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# KEYNOTE SPEAKER 2

## *Understanding Plant Life: A Perspective From Plant Isoprenoid*

**Dr. Nurul Jadid, M.Sc.**

## Understanding plant life : a perspective from plant isoprenoid

Disampaikan dalam
Seminar Nasional SIMBIOSIS IV
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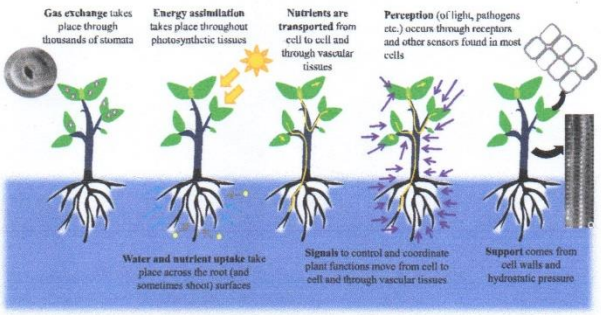
## Plant physiology: Same functions, more broadly distributed

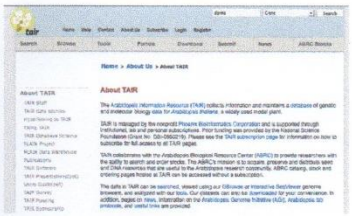



## Model plant : understanding biological system for future perspective on both fundamental science and biotechnology

- Easy to grow
- Small size : no large space, easy maintenace
- Short life cycle : 6-8 weeks
- Lot of seed production (up to 10,000 seeds per plant)
- Self-fertilize ability
- Small genome size : 25,700 genes

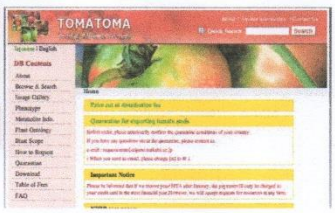
*Arabidopsis thaliana*

## Model plant : understanding biological system for future perspective on both fundamental science and biotechnology



*Arabidopsis thaliana*

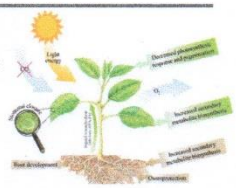
## Model plant : understanding biological system for future perspective on both fundamental science and biotechnology

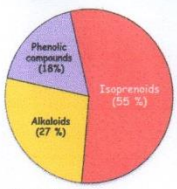



- Easy to grow
- Small size (dwarf) : 10–20 cm height
- Short life cycle : 70-90 d after sowing
- Small genome size : 950 Mb

## Secondary metabolites

- 15-25 % of plant genes encode for proteins and enzymes necessary for the synthesis of secondary metabolites
- Isoprenoids (55%) : > 35,000 compounds






(Croteau et al., 2000)

- *Photosynthesis*
- *Membrane structure*
- *Phytohormones*
- *Pollination attractors & seed dispersal agents*
- *Protein posttranslational modification*
- ...

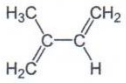
## Isoprenoids/terpenoids

The most diverse groups of natural products


Commercially important

## Terpenoids = isoprenoids : organic compounds produced by plants, which derived from isoprene

- Wide application in industries :
  - Perfumeries
  - Pharmaceuticals
  - Flavouring agents
  - Plant defense compounds
  - Anti-microbial agents




- Many of them are associated with PM :
  - Phytol side chain of chlorophyll
  - Plant hormones
  - Carotenoid pigments

- **The oldest known of biomolecules**
  - Hopanoids (triterpenoid-asscodiated membrane) have been recovered from sediments ≈ 2.5 billion years

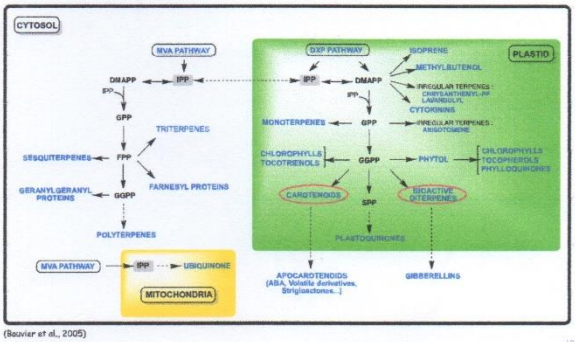
## Terpenoids = isoprenoids

- Build up of $C_5$ isoprene units

| Carbon atoms | Name | Parent isoprenoid |
|---|---|---|
| 10 | Monoterpenoids | GPP |
| 15 | Sesquiterpenoids | FPP |
| 20 | Diterpenoids | GGPP |
| 30 | Triterpenoids | Squalene |
| 40 | Tetraterpenoids | Phytoene |
| > 40 | Polyterpenoids | GGPP + $(C_5)_n$ |

- They are synthesized ubiquitously in eubacteria, archaebacteria, eukaryotes

## Plant Isoprenoids Biosynthesis & Compartmentation




(Bouvier et al., 2005)

**Plant Isoprenoids and food security.....**

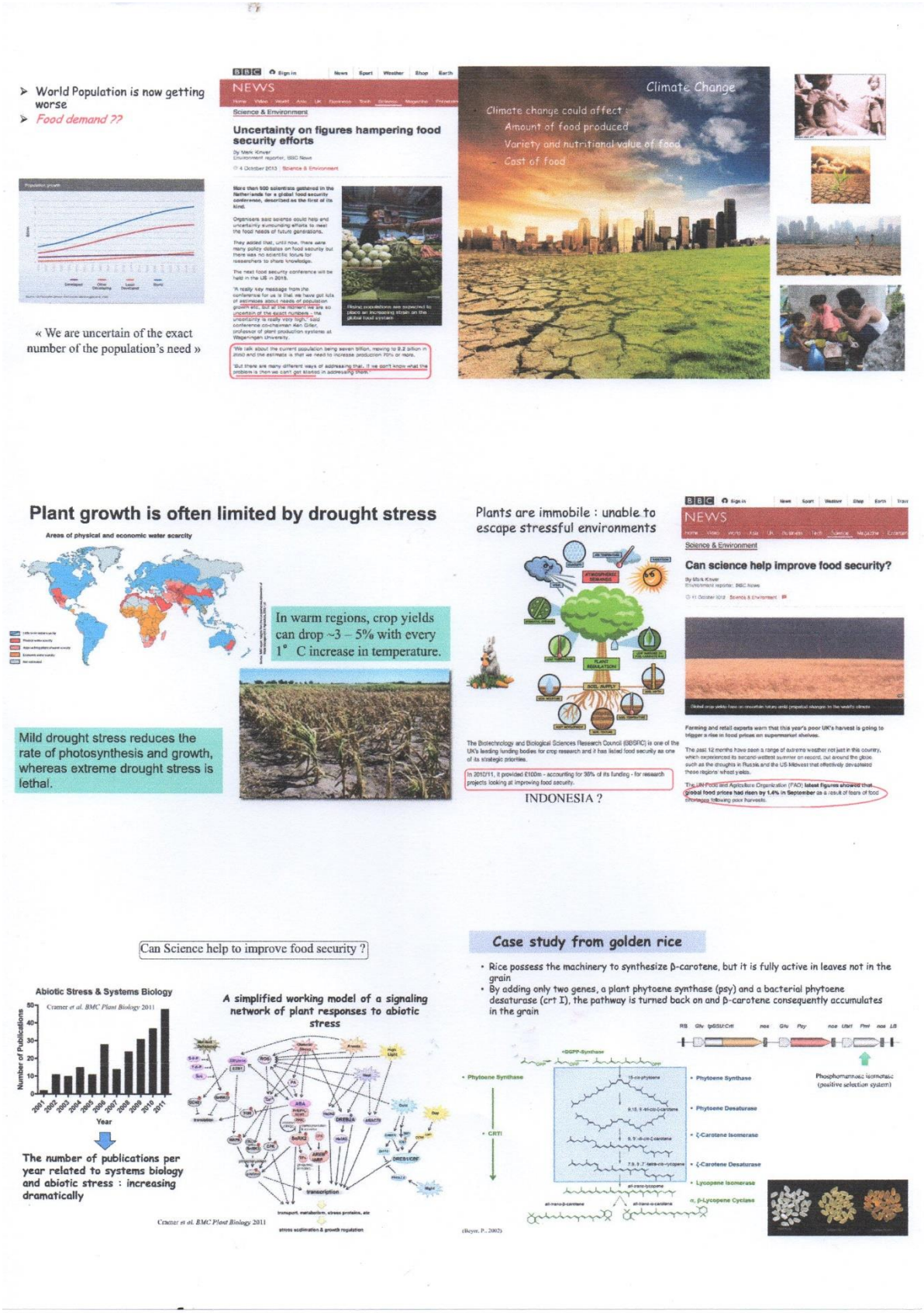
## Food Security ??

1. Food safety : conditions that preserve the quality of food, to prevent contamination
2. **Amount of food** — Number of food ? Variety of food ? Access to food ?

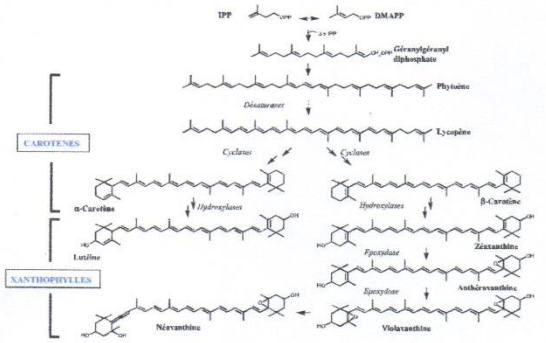
*"all people, at all times, have both physical and economic access to sufficient, safe and nutritious food to maintain a healthy and active life"*

- World Health Organization

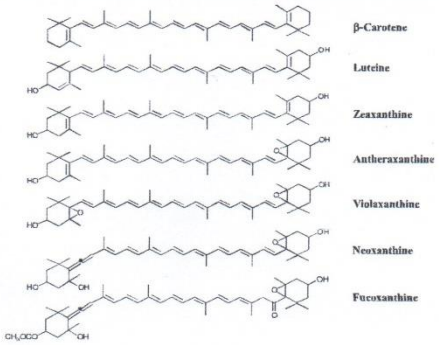
(WHO, 2013)


World Population is now getting worse
Food demand ??
« We are uncertain of the exact number of the population's need »
Uncertainty on figures hampering food security efforts
Climate Change
Climate change could affect :
Amount of food produced
Variety and nutritional value of food
Cost of food
Plant growth is often limited by drought stress
Areas of physical and economic water scarcity
In warm regions, crop yields can drop ~3 – 5% with every 1° C increase in temperature.
Mild drought stress reduces the rate of photosynthesis and growth, whereas extreme drought stress is lethal.
Plants are immobile : unable to escape stressful environments
INDONESIA ?
Can science help improve food security?
Can Science help to improve food security ?
Abiotic Stress & Systems Biology
Cramer et al. BMC Plant Biology 2011
Number of Publications
Year
The number of publications per year related to systems biology and abiotic stress : increasing dramatically
A simplified working model of a signaling network of plant responses to abiotic stress
Case study from golden rice
Rice possess the machinery to synthesize β-carotene, but it is fully active in leaves not in the grain
By adding only two genes, a plant phytoene synthase (psy) and a bacterial phytoene desaturase (crt I), the pathway is turned back on and β-carotene consequently accumulates in the grain
Phytoene Synthase
CRTI
Phytoene Synthase
Phytoene Desaturase
ζ-Carotene Isomerase
ζ-Carotene Desaturase
Lycopene Isomerase
α, β-Lycopene Cyclase
Phosphomannose Isomerase (positive selection system)

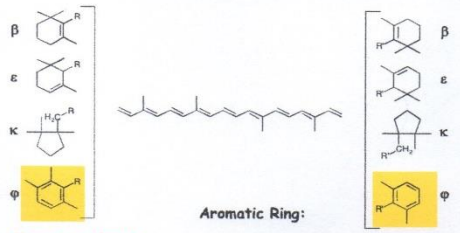
## Lesson from Carotenoid




## Principal Plant Carotenoids



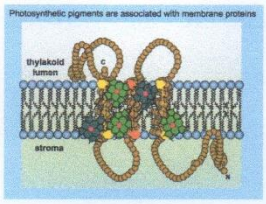

## Cycle rings found in carotenoids




- Absent in plants
- Present in some Cyanobacteria and bacteria
- Aromatic carotenoid is very stable and could integrated in the plastids

(Judid *et al., manuscript in preparation*)

## OBJECTIVES ????




*Isolation of CRTU gene encoding protein involved in the synthesis of aromatic*

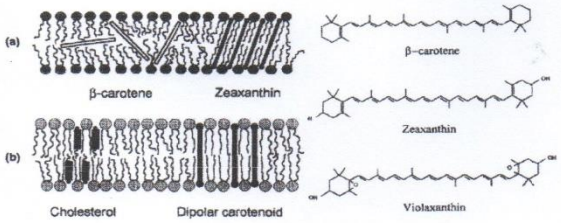
*Integration of CRTU gene in the genome of Arabidopsis thaliana*

The idea : integration of hydroxy-isorenieratene

Increase of chloroplast membran integrity → plants more tolerant to stress ?

(Judid *et al., manuscript in preparation*)

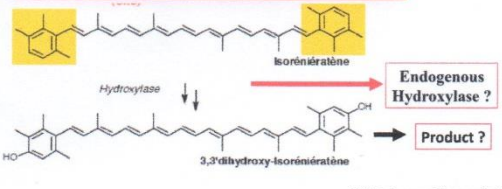
## The importance of carotenoid




Carotenoid = membrane stabilizer

## Gene is functional

Plant Transformation *via* floral dip




(Judid *et al., manuscript in preparation*)

## PERSPECTIVES

CRTU ⇒ Isorenieratene → ? Xanthophylles ?

Endogenous Hydroxylases

- Pigment analysis of 2nd et 3rd generation by: TLC, HPLC, LC/MS/MS
- Localisation of isorenieratene or their derived-molecules in cells?
- Effect of introduction of *CRTU* in plant resistance againts different environmental stress ?

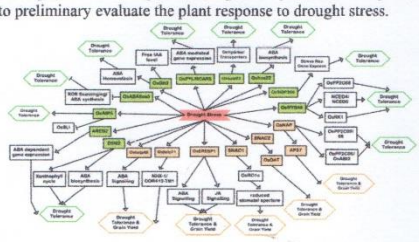
(Jadid *et al., manuscript in preparation*)

**Plant Isoprenoids and plant response to drought stress.....**

## Plant isoprenoids and drought Stress effects

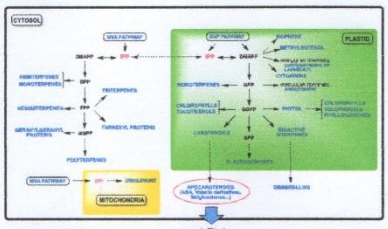
Plant resistance to drought stress involves cellular metabolism, hormone signalling, global transcriptional reprogramming

Profiling the pattern of drought-related gene expression is a promising strategy to preliminary evaluate the plant response to drought stress.



Basu *et al. F1000 Research*, 2016

## Isoprenoids-derived marker for drought stress




ABA

Marakot Jepon Mawar MKY

Tobacco : high economic value
Already become plant model
Marakot : 140 cm height
JP : 130 cm
MKY : 207 cm height

Erma Estiasih

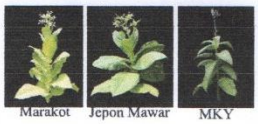
Erlyta Vivi P.

Wahyu

(Jadid *et al.*, 2018)

- Tobacco : « superstar » crop that play central role in both regional and national economic development (Disbun Prov Jatim, 2012).
- Eventhough, tobacco is now developped as a new biofactory for biopesticides, biofuels and also for healing some diseases
- Burley tobacco : a light air-cured tobacco used primarily for cigarette production, was introduced to Indonesia since 1957.

Typically high oil content, low sugar and high nicotine

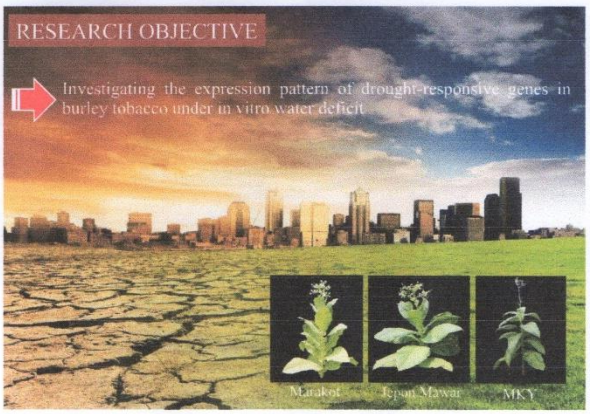


Marakot Jepon Mawar MKY

Tobacco : high economic value
Already become plant model
Marakot : 140 cm height
JP : 130 cm
MKY : 207 cm height

## RESEARCH OBJECTIVE

Investigating the expression pattern of drought-responsive genes in burley tobacco under in vitro water deficit



## METHODS

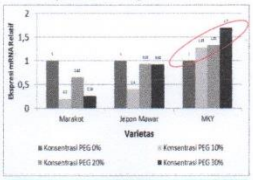
Drought-responsive genes used include :
- *NtLTP1* (AAT45202),
- Nt*ABA2* (*EU123520*),
- *NtERD10B* (AB049336)
- Reference gene *NtEF-1α* (AF120093)

Primers used in this study were designed using PrimerQuest Tool (Integrated DNA Technologies)

| Gen | Primer Forward (5' → 3') | Primer Reverse (5' → 3') |
|---|---|---|
| *NtLTP1* | CATTGTTGGTGGTGGTGTG | TGGAAGGGCTAATCTTGTAGG |
| *NtABA2* | GTGGGATTGGAGGGTTAGTT | GGTCCTCTATATTGCCCTTCTC |
| *NtERD10B* | ACGGACGAATACGGCAATC | TCTCCTTAATCTTCTCCTTCATCC |
| *NtEF-1α* | TGAGATGCACCACGAAGCTC | CCAACATTGTCACCAGGAAGTG |

## Pattern of Nt*ABA2* expression

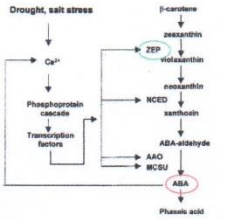
Gene expression analysis of Nt*ABA2*




var. MKY : Nt*ABA2* expression levels increased (almost 2 fold compared to control) following the augmentation of PEG concentrations

Overexpression of *ABA2* enhances drought tolerance (Yue *et al.*, 2012)

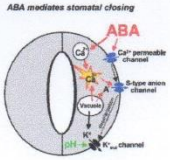
Nt*ABA2* **encodes** Zeaxanthin Epoxidase, converting zeaxanthin into violaxanthin



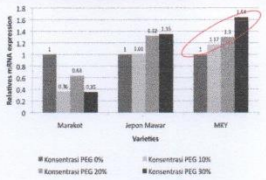

(Jadid *et al.*, 2018)

## Pattern of Nt*ABA2* expression

- Overexpression of *ABA2* >> enhances the production of Abscisic acid (Xiong *et al.*, 2002) → better resistance capacity to drought stress (Yue *et al.*, 2012)
- Roles of ABA during drought stress :
- Maintain water balance through stomatal closure
- ABA also regulates many drought-responsive genes (Singh and Laxmi, 2015)




## Pattern of Nt*ERD10B* expression

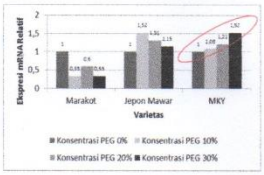



- Nt*ERD10B* expression is PEG-dependent → increased following the concentration of PEG

- *ERD (Early Response to Drought)* : gene family which is induced during drought stress (Alves *et al.*, 2011).
- *ERD10* responsible for the accumulation of LEA (Late Embryogenesis Abundant) which functions to protect other necessary growth protein from degradation
- Overexpression of *ERD10B* enhance plant tolerance against drought stress (Cushman & Bohnert, 2000).

(Jadid *et al.*, 2018)

## Pattern of Nt*LTP1* expression

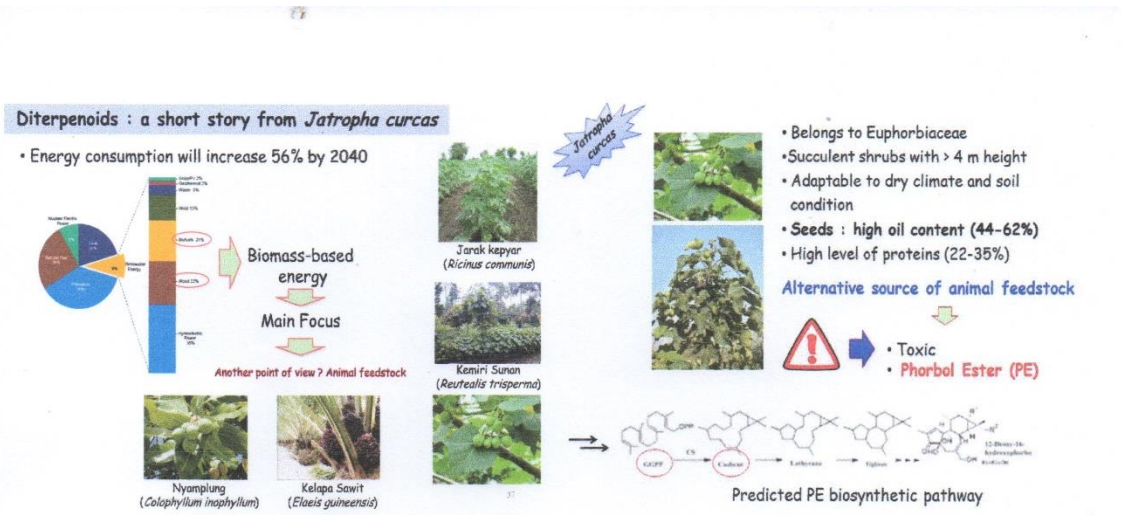


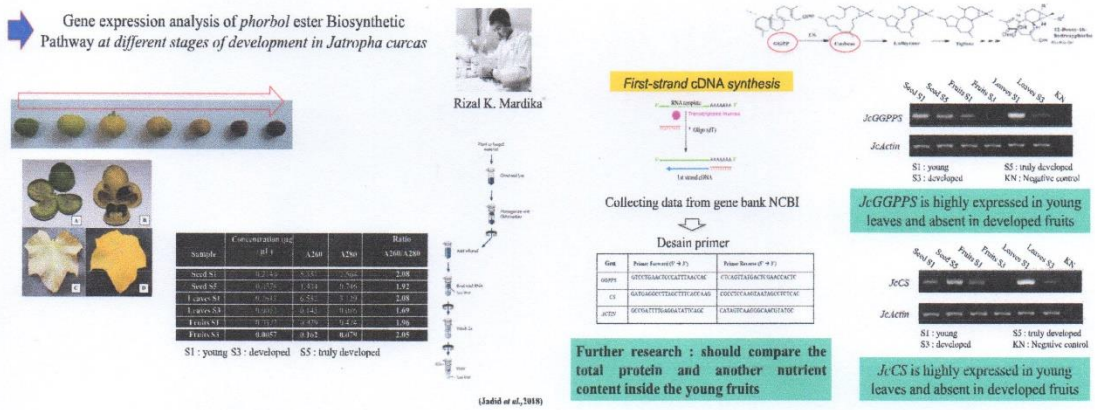

- The expression of *NtLTP1* in var. MKY exhibited similar pattern of expression than the other responsive genes
- Expression of *NtLTP1* in var. Jepon Mawar decreased following augmentation of PEG concentration

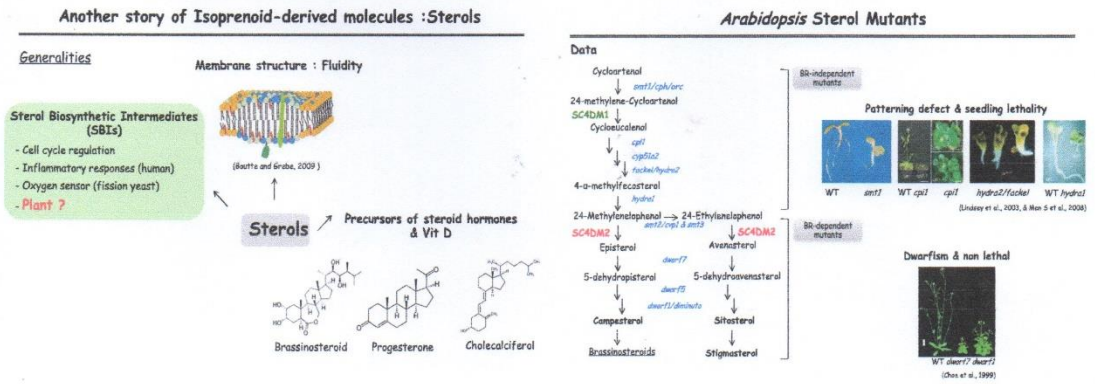
- *NtLTP1* : encodes lipid transfer protein functioning in wax deposition → eliminating water loss risk during drought stress
- Upregulation of this gene confers drought resistance (Cameron *et al.*, 2006).

**Diterpenoids and animal feedstocks.....**

(Jadid *et al.*, 2018)


Diterpenoids : a short story from Jatropha curcas
• Energy consumption will increase 56% by 2040
Biomass-based energy
Main Focus
Another point of view ? Animal feedstock
Jarak kepyar (Ricinus communis)
Kemiri Sunan (Reutealis trisperma)
Nyampiung (Colophyllum inophyllum)
Kelapa Sawit (Elaeis guineensis)
Jatropha curcas
• Belongs to Euphorbiaceae
•Succulent shrubs with > 4 m height
• Adaptable to dry climate and soil condition
• Seeds : high oil content (44-62%)
• High level of proteins (22-35%)
Alternative source of animal feedstock
• Toxic
• Phorbol Ester (PE)
Predicted PE biosynthetic pathway


Gene expression analysis of phorbol ester Biosynthetic Pathway at different stages of development in Jatropha curcas
Rizal K. Mardika
S1 : young S3 : developed S5 : truly developed
First-strand cDNA synthesis
Collecting data from gene bank NCBI
Desain primer
Further research : should compare the total protein and another nutrient content inside the young fruits
JcGGPPS is highly expressed in young leaves and absent in developed fruits
JcCS is highly expressed in young leaves and absent in developed fruits

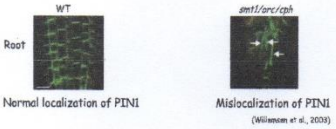

Another story of Isoprenoid-derived molecules :Sterols
Generalities
Membrane structure : Fluidity
Sterol Biosynthetic Intermediates (SBIs)
- Cell cycle regulation
- Inflammatory responses (human)
- Oxygen sensor (fission yeast)
- Plant ?
Sterols
Precursors of steroid hormones & Vit D
Brassinosteroid
Progesterone
Cholecalciferol
Arabidopsis Sterol Mutants
Data
Cycloartenol
24-methylene-Cycloartenol
Cycloeucalenol
4-α-methylfecasterol
24-Methylenelophenol
24-Ethylenelophenol
Episterol
Avenasterol
5-dehydropisterol
5-dehydroavenasterol
Campesterol
Sitosterol
Stigmasterol
Brassinosteroids
BR-independent mutants
BR-dependent mutants
Patterning defect & seedling lethality
Dwarfism & non lethal

## II. Problematic

**BR-independent mutants**

Data

- Membrane structure alteration (changes in regular sterol composition)
- Perturbation of endocytic trafficking of auxin efflux carrier (PIN1) in some BR-independent mutants




- Potential role of unidentified sterol biosynthetic intermediates (SBIs) ?

## Problematic

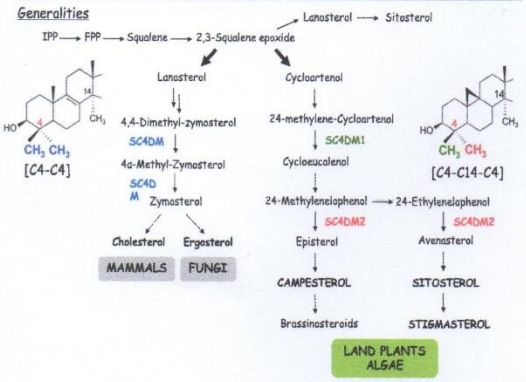
Data

**Role of SBIs in organisms**

C4-methyl SBIs have biological function beyond sterol synthesis

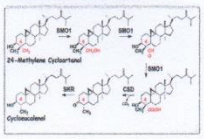
| Organisms | C4-methyl SBIs | Biological roles |
|---|---|---|
| Mammals | MAS (dimethylsterol) | Cell cycle regulation |
| | | Cellular proliferation |
| | | Inflammatory regulation |
| *Schizosaccharomyces pombe* | 4-methyl sterols | Oxygen sensor |
| *Cryptococcus neoformans* | 4-methyl sterols | Oxygen sensor |
| PLANTS | ? | ? |

## Simplified Overview of Sterol Biosynthetic Pathway




## Objective

➢ **Sequential steps in SC4DM**

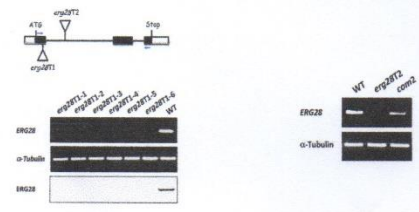



- The sequential steps in SC4DM involve 3 enzymes :
  - **SMO** : Sterol 4α-methyl oxidase (identified)
  - **CSD** : 4α-carboxysterol-C3-dehydrogenase/C4-decarboxylase (identified)
  - **SKR** : Sterone ketoreductase (identified)
  - Unlike in yeast & mammals, plants have 2 SMO involved in 2 SC4DM


- Yeast and mammals ERG28 ≠ an enzyme (characterized)
- Data from the *Arabidopsis* genome : AtERG28 is encoded by 1 gene ➔ no functional redundancy

**The function of AtERG28 in plant development ?**

## Characterization of *Aterg28* T-DNA and Complementation lines

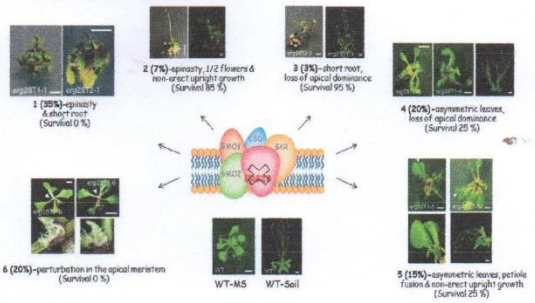



**Expression of *AtERG28* in knockout and complementation lines are confirmed by RT-PCR & western blot**

(Mialoundama, Jadid *et al.*, 2013)

## Pleiotropic phenotypes in *erg28* T-DNA lines

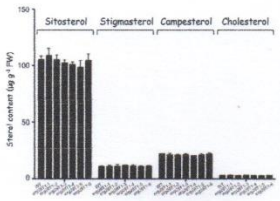
➢ Phenotypic analysis of *erg28* T-DNA lines (35-40 d)




**Loss of function of AtERG28 generates severe pleiotropic development**

(Mialoundama, Jadid *et al.*, 2013)

## Sterol composition in *erg28* T-DNA lines




*erg28*T-DNA lines

**Sterol content is not significantly affected in *erg28*T-DNA lines**

Severe pleiotropic phenotypes in *erg28* T-DNA lines are not caused by sterols deficiency

(Mialoundama, Jadid *et al*, 2013)

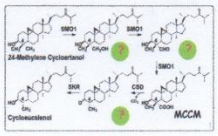
## Conclusion

➢ Problematic : AtERG28 Function in Development ?


- Loss of function of AtERG28 causes severe pleiotropic developmental defects, including :
  - Loss of apical dominance
  - Leaves Epinasty
  - Root inhibition
  - Perturbation of apical meristem
- Sterol composition in A*terg28* T-DNA lines is not affected
- Another pathway responsible for these phenomenon ?
  - Brassinosteroid feeding ?
  - Auxin, cytokinins, GA application ?

## Perspectives

1. Modulating individually of genes involved in the SC4DM complex
2. Identification of other SBIs & their conjugated forms & their biological roles in plant development


**These perspectives will offer an additional challenge to better understand the role of SBIs in plant development**

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