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# EVALUATION OF A FULL $^{15}N$ METABOLIC LABELLING PHOSPHOPROTEOMIC APPROACH TO HIGHLIGHT DEFENCE PATHWAYS AFTER PLANT CELL ELICITATION

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## INTRODUCTION

The plant cell wall is a dynamic structure and the first target of invading pathogens. Oligogalacturonides (OGAs) are enzymatically released from the plant cell wall pectin during pathogen attack. OGAs in a calcium-induced dimer conformation ("egg-box") are signalling molecules that inform the protoplast on cell wall integrity and that trigger cell defence responses. The exact sensing system of OGAs remains unknown. Chitosans, the deacetylated form of chitin present in the cell wall of invading pathogens like fungi also constitute a "warning signal".

## AIMS

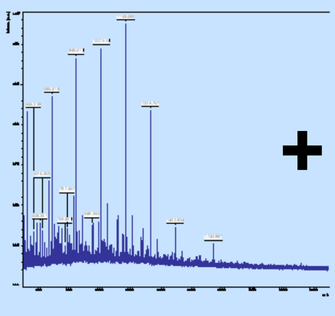
Characterization of signal transduction pathways and especially phosphoproteins involved in plant-pathogen interactions is still challenging.
The main aim of this work is to set up a full $^{15}N$ metabolic labelling gel-free quantitative phosphoproteomic approach.
The second objective is to evaluate the experimental procedure to study biotic and/or abiotic effects on cells at the phosphoproteome scale and to highlight key proteins involved in signal transduction pathways.

## THE ELICITATION

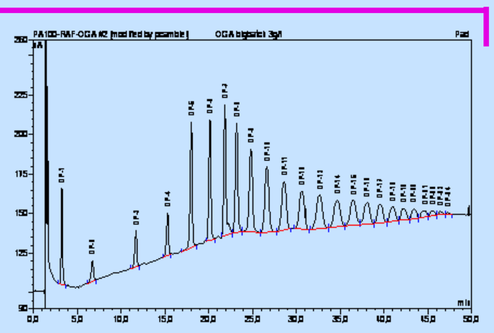
The combination between OGA and COS in a buffer allowing adoption of the egg box conformation (OGA) induces defence responses.

Cell respond to elicitation by increasing the extracellular medium, among others.

For each isotopic labelling, one batch of cells was elicited (test) and one was the control.



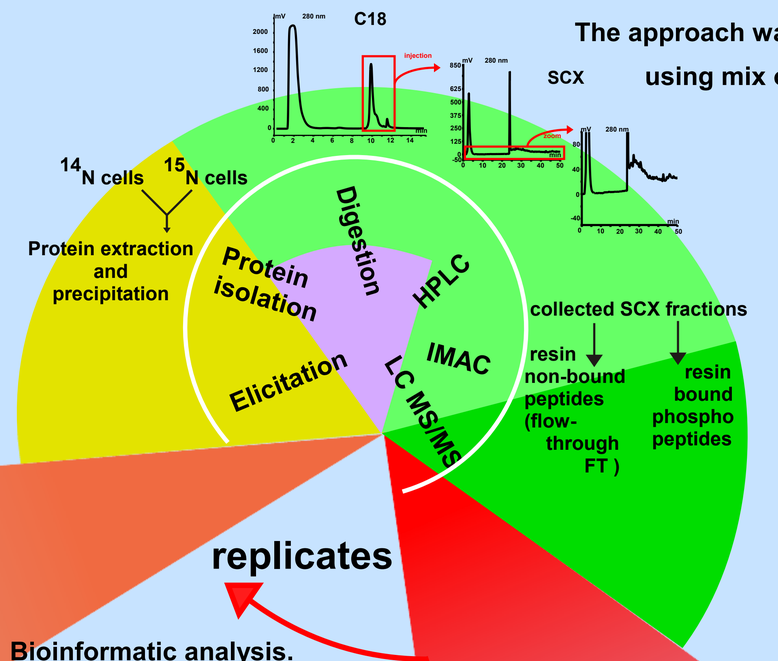
Low DP
Oligochitosans
(MALDI-TOF)



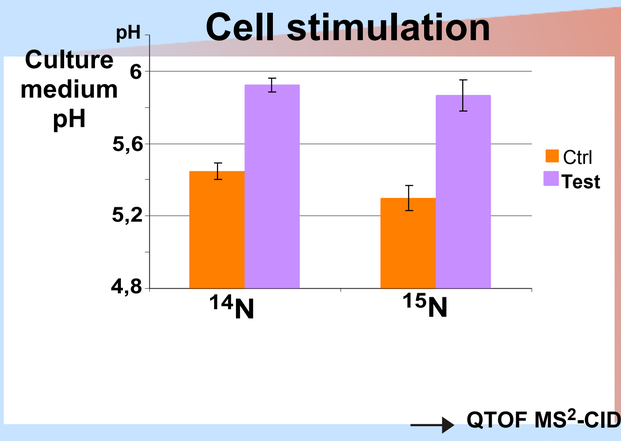
High DP
Oligopectates
(HPAEC-PAD)

## GEL-FREE (PHOSPHO)PROTEOMIC APPROACH

The approach was first validated using mix of caseins.




### Cell stimulation



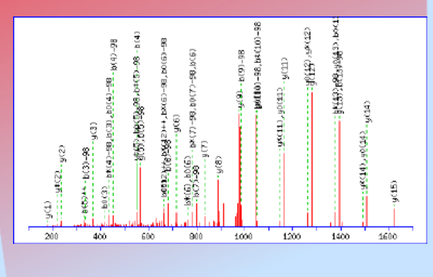

1 sample → Trypsin digestion → HPLC steps → $Fe^{3+}$ IMAC
- FT → QTOF $MS^2$-CID-DDA
- FT → LTQ $MS^3$-CID-Top 10 Full MS Enhanced
- Phosphos → QTOF $MS^2$-CID-DDA
- Phosphos → LTQ $MS^3$-CID-MSA

1 sample → Chymotrypsin digestion → HPLC steps → $Fe^{3+}$ IMAC
- FT → QTOF $MS^2$-CID-DDA
- FT → LTQ $MS^3$-CID-Top 10 Full MS Enhanced
- Phosphos → QTOF $MS^2$-CID-DDA
- Phosphos → LTQ $MS^3$-CID-MSA
- Phosphos → LTQ $MS^3$-ETD

replicates

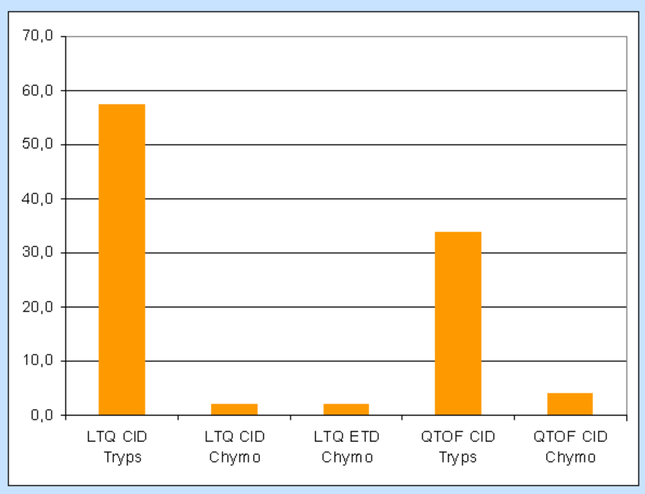
Bioinformatic analysis.
The average of the ratios of non-phosphorylated peptides is usefull to normalize $^{14}N$ versus $^{15}N$ data.
NCBI Arabidopsis database is used with the Mascot Software ($p<0.05$).
The false positive rate is ~2%

| Score | Expect | Rank | Peptide |
|---|---|---|---|
| 4 | 2.9e+002 | 8 | K.QYTESTDGSSIEIK.E [light] |
| (130) | 2.4e-011 | 1 | R.SYTNLLDLASGNFPVMGR.E [light] |
| 141 | 1.6e-012 | 1 | R.SYTNLLDLASGNFPVMGR.E [light] |
| (125) | 1.9e-010 | 1 | R.SYTNLLDLASGNFPVMGR.E [heavy] |
| (134) | 2.7e-011 | 1 | R.SYTNLLDLASGNFPVMGR.E [heavy] |



## MS ANALYSIS

## RESULTS




*100% = total of phosphopeptides, all results included

- LTQ $MS^3$ CID (Multistage Activation) highlighted ~30% more phosphopeptides than QTOF MS2 CID
- Digestion with chymotrypsin didn't improve the number of phosphopeptides identified
- LTQ-$MS^3$ and QTOF $MS^2$ didn't identify the same phosphopeptides. The two methods seem to be complementary
- Mascot and the quantitative toolbox (Matrix Science) seem to be the more convenient way to statistically analyze results.

## RUNNING EXPERIMENTS...

- Comparison between proteins identified by our protocol (2D-LC) and by 2-DIGE
- Characterization of the phosphoproteome of *Arabidopsis* cells challenged for 10 min and 4 h
- We are planning to look for membrane proteins involved in induced defense pathway.

DP: Degree of Polymerisation; SCX: Strong Cation eXchange chromatography; CID: Collision Induced Dissociation; DDA: Data Directed Analysis; LTQ: Linear Ion Trap Quadrupole; MSA: MultiStage Activation