MOLECULAR PHYSIOLOGY STUDIES OF BANANA FRUIT RIPENING
Identification of candidate genes for improvement of fruit quality traits throughout breeding

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Abstract
Banana is a complex species and attempts to improve the fruit quality through breeding strategies are complex and difficult to set up. Identification of the main fruit quality traits and gaining further insight into the molecular mechanisms that govern their elaboration and their regulation are a key prerequisite for the development of strategies to improve banana quality traits like postharvest technology, breeding and/or biotechnology approaches. In this way, access to functional molecular markers derived from relevant genes associated with main fruit quality traits should therefore provide a valuable and helpful resource for the development of these strategies.

In the present study, we report the cloning and sequencing of genes that are differentially expressed during fruit ripening, as tools for functional genomic studies and putative molecular marker developments. Different molecular biology approaches have been used to isolate these ripening related-genes. They include cDNA amplification (RT- and RACE-PCR) and construction of complete and subtractive suppressive cDNA libraries (SSH). Sequencing and BLAST analysis of some of these isolated cDNA clones revealed that 205 of them presented a high homology with different genes in database. Many of the predicted proteins encoded by these genes are putatively involved in the regulation of gene expression, hormonal metabolism, hormonal-signal transduction, sugar metabolism and other ripening process. Among these 205 genes, 11 are still unclassified since presenting homology to unknown proteins of rice or Arabidopsis. Finally, thirteen additional clones were putatively novel, since they failed to match with database sequences.

Furthermore, expression of genes involved in some fruits quality traits was examined in order to identify those of which expression could be correlated with the related physiological processes and thus considered as candidates.

These genes will contribute to increase pools of public EST collections of banana, one of the weakest public EST collection among those of the most consumed fruits. Moreover our data led us to gain more insights into the banana ripening process and to suggest some candidates.

Keywords: Banana, Ethylene, Fruit, Musa, Ripening, finger drop, Gene cloning
MOLECULAR PHYSIOLOGY OF BANANA FRUIT RIPENING AND QUALITY IMPROVEMENT

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INTRODUCTION

● For fruit, quality traits including nutritional and organoleptic aspects can be considered as a source of biodiversity and increase the added value of the product and consequently the incomes of the producers.

● Fruit Quality is a complex criteria (external and internal aspects as color, flavour, texture, aroma etc.) that is highly affected by both biotic and abiotic cues, some of them being antagonistic.
Elaboration of fruit quality traits, mainly set up during ripening, involved number of complex physiological processes resulting from the coercitive action of different genes.

Banana fruit undergoes a ripening climacteric process characterized by a peak of respiration and a burst of an autocatalytic production of ethylene, concomitantly with molecular and biochemical changes leading to fruit ripening (peel degreening, aroma volatiles, sugar accumulation or fruit softening).
OBJECTIVES

● Evaluate the variability of quality traits within banana species

● Investigate the physiological mechanisms that govern the elaboration of these quality traits

● Generate molecular tools for improvement of banana fruit quality throughout conventional breeding
Characterization of banana fruit quality traits

Quality biodiversity
Identification of contrasted varieties

Getting genome resources from banana
Genes - antibodies

Studies of Gene-Function-Phenotype relationship
Identification of candidate genes

Development of molecular markers

Genetic studies
Breeding program
Biotechnology approach
Improvement of banana fruit quality traits
Understanding banana fruit ripening process
A core collection of more than 30 different banana species was constituted and fruits are currently under characterization.

This collection includes cooking and dessert banana and, cultivated, wild types and hybrids cultivars.
RESULTS

Genomic resources

Distribution and frequency of putative functions of cDNA clones isolated from banana fruit:

- Hormonal metabolism: 17%
- Sugar metabolism: 14%
- Stress response gene: 3%
- Other metabolic gene: 11%
- Novel genes: 13%
- Unidentified functions: 9%
- Regulation of gene expression: 33%
Three ripening processes involved in fruit quality traits elaboration are currently under investigation in order to identify the related candidate genes.

- Ethylene fruit responsiveness (ripening initiation process)
- Finger drop phenomenon (commercial value of fruit)
- Sucrose metabolism (sensory properties of fruit)
### RESULTS

**Ripening initiation**

<table>
<thead>
<tr>
<th>Name</th>
<th>Size (pb)</th>
<th>Accession n°</th>
<th>Blast best hits description</th>
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<tbody>
<tr>
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<td>DV270719</td>
<td>MADS-Box transcription factor</td>
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<td>DV270722</td>
<td>IAA-amino acid hydrolase</td>
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<td>AF445195</td>
<td>Ethylene receptor (ERS-like) protein</td>
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<td>pCav45</td>
<td>1676</td>
<td>AF445196</td>
<td></td>
</tr>
<tr>
<td>AB266316</td>
<td>2210</td>
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</tr>
<tr>
<td>MaEIL1</td>
<td>1817</td>
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<td></td>
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<tr>
<td>MaEIL2/AB266318</td>
<td>1841</td>
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<td>EIN3-Like protein</td>
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<tr>
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<td>MaEIL4/AB266320</td>
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<td>AB266321</td>
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<tr>
<td>pCav53</td>
<td>1925</td>
<td>Unregistered</td>
<td>CTR-Like protein</td>
</tr>
</tbody>
</table>

*cDNA isolated from Cavendish fruit and putatively involved in ethylene fruit responsiveness and ripening initiation processes. AB266316 - 20 have been already published in Genbank database*
ERS-like, EIN3 and EIN3-Like (EIL) protein

- ERS are transmembrane protein and EIN3/EIL protein are primary and nuclear transcription factors

- EIN3/EIL protein downstream of the ethylene receptor, redundantly as positive regulators of ethylene signaling

- EIN3/EIL and ERS proteins are encode by a small multigene family

- Regulation EIN3/EIL and ethylene receptor protein gene expression occurs at both mRNA and protein levels
RESULTS

Ripening initiation process

3 days after treatment

6 days after treatment

ppm of acetylene applied at 20°C

DAF: days after flowering
RESULTS

Ripening initiation process

Banana fruit undergoes changes in ethylene responsiveness during green development stage and between 40, 60 and 90 days after flowering.

Ripening conditions: 20°C in air - ambient humidity
Ripening induction: $10^4$ ppm of acetylene at 20°C
Expression of ethylene receptor genes during green development (A, B, C, D) and late ripening (E, F, G, H) stages of Cavendish banana indicated in the x-axis. $\Delta \Delta Ct$ on the y-axis refers to the fold difference in gene expression to fruit taken at stage D (calibrator) and a housekeeping actin gene (reference). A = 30 DAF; B = 40 DAF; C = 60 DAF; D = 90 DAF; E, F, G and H = 90 DAF-fruits taken 1, 3, 6 and 11 days after initiation of ripening by exogenous acetylene treatment (10000ppm/20°C/24h), respectively.

AB266316 considered as candidate for ripening initiation process.
RESULTS

EIN3-Like gene expression

Expression of MaEIL genes during green development (A, B, C, D) and late ripening (E, F, G, H) stages of Cavendish banana indicated in the x-axis. $\Delta \Delta Ct$ on the y-axis refers to the fold difference in gene expression to fruit taken at stage D (calibrator) and a housekeeping actin gene (reference). A = 30 DAF; B = 40 DAF; C = 60 DAF; D = 90 DAF; E, F, G and H = 90 DAF-fruits taken 1, 3, 6 and 11 days after initiation of ripening by exogenous acetylene treatment (10000ppm/20°C/24h), respectively.

MaEIL1, MaEIL3/AB266319 and MaEIL2/AB266318 are putative candidates for ripening initiation process and control of late ripening process, respectively.
RESULTS

Finger drop process – Physical measurement

\[ \text{CZ} = \text{Control Zone} \]
\[ \text{DZ} = \text{Drop Zone} \]

(Mbeguié-A-Mbéguié et al., J. Exp. Bot 60 (7):2021-2034)

Ripening conditions: 20°C in air - ambient humidity
Ripening induction: 10^4 ppm of acetylene at 20°C
### RESULTS

**Finger drop process – Genomic resources**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Number of genes isolated</th>
<th>Function</th>
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</thead>
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<tr>
<td>Pectin methylesterase (PME)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
<td>Pectin degradation</td>
</tr>
<tr>
<td>Polygalacturonase (PG)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>Pectin degradation</td>
</tr>
<tr>
<td>Pectate lyase (PEL)&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Xyloglucan endotransglycosylase hydrolase (XTH)&lt;sup&gt;a,e&lt;/sup&gt;</td>
<td>10</td>
<td>Xyloglucan metabolism</td>
</tr>
<tr>
<td>Expansin&lt;sup&gt;f,g&lt;/sup&gt;</td>
<td>4</td>
<td>Cell wall loosening</td>
</tr>
</tbody>
</table>

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<sup>c</sup> Dominguez-Puigjaner et al., Plant Physiol. 114:1071–1076; <sup>d</sup> Pua et al., Physiol. Plant. 113: 92–99.<br>
<sup>e</sup> Lu et al., Acta Botanica Sinica 46, 355–362; <sup>f</sup> Trivedi and Nath. Plant Science 167, 1351–1358.<br>
<sup>g</sup> Sane et al., Post-harvest Biol. Technol. 45, 184–192.
RESULTS

Finger drop process – MaPME Genes expression

MaPME2 can be considered as candidate

Other candidates:
- MaPG4,
- MaEXP1, 4 and 5
- MaXTH1, 2, 6, 8 and 9
- MaPEL1 and 2
CONCLUSIONS

- Elaboration of quality traits of banana fruit and as probably other tropical fruits implies a complex physiological processes and different genes.

- Therefore improvement of genomique resources quality as well as getting more information on their function on fruit quality traits elaboration remain a challenge.
Investigations are currently undergoing at different levels on the candidate genes identified in this study:

- Getting more information on MaEIL1, 2 and 3 protein.

- Getting more information on the relationship between environmental cues (cultural practices, postharvest technologies on candidate gene expression).

- Exploiting of banana collection available at CIRAD (Guadeloupe, FWI) to examine the relationship between the structural genomique sequences and expression.
TEAMS

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