Supplementary data

Potato Tuber-inducing Activities of Jasmonic acid and Related-Compounds (II)

Kanji Miyawaki *, Shiro Inoue *, Naoki Kitaoka, and Hideyuki Matsuura ‡

Laboratory of Natural Product Chemistry, Division of Fundamental AgriScience Research, Research Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

* The authors contributed equally to this work.

‡ Corresponding author.

H. Matsuura (matsuura@chem.agr.hokudai.ac.jp)

- **Contents**

I. General Experimental Procedures

Optical rotations were obtained with a JASCO P-2200 polarimeter. NMR spectra were recorded in $CDCl_3$ using a JNM-EX 270 FT-NMR spectrometer (JEOL, ^1H NMR: 270 MHz, ^{13}C NMR: 67.5 MHz) and AMX 500 (Bruker, ^1H NMR: 500 MHz, ^{13}C NMR: 126 MHz). FDMS and FIMS analyses were performed on a JMS-T100GCV (JEOL). Silica gel 60 F_{254} TLC plates (0.25 and 0.5 mm thickness: Merck, Darmstadt, Germany) were used for analytical and preparative TLC, respectively, and silica gel column chromatography was carried out using silica gel (Wako-gel C-200) provided by Wako Pure Chem. (Japan).

II. Synthesis of *cis*-(−)-OPDA

Synthesis of *cis*-(−)-OPDA was performed according to a previously reported method (Kajiwara *et al.* 2012).

III. Synthesis of *iso*-OPDA

Synthesis of *cis*-OPDA was performed according to a previously reported method (Dabrowska and Boland 2007).

IV. Synthesis of *cis*-(+)-OPDA-L-Ile

The synthesis of *cis*-(+)-OPDA-L-Ile was performed according to a previously reported method (Uchiyama *et al.* 2018).

V. Synthesis of (+)-4,5-didehydroJA

The synthesis of (+)-4,5-didehydroJA was performed according to a previous paper (Monte *et al.* 2019) using (-)-JA isolated from the culture filtrate of *Lasiodiplodia theobormae* (Aldridge *et al.* 1971; Nakamori *et al.* 1994).

In brief, diethyl allyl phosphate (100 μL, 0.56 mmol), Na_2CO_3 (71 mg, 0.67 mmol), and palladium acetate (7.56 mg, 0.036 mmol) were added to a stirred solution of (+)-MeJA（67 mg, 0.3 mmol） originated from the culture filtrate of *L. theobromae* in DMF (3 mL) at 80 ℃, and the reaction mixture was further stirred for 12 hours. A usual work-up was performed for purification to give methyl (+)-4,5-didehydrojasmonate (35 mg, 0.17 mmol, 56%).

(+)-Methyl 4,5-didehydrojasmonate:

^{1}H NMR ($CDCl_3$, 270 MHz) δ_H: 7.61 (1H, dd, *J*=5.8, 2.5 Hz), 6.19 (1H, dd, *J*=5.8, 1.1 Hz), 5.45 (1H, m), 5.21 (1H, m), 3.64 (3H, s), 2.60-1.98 (8H, m), 0.94 (3H, t, *J*=7.9 Hz).

To a stirred mixture of methyl (+)-4,5-didehydrojasmonate (8 mg, 0.036 mmol) in 0.1 M phosphate buffer (8 mL, pH 8.0) and MeOH (1.5 mL), esterase was added from porcine liver (Sigma), and the reaction mixture was further stirred for 5 hours at 30°C. A usual work-up was performed for purification to generate (+)-4,5-dhidehydroJA (4.6 mg, 0.022 mmol, 61%).

(+)-4,5-DihidehydroJA:

FD-MS: *m/z* 208 $[M]^+$; ^{1}H NMR ($CDCl_3$, 270 MHz) δ_H: 7.61 (1H, dd, *J*=5.9, 2.1 Hz), 6.19 (1H, dd, *J*=5.9, 1.9 Hz), 5.45 (1H, m), 5.21 (1H, m), 2.99-2.0 (8H, m), 0.95 (3H, t, *J*=6.9 Hz).

VI. Synthesis of 3,7-didehydroJA

The synthesis of 3,7-didehydroJA was performed according to a previous paper (Monte *et al.* 2018) using MeJA as a starting compound. To a solution of methyl 4,5-didehydrojasmonate (93 mg, 0.41mmol) synthesized from MeJA in MeOH (15 mL) in a pressure-resistant glass reaction vessel, NaOMe (44 mg, 0.82 mmol) was added, and the reaction mixture was further stirred for 12 hours at 80°C. To quench MeONa, NH_4Cl powder was directly added to the solution, and a usual work-up was performed for purification to give methyl 3,7-didehydrojasmonate (55 mg, 0.24 mmol, 59%).

Methyl 3,7-didehydrojasmonate:

^{1}H NMR ($CDCl_3$, 270 MHz) δ_H: 7.61 (1H, dd, *J*=5.8, 2.5 Hz), 6.19 (1H, dd, *J*=5.8, 1.1 Hz), 5.45 (1H, m), 5.21 (1H, m), 3.64 (3H, s), 2.60-1.98 (8H, m), 0.94 (3H, t, *J*=7.9 Hz).

To a stirred mixture of methyl 3,7-didehydrojasmonate (30 mg, 0.14 mmol) in 0.1 M phosphate buffer (10 mL, pH 8.0) and MeOH (2.0 mL), esterase from porcine liver (5 mg) (Sigma) was added, and the reaction mixture was further stirred for 3 days at 25°C. A usual work-up was performed for purification to generate 3,7-dhidehydroJA (29 mg, quantitative).

3,7-dhidehydroJA:

FD-MS: *m/z* 208 $[M]^+$; ^1H NMR ($CDCl_3$, 270 MHz) δ_H: 7.61 (1H, dd, *J*=5.8, 2.5 Hz), 6.19 (1H, dd, *J*=5.8, 1.1 Hz), 5.45 (1H, m), 5.21 (1H, m), 3.64 (3H, s), 2.60-1.98 (8H, m), 0.94 (3H, t, *J*=7.9 Hz).

VII. Synthesis of the JA amino acid conjugates

The synthesis of JA amino acid conjugates was performed according to a previously reported method (Kramell *et al.* 1988).

VIII. Evaluation of the potato tuber inducing activity

The evaluation of potato tuber-inducing activity was performed according to the reported method using potato single node stems (Koda and Okazawa 1988).

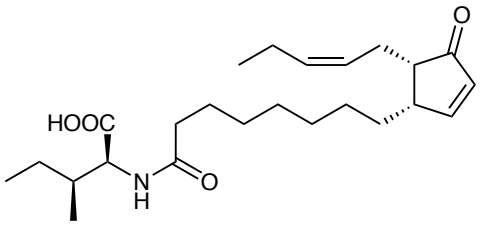
IX. Evaluation of endogenous amounts of JA, 12-hydroxyJA, and 12-*O*-β-D-glucopyranosyloxyJA

Evaluation of endogenous amounts of JA, 12-hydroxyJA, and 12-*O*-β-D-glucopyranosyloxyJA were accomplished according reported method (Matsuura *et al.* 2009).

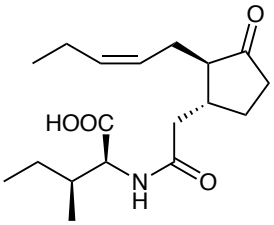
Table S1. Endogenous amounts of JA, 12OHJA, and 12OGlcJA in the single node stems (pmol/g fresh weight).

Treated compounds to single node stems	Endogenous amounts		
	JA	12OHJA	12OGlcJA
(−)-JA	3436 ± 2217	77236 ± 19205	45081 ± 12348
(+)-4,5-didehydro JA	689 ± 107	18042 ± 3102	8400 ± 1023
iso-OPDA	9 ± 3	62 ± 19	205 ± 53
3,7-didehydro JA	17 ± 1	138 ± 39	192 ± 47
control (non-treated)	10 ± 1	60 ± 34	180 ± 41

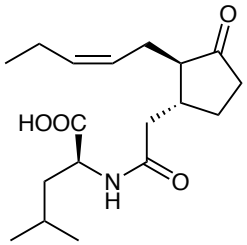
Three node stems of *S. tuberosum* L. cv Dansyaku were implanted in one flask, and the endogenous amounts of target compounds in the nodes were calculated using UPLC MS/MS system. The values represented average ± STDEV using 4 replicates (n= 4).



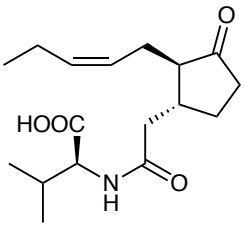
cis-(+)-OPDA-L-Ile



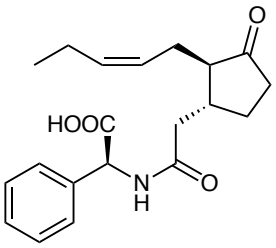
(–)-JA-L-Ile



(–)-JA-L-Leu



(–)-JA-L-Val



(–)-JA-L-Phe

Figure S1. Chemical structures of (-)-JA-L-Ile, -Leu, -Phe, -Val, and *cis*-(+)-OPDA-L-Ile.

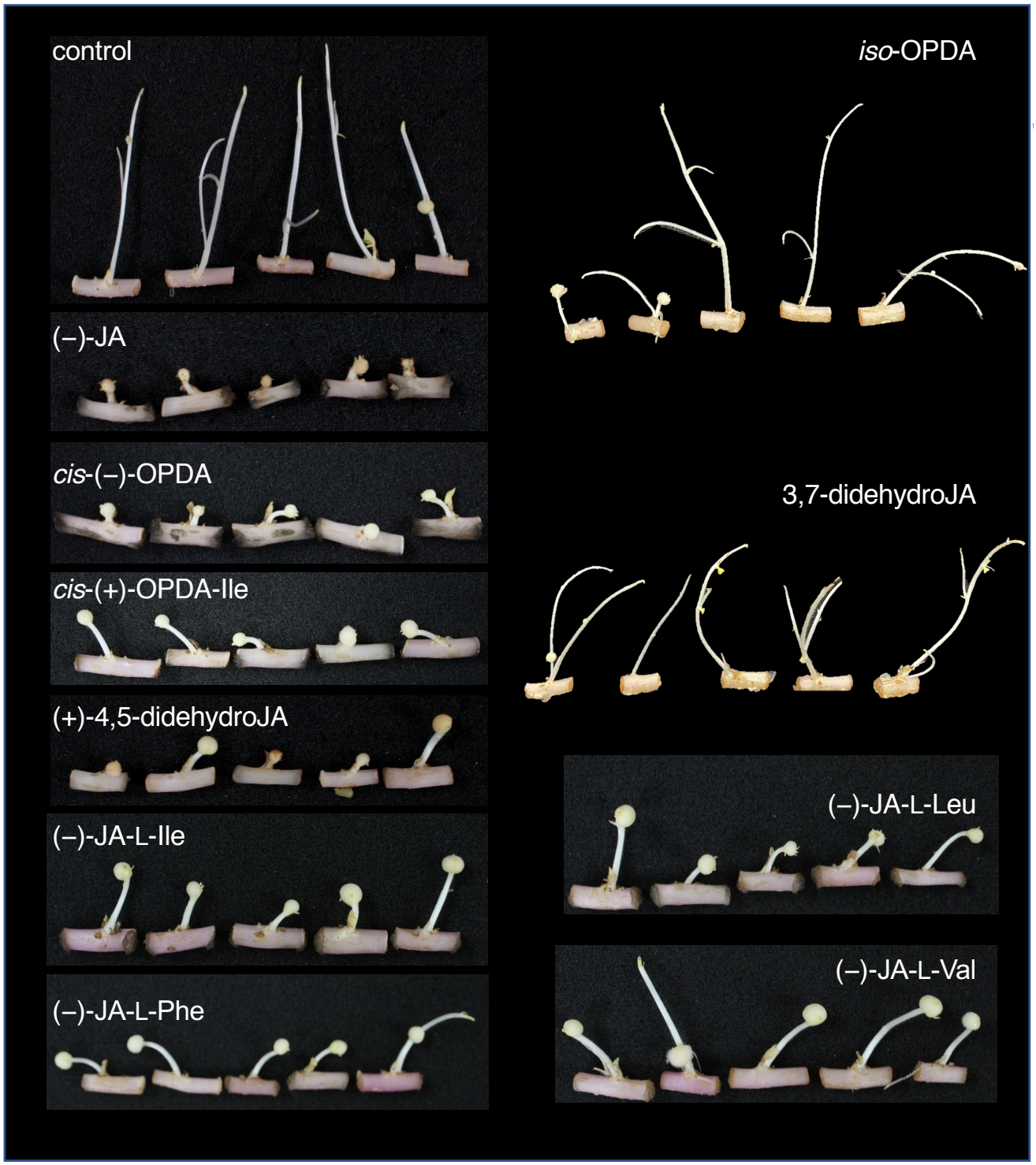


Figure S2. Potato tuber inducing activities.

Single node stems of *S. tuberosum* cv. Dansyaku were transplanted onto modified 1/2 white medium containing of target compound (1 x 10^{-4}M), and left for 3 weeks at 22 °C under the dark.

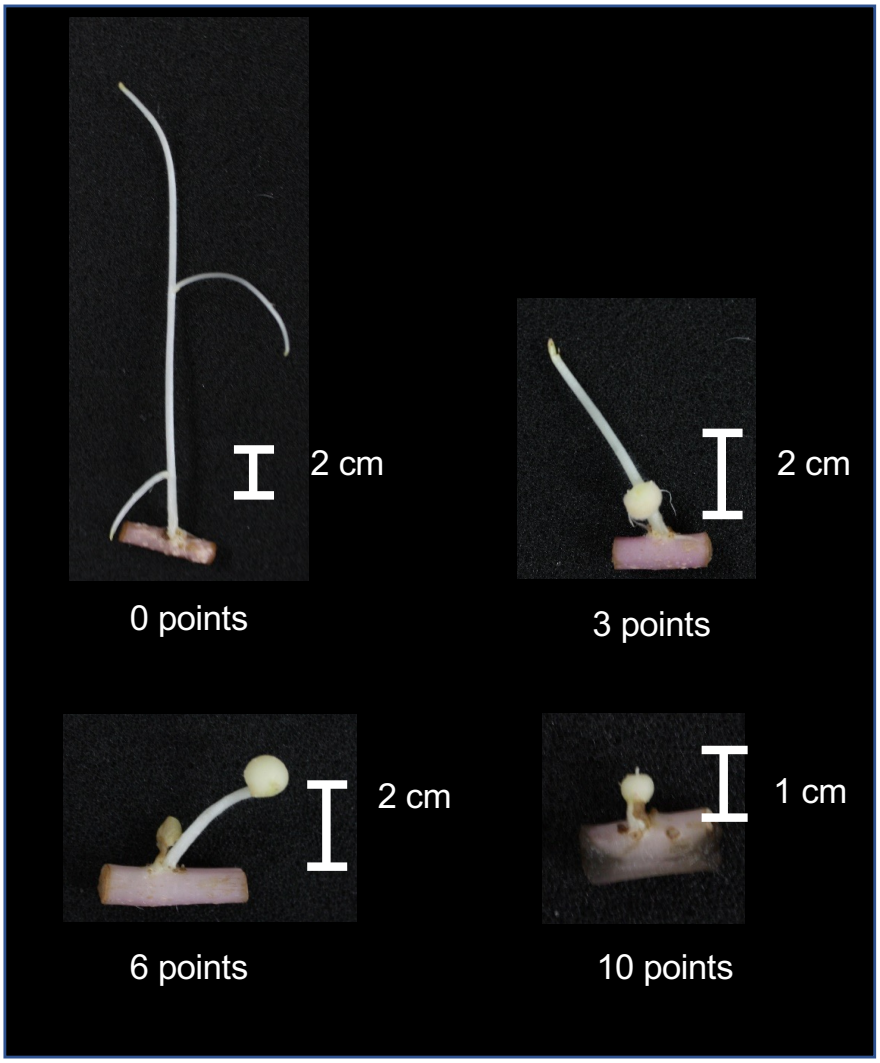


Figure S3. Grading scales to evaluate potato tuber inducing activity.

References

Aldridge DC, Galt S, Giles D et al. Metabolites of Lasiodiplodia-Theobromae. J Chem Soc C. 1971 (9):1623-27.

Dabrowska P, Boland W. iso-OPDA: An early precursor of cis-jasmone in plants? Chembiochem. 2007;**8**:2281-85.

Kajiwara A, Abe T, Hashimoto T et al. Efficient Synthesis of (+)-cis-12-Oxo-phytodienoic Acid by an in Vitro Enzymatic Reaction. Biosci Biotech Biochem. 2012;**76**:2325-28.

Koda Y, Okazawa Y. Detection of Potato Tuber-Inducing Activity in Potato Leaves and Old Tubers. Plant and Cell Physiol. 1988;**29**:969-74.

Kramell R, Schmidt J, Schneider G et al. Synthesis of N-(Jasmonoyl)Amino Acid Conjugates. Tetrahedron. 1988;**44**:5791-807.

Matsuura H, Aoi A, Satou C et al. Simultaneous UPLC MS/MS analysis of endogenous jasmonic acid, salicylic acid, and their related compounds. Plant Growth Regul. 2009;**57**:293-301.

Monte I, Ishida S, Zamarreno AM et al. Ligand-receptor co-evolution shaped the jasmonate pathway in land plants. Nat Chem Biol. 2018;**14**:480-88.

Nakamori K, Matsuura H, Yoshihara T et al. POTATO MICRO-TUBER INDUCING SUBSTANCES FROM LASIODIPLODIA-THEOBROMAE. Phytochem. 1994;**35**:835-39.

Uchiyama A, Yaguchi T, Nakagawa H et al. Biosynthesis and in vitro enzymatic synthesis of the isoleucine conjugate of 12-oxo-phytodienoic acid from the

isoleucine conjugate of α-linolenic acid. Bioorg Med Chem Lett. 2018;**28**:1020-23.