

# Correlation between endoreduplication and metabolism in *Arabidopsis thaliana*: an LC-MS based metabolomics approach

T'Kindt Ruben

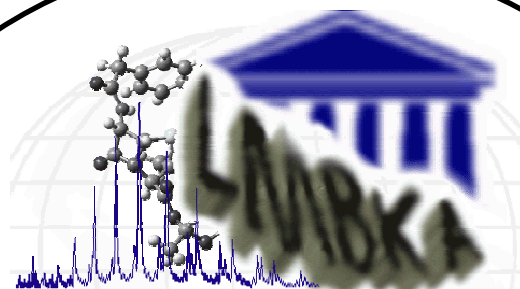
# Correlation between endoreduplication and metabolism in *Arabidopsis thaliana*: an LC-MS based metabolomics approach

- Introduction
- Analytical approach
- Biological approach
- Analytical performance
- Trial LC-MS metabolome comparison
- Future?

# Introduction



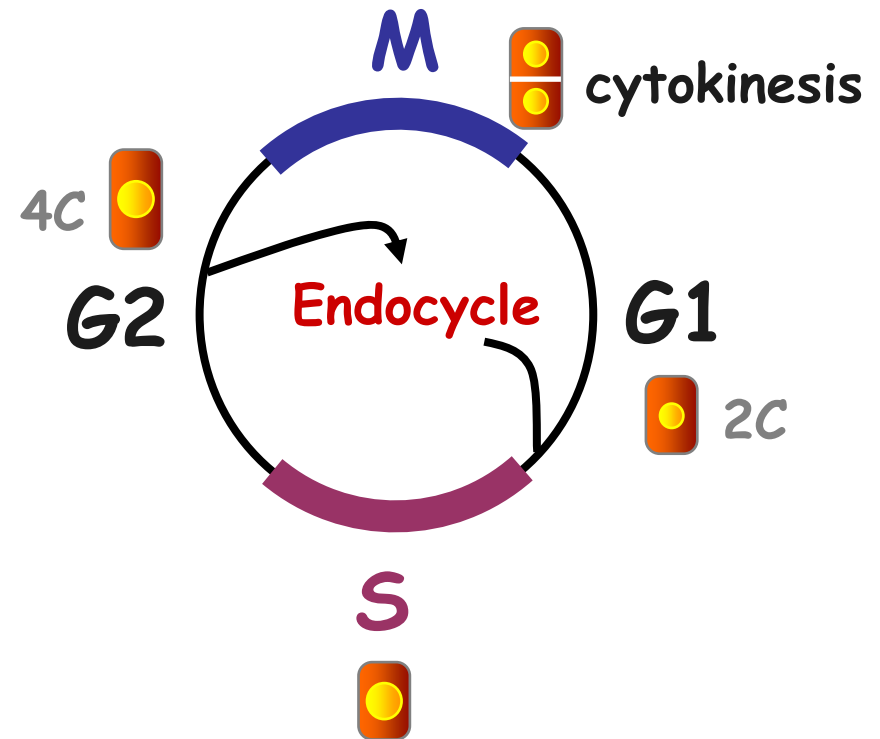
Department of Plant Systems Biology  
Flanders Interuniversity Institute  
for Biotechnology (VIB)  
Ghent University



Laboratory of Medical Biochemistry  
and Clinical Analysis  
Faculty of Pharmaceutical Sciences  
Ghent University

# Introduction

- Endoreduplication?
  - o No mitosis and/or cytokinesis between successive rounds of DNA replication
  - o DNA content of cell is doubled with every round of DNA replication
  - o Formation of cells with DNA ploidy level of 2C, 4C, 8C, 16C, 32C...
    - => polyteny



# Introduction

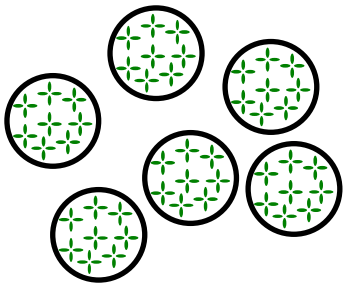
- Function of endoreduplication?
  - *Arabidopsis thaliana*: present in practically all tissues
    - Hypotheses:
      - Endoreduplication ~ fast life cycle and improved production stability
      - Buffering of mutations to preserve functional copies of the genome (Inzé et al., 2006)
      - Micro-array experiments: upregulation of genes involved in nitrogen assimilation and metabolism (Vlieghe et al., 2003)
  - Level of endoreduplication ~ developmental stage

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# Analytical approach

Harvesting  $\Rightarrow$  Homogenization  $\Rightarrow$  Weighing



( 100mg  
± 5% )

LC-MS analysis



E  
x  
t  
r  
a  
c  
t  
i  
o  
n

- o + 200ml 80/20 MeOH/H<sub>2</sub>O
- o Thermomixer (15', 1250rpm)



- o Sonication (15')
- o Centrifugation (15', 15000rpm)
- o Supernatant

# Analytical approach

- **LC-MS analysis** (*J. Sep. Sci* 2007, 30, 2002-2011)
  - Alliance 2690 LC system (Waters, Milford, MA)
    - Eluent A: H<sub>2</sub>O + 0.1% FA
    - Eluent B: AcN/H<sub>2</sub>O 90/10 + 0.1% FA
    - Atlantis dC18 (2.1 × 150mm) column
  - QTOF micro (Waters, Milford, MA)
    - Lockspray

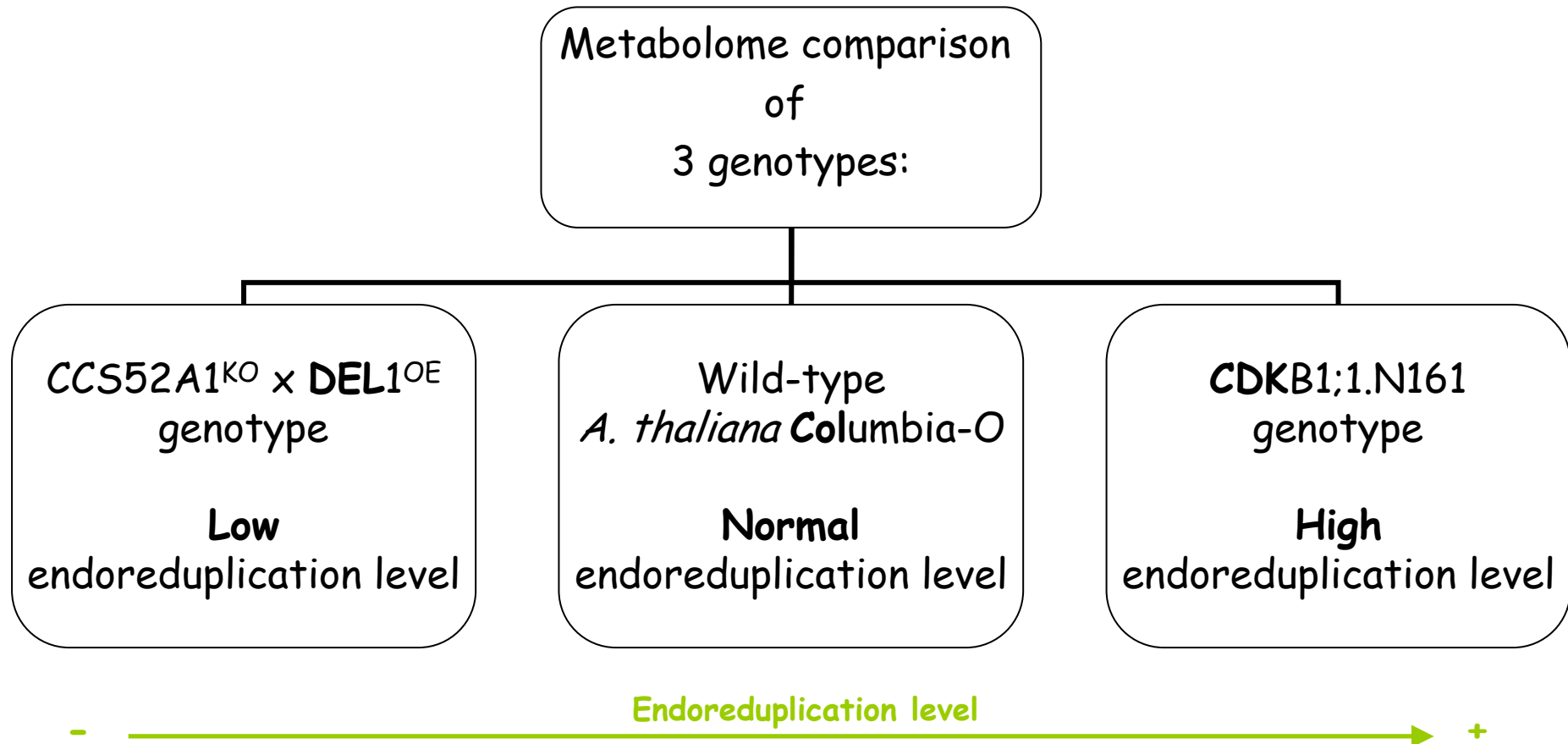




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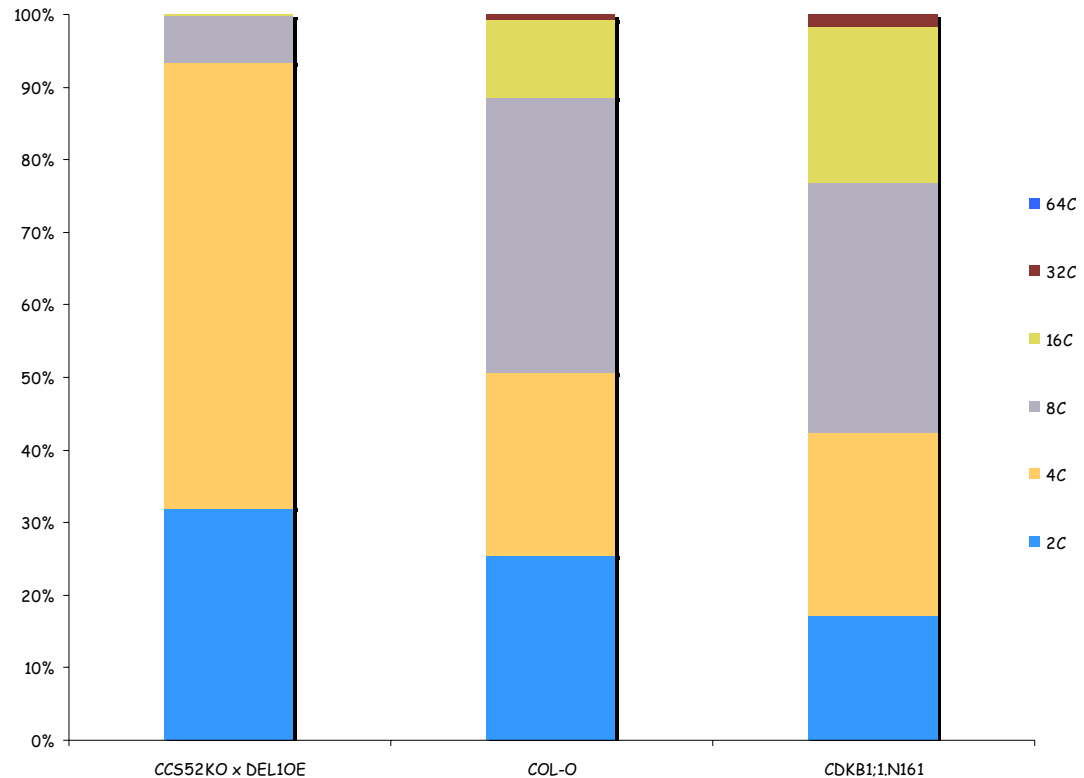
# Biological approach



# Biological approach

- Flow cytometry:

DNA content  
&  
ploidy level



Endoreduplication level

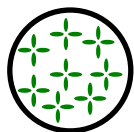


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# Analytical performance

- LC-MS precision
  - Variability of the LC/MS-tool through consecutive injections of the same extract
  - 25  $m/z$ - $tR$  combinations were randomly chosen out of the chromatogram, covering the  $tR$ -range and the  $m/z$ -range of our LC/MS tool and peak area deviation was evaluated
  - Primary leaf pairs extract (n=5)



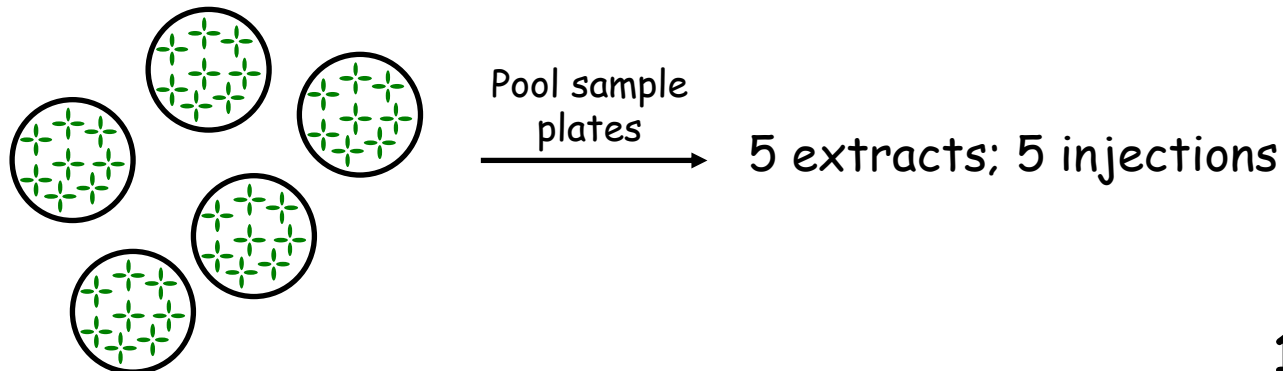
→ 1 extract; 5 injections

**8.88 ± 5.16%**

# Analytical performance

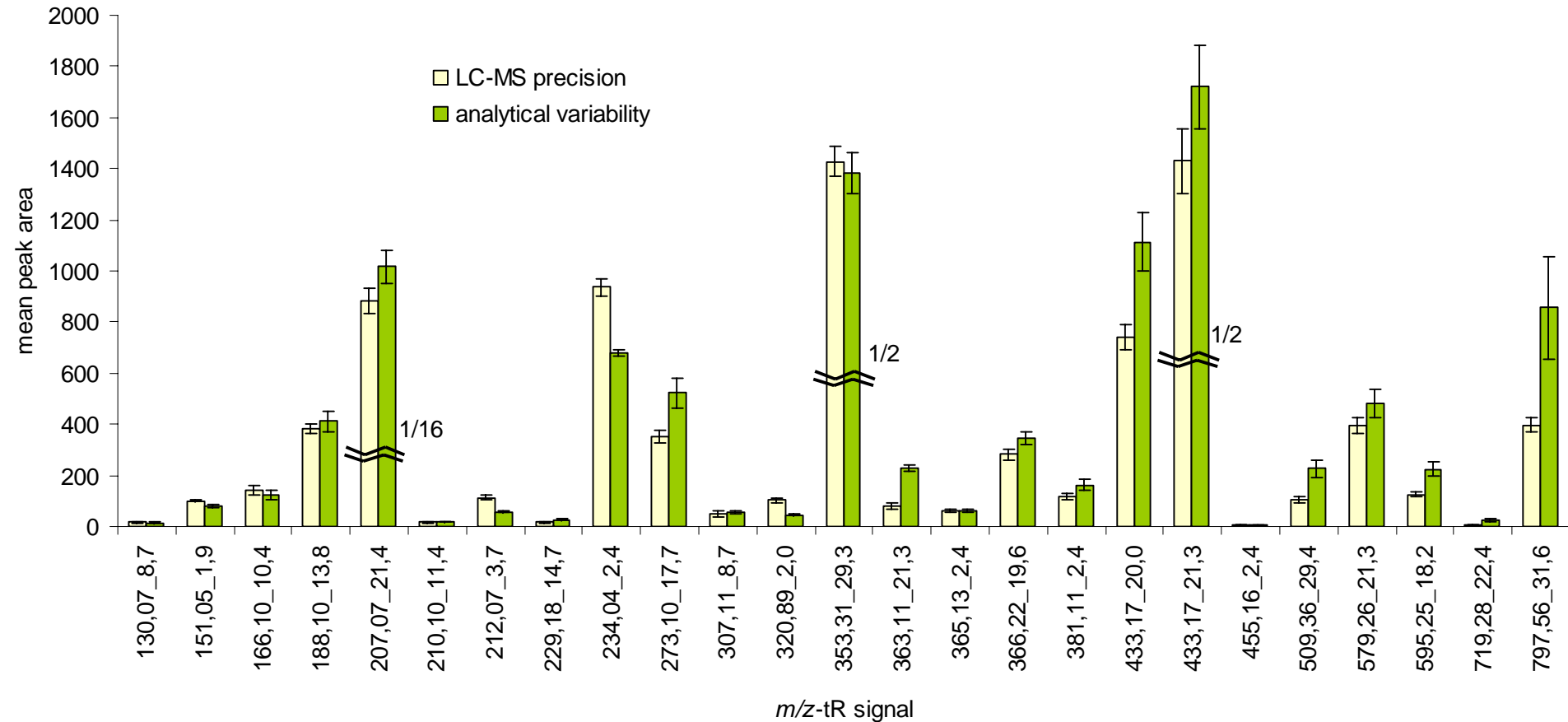
- Analytical variation

- includes the extra variability of the pre-LC/MS procedure
- Pooled primary leaf pairs extract, 5 samples



**12.53 ± 11.21%**

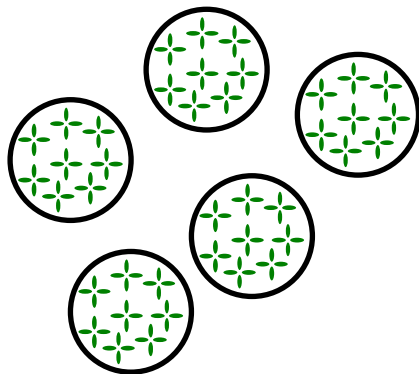
# Analytical performance



# Analytical performance

- Biological variation

- variability in plant growth conditions, development etc. on sample plates within primary leaf pairs of wild-type *Arabidopsis thaliana*

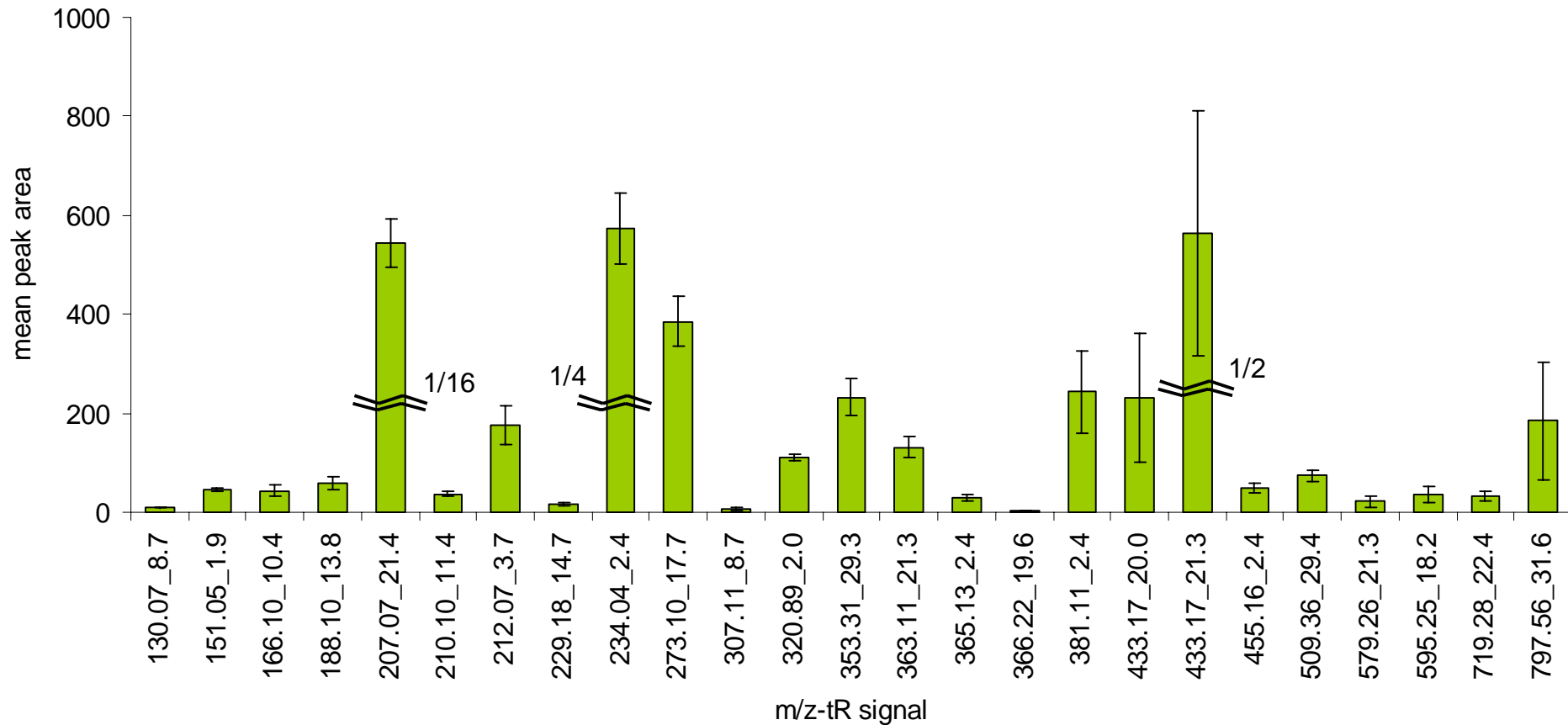


→ 5 extracts; 5 injections

**24.96 ± 16.60%**



# Analytical performance



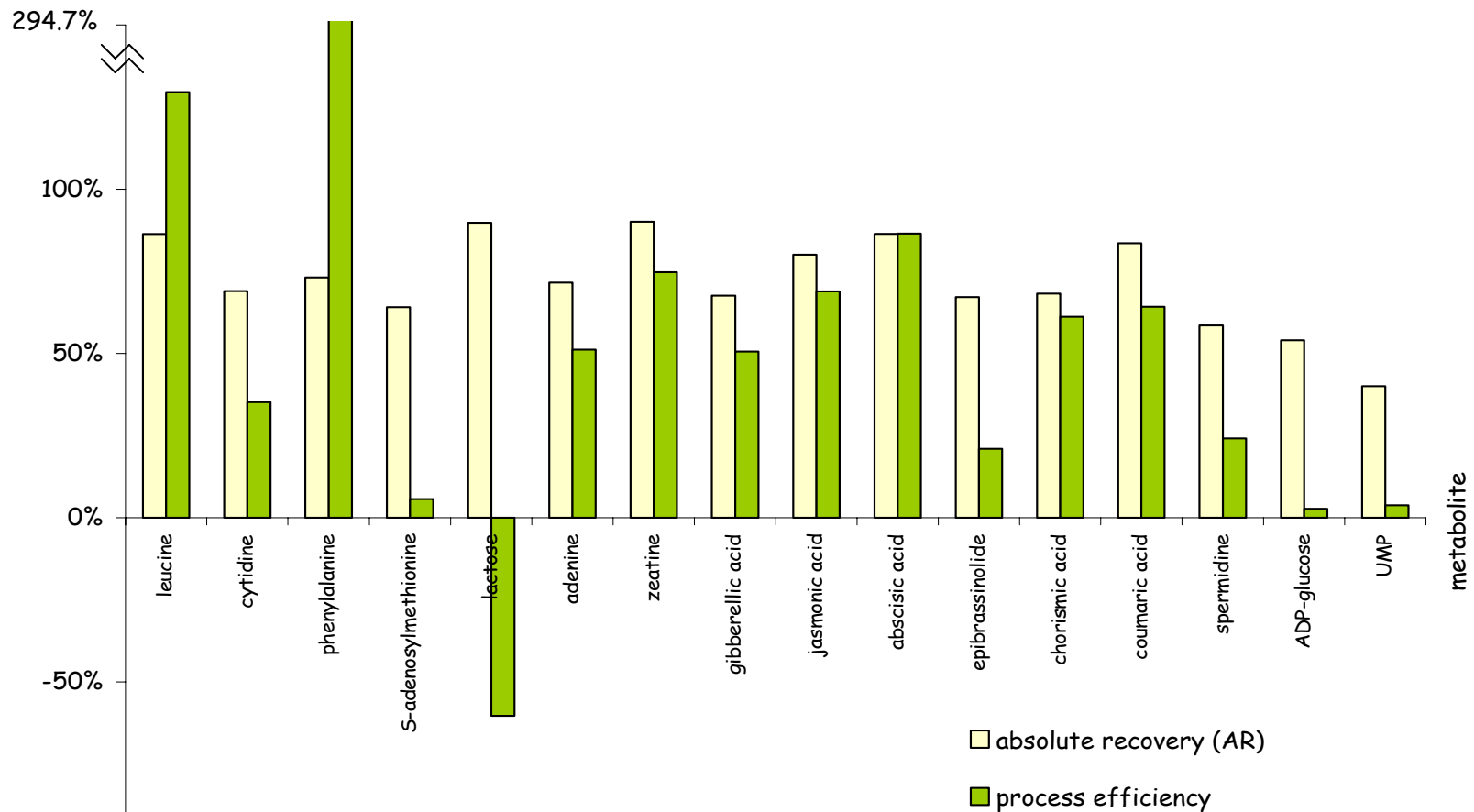
# Analytical performance

- Absolute recovery (AR) & Process efficiency (PE)
- Out of the broad range of *Arabidopsis thaliana* metabolites, we selected a group of 16 metabolites, representative for different chemical compound classes
- Analysis of 6 extracts with metabolites spiked before extraction (PRE), 6 extracts with metabolites spiked after extraction (POST), 6 blank extracts (BLANC) and 6 pure metabolite mixtures (in solvent, PURE).

$$AR = \% [ \text{peak area PRE} / \text{peak area POST} ]$$

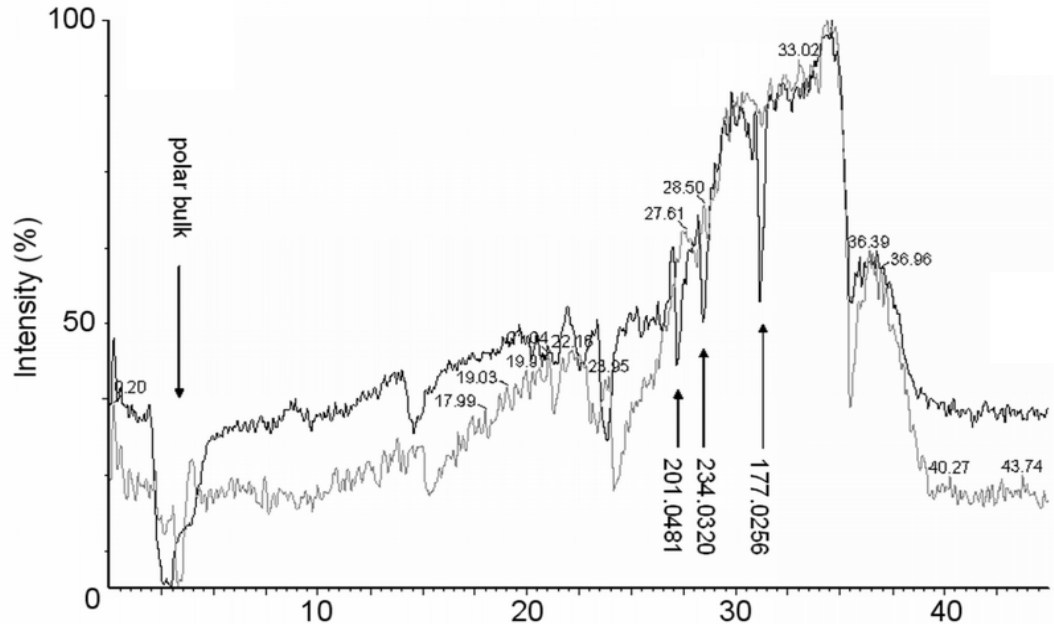
$$PE = \% [ ( \text{peak area PRE} - \text{peak area BLANC} ) / \text{peak area PURE} ]$$

# Analytical performance



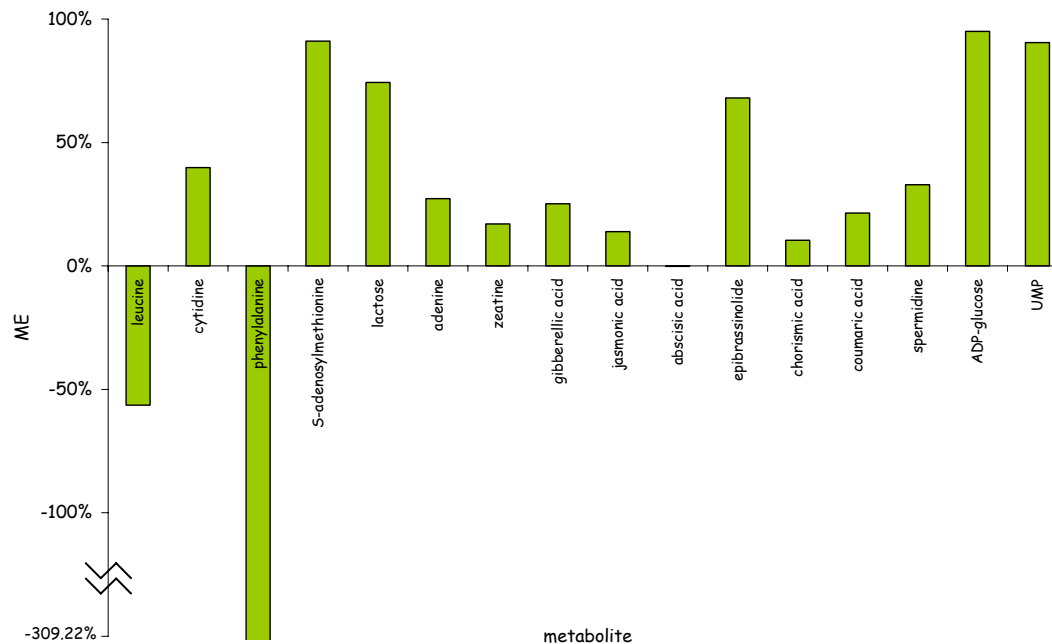
# Analytical performance

- Matrix effect (ME)
  - o Post-column infusion of leu-enk
  - o Blank run vs plant extract



# Analytical performance

- Matrix effect (ME)
  - Post-extraction addition
  - $ME = 100\% - \%[(\text{peak area POST} - \text{peak area BLANC}) / \text{peak area PURE}]$

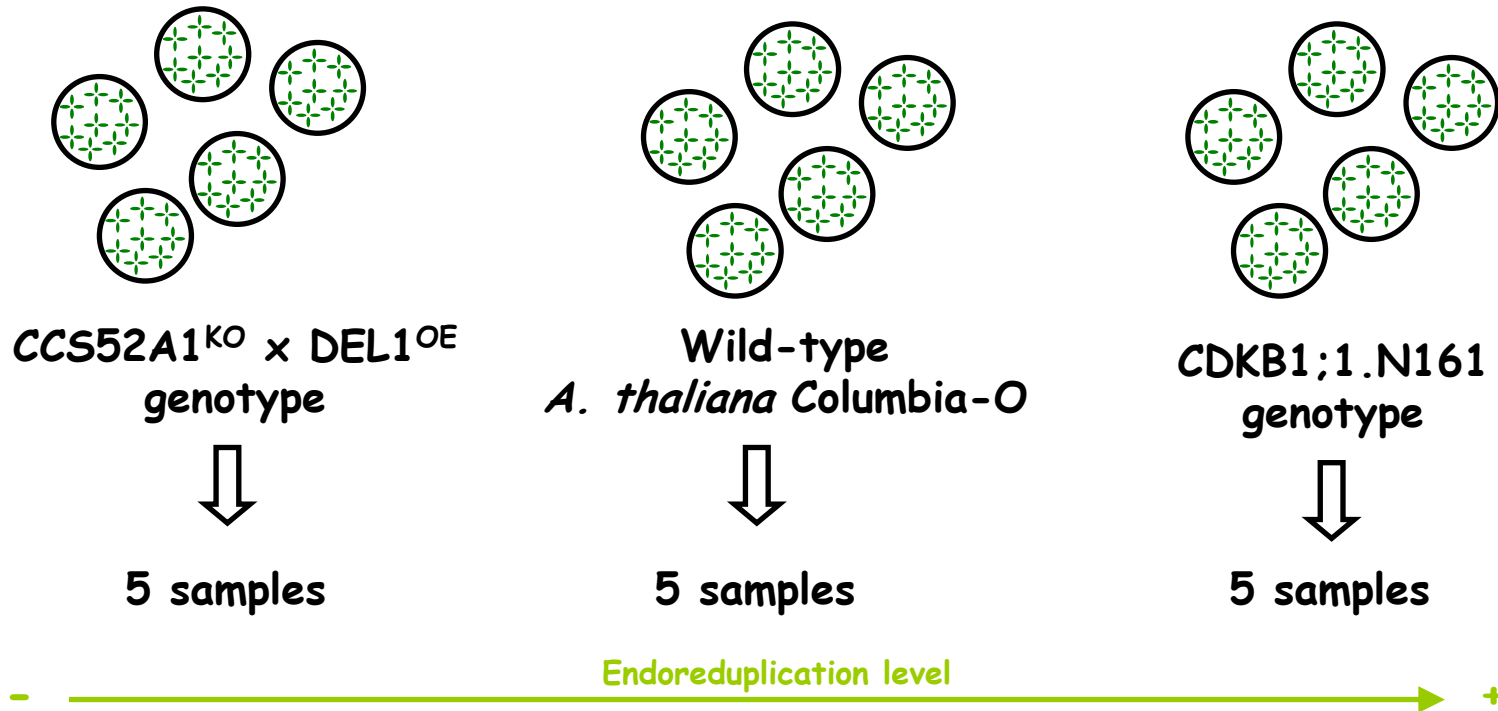


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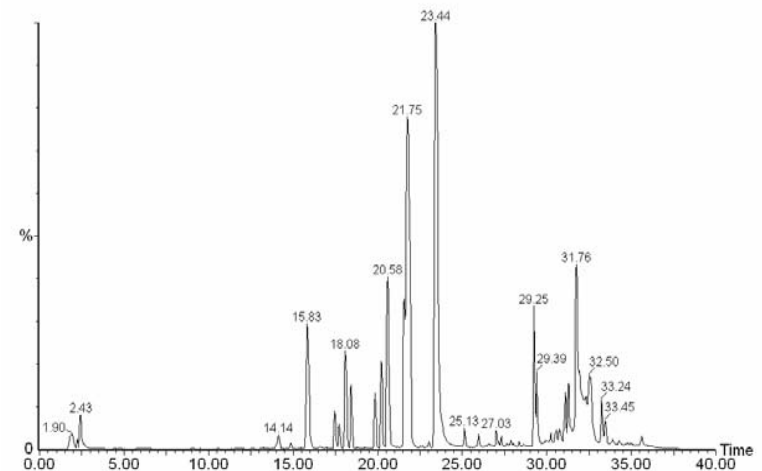
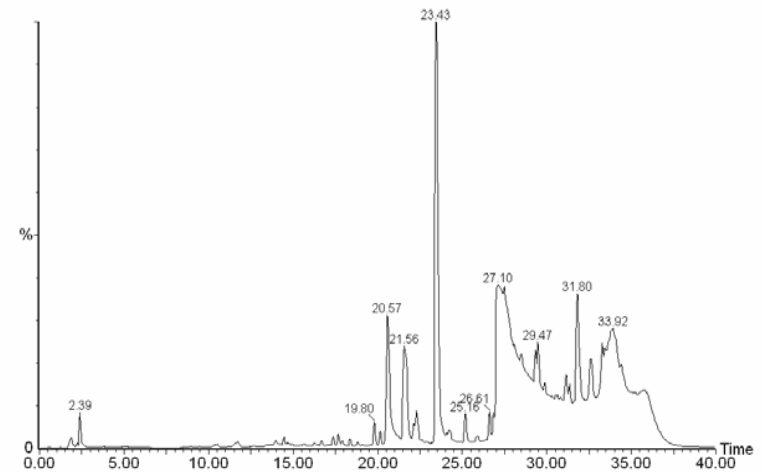
# Trial LC-MS metabolome comparison

- Primary leaf pairs of:



# Trial LC-MS metabolome comparison

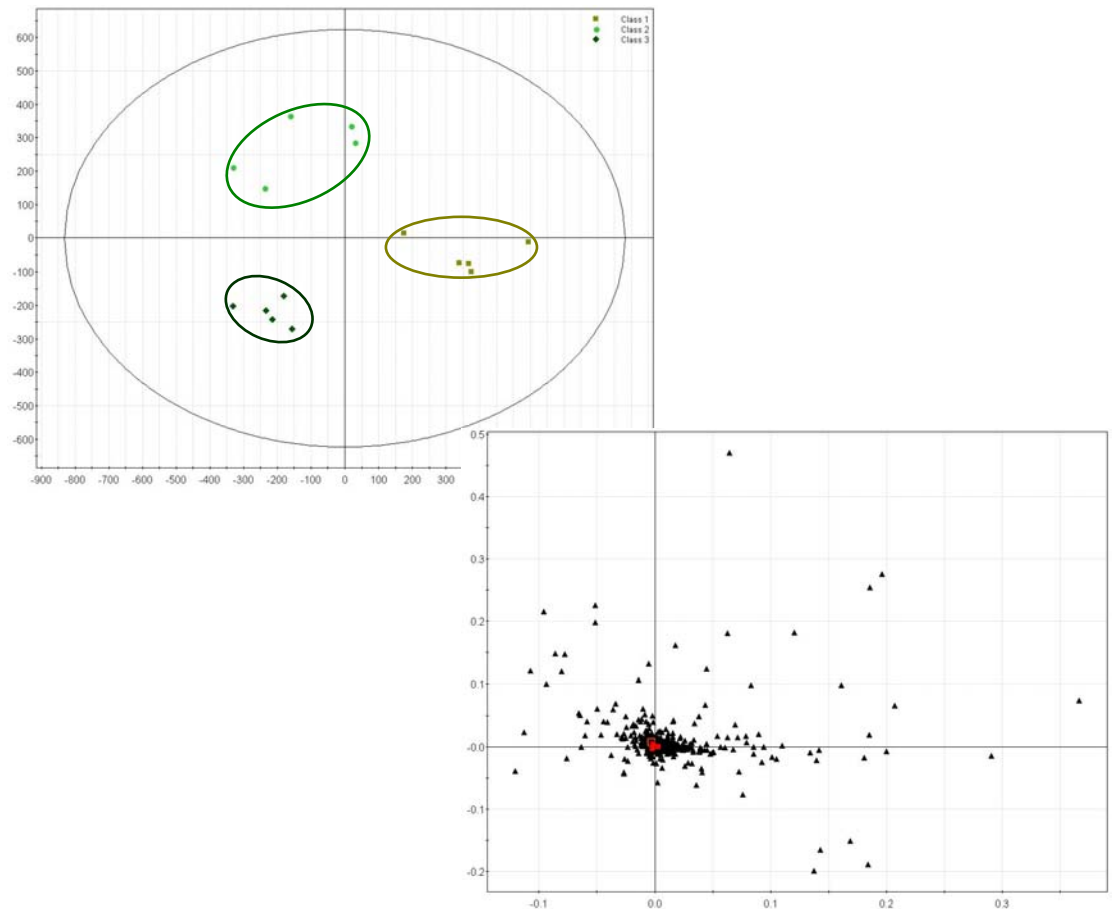
- Analysis in both + and - ESI mode
- $m/z$ -range 100-1000
- Data processing
  - MarkerLynx
  - Simca-P





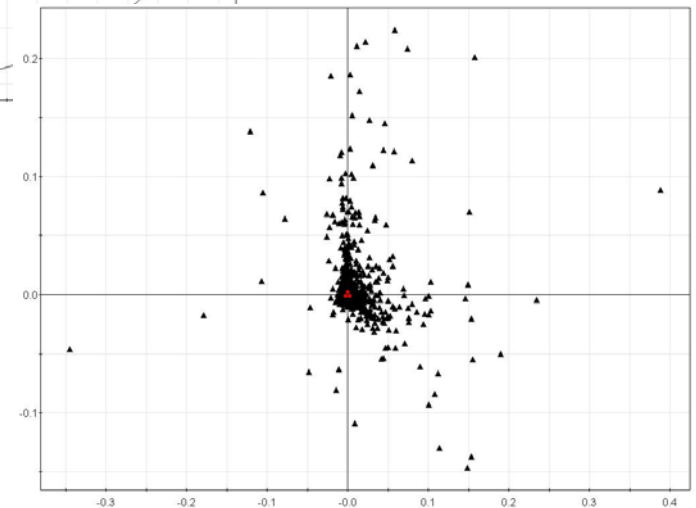
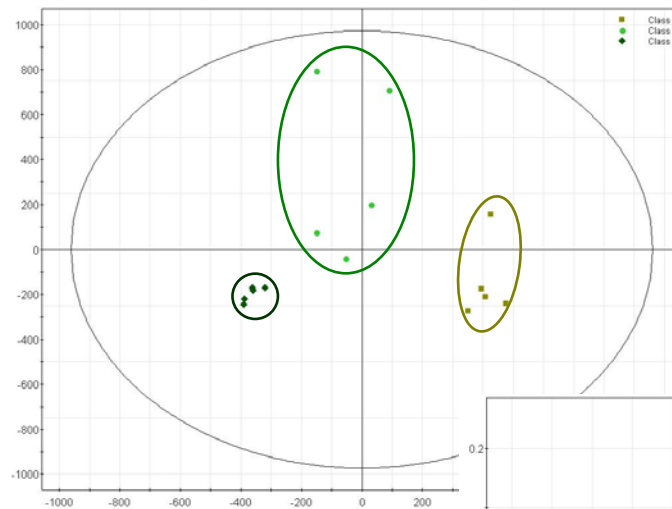
# Trial LC-MS metabolome comparison

- PLS-DA (supervised)
- Negative ESI mode:
  - 15 observations
  - 822 variables
  - $R^2X = 0.836$
  - $R^2Y = 0.986$



# Trial LC-MS metabolome comparison

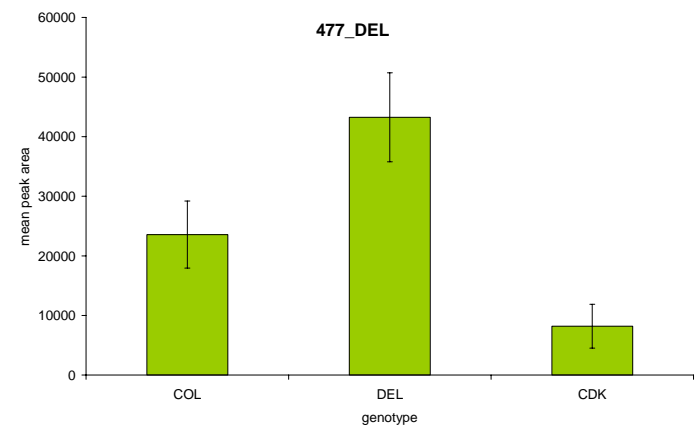
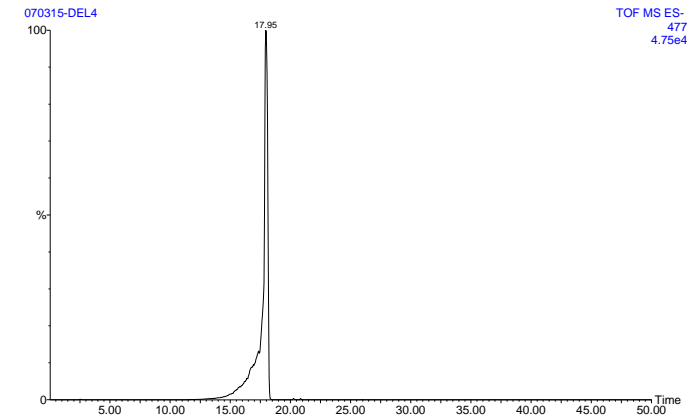
- PLS-DA (supervised)
- Positive ESI mode
  - 15 observations
  - 2034 variables
  - $R^2X = 0.730$
  - $R^2Y = 0.977$

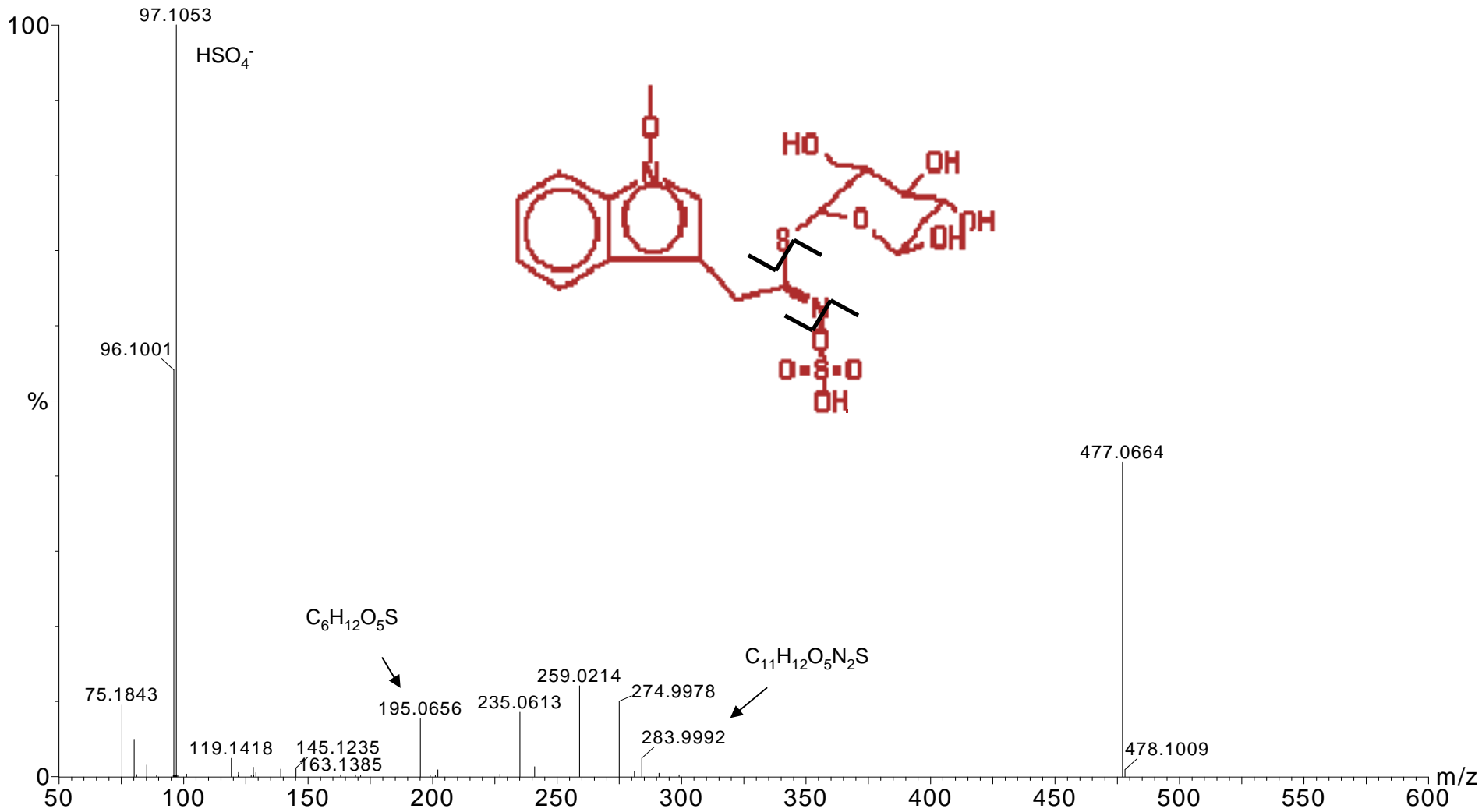


# Trial LC-MS metabolome comparison

Genotype	Retention time	m/z
COL-0	18.8493	447.1828
	22.8871	896.8694
	29.2287	397.2948
	29.2921	849.2389
	29.8268	878.8181
	21.7918	223.8778
	22.4221	784.8648
	21.6788	289.1349
	21.7820	878.8128
	28.8910	481.4118
	21.8603	877.2688
	22.2208	898.2671
	22.4223	878.8642
19.8432	281.1878	
21.7084	128.8894	
21.8186	888.8788	
CC88A1 <sup>DEL</sup> x DEL1 <sup>DEL</sup>	14.0708	477.1611
	14.8441	888.1988
	17.4463	288.2994
	2.4888	194.8891
	17.4464	288.8982
	22.1883	847.7117
	22.1833	281.2961
	28.9810	982.8527
CDK1:1.N161	27.2860	889.8187
	27.0818	288.2188
	21.1892	791.8449
21.7177	291.2988	
29.4648	887.8871	

Marker of  
endoreduplication  
process?





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## Future

- LC-MS metabolome comparison of a large batch of different genotypes
- QC inclusion (Gika et al., 2007)
- HILIC approach for polar metabolite fraction
- Additional GC-MS analysis
- FT-MS analysis towards identification

Thank you !