

Cloning and differential expression of banana genes encoding for EIN3-Like proteins involved in ethylene mode of action

Mbégué-A-Mbégué D.^{1*}, Hubert O.², Chillet M.², Fils-Lycaon B.³

¹CIRAD-FLHOR, UMR 1270 QUALITROP, Station de Neufchâteau, Sainte-Marie, 97130 Capesterre-Belle-Eau, Guadeloupe, French West Indies,

²CIRAD-FLHOR, UR 24 TROPICAL, Station de Neufchâteau, Sainte-Marie, 97130 Capesterre-Belle-Eau, Guadeloupe, French West Indies,

³INRA-CEPIA, UMR 1270 QUALITROP, Domaine de Duclos, Prise d'Eau, 97170 Petit-Bourg, Guadeloupe, French West Indies,

*Corresponding Author, mbeguie@cirad.fr

1. Introduction

Export bananas (*Musa acuminata*, cv Cavendish) of French West Indies are usually subjected to the “mixed-ripe” and/or “ship-ripe” processes during transit with a rapid deterioration of their quality. We hypothesized this post harvest process as associated to the level of fruit ethylene responsiveness at harvest time. We attempted to get more insight at molecular level, into the related mechanism in order to identify the related candidate genes usable as marker to improve post harvest quality of fruit.

Here we report the isolation of four banana cDNAs (MaEIL1-4) encoding for EIN3-Like (EIL) protein, the primary trans-acting factor that regulates positively the multiple ethylene responses (Tieman et al., 2001). Gene expression analysis of MAEIL3 and 4 shows these genes displaying a different expression pattern during fruit development, ripening, and during leaf development and after wounding.

2. Material and Methods

Materials used in this study were: fruits harvested at immature green (iMG), early mature green (eMG), late mature green (IMG), turning (Tr), Yellow (Yw) and Yellow Tiger (Yt); others banana tissues, flower (Fw), bract (Bc), root (Rt), intact roll leaf (iRL), wound roll leaf (WRL producing $15 \mu\text{l.kg}^{-1}.\text{h}^{-1}$ of C_2H_4), intact unroll leaf (iURL) and wound unroll leaf (WURL producing $3 \mu\text{l.kg}^{-1}.\text{h}^{-1}$ of C_2H_4) (fig. 1).

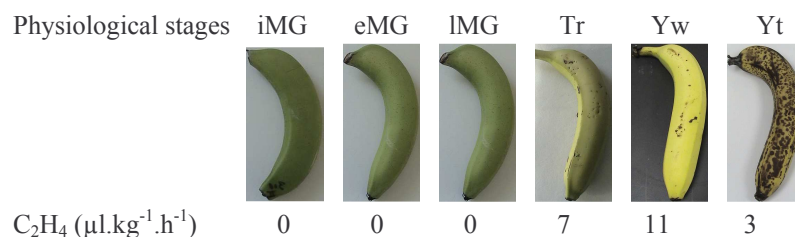


Fig. 1: Fruits that were used in these studies

MaEIL cDNAs were isolated from fruit peel and pulp tissues using both 5'- and 3'-RACE-PCR, and degenerate primers designed from conserved region of EIL-polypeptide sequences. For gene-expression analysis, the Real-time PCR was performed using SYBR Green PCR master mix (Applied Biosystem, France), total cDNA 10-fold diluted, and specific primers designed within the 3'UTR of each MaEIL cDNA and actin (internal standard).

3. Results and Discussion

3.1. MaEIL cDNAs sequences analysis

The partial MaEIL cDNAs, namely MaEIL1 (1817bp), 2 (1840bp) and 3 (1920 bp) encode for a protein of 495, 517 and 517 amino acids, respectively. These clones are 51 to 70 % and 35 to 70 % identical at nucleotide level and at polypeptide level, respectively. The full length cDNA (MaEIL4) is 2435 bp long and encodes for a protein of 635 amino acids. It is 40 to 70 % and 32 to 75 % identical to the three others at nucleotide level and protein level, respectively. The phylogenetic tree analysis shows that

MaEIL1, 3 and 4 belong to the same cluster; however; they constitute a quite different subgroup. MaEIL2 appears to be the most distantly related to this cluster.

3.2. MaEil3 and 4 gene expression in fruit and in other banana tissues and effect of wounding ethylene in banana leaf

MaEIL3 mRNA level decreased in both peel and pulp tissues since the iMG stage to late ripening stages (fig 3A and B). This decrease is more important in pulp that in peel tissue. MAEIL4 mRNA level increased in both peel and pulp tissues during the early developmental stages (iMG, eMG and IMG) and decreased during the late ripening stage (Tr, Yw and Yt). As for MaEIL3, this decrease is more important in pulp that in peel tissue. In pulp tissue, both MaEIL3 and MaEIL4 mRNA level decreased drastically at turning stage (Tr), the beginning of ripening.

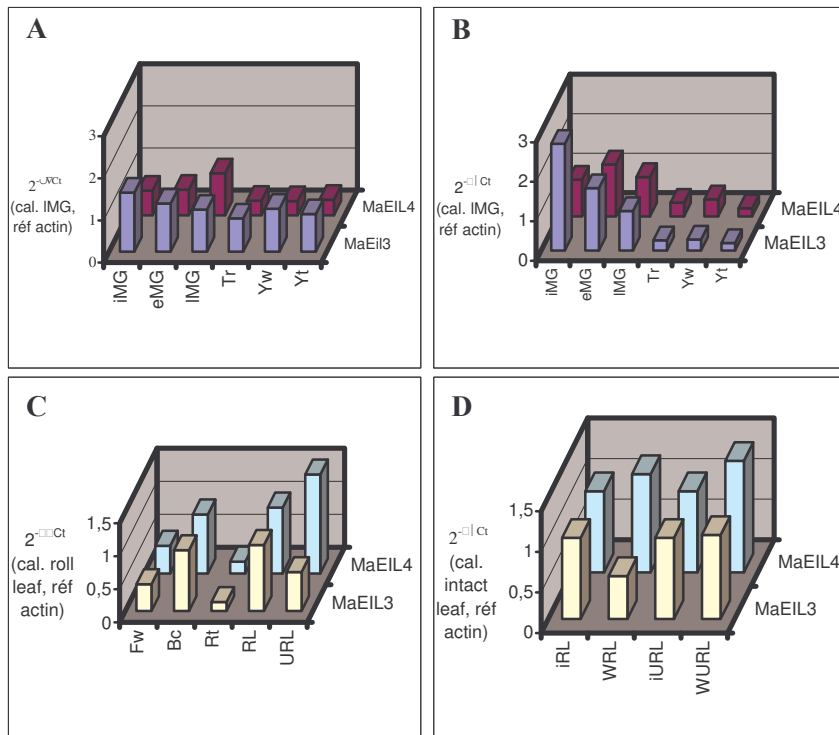


Fig. 3: MaEIL3 and 4 gene expression during ripening of banana in peel (A) and Pulp (B) tissues and in other tissues (C) and effect of wounding ethylene in young (roll) and old (unroll) banana leaves (D).

Both MaEIL3 and MaEIL4 mRNA are detected at different level in other banana tissues including flower, bract, and roots, young and old leaves. From the young to the old leaf, the MaEil3 mRNA level was reduced by half while that of MaEil4 was 2-fold increased (fig 3C).

Wounding reduced by half the MaEIL3 mRNA level in roll (young) leaf but have no effect in unroll leaf. For MaEIL4, wounding induced the mRNA whatever the developmental (fig 3D).

4. Conclusions

- MaEIL3 and 4 genes expression are differentially regulated in peel and pulp tissues fruit by development and ripening, and in leaf by development and wounding.
- The drastically decrease of both MaEIL3 and 4 mRNA level at the beginning of ripening (turning stage) rises the question on their putative role during the late ripening stage.
- Pattern of both MaEIL3 and MaEil4 genes expression are negatively correlated with ripening ethylene production. It should be interesting to assess the regulation of expression of these genes by exogenous ethylene on fruit.

5. References

- Adams-Phillips L, Barry C, Giovannoni J (2004). Signal transduction systems regulating fruit ripening. *Trends Plant Sci* 9(7):331-8.
- Tieman DM, Ciardi JA, Taylor MG, Klee HJ (2001). Members of the tomato LeEil (EIN3-like) gene family are functionally redundant and regulate ethylene responses throughout plant development. *Plant Cell* 13(1):47-58.