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Monogalactosyldiacylglycerol deficiency affects jasmonic acid biosynthesis and defense responses to insect herbivores in *Nicotiana tobacum*

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ABSTRACT

Jasmonic acid (JA) plays critical roles in plant development and defense. Linolenic acid (18:3) and hexadecatrienoic acid (16:3) released from chloroplast lipids are now known to be precursors for JA biosynthesis, but the relationship between chloroplast lipids, especially galactolipids and JA biosynthesis still remains unclear. Here, this question was addressed by characterizing the transgenic tobacco plants, which had reduction in monogalactosyldiacylglycerol (MGDG) and trienoic fatty acids owing to the fact that MGDG synthase activity was down-regulated by using RNA interference technology. In response to wounding, the transgenic plants produced lower levels of JA than wild-type plants. Moreover, the expression of genes encoding lipoxygenase (*LOX1*), allene oxide cyclase (*AOC*), hydroperoxide lyase (*HPL*) and proteinase inhibitor (*PI-I* and *PI-II*) was strongly activated by mechanical wounding in wild-type plants but was diminished in transgenic plants. In addition, the transgenic plants were shown to be more susceptible to attack by *Helicoverpa armigera* larvae. Treatment of transgenic plants with methyl jasmonate restored resistance to *H. armigera* and expression of *HPL*, *PI-I* and *PI-II*. Our results suggest that MGDG plays important roles as source of 18:3 and 16:3 in JA biosynthesis and JA-mediated defense responses to insect herbivores in tobacco.

1. Introduction

Jasmonic acid (JA) and its cyclic precursors and derivatives are members of a lipid-based signaling cascade originating from polyunsaturated fatty acids. They are growth regulators in plants that play dual roles in development and defense including pollen viability, fruit ripening, root growth, tendril coiling, plant response to wounding and abiotic stress, and defenses against insects and pathogens [1,2].

It is essential for resistance to insects and pathogens that plants acquire the ability to produce or perceive JA. The defense responses of plants to wounding and herbivores by activation of JA-mediated defenses have been characterized [3–5]. There is sufficient evidence that a number of JA-related genes and proteins can be activated by mechanical wounding and herbivore attack [3,6]. In the *Arabidopsis–Pieris rapae* interaction, between 67% and 84% of insects-regulated gene expression was under the control of the JA signaling pathway [7]. When plants encounter the chewing insects or mechanical wounding, the JA signaling pathway and defense genes are activated rapidly. For example, JA-mediated genes encoding proteinase inhibitor (PI) are expressed in response to mechanical wounding [8]. PI can play important roles in defense against chewing insects and pathogens [9]. The JA-deficient plants accumulated reduced levels of PI in wounded and unwounded leaves compared with wounded wild-type plants and received more damage by insects than wild-type plants [3,10].

The biosynthesis of JA is initiated in chloroplasts by the release of trienoic fatty acids-linolenic acid (18:3) from membrane glycerolipids, and lipoxygenase (LOX) catalyzes 18:3 leading to the formation of 13-hydroperoxy-9(Z),11(E),15(Z)-octadecatrienoic acid (13-HPOT). The next steps are catalyzed by allene oxide synthase (AOS) and allene oxide cyclase (AOC), forming 12-oxophytodienoic acid (OPDA), which is subsequently reduced to 3-oxo-2(2′[Z]-pentenyl)-cyclopentane-1-octanoic acid (OPC 8:0) by OPDA reductase (OPR), and then converted to JA by three cycles of β-oxidation [11,12]. Weber et al. demonstrated that a parallel "hexadecanoid" pathway derived from the unsaturated fatty acid-hexadecatrienoic acid (16:3) is present in a '16:3' plant, *Arabidopsis* [13] (Fig. 1).

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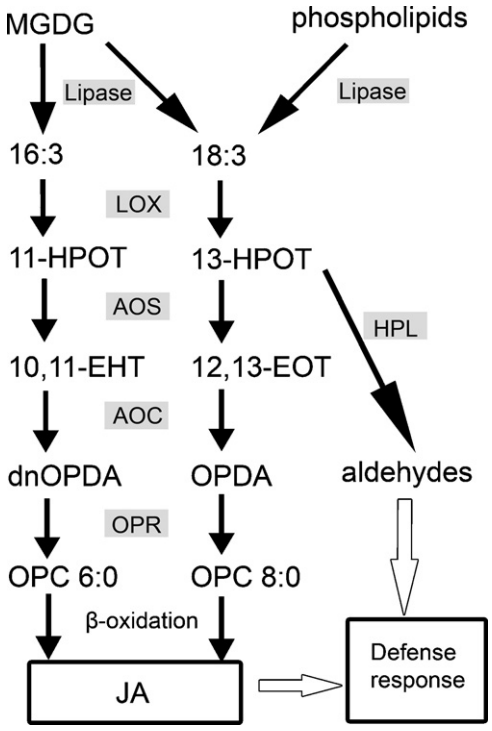

Fig. 1. The JA pathway. MGDG, Monogalactosyldiacylglycerol; 16:3, hexadecatrienoic acid; 18:3, linolenic acid; LOX, lipoxygenase; 11-HPOT, 11-hydroperoxy-7(Z),9(E),13(Z)-hexadecatrienoic acid; 13-HPOT, 13-hydroperoxy-9(Z),11(E),15(Z)-octadecatrienoic acid; HPL, hydroperoxide lyase; AOS, allene oxide synthase; 10,11-EHT, 10,11-epoxyhexadecatrienoic acid; 12,13-EOT, 12,13-epoxyoctadecatrienoic acid; AOC, allene oxide cyclase; OPDA, 12-oxophytodienoic acid; dnOPDA,dinor-OPDA; OPR, OPDA-reductase; OPC-6:0, 3-oxo-2-(2(Z)-pentenyl)-cyclopropane-1-hexanoic acid; OPC-8:0, 3-oxo-2-(2(Z)-pentenyl)-cyclopropane-1-octanoic acid.

The precursors of JA biosynthesis are 18:3 and 16:3, which released from plastid lipids by lipases in response to environmental and developmental cues. Mutational analysis using the model plants *Arabidopsis* and tomato has provided effective evidence to determine this step. The *Arabidopsis* triple mutant *fad3-2fad7-2fad8* is deficient in 18:3 and 16:3 and lacks JA. The growth of the mutant is unaffected, however, the mutant is male sterile and defective in resistance to insects and pathogens, and its phenotype can be rescued by exogenous applications of jasmonate [4,14]. The tomato *suppressor of prosystemin-mediated responses2* (*spr2*) mutation impairs wound-induced JA biosynthesis and expression of defensive *PI* genes. Loss of *spr2* function reduced the 18:3 content of leaves to 10% of wild-type levels, and abolished the accumulation of 16:3. The results suggest that *spr2* encodes a chloroplast fatty acid desaturase involved in JA biosynthesis [10].

Since most of the data support the lipolytic release of 18:3 and 16:3 as the initial step in JA biosynthesis, the major lipids releasing these trienoic fatty acids are worthy to be investigated. The *dad1* mutant of *Arabidopsis* is defective in anther dehiscence and pollen maturation. Also, 18:3 or JA can recover its fertility phenotype [15]. It is showed that *DAD1* encodes a novel class of phospholipase A1 (PLA1), whose activity is highly specific to the *sn*-1 position of lipids, releasing free fatty acids from phospholipids. However, compared to phospholipids, little is known about the function of galactolipids in JA biosynthesis. Galactolipids account for more than 70% of the lipids in the inner membrane of chloroplast envelope, whereas the contribution of phospholipids, mostly as phosphatidylcholine (PC) in the outer envelope, is less than 20% [16]. As much of the trienoic fatty acids, precursors of JA, are localized in galactolipids, it could be postulated that galactolipids are required for JA biosynthesis. A rapid accumulation of high levels of galactolipid species containing OPDA-OPDA and OPDA-dnOPDA (dinor-OPDA) in wounded leaves of *Arabidopsis* raises the possibility that these complex lipids are the primary products of plastidic oxylipin biosynthesis [17].

Monogalactosyldiacylglycerol (MGDG) accounts for approximately 50 mol% of the lipid matrix in plants, and it is rich in 18:3 and 16:3. Especially, 16:3 is exclusively in MGDG in "16:3" plants such as *Arabidopsis* and tobacco [16,18]. However, the actual participation of MGDG as a source of precursors of JA biosynthesis has not been confirmed experimentally. Recently, Luo et al. showed that the transgenic tobacco plants had reduced MGDG, 18:3 and 16:3 compared to wild-type plants as a result of MGDG synthase activities were down-regulated by transferring a gene-silencing construct against *NtMGD1* [19]. To study the function of MGDG in JA biosynthesis, we analyzed the transgenic tobacco plants in further detail. Our findings demonstrate that an alteration in MGDG content and unsaturated fatty acid composition could have affected wound-induced JA biosynthesis and defense responses. These results are consistent with the hypothesis that MGDG is the pool required for JA biosynthesis.

2. Materials and methods

2.1. Plant materials

Identification of original transformants of tobacco (*Nicotiana tabacum* L. cv. Wisconsin-38) was conducted as described by Luo et al. [19]. The transgenic line M18 was used as materials in this paper. The tobacco seedlings were grown in a growth chamber maintained under a 16 h light/8 h dark photoperiod at 25 °C. The 4th or 5th leaves numbered starting with the apical bud (position 1) of 6-week-old M18 plants as well as from corresponding wild-type plants were used as materials for analysis.

2.2. Wound and MeJA treatment

Tobacco plants were wounded by pressing the surface of the leaf with a sterile hemostat, perpendicular to the main vein, which effectively wounded about 30% of the leaf area. The wounded and unwounded plants were continuously illuminated for various periods, after which the leaves tissue were pooled at the indicated time points, weighed, and immediately frozen in liquid nitrogen. The 0 h time point indicates harvest of leaves from unwounded plants.

For methyl jasmonate (MeJA) treatment, the plants were sprayed with a solution of 0.01% (by vol.) ethanol and 0.1% (by vol.) Triton X-100 (Sigma–Aldrich, St. Louis, MO) in water containing 100 μM MeJA (Sigma–Aldrich, St. Louis, MO) until run-off occurred. Control plants were sprayed with a solution containing only 0.01% ethanol and 0.1% Triton X-100 in water.

2.3. Measurement of JA

JA was extracted using the method of Weber et al. [13] with some modification. Leaf materials (0.5–1.0 g fresh weight) were ground in liquid nitrogen as finely as possible and extracted with 3 ml of 80% (by vol.) methanol containing 60 ng of 9,l0-dihydro-JA (DHJA) as an internal standard. After the organic solvent was removed by rotary evaporation, the aqueous solution was adjusted to a volume of 5 ml and pH 2.5–3.0 with 2 M HCl, and then carefully extracted with ethyl acetate (3×5 ml). The ethyl acetate layers were collected, combined and evaporated in vacuo. The residue was dissolved with 0.1 M acetic acid and passed through a