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Potato tuberization activities of JA related compounds

Title:

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

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Abstract

New information is being accumulated for plant-derived oxylipins, such as JA amino acid conjugates. However, these compounds have not being examined for their activity in promoting potato tuber formation. It was found that (−)-JA had the highest activity followed *cis*-(−)-OPDA, (+)-4, 5-didehydroJA, *cis*-(+)-OPDA-L-Ile, and (−)-JA-L-Ile, -Leu, -Phe, -Val, although *iso*-OPDA and 3,7-didehydroJA did not exhibit activity.

Keywords: jasmonic acid, jasmonoyl L-isoleucine, *cis*-OPDA, *iso*-OPDA, 3,7-didehydrojasmonic acid

Tuberization in potato plants is regulated predominantly by photoperiods. Short-day conditions promote tuberization, whereas long-day conditions inhibit the tuberization process. Using a grafting experimental procedure, the biological role of a specific tuber-inducing stimulus in leaves exposed to short days was discovered to be translocated from leaves into the stolon tip to stimulate potato tuber formation by Gregory et al. (Gregory 1956) and Chapman et al. (Chapman 1958). An attempt was made to isolate and identify this unknown factor, resulting in the discovery of (+)-7-*iso*-12-*O*-β-D-glucopuranosyloxyjasmonic acid (12OGlcJA) from leaflets of potato plant (*Solanum tuberosum* L.) (Koda and Okazawa 1988; Yoshihara *et al.* 1989), and the aglycone moiety of the compound, (+)-7-*iso*-12-hydroxyjasmonic acid (12OHJA), was named as tuberonic acid. Subsequent studies showed that jasmonic acid (JA) also possessed potato tuber inducing activity (Koda *et al*. 1991). It is generally accepted that JA and its derivatives are important signalling compounds in plant development and stress response pathways, such as senescence (Miyamoto *et al.* 1995), wound response (Farmer and Ryan 1992) and reproductive development (Falkenstein *et al.* 1991; McConn and Browse 1996). JA is biosynthesized from the α-linoleinic acid released by lipase activity; the intermediate is converted by sequential peroxidation, dehydration and cyclization to form *cis*-12-oxo-phytodienic acid (*cis*-OPDA). *cis*-OPDA is converted to OPC 8:0 via OPR3 (Schaller *et al.* 2000; Strassner *et al.* 2002), which was named as OPDA reducing pathway (Figure 1), and furthermore, by three successive reduction and β-oxidation steps that shorten the acid side chain to produce (+)-7-*iso*-JA, which is spontaneously epimerized into (−)-JA. Different biosynthetic pathways using OPR2 to reduce 4,5-didehydro-7-*iso*-JA to JA have also

been reported using *opr3* mutant *Arabidopsis thaliana* (Chini *et al.* 2018). (+)-7-*iso*-JA is enzymatically converted into many kinds of conjugates and derivatives, including MeJA (Seo *et al.* 2001) and JA amino acid conjugates (Staswick and Tiryaki 2004). Among the amino acid conjugates, jasmonoyl-L-isoleucine (JA-L-Ile) is considered as the most important compound that shows biological activity, especially for wound response (Chini *et al.* 2007). However, it was found that each jasmonate has its own unique biological activity, thus *cis*-OPDA possesses its own unique biological activity that is needed for withstanding the wound response (Taki *et al.* 2005) and for the tendril-coiling response of *Bryonia dioica* Jacq. (Blechert *et al.* 1999). Furthermore, new types of JA-related compounds have been reported, such as death acids from *Cochliobolus heterostrophus* infected leaves of maize (*Zea mays*) (Christensen *et al.* 2015), *cis*-OPDA glutathione conjugates (Ohkama-Ohtsu *et al.* 2011), and *cis*-OPDA amino acid conjugates form *A. thaliana* (Flokova *et al.* 2016; Uchiyama *et al.* 2018), and wound response pathways involving interactions with COI1 proteins other than JA-L-Ile have been reported, which are mediated via *cis*-dinor-OPDA, *iso*-dinor-OPDA using *Marchantia polymorpha* as an experimental plant (Monte *et al.* 2018) and (+)-7-*iso*-12-hydroxyJA-L-Ile in *A. thaliana* (Poudel *et al.* 2019). Recent studies have also revealed the relationship between JA and *cis*-jasmone (Dabrowska and Boland 2007), and the details of their biosynthetic pathway have been clarified, but it is still debated whether JA is a precursor that is converted *cis*-jasmone. The key compounds for this topic are *iso*-OPDA and 3,7-didehydroJA (Koch, Bandemer and Boland 1997; Matsui *et al.* 2017; Matsui *et al.* 2019; Matsui *et al.* 2020).

Therefore, from the above knowledge it can be concluded that new information is being accumulated for plant-derived oxylipins, which is a general term for a group of

compounds originating from α linolenic acid (Figure 1). A paper regarding potato tuber-inducing activities using several kinds of JAs was published (Koda *et al.* 1991), although new types of JA were discovered after the report, such as JA amino acid conjugates, which did not undergo tests regarding their ability to promote potato tuber formation. In this paper, the structure-activity relationship activities for *cis*-(−)-OPDA, (+)-OPDA-L-Ile, *iso*-OPDA, (−)-JA-L-Leu, (−)-JA-L-Phe, (−)-JA-L-Val, (−)-JA-L-Ile, (+)-4,5-didehydroJA, and 3,7-didehydroJA promoting potato tuber formation are evaluated.

JA was used for a positive control in this study, since it was reported that JA showed potato tuber induing activity (Koda *et al.* 1991), and optically pure (−)-JA was isolated from the culture filtrate of *Lasiodiplodia theobromae* (Aldridge *et al.* 1971). Optically pure *cis*-(−)-OPDA (Figure 1) and *cis*-(+)-OPDA-L-Ile (Figure S1) were synthesized according to the reported method (Kajiwara *et al.* 2012, Uchiyama *et al.* 2018) using acetone powder prepared from flax seeds (Zimmerman and Feng 1978) and a recombinant AOC derived from *Physcomitrella patens* (Stumpe *et al.* 2010). *iso*-OPDA (Figure 1) was synthesized according to a previously reported method (Lauchli and Boland 2003). Optically pure JA amino acid conjugates, (−)-JA-L-Ile, -Phe, Leu, and Val (Figure S1), were obtained using (±)-MeJA as a starting compound, and (+) and (−) forms were separated at the final step by conventional silica gel column chromatography (Kramell *et al.* 1988). Optically pure, (+)-4,5-didehydroJA (Figure 1) was prepared according to the reported method (Monte *et al*., 2018) using (−)-JA as a starting compound. (+)-3,7-DidehydroJA (Figure 1) was prepared according to the

reported method (Monte *et al*., 2018) using (±)-MeJA as a starting compound. All data for synthesized compounds are given in Supplementary data.

The synthesized compounds were subjected to a bioassay to evaluate potato tuber-inducing activity using potato single node stems (Koda and Okazawa 1988) accorging to the described method in Supplementary data, in which the concentrations of test compounds and (−)-JA (positive control) were set at 10^{-4} M. Photographs showing representative biological activities are given in Figure S2. In the control plants, the stems extending from transplanted node stems had just grown out, and the emergences of induced potato tubers were very rare. In contrast, the induced potato tubers on the tip from transplanted node stems were observed in (−)-JA-treated stems, in which case the length of the extending stems from transplanted node stems was less than 1 cm. When the transplanted node stems were planted in agar medium containing (−)-JA at 10^{-4} M, the emergence of the stem from the transplanted stem was delayed compared with that of the control, and stems already bearing potato tuber at the tip suddenly appeared almost 12 days post-transplantation into the medium. The results of the bioassay using *cis*-OPDA were similar with that of (−)-JA at 10^{-4} M. However, the length of the extending stem from transplanted node was slightly longer than those treated with (−)-JA at 10^{-4} M, although the lengths of the extending stems from transplanted node stems were less than 1 cm. Similar to the trends of *cis*-OPDA, active potato tuber inductions were observed in *cis*-(+)-OPDA-L-Ile and (+)-4,5-didehydroJA, but the lengths of the extending stems from transplanted node stems were slightly longer than those treated with *cis*-OPDA. In the case of testing (−)-JA amino acids, potato tubers were able to be detected at the tips of the extending stems from transplanted node stems, but in some cases, potato tubers were found to have formed in

the middle of the extending stems from transplanted node stems. Generally, the stem length of (−)-JA amino acid-treated plants were over 1 cm but less than 2 cm. However, it was very rare to observe the potato tuber-inducing activity using *iso*-OPDA and 3,7-didehydroJA as test compounds. The induced potato tubers, if observed at all, were only found on stems that were more than 2 cm long. To evaluate the results of the bioassay numerically, the degree of activity was quantified. Judged by the previous description, the assessment of potato tuber-inducing properties can be roughly determined by the lengths of the extending stems from transplanted node stems and the location where the tuber arises. In accordance with this view, numerical values were assigned to the following plant states: 10 points, potato tubers formed at the tip of the extending stem less than 1 cm of the stem length; 6 points, potato tubers formed at the tip of the extending stem less than almost 2 cm of the stem length; 3 points, potato tubers formed on the extending stem more than 2 cm in length; and 0 points, potato tubers were not formed on the extending stem (Figure S3). We evaluated the biological activity of the potato tuber-inducing activity of each compound based on its numerical values using the evaluation method shown in Figure S3, and the results are given in Table I, and the schematic illustration to show relative activity for each compound is given in Figure 2. The results were similar to those of the other two independent experiments.

Since it was reported that JA showed potato tuber inducing activity (Koda *et al.* 1991) and exogenously applied JA was converted into 12OHJA and 12OGlcJA using potato single node stems (Matsuura and Yoshihara, 2003), it was hypothesized that exogenously applied *iso*-OPDA and 3,7-didehydroJA would not be converted to afford enough amounts of the compounds to induce potato tubers showing activity. Therefore,

the endogenous levels of those compounds in JA, (+)-4,5-didehydroJA, *iso*-OPDA, and 3,7-didehydroJA treated and the non-treated single node stems were evaluated according to the described method in Supplementary data. It was cleared that the endogenous levels of those compounds in *iso*-OPDA, 3,7-didehydroJA treated and the non-treated single node stems were almost same (Table S1).

In this paper, potato tuber-inducing activities of (−)-JA and the related compounds, *cis*-(−)-OPDA, (+)-OPDA-L-Ile, *iso*-OPDA, (−)-JA-L-Leu, (−)-JA-L-Phe, (−)-JA-L-Val, (−)-JA-L-Ile, (+)-4,5-didehydroJA, and 3,7-didehydroJA, were evaluated, which showed that (−)-JA gave the highest activity following *cis*-(−)-OPDA, (+)-4,5-didehydroJA and (−)-JA-L-Ile, although *iso*-OPDA and 3,7-didehydroJA did not show activity. Interestingly, the potato tuber-inducing activity of (−)-JA-L-Ile was weaker than that of (−)-JA. Since (−)-JA-L-Ile is thought to be the active form for JA-mediated biological activity, it was supposed that there might be a different mode of action to induce potato tuber other than that of (−)-JA-L-Ile signaling system. However, the weaker activity of (−)-JA-L-Ile might be due to the difference in physical properties between (−)-JA and (−)-JA-L-Ile, such as permeability to cell membrane of plants, but there is not enough accumulated knowledge to discuss this point. The involvement of the FT homologue protein, SPL6 (Navarro *et al.* 2011) and the mobile mRNA, BELL5 (Lin *et al.* 2013) in potato tuber induction has been reported, although the relationship between these mobile factors and the oxylipins has not been established yet. Furthermore, there are no data or reports on the involvement of the COlI-JAZ complex in the regulation of tuber induction and real form of the compound to induce potato tubers. To clarify the unclear points, further research is needed.

Acknowledgements

We acknowledge the assistance of Dr. Eri Fukushi and Mr. Yusuke Takata (Research Faculty of Agriculture, Hokkaido University) in obtaining the spectroscopic data. We used UPLC MS/MS systems at the Research Faculty of Agriculture, Hokkaido University.

Supplementary material

Supplementary material is available at Bioscience Biotechnology and Biochemistry **********.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Author contribution

H.M. shared the responsibility of writing the manuscript with K.M., S.I. and N.K. All authors were responsible for the study concept and design. K.M., S.I. performed all the experiments. All authors contributed to the critical revision of the manuscript.

Funding

This work was supported by the Japan Society for the Promotion of Science (JSPS) [KAKENHI Grant Number 20H04755 to HM].

Declaration of Competing Interests

No potential conflict of interest was reported by the authors.

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Table I. Evaluation of the potato tuber inducing activities.

Entry	Average ± STDEV
(−)-JA	29.0 ± 1.7 [a)]
(−)-*cis*-OPDA	25.5 ± 3.6 [a)]
cis-(+)-OPDA-L-Ile	25.3 ± 1.3 [a)]
(+)-4,5-didehydroJA	24.0 ± 4.0 [a)]
(−)-JA-L-Ile	18.8 ± 1.6 [b)]
(−)-JA-L-Phe	17.3 ± 1.3 [a)]
(−)-JA-L-Leu	16.8 ± 1.5 [b)]
(−)-JA-L-Val	16.2 ± 1.5 [b)]
iso-OPDA	6.8 ± 2.5 [a)]
3,7-didehydroJA	3.6 ± 2.9 [a)]
control	3.8 ± 1.3 [a)]

Three node stemstems of *S. tuberosum* L. cv Dansyaku were transplanted in one flask, and the mean and standard deviation values were calculated from the points given according to Figure S3.

a) n=4, b) n=5.

Figure Legends

Figure 1. Biosynthetic pathway of JA and its derivatives.
cis-OPDA: *cis*-12-oxo-phytodienoic acid, OPC 8:0:
3-oxo-2-(2‘-[*Z*]-pentenyl)-cyclopentane-1-octanoicacid, OPC 6:0;
3-oxo-2-(2’-[*Z*]-pentenyl)-cyclopentane-1-hexanoic acid, OPC 4:0:
3-oxo-2-(2‘-[*Z*]-pentenyl)-cyclopentane-1-butanoicacid, LOX: lipoxygenase, AOS:
alene oxide synthase, AOC: alene oxide cyclase, OPR3: *cis*-12-oxophytodienoate
reductase 3, OPR2: *cis*-12-oxophytodienoate reductase 2, JA: jasmonic acid.

Figure 2. Order of strength of biological activity to induce potato tubers.

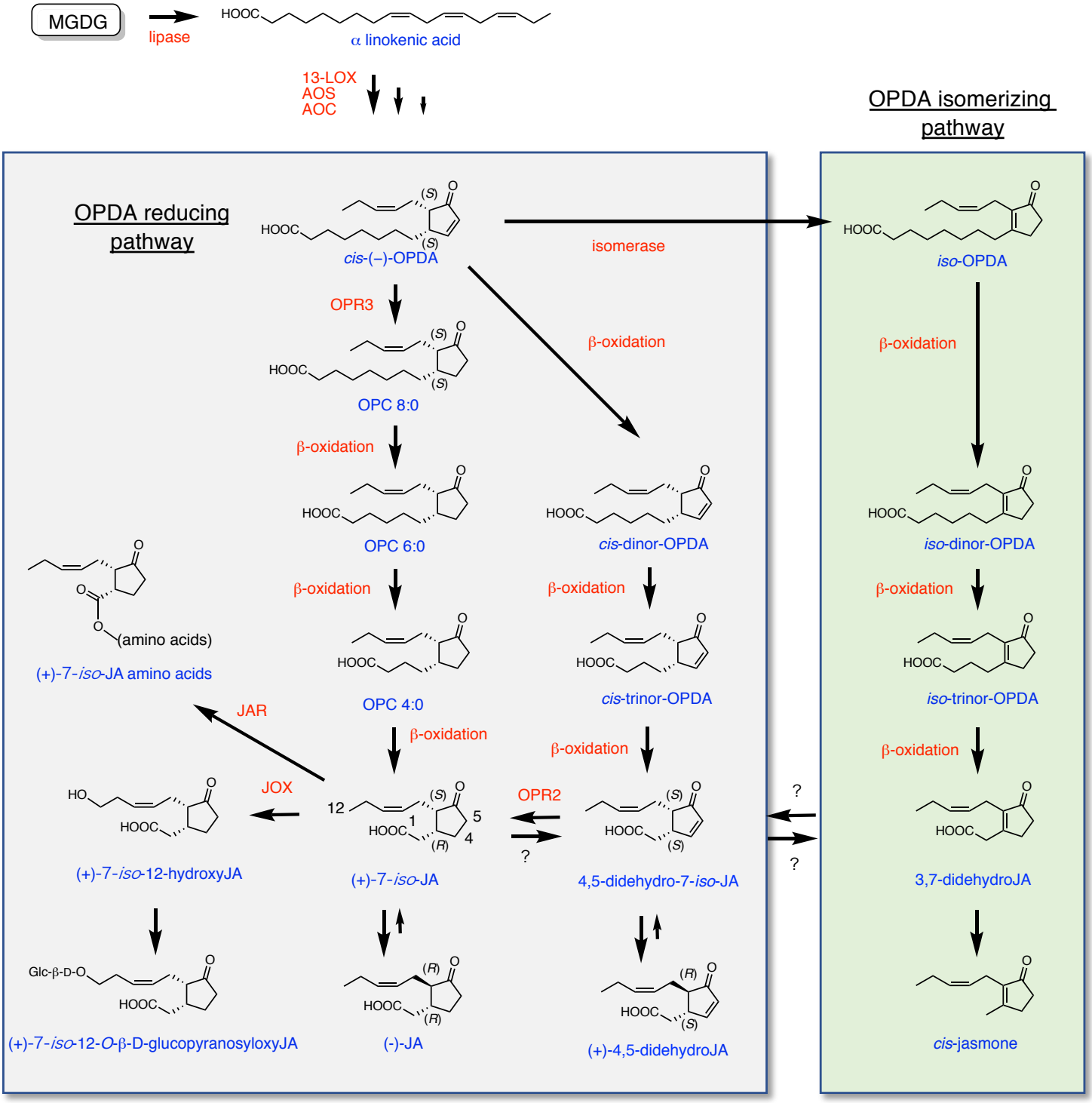
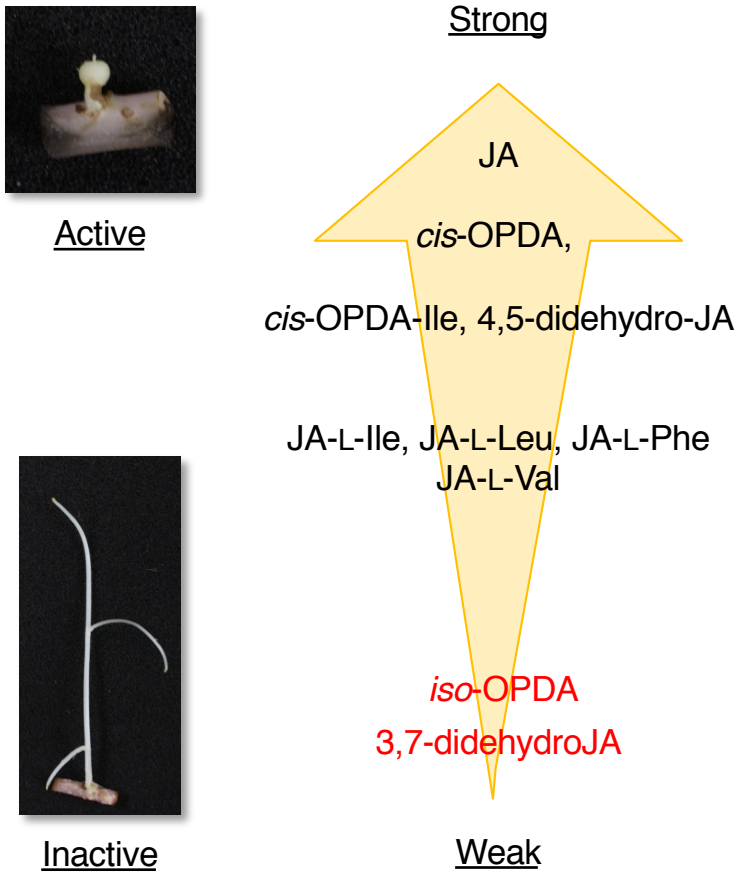


Figure 1. Biosynthetic pathway of JA and its derivatives.

cis-(–)-OPDA: *cis*-12-oxo-phytodienoic acid, OPC 8:0: 3-oxo-2-(2‘-[*Z*]-pentenyl)-cyclopentane-1-octanoicacid, OPC 6:0; 3-oxo-2-(2’-[*Z*]-pentenyl)-cyclopentane-1-hexanoic acid, OPC 4:0: 3-oxo-2-(2‘-[*Z*]-pentenyl)-cyclopentane-1-butanoicacid, LOX: lipoxygenase, AOS: alene oxide synthase, AOC: alene oxide cyclase, OPR3: *cis*-12-oxophytodienoate reductase 3, JA: jasmonic acid.



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