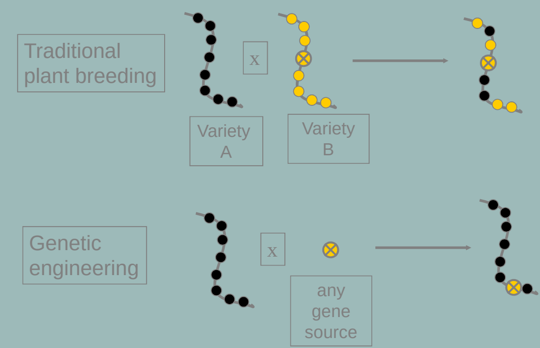
How are genetically engineered plants produced?
S. Strauss

Genetic engineering of plants



What are GMOs?

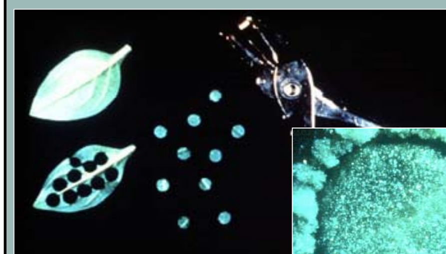
- GMO = genetically modified organism
  - Same as GE or GEO = genetic engineering *= creation of recombinant DNA modified organisms*
- It's the <u>method</u>: Native or "foreign" genes, modified traits or new traits
- **Genes isolated in chemical form, changed in a test tube, and re-inserted asexually**
  - Vs. making crosses or random mutations in conventional breeding
- Powerful breeding tool – BUT relatively <u>simple</u> traits can be <u>designed</u>…but without constraints from <u>native gene pools</u>
  - That's why its called genetic engineering, though we are slightly modifying, not building, a new organism

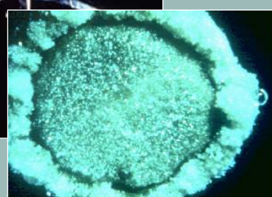
How are GMO crops produced

<u>Step 1</u>
Getting whole plants back from cultured cells = <u>organismal cloning</u>

Differentiation of new plant organs (shoots, roots, embryos) from single cells

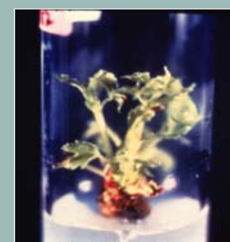
First step is de-differentiation into “callus” after treatment with the plant hormone auxin



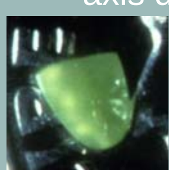


Leaf-discs

Shoots produced first, then roots, using specific plant hormones for each step

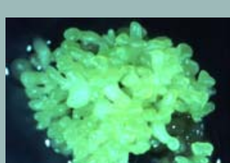


Somatic embryogenesis – shoot-root axis differentiated as a unit



Immature cotyledon

Somatic embryos



Repetitive embryogenesis = cloning

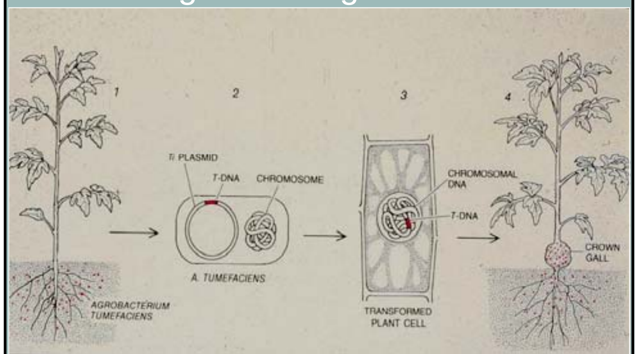
Step 2

Getting DNA into plant cells

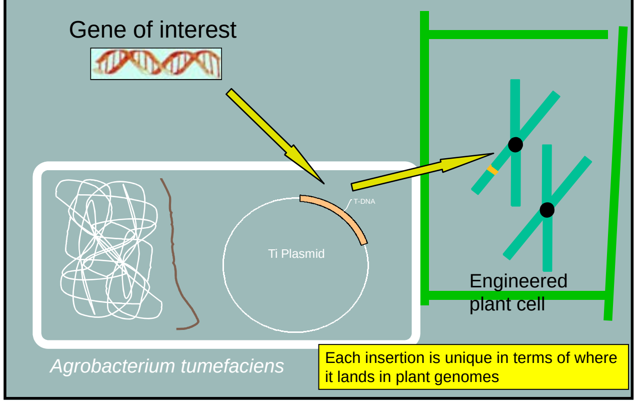
Main methods

- *Agrobacterium tumefaciens*
- Biolistics [gene gun]

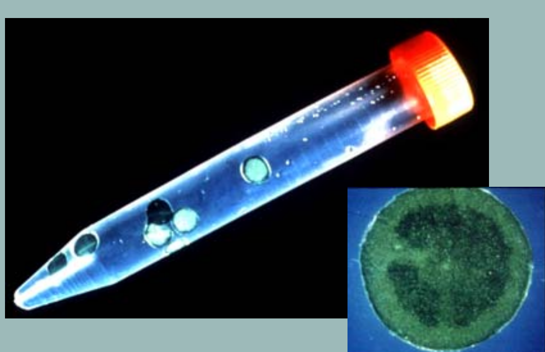
Agrobacterium is a natural plant genetic engineer



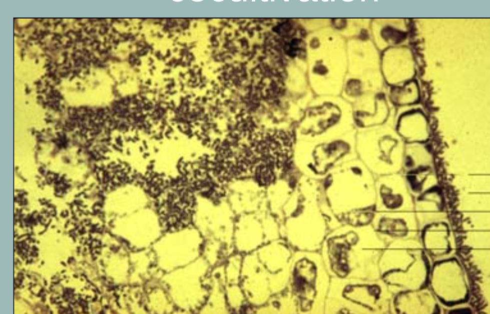
Agrobacterium engineering



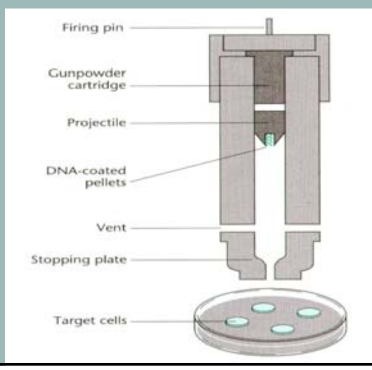
Cocultivation of Agrobacterium with plant tissues



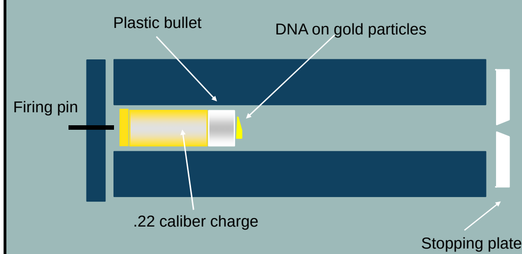
Agrobacterium in contact with wounded plant tissues during cocultivation



Gene gun bombardment of plant tissues in Petri dish

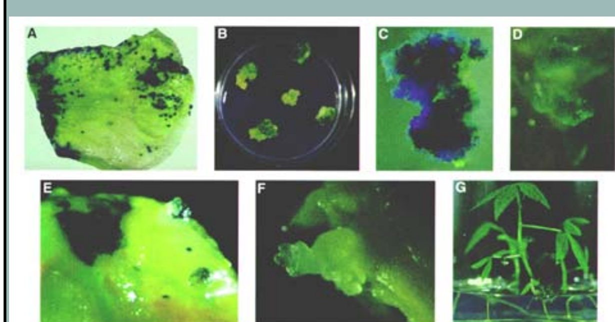


The gene gun



DNA coated metal particles after "gene-gun" insertion into tissues

Transgenic cassava via biolistics - GUS reporter gene

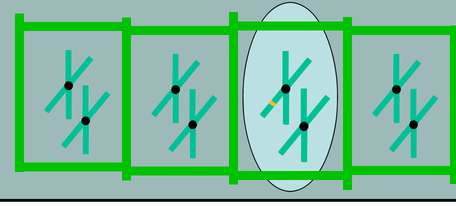


Step 3

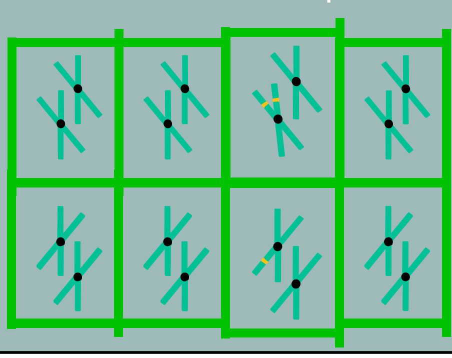
Selection of transgenic cells

Only a few cells get engineered

Challenge: Recover plants from that one cell so new plant is not chimeric (i.e., not genetically variable within the organism)

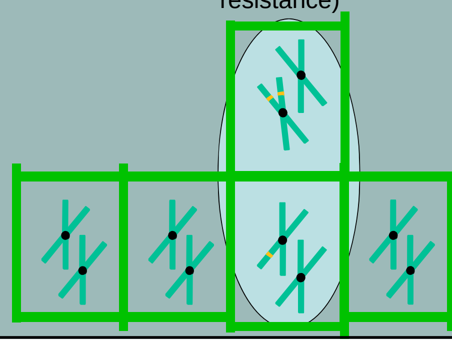


Hormones in plant tissue culture stimulate division from plant cells



Antibiotics in plant tissue culture limit growth to engineered cells

Other kinds of genes can also be used to favor transgenic cells (e.g., sugar uptake, herbicide resistance)



Antibiotic selection of transgenic tissues

Then plants are propagated normally (seeds, cuttings) and tested for health and new qualities

Propagation of poplars in tissue culture

Growth in the field

Poor control over transgene insertion

- Location in genome ~random
  - Chromosomal environment important to level and pattern of expression, *not just promoters*
  - Expression varies a great deal among gene insertion events
- Also varying among events
  - Number of copies varies from one to dozens
  - Orientation of gene varies
  - Genes can be turned off, or be unstable
- Gene gun with more random variation then Agrobacterium, thus gun little used today

Large consequences of poor transgene/transformation control for biotechnology

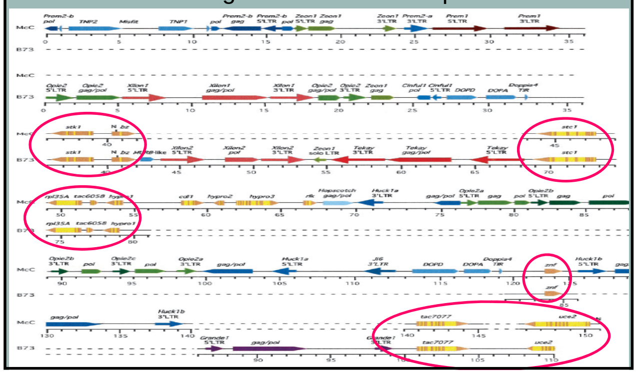
- Gene transfer and tissue culture process is itself mutagenic
  - Insertion into/near genes
  - Increased random mutation in genome from stress of hormones/new development path
- Thus, extensive selection after gene insertion for desirable events prior to commercial use
  - Dozens to hundreds screened, over many generations where possible
- **Event = unit of regulation**

Interpreting significance of GE's unintended effects on genome

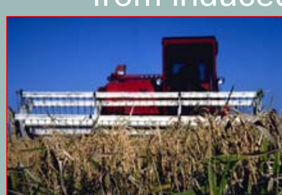
- How does it compare to conventional breeding
  - Lots of unintended genetic change in making hybrids, inbreeding, random mutagenesis
- Lots of genetic variation in gene content and organization
- No urgency to regulate traditional breeding at all in spite of this

Extensive natural genetic diversity in gene structure/content (maize)

Massive rearrangements in DNA sequences



Many plant varieties derived from induced mutations



Calrose 76 semi-dwarf rice

Over 2,000 crop varieties derived from mutagenesis have been commercialized.

High oleic sunflower

Rio Red grapefruit

Changes in chromosome number, loss/gain in wheat breeding

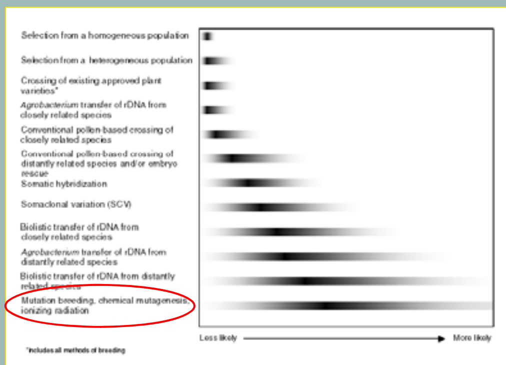
- Wheat, *Triticum aestivum*

Triticum urartu 2n=14 X *Aegilops speltoides* 2n=14

Triticum turgidum 2n=28 X *Aegilops tauschii* 2n=14

Triticum aestivum 2n=42

Expert views on unintended consequences for food quality from breeding methods



National Research Council (2004) http://books.nap.edu/execsumm_pdf/10977.pdf

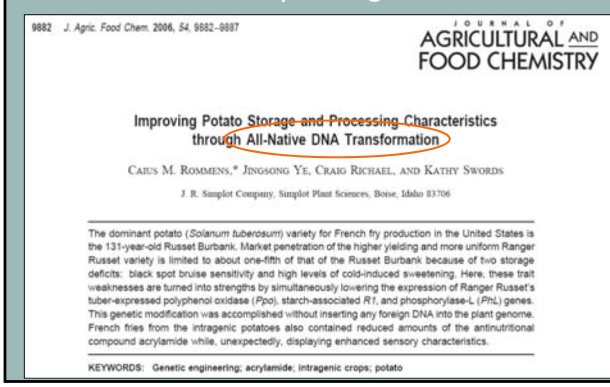
Gene silencing and cisgenics as "natural" GE methods ?

- Increasing use of RNAi (RNA interference), as a general means of gene suppression in research and commerce
  - Common mechanism for gene expression control in all genomes
- A way to knock down specific genes' expression, inhibit viruses
- Genes with inverted repeat DNA create double-stranded RNA, which induces sequence-specific RNA degradation or inhibition of translation

Cisgenics and intragenics

- Cisgenic: Transfer of intact gene from one species to a sexually compatible or closely related species
  - Disease resistance genes often targets
- Intragenic: Use of modified gene but only from sexually compatible or closely related species
- Much higher public approval/comfort compared to wide-cross GMOs (e.g., Bt crops)

Recombinant DNA modification of native plant genes



9882 *J. Agric. Food Chem.* 2006, *54*, 9882–9887

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Improving Potato Storage and Processing Characteristics through All-Native DNA Transformation

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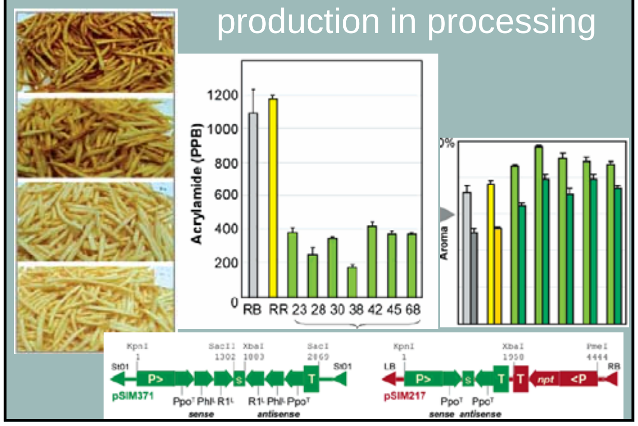
The dominant potato (*Solanum tuberosum*) variety for French fry production in the United States is the 131-year-old Russet Burbank. Market penetration of the higher yielding and more uniform Ranger Russet variety is limited to about one-fifth of that of the Russet Burbank because of two storage deficits: black spot bruise sensitivity and high levels of cold-induced sweetening. Here, these trait weaknesses are turned into strengths by simultaneously lowering the expression of Ranger Russet's tuber-expressed polyphenol oxidase (*Ppo*), starch-associated *R1*, and phosphorylase-L (*PhL*) genes. This genetic modification was accomplished without inserting any foreign DNA into the plant genome. French fries from the intragenic potatoes also contained reduced amounts of the antinutritional compound acrylamide while, unexpectedly, displaying enhanced sensory characteristics.

KEYWORDS: Genetic engineering; acrylamide; intragenic crops; potato

Reduced browning and acrylamide production in processing



Discussion questions

- What is unclear in basic concepts and methods?
- What is mutagenesis? How does it differ from natural genetic diversity?
- Should mutagenesis from breeding and/or GE be regulated?
- Should RNAi and cis/intragenics be regulated, or regulated differently, from other kinds of GE crops?