BIOTECHNOLOGY AND POSTHARVEST QUALITY

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Biotechnology

- The application of molecular biology and novel analytical tools to:
  - **Identify** the ‘key’ genes that underscore important traits in living organisms.
  - **Modify** these genes to enhance selected traits.
What Determines Traits?

DNA → Gene → mRNA → Protein → Metabolite

Traits (Postharvest)
- Sweetness
- Texture
- Aroma
- Shelf-life
- Low microbial adherence

Postharvest Quality (PHQ)
- "The factors that ensure maximum income for producers and meet the nutritional and aesthetic needs of the consumer after horticultural crops are harvested." (Kader, 2002).

CONSUMERS
- Sweetness
- Texture
- Aroma
- Acidity
- Color
- Appearance
- Flavour
- Nutrition

PRODUCERS
- Shelf-life
- Reduced Browning
- Reduced Microbes
- Firmness
- Disease resistance
- Chilling-tolerance

Producers and Consumers sometimes have opposing wants
Traits Are Determined By The Genetic Make-Up (Genotype) Of The Organism And The Environment

Different Genotype

Normal tomato

Ripening mutant

Different Environment

Normal tomato

Using Biotechnology to Create Novel Postharvest Traits

Genetic modification of Plants
All Crop Plants Have Been Genetically Modified

**Biotechnological sophistication**

- Domestication
- Mutagenesis
- T.I.L.L.I.N.G
- Scientific Breeding
- Transgenic Technology
- Marker-Assisted Breeding

- 10,000
- 1903
- 1965
- 1994
- 2005
- 2009

*These timelines are approximate only*
Induced Mutations In Genes Can Also Lead to Novel Traits

```
Mutation
↓
Gene
↓
DNA
↓
mRNA
↓
Protein
↓
Metabolite
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New Trait ← Trait

Natural mutations creates diversity

The effects of natural genetic mutations in carrots

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Mutations are permanent changes in the DNA sequence. Mutations to the order of the A, T, G, C bases can result in different versions of a particular gene. The different versions of a gene are called alleles. One reason individuals of the same species do not have exactly the same traits is because they have different alleles. The color of fruits and vegetables is usually controlled by more than one gene, and there may be several alleles for each gene. The first cultivated carrots came from the area of Afghanistan and were purple or yellow. Traders carried them to Europe and the Mediterranean, where mutations occurred or they were crossed with wild varieties. This resulted in the orange carrots we are familiar with. Carrots may be white, yellow, orange, red, or purple, depending on the combination of alleles that they inherit.
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1. Domestication of Plants

5-10,000 years ago, natural mutations, changes in DNA, occurred in wild plants creating desirable traits. These 'mutants' were propagated by early farmers.

- Wild banana with seeds
- Cultivated banana - sterile

<table>
<thead>
<tr>
<th>Wild banana</th>
<th>Domesticated banana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild</td>
<td>Domesticated</td>
</tr>
<tr>
<td>Tawsin</td>
<td>Maize</td>
</tr>
</tbody>
</table>

2. Selective Breeding

- **Recipient Parent**
  - *Solanum lycopersicum*
- **Donor Parent**
  - *Solanum peruvianum*

Percent of "wild" genes:
- 50%
- 25%
- 12.5%
- 6.25%
- 3.125%
- 1.5%
- 0.75%

Using the wild tomato species as a source of novel genes.

Kent Bradford, Department of Plant Sciences, UC Davis
Postharvest traits developed through selective breeding

**Uniform Ripening**

1) A mutant 'uniform ripening' gene was bred into most tomatoes in 1920s (US).
2) Selection for harvesting was easier.
3) However the gene reduced sugars (10-15%) and flavor compounds.
4) Most heirloom tomatoes were not altered in this way.

**Longer Shelf-life**

- **Rin** is a natural Ripening mutant
- Poor organoleptic Quality but long shelf-life

**Normal tomato**
- With short shelf-life and good quality

- **Tomato with Longer shelf-life**
  - Good quality

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3. **Induced Mutations for Breeding**

Seeds* are exposed to a chemical or physical agent that causes a high frequency of changes in the DNA sequence of that organism. A mutation in a gene that determines an important trait will create a line with a new, desirable phenotype.

- 3088 mutant varieties worldwide from 170 different plant species including improved postharvest traits

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http://www-naweb.iaea.org/nafa/pbg/  *Other plant parts may be mutagenized as well
4. Transgenic Manipulation

- DNA sequence is introduced or transformed into an organism.
- The action of that gene, normally present in the plant, may be suppressed, overexpressed or modified.
- A new gene from another species may also be introduced.
- Crops so produced are often (but inaccurately) described as Genetically Modified Organisms (GMOs).
- Can create more novel traits than by Selective Breeding or Mutagenesis.

The Gene to be Modified Is Introduced into the Plant Using Bacterial Plasmid as Vectors

Plasmids are circular molecules of DNA naturally found in bacterial cells.
The Construct Is Integrated Into A Cell From Which A Whole Plant May Be Regenerated

DNA inserted in plant chromosome.

Only cells containing recombinant DNA survive and divide in culture.

Cells regenerate into transgenic plant.

Plantlet grow into plants with new traits.

Which method creates more genetic changes in a plant?

A. Mutagenesis
B. Transgenic manipulation.
C. Conventional Breeding.

Transgenic Manipulation Of Plants Offers Many Possibilities and has created a lot of angst among consumers

Plants with animal genes have **not** been marketed.

How many inaccuracies can you spot in this product description?

A. Two
B. Three
C. Four
D. Five
TRUTH: Few fruits and vegetables are transgenic i.e. ‘GMO’

**Transgenic Papaya Resistant To Papaya Ringspot Virus**

<table>
<thead>
<tr>
<th>Normal</th>
<th>Transgenic</th>
</tr>
</thead>
</table>

- Transgenic papaya accounts for 90% of all grown in Hawaii.
- Cultivated since 1999.

*California Agriculture vol 58 #2; http://CaliforniaAgriculture.ucop.edu*
Other Commercialized Transgenic Horticultural Crops

- **Ear Worm Resistant Sweet Corn**
- **Virus Resistant Squash**
- **Florigene Moonshadow carnations**
- **Roundup Ready Sugarbeet**

Other examples: Flavr Savr tomatoes – Extended Shelf-life. Discontinued in 1999

- Polygalacturonase a ‘fruit softening’ gene was suppressed.
- Transgenic fruits could be harvested ripe but withstand shipping and handling.
- Processing costs were 20% lower.

Chrispeels & Sadava, 2002 Plant, Genes and Crop Biotechnology.
Lab GMOs: Enhanced Shelf-life In Transgenic Apples: Ethylene Biosynthetic Gene ACO* Was Suppressed


Lab GMOs: Reduced Browning In Transgenic Apples With Low Levels Of PPO*


*ACO = 1-aminocyclopropane-1-carboxylic acid oxidase

*Polyphenol oxidase (PPO)
Lab GMOs: Transgenic Tomato Expressing An Anthocyanin gene.

Normal Red Tomato

Transgenic Purple Tomato

http://www.norfolkplantsciences.com/projects/ripening-tomatoes/

Database Of Plant GMOs Produced Worldwide
Transgenic Fruits & Vegetables – Where Are We?

• Between 2003-2008:
  • 313 publications on transgenic research of produce.
  • Research in diverse countries: USA, Europe, India, Japan, Brazil, South Korea, Israel, Tunisia among others.
  • 77 specialty type crops transformed with 206 traits altered.
  • Still only 4 transgenic lines currently on market for consumption: sweet corn, papaya, zucchini squash and sugar beet.

Little translation of technology from lab-to-table.

Limitations To Marketing GM Horticultural Crops

• **Expensive.**
  • Estimates of regulatory costs:
    • US$15 M per transgenic line.
    • Time to market: 10 - 17 years.
    • Total cost can be USD $100-150 million
  • Fruits and vegetables are niche crops, no economies of scale.

• **Public resistance.**
  • More intimate ‘association’ with fruit and vegetables. Not so with processed maize, soybean.
  • ‘Fear’ of the technology.
While some argue that transgenic plants are a cure-all, public opinion has been less than enthusiastic.

**Unresolved Issues**

- Gene transfer to non-GMO crops.
  - Disturbing evidence that native maize landraces in Mexico pollinated by GMO crops*
- Use of markers, bacterial plasmid; tissue culture.
- Not naturally found in plants; somaclonal variation.
- ‘Monopolization’ of currently used GMOs by seed companies.
  - Perception: Profits over humanity and the environment

Solution: Transgrafting. Use Transgenic Rootstock – Harvest Fruit From Non-Transgenic Scion...

Do you consider this plant to be GMO?

A. Yes
B. No
C. Not Sure


Solution: Engineer Plants With No Plasmid, No Antibiotic Selectable Gene And No Tissue Culture.

Normal

Transgenic

12 days at room temperature

Melon with the ACO gene silenced showed enhanced shelf-life. Process worked but was inefficient.

Mutant ACC Oxidase Melons Found By TILLING Have A Longer Shelf-life

Mutagenesis: Mutant plants are screened for altered trait
TILLING: Mutant DNAs are screened for altered gene.

Arcadia Biosciences (Davis, CA):
- Long shelf-life:
  - Strawberry
  - Lettuce
  - Tomato

http://www.arcadiabio.com/extendedshelflife
Smart Breeding: Finding DNA Markers Among Diverse Genotypes To Serve As ‘Tags’ for a Trait

Using DNA Markers In Plant Breeding: Marker Assisted Selection (MAS)
Potential Efficiency Of MAS

Collard et al 2005 Euphytica vol 142: 169

Several Products Have Been Bred by Marker Assisted Selection – Example of One With Enhanced Postharvest Trait - Beneforte

Broccoli were bred with 2-3 times the amount of glucoraphanin, a compound that stimulates the body’s antioxidant (defense) system.

http://www.superbroccoli.info/why-beneforte
Finding DNA Markers Is Feasible Because Genome Sequencing Is Relatively Cheap

1 Million bp or units of DNA sequence cost $5000 in 2001 now it costs just 6 cents

Whole Genome sequencing projects of several Horticultural crops are complete (this list will soon to be outdated)
Summary

• A repertoire of sophisticated tools have been developed that can be used to identify key ‘Postharvest’ genes and to alter the genetic makeup of crop plants.

• Transgenic manipulation is a successful way to introduce new traits but has not gained much traction.

• Marker Assisted Selection and TILLING may be viable alternatives.

• Genomics of horticultural crops has been revolutionized by cheap sequencing. This will help to select fruits, vegetables and nuts with improved postharvest traits.

• It still remains difficult to define “quality’ for many crops which hampers identifying gene and gene networks to permit targeted breeding approaches.

Online Resources

• http://sbc.ucdavis.edu/Outreach/Biotechnology_Tutorials_Online.htm
• http://www.agbioworld.org/
• http://californiaagriculture.ucop.edu/0402AMJ/toc.html
• http://californiaagriculture.ucanr.org/collectionview.cfm?collection=13786
• http://www.pacificbiosciences.com/
• http://www.nanoporetech.com
• http://www.gmo-compass.org/eng/home/
• http://tiling.ucdavis.edu/index.php/Main_Page
• http://solgenomics.net/
• http://www.washingtonpost.com/local/scientists-breed-a-better-seed-trait-by-trait/2014/04/16/ec8ce8c8-9a4b-11e3-80ac-63a8ba7f7942_story.html
• http://mvgs.iaea.org/PDF/Mutation%20Induction%20for%20Breeding%20101.pdf
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  - http://ubibiotech.org/resources/presentations/presentations.html retrieved 6/11/12
  - “What’s for dinner – Genetic engineering from the lab to your plate.
- Prakash C.S. : Agricultural Biotechnology”
- www.agbioworld.org retrieved 6/11/12
- US Regulatory Agencies Unified Biotechnology website
  - http://usbiotechreg.nbii.gov/lawsregsguidance.asp (No longer active)

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- Collard et al 2005 Euphytica vol 142: 169
- Colbert et al 2001 Plant Physiol. 126: 480-484