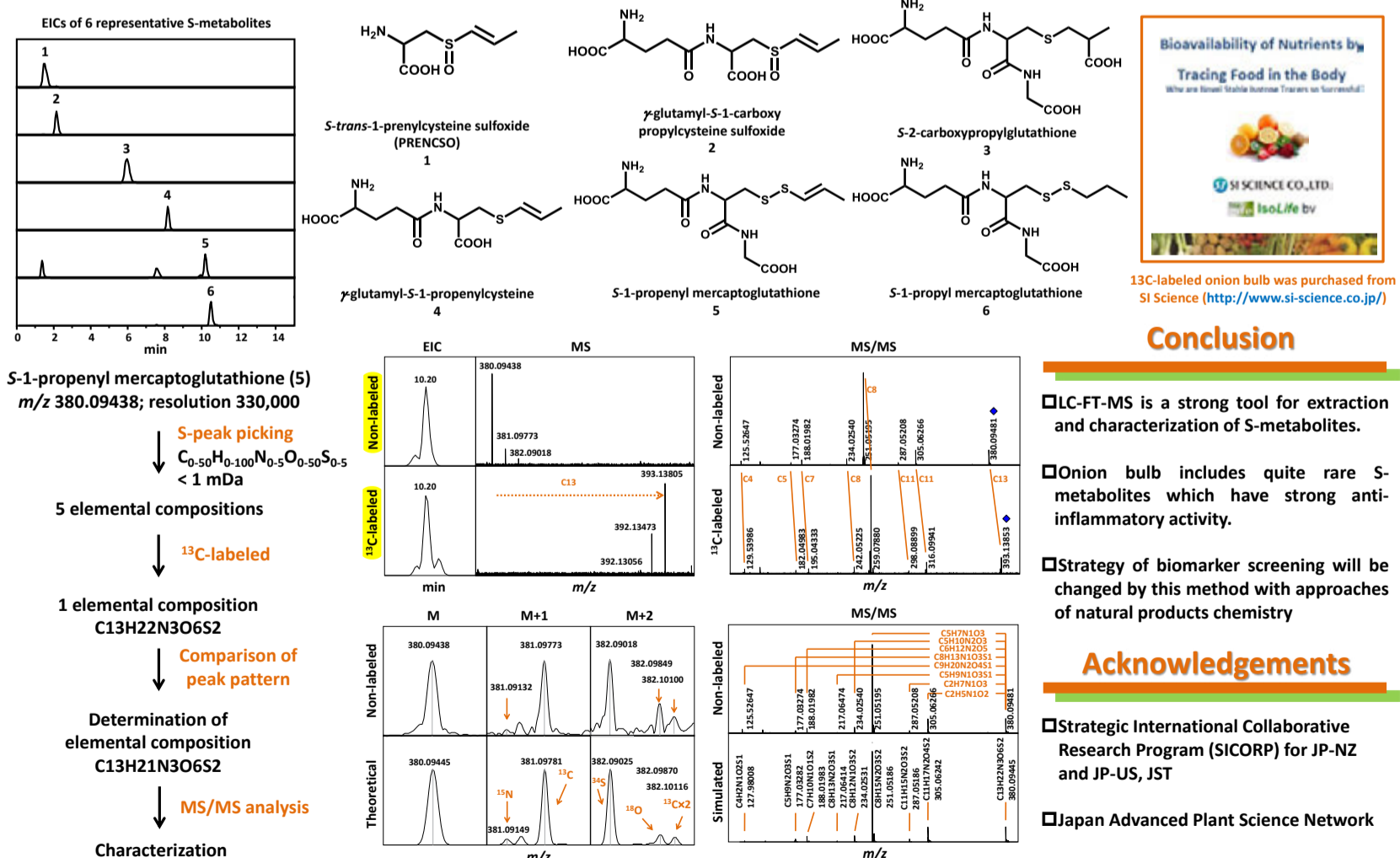
Summary

Plants accumulate human beneficial metabolites such as amino acids, flavonoids and sulfur-containing metabolites (S-metabolites) with a wide range of biological activities related to antioxidation, platelet aggregation inhibition, and anticancer benefit. Profiling methods of S-metabolites with high accuracy and throughput are being required for efficient phytochemical functional genomics and crop breeding. Here, we describe the S-metabolite-targeted analysis using liquid chromatography (LC)-fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) with U-^{13}C-labeled onion bulb. Natural abundance of ^{32}S (31.972072 Da) and its isotope ^{34}S (33.967868 Da) is 95.02 % and 4.21 %, respectively. These facts are obviously reflected on molecular ion peaks of S-metabolites. Thus, the use of such information allows us to efficiently and accurately extract the peaks related to S-metabolites. By using this method, finally, *S*-alk(en)ylcysteine sulfoxides together with their intermediates were chemically annotated in onion bulb. It means to realize S-omics using LC-FT-MS for screening of biomarkers in any other organisms.

Health-promoting crops

Genomics Transcriptomics
Next generation sequencer etc

Phytochemical genomics Crop breeding
Diverse population of plants/chromosomes

Integration

mQTL

Metabolomics
LC-MS etc
High throughput analysis
Highly accurate analysis

Selection of potential inbred parents of higher-yielding hybrids

Crossing of inbreds to make hybrids

Selection and evaluation of heteroic hybrids for commerce

Amino acids
Vitamines
Lipids
Terpenoids
Phenylpropanoids
Flavonoids
Sulfur-metabolites
Alkaloids

Peak picking of S-metabolite

Theoretical ion peak pattern

Monoisotopic ion   M+1 ion   M+2 ion
$^{12}C_x{}^1H_w{}^{14}N_x{}^{16}O_y{}^{32}S_z$

1.99579 Da
0.99703 Da
0.0063 Da
0.0084 Da
0.0024 Da

^{15}N-substituted
^{13}C-
^{34}S-
^{18}O-
^{13}Cx2-

m/z

Image of picking S-metabolite peaks
BPI
Extraction of S-peaks
min

FT-ICR-MS (Solarix 7.0 T)

Hexapol ion transfer   Magnet

ICR cell

Dual ion funnel   Q   Q   Magnet

Profiling of S-metabolites using LC-FT-MS in non-labeled and ^{13}C-labeled onion bulb

EICs of 6 representative S-metabolites

1
2
3
4
5
6

0 2 4 6 8 10 12 14
min

S-trans-1-prenylcysteine sulfoxide (PRENCSO)
1

γ-glutamyl-*S*-1-carboxy propylcysteine sulfoxide
2

S-2-carboxypropylglutathione
3

γ-glutamyl-*S*-1-propenylcysteine
4

S-1-propenyl mercaptoglutathione
5

S-1-propyl mercaptoglutathione
6

Bioavailability of Nutrients by Tracing Food in the Body

SI SCIENCE CO.,LTD.
IsoLife bv

13C-labeled onion bulb was purchased from SI Science (http://www.si-science.co.jp/)

S-1-propenyl mercaptoglutathione (5)
m/z 380.09438; resolution 330,000

S-peak picking
$C_{0-50}H_{0-100}N_{0-5}O_{0-50}S_{0-5}$
< 1 mDa

5 elemental compositions

^{13}C-labeled

1 elemental composition
C13H22N3O6S2

Comparison of peak pattern

Determination of elemental composition
C13H21N3O6S2

MS/MS analysis

Characterization

EIC   MS
Non-labeled
10.20
380.09438
381.09773
382.09018

^{13}C-labeled
10.20
393.13805
392.13473
392.13056

min   *m/z*

MS/MS
Non-labeled
125.52647   177.03274   188.01982   234.02540   234.05225   287.05208   305.06266   380.09481
C4   C5   C7   C8   C11   C11   C13

^{13}C-labeled
125.53986   182.04983   195.04333   242.05225   259.07880   298.08899   316.09941   393.13853
C4   C5   C7   C8   C11   C11   C13

M   M+1   M+2
Non-labeled
380.09438   381.09773   382.09018
381.09132   382.09849   382.10100

MS/MS
Non-labeled
125.52647   177.03274   188.01982   217.06474   251.05195   287.05208   305.06266   380.09481
C5H7N1O3
C8H10N2O5
C5H12N2O5
C8H13N1O3S1
C9H20N2O4S1
C2H7N1O5
C2H5N1O2

Theoretical
380.09445   381.09781   382.09025   382.09870   382.10116
381.09149
^{15}N ^{13}C ^{34}S ^{18}O ^{13}Cx2

Simulated
C4H2N1O3S1   127.98008   C5H9N2O3S1   177.03282   C7H10N1O1S2   188.01983   C8H13N3O3S1   217.06414   C9H15N2O3S2   234.02540   234.05193   251.05195   251.05186   C11H15N2O2S2   287.05208   287.05186   305.06242   C11H17N2O4S2   305.06266   C13H22N3O6S2   380.09445

m/z

Conclusion

- LC-FT-MS is a strong tool for extraction and characterization of S-metabolites.

- Onion bulb includes quite rare S-metabolites which have strong anti-inflammatory activity.

- Strategy of biomarker screening will be changed by this method with approaches of natural products chemistry

Acknowledgements