Biotechnology and Postharvest Quality

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Overview of Topics

- Biotechnology.
- Postharvest Quality (PHQ).
- Transgenic manipulation.
- Targeted Induced Local Lesions IN Genomes (TILLING).
- Marker Assisted Selection.

Biotechnology

- A set of tools used to modify the genetic makeup of an organism.
- Produces new product
- Product perform new function(s)

Postharvest Quality

- The factors that ensure maximum income for producers as well as meeting the nutritional and aesthetic needs of the consumer after horticultural crops are harvested.
- Producers and consumers often have opposing needs

Postharvest traits are due to the interaction of the environment and genotype

Selective breeding for improved traits

Using the wild tomato species as a source of genes for nematode resistance

Six or more generations of backcrosses to the cultivated parent, selecting for resistance at each generation

Kent Bradford, Dept. Plant Sciences, UC Davis
All crop plants have been genetically modified

Transgenic manipulation
- Allows the transfer of genes between different organisms.
- Crops so produced are said to be Genetically Modified Organisms (GMOs).
- Native gene may be suppressed, overexpressed or modified.

Transgenes are expressed in the plant using bacterial plasmid as vectors.

Plasmids are circular molecules of DNA found in bacterial cells. They confer selective advantage e.g. survival on antibiotics, fertility etc to bacteria

Different methods of introducing the vector construct into the plant
1. Particle bombardment (random insertion of genes).
2. Agrobacterium tumefaciens (more precise).

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

Transgenic manipulation of plants offers many possibilities
- Can cross species barrier.
- NOTE: The famous “fishberry” was never pursued.
Transgenic papaya resistant to papaya ringspot virus

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

Other commercialized transgenic horticultural crops

Sweet corn resistance to earworm

Florigene Moonshadow carnations

Examples: Flavr Savr tomatoes – extended shelf-life

Transgenic plums resistant to plum pox virus

Non-transgenic fruit

Transgenic fruit

Delayed softening/ripening in ACC Oxidase (ACO) silenced transgenic apples

ACO silenced

At harvest

Control

ACO silenced

After 3 months at room temperature

Control


Database of GMO produced worldwide:

GMO Compass

Transgenic fruit & vegetables – where are we?

- From 2003-2008:
  - 313 publications on transgenic research of produce.
  - USA, Europe, India, Japan, Brazil, South Korea, Israel, Tunisia among others.
- 77 specialty type crops.
- 206 traits.
- Still only 4 transgenic lines currently on market: sweet corn, papaya, zucchini squash, carnations


Limits to marketing GMO Horticultural crops

- Several postharvest traits are complex
  - Multigenic; strongly affected by the environment. Difficult to engineer.
- Public resistance to the idea.
  - More intimate ‘association’ with fruit and vegetables than maize or soybean products
- Economies of scale.
  - Expensive to apply to niche crops and cultivars
  - Estimates of cost: US$15 M per transgenic line
- Technologies not sufficiently efficient for some crops.
  - Poor transformation efficiencies; long generation times - not tractable.

Grafting: Use transgenic rootstock – harvest fruit from control scion

- Limitations. Trait must be determined by:
  - Activity in root.
  - A transgene that moves systemically through the plant.
- But is the fruit really non-GMO?


Engineered melons: ACC Oxidase (ACO) knockdown – no plasmid, no markers and no tissue culture used.

Control  ACO1 knock down

Stored at room temperature for 12 days


While some argue that transgenic plants are a cure-all Public opinion has been less than enthusiastic

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Unresolved Issues

- Gene transfer to non-GMO crops.
  - Disturbing evidence that native maize landraces in Mexico pollinated by GMO crops*
- Positional Effects.
  - Disruption of native genes at the site where the construct is inserted**
- Use of markers, bacterial plasmid; tissue culture.
  - Not naturally found in plants; somaclonal variation.
- Monopolization/Concentration power by seed companies
  - They determine the traits worthy of investment. Humanitarian interests may not be prioritized.

* http://www.plantbiosciences.ucdavis.edu/techreports/mec_3993_LOW.pdf
Still Alternative approaches necessary!

What lessons can we learn from nature?

Spontaneous mutations in wild have created novel/useful traits

Wild banana with seeds Cultivated banana- sterile

- We can accelerate this natural process by subjecting seeds to mutagens. The mutant seeds are grown and the plants screened for useful traits.
- DNA may also be extracted from mutants and screened for defects in gene of interest (TILLING)

Targeted Induced Local Lesions In Genomes (TILLING)

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

Mutant population TILLED for lines with ACC Oxidase defective gene

Great genetic diversity exists - can we exploit it?

Breeding using Genomics

- Part of the variation in the population is due to differences in gene sequence.
- Ideally – identify the gene sequence that is the basis of the trait.
- Alternately – identify a marker sequence – one that is associated with, and can act as a proxy for the trait.
- Chances of finding good markers increases if the whole genome of a species is sequenced.
Cost of sequencing now cheap:
Whole genome sequencing of plants feasible.

Finding molecular markers for a trait occurs by trial and error

Marker-Assisted Selection (MAS)

Summary

- A repertoire of sophisticated tools have been developed to alter the genetic makeup of crop plants.
- Transgenic manipulation has proven to be very successful but has not gained much traction.
- Alternate approaches such as Marker Assisted Selection and TILLING may be viable alternatives.
- Genomics of horticultural crops will be revolutionized by Next-Gen sequencing.
Online Resources

- http://sbc.ucdavis.edu/Outreach/Biotechnology_Tutorials_Online.htm
- http://www.agbioworld.org/
- http://californiaagriculture.ucop.edu/0402AMJ/toc.html
- http://www.454.com/
- http://www.pacificbiosciences.com/
- http://www.nanoporetech.com
- http://www.gmo-compass.org/eng/home/
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