Biotechnological approaches in post harvest management: an overview
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Post harvest physical and pathological damage:
Mechanical injuries causing bruising lead to water loss, fungal infection and stimulate respiration and ethylene production leading to a loss in quality.

Environmental Factors Influencing Deterioration:
- Temperature
- Relative humidity
- Atmospheric composition
- Amounts of O\textsubscript{2} or CO\textsubscript{2}
- Ethylene
- Light

India is the largest producer of choicest varieties of mangoes in the world.
- India's mango annual production is over 10 million tons.
- Post harvest losses are estimated to be around 25 -30 per cent of total produce due to improper handling and storage practices, which amounts to over Rs. 250 crore (~US$ 50 million).
- Remarkable improvements have been made in the post harvest handling and storage of mangoes over past years.

Ripening Process
The ripening process has evolved as a seed dispersal mechanism. In the case of fleshy fruits, as the seeds mature the surrounding tissues undergo profound changes in composition that alter the texture, flavour, aroma and nutritional value. These changes serve as attractants to seed dispersing organisms by converting an unpalatable tissue into one that is attractive and nutritionally rich.

Ripening
Ripening is a normal phase in the maturation process of fruits and vegetables. Upon its onset, it only takes about a few days before the fruit or vegetable is considered inedible. This unavoidable process brings significant losses to both farmers and consumers alike. Efforts are being made to delay fruit ripening so that farmers will have the flexibility in marketing their goods and ensure consumers of "fresh-from-the-garden" produce.

Typical changes that occur during ripening
- Changes in colour
- Softening and associated alteration in texture
- Production of volatiles and flavour compounds
- Altered sugar and organic acid metabolism
- Increase in pathogen susceptibility
- Increase in membrane permeability which releases compartmentalized enzymes.
- Increase in protein (enzyme) synthesis
Carbohydrate Changes

- Conversion of starch to sugar - not desirable in potato but very desirable in apple, banana.
- Conversion of sugar to starch - not desirable in sweet corn but very desirable in potato.
- Conversion of starch and sugars to CO$_2$ and water during respiration - not desirable because it leads to a reduction in quality.

Pigment Changes

- Chlorophyll - a loss of chlorophyll in tomatoes is desirable but a loss in chlorophyll in broccoli is undesirable.
- Carotenoids (yellow, orange and red colour) - are desirable in fruits such as apricots, peaches and citrus giving them their yellow and orange colour. In tomatoes and pink grapefruit, a specific carotenoid, lycopene gives them their red colour.
- Anthocyanins (red and blue colour) - give red and blue colour to apples, berries, cherries and other fruits.
- Phenolic compounds - are responsible for tissue browning.

Other Changes

- Organic acids (affects sweetness)
- Proteins (affects texture)
- Amino acids (affects flavour)
- Lipids (affects flavour)

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Fruit Ripening

Ripening is a process in fruits that causes them to become edible. The fruit becomes sweeter, less green and softer. Ripening is associated with change in composition i.e. conversion of starch to sugar.

However, all fruits do not ripen in the same manner. On the basis of their ripening process, fruits are classified as:

- Climacteric Fruits
- Non-Climacteric Fruits

Climacteric Fruits

- Climacteric Fruits are defined as fruits that enter 'climacteric phase' after harvest i.e. they continue to ripen off the tree/plant.
- Most ripened climacteric fruits are too soft and delicate to withstand rigour of transport and repeated handling.
- These are harvested hard and green, but fully mature.
- Ripening is done near the consumption areas.
Classification on the basis of the mechanism of ripening

- Climacteric fruits
  - Fruits that will ripen in response to ethylene.
  - Examples: Mango, Banana, Papaya, Guava, Sapota, Kiwi, Fig, Apple, Passion fruit, Apricot, Plum, Pear

- Non-climacteric fruits
  - Fruits that do not ripen in response to ethylene, once harvested, they do not ripen further.
  - Examples: Orange, Mosambi, Kienow, Grape fruit, Grapes, Pomegranate, Litchi, Watermelon, Cherry, Raspberry, Blackberry, Strawberry, cas hew, lemon

How is ripening controlled in non-climacteric fruit?

Far less is known partly because some of the systems are not easily studied.

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Major changes during tomato fruit development and ripening:
Relative changes in cell division, cell expansion, respiration, ethylene synthesis, fruit softening, and carotenoid accumulation are shown over the course of fruit development. The time from anthesis (a) to mature green (MG; fully expanded immature fruit with mature seed), breaker (BR; first visible carotenoid accumulation), and red ripe (RR) can vary substantially among cultivars.

Giovannoni, Plant Cell 2004
Ethylene plays a active role in plant growth development

- Shoot and root growth and differentiation
- Dormancy
- Adventitious root formation
- Stimulates leaf and fruit abscission
  - Flower induction
  - Stimulates flower opening
  - Flower and leaf senescence
  - Fruit ripening

Ethylene
Ethylene is a natural plant hormone associated with the growth, development, ripening and aging of many plants. This phytohormone is said to promote ripening in a variety of fruits including bananas, pineapples, tomatoes, mangoes, melons, and papayas. It is produced in varying quantities depending on the type of fruit. But when the concentration of ethylene reaches 0.1-1.0 ppm (parts per million), the ripening process in climacteric fruits is considered irreversible.

Effects of Ethylene in Ripening

- Conversion of starch to sugars via hydrolysis
- Cell wall degradation and tissue softening
- Synthesis of pigments
- Synthesis of flavours

Factors that Increase Ethylene

- Maturity at harvest (Ripening signals lead to burst of ethylene production)
- Physical injury (wounding)
- Picking fruit
- Infection by pathogens such as bacteria, fungi etc.
- High or low temperature
- Water stress

Control of Ethylene Production

- Reducing the storage temperature
- Treating with silver thiosulphate (commonly used in flowers)
- Reducing O₂ levels to less than 8%
- Treating with enzyme inhibitors of ACC synthase and ACC oxidase
- Genetic engineering (for example use of antisense technology) to prevent enzyme expression and in turn slowing the processes involved in ripening resulting in increased shelf-life.

Climacteric fruits are usually harvested once they have reached maturity which then undergoes rapid ripening during transit and storage. Important tropical fruits such as banana, mango, papaya, pineapple and guava are examples of these fruits. Non-climacteric fruits do not ripen after harvest. Thus, in order to attain full ripeness and flavor, these fruits such as strawberries and oranges, are often harvested once they have fully ripened.

Normally, farmers pick their produce while they are still green. The ripening process is then induced by spraying the fruits or vegetables with ethylene gas when they reach their destination. For long hauls, fruits and vegetables are refrigerated to prevent damage and delay their ripening. However, there are drawbacks to these postharvest practices. Fruits that have been harvested prematurely may result in poor taste and quality despite appearing as fully ripened ones. Fruits transported for long periods under refrigeration also have the tendency to lose their quality.
Regulation of Ethylene Production
The amount of ethylene produced can be controlled primarily by "switching off" or decreasing the production of ethylene in the fruit and there are several ways to do this. They include:

- **Suppression of ACC synthase gene expression:** ACC (1-aminocyclopropane-1-carboxylic acid) synthase is the enzyme responsible for the conversion of S-adenosylmethionine (SAM) to ACC; the second to the last step in ethylene biosynthesis. Enzyme expression is hindered when an antisense ("mirror-image") or truncated copy of the synthase gene is inserted into the plant's genome.

- **Insertion of the ACC deaminase gene:** The gene coding for the enzyme is obtained from *Pseudomonas chlororaphis*, a common nonpathogenic soil bacterium. It converts ACC to a different compound thereby reducing the amount of ACC available for ethylene production.

Control of Ethylene Perception:
Since ethylene signals the onset of fruit ripening, delayed ripening on some plants can be achieved by modifying their ethylene receptors. The gene ETR1 is one example, and it has been shown to encode an ethylene binding protein. Plants with modified ETR1 lack the ability to respond to ethylene.

**Suppression of Polygalacturonase Activity:**
Polygalacturonase (PG) is the enzyme responsible for the breakdown of pectin, the substance that maintains the integrity of plant cell walls. Pectin breakdown occurs at the start of the ripening process resulting in the softening of the fruit. To produce a fruit with DR trait using this method, scientists insert an anti-sense or a truncated copy of the PG gene into the plant's genome resulting in a dramatic reduction of the amount of PG enzyme produced thereby delaying pectin degradation.

Ethylene biosynthesis
Ethylene is synthesized from methionine with ACC synthase as the rate limiting enzyme

\[
\text{Met} \rightarrow \text{SAM} \rightarrow \text{ACC} \rightarrow \text{H}_2\text{C} = \text{CH}_2
\]

AM synthetase \( \rightarrow \) ACC oxidase

\[
\text{SAM} = \text{S}-\text{adenosyl-methionine}
\]

\[
\text{ACC} = \text{1-aminocyclopropane-1-carboxylic acid}
\]

- **Insertion of the SAM hydrolase gene:** This approach is similar to ACC deaminase wherein ethylene production is hindered when the amount of its precursor metabolite is reduced; in this case SAM is converted to homoserine. The gene coding for the enzyme is obtained from *E. coli* T3 bacteriophage.

- **Suppression of ACC oxidase gene expression:** ACC oxidase is the enzyme which catalyzes the oxidation of ACC to ethylene, the last step in the ethylene biosynthetic pathway. Through anti-sense technology, down regulation of the ACC oxidase gene results in the suppression of ethylene production, thereby delaying fruit ripening.

Flavr Savr (also known as CGN-89564-2), a genetically modified tomato, was the first commercially grown genetically engineered food to be granted a license for human consumption. It was produced by Calgene, and submitted to the U.S. Food and Drug Administration (FDA) in 1992. On May 17, 1992, the FDA completed its evaluation of the Flavr Savr tomato by concluding that the tomato "is as safe as tomatoes bred by conventional means." It was first sold in 1994, and was only available for a few years before production ceased in 1997. The tomato was made more resistant to rotting by adding an antisense gene which interferes with the production of the enzyme polygalacturonase. The enzyme normally degrades pectin in the cell walls and results in the softening of fruit which makes them more susceptible to being damaged by fungal infections. The modified tomatoes are picked before fully ripe and are then artificially ripened using ethylene gas which acts as a plant hormone. Picking the fruit while unripe allows for easier handling and extended shelf life. Flavr Savr tomatoes could be allowed to ripen on the vine, without compromising their shelf life. The intended effect of slowing down the softening of Flavr Savr tomatoes would allow the vine-ripe fruits to be harvested like green tomatoes without greater damage to the tomato itself. In this respect the Flavr Savr turned out to be a disappointment, as the antisense PG gene had a positive effect on shelf life, but not on the fruit's firmness, so the tomatoes still had to be harvested like any other unmodified vine-ripe tomatoes.
Grape production in India

- Grape occupies fifth position amongst fruit crops with a production of 1.28 MT from an area of 107,500 acres (43,000 ha).
- Grape is grown in three distinct agro-climatic zones: Sub-tropical, hot tropical and mild tropical climatic regions in India.
- Major varieties of grapes produced in India are: Thomson seedless, Ponaka, Anab-e-shah, Perlette, Bangalore blue, Pusa seedless, Beauty seedless etc.
- Maharashtra is a major producer followed by Karnataka.

Economic importance of Grapes

- **Wine Industry:**
  - Grapes are an important fruit for wine industry.
- **Medicinal Value of grapes:**
  - Leaves are used to stop bleeding, pain and inflammation of hemorrhoids.
  - Unripe grapes are used in treatments for TB and constipation and thirst.
  - Resveratrol, an alkaloid found in grapes skin shows anti-carcinogenic and anti-hypertension activities.
  - Ellagic acid found in grapes acts as a free radical scavenger.
A graph showing chitinase enzyme assay of different transgenic lines of grapevine cv. Pusa Seedless

A graph showing bacterial chitinase enzyme assay of different transgenic lines of grapevine

Chitinase Enzyme Activity
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
Control
PS-Chi4
PS-Chi9
PS-Chi15
PS-Chi18
Transgenic lines
Relative chitinase activity
(µ mol/min/mg protein)

Chitinase Enzyme Activity
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
Control
PS-ChiB-3
PS-ChiB-4
PS-ChiB-5
PS-ChiB-9
PS-ChiB-19
Transgenic lines
Relative chitinase activity
(µ mol/min/mg protein)

Challenges and Opportunities

- Fruit plants/trees are difficult to regenerate and transform
- Generation time is much longer
- Controversies associated with application of GM technology

Eighteen plant genomes have been sequenced and analyzed.

Since 2004, Brazil, South Africa and Canada have the highest average annual growth rate in hectare: 33%, 38% and 76%, respectively.

Source: International Service for the Acquisition of Agri-Biotech Applications.

Wayne Peng Nature Biotech 29: 302 April 2011
Crops with two or more stacked traits continue to be popular.

Source: International Service for the Acquisition of Agri-Biotech Applications.

CASE STUDY – INDIA
Adoption and Impact of Bt cotton (2002-08)

Source: Clive James, 2009
Conclusions

- Genetically modified crops are a reality and are being extensively grown in many countries.
- Total area under cultivations of GM crops in 2010 was ~148 (134) million hectares, and five countries i.e. USA (66.8), Brazil (25.5), Argentina (22.9), India (9.4) and Canada (8.8) accounted for ~ 133.3 (123.3) million hectares. India had around ~ 9.4 (8.4) million hectares under GM cotton. China (3.5), Paraguay (2.6), Pakistan (2.4), South Africa (2.2) and Uruguay had with 1.1 million hectares.
- Four crops namely soybean, maize, cotton and canola (with genes of herbicide or insect resistance or both) account for a major area.
- Many other GM crops are in pipeline or under field trials.
- The adoption of this technology has raised many ethical and social issues which have implications on human health, environmental sustainability, rights of consumers, intellectual property rights, farmers’ rights, commercialization of agriculture etc.
- The technology has a great potential but like other new technologies, apprehensions and issues of concern need to be addressed.

Thank you