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Role and functions of phosphooxylipins in lipid signalling and biotic defence in *Arabidopsis thaliana*

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1. Introduction

Oxylipins are **oxidised compounds derived from polyunsaturated fatty acids** (PUFAs), produced by the action of enzymes such as lipoxygenases. They constitute a large family of lipid metabolites involved in many physiological processes in plants, including growth, development and **stress response**. Their production is rapidly induced during biotic or abiotic stress, where they participate in signalling and the establishment of defences. While some oxylipins exist in free form, the vast majority are found in **esterified form**, integrated into membrane lipids such as galactolipids and phospholipids. These esterified forms, called **phosphooxylipins** (POLs), represent a potential reservoir of bioactive molecules that can be released during the degradation of membranes under stress.

(a) **α-Linolenic acid (18:3)** –13-LOX→ **13-HPOT** –AOS→ **12,13-EOT** –AOC→ **12-OPDA**
(b) **hexadecatrienoic acid (16:3)** –13-LOX→ **11-HPHT** –AOS→ **10,11-EHT** –AOC→ **dn-OPDA**

Figure 1. (a) 12-OPDA and (b) dn-OPDA biosynthetic pathway

Among oxylipins, **jasmonates** are the most studied and best characterised class for their role in plant defence mechanisms. They include **jasmonic acid** (JA) and its precursors, such as **OPDA** (12-oxo-phytodienoic acid) and **dn-OPDA** (dinor-OPDA). These molecules act as key lipid hormones, regulating the expression of defence genes, the production of secondary metabolites and the **response to biotic and abiotic stresses**. To date, jasmonates are oxylipins whose biological activity in defence signalling has been demonstrated.

2. Oxylipin-containing phospholipids

Among the oxylipins identified in membrane lipids, OPDA (18:4-O) and dn-OPDA (16:4-O) are **talk to be found exclusively** in plastid lipids — notably **phosphatidylglycérol** (PG), and galactolipids (MGDG, DGDG and acMGDG). **Arabidopsides**, discovered in *Arabidopsis thaliana*, are the first forms of oxylipin-containing lipids to be identified, corresponding to galactolipids carrying OPDA and dn-OPDA.

Conversely, extraplastidial phospholipids, such as **phosphatidylcholine** (PC) and **phosphatidylethanolamine** (PE), mainly carry 18:3-O chains, corresponding to 13-HPOT-type hydroperoxides. These marked differences between plastidial and extraplastidial lipids highlight the possible compartmentalisation of jasmonate-derived oxylipin biosynthesis and suggest that plastidial lipids are mainly involved in the production of hormone precursors such as OPDA and dn-OPDA. To date, no studies have reported the presence of oxylipins in other classes of phospholipids, such as **phosphatidic acid** (PA), **phosphatidylserine** (PS), **phosphatidylinositol** (PI) or its phosphorylated forms (**PIP** and **PIP$_2$**).

Nilsson & al. (2014)

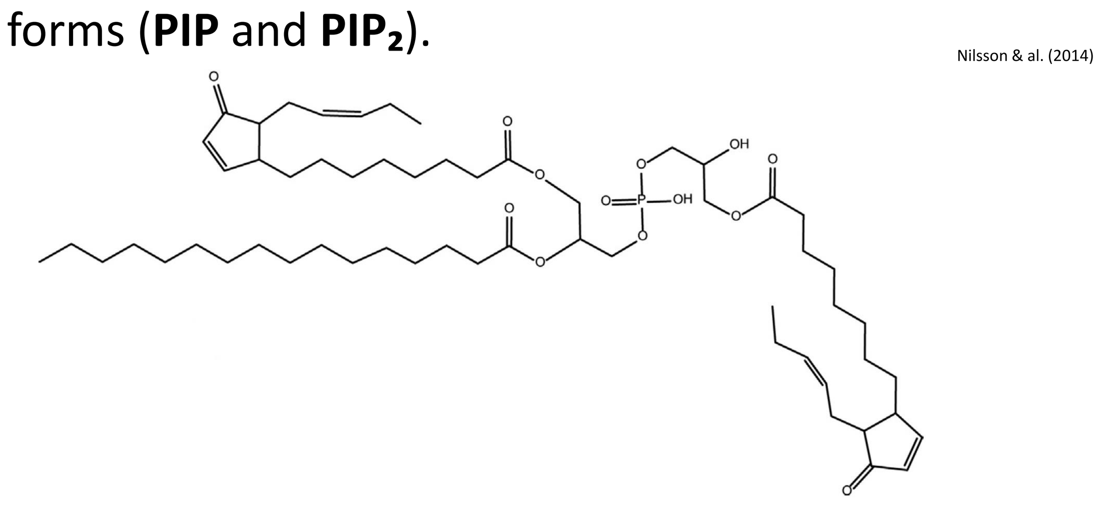

Figure 2. Putative structure of an acylated OPDA containing PG (16:0, OPDA, PG-OPDA)

Objectives

The main objective of this thesis is to better understand the **metabolic role** of phosphooxylipins (POLs) in the response of *Arabidopsis thaliana* to various biotic stresses, including the bacterium ***Pseudomonas syringae*** and the insect ***Myzus persicae***, according to different lines of study:

- **POLs production** under stress: analysing **POLs** synthesis by *A. thaliana* in response to various stresses, using a semi-quantitative approach.
- **Biosynthesis of esterified jasmonic acid precursors** in POLs (**POL-JAPs**): characterising the enzymatic pathways involved in the formation of these molecules under stress.
- **Subcellular localisation and systemic production** of POL-JAPs: determine where these molecules are produced and whether they appear in distal leaves, suggesting a role in distant signalling.
- **Potential protective role of POL-JAPs**: evaluate the impact of these molecules on plant resistance to biotic stresses.

3. Plant infection

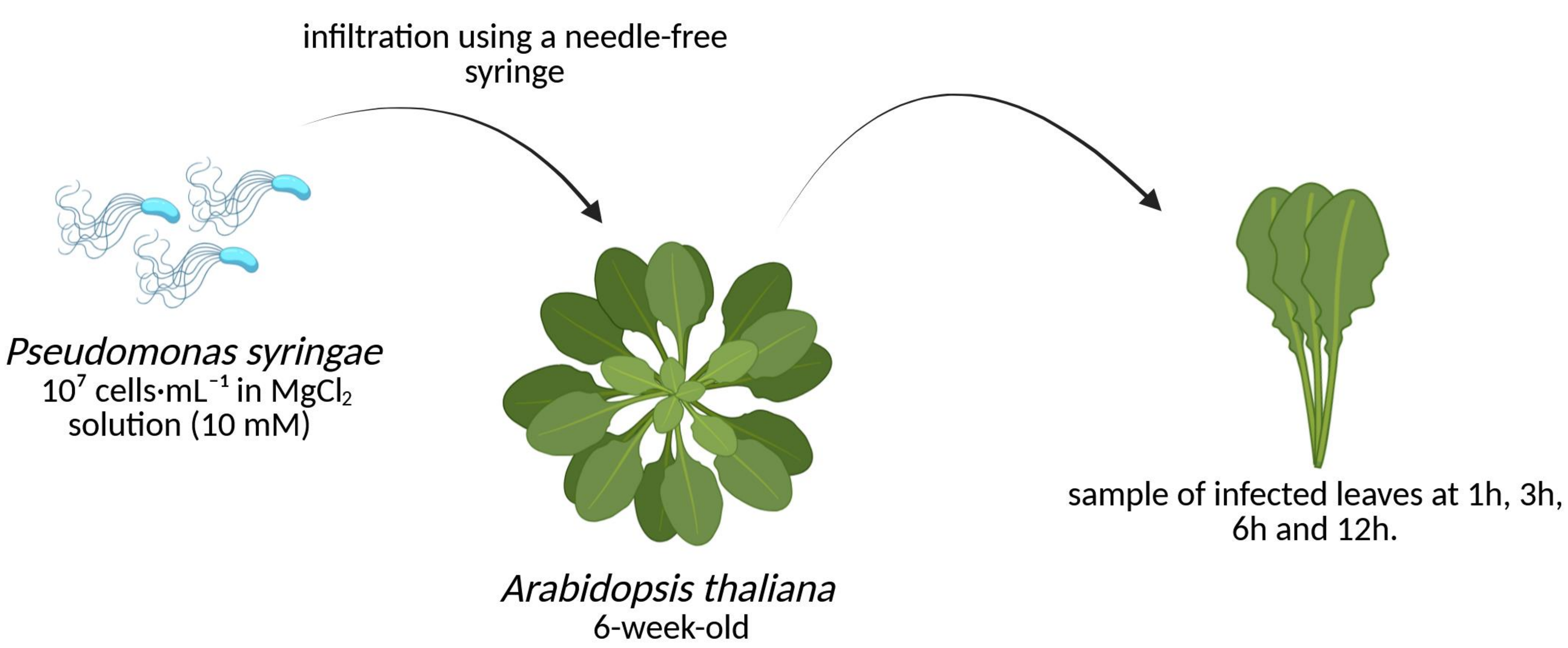

Figure 3. Inoculation of *Arabidopsis thaliana* with *Pseudomonas syringae*

4. Total lipid extraction

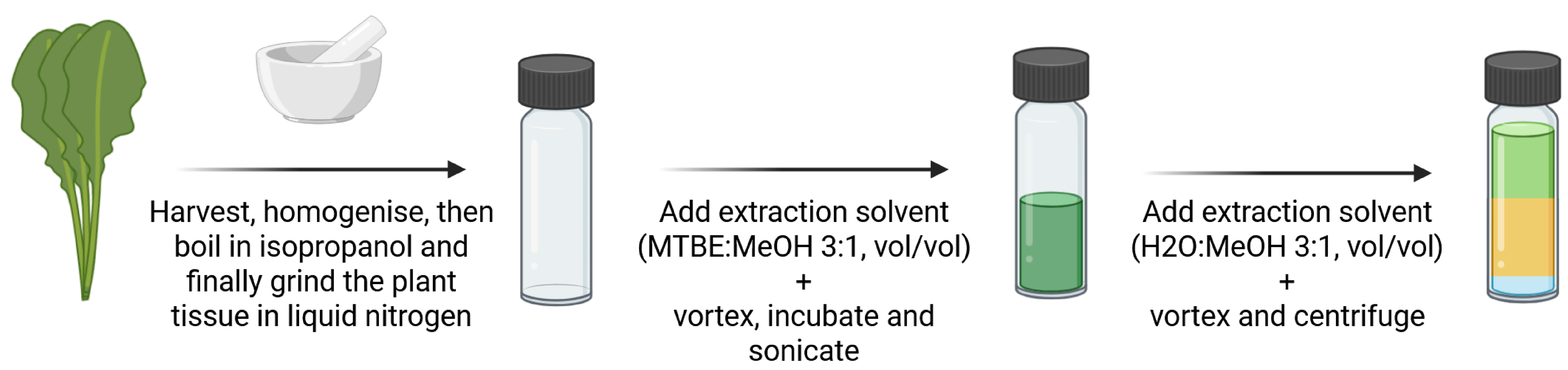

Figure 4. One-step rapid lipid extraction method

This extraction method based on the MTBE/MeOH/H_2O mixture allows, after phase separation, three distinct fractions to be obtained according to their density: the **upper phase** contains **non-polar metabolites**, mainly **lipids**. The intermediate phase contains polar and semi-polar metabolites, and finally, the pellet contains proteins, starch and cell wall polymers.

5. Preparative TLC and GC-MS

Total lipids will be separated by **preparative TLC** on activated silica plates. The main lipid classes (**phospholipids** and galactolipids) will be visualised with iodine, scraped off, then transesterified into fatty acid methyl esters (FAMEs). These FAMEs will then be analysed by **GC-MS** to determine the fatty acid composition and evaluate the variations induced by bacterial stress, with a view to developing the LC-MS/MS method and guiding the choice of analytical targets.

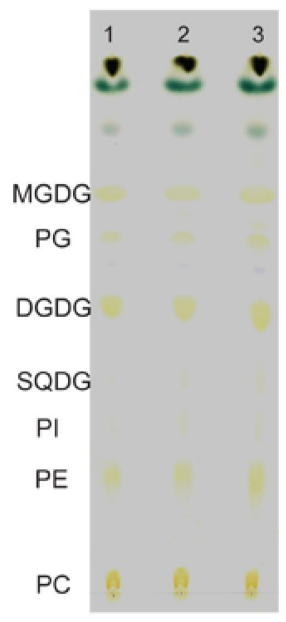

Figure 5. Separation of lipids by TCL and iodine vapour detection

Wang & Benning (2011)

6. Development of LC-MS/MS method

An analytical method dedicated to the detection and characterisation of **phosphooxylipins** (POLs) will be developed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) on a **triple quadrupole** system. The objective will be to specifically identify and quantify oxidised **phospholipids derived from the jasmonate pathway**, particularly those containing OPDA (18:4-O), dn-OPDA (16:4-O) and 13-HPOT (18:3-O) chains. The development of this method will include the optimisation of chromatographic conditions, the selection of characteristic **MRM** (Multiple Reaction Monitoring) transitions, and the development of a semi-quantitative approach to compare POLs production between infected plants and controls.

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