LIÈGE université Gembloux Agro-Bio Tech

fnrs LA LIBERTÉ DE CHERCHER

Laboratory of Chemistry of Natural Molecules

FRIA grantee of the Fonds de la Recherche Scientifique – FNRS

Contact: louise.vilain@uliege.be or +32 (0) 81 62 22 29

Development of a LC-MS/MS method for the profiling of free and esterified jasmonates in plant samples

VILAIN Louise[1], LEFEBVRE Thibault[1,2], FAUCONNIER Marie-Laure[1], GENVA Manon[1]

[1] Laboratory of Chemistry of Natural Molecules, Gembloux Agro-Bio Tech, Passages des Déportés 2, 5030 Gembloux, Belgium

[2] Haute Ecole de la Province de Namur (HEPN), Avenue de Namur 61, 5590 Ciney, Belgium

1. Introduction

Because jasmonates regulate many aspects of plant development and stress responses, they are best known to form an important class of **plant hormones**.[1]

Jasmonates are endogenous plant compounds deriving from galactolipids. They are divided into free and esterified jasmonates, depending on whether the oxidation of fatty acids occurs after their release from galactolipids (**free jasmonates**) or while they are still attached to the galactose headgroup (**esterified jasmonates**).[2]

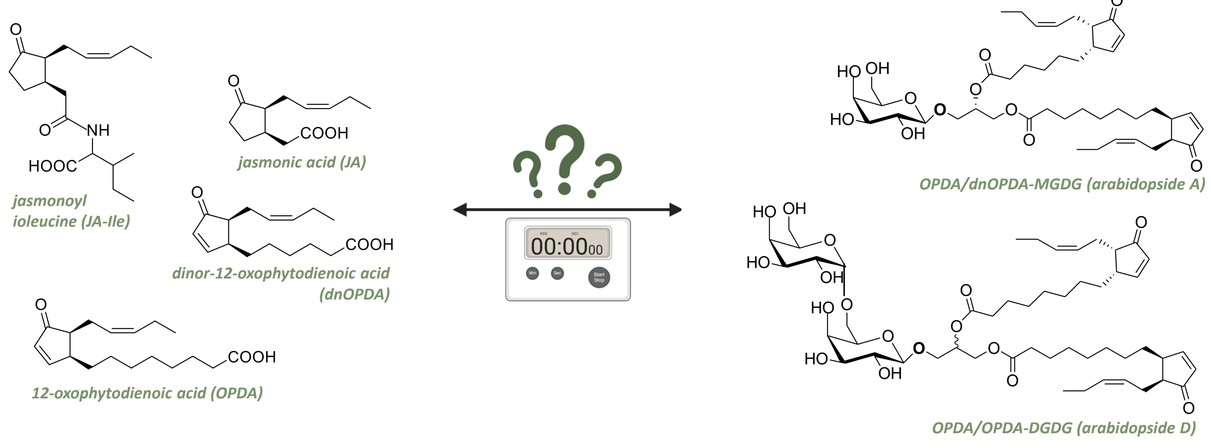

Figure 1. Chemical structures of some free jasmonates (left side) and esterified jasmonates (right side).

However, the dynamics between free and esterified jasmonates remain largely unexplored. To unravel them, we are developing a **LC-MS/MS method** to analyze a wide range of free and esterified jasmonates in plant samples. Such a comprehensive analysis has indeed not been described yet, notably due to low endogenous concentrations and lack of standards.

2. Methodology

After induction of jasmonate production by **mechanical wounding**, jasmonates were extracted[3] and purified[4] for LC-QTOF then LC-QQQ analysis.

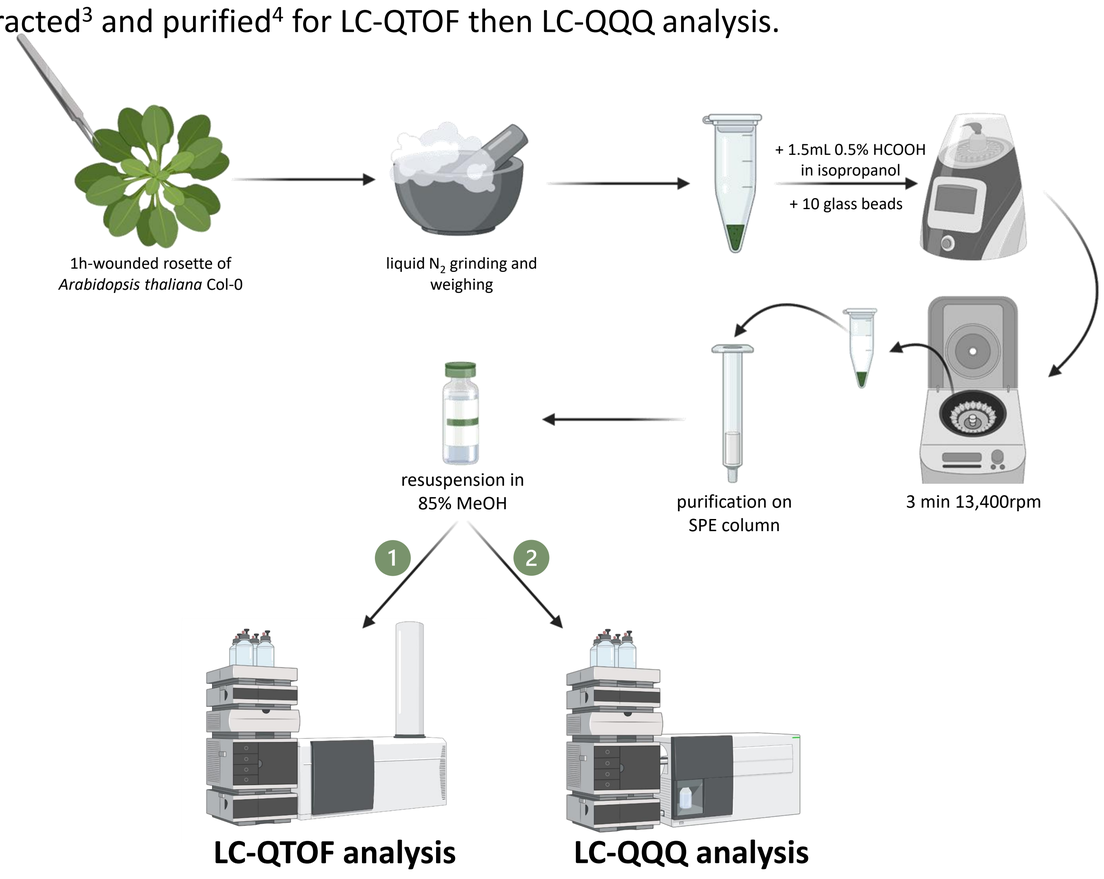

Figure 2. Overview of plant harvest, extraction of jasmonates, purification of jasmonates and analysis of jasmonates. Jasmonates were analyzed first in LC-QTOF for their identification, then in LC-TQ for their routine analysis.

3. LC-QTOF for identification

Few standards of jasmonates are commercially available and hence, it is not possible to confirm this way the retention times and the MRM transitions of all existing jasmonates. Instead, the monoisotopic mass of each analyzed jasmonate was calculated and their fragmentation patterns in negative ionisation were investigated. A plant sample was then injected in LC-QTOF to obtain for each jasmonate accurate mass measurements of its precursor ion ($[M-H]^-$ or $[M+HCOO]^-$) and of its product ions. Of note, a mass error of ± 30ppm between predicted and measured m/z is typically accepted in LC-QTOF[5].

Table 1. Jasmonates detected in LC-QTOF. Retention time (RT) in minutes (min) and the mass errors calculated for the precursor ion as well as its product ion(s) in parts per million (ppm) are presented for each jasmonate. Mass errors were calculated this way: (measured m/z – predicted m/z)/(1,000,000*predicted m/z). Major free and esterified jasmonates of the plant sample are highlighted in bold.

Analyzed jasmonate	Chemical formula	RT (min)	Mass error of precursor ion (ppm)	Mass error of product ion 1 (ppm)	Mass error of product ion 2 (ppm)
12-HSO_4-JA		2.2	9.33558	4.77516	7.87952
12-OH-JA		2.45	-2.11005	12.45466	/
12-COOH-JA		2.5	-20.57786	-2.11817	/
JA-Gln		3.2	-2.98953	-10.8093	/
JA-Glc		3.4	14.22900	-4.68638	5.67665
12-O-Glc-JA		3.4	-2.73785	3.66967	3.81268
12-OH-JA-Ile		3.5	-1.06151	1.96791	-7.43189
12-COOH-JA-Ile		3.5	-5.49157	-3.33624	4.17278
OPC-4-Gln		3.85	-17.49142	10.14733	/
OPC-4-Glc		4.1	-3.64774	1.85537	/
JA		4.35	-4.11252	6.18501	2.30124
dnOPDA-Glc		4.5	-1.15444	-0.07600	/
JA-Val		4.7	-6.47011	0.65477	/
dh-JA		4.75	1.13672	-11.60769	/
OPC-4		5.1	-7.04198	-0.36335	0.59309
JA-Ile		5.2	-3.11606	1.66043	2.44628
dnOPDA		5.55	-7.18181	3.75465	-9.44460
OPDA-Ala		5.8	-21.26807	2.56701	-16.80671
OPDA		6.5	-7.03993	0.00001	-2.30577
OPDA/dnOPDA-DGDG		7	36.62841	-1.57969	0.30399
OPDA/OPDA-DGDG		7.3	-7.84419	0.30907	/
OPDA/OPDA-MGDG		8.1	-12.05214	0.92721	/
18:2/dnOPDA-MGDG		9	-16.76292	-0.68398	-27.01765
OPDA/dnOPDA-MGDG-18:3		9.15	-50.19047	-2.30086	-1.55796
OPDA/18:3-DGDG		9.2	-7.94931	-1.54535	-3.15638
OPDA/16:3 MGDG		9.4	-5.61036	-0.85853	-0.50163
OPDA/dnOPDA-MGDG-18:1		9.45	-38.84174	-2.91900	1.13997
OPDA/16:2-MGDG		9.5	-13.98037	-2.60993	-30.66960
OPDA/OPDA-DGDG-OPDA		9.5	-12.42395	-0.44643	/
OPDA/dnOPDA-MGDG-OPDA		9.85	-8.78163	0.54946	-0.19000
OPDA/18:3-MGDG		10	-12.84815	-1.20194	-1.96597
OPDA/18:2-MGDG		10.1	-18.35861	-1.09892	-29.81118
OPDA/16:0-DGDG		10.2	-11.16916	-2.91900	-2.29203
OPDA/OPDA-MGDG-OPDA		10.3	-15.88157	-1.68272	/
16:0/OPDA-MGDG		10.4	-12.58008	-6.42183	-7.46384

4. LC-QQQ for routine analysis

Once the identity of jasmonates was confirmed in LC-QTOF, the plant sample was re-injected in LC-QQQ to evaluate its potency for routine analysis. MRM transitions were established thanks to previous work in LC-QTOF and the retention order was maintained as the same column was used.

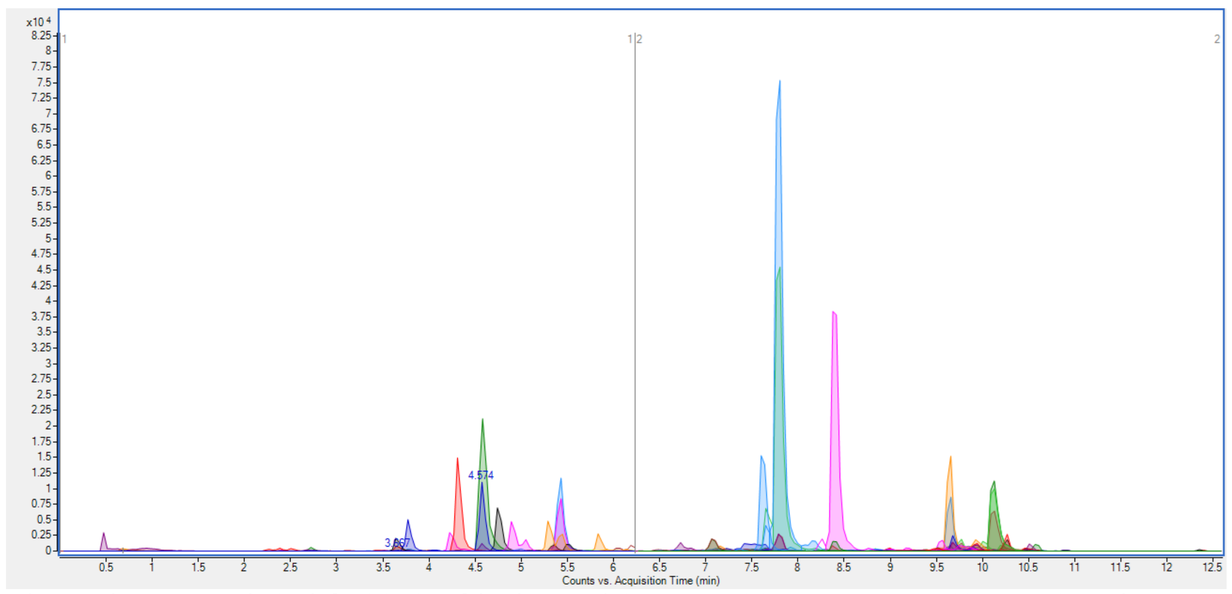

Figure 3. Chromatogram obtained after injection of the plant sample in LC-QQQ. Minimum one MRM transition to maximum three MRM transitions were monitored per jasmonate. The analysis is performed in two time segments for improved sensitivity.

5. Conclusion and perspectives

Our work highlights the complementarity of LC-QTOF and LC-QQQ for the identification and quantification of jasmonates respectively. Indeed, 20 free jasmonates and 16 esterified jasmonates were identified in LC-QTOF, and all of them were successfully detected in LC-QQQ with better resolution and sensitivity.

The next steps in this method development are:

- **Optimizing MS/MS parameters** for improved sensitivity,
- Testing **more selective extractions** of for jasmonates,
- Verifying adequate elution of jasmonates during **purification**,
- Adding **deuterated internal standards** for relative quantification,
- Building **calibration curves** with available standards for absolute quantification.

This method will enable us to unveil the dynamics between free and esterified jasmonates, and more generally to better understand the occurrence and functions of jasmonates in plants.

References:

1 Wasternack, C. & Hause, B. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. *Annals of Botany* **111**, 1021–1058 (2013).

2. Genva, M. *et al.* New insights into the biosynthesis of esterified oxylipins and their involvement in plant defense and developmental mechanisms. *Phytochem Rev* **18**, 343–358 (2019).

3. Glauser, G. & Wolfender, J.-L. A non-targeted approach for extended liquid chromatography-mass spectrometry profiling of free and esterified jasmonates after wounding. in *Jasmonate Signaling* (eds. Goossens, A. & Pauwels, L.) vol. 1011 123–134 (Humana Press, Totowa, NJ, 2013).

4. Genva, M., Andersson, M. X. & Fauconnier, M.-L. Simple liquid chromatography-electrospray ionization ion trap mass spectrometry method for the quantification of galacto-oxylipin arabidopsides in plant samples. *Sci Rep* **10**, 11957 (2020).

5. Zhu, Z.-J. *et al.* Liquid chromatography quadrupole time-of-flight mass spectrometry characterization of metabolites guided by the METLIN database. *Nat Protoc* **8**, 451–460 (2013).

BioRender was used for some illustrations of this poster.