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Metabolomic Approach for Carotenoids Analysis of Chrysanthemum flower by Liquid Chromatograph Tandem Mass Spectrometry

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Junichi Masuda[1], Satoshi Yamaki[1], Mami Okamoto[2], Muneo Sato[2], Yoshihiro Hayakawa[1], Yuji Sawada[2], Masaru Furuta[1], Masami Y. Hirai[2]

[1] Shimadzu Corporation. 380-1 Horiyamashita, Hadano, Kanagawa 259–1304,

[2] RIKEN CSRS. . 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

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Introduction

Here we demonstrate the metabolomic analysis for carotenoids in Chrysanthemum flower as a model samples by a liquid chromatograph tandem mass spectrometry. Chrysanthemum flower (*Chrysanthemum morifolium*) is one of the most important horticulture plant and the color of flower gains control of the market value. To enhance the market vale, breed improvement has been performed by hybridizing or mutation since it is known that major determinant factors of the colors are several metabolites such as carotenoids or flavonoids.
Recently, systematical metabolomic approach has been applied to improve the breeding of plants with efficiency. Under this situation, a metabolomic approach of carotenoids was investigated to establish the metbolomic platform using liquid chromatograph tandem mass spectrometry in order to develop novel and efficient breeding process for horticulture plant.

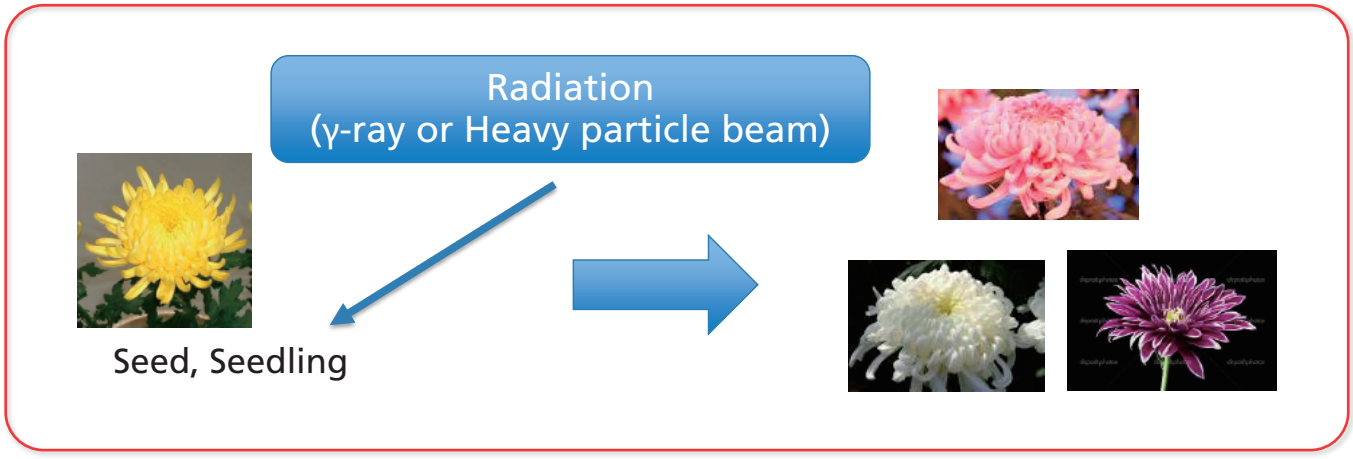

Figure 1. Image of the Radiation breeding

Experimental

Standard sample and Analytical condition

Standard regents of major carotenoids were purchased from Wako Chemical (Japan). Specified carotenoids in Chrysanthemum such as epoxides compounds were obtained from the Institute of Vegetable and Floriculture Science, NARO (Tsukuba, Japan). The analysis of carotenoids was performed by a reversed phase chromatography followed by the detection using a triple quadrupole mass spectrometer, LCMS-8050 (Shimadzu, Japan) equipped with a APCI under SIM or MRM mode. Analytical condition is shown in Table 1.

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Table 1. Analytical condition

HPLC : Nexera UHPLC system	
Column	: SunShell C18, 2.6um, 2.1 x 50mmL.
Mobile phase	: A - Water
	: B - Acetonitrile / 2-propanol (2/1)
Gradient program	: 70%B(0 min) -100%B(50 - 9 min) - 100%B(9 - 14 min) - 70%B(14.01 - 20min)
Flow rate	: 0.3 mL / min
Column temperature	: 40 °C
Injection volume	: 1.5 µL as loop injection
MS : LCMS-8050 Triple quadrupole mass spectrometer	
Ionization	: APCI (Positive / Negative)
Ion spray voltage	: +4.5 kV, -3.5 kV
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 5 L/min
IF Temp.	: 350 °C
DL Temp.	: 200 °C
HB Temp.	: 300 °C
SIM and MRM	:

Triple Quadrupole LC/MS/MS [LCMS-8050]

SIM or MRM parameters were optimized by flow injection analysis of each carotenoid standard. Though both positive and negative ions were observed for some carotenoid, the SIM or MRM parameter was determined depending on the sensitivity and CID spectrum of each carotenoids. Optimized SIM or MRM parameter is in Table 2 with each standard retention time.

Table 2. SIM or MRM parameter for each carotenoid

Compounds		+／−	Transition	CE	Retention	Start	End
ζ-carotene	met. 1	+	541.50>81.05	-39	11.069	10.158	12.158
Phytofluene	met. 2	+	543.45		11.687	10.700	12.700
Neurosporene	met. 3	+	539.50>81.00	-42	10.638	9.670	11.670
(3S,5S,6R,3'R,6'R)-5,6-Dihydro-5,6-Dihydroxylutein	met. 4	-	730.50>326.25	31	7.946	6.944	8.944
(all-E)-Lutein-5,6-epxide	met. 5	-	584.4		4.369	3.465	5.465
(9'Z)-Lutein-5,6-epxide	met. 6	-	584.35		5.338	4.338	6.338
(9Z)-Lutein	met. 7	-	568.40>550.35	23	6.354	5.357	7.357
(9'Z)-Lutein	met. 8	-	568.2		6.363	5.357	7.357
β-carotene	met. 9	+	537.40>177.35	-20	11.654	10.678	12.678
α-carotene	met. 10	+	537.40>123.15	-27	11.5	10.503	12.503
Zeinoxanthin	met. 11	-	552.35		8.989	7.990	9.990
Zeaxanthin	met. 12	-	568.35		5.327	4.322	6.322
Anthraxanthin	met. 13	-	584.35		4.371	3.305	5.305
Violaxanthin	met. 14	-	600.45		3.441	2.435	4.435
Neoxanthin	met. 15	-	600.4		3.104	2.104	4.104
Cantaxanthin	met. 16	-	564.20>472.20	22.0	6.531	5.532	7.532
Fukoxanthin	met. 17	-	658.45>59.15		2.964	1.970	3.970

Although ESI or APCI can be used for carotenoids analysis, we found that APCI can give the stable result than ESI due to ion-suppression.
Thus, APCI was used for this study.

Extraction of carotenoids

Carotenoids in each flower, which was provided by the Technology Center for Agriculture, Forestry and Fisheries in Hyogo, Nagasaki and Kagoshima prefectures, were extracted with n-Hexane following the procedure shown in Figure 2. The final supernatants obtained were injected onto the system.

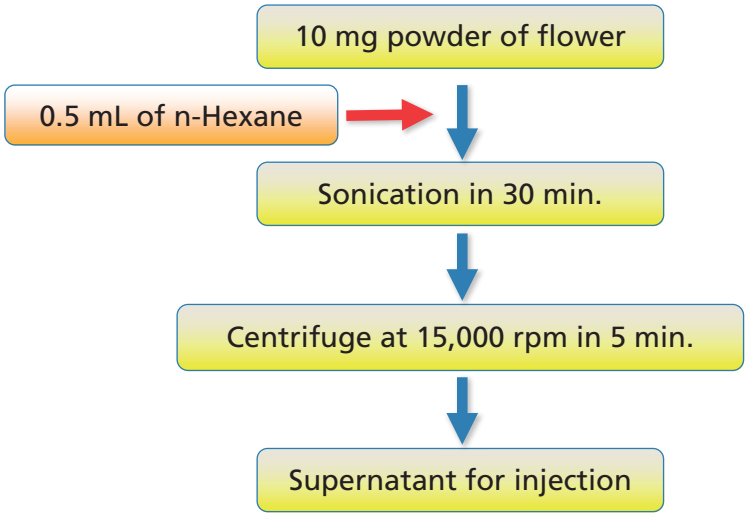

Figure 2. Procedure of extraction for carotenoids

Result and Discussion

Difference of carotenoids in Wild type or Mutant

Obtained chromatograms of standard carotenoids and breeds (Wild type and mutants) are shown in Figure 3.
Divergence in chromatograms among the breeds was found such as Zeinoxanthn, Lutain-related carotenoids as below.

17 of standard
Carotenoids
(Peak# is in Table 2 as met#.)

Wild type:
White colored flower

Mutant Breed (1):
Yellow colored flower

Mutant Breed (2):
Yellow colored flower

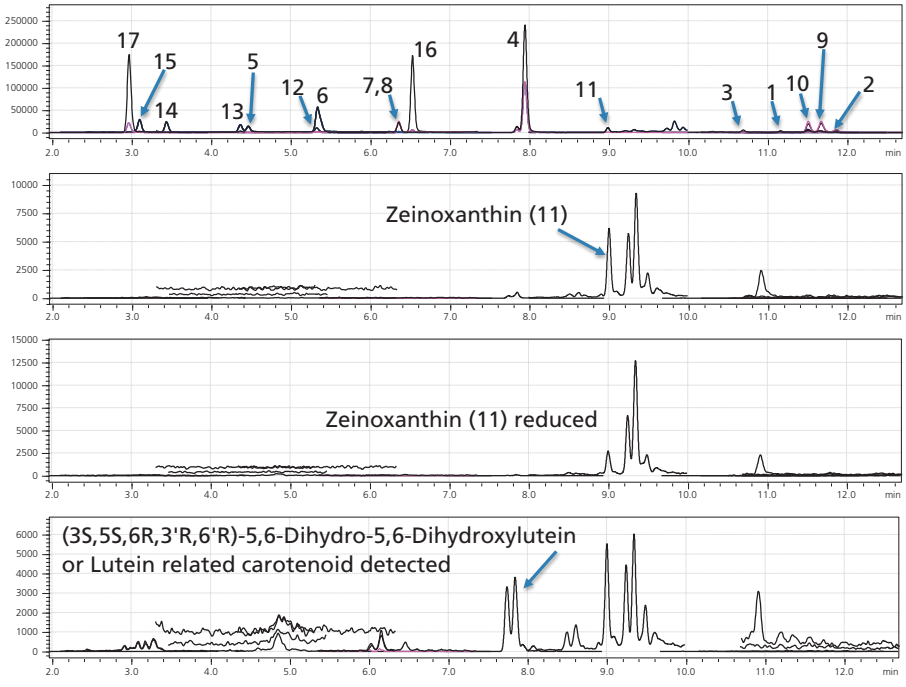

Figure 3. MRM Chromatograms of carotenoids

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The results suggest that radiation may have an influence for the pathway of carotenoids such as hydroxylation and epoxidation, especially for the pathway from Zeinoxanthin to Lutein-epxide in the map shown as Figure 4.

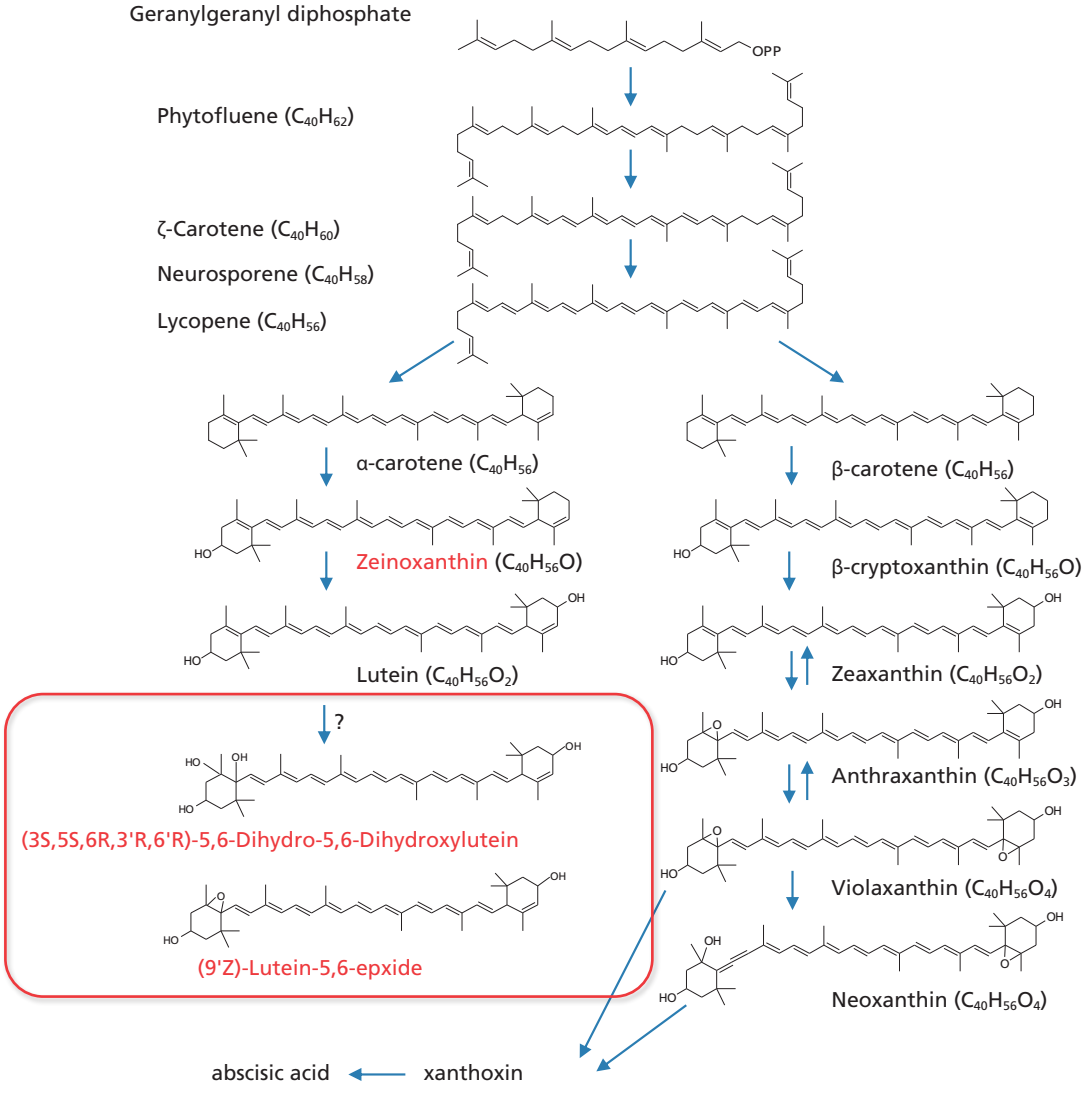

Figure 4. Metabolic pathway of major carotenoids

Cluster analysis for the breed vs. carotenoids

Cluster analysis was performed for the series of result (breeds vs. carotenoids) by the self-developed program using the R language.
This heatmap shown in Figure 5 suggests that there are some mutants which may have several specific carotenoids. The result indicates the possibility for screening of mutant breeds using the developed analytical procedure.

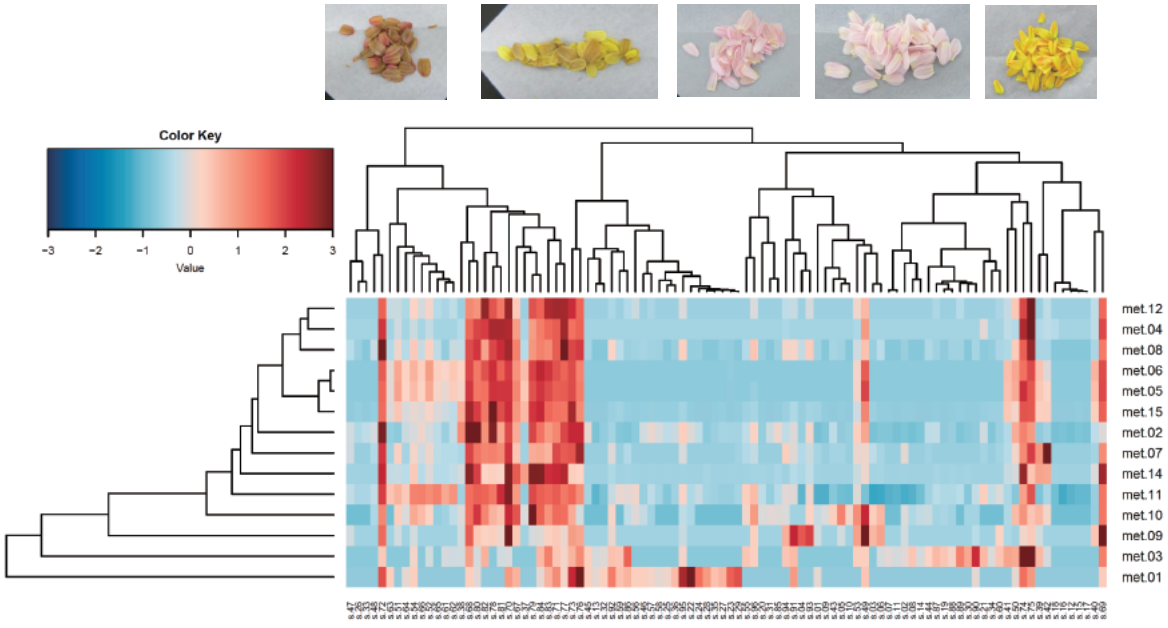

Figure 5. Heatmap of correlation for breeds vs. carotenoids

Conclusions

- Major carotenoids in Chrysanthemum flower were simultaneously identified using a LC-MS/MS system with a convenient procedure of extraction.
- Difference of the specific carotenoids in mutant suggested the influence for the metabolic pathway.
- The result of cluster analysis suggests the potential for evaluation of breeding such as mutants vs. flower color with metabolic approach of carotenoids identification.
- Further, we have been investigating the relation between color of flowers and difference of metabolic carotenoids to establish the screening frame work

Reference

"Diversity of Carotenoid Composition in Flower Petals" A. Omiya; JARQ 45 (2), 163 – 171 (2011)

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