Role of differentially expressed ripening related genes and promoters in banana fruit: Identified by mRNA DDRT-PCR

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Abstract: It is now becoming increasingly evident that plants have a tight regulation of gene expression during fruit ripening which is brought about through differential expression of hundreds of genes. This review deals with the identification of several ripening related and ethylene induced/suppressed genes in banana pulp using mRNA differential display reverse transcription (DDRT-PCR). It also highlights recent developments in identifying and assigning role to various ripening related genes and promoters during ripening that may help to better understand these phenomena at the molecular level and identify candidate genes for genetic manipulation to improve the postharvest life of banana fruits.

Key words: Fruit ripening, Differential gene expression, Banana, Ripening related gene and promoters, DDRT-PCR, Ethylene signalling

Introduction

Fruit ripening is highly complex final phase of fruit development, governed both developmentally and hormonally and involves progressive changes in several physical and biochemical parameters (Giovannoni, 2001). The main changes associated with ripening include those in colour, loss in firmness, taste and flavors so as to make it more attractive and edible thus facilitating seed dispersal (Amoros et al., 2003). Each of the characters that undergo a change during ripening is governed by several genes. Fruit ripening thus involves the expression and regulation of hundreds of genes.

Ripening has been studied in great detail in tomato and to some extent in apples, kiwifruit, peach, melon and avocado but to a lesser extent in banana. Most studies in all these fruits have involved identification of genes involved in ethylene biosynthesis [1-amino cyclopropane-1-carboxylate synthase (ACS), 1-amino cyclopropane-1-carboxylate oxidase (ACO)], the ethylene signal cascade [Ethylene receptor (ETR), Constitutive response (CTR), Ethylene insensitive (EIN)] and several downstream target genes involved in cell wall degradation, pigment biosynthesis and sugar metabolism. However, apart from these genes, ripening involves the up-regulation and down-regulation of hundreds of other genes, most of which are yet to be identified. Approaches such as subtractive hybridization, micro-array and differential library screening have been employed in climacteric fruits like avocado (Dopico et al., 1993), kiwifruit (Ledger and Gardner, 1994) and banana (Clendennen and May, 1997; Medina-Suarez et al., 1997) and in non-climacteric fruits like strawberry (Nam et al., 1999) and grapes (Davies and Robinson, 2000) for identifying differentially expressed genes related to fruit ripening whose action in the ripening process was not very obvious. The identification of some of these novel genes has been made possible by the technique of mRNA differential display reverse transcription-PCR (DDRT-PCR). This technique developed by Liang and Pardee (1992) is a powerful tool to study differential gene expression and has been employed recently for studying changes associated with climacteric fruit ripening and other developmental processes in plants.

Banana is an important tropical fruit as it is fourth most important staple crop in the world and India contributes about 20% of the total edible banana production across the world. Banana fruit do not usually ripen on the plant and are artificially ripened after harvesting. Unlike many other fruits it cannot be stored at low temperature as low temperature induces chilling injury and high temperatures result in boiling of the pulp, a condition in which the taste and texture of the pulp are not of edible quality. Thus controlling the rate of ripening in banana fruit is of prime importance to increase its shelf life. The ripening process in banana could not be controlled efficiently by various means such as modern storage facility, cool temperature storage, chemical sprays etc., once ripening has been initiated. Therefore, molecular genetic approach could be an ideal approach to control the ripening in banana even after ripening has set in. At the gene level the process of banana ripening has not been studied to a great extent in spite of its importance as a major food crop.

This review deals with the identification of several ripening related and ethylene induced/suppressed genes in banana pulp using mRNA DDRT-PCR. Though genes such as ACS, ACO and polygalacturonase (PG) have already been used to control shelf life of climacteric fruit like tomato, melon and avocado and technology patented. However, there is constant need to identify newer genes and promoters, which are ripening related and ethylene regulated in order to improve quality of the fruit. Moreover, fruit specific promoters can be utilized to express desired gene in tissue specific manner. Such study would also be useful in selecting better candidate genes for delaying ripening in banana.

Role of ethylene in fruits ripening

Ethylene is a gaseous phytohormone that regulates the variety of developmental and stress responses in plants (Johnson and Ecker, 1998). It plays important role in the initiation and progression of ripening in climacteric fruits such as tomato, banana, apple, mango, avocado, kiwifruit, melon etc. At the onset of ripening, climacteric fruits exhibit a burst in CO₂ production termed as the respiratory climacteric along with a concomitant rise in ethylene biosynthesis and evolution.
Ethylene biosynthesis in fruits

Endogenous ethylene is produced in plants by the Yang cycle (Fig. 1). Ethylene is synthesized from methionine by the conversion of S-adenosyl-L-methionine (SAM) to 1-aminocyclopropane-1-carboxylate (ACC), catalysed by ACC synthase (ACS), and the subsequent oxidation of ACC to ethylene by ACC oxidase. The rate limiting step of this pathway is the conversion of AdoMet to 1-aminocyclopropane-1-carboxylate (ACC) and methylthioadenosine (MTA) by ACC synthase (Yang and Hoffman, 1984). Methionine is recycled into the pathway by the conversion of MTA to methionine thus ensuring high rates of ethylene biosynthesis even when the pool of free methionine is small.

Depending on the level and nature of ethylene produced in plants McMurchie et al. (1972) introduced the concept of system I and system II ethylene production. System I operates in vegetative tissues and fruits (climacteric and non-climacteric). It is responsible for the basal and wound induced ethylene production, and is auto-inhibitory. System II is responsible for the upsurge of ethylene production during ripening in climacteric fruits, and during petal senescence. It is auto-stimulatory and requires the induction of both ACS and ACO genes for its biosynthesis (Alexander and Grierson, 2002).

Ethylene signal transduction

Ethylene responses are mediated through a signal transduction cascade that has been partially defined in Arabidopsis using genetic and molecular approaches (Chen et al., 2005) (Fig. 1). Ethylene is perceived by a family of receptors that have similarity to histidine kinases and are negative regulators of ethylene signal transduction (Kevany et al., 2007). In tomato six ethylene receptors viz., LeETR1-6 has been identified (Bleecker, 1999). These receptors interact with and apparently regulate the activity of CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1), a Ser/Thr kinase that is also a negative regulator of ethylene signaling. CTR1 has sequence similarity to Raf, a mitogen-activated protein kinase kinase kinase (MAPKKK); consequently, a MAP kinase cascade has been postulated to act downstream of CTR1, although the components of this cascade have been the subject of recent debate (Ouakad et al., 2003). When ethylene binds to the receptors, CTR1 is thought to be inactivated. This relieves the suppression on downstream signaling elements, resulting in the activation of ethylene insensitive 2 (EIN2), a membrane-bound protein with similarity to Nramp metal-ion transporters (Alonso et al., 1999), this in turn activates downstream transcriptional regulators (Solano et al., 1998) such as EIN3 and EIN3-like (EIL) proteins, of which EIN3 appears to play the predominant role in regulating the ethylene response (Chao et al., 1997). The members of EIN3/EIL family regulates expression of a variety of genes, including another group of trans-factors, ethylene-responsive factor 1 (Solano et al., 1998). The pathway between CTR1 and EIN3 is also modulated by the EINS1/XRN4 59-39 exonuclease, but its direct RNA target(s) is currently unknown (Potuschak et al., 2006). Two additional proteins, EIN3 BINDING F-BOX1 (EBF1) and EBF2 were recently shown to function in ethylene perception by regulating EIN3/EIL turnover. Ethylene appears to block ubiquitination and proteosomal degradation of EIN3, allowing EIN3/EIL levels to rise and mediate ethylene signaling. It has been shown that EIN3 and EIL1 are the main targets of EBF1/2. EBF1 plays main role in air and during the initial phase of signaling, EBF2 plays a more prominent role during the latter stages of the response and the resumption of growth following ethylene removal (Binder et al., 2007).

Techniques employed to isolate and analyze differentially expressing genes

Several methods have been developed over the period of time to identify and analyze differentially expressing genes from a number of species, tissues and fruits. Some of the important methods and their applications are briefly discussed.

Differential display reverse transcriptase PCR (DDRT-PCR): Differential display reverse transcriptase PCR (DDRT-PCR) is a powerful tool developed by Liang and Pardee (1992) to isolate differentially expressing genes. The advantage of differential–display as compared to alternative methods for differential screening lies in its ability to compare numerous systems for both induction and repression of specific gene expression simultaneously. In addition, differential display is likely to permit the identification of rare transcripts via the PCR component of this technique. Using this technique Boung-jun and Giovannoni (1995), identified eight ripening related transcripts from tomato. Zegzouti et al. (1999), identified 19 ethylene responsive and ripening related genes differentially expressed in tomato.

We have used this technique to amplify differential cDNA fragments from banana treated with ethylene from 0 day, 2 days and 4 days (Fig. 2). Of the 22 differential bands (Fig. 3) obtained, 16 were up-regulated while 6 were down-regulated. As shown in Table 1 of the 22 ripening related genes sequenced, only two (Pectate lyase and β-1,3-glucanase) have previously been reported in banana (Gupta et al., 2006).

Suppression subtractive hybridization-PCR (SSH-PCR): SSH is a rapid and effective method for cloning differentially expressed cDNAs (Diatchenko et al., 1996). It has also been found to give a low background and has been successfully applied for the isolation of differentially expressed ripening related genes from various fruits such as capsicum (Liu et al., 2005), grape berry (Ageorges et al., 2006) and banana (Kesari et al., 2007).
Serial analysis of gene expression (SAGE): Serial analysis of gene expression (SAGE) is a method which allows the quantitative analysis of thousands of transcripts at once, therefore generating important information on the relative abundances of mRNA transcripts between samples (Velculescu et al., 1995). In addition to providing qualitative data on gene expression (i.e. transcript identity), SAGE also provides quantitative data in that the abundances of different tag sequences correspond to the relative abundances of the mRNA transcripts. It can also be used to identify novel genes by using unidentified tags as probes for screening cDNA libraries. However, the major disadvantages of this technique are that a large number of tags need to be sequenced in order to detect mRNA species present in low abundance and relatively a large amount of purified poly(A) mRNA (5 µg) is required.

Microarray technologies: Microarray is an advanced and high throughput method based on DNA chips which consists of very high density arrays of short oligonucleotides synthesized on a silicon wafer by a combination of conventional oligonucleotide synthesis chemistry and photolithography (Schena et al., 1995). There are two parallel methods to monitor the genes which are differentially expressed in a particular sample, oligonucleotide microarrays and cDNA microarrays. Both of these methods offer tremendous advantages in speed of analysis, allowing thousands of cDNA sequences to be surveyed in a single set of experiments. This method has been applied for profiling of differentially expressed genes from various plant samples and recently there are also some reports for there usefulness in profiling of ripening related genes (Cercós et al., 2006).

Northern and real-time PCR analysis: Although the aforementioned techniques are powerful tools to analyze the genes, expression results have to be confirmed by other techniques such as real-time polymerase chain reaction (RT-PCR), Northern blot etc. Real-time PCR allows the determination of the mRNA levels of specific genes precisely, reliably and relatively fast. Northern analysis is one of the most cost effective and reliable methods to analyze the expression of genes, however sensitivity of this method is lesser than that of RT-PCR.

Genes known to participate in ripening of climacteric fruits: In this section some of the known ripening genes that play role in ethylene biosynthesis, signal transduction, softening, colour, aroma and sweetening are discussed.

Genes related to ethylene biosynthesis: Small multi-gene families code for the ACS and ACO enzymes involved in ethylene biosynthesis. Their expression is differentially and tightly regulated by various developmental, environmental, and hormonal signals (Fluur and Mattoo, 1996). Some members are involved in ethylene biosynthesis in the fruits that initiate ripening. In tomato, eight ACS genes (LeACS1A, LeACS1B, and LeACS2-7) have been identified among which LeACS2 and LeACS4 are highly expressed during tomato fruit ripening (Oetiker et al., 1997). ACS genes have been also identified from apple (Dong et al., 1992) and melon (Yamamoto et al., 1995). ACO gene family members have been reported in tomato (Barry et al., 1996), apple (Ross et al., 1992) and melon (Bouquin et al., 1997). Of the three ACOs in tomato LeACO1 mainly expresses in ripening fruit while other two ACOs play a role in other aspect of plant development.

Genes related to ethylene signal transduction pathway: The ethylene receptors in all the species studied so far are members of a multigene family. Several homologues of ETR and ERS have also been identified from different fruits and include NR, LeETR1, LeETR2, LeETR4-7 from tomato (Tieman and Klee, 1999), CS-ETR1, CS-ETR2 and CS-ERS from cucumber (Yamasaki et al., 2000), PE-ETR1 and PE-ERS1 from passion fruit (Mita et al., 2002), Cm-ETR1 and Cm-ERS1 from melon (Takahashi et al., 2002). All the above studies suggest that the ethylene signal transduction pathway is well conserved among other species as well. Recently, REVERSION-TO-ETHYLENE SENSITIVITY1 (RTE1) gene was identified as a positive regulator of ETR1 signal transmission (Resnick et al., 2006) that co-localizes with ETR1 in ER membranes thus supporting a model where RTE1 modifies ETR1 function (Chun-Hai et al., 2008). Over-expression of RTE1 confers reduced ethylene sensitivity that is largely dependent on the ETR1 locus (Zhou et al., 2007). Similarly, over-expression of the tomato RTE1 homolog, GREEN-RIPE (GR) provides a tool to assess apparent tissue specific ethylene signal transduction (Barry and Giovannoni, 2006).

CTR1 was isolated from Arabidopsis mutants that displayed a constitutive triple response even in the absence of ethylene and is considered as negative regulator of ethylene signal transduction pathway (Huang et al., 2003). CTR1 homologs have not been reported from other plant species except tomato where DDRT-PCR results point out to a gene having similarity to the CTR1 (Zegzouti et al., 1999). Tomato CTR1 gene family is differentially regulated at the mRNA level by ethylene during ripening (Adams-Phillips et al., 2004).

Genes related to cell wall softening: One of the major changes taking place during ripening is the softening of fruits. The action of various cell wall hydrolyses leads to rapid softening which in turn makes the fruit susceptible to cracking and pathogen attack. The tomato polygalacturonase (PG) is a major cell wall polyuronide degrading enzyme. It is transcriptionally activated during ripening and induction of PG mRNA occurs at very low ethylene levels (Sitrit et al., 1999). Pectin methylesterase (PME) is responsible for de-esterification of the highly methyl-esterified polygalacturonans in the cell wall during ripening. Esterification drops from 90% in mature green tomato fruit to 35% in red ripe fruit and this makes the polyuronides susceptible to degradation by PG (Carpita and Gibeaut, 1993). Tomato plants expressing a ripening-related antisense Rab11 GTPase gene show reduced levels of PG and PME and reduced fruit softening, indicating that Rab11 GTPase plays a role in trafficking of cell-wall modifying enzymes (Lu et al., 2001). Apart from these, β-galactosidase plays a role in breakdown of polymeric galactose into free galactose. This enzyme is encoded by multi gene family, all
S-adenosyl methionine

1-amino-cyclopropane-1-carboxylic acid

ETHYLENE

Perception by receptor (ETR)

unnamed

i) Housekeeping genes remain unchanged

ii) Some genes switched off

iii) Ripening genes switched on

Changes in cell wall metabolism

Production of coloured carotenoids

Synthesis of new flavoured and aroma volatiles

Respiratory climacteric

Loss of chlorophyll + chloroplast thylakoids

Further stimulation of gene expression

Enhanced (autocatalytic) ethylene biosynthesis

Fig. 1: Schematic representation of interactions between ethylene biosynthesis, signal transduction and fruit ripening with tomato as a model system (Gray et al., 1994).

Fig. 2: Changes in appearance of banana fruit during ethylene induced ripening. Change in peel colour from day 0, 2 and 4 during ethylene induced ripening is similar to peel colour index (PCI) given by Von Loesecke (1950)
of which display different patterns of expression during fruit ripening (Smith and Gross, 2000). Analysis of β-galactosidase suppressed transgenics and ripening mutants has shown that member, TBG4 may be regulated by ethylene and that strong suppression of β-galactosidase activity early in ripening can reduce fruit softening by up to 40% (Carey et al., 1995).

Database searches identified 25 tomato XTHs among which six viz. SIXTH1, 2, 3, 5, 9 and 10 were expressed during fruit ontogeny. Of the six XTHs in tomato SIXTH5 abundantly expressed at the onset of, and throughout, fruit ripening (Saladie et al., 2006). Expansins are cell wall localized proteins that are thought to cause cell wall loosening by reversibly disrupting the hydrogen bonds between cellulose microfibrils and matrix polysaccharides (Cosgrove, 2000). LeExp1 in tomato, MaExp1 in banana and MiExpA1, in mango, are under transcriptional control of ethylene and rapidly accumulate after ethylene treatment (Trivedi and Nath, 2004; Sane et al., 2005). An ethylene induced cDNA (LeCRK1), encoding a novel isoform of calcium-dependent protein kinase (CDPK) has been isolated from tomato cDNA library having role in the ripening (Leclercq et al., 2005). These studies suggest that several enzymes responsible for softening of fruits during ripening are regulated by ethylene, but their precise role in fruit softening remains to be elucidated.

Genes related to sweetening of the fruits: Different genes play predominant role in the massive conversion of starch into hexose sugar which leads to sweetening of the fruit. Among them, Sucrose phosphate synthase (SPS) catalyzes the synthesis of sucrose from UDP-glucose and fructose-6 phosphate (Winter and Huber, 2000). The conversion of starch to sucrose was associated with SPS activity. Invertase (β-fructofuranosidase) is a sucrase enzyme that catalyzes the hydrolysis of sucrose to fructose and glucose imparting sweetness to fruits. In addition, β-fructosidase also causes the conversion of sucrose into hexose sugar leading to sweetening of ripening fruits. This gene has been isolated from cherry (Krishnan and Pueppke, 1990) and tomato (Kiann et al., 1993).

Genes related to volatile production: The characteristic flavour of edible fruits results from the aroma volatiles produced within the fruit during ripening. These aroma volatiles are formed by several different pathways that includes the action of lipoxygenase (LOX), alcohol dehydrogenase, alcohol acyl transferase and aldehyde dehydrogenase. Tomato LOXs consists of at least five genes, TomloxA, TomloxB, TomloxC TomloxD and TomloxE that are involved in flavour volatile production (Griffiths et al., 1999). Alcohol dehydrogenase (ADH) has also been shown to play a role in hexanol and hexenol accumulation in tomato fruit (Speirs et al., 1998). Two ADHs have been identified in tomato among which ADH2 accumulates during the later stages of ripening (Longhurst et al., 1994). Alcohol acyl transferase is capable of producing esters from a wide range of combinations of alcohols and acyl-CoAs, responsible for production of distinct flavour of different fruit during ripening (Souleyre et al., 2005). AATs have been isolated from ripening fruits of strawberry (Aharoni et al., 2000), melon (El-

Role of other differentially expressed genes involved in fruit ripening: Apart from the genes that are involved in ethylene biosynthesis and signal transduction, cell wall hydrolysis, volatile formation and those related to sweetening of the fruit as discussed in earlier section. There are still thousands of genes that are differentially expressed during the course of ripening but their roles are not very well elucidated in the process. These additional genes may be involved...
in transcriptional and post-transcriptional regulation, stress-related responses, pigment biosynthesis and several with unidentified functions whose action in the ripening process was not very obvious but which are now known to play a crucial role in ripening.

Some of these are--HyPRP from strawberry, involved in polyphenol anchoring (Blanco-Portales, 2004); GH3-like gene from pungent pepper expressed in fruit when auxin levels were decreasing (Liu et al., 2005); dioxygenase1 genes from tomato involved in formation of terpenoid compounds (Simkin et al., 2004); E,E-alpha-farnesene synthase gene from apple involved in biosynthesis of terpenes (Pechous and Whitaker, 2004); phytoene synthase (PSY1) gene catalyzes the formation of phytoene (Bartley and Scolnik, 1993); Myb-related genes from grape regulates anthocyanin biosynthesis (Kobayashi et al., 2003); E4/E8 genes are transcriptionally activated by ethylene (Martinez et al., 2004); ACO from apple (Atkinson et al., 1998); cucumisin from melon (Yamagata et al., 2004); E8 genes are transcriptionally activated by ethylene (Deikman et al., 1998); DNA helicases and DNA methyl transferases, involved in carrying out changes in DNA structure and function (Jones et al., 2000); MADS-box gene (LeMADS-RIN) from tomato providing molecular insight into developmental regulation of ripening (Vrebac et al., 2002) and a transcription factor ASR (ABA stress ripening related) protein from apricot (Mbegue-A-Mbegue et al., 1997) has been identified during ripening. In addition to above, several other novel genes were isolated by various workers such as, dehydrin cDNA from orange and grape fruits (Porat et al., 2004), L-galactono-1,4-lactone dehydrogenase, melon (Paterson and Kanelis, 2004), for improvement of fruit quality. These additional genes could also be utilized to produce antisense transgenic plants with delayed ripening as was demonstrated using the Rab11 GTPase in tomato (Lu et al., 2001).

**Fruit specific and ripening related promoters/cis-elements:** The study of the promoters of fruit specific and ripening related gene would help in the expression of the gene of interest in tissue specific manner. Regulation of the expression of a particular gene involves the binding of specific trans-acting factors to the cognate cis-elements. This constitutes a crucial step in transcriptional initiation and governs the spatial and temporal expression of a number of inducible genes. Promoters from some of ripening related genes have been isolated from many fruits such as E8 (Deikman et al., 1998) and 2A11 (Van Houck et al., 1993) from tomato; ACO from apple (Atkinson et al., 1998); cucumisin from melon (Yamagata et al., 2004); E4/E8 genes are transcriptionally activated by ethylene (Deikman et al., 1998); DNA helicases and DNA methyl transferases, involved in carrying out changes in DNA structure and function (Jones et al., 2000); MADS-box gene (LeMADS-RIN) from tomato providing molecular insight into developmental regulation of ripening (Vrebac et al., 2002) and a transcription factor ASR (ABA stress ripening related) protein from apricot (Mbegue-A-Mbegue et al., 1997) has been identified during ripening. In addition to above, several other novel genes were isolated by various workers such as, dehydrin cDNA from orange and grape fruits (Porat et al., 2004), L-galactono-1,4-lactone dehydrogenase, melon (Paterson and Kanelis, 2004), for improvement of fruit quality. These additional genes could also be utilized to produce antisense transgenic plants with delayed ripening as was demonstrated using the Rab11 GTPase in tomato (Lu et al., 2001).

**Table - 1:** Transcript size and sequence homology of various genes isolated by differential display from banana fruit pulp RNA (Gupta et al., 2006)

<table>
<thead>
<tr>
<th>Clone</th>
<th>Regulation</th>
<th>Transcript size (Kb)</th>
<th>Expression (Specificity)</th>
<th>Best hits (BLASTx)</th>
<th>Acc. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1-4</td>
<td>Up</td>
<td>1.5</td>
<td>FS, RS</td>
<td>Iroquois homeobox protein like (rat and human)</td>
<td>DQ298191</td>
</tr>
<tr>
<td>H1-26</td>
<td>Up</td>
<td>1.1</td>
<td>FS, RS</td>
<td>No significant homology</td>
<td>CF542264</td>
</tr>
<tr>
<td>H1-35</td>
<td>Up</td>
<td>1.2</td>
<td>ND</td>
<td>ABA stress ripening protein like</td>
<td>DQ298189</td>
</tr>
<tr>
<td>H2-10</td>
<td>Down</td>
<td>1.2</td>
<td>FS</td>
<td>Unknown (Arabidopsis)</td>
<td>DQ298195</td>
</tr>
<tr>
<td>H3-5</td>
<td>Up</td>
<td>1.35</td>
<td>FS, RS</td>
<td>β-1,3-glucanase of M. acuminata</td>
<td>CF519299</td>
</tr>
<tr>
<td>H3-15</td>
<td>Down</td>
<td>1.45</td>
<td>FS, RS</td>
<td>No significant homology</td>
<td>CF542224</td>
</tr>
<tr>
<td>H4-1</td>
<td>Up</td>
<td>1.4</td>
<td>FS, RS</td>
<td>Isoflavone reductase like</td>
<td>CF519305</td>
</tr>
<tr>
<td>H4-11</td>
<td>Up</td>
<td>0.9</td>
<td>FS, RS</td>
<td>Conserved hypothetical protein (C. perfringens)</td>
<td>CF542265</td>
</tr>
<tr>
<td>H6-1</td>
<td>Down</td>
<td>1.3</td>
<td>ND</td>
<td>Auxin/Al responsive protein-like</td>
<td>CF519304</td>
</tr>
<tr>
<td>H6-9</td>
<td>Down</td>
<td>1.4</td>
<td>FS</td>
<td>Auxin/Al responsive protein-like (B. napus)</td>
<td>CF519300</td>
</tr>
<tr>
<td>H6-10</td>
<td>Down</td>
<td>0.75</td>
<td>ND</td>
<td>Unnamed product from H. sapiens</td>
<td>CF542263</td>
</tr>
<tr>
<td>H6-19</td>
<td>Down</td>
<td>0.8</td>
<td>FS</td>
<td>Unknown (Arabidopsis)</td>
<td>DQ298194</td>
</tr>
<tr>
<td>H8-19</td>
<td>Up</td>
<td>0.8</td>
<td>RR</td>
<td>Invertase/PME inhibitor from rice</td>
<td>DQ298193</td>
</tr>
<tr>
<td>H9-2</td>
<td>Up</td>
<td>2.0</td>
<td>RR</td>
<td>Unknown protein (Bos taurus)</td>
<td>DQ298190</td>
</tr>
<tr>
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<td>RR</td>
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<tr>
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<td>FS, RS</td>
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<td>DQ298188</td>
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<td>ND</td>
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<td>H10-1</td>
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<td>1.6</td>
<td>RR</td>
<td>Pectate lyase of M. acuminata</td>
<td>CF519302</td>
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<tr>
<td>H12-5</td>
<td>Down</td>
<td>1.8</td>
<td>FS</td>
<td>No significant homology</td>
<td>CF569230</td>
</tr>
<tr>
<td>H12-8</td>
<td>Down</td>
<td>1.4</td>
<td>RR</td>
<td>No significant homology</td>
<td>CF542267</td>
</tr>
<tr>
<td>H13-2</td>
<td>Up</td>
<td>1.7</td>
<td>RR</td>
<td>Transcriptional co-repressor like/regulatory proteins from Arabidopsis, rice, sorghum</td>
<td>DQ298192</td>
</tr>
<tr>
<td>H14-4</td>
<td>Up</td>
<td>0.65</td>
<td>ND</td>
<td>No significant homology</td>
<td>CF542268</td>
</tr>
</tbody>
</table>

FS: Fruit specific expression; RS: Ripening specific expression; RR: Ripening related; ND: Not studies in detail
element necessary for fruit-specific expression of the cucumisin gene. The ethylene responsive promoters from tomato, apple, strawberry and peach do not share homology in their ethylene responsive elements, which suggest that these elements might be species specific. Moreover, the detailed mechanisms that regulate the expression of differentially expressing genes during fruit ripening are poorly understood, as many of the essential cis-elements and trans-factors have not been identified yet. Promoters of fruit-specific genes may then only be utilized for use in strategies to manipulate fruit ripening and produce valuable proteins such as antibody and edible vaccines through methods of genetic engineering (Daniell et al., 2001).

Need for further investigation of ripening related novel genes and promoters in banana: The complete understanding to the hormonal control of ripening of banana is lacking due to insufficient data on endogenous phytohormone levels and the effect of exogenously applied hormones on the regulation of banana ripening (Mc Glasson et al., 1978; Yang and Hoffman, 1984). The banana ripening is far more complicated and cannot be explained only on the basis of few genes and promoters that have so far been identified. Thus there is constant need to identify newer genes and promoters, which are ripening related in order to make out a clear cut picture about how the process of banana ripening starts, proceeds and culminates in senescence and also to improve quality of the fruit in a better way. Some of the important studies carried out with respect to banana ripening are discussed below.

Changes associated with banana ripening: A sharp rise in respiratory rate (Beaudry et al., 1987) and ethylene biosynthesis (Pathak et al., 2003) were observed during banana fruit ripening. Other characteristic changes during ripening in banana are conversion of starch to sugars mainly sucrose, fructose and glucose, gradual degreening of the peel as a result of chlorophyll breakdown by chlorophyllase (Druy et al., 1999) that is used to define the ripening stages and indicated as PCI number, 1 (very green) to 7 (yellow flecked with brown), increase in the activity of alcohol acetyl transferase, which catalyzes the synthesis of isoamyl acetate, a major characteristic aroma compound of banana fruit (Wylie and Fellman, 2000).

Ethylene biosynthetic enzymes during banana ripening: Studies on ethylene biosynthetic genes in banana have revealed the presence of at least 3 ACS genes (MaACS1, 2 and 3) and more than six ACO genes (Lopez-Gomez et al., 1997). Of the three ACS genes, only MaACS1 was expressed in fruit while MaACS2 was induced by wounding. Of the nine sequences ACO genes studies only on two of them have been published. The levels of mRNA corresponding to both Mt-ACO1 and Mt-ACO2 were increased during ethylene induced fruit ripening (Do et al., 2005).

Cell wall degrading enzymes during banana ripening: In banana, extensive softening of the pulp occurs during ripening by various cell wall degrading enzymes that may either act sequentially or synergistically. The enzyme activities of PME, PL and PG (Lohani et al., 2004) have been studied in banana fruits and except for PME, the genes for Pectate lyase (PL) (Dominguez-Puigjaner et al., 1997) and PG (Asif and Nath, 2005) have been cloned from banana. Four cDNA clones of PG, MAPG1, MAPG2, MAPG3 and MAPG4 have been reported and their differential expression during ripening in banana was studied (Asif and Nath, 2005). Different forms of expansins like MaExp1 (Trivedi and Nath, 2004), MaEXP2, MaEXP3, MaEXP4 and MaEXP5 (Asha et al., 2007) from banana fruit were identified. All these expansins were upregulated during ripening and maximum transcript accumulation was observed for MaEXP2. Other enzymes like chitinase, endochitinase, β-1-3-glucanse and β-glucosidase like protein (Medina-Suarez et al., 1997) have been shown to be expressed in ripe fruit but have not been studied in detail.

Other enzymes playing role in banana ripening: A number of other enzymes associated with banana ripening have been identified and characterized. Most of these enzymes are involved in carbohydrate metabolism and softening (Mota et al., 2002), whereas others showed antifungal activity (Peumans et al., 2002).

Differential screening of banana cDNA library led to the identification of many ripening related genes encoding enzymes such as malate synthase (Pua et al., 2003); cytochrome P450 (Pua and Lee, 2003); acidic chitinase type III (Peumans et al., 2002); polyphenol oxidase (Gooding et al., 2001); BanLec (Jin et al., 2004); SPS (Nascimento et al., 1997); isoamylase-type starch-debranching enzyme (Bierhals et al., 2004); starch synthase and granule bound starch synthase (GBSS) (Mota et al., 2002); β-1,3-glucanase (Peumans et al., 2000); β-glucosidase like protein (Medina-Suarez et al., 1997) and a thiamatin like protein (Clendennen and May, 1997). Recently, many differentially expressed ripening related genes were isolated from banana fruit using suppression subtractive hybridization (SSH) technique (Manrique-Trujillo et al., 2007; Kesari et al., 2007) and differential display reverse transcription-PCR (Gupta et al., 2006). The role of ethylene in the regulation of these transcripts has been well documented.

Conclusion

Identification of additional components involved in various aspects of ripening and their characterization is important not only to understand how a complex process such as ripening is governed but also because of the tremendous potential these studies have for biotechnological application. Identification of fruit specific promoter/cis elements responsible for ripening may be utilized to develop transgenics with delayed fruit ripening which in turn will reduce the monetary loss that at present is approximately to the tune of Rs 15,000 crores per annum in our country due to the over-ripening of fruits like tomato, banana, papaya and mango. An added advantage of expressing genes in targeted tissue is that it reduces the impact of transgene expression on the normal growth and development of the plant while enabling the production of desired product in easily harvested tissue such as fruit. Such study may contribute in understanding the gap area of fruit ripening and in long run it may also contribute in addressing the problems being faced in fruit spoilage.
References


Méditerranéens


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Research in Environment and Life Sciences 88 November, 2008
Role of differentially expressed ripening related genes


