

# Expression analysis of a set of genes related to the ripening of bananas and mangoes

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**Abbreviations:** cDNA-AFLP, cDNA-Amplified Fragment Length Polymorphism; ACC, Amino Cyclo Carboxylic Acid; ACO1, Amino Cyclo Carboxylic Acid Oxidase 1; ACS1 Amino Cyclo Carboxylic Acid Synthase 1; ACS2, Amino Cyclo Carboxylic Acid Synthase 2; ACS3, Amino Cyclo Carboxylic Acid Synthase 3; ACT,  $\beta$ -Actin; AMY, Alpha-amylase; AOX, Ascorbate oxidase; APX, Ascorbate peroxidase; BGAL, Beta-galactosidase; CHS1, Chalcone synthase 1; CHS2, Chalcone synthase 2; EXP, Expansin; FFT, Fructan fructan 1-fructosyltransferase; FGT, UDP glucose: flavonoid 3-O-glucosyltransferase; PG, Polygalacturonase; PME, Pectinmethylesterase; PHO, Alpha 1,4-glucan phosphorylase; R1, Starch debranching enzyme;

## ABSTRACT

During ripening many important physico-chemical changes contribute to fruit quality, and they are precisely determined by gene expression. Specific genes are essential to normal ripening; however, information on gene expression about the majority of tropical fruit, such as bananas and mangoes is limited. In this way, the present study was undertaken with the objective to provide preliminary access to the changes in expression of some genes potentially relevant to banana and mango ripening. To simultaneously evaluate the changes in gene expression, a small collection of genes related to ethylene biosynthesis, starch mobilization, cell wall disassembly, pigment synthesis and ascorbate metabolism was assembled in nylon membranes and probed with cDNA from unripe and ripe fruit. Some interesting differences were observed between gene expression in bananas and mangoes. In relation to starch metabolism, banana  $\alpha$ -amylase was induced during ripening while phosphorylase was more induced in mangoes. Similarly, expression of cell wall-related genes for polygalacturonase and expansin were also different in those fruits. Fructanfructosyltransferase, chalcone synthase, and ascorbate oxidase genes were also induced in ripening mangoes, but not in bananas. Although the number of sequences involved was relatively small, this simple and feasible approach provided interesting preliminary data that can be starting points for more in depth studies.

**Key words:** Fruit ripening; reverse northern blotting.

## INTRODUCTION

Fruit ripening is complex phenomenon with drastic changes in chemical composition, which enhance important quality attribute such as flavor, softness, sweetness and color (Fraser

et al., 2007). Besides sensorial characteristics, the amounts of compounds with biological activity, as is the case of vitamins, is also affected during ripening, contributing to improve the nutritional value of fruit (Davuluri et al., 2005). These changes

are very coordinated and involve many biochemical steps, which are upon a stringent and integrated control played by plant hormones and gene expression, including epigenetic mechanisms (Speirs and Brady 1991, Seymour et al., 1996, Seymour et al., 2008).

The expression of specific genes is essential to normal ripening and the *de novo* synthesis of enzymes involved in the autocatalytic ethylene burst during climacteric fruit ripening is a classical example (Alba et al., 2005). However, several other metabolic pathway are dependent on the modulation by gene expression, and some genes of enzymes putatively controlling the most important physiological changes associated to fruit ripening have been pointed. Based on the studies on tomatoes, there is already some understanding about the genes involved in the most important physiological changes, such as the accumulation of soluble sugars, cell wall disassembly, and synthesis of pigments during fruit ripening (Alba et al., 2005, Bennett et al., 2008, Cara et al., 2008, Mounet et al., 2009). In this way, the identification of genes induced during ripening could provide important clues about the activation of biochemical steps and its relationship to fruit quality.

Additionally, important information on some potentially relevant sites for genetic manipulation would be provided. In this way, there is interest on the application of some non-targeted approaches to study fruit ripening, from the low-budget library screening, differential and cDNA-AFLP techniques to the more sophisticated and high throughput microarray (Jiang et al., 2000, Kuhn, 2000). Most of the available information on gene expression during ripening comes from the model fruit tomato (Giovannoni, 2001; Moore et al., 2002), and much less is known about some relevant tropical fruit, as is the case of bananas (*Musa acuminata* Colla) and mangoes (*Mangifera indica* L.). In tomato the high throughput analysis revealed a dynamic and highly coordinated change in expression of several transcription factors and regulatory elements putatively implicated in the control of fruit ripening (Alba et al., 2005, Mounet et al., 2009).

In spite of the information on those fruit is very limited, important data can derive from the use of heterologous gene sequences in studies of gene expression during fruit ripening. The large

amount of sequence data provided by genome sequencing projects or even by other independent laboratories can be used in the screening of prospective candidates for more in depth, specie-specific studies on gene expression during the ripening of not well known fruit. In this way, the present study was undertaken to provide preliminary access to the changes in expression of some genes potentially relevant to banana and mango ripening. In order to simultaneously evaluate the changes in gene expression during ripening of those fruits, a small collection of genes was assembled in nylon membranes and probed with cDNA from unripe and ripe fruit. The sequences were selected among those that would be more related to the biochemical pathways potentially relevant to the fruit quality, namely those involved in ethylene biosynthesis, starch mobilization, cell wall disassembly, pigment synthesis and ascorbate metabolism.

## MATERIAL AND METHODS

**Plant material:** Mature green unripe bananas (*Musa acuminata* Colla, cv. Nanicão) and mangoes (*Mangifera indica* L., cv. Keitt) were obtained directly from the producers in the same day of harvest. Banana fruits were cultivated in Registro (São Paulo State, Brazil) and mangoes were from a plantation located in Joanópolis (São Paulo State, Brazil). Part of the fruit was immediately peeled, sliced, frozen in liquid N<sub>2</sub> and stored at -80 °C, constituting the samples of unripe bananas or mangos. The remaining fruit were stored at 20 °C and let to ripe naturally. Full ripe bananas were sampled 22 days after harvest and mangoes were sampled 9 days after harvest. After peeling, slicing and freezing the samples of ripe fruits were frozen in liquid N<sub>2</sub>, and stored at -80°C.

**DNA material and bacteria:** Sequences of the genes of interest were kindly provided by several different research groups mainly as plasmid cloned samples. The accession numbers of the sequences as well as the plant source and insert size, are presented in Table 1. Plasmid DNA was propagated by culturing transformed cells of *Escherichia coli* Xl1-Blue. Bacteria was made competent by calcium chloride and transformed according to standard protocols (Clark 1994).

**Table 1.** DNA sequences used for the reverse northern blot.

Gene	Access number	Size (bp)	Organism	Identity	Reference
ACC	AF179249	1,777	<i>Lycopersicon esculentum</i>	ACC synthase 6 (ACS6)	Haudenschild & Theologis (*)
ACO1	X91076, Z93121, U86045	2,496	<i>Musa acuminata</i>	ACC oxidase (Ma-ACO1)	Liu et al 1999
ACS1	AB021906	1,604	<i>Musa acuminata</i>	ACC synthase (Ma-ACS1)	Liu et al 1999
ACS2	AB021907	1,089	<i>Musa acuminata</i>	ACC synthase (Ma-ACS2)	Liu et al 1999
ACS3	AB021908	1,089	<i>Musa acuminata</i>	ACC synthase (Ma-ACS3)	Liu et al 1999
FFT	AJ000481	2,073	<i>Cynara scolymus</i>	Fructan fructan 1-fructosyltransferase	Hellwege et al 1998
AMY	AF533648	1,953	<i>Musa acuminata</i>	Alpha-amylase	Vieira Jr et al 2006
PHO	AY463025	358	<i>Musa acuminata</i>	Alpha 1,4-glucan phosphorylase	Mainardi et al 2006
R1	AF295654	321	<i>Beta vulgaris</i>	Starch debranching enzyme (R1)	Schneider et al 2001
B-Gal	AF508799	2,925	<i>Oryza sativa</i>	Beta-galactosidase	Chantarangsee et al 2007
EXP	AF096776	1,147	<i>Lycopersicon esculentum</i>	Expansin (LeEXP2)	Catala et al 2000
PME	Y07899	648	<i>Carica papaya</i>	Pectinmethylesterase	Gouveia et al (*)
PG	FJ007644	1,444	<i>Carica papaya</i>	Polygalacturonase	Fabi, et al (*)
CHS1	AB009350	1481	<i>Citrus sinensis</i>	Chalcone synthase 1 (CitCHS1)	Moriguchi et al 1999
CHS2	AB009351	1,412	<i>Citrus sinensis</i>	Chalcone synthase 2 (CitCHS1)	Moriguchi et al 1999
FGT	CD800813	532	<i>Vitis vinifera</i>	UDP glucose: flavonoid 3-O-glucosyltransferase	Abbal et al (*)
APX	BF459601	482	<i>Solanum tuberosum</i>	Ascorbate peroxidase	Crookshanks et al (2001)
AOX	AF233594	2,122	<i>Cucumis melo</i>	Ascorbate oxidase (Cm-AO4)	Sanmartin et al (*)
ACT	AB022041	516	<i>Musa acuminata</i>	Beta-actin (MA-Actin)	Liu et al (1999)

\* Unpublished

**Cloning and PCR amplification of DNA inserts:** The propagated plasmids were purified by using the QIAprep Spin Miniprep (Qiagen), and used for the isolation of the inserts. The fragments of the genes were PCR amplified by using vector flanking sense and antisense oligonucleotides, mostly M13 primers. A typical 100  $\mu$ L amplification reaction was composed of 1  $\mu$ M of each sense and reverse primers, dNTP (0,2  $\mu$ M each), 1 unit recombinant *Taq* DNA Polymerase (Invitrogen), 1.5 mM MgCl<sub>2</sub>, 10mM Tris-HCl buffer (pH 8.0) and 1  $\mu$ L DNA (10 – 100 ng). PCR conditions were: 29 cycles of incubation at 94 °C/5 min, 55 °C/30 sec, 72 °C/2.5 followed for final incubation at 72 °C for 5 min. The PCR products were separated by electrophoresis on agarose gels (1.5%) and purified using GFX PCR DNA and Gel Band Purification (Amersham Biosciences).

**Membrane preparation:** Purified samples of PCR products were transferred to Hybond N+ nylon membranes (Amersham Biosciences) using the Bio-Dot® apparatus (Bio-Rad), according to the manufacturer instructions. Just before transferring, the DNA solutions were heated at 95 °C by 5 min and after chilling on ice an equal volume of 20X SSC was added. In order to evaluate local differences in hybridization signal, each sample was divided in four sub-samples and spotted in replicates onto the membranes. In this way, each replica filter

presented a duplicate of each spotted sample. After applying the DNA samples the membranes were denatured with 1.5M NaCl/0.5M NaOH solution for 5 min followed by neutralization with 1.5M NaCl/ 10mM EDTA in 0.5M Tris-HCl buffer (pH 7.2). After drying on air the spotted DNA was permanently fixed to the nylon filters by UV cross-linking.

**RNA extraction and probe preparation:** Total RNA were extracted from 3g of frozen ripe and unripe fruit samples (banana and mango) as described previously (Nascimento et al., 1997). RNA integrity was checked by electrophoresis on 1.5% agarose gels (Sambrook et al., 1989). Twenty-five micrograms of total RNA were used to prepare the <sup>32</sup>P-labelled cDNA probe from ripe or unripe fruits. The RNA was brought into solution with 2 $\mu$ g de Oligo (dT)<sub>12-18</sub> in 25 $\mu$ L DEPC water and denatured at 65 °C for 3 min. The denatured RNA was reverse transcribed at 42 °C for 50 min in a 35  $\mu$ L reaction containing 2.8 units of reverse transcriptase in 20mM Tris-HCl (pH 8.4) buffer, 50mM KCl, 7 mM MgCl<sub>2</sub>, 1.7 units of RNAout™, 1.5 mM DTT, 1.5 mM of each dATP, dGTP, dCTP and 50  $\mu$ Ci  $\alpha$ -[<sup>32</sup>P]-dCTP. After that period, a 2  $\mu$ L aliquot of 25mM dCTP was added to the solution and incubated at 42 °C for 45 min. The reaction was terminated at 70°C for 15 min and after chilling on ice and addition of 3 units of *E. coli* RNAase H, a new incubation was performed at 37 °C

for 20 min to eliminate the remaining RNA. Unincorporated labeled nucleotides were removed by filtration on MicroSpin™ G-50 Columns (Amersham Biosciences). The probes were denatured for five minutes at 100 °C, chilled on ice and used for membranes hybridization.

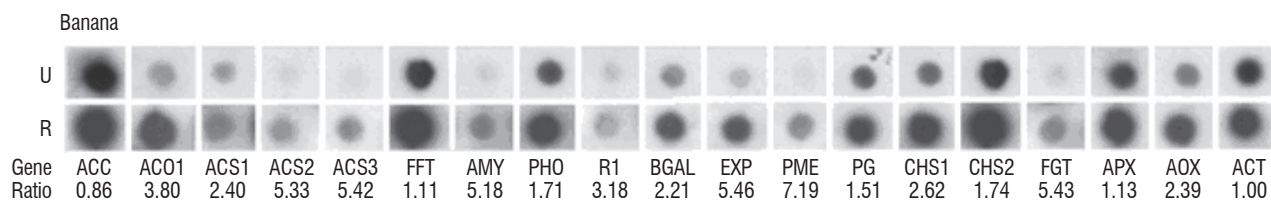
**Reverse northern-blotting:** Previous to the addition of the labeled probes the membranes were blocked by incubation with 10 ml of 'Hybridization Solution' (Sigma-Aldrich) for 1h at 68°C. For probing with the labeled cDNA a pair of replicate membranes was used: one was hybridized with the probe from unripe fruit while the other was hybridized with the probe from ripe fruit, under identical conditions. After blocking of the membranes the denatured probed were added to the solutions and incubated at 68°C for 15 hours. The membranes were washed under stringent conditions according to the instructions of the manufacturer and exposed to radiographic films 'Hiperfilm MP' (Amersham Biosciences). The signals of the autoradiographies were let to develop under standard conditions (Sambrook et al., 1989). Several different exposition times were let to develop in order to provide a good, reproducible signal from the hybridization of the probes to each spotted DNA sample.

**Densitometry analysis:** The exposed films were scanned in a GS-600 Densitometer (Bio-Rad) and the signals of the hybridized spots were analyzed by densitometry using the 'Molecular Analyst Software' (Bio-Rad). The hybridization signals were estimated as the 'signal volume', which was the product of optical density by the spotted area. The values were corrected by the local background and the signals from non-

spotted regions of the membranes, corresponding to void wells, were subtracted. Saturated hybridization signals were avoided by using data from films revealed after shorter exposure times. Since each DNA sample was spotted in duplicate, the average value was calculated from the two corrected 'signal volume'. In order to compare the data obtained from the membranes hybridized to the two different probes, and to minimize differences caused by the experimental conditions, the average corrected 'signal volume' of each gene was normalized against the average corrected 'signal volume' for the actin. Finally, the changes in gene expression during ripening were estimated by the calculation of the ripe to unripe ratio (R/U) of the normalized, corrected 'signal volume' of hybridizations obtained for each gene of interest. So, the ratios presented in the figures were calculated by dividing the individual values from the two hybridization conditions by the corresponding value of the actin gene. In this way, the actin expression ratio was equal to 1.0. Only those genes that were above the threshold ratio of 2.0 were considered as ripening induced.

## RESULTS

The signals obtained for the hybridization of the selected genes with the banana probes are presented in figure 1. The genes related to ethylene biosynthesis presented a large increase in expression reaching more than threefold for ACO1 and fivefold for ACS2 and ACS3. Surprisingly, no significant change was noticed for one of the ACC sequence (ACC), which was almost constant.



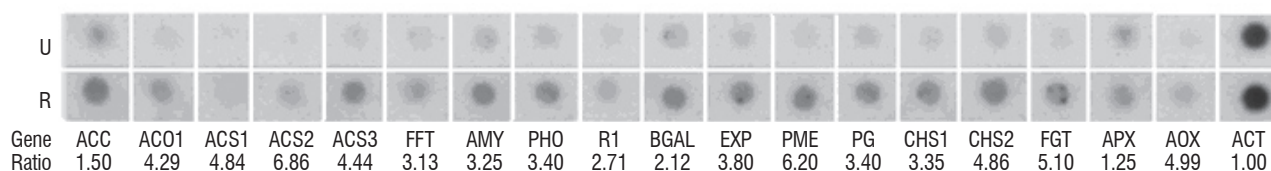
**Figure 1.** Reverse northern-blotting analysis of banana fruit gene expression. The sequences of aminocyclohexanoic acid synthase (ACC, ACS1, ACS2 and ACS3), aminocyclohexanoic acid oxidase (ACO), fructanfructosyltransferase (FFT),  $\alpha$ -amylase (AMY), starch-phosphorylase (PHO),  $\alpha$ -glucan-water-dikinase (R1),  $\beta$ -galactosidase (BGAL), expansine (EXP), pectinmethylesterase (PME), polygalacturonase (PG), chalcone synthase 1 (CHS1), chalcone synthase (CHS2), UDP-glucan-4-epimerase (UDGP), flavonolglucosyltransferase (FGT), ascorbate peroxidase (APX), ascorbate oxidase (AOX) and actin (ACT) genes were spotted onto nylon membranes and probed against <sup>32</sup>P-labeled cDNA from unripe (U) and ripe (R) banana fruit (Column on the left). The values on the right column correspond to the ripe to unripe ratio (R/U) of the normalized, corrected 'signal volume' of hybridizations obtained for each gene of interest.

In relation to the genes of enzymes related to the carbohydrate metabolism, no difference was noticed for the fructan metabolism FFT sequence, while those involved with starch degradation, such as  $\alpha$ -amylase (AMY), were clearly stimulated. Among the genes of cell wall metabolism, pectin methyl esterase was largely affected, while those of BGAL and PG were almost invariable. The non enzymatic protein expansine (EXP) was also induced during ripening, with a fivefold increase.

An apparent increase in expression for the pigment related genes was noticed for the chalcone synthase 2

(CHS2), but not for the chalcone synthase 1 (CHS1). Clear differences were also observed between the ascorbate metabolism enzymes, since the oxidase (AOX) was induced while the peroxidase (APX) was almost invariable.

Similarly to what was observed at the banana experiment, all the sequences tested hybridized to the mango cDNA probes (Figure 2). The ethylene-related enzymes were strongly induced, with more than fourfold increases for the oxidase (ACO1) and the synthases (ACC1, ACC2 and ACC3), except the one from tomato (ACC).



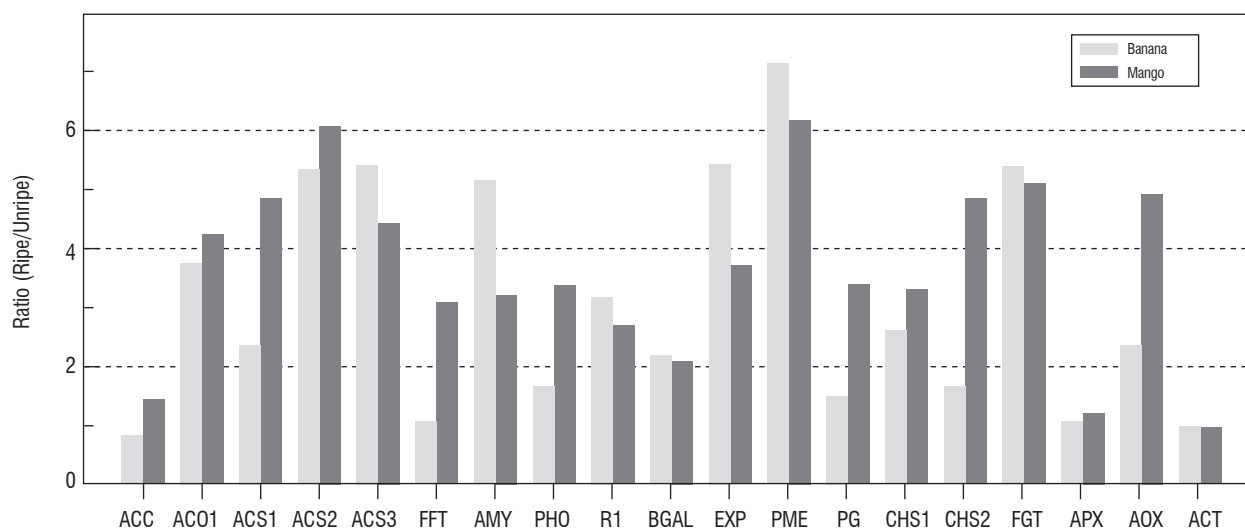
**Figure 2.** Reverse northern-blotting analysis of mango fruit gene expression. The sequences of aminocyclohexanoic acid synthase (ACC, ACS1, ACS2 and ACS3), aminocyclohexanoic acid oxidase (ACO), fructanfructosyltransferase (FFT),  $\alpha$ -amylase (AMY), starch-phosphorylase (PHO),  $\alpha$ -glucan-water-dikinase (R1),  $\beta$ -galactosidase (BGAL), expansine (EXP), pectinmethylesterase (PME), polygalacturonase (PG), chalcone synthase 1 (CHS1), chalcone synthase (CHS2), UDP-glucose-4-epimerase (UDGP), flavonolglucosyltransferase (FGT), ascorbate peroxidase (APX), ascorbate oxidase (AOX) and actin (ACT) genes were spotted onto nylon membranes and probed against  $^{32}$ P-labeled cDNA from unripe (U) and ripe (R) banana fruit (Column on the left). The values on the right column correspond to the ripe to unripe ratio (R/U) of the normalized, corrected 'signal volume' of hybridizations obtained for each gene of interest.

Regarding the carbohydrate enzymes, significant stimulation during fruit ripening could be observed for the three sequences related to starch metabolism and also for that corresponding to fructanfructosyltransferase (FFT), which showed a threefold increase. The same could be said for the proteins that would contribute for cell wall disassembly, mainly PME and PG.

In contrast to banana fruit, all the three sequences involved in pigment synthesis were strongly stimulated during mango ripening. On the other hand, only the ascorbate oxidase was significantly induced.

Figure 3 presents the comparison between the expression patterns of the selected genes for both banana

and mango fruits. It is possible to notice that some sequences were almost invariable or their threshold ratios were very close to the value of 2.0, for both banana and mango. That was the case of the ACC, BGAL, and APX. In contrast, all the other sequences indicated changes in gene expression during fruit ripening, although it is possible to discriminate between those that changed similarly in both fruit from those sequences that presented contrasting patterns. Among the first group of sequences there were ACO1, ACS2, ACS3, R1, and CHS, which presented similar ratios in bananas and mangoes. On the opposite, ACS1, FFT, AMY, PHO, EXP, PG, CHS2 and AOX were the sequences that changed differently between the two fruits.



**Figure 3.** Comparison of gene expression in bananas and mangoes. The values of ripe to unripe ratio of gene expression for the aminocyclohexanoic acid synthase (ACC, ACS1, ACS2 and ACS3), aminocyclohexanoic acid oxidase (ACO), fructanfructosyltransferase (FFT),  $\alpha$ -amylase (AMY), starch-phosphorylase (PHO),  $\alpha$ -glucan-water-dikinase (R1),  $\beta$ -galactosidase (BGAL), expansin (EXP), pectinmethylesterase (PME), polygalacturonase (PG), chalcone synthase 1 (CHS1), chalcone synthase (CHS2), UDP-glucan-4-epimerase (FGE), UDP-glucan-6-phosphate 4-epimerase (FGE), UDP-glucan-6-phosphate 4-epimerase (FGE), UDP-glucan-6-phosphate 4-epimerase (FGE), ascorbate peroxidase (APX), ascorbate oxidase (AOX) and actin (ACT) genes from bananas and mangoes were plotted in order to compare the relative values of gene expression.

## DISCUSSION

All the spotted sequences hybridized to the banana and mango cDNA probes at both ripening stages, even those isolated from other plant species. This indicates that in spite of the stringent conditions used for membrane hybridization and washing, there is high similarity between the probed sequences and those from bananas and mangoes.

Most of the sequences analyzed were expected to indicate significant changes in gene expression during ripening. That was the case of the ethylene enzymes, which account for the autocatalytic synthesis during ripening of climacteric fruit, such as bananas and mangoes. Although the observed changes were in overall agreement to the previous results by Liu et al., (1999), the unexpected lower increase in ACS1 could be explained by the differences in sampling points taken for the analysis, since the authors mentioned that ACS1 underwent a sharp and transitory induction, or could be explained by the lower similarity level to the equivalent mango sequence. This would explain why the expected increase in aminocyclohexanoic acid synthase expression was observed when using the ACS1, ACS2 and ACS3 probes, but not with the ACC sequence.

Both bananas and mangos are considered starch-rich fruits, which is the substrate for soluble sugar synthesis during ripening. In this way, starch mobilization is a crucial step in the process of fruit sweetening, and the three sequences evaluated are supposed to play a role depolymerization (Silva et al, 2008). R1 has  $\alpha$ -glucan-water-dikinase activity which is thought to be essential in phosphorylation of starch polymers both at development and degradation of starch granules (Ritte et al., 2002, Ritte et al., 2006). Although the enzyme can potentially contribute to granule disassembly during fruit ripening, no information on its induction had already been reported in bananas or mangoes. The differences between the ratios of  $\alpha$ -amylase and phosphorylase in bananas and mangoes can also provide some interesting clues about the metabolism of starch in those fruits, because it could be an indication whether the starch degradation process would be more operative through the amylolytic or phosphorolytic pathways. The relative change in  $\alpha$ -amylase expression would in agreement to the previous observation of enzymatic changes during ripening of both fruit (Basinello et al., 2002, Simão et al., 2008). On the other hand phosphorylase activity seemed to be more induced in mangoes, which would not be expected considering that changes in enzyme activity during

fruit ripening were not so significant (Simão et al., 2008). It is possible that the changes in activity of mango phosphorylase would be more dependent on gene expression than that of banana enzyme. The hybridization of FFT sequence to the cDNA from bananas and mangoes opens the possibility of occurrence of a similar enzyme in those fruits. In fact, although not considered as a relevant source of fructose polymers, the biosynthesis of fructans seems to be an operative pathway in banana fruit pulp (Agopian et al., 2008). Similarly, it is likely that it occurs in mango, as denoted by the threefold increase in FFT ratio, especially if one considers that fructose is the predominant monosaccharide in this fruit (Simão et al., 2008).

Fruit softening is another important change observed during fruit ripening. However, its dependence on cell wall disassembly seems to be different for bananas and mangoes (Fioravante et al., 2008). The texture of banana pulp seems to be more dependent on the starch, which is stored at amounts as high as 25% of fresh weight (Cordenunsi and Lajolo 1995). This could explain why PG expression in mango fruit was almost twofold that seem for banana fruit. The observed differences could indicate that pectin depolymerization by PG would be a more relevant event for cell wall disassembly during mango ripening, while relaxation of the structure by changes in pectin methylation by PME and expansin action would predominate in starchy banana fruit.

Besides carotenoid, anthocyanins are main contributors to the color of ripe fruits (Ronen et al., 2000), and the biosynthesis of anthocyanins, which are compounds of the class of flavonoids, is catalyzed by chalcone synthase. Although none of the fruit studied are recognized as source of anthocyanins, at least in the pulp, there were expressive changes in gene expression related to these two sequences. Both CHS1 and FGT increased similarly in bananas and mangoes, but the last fruit had a fivefold increase in CHS2. It is possible that, in spite of the large stimulation on the expression of these enzymes, the availability of precursor compounds is limiting, precluding the accumulation of appreciable amounts of anthocyanins.

The analysis of APX and AOX, which would be potentially relevant to the levels of ascorbate in the pulp of the fruits, suggests that oxidative steps are favored during ripening of mango fruit. Since the ascorbate metabolism is a component of the cell redox system and it is implicated in some important

changes during ripening, such as cell wall disassembly (Belfield et al., 2005), it is possible that AOX induction in mango fruit would not be only important in relation to the vitamin C levels in the pulp, but also for fruit softening.

The small collection of genes employed in this study was composed of homologous and heterologous sequences related to the some biochemical pathways potentially relevant to the fruit quality. It was possible to evaluate the expression of proteins involved in ethylene biosynthesis, starch mobilization, cell wall disassembly, synthesis of anthocyanins and ascorbate metabolism in both bananas and mangoes. Some presented results were in agreement to previous observations, and they can be taken as validating data to the approach described here. Additionally, new data on the changes in expression of proteins or enzymes that have not yet been described for those fruits were also presented, and they can be useful starting points for more specific studies. Overall, the results presented here are an evidence of the feasibility of the reverse northern-blotting approach based on heterologous sequences, which was a quick and simple way to provide access to changes in gene expression. This approach could be scalable to include more genes or be applied to study other fruit species or fruit at different postharvest conditions.

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Aalborg Universitet, Denmark (*S. tuberosum* APX clone); 10) Dr. A. Kanelis and Dr. I. Pateraki from the Aristotle University of Thessaloniki, Greece (*C. melo* AOX clone).

## REFERENCES

- Agopian RGD, Soares CA, Purgatto E, Cordenunsi BR, Lajolo FM (2008) Identification of fructooligosaccharides in different banana cultivars. *J. Agri. Food Chem.* 56: 3305-3310.
- Alba R, Paxton P, Fei Z, McQuinn D, Martin P, Tanksley SD, Giovannoni JJ (2005) Transcriptome and selected metabolite analyses reveal multiple points of ethylene control during tomato fruit development. *Plant Cell* 17: 2954-2965.
- Albelda SM, Sheppard D (2000) Functional genomics and expression profiling. Be there or be square. *Am. J. Respiratory Cell and Mol. Biol.* 23: 265-269.
- Basinello PZ, Cordenunsi BR, Lajolo FM (2002) Amylolytic activity in fruits: comparison of different substrates and methods using banana as model. *J. Agri. Food Chem.* 50: 5781-5786.
- Belfield EJ, Ruperti B, McQueen-Mason JARS (2005) Changes in expansin activity and gene expression during ethylene-promoted leaflet abscission in *Sambucus nigra*. *J. Exp. Bot.* 56: 817-823.
- Bennett AB, Labavitch JM (2008) Ethylene and ripening-regulated expression and function of fruit cell wall modifying proteins. *Plant Sci* 175: 130-136.
- Cara B, Giovannoni JJ (2008) Molecular biology of ethylene during tomato fruit development and maturation. *Plant Sci* 175: 106-113.
- Catala C, Rose JK, Bennett AB (2000) Auxin-regulated genes encoding cell wall-modifying proteins are expressed during early tomato fruit growth. *Plant Physiol.* 122: 527-534.
- Chantarangsee M, Tanthanuch W, Fujimura T, Fry SC, Ketudat-Cairns J (2007) Molecular characterization of beta-galactosidases from germinating rice (*Oryza sativa*). *Plant Sci.* 173: 118-134.
- Clark MD, Panopoulou GD, Cahill DJ, Büssov K, Lehrach H (1999) Construction and analysis of arrayed cDNA libraries. *Methods in enzymology* 303: 205-233.
- Cordenunsi BR, Lajolo FM (1995) Starch Breakdown During Banana Ripening: Sucrose Synthase And Sucrose Phosphate Syntase Behavior. *J. Agri. Food Chem.* 43: 347-351.
- Crookshanks M, Emmersen J, Welinder KG, Nielsen KL (2001) The potato tuber transcriptome: analysis of 6077 expressed sequence tags. *FEBS Lett* 506: 123-126.
- Davuluri GR, Tuinen A, Fraser PD, Manfredonia A, Newman R, Burgess D, Brummell DA, King SR, Palys J, Uhlig J, Bramley PM, Penning HMJ, Bowler C (2005) Fruit-specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes. *Nature Biotech.* 23: 890-895.
- Drmanac R, Drmanac S (1999) cDNA screening by array hybridization. *Meth. Enzymol.* 303: 165-178.
- Eisen MB, Brown PO (1999) DNA arrays for analysis of gene expression. *Meth. Enzymol.* 303: 179-204.
- English D, Parker B (1999) Methods for preparing PCR based arrays on Nytran N and SuPerCharge Nylon Membranes. *Applications Notes for Biomolecular Screening.*
- Fioravante AP, Nascimento JRO, Lajolo FM, Cordenunsi BR (2008) Starch mobilization and sucrose accumulation in the pulp of Keitt mangoes during postharvest ripening. *J. Food Biochem.* 32: 384-395.
- Fraser PD, Enfissi EMA, Halket JM, Truesdale MR, YU D, Gerrish C, Bramley PM (2007) Manipulation of phytoene levels in tomato fruit: Effects on Isoprenoids, Plastids, and Intermediary Metabolism. *Plant Cell* 19: 3194-3211.
- Giovannoni J (2001) Molecular biology of fruit maturation and ripening. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52: 725-749.
- Gray J, Pictou S, Shabbeer J, Schuch W, Grierson D (1992) Molecular biology of fruit ripening and its manipulation with antisense genes. *Plant Mol. Biol.* 19: 69-87.
- Hellwege EM, Raap M, Gritscher D, Willmitzer L, Heyer AG (1998) Differences in chain length distribution of inulin from *Cynara scolymus* and *Helianthus tuberosus* are reflected in a transient plant expression system using the respective 1-FFT cDNAs. *FEBS Lett.* 427: 25-28.
- Hulme AC (1971) *The biochemistry of fruits and their products.* Academic Press, London.
- Jiang H, Kang D, Alexandre D, Fisher PB (2000) Rash, a rapid subtraction hybridization approach for identifying and cloning differentially expressed genes. *PNAS* 97: 12684-12689.
- Kuhn E (2001) From library screening to microarray technology: strategies to determine gene expression profiles and to identify differentially regulated genes in plants. *Annals of Botany* 87: 139-155.
- Liu X, Shiomi S, Nakatsuka A, Kubo Y, Nakamura R, Inaba A (1999) Characterization of Ethylene Biosynthesis Associated with Ripening in Banana Fruit. *Plant Physiol.* 121: 1257-1265.
- López-Gómez R, Gómez-Lim MA (1992) A method for extracting intact RNA from fruits rich in polysaccharides using ripe mango mesocarp. *Hortscience* 27: 440-442.
- Mainardi JA, Purgatto E, Vieira A Jr, Bastos WA, Cordenunsi BR, Nascimento JRO, Lajolo FM (2006) Effects of ethylene and 1-methylcyclopropene (1-MCP) on gene expression and activity profile of alpha-1,4-glucan-phosphorylase during banana ripening. *J. Agric. Food Chem.* 54: 7294-98.
- Moore S, Vrebalov J, Payton P, Giovannoni J. (2002) Use of genomics tools to isolate key ripening genes and analyse fruit maturation in tomato. *J. Exp. Bot.* 53: 2023-2030.
- Moriguchi T, Kita M, Tomono Y, Endo-Inagaki T, Omura M (1999) One Type of chalcone synthase gene expressed during embryogenesis regulates the flavonoid accumulation in citrus cell cultures. *Plant Cell Physiol.* 40: 651-655.
- Mounet F, Moing A, Garcia V, Petit J, Maucourt M, Deborde C, Bernillon S, Le Gall G, Colquhoun I, Defernez M, Giraudel JL, Rolin D, Rothan C, Lemaire-Chamley M (2009) Gene and Metabolite Regulatory Network Analysis of Early Developing Fruit Tissues Highlights New Candidate Genes for the Control of Tomato Fruit Composition and Development. *Plant Physiol.* 149: 1505-1528.
- Nascimento JRO, Cordenunsi BR, Lajolo FM, Alcocer MJC (1997) Banana sucrose-phosphate synthase gene expression during banana ripening. *Planta* 203: 283-288.
- Overmyer K, Tuominen H, Kettunen R, Betz C, Langebartels C, Sandermann H, Kangasjärvi J (2000) Ozone-sensitive Arabidopsis rcd1 mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. *Plant Cell* 12: 1849-1862.
- Rast JP, Amore G, Calestani C, Livi CB, Ransick A, Davidson EH (2000) Recovery of developmentally defined gene sets from highdensity cDNA macroarrays. *Dev. Biology* 228: 270-285.
- Ritte G, Lloyd JR, Eckermann N, Rottmann A, Kossmann J, Steup M (2002) The starch-related R1 protein is an  $\alpha$ -glucan, water dikinase. *PNAS* 99: 7166-7171.
- Ritte G, Heydenreich M, Mahlow S, Haebel S, Kottling O, Steup M (2006) Phosphorylation of C6- and C3-positions of glucosyl residues in starch is catalysed by distinct dikinases. *FEBS Lett.* 580: 4872-4876.
- Ronen G, Carmel-Goren L, Zamir D, Hirschberg J (2000) An alternative pathway to  $\beta$ -carotene formation in plant chromoplasts discovered by map-based cloning of Beta and old-gold color mutations in tomato. *PNAS* 97: 11102-11107.



Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York USA.

Schneider K, Weisshaar B, Borchardt DC, Salamini F (2001) SNP frequency and allelic haplotype structure of *Beta vulgaris* expressed genes. *Mol. Breed.* 8: 63-74.

Silva APFB, Nascimento JRO, Lajolo FM, Cordenunsi BR (2008) Starch mobilization and sucrose accumulation in the pulp of Keith mangoes during postharvest ripening. *J. Food Biochem.* 32: 384-395.

Simão RA, Silva AP, Peroni FH, Nascimento JRO, Lajolo FM, Cordenunsi BR (2008) Mango starch degradation II The binding of alpha-amylase and beta-amylase to the starch granule. *J. Agric. Food Chem.* 56: 7416-21.

Smith H, Billett E, Giles AB (1977) The photocontrol of gene expression in higher plants In: Smith H., ed. *Regulation of enzyme synthesis and activity in higher plants*. San Diego, Academic Press, p. 93-125.

Speirs J, Brady CJ (1991) Modification of gene expression in ripening fruit. *Aust. J. Plant Physiol.* 18: 519-32.

Tucker G.A., Grierson D. Fruit ripening. (1987) In: *The biochemistry of plants* 12: 265-318.

Vieira-Jr A, Nascimento JR, Lajolo FM (2006) Molecular cloning and characterization of an alpha-amylase occurring in the pulp of ripening bananas and its expression in *Pichia pastoris*. *J. Agric. Food Chem.* 54(21): 8222-8228.