

Metabolomics Reveals Rubiadin Accumulation and the Effects of Methyl Jasmonate Elicitation in *Damnacanthus major* Calli

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ABSTRACT

Callus suspension techniques have been considered attractive for improving bioactive metabolite productivity; methyl jasmonate (MeJA) is a widely used elicitor for stimulating synthetic pathways. In this study, a multivariate analysis-based metabolomics approach was employed to investigate the primary and specialized metabolites in the leaves, unelicited calli, and 100 or 200 μM MeJA elicited calli of *Damnacanthus major*. Rubiadin, a powerful anthraquinone with various therapeutic properties, was only identified in *D. major* calli, accumulating in a MeJA elicitation concentration-dependent manner. Callus cultures also contained high levels of amino acids, sugars, and phenolic compounds, indicating energy metabolism and metabolic adaptation responses for proliferation and stabilization. Regarding MeJA application, elicited calli contained higher amounts of quinic acid, kaempferol, and glucose with lower amounts of sucrose and raffinose than those in the unelicited control, which were closely related to protective mechanisms against MeJA. Moreover, excessive elicitation increased the asparagine, fructose, and raffinose levels and decreased the glucose and sucrose levels, which was ascribed to increased activation of the aminoacyl-tRNA biosynthesis pathway and wider utilization of glucose than of fructose after sucrose degradation. These results will be useful for optimizing plant cell culture techniques to achieve high production rates for valuable specialized metabolites.

INTRODUCTION

➤ *Damnacanthus major*

❖ Anthraquinones produced by Rubiaceae family are biologically active with anti-inflammatory, antioxidant, anticancer, and antibacterial activities.

➤ Callus suspension techniques

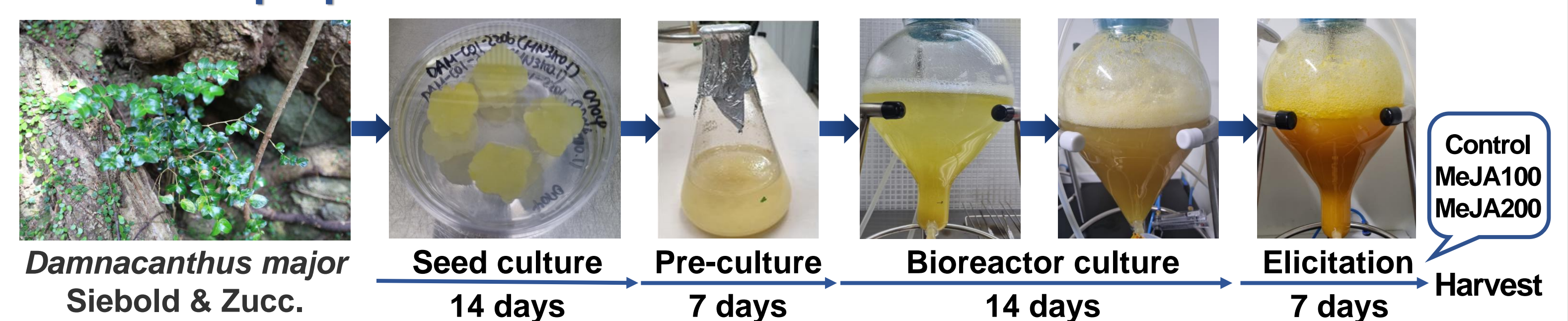
❖ Callus cultures can produce specialized metabolites at a faster rate than that of traditional cultivation and are performed under controlled conditions without being affected by various environmental factors.

OBJECTIVES

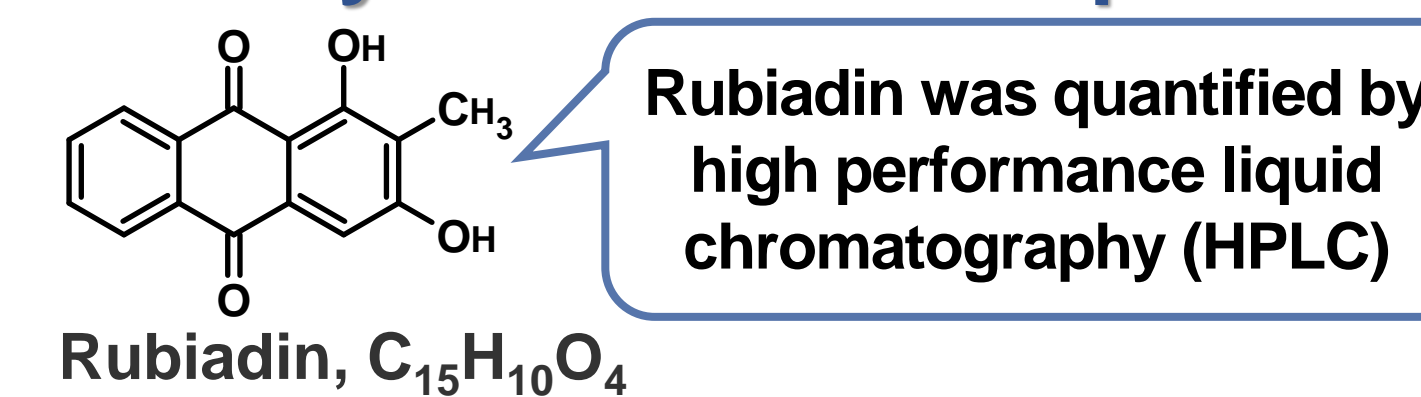
➤ Description of the metabolic differences between *D. major* leaves and callus cultures treated with different concentrations of MeJA.

MATERIALS & METHODS

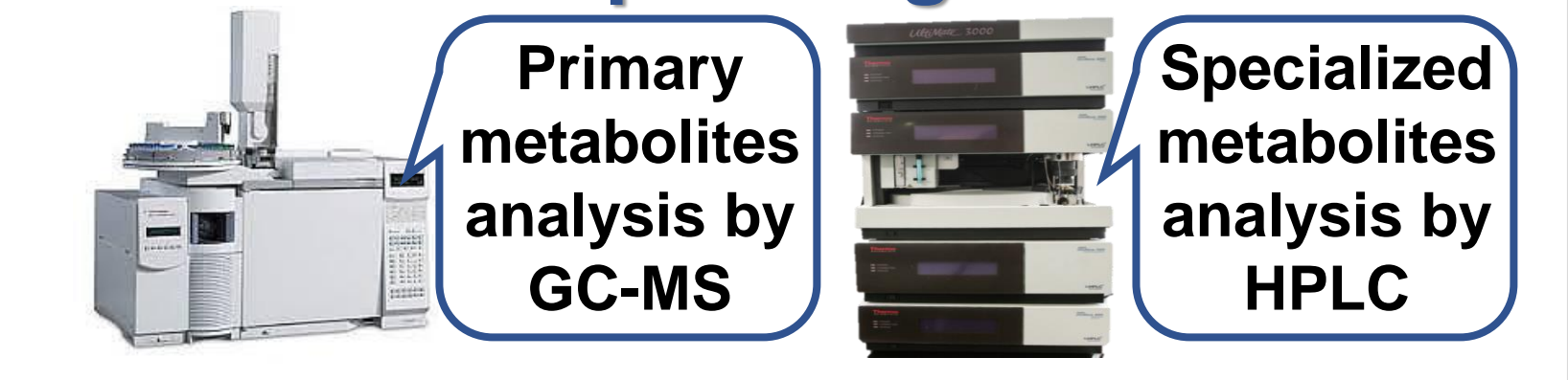
➤ Material preparation



➤ Analysis of an anthraquinone



➤ Metabolite profiling



RESULTS & DISCUSSION

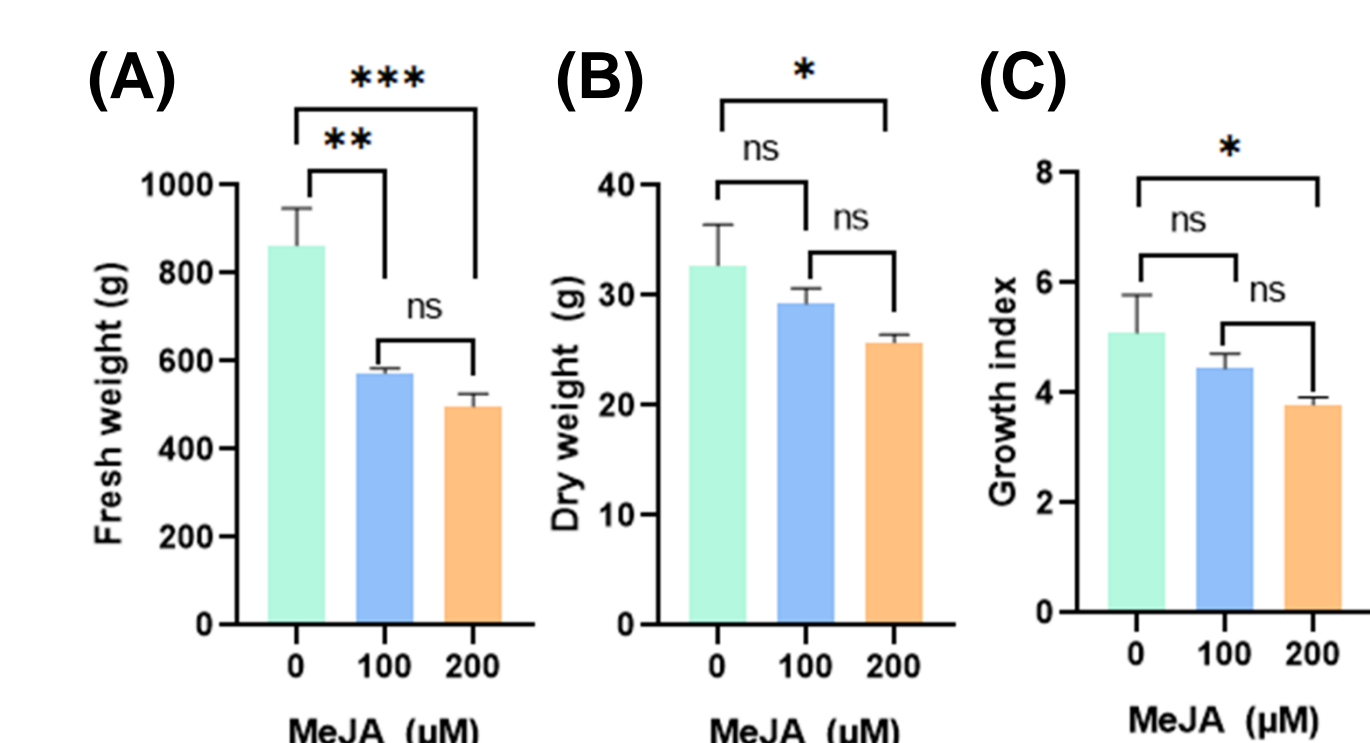


Figure 1. Biomass accumulation in response to different MeJA concentrations in the calli of *D. major*. (A) Fresh weight, (B) dry weight, (C) growth index (not significant, ns; significant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

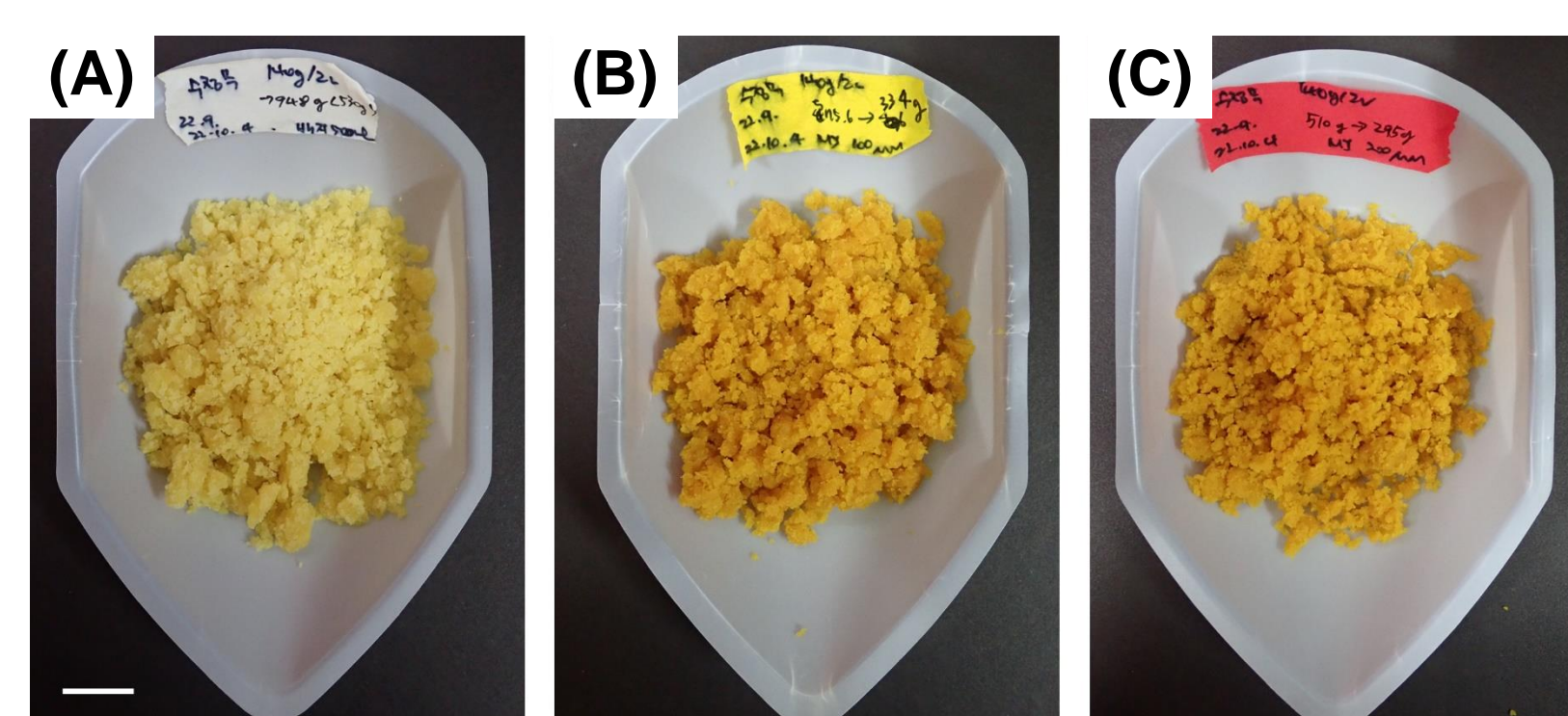


Figure 2. Effect of MeJA concentration on *D. major* callus browning for 3 weeks. (A) Unelicited (control), (B) 100 μM MeJA, (C) 200 μM MeJA (Scale bar = 1 cm).

The 100 μM treated group did not show significant differences in the biomass reduction from control group, and the color darkened depending on the MeJA concentration.

Table 1. One-way analysis of variance (ANOVA) results for the content of rubiadin in *D. Major* leaves and calli treated with different MeJA concentrations.

(mg/g Dry weight)	Leaves	Unelicited Control	Elicited MeJA 100	Elicited MeJA 200
Rubiadin	* N.D. ^a	0.121 \pm 0.007 ^b	1.458 \pm 0.008 ^c	1.961 \pm 0.143 ^d

* N.D. Not detected; ^{a,b,c,d} Different superscript letters indicate statistical differences with $p < 0.05$ (Tukey's multiple-range test).

Rubiadin was detected only in the calli of *D. major*, and higher levels of rubiadin were observed with increasing MeJA concentration.

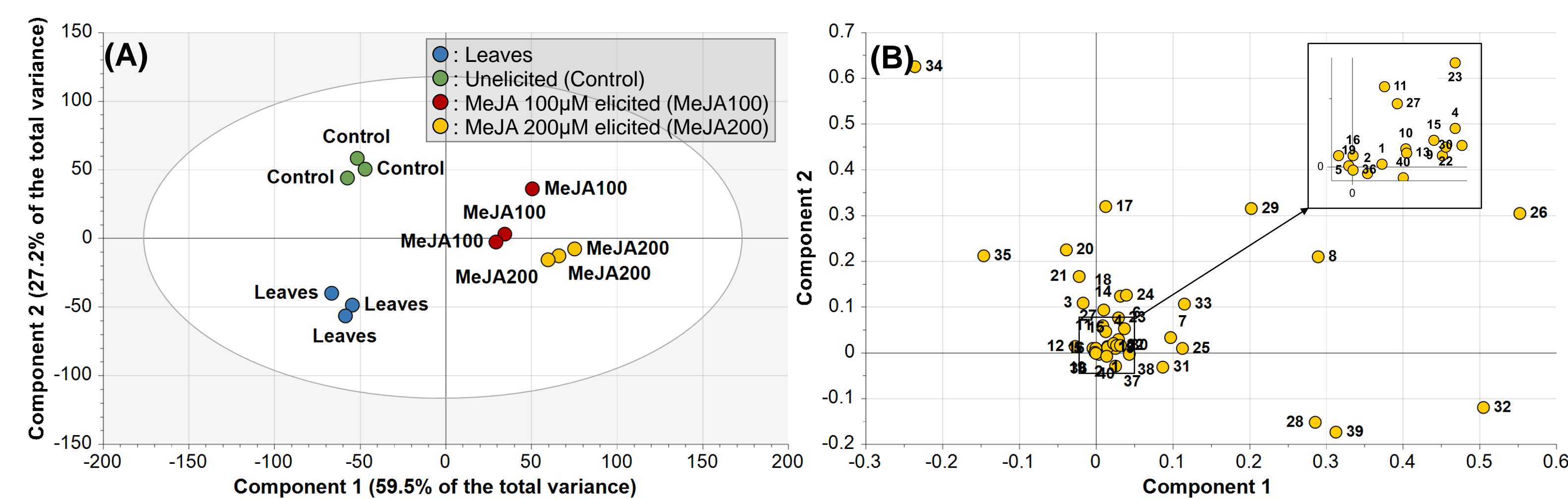
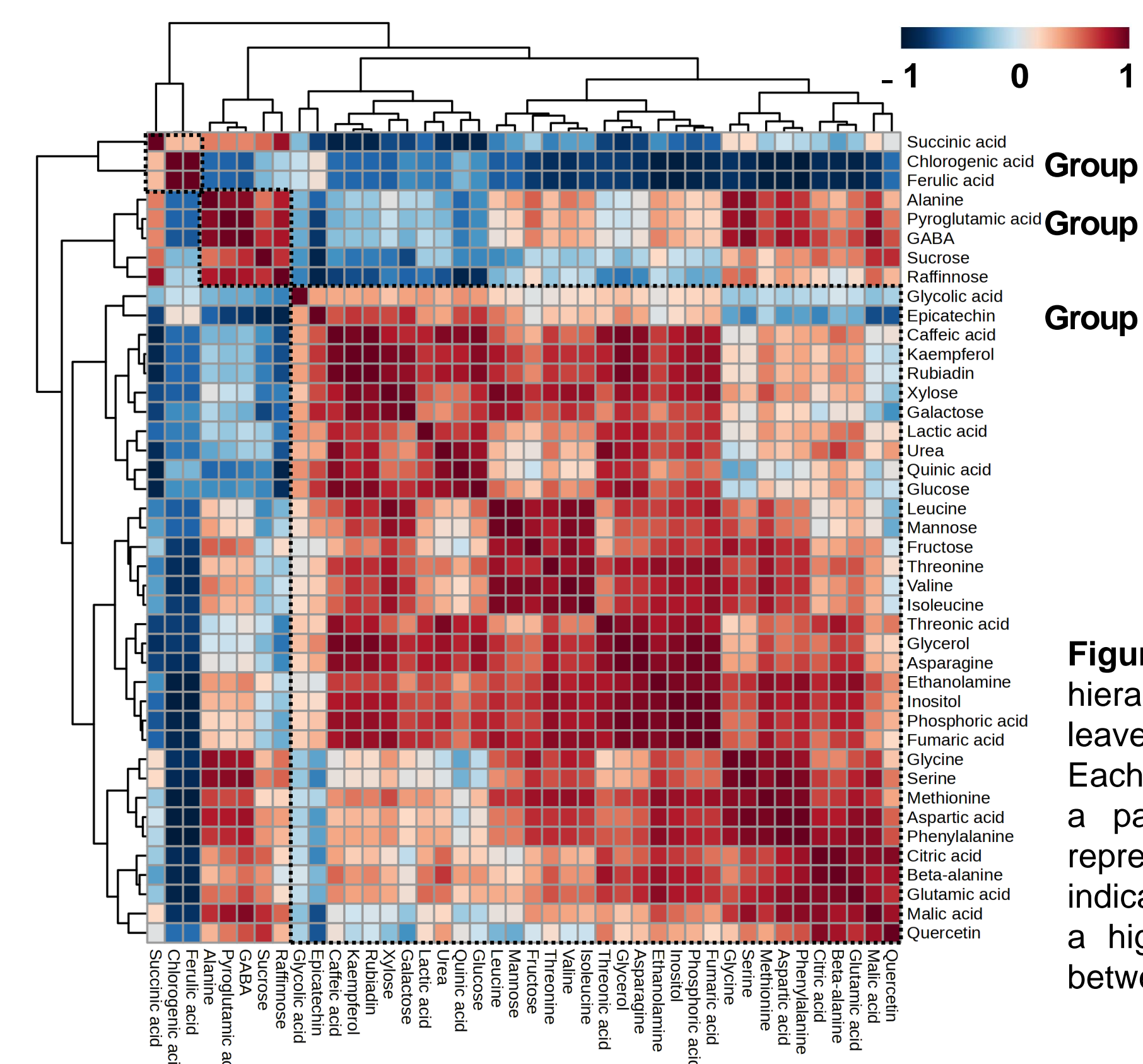


Figure 3. The score (A) and loading (B) plots of principal components 1 and 2 in the principal component analysis (PCA) results obtained from *D. major* leaves and calli treated with different MeJA concentrations. Plot annotations: 1, Lactic acid; 2, Glycolic acid; 3, Alanine; 4, Valine; 5, Urea; 6, Ethanolamine; 7, Glycerol; 8, Phosphoric acid; 9, Leucine; 10, Isoleucine; 11, Glycine; 12, Succinic acid; 13, Fumaric acid; 14, Serine; 15, Threonine; 16, β -Alanine; 17, Malic acid; 18, Aspartic acid; 19, Methionine; 20, Pyroglutamic acid; 21, GABA 22, Threonic acid; 23, Glutamic acid; 24, Phenylalanine; 25, Xylose; 26, Asparagine; 27, Citric acid; 28, Quinic acid; 29, Fructose; 30, Mannose; 31, Galactose; 32, Glucose; 33, Inositol; 34, Sucrose; 35, Raffinose; 36, Caffeic acid; 37, Epicatechin; 38, Quercetin; 39, Kaempferol; 40, Rubiadin.



Most of the primary and specialized metabolites were present at lower levels in *D. major* leaves than in *D. major* calli, and those were closely related to biological pathways.

Figure 4. Metabolite to metabolite correlation matrix and hierarchical cluster analysis (HCA) obtained from *D. major* leaves and calli treated with different MeJA concentrations. Each square indicates the Pearson's correlation coefficient of a pair of compounds with the value of this coefficient represented by blue or red colors. A higher intensity of red indicates a positive correlation between metabolites, whereas a higher intensity of blue indicates a negative correlation between metabolites. Each group is marked by a dotted box.

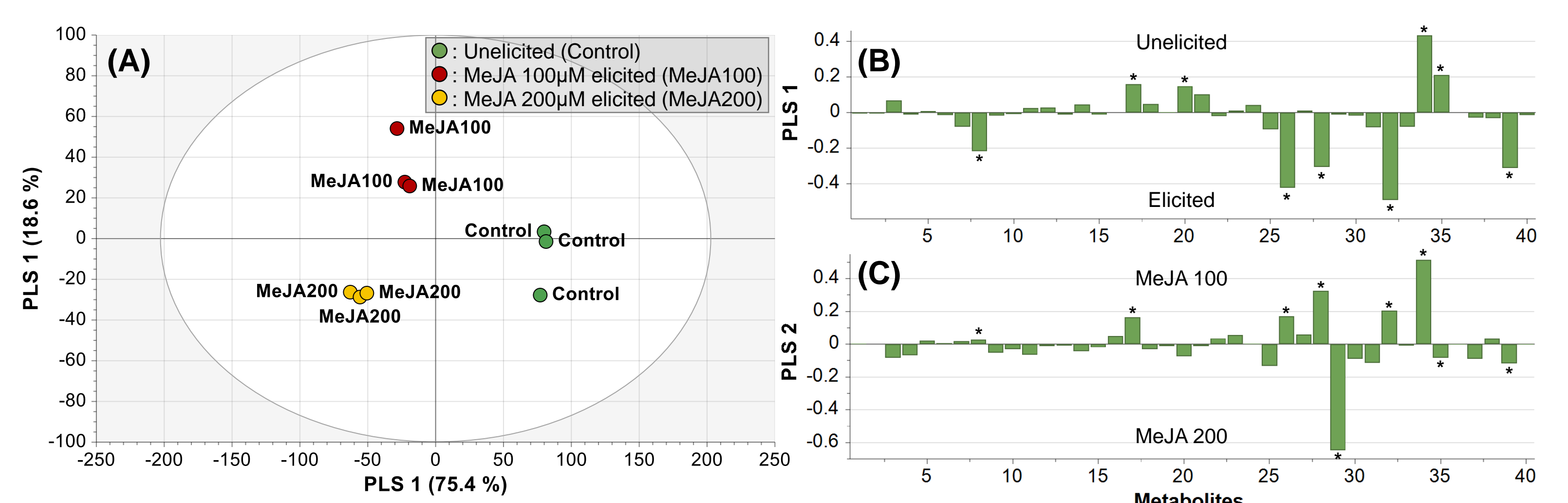


Figure 5. Score plots (A) and loading column plots of partial least squares (PLS) 1 (B) and 2 (C) from the partial least squares discriminant analysis (PLS-DA) of *D. major* leaves and calli treated with different MeJA concentrations. Asterisk symbols in the loading column plots represent significantly different and influential metabolites with $p < 0.05$ and VIP values ≥ 0.8 . Plot annotations: 1, Lactic acid; 2, Glycolic acid; 3, Alanine; 4, Valine; 5, Urea; 6, Ethanolamine; 7, Glycerol; 8, Phosphoric acid; 9, Leucine; 10, Isoleucine; 11, Glycine; 12, Succinic acid; 13, Fumaric acid; 14, Serine; 15, Threonine; 16, β -Alanine; 17, Malic acid; 18, Aspartic acid; 19, Methionine; 20, Pyroglutamic acid; 21, GABA 22, Threonic acid; 23, Glutamic acid; 24, Phenylalanine; 25, Xylose; 26, Asparagine; 27, Citric acid; 28, Quinic acid; 29, Fructose; 30, Mannose; 31, Galactose; 32, Glucose; 33, Inositol; 34, Sucrose; 35, Raffinose; 36, Caffeic acid; 37, Epicatechin; 38, Quercetin; 39, Kaempferol; 40, Rubiadin.

MeJA elicitation reorganized various primary metabolites (including central carbons, amino acids, and sugars) as well as specialized metabolites in *D. major* calli, and that these metabolic strategies were significantly linked to defensive mechanisms against elicitation.

CONCLUSION

- ❖ Using the callus culture technique with MeJA elicitation, we produced rubiadin, an anthraquinone with various biological activities.
- ❖ Our results revealed that MeJA elicitation activates the production of specialized metabolites as defensive mechanisms, followed by degradation of sugars as energy sources.
- ❖ In conclusion, 100 μM MeJA treatment of *D. Major* calli would be the best strategy for increasing valuable specialized metabolites with maintained production rates.

ACKNOWLEDGEMENT

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