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Plant esterified oxylipins: structure – function relationship

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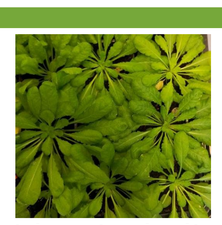
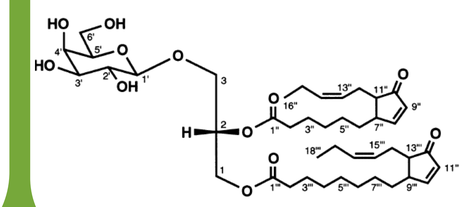
Context and objectives

Plant oxylipins produced by the oxidation of unsaturated fatty acids play important roles in plant metabolism and protection against pathogens. Recently, it has been discovered that *Arabidopsis thaliana* L. produces high quantities of oxylipins esterified to galactolipids under stress[2]. Those molecules are called arabidopsides and are produced following oxidation of monogalactosylglycerol and digalactosylglycerol found in high quantities in thylakoïd membranes. Moreover, arabidopsides pattern is different depending on the nature of the stress[3], suggesting an involvement of those molecules in plant protection responses.

Unfortunately, those compounds are not commercially available yet. In the present work, high quantities of arabidopsides were extracted and purified from *Arabidopsis thaliana* L.



Arabidopsis thaliana L.



Arabidopside A[1]

Materials and methods

1. Lipids extraction

a. Arabidopsis thaliana growing under short day conditions (8H day/ 14H night)
b. Leaves harvesting and stress induction with liquid nitrogen. Leaves need to be frozen
c. Leaves thawing at room temperature
d. Leaves blending and lipid extraction with butanol : methanol (3:1)
e. Liquid : liquid extraction of lipids
   50% aqueous phase: 2% acetic acid in water
   50% organic phase: heptane : ethyl acetate (3:1)
f. Organic phase drying and storage in $CHCl_3$

2. Arabidopsides purification

a. Silica column purification of glycolipids
   Apolar lipids elution with $CHCl_3$: aceton (9:1)
   Glycolipids elution with aceton : methanol (9:1)
b. Semi-preparative HPLC purification of arabidopsides
   C18 column (4,6 mm ; 5 µm)
   isocratic elution of acetonitril : water (85:15)
   UV detection at 220 nm

3. Arabidopsides characterization

- HPLC-mass spectrometry
- NMR (^{1}H & HSQC)
- UV spectrometry

Results: four arabidopsides highly purified

NMR (HSQC & ^{1}H), mass spectrometry and UV spectrometry confirmed arabidopsides identification. For example, UV highest absorbance of molecules was closed to 220 nm, as described in literature[4] (**Fig 2.**).

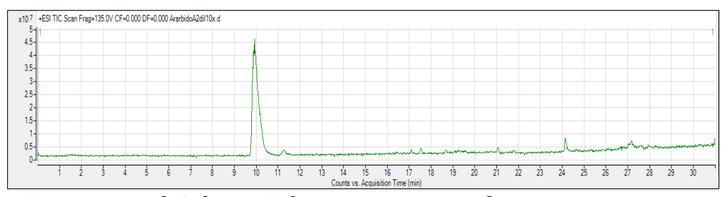


Fig 1. Arabidopside A LC-MS chromatogram

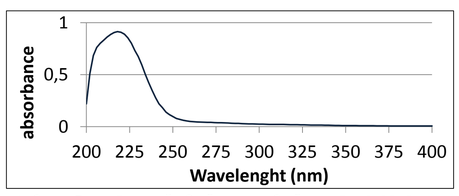


Fig 2. Arabidopside A UV-spectra

Otherwise, HPLC-MS results (**Fig 1.**) showed that arabidopsides A and B purities are very high (respectively 97,4 and 91,28%) and that arabidopsides D and E purities are quite lower (respectively 83,4 and 76,9 %) (**Fig 3.**).

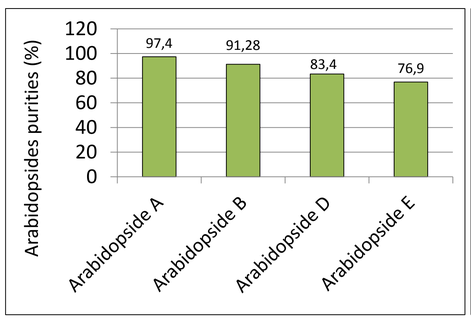


Fig 3. Arabidopsides purities

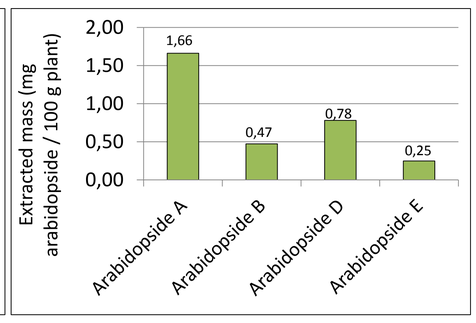


Fig 4. Arabidopsides extraction and purification yield

Finally, **Fig 4.** shows that arabidopside A was extracted and purified with the highest yield.
Contrariwise, arabidopside E was extracted and purified in lowest quantities.

Perspectives

Arabidopsides are lipidic compounds produced by *Arabidopsis thaliana* L. under stress

OPDA containing molecules (ex. Arabidopsides) are present in plant thylakoïd membranes[5]

Are arabidopsides present in other membranes (ex. Plasma membrane)?

IN PROGRESS

Does arabidopsides modify membranes organization?

In-silico: modelization

In-vitro: membrane models

Can different membrane organization be a signal for defence mechanism activation?

Conclusion

In this study, arabidopsides production was easily induced in *Arabidopsis thaliana* L. Four different molecules were then extracted and highly purified

For further information

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Literature

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Acknowledgments

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