Introduction

The term ‘genetic engineering’ is coined by Jack Williamson. It is different from traditional breeding. It involves directly manipulating/altering the structure and characteristics of genes using molecular cloning & transformation.

Needs

1. Improve value of plant
   - Increase yield (herbicide-resistance, pest-resistance)
   - Enhance nutrition
   - Enhance taste
2. Plants can produce proteins for human needs (pharmaceutics)
3. Modified plants can be used to study effects of genes

Engineering for

- Disease resistance
- Insect-pest resistance
- Parthenocarpic plants
- Post-harvest traits
- Male sterility and fertility restoration
- Herbicide tolerance
- Salt tolerance
- Abiotic stress

Common Plant Transformation Techniques

- Agrobacterium mediated gene transfer - transfer of DNA from bacteria to plants.
- Biolistics - rapidly propelled tungsten or gold micro projectiles coated with DNA are blasted into cells.
- Electroporation - Electrical impulses are used to increase membrane and cell wall permeability to DNA contained in the
surrounding solution.
- **Microinjection** - Injection of DNA directly into the cell nucleus using an ultrafine needle
- **Polyethylene glycol mediated gene transfer** - Plant cell protoplasts treated with PEG are momentarily permeable, allowing uptake of DNA from the surrounding solution.

**Agrobacterium Mediated Gene Transfer**

- This method of transformation is the most widely used to introduce foreign genes into plant cells.
- *A. tumefaciens* contains a Ti plasmid (tumor-inducing) which normally infects dicotyledonous plant cells, making the bacteria an excellent vector for the transfer of foreign DNA.
- By removing the tumor inducing genes and replacing them with the genes of interest, efficient transformation can occur “Gene gun” method.
- DNA- or RNA-coated gold/tungsten particles are loaded into the gun and pull the trigger.
- A low pressure helium pulse delivers the coated gold/tungsten particles into virtually any target cell or tissue.
- The particles carry the DNA as cells do not have to be removed from tissue in order to transform the cells.
- As the cells repair their injuries, they integrate their DNA into their genome, thus allowing for the host cell to transcribe and translate the transgene.

<table>
<thead>
<tr>
<th>Table 1: Examples of plant disease resistant genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purpose of gene manipulation</strong></td>
</tr>
<tr>
<td>Potato virus X (PVX)</td>
</tr>
<tr>
<td>Potato virus Y (PVY)</td>
</tr>
<tr>
<td>Tomato yellow leaf curl virus</td>
</tr>
<tr>
<td>Tomato spotted wilt virus</td>
</tr>
<tr>
<td>Tomato yellow leaf curl virus</td>
</tr>
<tr>
<td>Cucumber mosaic virus</td>
</tr>
<tr>
<td>Cucumber mosaic virus</td>
</tr>
</tbody>
</table>

**Table 2: Examples of successful engineered vegetables against bacteria**

<table>
<thead>
<tr>
<th>Resistant against</th>
<th>Transgene product</th>
<th>Origin of gene</th>
<th>Transformed plant</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Erwinia carotovara</em></td>
<td>Lysozyme</td>
<td>T4 bacteriophage</td>
<td>Potato</td>
</tr>
<tr>
<td><em>Erwinia carotovara</em></td>
<td>Tachyplesin</td>
<td>Horseshoe crab</td>
<td>Potato</td>
</tr>
<tr>
<td><em>Pseudomonas syringae pv.</em></td>
<td>Phaseolotoxin insensitive OCTase</td>
<td><em>Pseudomonas syringae pv.</em></td>
<td>Bean</td>
</tr>
<tr>
<td>Phaseolicola</td>
<td></td>
<td>Phaseolicola</td>
<td></td>
</tr>
<tr>
<td><em>Erwinia carotovara</em></td>
<td>Pestate lyase</td>
<td><em>Erwinia carotovara</em></td>
<td>Potato</td>
</tr>
<tr>
<td><em>Erwinia carotovara</em></td>
<td>Glucose oxidase</td>
<td>Aspergillus niger</td>
<td>Potato</td>
</tr>
</tbody>
</table>

**Insect and Pest Resistance**

*Bacillus thuringiensis* is a soil bacterium that produces proteins that kill insects and nematodes.

**Mode of Action**

*There are three mechanisms*

- Insect eats *Bt* crystals with foliage
- The toxin binds to specific receptors in the gut and the insect stops eating
- The crystals cause the gut wall to break down, leading to leaching of ingested material
- The insect dies in 1-2 days

When insects ingest toxin crystals, the alkaline pH of their digestive tract activates the toxin.

<table>
<thead>
<tr>
<th>Table 3: Insect and Pest resistance in vegetable crops</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tomato</strong></td>
</tr>
<tr>
<td><em>Resistant to lepidopteran pests</em></td>
</tr>
<tr>
<td>c r y I A c</td>
</tr>
<tr>
<td><em>Resistant to Heliocoverpa armigera</em></td>
</tr>
<tr>
<td>c r y I A c</td>
</tr>
<tr>
<td><em>Resistant to tobacco hornworm, Manduca sexta</em></td>
</tr>
<tr>
<td>c r y</td>
</tr>
<tr>
<td><em>Brinjal</em></td>
</tr>
<tr>
<td><em>Resistant to Colorado potato beetle, Leptinotarsa decemlineata</em></td>
</tr>
<tr>
<td>c r y I b</td>
</tr>
<tr>
<td><em>Resistant to fruit borer, Leucinodes orbonalis</em></td>
</tr>
<tr>
<td>c r y</td>
</tr>
<tr>
<td><em>Broccoli</em></td>
</tr>
<tr>
<td><em>Resistant to diamondback moth, Plutella xylostella, cabbage looper, Trichoplusia ni, and cabbage butterfly, Pieris rapae</em></td>
</tr>
<tr>
<td>c r y I C</td>
</tr>
<tr>
<td><em>Chinese cabbage</em></td>
</tr>
<tr>
<td><em>Resistant to diamondback moth, Plutella xylostella</em></td>
</tr>
<tr>
<td>c r y I A c</td>
</tr>
</tbody>
</table>
Herbicide Resistance

- Modification of target in which target has been modified for developing resistance against at least three herbicides (glyphosate, sulphonylureas and imidazolines).
- Deoxification by degradation of herbicide in which number of detoxifying enzymes have been identified in plants as well as microbes. e.g. Glutathione – S-transferase, which detoxifies the herbicide atrazine.

Engineering for Salt Resistance

- Salt tolerant lines have been isolated in Lycopersicum by tissue culture technique.
- S.peruvianum and S.pennelli have been found tolerant to sodium chloride.
- Similarly, applying selection pressure to suspension culture has isolated salt tolerant lines of Capsicum annuum.
- Such lines could grow in liquid media containing 1-2 w/v sodium chloride.

Engineering for Post-harvest Traits

- Tomato plants have been engineered for reduced synthesis of polygalacturonase, which is responsible for softening the cell wall during ripening of some fruits.
- Using antisense technology, antisense RNA from polygalacturonase has been inserted.

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<th>Origin of gene</th>
<th>Transformed plant</th>
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</thead>
<tbody>
<tr>
<td>Improved storage/shelf-life</td>
<td>Antisense polygalacturonase</td>
<td>Tomato</td>
<td>Tomato</td>
</tr>
<tr>
<td>Ripening</td>
<td>Antisense oxidase</td>
<td>ACC Tomato</td>
<td>Tomato</td>
</tr>
<tr>
<td>Fruit pigmentation</td>
<td>Phytoene synthase gene</td>
<td>Tomato</td>
<td>tomato</td>
</tr>
</tbody>
</table>

Table 4: Examples of engineering for post-harvest traits

<table>
<thead>
<tr>
<th>Crop</th>
<th>Gene</th>
<th>Nutrient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato (Protato)</td>
<td>AmA1</td>
<td>Protein</td>
</tr>
<tr>
<td>Potato</td>
<td>crtB</td>
<td>β-carotene</td>
</tr>
<tr>
<td>Tomato</td>
<td>CHI-A</td>
<td>Flavonoid content</td>
</tr>
</tbody>
</table>

Genetic Engineering for Quality Improvement

Seed less vegetable development

- Expression of iaaM gene driven by ovule specific promoter
- DefH9 has been shown to confer parthenocarpy to transgenic tomato.
  - It also has high yield and quality in egg plant.
  - Transgene tomatos transferred with rolB gene under control of ovary and young fruit specific promoter TPRP-F1 developed parthenocarpy fruits.

Table 6: Examples of transgene for producing transformed plants

<table>
<thead>
<tr>
<th>Transgenic against</th>
<th>Transgene origin</th>
<th>Transformed plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>Rol B gene</td>
<td>Rhizogens Tomato</td>
</tr>
<tr>
<td>Brinjal</td>
<td>laah gene</td>
<td>Pseudomonas syringae pv. savas Brinjal</td>
</tr>
</tbody>
</table>

Table 7: Some commercial release of vegetable varieties

<table>
<thead>
<tr>
<th>Trait</th>
<th>Crops</th>
<th>Company</th>
<th>Product status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality (vine-ripen flavour, shelf life)</td>
<td>Tomato Flavr-savr</td>
<td>Calgene</td>
<td>Released 1994</td>
</tr>
<tr>
<td>Quality (vine-ripen flavour, shelf life)</td>
<td>Tomato Endless summer</td>
<td>D N A plant technology</td>
<td>Blocked by patent claims</td>
</tr>
<tr>
<td>Virus resistance</td>
<td>Tomato</td>
<td>- Zenga</td>
<td>Released in 1993-94</td>
</tr>
<tr>
<td>Virus resistant</td>
<td>Tomato - cap-sicium</td>
<td>- China</td>
<td>Released in 1993-94</td>
</tr>
</tbody>
</table>

Table 8: Vegetable transgenic developmental activities in India

<table>
<thead>
<tr>
<th>Institute</th>
<th>Vegetable crop</th>
<th>Transgene</th>
<th>Target trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPRI, Shimla</td>
<td>Potato</td>
<td>Bt cry 1 Ab</td>
<td>Develop plant resistant to potato tuber moth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Osmotin</td>
<td>Develop water stress tolerant plants</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coat protein</td>
<td>Develop plants resistant to potato</td>
</tr>
<tr>
<td>IARI, New Delhi</td>
<td>Brinjal</td>
<td>Cry 1 Ab</td>
<td>Generate lepidopteron resistant plants</td>
</tr>
<tr>
<td></td>
<td>Tomato</td>
<td>Cry 1 Ab</td>
<td>Generate lepidopteron resistant plants</td>
</tr>
<tr>
<td></td>
<td>Cauliflower</td>
<td>Cry 1 Ab</td>
<td>Develop diamond baco moth resistant plants</td>
</tr>
</tbody>
</table>
Future Aspects

Genetic engineering has the potential to enhance the levels of phytonutrients in our food chain so as to reduce the risk of many chronic diseases. Biofortification can be achieved by metabolic engineering, either using the natural genetic variations present in food crops or through genetic engineering. However, a significant fundamental research is needed to clarify what are phytonutrients, their physiological effects when consumed by humans, their mechanism of action in preventing diseases, impact on the food matrix, as well as the synergistic interactions among various phytonutrients.

References
