

MOLECULAR PHYSIOLOGY OF BANANA FRUIT RIPENING: Improvement of fruit quality

Didier MBEGUIE-A-MBEGUIE

CIRAD, Mixt Unit Research 94 QUALITROP, Guadeloupe, FWI





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 involved number of complex physiological processes resulting from the coercitive action of different genes

ABSTRACT

It is now assumed that the quality of fruit is a complex trait in which a lot of mechanisms are involved, some of them being antagonistic. All of these mechanisms are the results the coercive action of differentially regulated genes. Understanding at molecular level of the mechanisms that control the target quality trait is an essential work beforehand to any plan of improvement of fruit quality trait using plant genetic strategies such as candidate-gene approach and or marker assisted selection. Presently we investigate at molecular level the ethylene responsiveness, and sugar and phenylpropanoid metabolisms, three ripening aspects involved in functional, nutritional and organoleptic qualities of banana fruit. As a first part of this project, we report here 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 ripening 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. Expressions of a few of the matching clones have been followed in relation with ethylene responsiveness of fruit. Our results show that the expression of these genes is under ethylene and development (or both) control. These cDNA clones provide us with a basis for future work that will combine physiological, genomic and genetic approaches to identify key candidate-genes involved in the expression of banana quality trait.

Keywords: Banana, Ethylene, Fruit, Musa, Ripening, gene cloning



INTRODUCTION



- As other fruits, the main quality traits of banana are set up during an important phase of its development namely ripening
- Banana fruit undergoes a ripening climacteric process characterized by a peak of respiration and a burst of an autocatalytic production of ethylene
- Concomitantly, there are molecular and biochemical changes leading to fruit ripening. Some of these changes are ethylene-dependant (peel degreening, aroma volatiles), ethylene-independent (sugar accumulation) or both (fruit softening).



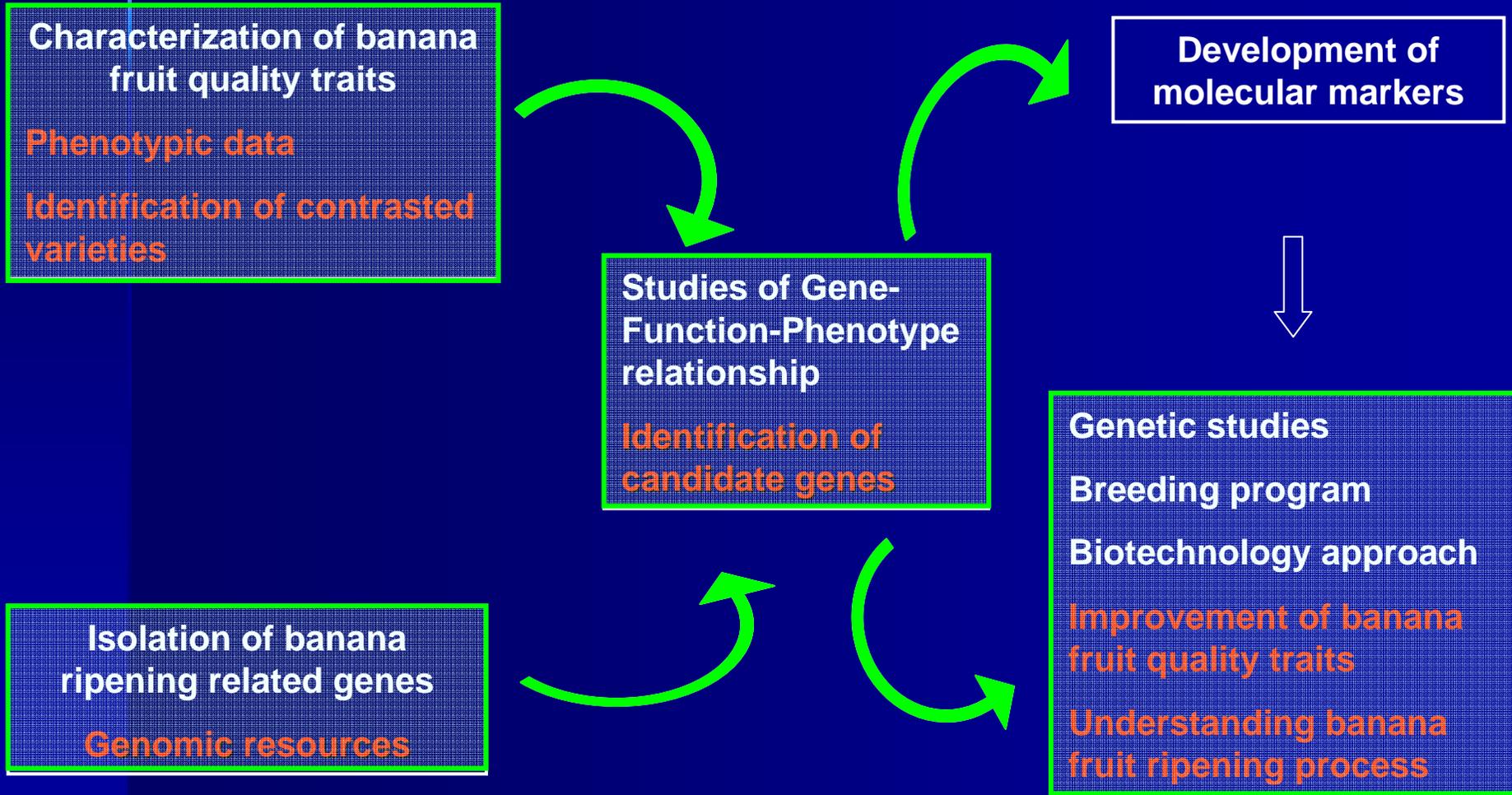
OBJECTIVES



- Get more insights into the variability of quality traits within the banana fruit species and thus identifying the contrasted varieties for functional genomic studies
- Investigate, throughout functional genomic, the physiological mechanisms that govern the elaboration of these quality traits and identifying the related- candidate genes
- Generate, from these candidates genes, the molecular markers usable as tools to improve banana fruit quality traits throughout conventional breeding, agricultural practices, post-harvested technology



STRATEGY





PRELIMINARY RESULTS

Characterization of banana quality traits



- 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



THE CORE COLLECTION



Diploids :

Fehi {
- Aata (2x)
- Asupina (3x)

AAw {
- Calcutta 4
- Cici (Brésil)
- Pa Rayong

BBw {
- Pisang Batu
- Pisang Klutuk Wulung

AAcv {
- Galéo
- IDN 110
- Kirun
- Sowmuk
...

Triploids:

AAAcv {
- Figue Rose
- Mossi
- N'Genge
- Yangambi Km5
- Cavendish

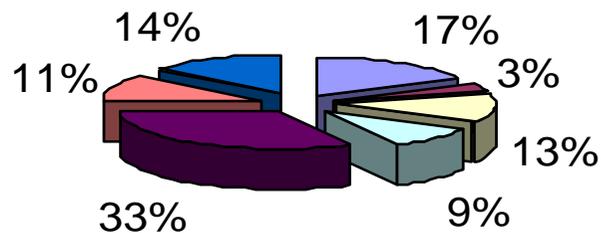
AABcv {
- French Clair
- Kingala 1
- Popoulou

ABBcv - Poteau Géant

AAAcy {
- Hybride 3A Guest
- Hybride 8F Guest
- Hybride 9D Guest

PRELIMINARY RESULTS

Genomic ressources



- Regualtion of gene expression
- Hormonal metabolism
- Stress response genes
- Sugar metabolism
- Other matobolic genes
- Unidenfied functions
- Novel genes

Material:

Peel and pulp of Cavendish
Different ripening stages

Methods for Genes isolation:

RT-, RACE-PCR, EST,
Construction of SSH and total
cDNA libraries

Methods for genes analysis:

Online BLAST program

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



PRELIMINARY RESULTS

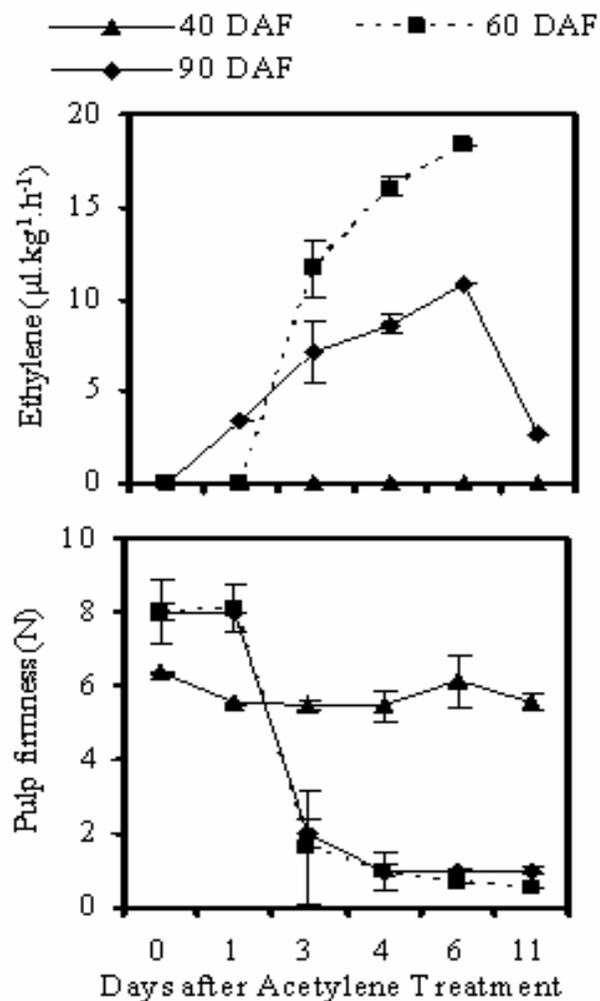
Gene-function studies



- Three mechanisms are currently under investigation:
 - Ethylene fruit responsiveness (ripening initiation process)
 - Sucrose metabolism
 - Fruit drop
 - Polyphenol metabolism

PRELIMINARY RESULTS

Ethylene fruit responsiveness



Legend: Physico-chemical characterization of Cavendish banana fruit in relationship with ethylene fruit responsiveness

Banana fruit were harvested at 40, 60 and 90 DAF (days after flowering) according to the Heat units concept (Ganry and Mayer, 1972). Fruit ripening was then induced by acetylene treatment (10000ppm/24/20°C). Ripening process was monitored throughout ethylene and pulp firmness measurement.

In Cavendish banana, fruit ethylene responsiveness undergoes changes between 40 and 60 DAF, and increased until 90 DAF (commercial harvesting time)



PRELIMINARY RESULTS

Genomic ressources



Sequence ID	Size	E-value	Accession n°	Blast Best Hit Description
SSH2e08	224	1,00E-18	DV270719	MADS-box transcription factor
SSH3e03	497	2,00E-28	DV270722	IAA-amino acid hydrolase
pCav22	765	171 1e-41	AF414128	calmodulin-like protein
pCav44	1293	654 0.0	AF445195	ethylene receptor-like protein
pCav45	1676	897 0.0	AF445196	
pCAV46	1800	585 E-159	DQ682615	EIN3-Like protein
pCAV47	1841	458 E-127	DQ682616	
pCav48	1920	602 E-171	DQ682617	
pCAV49	2440	690 0.0	DQ682618	
pCAV53	1925	0.0	Unregistered	CTR-Like protein

Legend: A part of cDNA isolated from Cavendish fruit and putatively involved in ethylene fruit responsiveness and ripening initiation processes

PRELIMINARY RESULTS

EIN3-Like genes expression - Ethylene fruit responsiveness

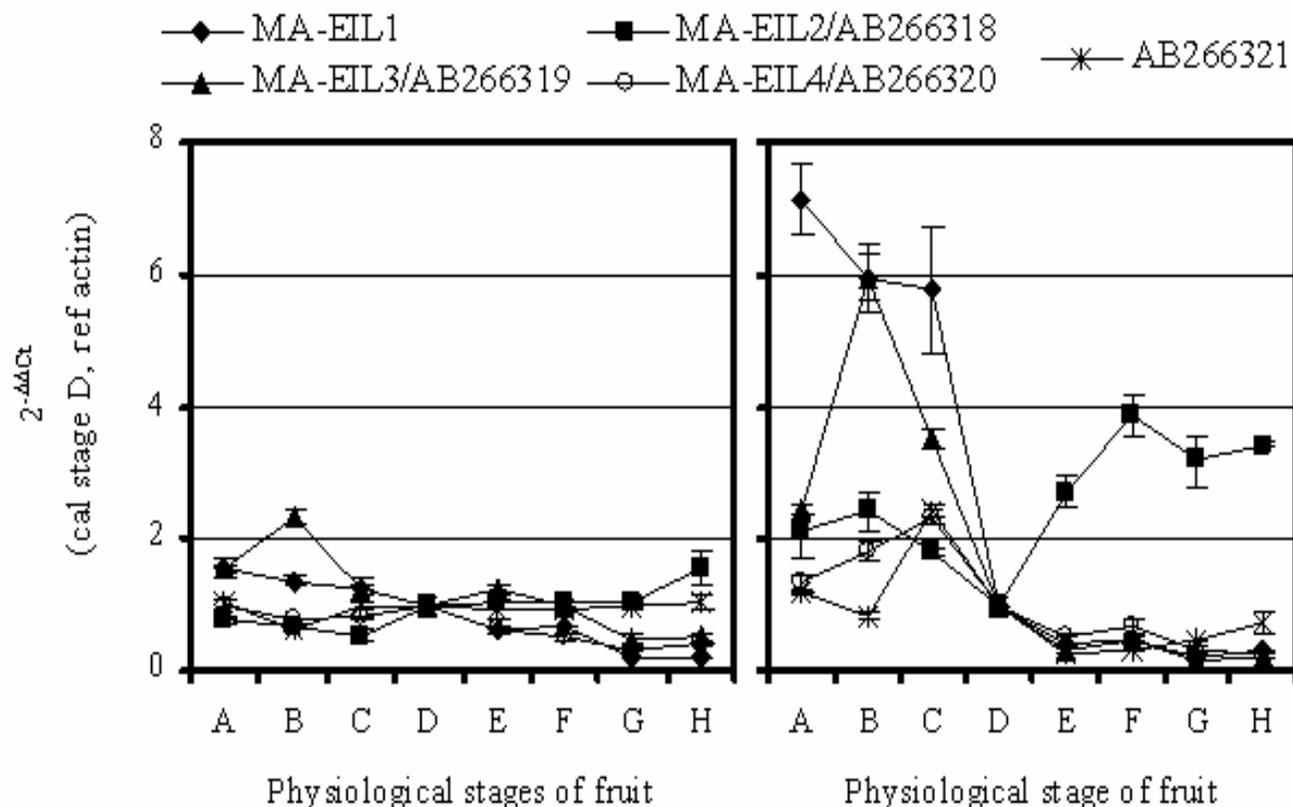


- EIN3 and EIN3-like (EIL) protein:
 - Key transcriptional factor of ethylene signalling
 - Function downstream of the ethylene receptor
 - Functionally redundant as positive regulators of multiple ethylene response
 - Protein encode by a small multi gene family from which mem
 - EIN3-Like genes were isolated and characterized from different plant species
 - Gene expression regulated at both transcriptionnal and traductionnal level

- Five EIL genes have been isolated from banana fruit but uncharacterized

PRELIMINARY RESULTS

EIN3-Like genes expression - Ethylene fruit responsiveness



- A: *immature green I (30 DAF)*
- B: *immature green II (40 DAF)*
- C: *Early mature green (60 DAF)*
- D: *Late mature green (90 DAF)*
- E: *90 DAF + 1 DAT (days after acetylene treatment)*
- F: *90 DAF + 3DAT*
- G: *90 DAF + 6 DAT*
- H: *90 DAF + 11 DAT*

Expression of banana EIN3-Like gene during ripening of Cavendish fruit by Real-Time quantitative RT-PCR. The relative fold difference of mRNA level and was calculated using the $2^{-\Delta\Delta C_t}$ formula (Livak and Schmittgen, 2001) with actin as reference and stage D as calibrator.

PRELIMINARY RESULTS

EIN3-Like genes expression - Ethylene fruit responsiveness



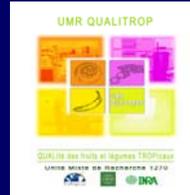
- Conclusions:

- Banana EIL genes are transcriptionnally regulated by fruit development and ripening
- There is drastic change on mRNA level of two EIL members (MA-EIL1 and MA-EIL3) between 40, 60 and 90 DAF concomitantly with changes of ethylene responsiveness of fruit
- Only one EIL member (MA-EIL2) was up-regulated by ripening

MA-EIL1, 2 and 3 appear as putative candidates for ethylene fruit responsiveness process and control of the late ripening stages of banana fruit

PRELIMINARY RESULTS

EIN3-Like genes expression - Ethylene fruit responsiveness



- Prospects:
 - Getting more informations on accumulation of MA-EIL1, 2 and 3 protein
 - Assessing the in vivo role of MA-EIL1, 2 and 3 gene throughout biotechnology approach and/or genetic studies



TEAM - COLLABORATIONS



INRA/CIRAD UMR 94 QUALITROP

C. GALAS

P. JULIANNUS

B. FILS-LYCAON

D. MBEGUIE-A-MBEGUIE

D. RINALDO

CIRAD UMR 95 QUALISUD

O. HUBERT

M. CHILLET

C. BUGAUD

CIRAD UMR 96 DAP

F.C. BAURENS

CIRAD UR 75 VEGETATIVELY PROPAGATED CROPS

I. HIPPOLYTE

C. JENNY