Conserved and novel mechanisms underlie oil palm (*Elaeis guineensis* Jacq.) fruit abscission: A model for studying fruit abscission in the palm family (Arecaceae).

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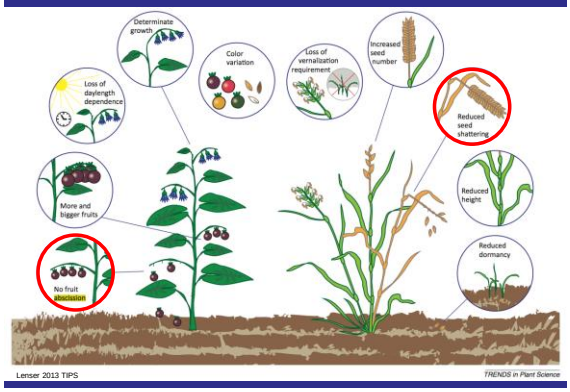
Why study organ abscission?

Fruit abscission is a domestication trait



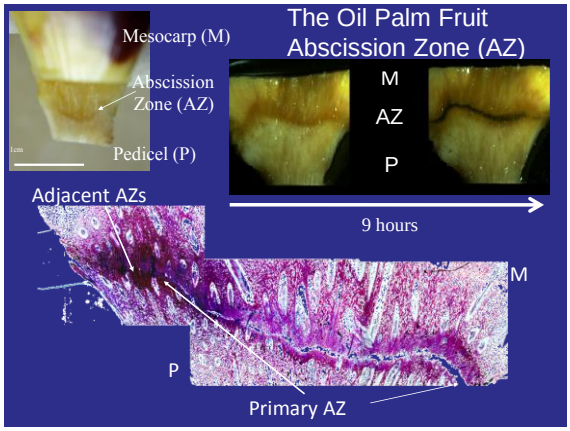
Unsynchronized fruit ripening and abscission of palm fruit cause economical losses

Elaeis guineensis (African Oil Palm) as a model

Increases harvest cost:

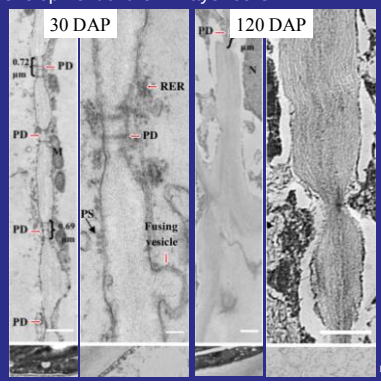
- ✓Labor to identify and harvest ripe bunches before fruit drop
- ✓Labor to collect dropped fruits

The Oil Palm Fruit Abscission Zone (AZ)



MRI 3D Imaging of palm fruit reveals H_20 abundant AZ

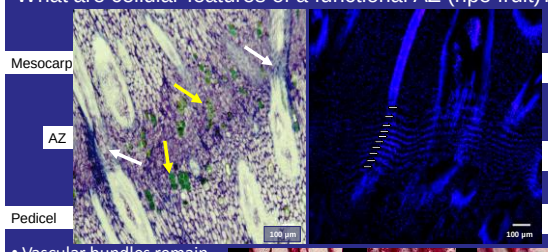
Cell wall striations appear and plasmodesmata increase in size during development of the AZ layer cells.



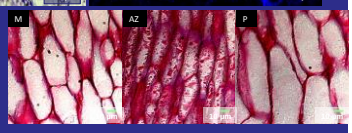
Peerapat Roongsattham (doctoral student 2008-2011)

Roongsattham et al. 2016

What are cellular features of a functional AZ (ripe fruit)?

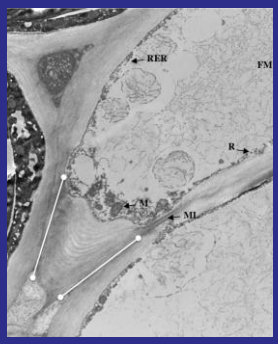


- Vascular bundles remain undifferentiated in AZ
- Phenolic cells
- Nuclear alignment (AZ organizes into cell layers)
- Intracellular pectin accumulate during development throughout AZ layers



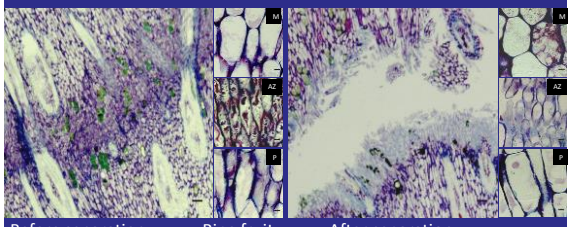
Roongsattham et al. 2016

Cell wall striations appear particularly at tip of AZ cells



120 DAP

What cellular features change during separation?

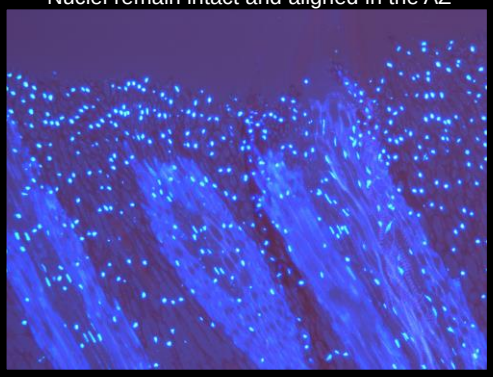


Before separation Ripe fruit After separation

- AZ toluidine blue color change after separation suggests cation changes in cation environment (eg. H^+, Ca^{2+})
- Intracellular pectins decrease

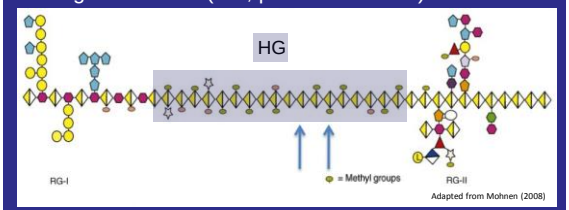
Roongsattham et al. 2016

Nuclei remain intact and aligned in the AZ



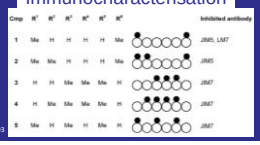
DAPI stained

Primary AZ of oil palm is rich in unmethylated homogalacturonan (HG, pectin backbone)



Adapted from Mohnen (2008)

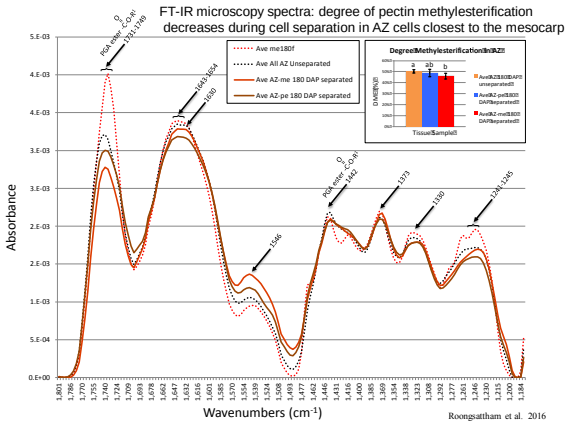
Immunocharacterisation

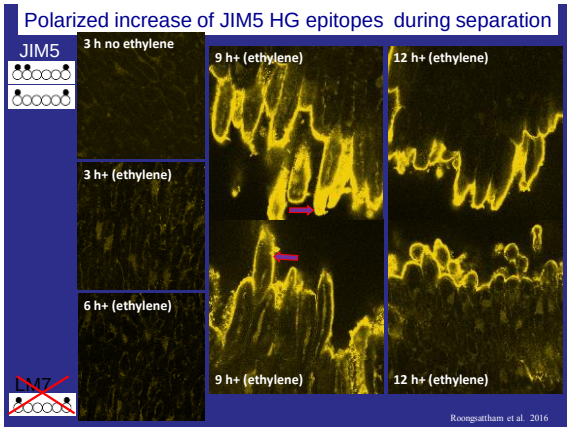


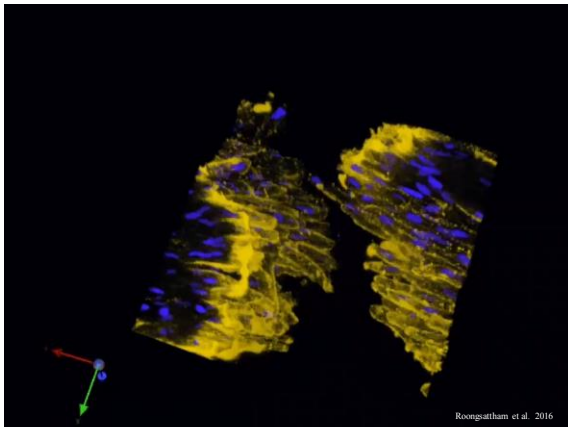
Clausen et al., 2003

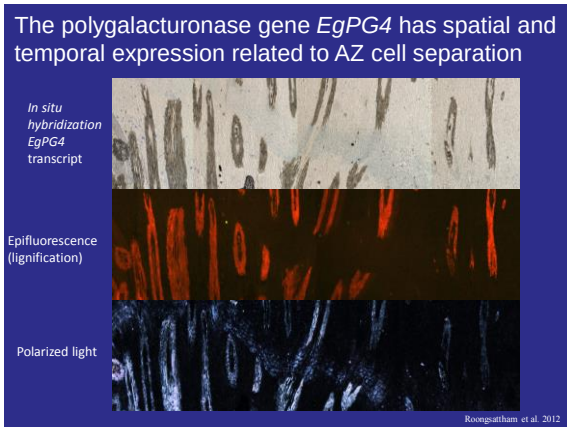
FT-IR microscopy

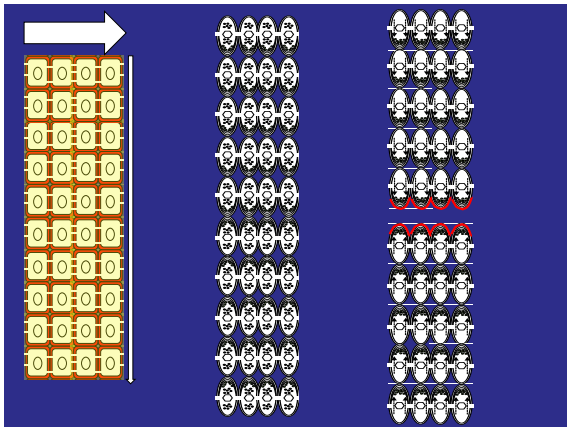
Are there changes in the methylation status of HG in the AZ?

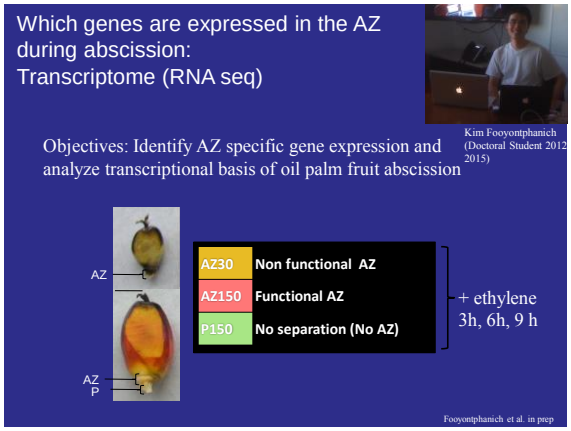

FT-IR microscopy spectra: degree of pectin methylesterification decreases during cell separation in AZ cells closest to the mesocarp
Ave me180f
Ave All AZ Unseparated
Ave AZ-me 180 DAP separated
Ave AZ-pe 180 DAP separated
Absorbance
Wavenumbers (cm-1)
Roongsattham et al. 2016

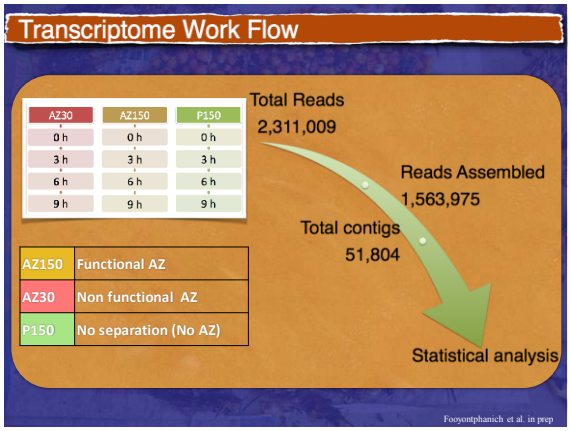

Polarized increase of JIM5 HG epitopes during separation
JIM5
3 h no ethylene
3 h+ (ethylene)
6 h+ (ethylene)
9 h+ (ethylene)
12 h+ (ethylene)
9 h+ (ethylene)
12 h+ (ethylene)
LM7
Roongsattham et al. 2016

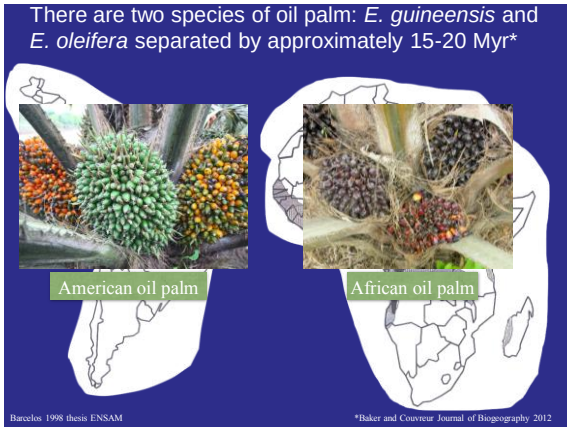

Roongsattham et al. 2016

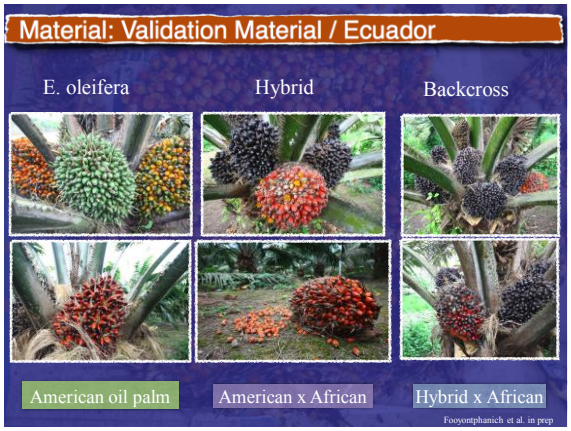

The polygalacturonase gene EgPG4 has spatial and temporal expression related to AZ cell separation
In situ hybridization EgPG4 transcript
Epifluorescence (lignification)
Polarized light
Roongsattham et al. 2012

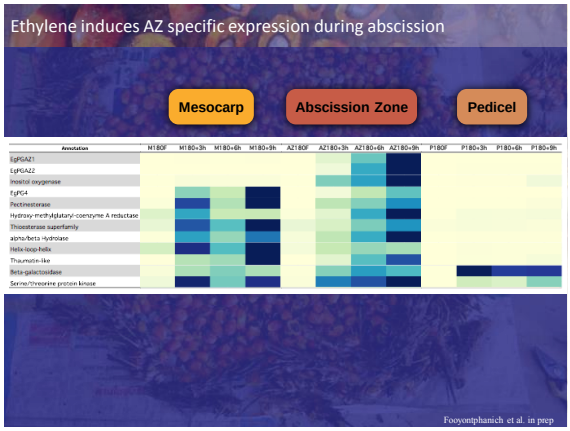


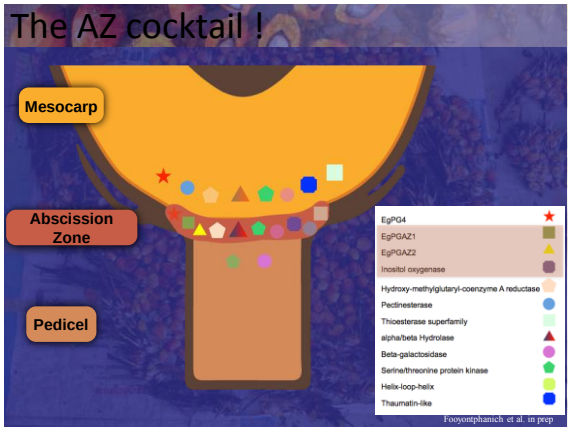

Which genes are expressed in the AZ during abscission:
Transcriptome (RNA seq)
Kim Fooyontphanich (Doctoral Student 2012 2015)
Objectives: Identify AZ specific gene expression and analyze transcriptional basis of oil palm fruit abscission
AZ
AZ
P
AZ30 Non functional AZ
AZ150 Functional AZ
P150 No separation (No AZ)
+ ethylene
3h, 6h, 9 h
Fooyontphanich et al. in prep


Transcriptome Work Flow
AZ30
AZ150
P150
0 h
3 h
6 h
9 h
Total Reads
2,311,009
Reads Assembled
1,563,975
Total contigs
51,804
Statistical analysis
AZ150 Functional AZ
AZ30 Non functional AZ
P150 No separation (No AZ)
Fooyontphanich et al. in prep


There are two species of oil palm: E. guineensis and E. oleifera separated by approximately 15-20 Myr*
American oil palm
African oil palm
Barcelos 1998 thesis ENSAM
*Baker and Couvreur Journal of Biogeography 2012


Material: Validation Material / Ecuador
E. oleifera
Hybrid
Backcross
American oil palm
American x African
Hybrid x African
Fooyontphanich et al. in prep


Ethylene induces AZ specific expression during abscission
Mesocarp
Abscission Zone
Pedicel
Fooyontphanich et al. in prep


The AZ cocktail !
Mesocarp
Abscission Zone
Pedicel
EgPG4
EgPGAZ1
EgPGAZ2
Inositol oxygenase
Hydroxy-methylglutaryl-coenzyme A reductase
Pectinesterase
Thioesterase superfamily
alpha/beta Hydrolase
Beta-galactosidase
Serine/threonine protein kinase
Helix-loop-helix
Thaumatin-like
Fooyontphanich et al. in prep


The search for non-shedding oil palm
Colé
Quito
Ecuador
Guayaquil
Taisha
Collected in Taisha 2006 (Phillipe Amblard and Claude Louis, PalmElit/CIRAD)
Planted in Colé by DANEC and Murrin Corp.

Is *E. oleifera* non-shedding (no abscission)?

Field phenotyping

Field notes
Confirm pollination
NS – not shedding
Sh – shedding
Sh (low) – a little shedding
IM – Immature fruit
NF – no fruit
IC – incomplete pollination
D - dead

Other Notes (e.g.)
Only one fruit
Signs of shedding

In vitro phenotyping

Before After

Fooyontphanich *et al.* 2016

E. oleifera Taisha that do not abscise fruit and have different AZ anatomy

Is non-shedding a character in wild populations?

Elaeis oleifera collections (AAU Herbarium)

How has the abscission behavior evolved in the Palm Family?

Palm diversity in Ecuador
32 genera
136 species of palms
20% of all palm species known to the Americas.

http://www.palmbase.org

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Thank you very much