

# Transcriptional regulation of Banana EIN3-like genes: correlation with ethylene fruit responsiveness and ripening processes

D. Mbégué-A-Mbégué<sup>1\*</sup>, O. Hubert<sup>2,3</sup>, B. Fils-Lycaon<sup>4</sup>, M. Chillet<sup>3,5,6</sup>, F.-C. Baurens<sup>7</sup>

<sup>1</sup>CIRAD, UMR QUALITROP, F-97130 Capesterre-Belle-Eau, Guadeloupe France; <sup>2</sup>CIRAD, UMR QUALISUD, F-97130 Capesterre-Belle-Eau, Guadeloupe, France

<sup>3</sup>CIRAD, UMR QUALISUD, F-34398 Montpellier, France; <sup>4</sup>INRA, UMR QUALITROP, F-97170 Petit-Bourg, Guadeloupe, France; <sup>5</sup>CIRAD, UMR QUALISUD, Sao Paulo, SP, Brazil; <sup>6</sup>Universidade de Sao Paulo, Dpto dos Alimentos e Nutricao Experimental USP/FCF, São Paulo, SP, Brazil; <sup>7</sup>CIRAD, UMR DAP, F-34398 Montpellier, France

\* Corresponding author : [didier.mbeugie-a-mbeugie@cirad.fr](mailto:didier.mbeugie-a-mbeugie@cirad.fr)

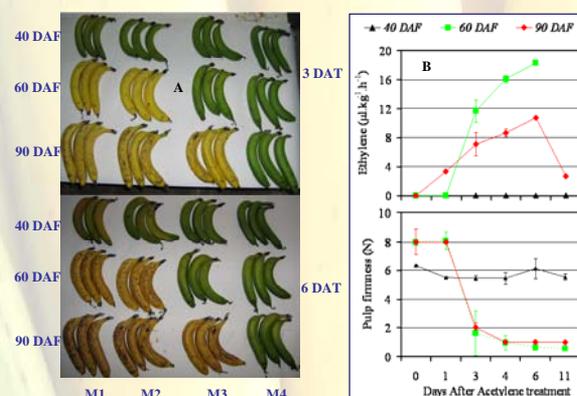


## Introduction and Objectives

- Banana fruit is one of tropical and climacteric fruits harvested and transported in green, treated with ethylene to induce ripening before delivery to the market.
- For such exported fruit, the competence of the fruit to respond to ethylene at harvesting time might be sufficient to avoid "mixed-ripe" after ethylene treatment but not achieved to avoid "ship-ripe" fruit during transit. "mixed-ripe" and "ship-ripe" are two postharvest disorders widely observed in export bananas (*Musa acuminata*, cv Cavendish) channels of French West Indies.
- The involvement of ethylene signal transduction pathway in the competence of the climacteric fruit to respond to ethylene is well studied in tomato but not in banana. In this mechanism, the EIN3 (ethylene insensitive 3) and EIN3-Like (EIL) proteins are the primary trans-acting factors that act as positive regulator of ethylene responses.
- In the prospect of improvement of banana fruit quality throughout conventional breeding and marker assisted selection, getting major or candidate gene(s) putatively involved on ethylene signal transduction pathway is an essential step for identification of related marker (s).
- In order to identify these candidate genes, we examined here expression of five banana EIN3-like genes in relationship with ripening and fruit ethylene responsiveness.

## Methods

- Relationship between *MaEIL* genes expression and ethylene fruit responsiveness was investigated in green fruits harvested between 30 and 90 DAF, a period during which fruit undergoes change on ethylene responsiveness (Fig. 1).
- The level of fruit ethylene sensitivity was estimated by treatment with different acetylene concentrations (see Fig. 1 legend) following by colour (Fig. 1A) or ethylene and firmness (Fig. 1B) evolution during postharvest ripening.
- To obtain late ripening stages, 90 DAF-fruits were subjected to M2 treatment (see Fig. 1 legend), kept in air and picked out one, 3, 6 and 11 DAT (days after ripening induction).



- Relationship between *MaEIL* genes expression and ethylene was investigated in 90 DAF-fruit taken at the end of different "T" treatments (see Fig. 3 legend).
- RACE-PCR and degenerated primers were used to isolate partial EIN3-Like cDNA (*MaEILs*) from banana and sequence comparison was performed using BLAST program.
- Real-time quantitative PCR was performed with each *MaEILs* specific primers for gene expression analysis.

Fig. 1. Evaluation of ethylene sensitivity of banana fruits harvested at different green development stages throughout colour evolution (panel A) and ethylene and firmness evolution during postharvest ripening (panel B) of fruit subjected to M2 treatment. M1 = 10<sup>0</sup> ppm of acetylene/20°C/24h; M2 = 10<sup>1</sup> ppm of acetylene/20°C/24h; M3 = 10<sup>2</sup> ppm of acetylene/20°C/24h; M4 = 10<sup>3</sup> ppm of acetylene/20°C/24h.

## Results and Discussions

	AB266318 (2251 bp)	AB266319 (2613 bp)	AB266320 (2439 bp)	AB266321 (2313 bp)
<b>A</b>				
<i>MaEIL1</i> (1817 bp)	77%	78%	76%	74%
<i>MaEIL2</i> (1841 bp)	<b>98%</b>	70%	74%	70%
<i>MaEIL3</i> (1924 bp)	70%	<b>99%</b>	84%	73%
<i>MaEIL4</i> (2448 bp)	74%	84%	<b>99%</b>	71%
<b>B</b>				
<i>MaEIL1</i> (495 aa)	69%	68%	67%	64%
<i>MaEIL2</i> (517 aa)	<b>96%</b>	61%	61%	60%
<i>MaEIL3</i> (517 aa)	61%	<b>99%</b>	76%	65%
<i>MaEIL4</i> (635 aa)	59%	84%	<b>92%</b>	63%

Table 1. Comparison between nucleotide (A) and amino acid (B) sequences of *MaEIL* genes isolated in this study and those already published in the Genbank data base under accession number AB266318, AB266319, AB266320, AB266321.11

**Five EIN3-like genes identified in Cavendish Banana: *MaEIL1*, *MaEIL2/AB266318*, *MaEIL3/AB266319*, *MaEIL4/AB266321*, and *AB266318***

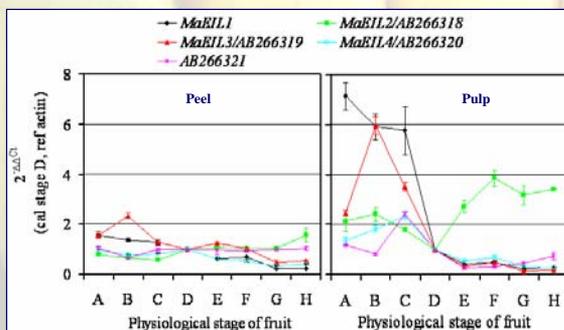


Fig 2. Expression of *MaEIL* genes during green development (A, B, C, D) and late ripening (E, F, G, H) stages of Cavendish banana indicated in the x-axis.  $\Delta\Delta C_t$  on the y-axis refers to the fold difference in gene expression to fruit taken at stage D (calibrator) and a housekeeping actin 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, respectively.

- MaEIL1* and *MaEIL4/AB266320* were an ethylene down-regulated genes in both peel and pulp tissues.
- MaEIL2/AB266318* was an ethylene-induced gene in both peel and pulp tissues.
- MaEIL3/AB266319* expression was ethylene-independent in peel tissue and down-regulated by ethylene together with other development cues in pulp tissue.
- In banana fruit, *MaEIL* genes are transcriptionally regulated by ethylene in different manner and according to the tissue.**

- A high expression of *MaEIL* genes observed in pulp tissue, *MaEIL1* and *MaEIL3/AB266319* being the most expressed.
- A **6-fold** decrease of *MaEIL1* and *MaEIL3/AB266319* mRNA level observed in pulp tissue of green fruit harvested between 60 DAF and 90 DAF, and 40 and 90 DAF, respectively.
- MaEIL2/AB266318* was the unique ripening-induced gene in pulp tissue
- No marked change on *MaEIL4/AB266320* and *AB266321* mRNA levels was observed in both peel and pulp tissues.
- MaEIL1* and *3* genes can be considered as candidates for ripening initiation process of banana**
- MaEIL2* can be considered as a putative candidate for the control of the late ripening process of banana.**

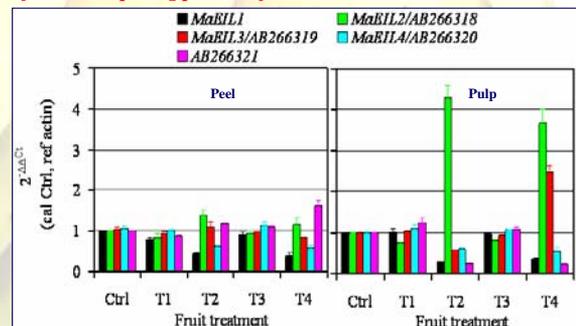


Fig 3. Expression of *MaEILs* genes in 90 DAF-fruits at the end of "T" treatments indicated in the x-axis.  $\Delta\Delta C_t$  on the y-axis refers to the fold difference in gene expression to control fruit (calibrator) and a housekeeping actin gene (reference). Ctrl: 48h/air (control fruits); T1: 1-MCP (0.5ppm)/24h + 24 h/air; T2: acetylene (104ppm)/24h + 24 h/air; T3: 1-MCP (0.5ppm)/24h + acetylene (104ppm)/24h; T4: acetylene (104ppm)/24h + 1-MCP (0.5ppm)/24h