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Additional evidence against jasmonate-induced jasmonate induction hypothesis

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ABSTRACT

Jasmonates are phytohormones involved in development and stress reactions. The most prominent jasmonate is jasmonic acid, however, the bioactive jasmonate is (+)-7-*iso*-jasmonoyl-*L*-isoleucine (JA-Ile). Biosynthesis of jasmonates is long time known; compartmentalization, enzymes and corresponding genes are well studied. Because all genes encoding these biosynthetic enzymes are jasmonate inducible, a hypothesis of jasmonate-induced-jasmonate-biosynthesis is widely accepted. Here, this hypothesis was revisited by employing the synthetic JA-Ile mimic coronalon to intact and wounded leaves, which excludes structural cross-contamination with endogenous jasmonates. At an effective concentration that induced various jasmonate-responsive genes in Arabidopsis, neither accumulation of endogenous jasmonic acid, JA-Ile, nor of their hydroxylated metabolites was detected. Results indicate that in spite of jasmonate-induced biosynthetic gene expression, no jasmonate biosynthesis/accumulation takes place supporting a post-translational regulation.

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1. Introduction

In higher plants, low molecular lipid-derived signal molecules are ubiquitously distributed and involved in many developmental processes as well as in many different stress-related physiological responses [1–3]. Fatty acid-derived octadecanoids such as *cis*-(+)-12-oxo-phytodienoic acid (*cis*-OPDA) and jasmonic acid (JA) are well known examples for the so-called jasmonates, an important class of oxylipin phytohormones. The biosynthesis of octadecanoids, starting from α-linolenic acid, was first reported by Vick and Zimmermann [4]. Today, the whole biosynthetic pathway of jasmonates is elucidated in detail including the identification and cloning of all enzymes involved, as described in many reviews [1,2,5,6]. Briefly, biosynthesis of jasmonic acid (JA) takes place in three different cell compartments. In the chloroplast, α-linolenic acid is released from membranes, activated by a 13-lipoxygenase (13-LOX) to a hydroperoxyoctadecatrienoic acid, which is further converted to an unstable epoxide by action of a 13-allene oxide synthase (13-AOS); followed by an allene oxide cyclase (AOC) catalyzed cyclization to *cis*-OPDA. After transport of *cis*-OPDA into peroxisomes the cyclopentenone ring is reduced by a *cis*-OPDA reductase 3 (OPR3) and subsequently the carboxylic acid side chain is shortened by β-oxidation to generate (+)-7-*iso*-JA, which is again released into the cytosol and epimerizes to the less active (−)-JA. It is worth to mention that the expression of all genes for JA biosynthesis is inducible by jasmonate treatment [7–9], suggesting a jasmonate-induced-jasmonate-biosynthesis. However, only in 2004 it became clear that not JA itself but its isoleucine conjugate represents the active phytohormone [10]. This conjugation is catalyzed by JASMONATE RESISTANT 1 (JAR1) using (+)-7-*iso*-JA as the JA substrate [10]. As the endogenous bioactive jasmonate (+)-7-*iso*-jasmonoyl-*L*-isoleucine (JA-Ile, Fig. 1) was identified [11]. Strikingly, JA-Ile is the only jasmonate that interacts with the corresponding SCF^{COI1}-JAZ co-receptor complex thereby initiating the jasmonate depending responses in a plant cell [12,13]. In detail, upon JA-Ile binding, the COI1-JA-Ile subunit of the SCF^{COI1} complex (acting as an E3 ubiquitin ligase) interacts with JAZ proteins and forms the whole co-receptor complex [14]; JAZ proteins, acting as repressors of jasmonate signaling, are subsequently ubiquitinated and targeted for 26S proteasome-mediated degradation [2]. This activates transcription factors such as MYC2, subsequently the expression of JA-responsive genes and, as a consequence thereof, the onset of defense reactions [2].

Interestingly, coronatine, a bacterial phytotoxin from *Pseudomonas syringae* consisting of the polyketide coronafacic acid and the rare cyclopropyl amino acid, coronamic acid [15],

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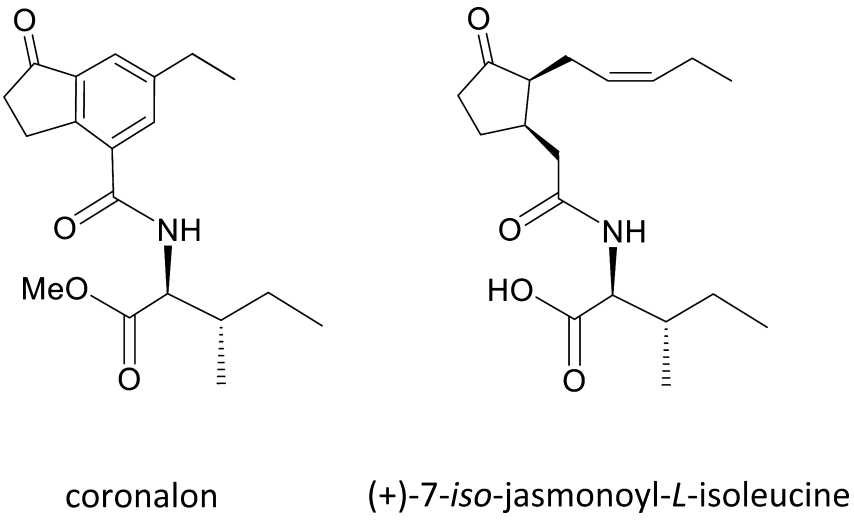

Fig. 1. Structures of (+)-7-*iso*-jasmonoyl-*L*-isoleucine (JA-Ile) and 6-ethyl indanoyl isoleucine (coronalon).

is also able to induce typical jasmonate-induced responses. The thereby suggested interaction between coronatine and the JA-Ile co-receptor complex has already been shown, demonstrating high affinity binding [14]. Because the synthesis of coronatine is complex and tedious, alternative compounds exhibiting the same biological activities have been designed and synthesized as structural mimics of coronatine, namely the 6-substituted 1-oxoindanoyl isoleucine conjugates [16–18]. In particular, a 6-ethyl-indanoyl-isoleucine conjugate (2-[(6-ethyl-1-oxo-indane-4-carbonyl)-amino]-3-methyl-pentanoic acid methyl ester), coronalon (Fig. 1), has been widely tested and established as efficient mimic of various jasmonate-induced responses in plants; among others, induction of secondary metabolites, volatiles and defense-related genes in various plant species, induction of intracellular calcium transients, pest resistance in field studies, root growth inhibition [16,17,19–21], for reviews: [22,23]. Only recently, based on modeling studies it was predicted for lima bean (*Phaseolus lunatus*) that coronalon can directly interact with the COI1-JAZ co-receptor as well [18].

In the present work we use the JA-Ile mimic coronalon to re-investigate the hypothesis of jasmonate-induced jasmonate accumulation in plant cells. By studying the effects of exogenous application of jasmonates – or their structural mimics like coronalon – it clearly has to be distinguished between a feedback loop in terms of activation of JA biosynthesis genes and expression of JA-responsive genes on one side, and accumulation of endogenous jasmonates on the other side. This work was motivated by the fact that very many reviews on jasmonate biosynthesis suggest a jasmonate-induced-jasmonate-biosynthesis regulation of JA biosynthesis and accumulation [1,2,24–26] although for tomato (*Solanum lycopersicum*), lima bean, and *Nicotiana attenuata* leaves it has been shown that such a positive feedback loop does not exist for endogenous JA accumulation [27–29]. In addition, for *Arabidopsis thaliana* leaves it has been demonstrated that coronatine application cannot induce JA-Ile accumulation in contrast to wounding [30]. Thus, in this study not only both jasmonates, JA and the bioactive JA-Ile, but also their first degradation metabolites are investigated in order to discover potential differences in JA versus JA-Ile accumulation. Moreover, in addition to earlier studies, the effect of wounding in combination with exogenous jasmonate application was studied to address the possibility of additive or synergistic effects with respect to jasmonate accumulation.

2. Material and methods

2.1. Plant material and treatment

Arabidopsis thaliana ecotype Columbia was used for all experiments and plants were grown as described before [31]. Four to five week old plants, grown under short-day conditions, were used. For wounding, each side of the leaf was treated with a pattern wheel (six vertical motions) followed by an immediate application of 10 μL of 50 μM of coronalon or solvent control (0.1% ethanol) on each side (20 μL per leaf, 1 nmol). For coronalon spray treatment, plants were sprayed with 1 mL of 50 μM coronalon (50 nmol) or solvent control. All plants were incubated for the indicated time points. To minimize evaporation of the applied solutions, plants were incubated with a translucent cover. Coronalon was synthesized as described [18]. For double treatment with coronalon and α-linolenic acid (LA; 18:3), plants were pretreated for 1 h with 500 μM LA spray followed by coronalon application and subsequent incubation (with cover) for 1 and 3 h, respectively.

2.2. RNA Extraction and Q-RT-PCR

For RNA extraction 100 mg of fresh plant material was used. Samples were homogenized for 1 min at 1000 rpm in the Genogrinder 2010 (Spex Sample Prep, Stanmore, UK) and extracted following the protocol described before [32]. Q-RT–PCR was carried out in 96-well plates on a Bio-Rad CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, USA) by the use of Brilliant II QPCR SYBR green Mix (Agilent, Böblingen, Germany). The obtained mRNA levels of the genes of interest were normalized to the *RPS18B* mRNA level in each cDNA sample and the dissociation curve was analyzed for all primer pairs. In the Bio-Rad CFX Manager Software (3.1), expression levels of genes of interest were calculated by use of the Normalized Expression (ΔΔCq). The primer pairs used are listed in Supplementary materials (Table A1).

2.3. Phytohormone analysis

For jasmonate phytohormones and derivatives extraction (jasmonic acid, JA; (+)-7-*iso*-jasmonoyl-*L*-isoleucine, JA-Ile; OH-JA; 12-OH-JA-Ile), 250 mg of fresh plant material was used. Samples were homogenized for 1 min at 1000 rpm in the Genogrinder 2010 (See Section 2.2), extracted and analyzed according to [31]. Briefly,

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