

Genetic Engineering of Plants

Chem 210

3/22/00

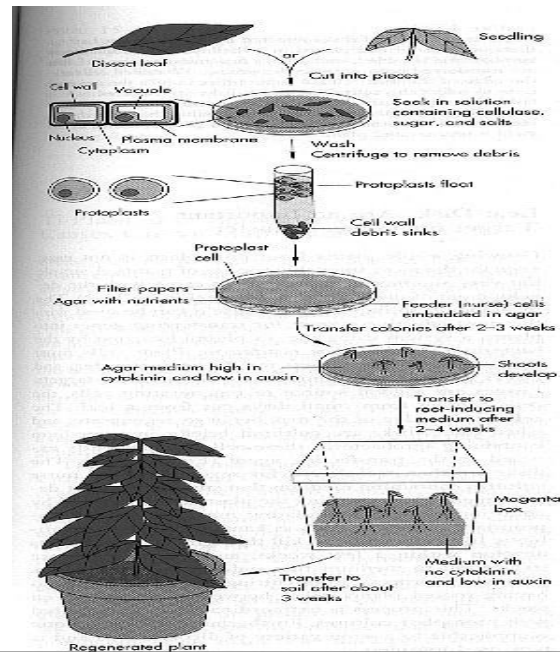
What we've learned so far...

- Genes can be cloned into vectors (such as plasmids).
- These vectors can be introduced into bacteria (such as *E. coli*).
- The “foreign” DNA can be expressed in the bacteria; in other words, bacteria can be modified to produce proteins from other sources.
- Question: can this be done with more complicated systems (I.e., eukaryotes? plants? animals?).

Basic information about plants

- Plants can be *self-fertilized*.
- Most plants can be classified into one of two classes, which differ in many properties:
 - *Monocotylenous* (“*monocots*”)--seed have a single leaf (wheta, rice, corn).
 - *Dicotyledonous* (“*dicots*”) - seed have two leaves.
- Plant cells are surrounded by a cellulose cell wall
- Whole plants can be regenerated from individual cultured cells.

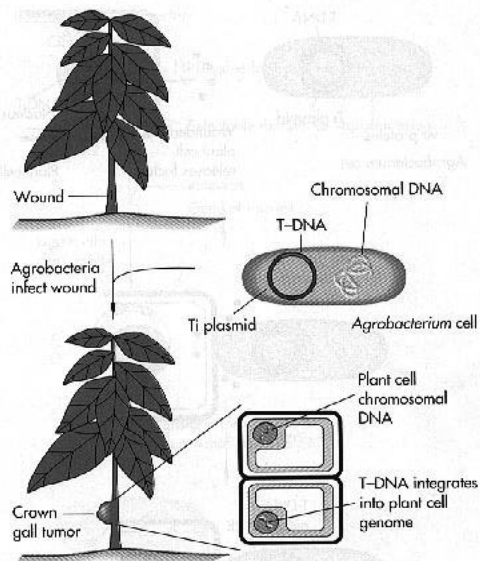
Regeneration of plants from cultured cells



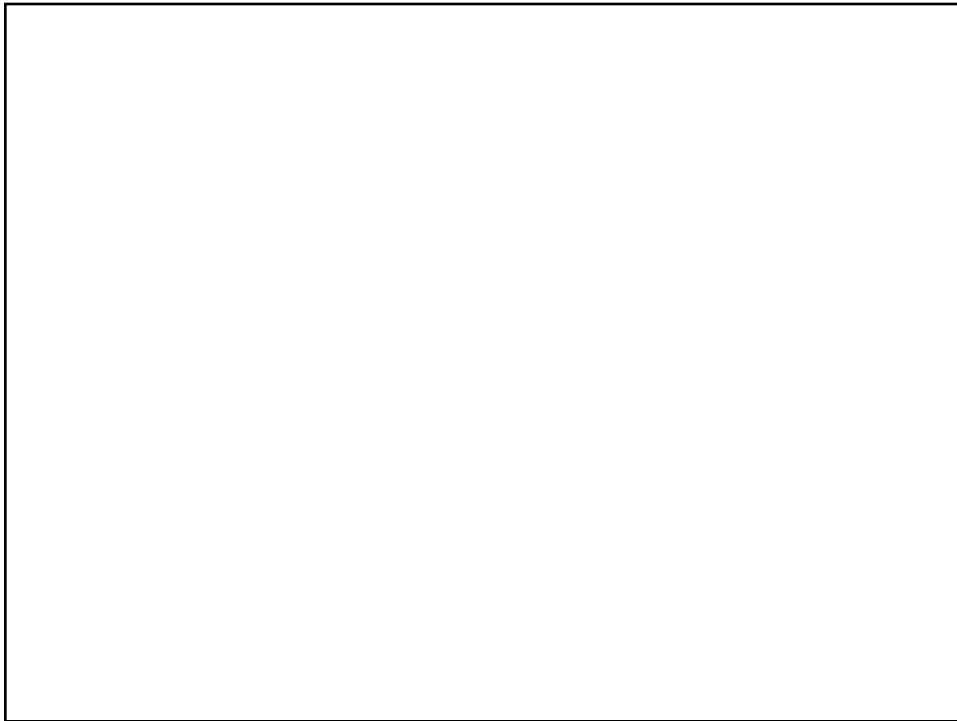
How to introduce foreign DNA into plants?

- Bacterial infection (*Agrobacterium tumefaciens*)
- Viral infections (tobacco mosaic virus)
- “Gunshot” introduction

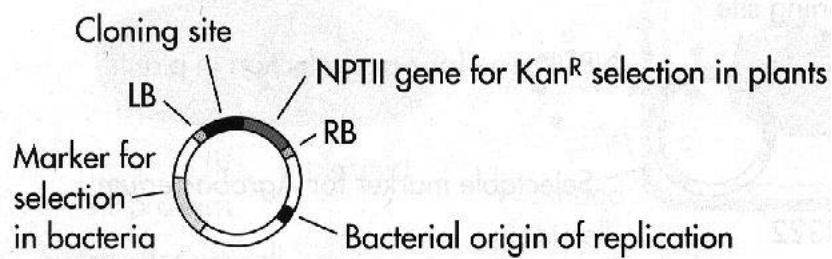
Agrobacterium infection causes crown gall tumors in plants



- Plants need to have a “wound”
- A piece of DNA (called *T-DNA*) from a plasmid (the *Ti plasmid*) in the *Agrobacterium* gets incorporated into the plant cell genome

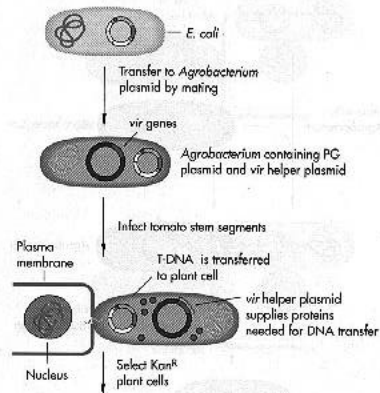


Integration of T-DNA into plant genome controlled by left- and right- border regions on Ti plasmid



- Any gene can be inserted between the LB and RB regions (replacing the T-DNA); this DNA can be incorporated into the plant genome.

Instead of the Ti plasmid, a synthetic binary vector system can be used for gene transfer



- Small plasmid containing gene of interest flanked by border regions.
- Larger plasmid containing vir genes.
- Agrobacterium can be transformed with both plasmids.
- Advantage; easy to generate small plasmids with cloned genes.

Summary

- The basic principles behind genetic engineering of plants the same as with bacteria--introduce foreign genes into the cells, express these genes to create new proteins.
- Genes/proteins can be selected based on desirable properties (pest resistance, herbicide resistance, etc.).
- This technology is widely applied in large scale, commercial/corporate agriculture

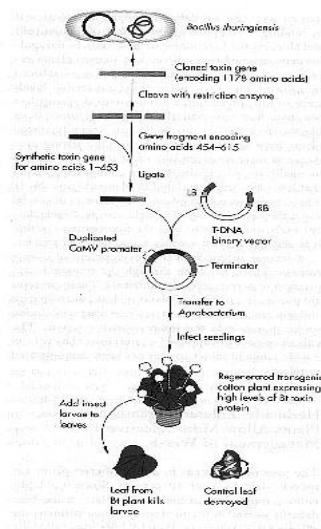
For monocots, agrobacterium infection not effective--introduce genes using “gene gun”

These technologies are being broadly applied in commercial agriculture

- Introduce pest resistance in crops
 - Viral resistance in tobacco
 - Insect resistance in cotton, corn, etc.
- Create herbicide resistant plants
 - Resistance to “Round-up” (glyphosphate)
- Create crops with new properties
 - Slow ripening tomatoes
 - Freeze resistant potatoes

Tobacco which expresses the coat protein from the mosaic virus is resistant to virus infection

Cotton can be engineered to produce a bacterial toxin which destroys insects



- *Bacillus thuringiensis* is a bacterium which produces a toxin (Bt toxin) to insects.
- The gene which expresses the functional portion of this toxin can be introduced into plants using a T-DNA vector.
- Plants expressing this gene are resistant to insects.

Round-up is a commonly used herbicide

- Round-up used to destroy weeds.
- The active ingredient is glyphosphate.
- Glyphosphate inhibits an enzyme 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) which is necessary for synthesis of aromatic amino acids.
- Effective at low doses; can be broken down by bacteria in the soil.
- Inhibits EPSPS in plants, but some bacterial EPSPS are not inhibited.

Introduction of a bacterial EPSPS into plants results in round-up resistant plants

Control over tomato ripening is a problem in large scale production

- Tomatoes taste best when “just ripened”
- But, ripe tomatoes are hard to transport (soft + mushy)
- And, tomatoes don't stay ripe for long.
- Problem: how to make tomatoes which can withstand transport process and end up ripe at the grocery store?

How to make tomatoes firmer longer

- As the tomato goes from green to red, tomatoes produce the enzyme polygalactonuronase (PG) which breaks down some cell wall components + softens fruit.
- Strategy: delay/inhibit PG action; fruit would still ripen, but remain firm (easier to transport, get non-runny Kethcups, etc.).
- One way to do that is to apply *anti-sense* technology: create a gene which codes for mRNA complementary to the mRNA of PG; this blocks translation, resulting in less protein.

Antisense technology can make firmer tomatoes

Some species show remarkable resistance to cold temperatures

- Plants made mainly of water; if water freezes, can damage the plant cells.
- Lots of crops lost to freezing each year.
- One solution: take advantage of anti-freeze proteins, such as those found in some ocean fish.
- These proteins lower the freezing temperature of water by altering solvent structure.
- Express these proteins in crops--get frost-resistant crops.