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STRUCTURE-ACTIVITY RELATIONSHIPS ON SENESCENCE-PROMOTING EFFECT OF ARABIDOPSIDES FROM *ARABIDOPSIS THALIANA*

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Abstract – Arabidopsides A-D and F isolated from *Arabidopsis thaliana* are rare oxylipins containing 12-oxophytodienoic acid (OPDA) and/or dinor-oxophytodienoic acid (dn-OPDA) which are known as precursors of jasmonic acid (JA) and methyl jasmonate (MeJA). The senescence-promoting effect of these arabidopsides A-D and F were examined by an oat (*Avena sativa*) leaf assay. Arabidopsides C and D, digalactosyldiacylglycerides containing OPDA and/or dn-OPDA, are more effective on leaf senescence than arabidopsides A, B, and F, monogalactosyldiacylglycerides containing OPDA and/or dn-OPDA. The effect of arabidopsides C and D is the almost same as that of JA, MeJA, and OPDA, which are well known to be senescence promoters. These results suggest that arabidopsides A-D and F play important roles in leaf senescence.

INTRODUCTION

In our recent search for bioactive substances of *Arabidopsis thaliana*, five new oxylipins named arabidopsides A-D and F containing 12-oxophytodienoic acid (OPDA) and/or dinor-oxophytodienoic acid (dn-OPDA), were isolated from the aerial parts of this plant.[1-4] On the other hand, *sn*1-*O*-

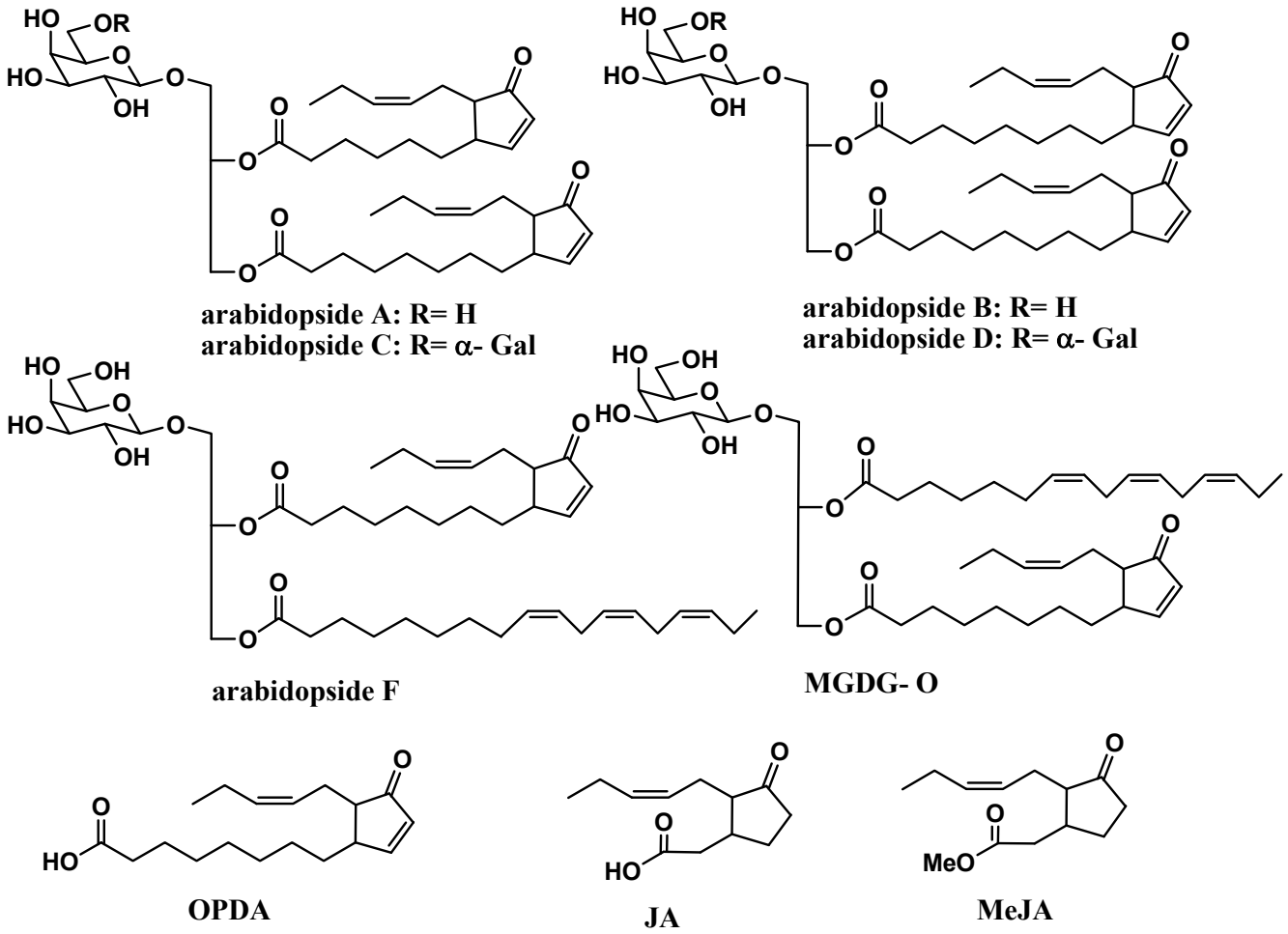

Figure 1. Structures of arabidopsides A- D and F and related compounds

(12-oxophytodienoyl)-*sn*2-*O*-(hexadecatrienoyl)-monogalactosyl glyceride (MGDG-O) containing OPDA was recently reported to accumulate after wounding of *A. thaliana*.[5] These oxylipins are monogalactosyldiacylglycerides (MGDG) or digalactosyldiacylglycerides (DGDG) containing OPDA and/or dn-OPDA which are known as precursors of jasmonic acid (JA) and have received much attention because they play important roles in regulation of developmental and defense gene expression of plants as JA and methyl jasmonate (MeJA).[6,7] For example, when MeJA was applied to oat (*Avena sativa*) leaf segments, the chlorophyll (Chl) content was less than that of the segments without exogenous JA.[8] It was clarified that chlorophyllase, a key enzyme involved in Chl degradation, was induced by MeJA in *A. thaliana*.[9] It was suggested that arabidopsides A-D and F may also act as plant growth regulators of plant senescence including especially Chl degradation, since these oxylipins containing OPDA and/or dn-OPDA may exist in the chloroplast membrane.[10,11] Our group previously reported that the senescence-promoting effect of arabidopside A was observed for the first time.[12] In this paper, we describe the structure-activity relationships on the senescence-promoting effect of arabidopsides A-D and F and related compounds under light condition in oat leaf segments.

Results and Discussion

In our senescence-promoting assay of oat leaf segments, the color of the leaf segments treated with arabidopsides C and D and MeJA at 10^{-5} M turned almost yellow after incubation for 4 days under continuous light conditions, while that of leaf segments incubated without test compounds was almost unchanged.

A B

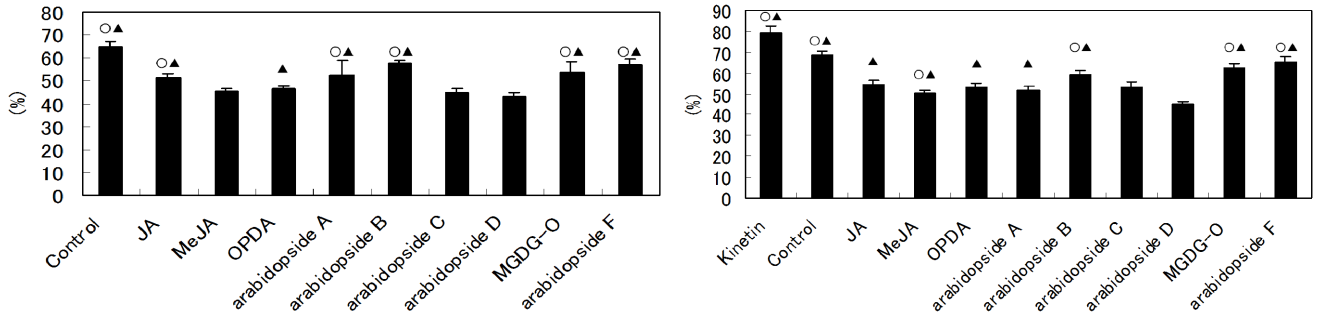

Figure 2. Effects of arabidopsides A-D and F and related compounds on Chl content in oat leaf segments under continuous light (A) and with 0.1 μM kinetin under continuous light (B). Means ± SD of results from 9 replicates of 10 leaves. Symbols ○ and ▲ in each panel indicate significant differences at P = 0.01 (Student's t-test). ○: $P < 0.01$ vs. arabidopside C, ▲: $P < 0.01$ vs. arabidopside D.

Figure 2A shows the effects of arabidopsides A-D and F and related compounds on the leaves of chlorophyll in detached oat leaves. Arabidopsides C and D significantly promoted the decrease in Chl content of the leaf segment. Chl content of the leaf segments treated with arabidopsides C and D at 10^{-5} M was about 45 % of the initial Chl content, while those of arabidopsides A, B, and F and MGDG-O at 10^{-5} M were about 50-60 % of the initial Chl content. The effect of arabidopsides C and D on the leaves of chlorophyll degradation was as strong as MeJA and OPDA.

Figure 2B shows the effects of arabidopsides A-D and F and related compounds on the leaves of chlorophyll with 0.1 μ M kinetin under continuous light condition. Kinetin is one of cytokinins which prevent chloroplast degradation by retarding the breakdown of chlorophyll in senescing leaves. In spite of the inhibitory activity of Chl degradation by kinetin, Chl content of the leaf segments treated with arabidopsides C and D at 10^{-5} M containing 0.1 μ M kinetin were about 53 % and 45 %, respectively. In particularly, arabidopside D promoted degradation of Chl content at the almost same extent with the case of the above assay (Figures 2A, B). Arabidopside D may also inhibit the activity of kinetin. On the other hand, Chl content of the leaf segments treated with arabidopsides A, B, and F and MGDG-O at 10^{-5} M containing 0.1 μ M kinetin were about 55- 65 % of the initial Chl content.

These results indicated that arabidopsides C and D, digalactosyldiacylglycerides containing OPDA and/or dn-OPDA were more effective than arabidopsides A, B, and F and MGDG-O, monogalactosyldiacylglycerides containing OPDA and/or dn-OPDA, in oat leaf senescence. Although it is not clear whether OPDA and dn-OPDA were released from arabidopsides C and D, these oxylipins exhibited higher activity in leaf senescence. Most of the fatty acids from which OPDA/dn-OPDA are derived are part of the plastid galactolipids. DAD1-lipase is the only lipase proven to be involved in the formation of jasmonic acid and hydrolyzes the plastidial phosphatidylcholine.[13] In addition, AtPLA I can use diverse lipid species including arabidopsides as substrates, and it displays a preference for arabidopsides to non-OPDA-containing MGDG or DGDG.[14] The lipase in oat leaves, such as DAD1 and AtPLA I, may hydrolyze arabidopsides into OPDA/dn-OPDA. This hydrolysis reaction may be important for Chl degradation induced by arabidopsides in oat leaves. In addition to the effect of the number of OPDA/dn-OPDA, digalactosylglycerol in arabidopsides C and D may make a larger contribution to membrane permeability than monogalactosylglycerol in arabidopsides A, B, and F and MGDG-O.

Since galactolipids containing OPDA and/or dn-OPDA were rare, their functions in the plants were not also clear.[1-5, 11-18] However, bioactivities and dynamics of these compounds in stressed plants were reported. For example, *sn*1-*O*-(12-oxophytodienoyl)-monogalactosyl glyceride isolated from *Ipomoea tricolor* showed stomatal opening activity.[15] Arabidopsides A-D and F and MGDG-O were increased in wounded *A. thaliana* leaves.[5,11,18] Recently, arabidopsides E and G containing three OPDA and/or dn-OPDA were isolated in *A. thaliana* leaves infected by *P. syringae*, which is a plant pathogen and infects a wide range of plant species. These compounds also had antifungal activity against *Botrytis cinerea*.[16,17] We also reported that arabidopsides A, B, and D inhibited the root growth of cress seedlings[1,2,4] and arabidopside A promoted the senescence of oat leaves.[12] In this paper, we reported arabidopsides C and D exhibited strong activity in oat leaf senescence. Therefore, galactolipids containing OPDA and/or dn-OPDA such as arabidopsides A-D and F may play important roles in various bioactivities of plants.

EXPERIMENTAL

Plant and Chemical Material and Isolation of Arabidopsides.

Seeds of *Arabidopsis thaliana* L. ecotype Col-0 (Brassicaceae) were purchased from LEHLE SEEDS (Round Rock Co., Ltd., USA). They were immersed in water for 2 days before sowing on rock wool (Rock fiber, NITTOBO Co., Ltd., Japan). They have been cultured under continuous light (24 h-light, ca. 3,500 lux) at 24 °C, until forming the flower bud. Aerial parts of *A. thaliana* (1.00 kg) were extracted with MeOH (9 L) and evaporated to dryness *in vacuo* at 30 °C. Then the residue (18.1 g) was partitioned between EtOAc (900 mL x 3) and H_2O (900 mL). The EtOAc-soluble portion (3.21 g) was subjected to

silica gel column chromatography (2.0 x 31 cm, $CHCl_3$/MeOH, 49:1→1:1). The fraction eluted with $CHCl_3$/MeOH (19:1) was applied to a C_{18} Sep-Pak cartridge (MeCN/H_2O, 3:1, Waters) and the fraction (112 mg) containing oxylipins was further separated by HPLC [Deverosil ODS HG-5, 10.0 × 250 mm, (Nomura Chemical, Japan), flow rate 2.8 mL/ min; solvent MeCN/ H_2O (3:1); detection UV (222 nm)] to yield arabidopsides A (8.3 mg, t_R 12.6 min), B (2.8 mg, t_R 18.4 min), and F (3.8 mg, t_R 51.1 min) and MGDG-O (4.1 mg, t_R 39.2 min). On the other hand, the fraction eluted with $CHCl_3$/MeOH (1:1) was applied to a C_{18} Sep-Pak cartridge (MeCN/H_2O, 1:1) and the fraction (89.1 mg) containing oxylipins was further separated by HPLC [TSK-gel, ODS-100V, 7.6 × 250 mm, TOSOH, Japan, flow rate 2.0 mL/min; solvent MeCN/ H_2O (55:45); detection UV (222 nm)] to yield arabidopsides C (2.1 mg, t_R 17.3 min) and D (3.8 mg, t_R 36.2 min). The isolated arabidopsides A-D and F and MGDG-O were identified by NMR and ESIMS.[1,3,4,5]

JA and OPDA were purchased from Sigma Chemical Co., Ltd.,USA, and from Cayman Chemical Co. Ltd, USA, respectively. MeJA was derived from JA by methylation with trimethylsilyldiazomethane.[19]

Bioassay.

The oat leaf assay was used with modifications according to Ueda *et al*.[8,20] Oat (*Avena sativa* cv. zenshin, Sapporosaishuen Co. Ltd., Japan) seeds were germinated in vermiculite moistened with water and seedlings were grown under continuous white fluorescent light of about 4,000 lux at 24 ºC for 7 days. Ten upper 3-cm leaf segments were placed on two layers of Toyo No.1-filter paper moistened with 5 mL of test solutions in a Petri dish. The test compounds were dissolved in water containing 0.5 mg/mL of the surfactants Tween 80:sorbitan monooleate (7:3) with or without 0.1 μM kinetin. The leaf segments incubated in the same medium without test compounds were used as control. The Petri dishes were kept in dark condition at 24 ºC, or placed under continuous white fluorescent light of about 4,000 lux at 25 ºC for 4 days. The Petri dishes in the presence of kinetin were placed under continuous light condition. After observation of the leaf coloring, the leaf segments were extracted with 80% cold acetone and their Chl contents were measured.

Measurement of Chlorophyll Content.

Each leaf segment was homogenized in 10 mL of 80% cold acetone, and Chl content was estimated according to the method of Arnon (1949).[21] The extract was centrifuged at 3,000 rpm for 5 min and then the resulting pellet was resuspended in 5 mL of 80% cold acetone and centrifuged at the same speed for 5 min twice. The three supernatants were combined and adjusted to 20 mL in a volumetric flask. The absorbances of each extract at 645 and 663 nm were spectrophotometrically measured and the Chl content was calculated. All experiments were repeated at least nine times. These results were averaged and

expressed as percentages of the Chl contents of oat leaf segments incubated without test compounds.

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