KEYNOTE SPEAKER 2

Understanding Plant Life: A Perspective From Plant Isoprenoid

Dr. Nurul Jadid, M.Sc.
Plant physiology: Some 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 maintenance
- 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

Secondary metabolites

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

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Isoprenoids/terpenoids

The most diverse groups of natural products

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

- Wide application in industries:
  - Perfumery
  - 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 biomoecules
  - Hopanes (triterpenoid-associated membrane) have been recovered from sediments 2.5 billion years

Commerially important

Terpenoids = isoprenoids:

- Build up of C5 isoprene units

<table>
<thead>
<tr>
<th>Carbon atoms</th>
<th>Name</th>
<th>Parent isoprenoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Monoterpenoids</td>
<td>GPP</td>
</tr>
<tr>
<td>15</td>
<td>Sesquiterpenoids</td>
<td>FPP</td>
</tr>
<tr>
<td>20</td>
<td>Diterpenoids</td>
<td>GGPP</td>
</tr>
<tr>
<td>30</td>
<td>Triterpenoids</td>
<td>Squalene</td>
</tr>
<tr>
<td>40</td>
<td>Tetraterpenoids</td>
<td>Phytoene</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>Polytiterpenoids</td>
<td>GGPP + C10</td>
</tr>
</tbody>
</table>

- They are synthesized ubiquitously in eubacteria, archaeabacteria, eukaryotes

Plant Isoprenoids Biosynthesis & Compartmentation

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
Lesson from Carotenoid

Cycle rings found in carotenoids

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

The importance of carotenoid

Gene is functional

Plant Transformation via floral dip

Carotenoid = membrane stabilizer
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.

Isoprenoids-derived marker for drought stress

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

Research Objective

Investigating the expression pattern of drought stress marker genes on barley tobacco model in vitro water stress.
**METHODS**

Drought-responsive genes used include:
- NILTP1 (AAT45202),
- NsAB42 (EU1243209),
- NsERD106 (AB0451636)
- Reference gene NsEF-1a (AF120093)

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Forward (5'→3')</th>
<th>Primer Reverse (5'→3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAB42</td>
<td>TGAAGTAGACGAAGAGAGCACTC</td>
<td>TTCCTGTGTTCTCTCTGTTCCTC</td>
</tr>
<tr>
<td>NsAB42</td>
<td>AGGACATCAAGAGCAGCA</td>
<td>TCTCATGAGAAGCAAGCAGATC</td>
</tr>
<tr>
<td>NILTP1</td>
<td>TGAAGTAGACGAAGAGAGCACTC</td>
<td>TTCCTGTGTTCTCTCTGTTCCTC</td>
</tr>
<tr>
<td>NsEF-1a</td>
<td>TCAAGACTGCAGCAAGGATCTC</td>
<td>GGACCTTACGCAAGGATCTC</td>
</tr>
</tbody>
</table>

**Pattern of NsAB42 expression**

Gene expression analysis of NsAB42

- var. MKC - NsAB42 expression levels increased (almost 2 fold) compared to control following the augmentation of PEG concentrations
- Overexpression of AB42 enhances drought tolerance (Xu et al., 2017)

**Pattern of NsERD106 expression**

- ERD (Early Response to Drought): gene family which is induced during drought stress (Alves et al., 2011)
- ERD106 responsible for the accumulation of LEA (Late Embryogenesis Abundant) which functions to protect other necessary growth protein from degradation
- Overexpression of ERD106 enhances plant tolerance against drought stress (Cushman & Bohnert, 2000)

**Pattern of NILTP1 expression**

- NILTP1 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)

The expression of NILTP1 in var. MKC exhibited similar pattern of expression than the other responsive genes

Expression of NILTP1 in var. Japan Mazur decreased following augmentation of PEG concentration

**Pattern of NsAB42 expression**

Gene expression analysis of NsAB42

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II. Problematic

**Defa**
- Membrane structure alteration (changes in regular sterol composition)
- Perturbation of endocytic trafficking of vacuolar affinity carriers (PINs) in some 8-B1-independent mutants

**Defa**
- Role of SEIs in organisms

**C4-methyl SBIs have biological function beyond sterol synthesis**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>C4-methyl SBIs</th>
<th>Biological roles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammals</td>
<td>MAS (1,6-dihydroxy)</td>
<td>Cell cycle regulation, Cellular proliferation, Inflammatory regulation</td>
</tr>
<tr>
<td>Schizosaccharomyces pombe</td>
<td>4-methyl sterols</td>
<td>Oxygen sensor</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>4-methyl sterols</td>
<td>Oxygen sensor</td>
</tr>
<tr>
<td>PLANTS</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

**Simplified Overview of Sterol Biosynthetic Pathway**

**Objective**
- The sequential steps in 5ΔH4C4 involve 3 enzymes:
  - SDO: 4-C4-sterol 4-dehydrase (identif)
  - CDO: 4-C4-sterol 4-dehydrogenase/C4-desaturase (identif)
  - DOR: 4-methylsterol 4-desaturase (identif)
- Unlike in yeast & mammal, plants have 2 SDO activated in 2 SOX7

**Characterization of Aterg2g8 T-DNA and Complementation lines**

**Plasmodic phenotypes in erg2 g8 T-DNA lines**

Expression of ATERG8 in knockout and complementation lines are confirmed by RT-PCR & western blot

A TERG8 gene encodes severe plasmodiophora development

[Images and text continued...]

xxiv
Sterol composition in *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 sterol deficiency

**Conclusion**

- Loss of function of *ATRB628* causes severe pleiotropic developmental defects, including:
  - Loss of apical dominance
  - Leaved epinasty
  - Root inhibition
  - Perturbation of organ meristems
- Sterol composition in *erg28*-T-DNA lines is not affected
- Another pathway responsible for these phenotypes?
- Smaller sized feeding?
  - Auxin, cytokinin, GA application?

**Perspectives**

1. Modulating individually of genes involved in the SRE16 complex
2. Identification of other SREs and their conjugated forms and their biological roles in plant development

**Acknowledgments**

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- Meliana Chang Wijaya

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

Thank you for your attention