BIOTECHNOLOGY AND POSTHARVEST QUALITY

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BIOTECHNOLOGY

The application of molecular biology and novel analytical tools to:

- Identify ‘key’ genes underscoring traits
- 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

- “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

Flavour
Aroma
Textural quality
Shelf-life
Reduced disease resistance
Reduced microbial contamination
Reduced chilling injury

PRODUCERS

Atmospheric fluctuations

It is often difficult to define quality and the causal genes

Traits are determined by the genetic make-up (genotype) of the organism and the environment

Different Genotype

Normal tomato

Ripening mutant

25°C

Different Environment

Normal tomato

Ripening tomato

25°C

All crop plants have been genetically modified

These are all the products of human intervention. These plants have modified genomes that make them unfit for surviving in the wild.
1. Domestication of Plants

Wild banana with seeds

Cultivated banana - sterile

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

2. Selective Breeding

**ADVANTAGES**
- Very successful.
- No regulatory hurdles.
- Technology accessible and relatively cheap.
- Potential may be untapped.

**DISADVANTAGES**
- Limited to sexually compatible crosses. Therefore the amount of variation that can be introduced is limited.
- Slow. Cumbersome and inefficient.

Postharvest traits developed through selective breeding

**Uniform Ripening**

1. A mutant ‘uniform ripening’ gene was bred into most tomatoes in 1920s (US).
2. This made selection of the correct maturation stage at harvesting easier.
3. However, the mutation also reduced sugars (10-15%) and flavor compounds.
4. Most heirloom tomatoes were not altered in this way.

**Longer Shelf-life**

- This is a natural Ripening mutant with good organoleptic quality but long shelf-life.
- Normal tomato with short shelf-life and good quality.
- Tomatoes with longer shelf-life and good quality.
3. Marker Assisted Selection (MAS)

By applying genomics i.e. using DNA markers for selection of desirable progeny instead of the phenotype, the breeding time can be cut in half or more.

**ADVANTAGES**
- No regulatory hurdles.
- Marker development is becoming more accessible and cheaper.
- Enhances the efficiency of conventional breeding.

**DISADVANTAGES**
- Limited to sexually compatible crosses. Therefore the amount of variation that can be introduced is limited.
- Cost of markers can be very high.
- Markers are not always reliable when plants are grown in different environments.

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

Whole Genome sequencing projects of several Horticultural crops are complete (this list is already outdated)

Now, 1000 bp of DNA sequence cost less than 5 cents to produce. Genotyping of all crops will soon be feasible and will enable efficient MAS.

4. Mutagenesis

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.

**ADVANTAGES**
- Can introduce genetic change quickly
- Technology accessible and relatively cheap.
- Little knowledge of the genome of the target plant needed.

**DISADVANTAGES**
- Inefficient: relies on random events.
- Screening for new phenotype is slow and expensive.
- High failure rate if the aim is to select for a particular phenotype.

Mutagenesis has led to the production of 3088 varieties from 170 different plant species including some with improved postharvest traits.

http://www-naweb.iaea.org/nafa/pbg/*Other plant parts may be mutagenized as well.
5. Targeted Induced Local Lesions In Genomes (TILLING).


Applying genomics to mutagenesis. Treated plants are first screened for genes with altered sequences i.e. gene mutations not for an altered phenotypes.

Mutant ACC Oxidase melons found by TILLING have a longer shelf-life

- http://www.arcadiabio.com/extendedshelflife

- Mutant ACC Oxidase melons found by TILLING have a longer shelf-life

2. T.I.L.L.I.N.G

ADVANTAGES
- Can introduce genetic change quickly.
- Technology accessible and relatively cheap.
- No DNA is added to the plant.

DISADVANTAGES
- Inefficient: relies on random events.
- Moderate-high failure rate.
- Limited in the types of changes that can be introduced i.e. mainly loss-of-function.
- Gene target must be known.

Plasmids are circular molecules of DNA naturally found in bacterial cells.

Transgenic manipulation

Step 1

1. Plasmid (Vector) → Gene of interest → Splice gene into Plasmid vector → Gene Construct

The gene to be modified is usually introduced into the plant using bacterial plasmid as vectors.

The gene construct is integrated into a cell from which a whole plant may be regenerated

Step 3

1. DNA inserted in plant chromosome

Only cells with recombinant DNA survive and divide in culture

Cells regenerate into transgenic plant

Plantlet grow into plants with new traits

Transgenesis: Introducing the gene into the plant

Step 2

1. Direct transfer

Agrobacterium tumefaciens

DNA construct physically introduced into tissue

A soil bacterium that naturally inserts its plasmid DNA into the plant genome.
Which method creates more genetic changes in a plant?

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

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Summary of how plants are genetically modified

- Conventional Breeding
- Mutagenesis
- Marker-Assisted Selection
- Transgenic modification

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2. Transgenesis

ADVANTAGES
- Novel phenotypic variation can be introduced rapidly.
- Minimal alteration to the genome compared to other processes.
- Technology accessible and relatively cheap.

DISADVANTAGES
- Regulatory process for approving transgenic products for sale is very expensive and slow.
- Traditionally relied on using bacterial (foreign) genes.
- Many important traits in plants are complex and modifying these by knocking out single or multiple genes have not been successful.
- Distrust of GMOs by the public.

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Problem: GMOs create angst among the public and has driven the desire for labeling products as non-GMO.

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’

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One of first plant GMOs: Flavr Savr tomatoes was altered in a postharvest trait. Extended shelf-life. Discontinued in 1999

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Problems: Transgenic manipulation of plants offers many possibilities but has created a lot of angst among consumers

Please note: Plants with animal genes have not been marketed.

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* Moving away from this.

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Beckles, Diane “Biotechnology and Postharvest Quality”  
Postharvest Technology of Horticultural Crops Short Course 2015  
(c) Postharvest Technology Center, UC Regents
Lab GMOs: enhanced shelf-life in transgenic apples: ethylene biosynthesis suppressed

Control


Transgenic

Normal

At harvest

After 3 months at room temperature

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


Lab GMOs: transgenic tomato expressing an anthocyanin gene.

Normal Red Tomato

Transgenic Purple Tomato

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

Transgenic papaya resistant to Papaya Ringspot Virus

Normal Transgenic

- 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

Problem: Steady increases in the proportion of biotech crops grown but few are horticultural crops

Between 2003-2008:
- More than 77 specialty type crops transformed with 206 traits altered.
- Still only 5 transgenic lines currently on market for consumption: sweet corn, papaya, zucchini squash, sugar beet and innate tomato.

http://isaaa.org/resources/publications/pocketk/16/default.asp


Why there are few GM Horticultural Crops are on the market?

- Getting GM crops to market is expensive.
- Estimates of regulatory costs:
  - US$15 M per transgenic line, Time to market: 10 - 12 years.
  - Fruits and vegetables are niche crops, no economies of scale.
- Public resistance to GMOs.
- More intimate "association" with fruit and vegetables. Not so with processed maize, soybean.
- "Fear" of the technology.
- Difficult to obtain transformative robust genetic changes in keys traits that would make GM produce economical

What are the legitimate problems with GMOs?

- Gene transfer to non-GMO crops (evidence in maize).
  - SOLUTION: Better ‘Buffer’ growth zones
- Use of antibiotic markers, bacterial plasmid (foreign DNA).
  - SOLUTION: Marker-free systems*, cisgenics and intragenics*
- Positional effects due to non-specific insertion of plasmid
  - SOLUTION: Genome editing*
- ‘Monopolization’ of currently used GMOs by seed companies.
  - SOLUTION: Greater public investment in traits with humanitarian goals.

- Molecular analysis of GMOs produced over 20 years and modifying
  100s traits show them to be substantially equivalent to
  conventionally produced crops.
- Further, while consumption of GMOs is a personal decision, there is
  no credible evidence that current products are harmful to human or
  animal health.

Solution: Transgrafting

Do you consider this plant to be GMO?

A. Yes
B. No
C. Not Sure

Use transgenic rootstock – harvest fruit from
non-transgenic scion. Limited to traits altered
by mobile systemic proteins.

Cisgenics & Intragenics

DEFINITIONS OF KEY TERMS IN RELATION TO PLANTS

Cisgenics is the genetic modification of a
recipient plant with a natural gene from a
compatible – usually compatible – plant. Such a
gene includes its natural traits. Bacteria do not
interact with nor reproduce in the normal
sense orientation. Cisgenic plants can harbor
many non-GMO traits, but they do not contain
any transgene.

Intragenics: gene silencing by RNAi (RNA interference)

Design a palindromic DNA sequence so that the RNA
produced forms a hairpin

Introducing a DNA sequence in a hairpin
conformation into the cell causes the
destruction of all RNA with that identical
sequence. We say that the gene is silenced.

Transformation can be done without a plasmid vector construct.

‘Intragenic’ GMO melon: Plant ACO gene silenced by RNAi
showed enhanced shelf-life.

Normal
Transgenic

12 days at room temperature

Melon transformed with melon ACO RNAi hairpin sequence. No plasmid DNA, antibiotic
selectable gene, and no tissue culture used. The process worked but was inefficient.
Postharvest GMO products close to market: reduced browning in transgenic apples (NA)* and potatoes (A)*

Genes suppressed by RNAi technology

- NA = Not approved by FDA
- *A = Approved by FDA


Genome Editing: The exact region of the genome to be modified can be defined using methods such as CRISPR-CAS*


The CAS9 protein cuts at the region of the genome desired by the Researcher. The resulting mutation can alter gene function, or, a fragment of new DNA can be introduced at that specific region. CRISPR is fast, easy, accurate and cheap.


A need to rethink GMOs. Gene transfer among species may be more common than we originally thought

The genome of cultivated sweet potato contains Agrobacterium T-DNAs with expressed genes: An example of a naturally transgenic food crop


Summary

- Crop improvement techniques have moved away from a phenotype-based approach for selection to a DNA or genotyping approach. Marker Assisted Selection and TILLING use genotyping to improve the efficiency of traditional breeding and mutagenesis respectively.
- Transgenic manipulation is a successful way to introduce new traits into commercial lines but has not gained much traction. Investments in improving PH quality of specialty crops may not be profitable in the short term.
- Recent genome editing discoveries, plus the use of intragenics and cisgenics will make gene manipulation more accurate with fewer unintended consequences.
- It still remains difficult to define quality and hence genes that underscore "postharvest quality" in specialty crops, which hampers genetic improvement efforts.

Online Resources

- http://www.pbworld.org/
- http://californiaagriculture.ucop.edu/i402AM.jsp?loc=cal.PNG
- http://californiaagriculture.ucop.edu/pastview.cfm?collection=1376
- http://www.pacificbiosciences.com/
- http://www.nanoporetech.com
- http://tilling.ucdavis.edu/index.php/Main Page
- http://poligenomics.net/
- http://www.washingtonpost.com/local/scientists-breed-a-better-root-plant-by-7fa21f1b6a8b5d4b-11e3-80ac-83fe8e21f9d2_story.html
- http://images.lawe.org/PDF/Mutation%20Induction%20for%20Breeding%20101.pdf

References

- Chrispeels and Sadava, 2002 Plant, Genes and Crop Biotechnology; ASPB.
- "What’s for dinner - Genetic engineering from the lab to your plate." http://mail.wecdsb.on.ca/~a_altenhof/FOV1-000460CD/S0595122D.3/geneticapplications.pdf retrieved 6/11/12
- US Regulatory Agencies Unified Biotechnology website http://usbiotechreg.nbii.gov/lawsregsguidance.asp (No longer active)
### References (cont’d)

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

### Commonly asked questions regarding GMOs

- Is it necessary to label food containing GMOs?  
- Is Labeling GMOs a good idea?  
- Do GMOs contribute to a loss of biodiversity?  
- Are we properly exploiting natural variation in wild species for crop improvement? Wild species are untapped source of genetic diversity
- Wasn’t there a study that showed GMOs caused cancer in rats?  
  That GMO Rat Study?
- Did GMOs cause Indian Farmer Suicide?  
  [http://www.theguardian.com/environment/2008/nov/05/gmcrops-india](http://www.theguardian.com/environment/2008/nov/05/gmcrops-india)
- Does Monsanto Sells Suicide Seeds?  
- Do GMOs cause allergies?  
  Twenty Year Study of GMOs
- Are Heirlooms substantially different and more ‘natural’ than conventional tomatoes?  