Imaging Mass Spectrometry: What Can Metabolite Location Suggest About Pathways of Lipid Metabolism in Oilseeds?

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Analyzing Lipid Composition and Distribution

Direct-Infusion (MS) Lipidomics

- Chemical Composition: ✔
- Spatial Information: Limited

In situ Visualization

- Chemical Composition: Limited
- Spatial Information: ✔

http://grandfathersmiles.blogspot.com/

**Chemical Composition**

- TAG
- FFA
- DAG
- StE
- PC
- PA
- PI
- PE
- PS

**Relative Abundance**

- 825
- 850
- 875
- 900
- 925

**TAG Content (mol %)**

- Bligh/Dyer
- DOMS

**Fluorescence**

- BODIPY 493/503
- 20 µM

**MRI**

- TEM
- LB
- PB

**2 µm**

- 0.15 mg/mm³
- 0.50 mg/mm³
Mass Spectrometry Imaging – Lipid Location
Matrix-assisted laser desorption ionization (MALDI)- MS

Specimen Preparation
Matrix Application → MALDI MS Imaging

Map Lipids in Two Dimensions

Mass Spectrum
Validation: MS/MS^n
Compare with Extracts

Advances in laser optics, Y-J Lee

Sturtevant D, Lee YJ, Chapman KD.
Three-Dimensional Reconstructions

PC Distribution \textit{in situ}  
- \textit{Arabidopsis thaliana} seeds

$\text{PC 38:3}$  
$[\text{M+H}]+ \ m/z \ 812.617$

$\text{PC 34:2}$  
$[\text{M+H}]+ \ m/z \ 758.571$

$\text{PC 34:3}$  
$[\text{M+H}]+ \ m/z \ 756.554$

Drew Sturtevant, Maria Duenas, Young-Jin Lee  
Sturtevant et al, BBA 2017 Feb;1862(2):268-281
Spatial Heterogeneity of Seed Lipids

How Prevalent is this Phenomenon? All seeds examined—Arabidopsis thaliana, Brassica napus, Simmondsia chinensis, Camelina sativa, Gossypium hirsutum, Ricinus communis, Glycine max

Are there Metabolic Explanations? Yes some; still much to learn. Examples in Camelina (Patrick Horn/ Ed Cahoon; Sophia Marmon, Ivo Feussner/ Drew Sturtevant)

Unforeseen Consequences? Yes-- Engineering Lipid Pathways-Example, High Oleic Camelina- Ed Cahoon/ Patrick Horn; Long Chain PUFAs in Camelina- Richard Haslam, Louise Michaelson and Johnathan Napier/ Mina Aziz, Drew Sturtevant)
Heterogeneity of PC Species in *Brassica napus*

Drew Sturtevant with Helen Woodfield and John Harwood

(cv. Westar)

Visualized same intensity

Varying Min/Max intensity

Spatial Heterogeneity
Common theme

Established early in Development- PC and TAG

Indication of Metabolic Precursor-Product Relationships

Woodfield et al., 2017, *Plant Physiol*
Heterogeneity Develops Early in *Brassica napus*

TAG molecular species are distributed differentially in seed parts, and these locations are generally similar through seed maturation. (cv. Westar)
Heterogeneity of PC/ TAG Molecular Species Supports Precursor-Product Relationships

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<td>18:1/18:2</td>
<td>0-35</td>
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<td>0-45</td>
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PC(-derived DAG species) differentially compartmentalized with TAG molecular species.

(cv. Westar)
Imaging Mass Spectrometry – Camelina Seeds

Patrick Horn
With Ed Cahoon, U Nebraska

Marked Heterogeneity of PC species
(maps of 20 different species)

Horn et al., 2013, Plant J 76(1):138-50
TAG Distribution in *Camelina*- Heterogeneous

Insights Into Spatial Differences in TAG Production?

Max = 15% TAG or 25% PC

COTYLEDONS

DGAT

G3P → LPA → PA → DAG 
16:0, 18:1 18:1

PDCT (ROD1)

PC

DGAT

PA → DAG

PDAT

TAG

LPC

PC

FAD2

18:1-CoA

CoA pool (18:2, etc.)

18:X (16:0, 20:1)

18:2

PC

Alternatives? = Low amounts
Insights Into Spatial Differences in TAG Production?

**EMBRYONIC AXIS**

- **DGAT**
  - G3P → LPA → PA → DAG
  - 16:0, 18:1 → 18:1
  - PDCT (ROD1)

- **PDAT**
  - 20:1-CoA
  - CoA pool (18:2, etc.)

- **PC**
  - 18:2
  - 18:1

- **TAG**
  - 20:1-CoA

- **FAD2**
  - 18:1-CoA
  - 18:X (16:0, 20:1)

- **FAE1**
  - 18:1-CoA
  - 18:X (16:0, 20:1)

- **Max** = 15% TAG or 25% PC
DGAT1 Suppression
Drew Sturtevant with Sofia Marmon and Ivo Feussner

<table>
<thead>
<tr>
<th>PC</th>
<th>Bright-Field Image</th>
<th>PC 34:3 (18:3/16:0)</th>
<th>m/z 756.554</th>
<th>PC 34:2 (18:2/16:0)</th>
<th>m/z 758.569</th>
<th>PC 36:6 (18:3/18:3)</th>
<th>m/z 778.538</th>
<th>PC 36:5 (18:3/18:2)</th>
<th>m/z 780.554</th>
<th>PC 36:4 (18:2/18:2)</th>
<th>m/z 782.569</th>
<th>PC 38:4 (20:1/18:3)</th>
<th>m/z 810.601</th>
<th>PC 38:3 (20:1/18:2)</th>
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PDAT1 Suppression
Drew Sturtevant with Sofia Marmon and Ivo Feussner

PC

Bright-Field Image
A

Control
B

amiPDAT.3

Bright-Field Image

TAG

Bright-Field Image
A

Control
B

amiPDAT.3

PC 34:3 (18:3/16:0)
m/z 756.554
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m/z 810.601
PC 38:3 (20:1/18:2)
m/z 812.616

TAG 52:6 (18:3/18:3/16:0)
m/z 873.694
TAG 54:9 (18:3/18:3/18:3)
m/z 895.678
TAG 54:8 (18:3/18:3/18:2)
m/z 897.694
TAG 54:7 (18:3/18:2/18:2)
m/z 899.709
TAG 54:6 (18:2/18:2/18:2)
m/z 901.725
TAG 56:7 (20:1/18:3/18:3)
m/z 927.741
TAG 56:5 (20:1/18:2/18:2)
m/z 929.756
Reverses some of the C18:3 species, but not those with long chains—
influence on C18:2 TAGs and accentuates distributions in WT or amiDGAT1
High Oleic Transgenics (66% oleic)

RNAi FAE1/FAD2-(66% 18:1)

***Increased 18:1 PC-

***New Distributions–

But Still Heterogeneity
High Oleic Transgenics (TAGs)

***Increased 18:1 TAG-
***New Distributions–Still Heterogeneity

Wild-type TAG 54:3

![Graph showing TAG distributions and molecular species](image)

- **TAG-52:4 (PLnO)**
- **TAG-52:3 (PLO)**
- **TAG-52:2 (POO)**
- **TAG-54:6 (LLL)**
- **TAG-54:5 (LnOO)**
- **TAG-54:4 (LOO)**
- **TAG-54:3 (OOO)**

Graph showing relative mol % of TAG molecular species for High Oleate MALDI, High Oleate TLE-ESI, and Wild-type MALDI.
Imaging Heterogeneity of Engineered Plant Oils

Spatial Heterogeneity of FAD2/FAE1 (RNAi) Knockdown

PC/TAGs with 18:1 enriched in cotyledons (relative to axis)

→ Spatially incomplete RNAi-mediated knockdown? Promoter?
→ room to further boost total 18:1?
→ Compartmentation suggests new strategy
Summary and Significance

• Imaging Mass Spectrometry- new tool for metabolomics-to map of chemical organization in plant tissues at tissue/cellular levels.

• MALDI-MS imaging indicates that heterogeneity of lipid metabolites (and metabolic pathways) in seed tissues is a widespread phenomenon. Enzymes/pathways responsible in part (FAE1, DGAT1, PDAT1, others?).

• Important to consider endogenous heterogeneity to help inform metabolic engineering strategies.

• Localization suggests that there is much more to be learned about the biochemistry, cell biology and functions of lipid metabolites from a spatial perspective.
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University of Nebraska
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