Genetically Modified Organisms (GMOs) in Plants: Golden Rice

Biol 3950

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Biol 3950: Research Methods in Genetic Biotechnology

MEMORIAL UNIVERSITY

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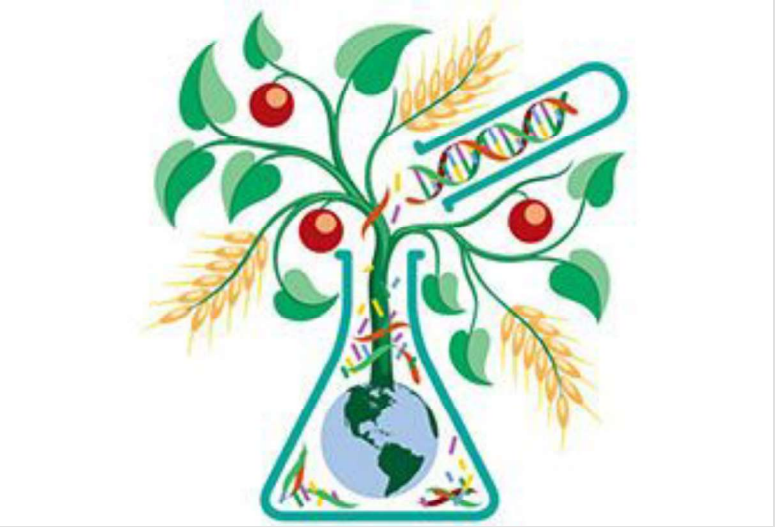
Overview

Production of genetically modified organisms (GMOs) in plants has a standardized methodology.

- Identification of trait
- Gene cloning
- Vector construction
- Plant transformation
- Selection of transgenic plants



- Regulation and monitoring

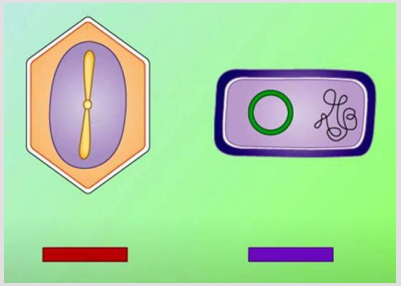
- Variation depending on target plant species/ intended genetic modifications.

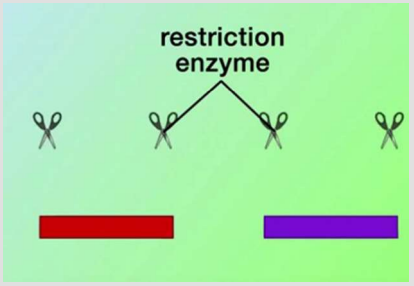
Identification of trait and gene cloning

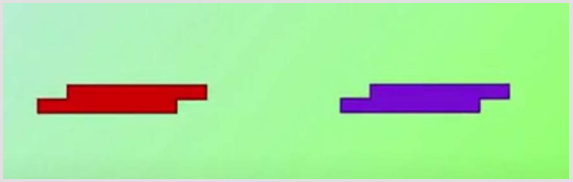
Identification of the desired trait.

Source genes also identified.

- Genes for enzymes, regulatory factors, or other proteins.





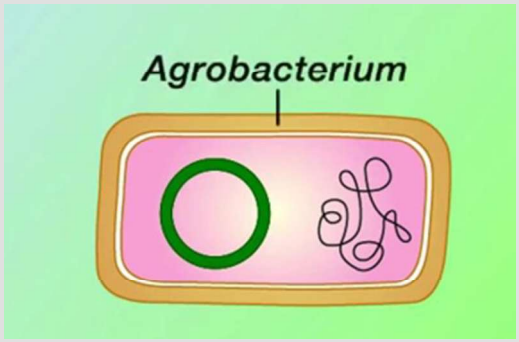


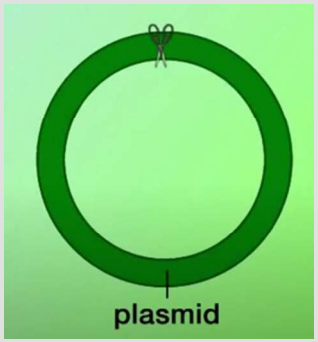
- ❑ Desired genes are isolated and amplified (PCR).
- ❑ Restriction enzymes and DNA ligase applied to construct a vector.

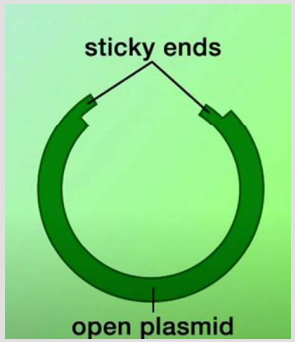
Vector Construction

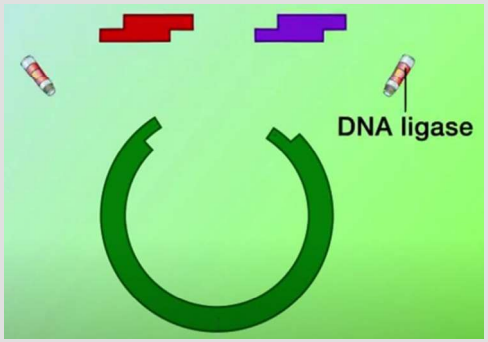
Vector containing the target gene is prepared

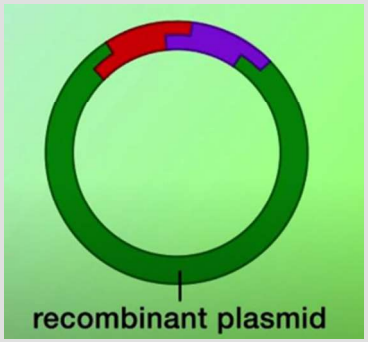
- Regulatory elements for gene expression (ie. promoters, enhancers, terminators, and selectable marker genes)





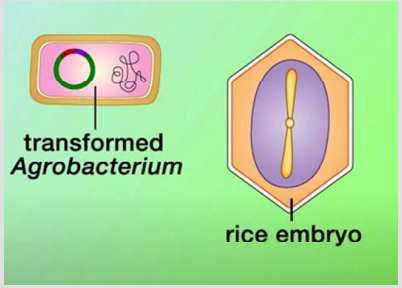


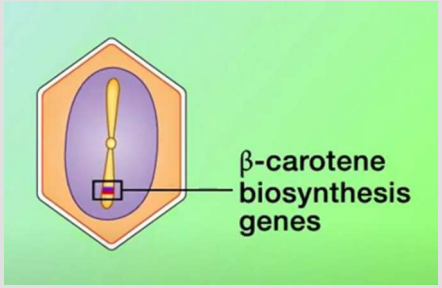




Plant transformation

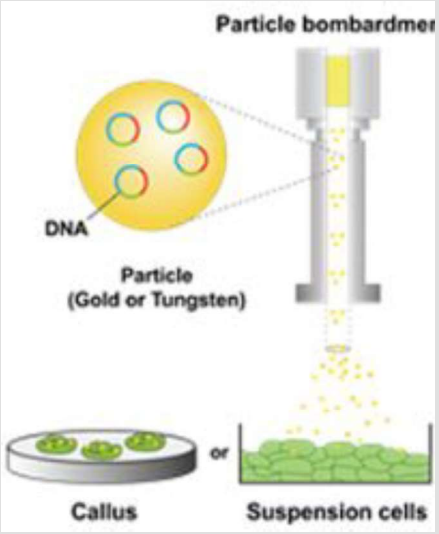
Vector is introduced into the target plant cell





Two methods:

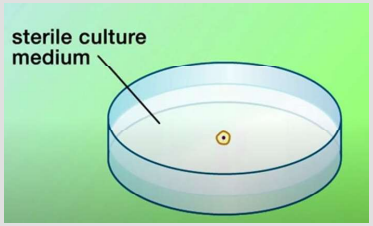
- Agrobacterium-mediated transformation
- Biolistic or particle bombardment

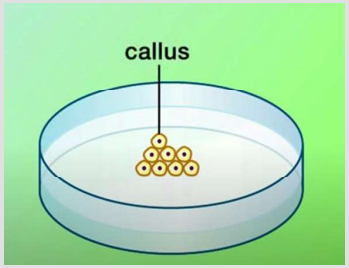


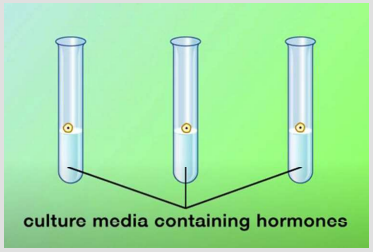
Selection and regeneration

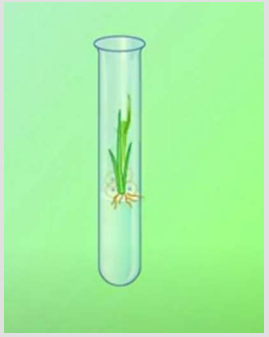
- Transformed plant cells are selected and regenerated using tissue culture techniques.
  - Selectable marker genes
    - Callus cells transferred to hormone-rich media.

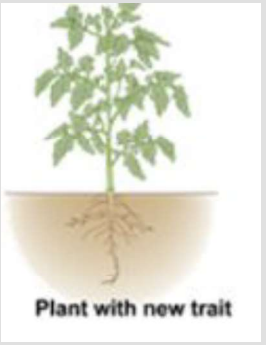
- Transgenic plants screened to confirm expression of the introduced genes.

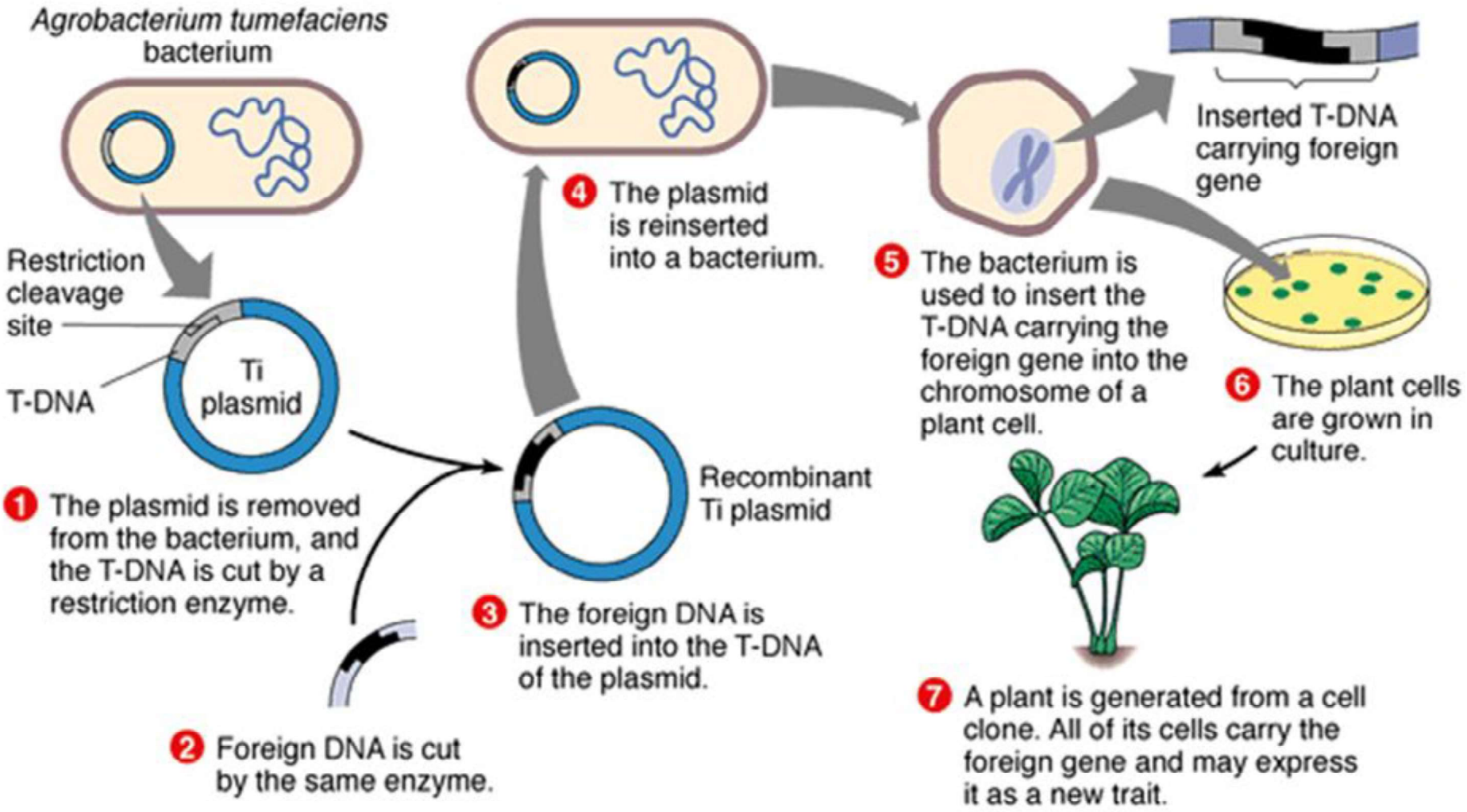











Agrobacterium tumefaciens bacterium
Restriction cleavage site
T-DNA
Ti plasmid
1 The plasmid is removed from the bacterium, and the T-DNA is cut by a restriction enzyme.
2 Foreign DNA is cut by the same enzyme.
3 The foreign DNA is inserted into the T-DNA of the plasmid.
Recombinant Ti plasmid
4 The plasmid is reinserted into a bacterium.
5 The bacterium is used to insert the T-DNA carrying the foreign gene into the chromosome of a plant cell.
Inserted T-DNA carrying foreign gene
6 The plant cells are grown in culture.
7 A plant is generated from a cell clone. All of its cells carry the foreign gene and may express it as a new trait.
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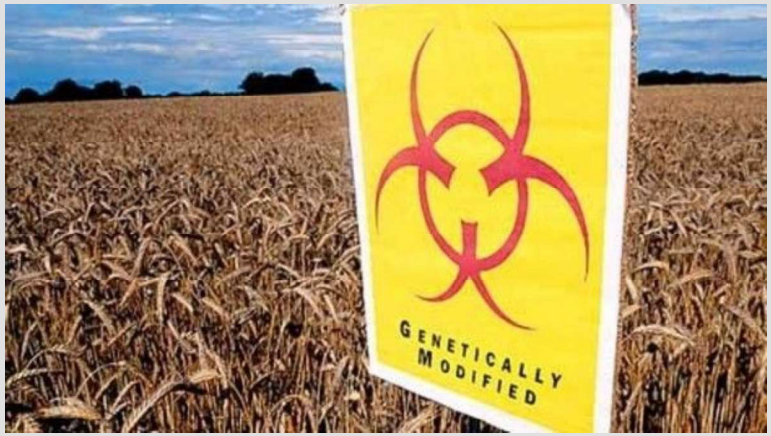
Regulation and monitoring

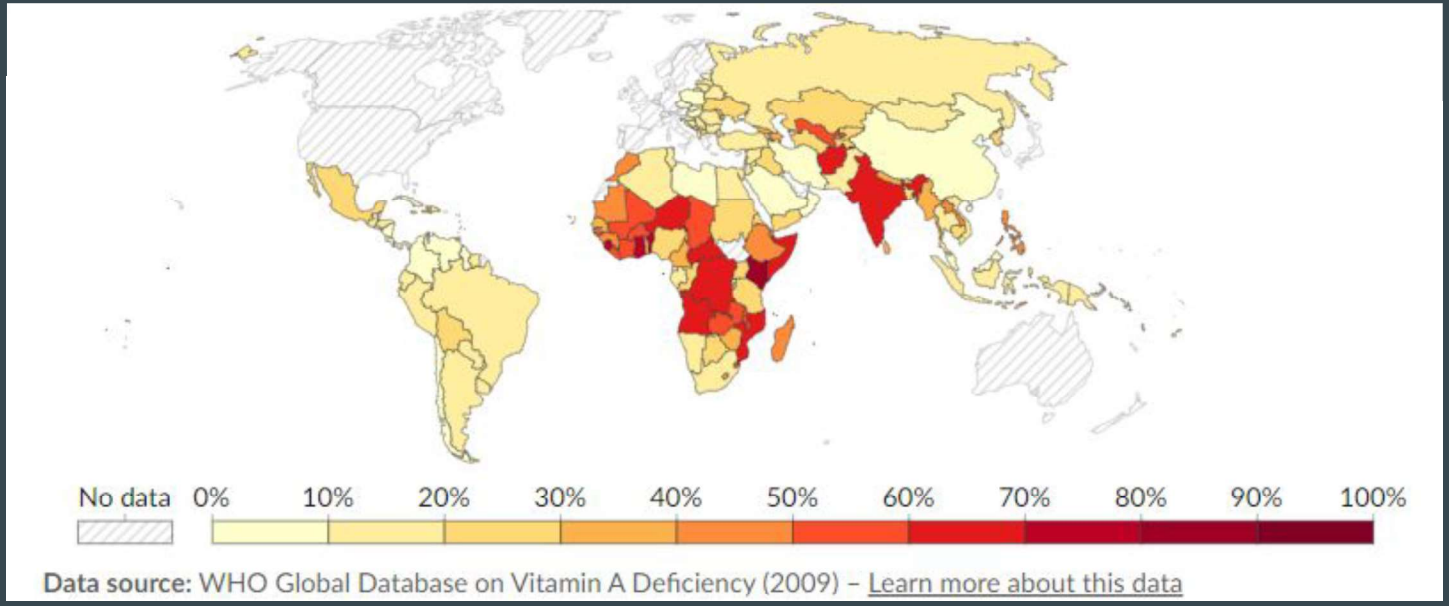
- Transgenic plants subjected to field trials.
- Regulatory agencies: safety, environmental impact, and efficacy of GMOs
  - Cultivation
- Monitoring programs over time.



An example of GMO: Golden Rice

Golden Rice: Overview

- Dr. Ingo Potrykus and Dr. Peter Beyer
- Motivated by Vitamin A deficiency (VAD)



- Micronutrient deficiency can lead night blindness and increased overall mortality.

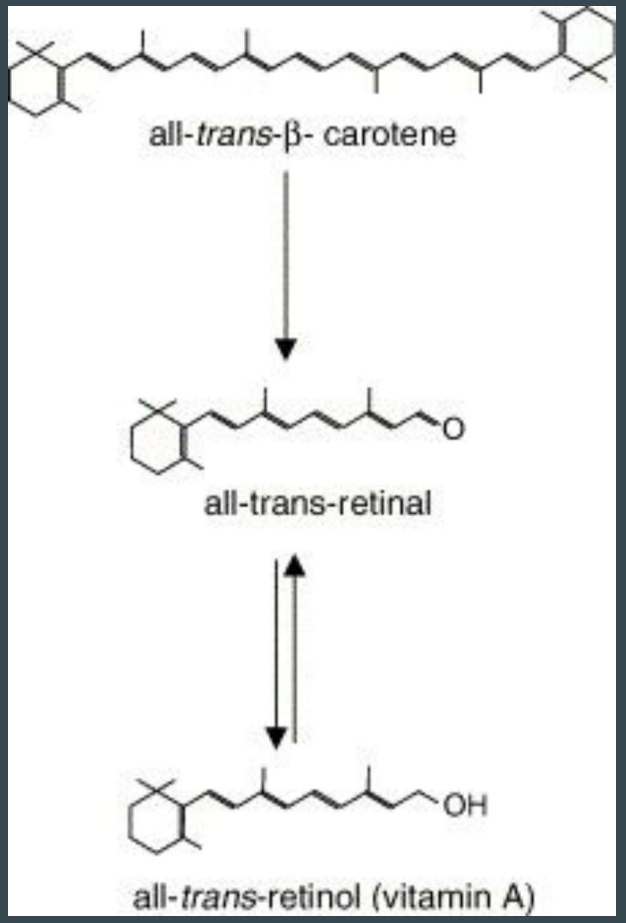
Overview

-Beta-carotene is a precursor of vitamin A.

- Enzyme beta-carotene dioxygenase and further metabolized into retinol.

-Rice does not naturally contain beta carotene.

- Biofortified rice as a solution to VAD.

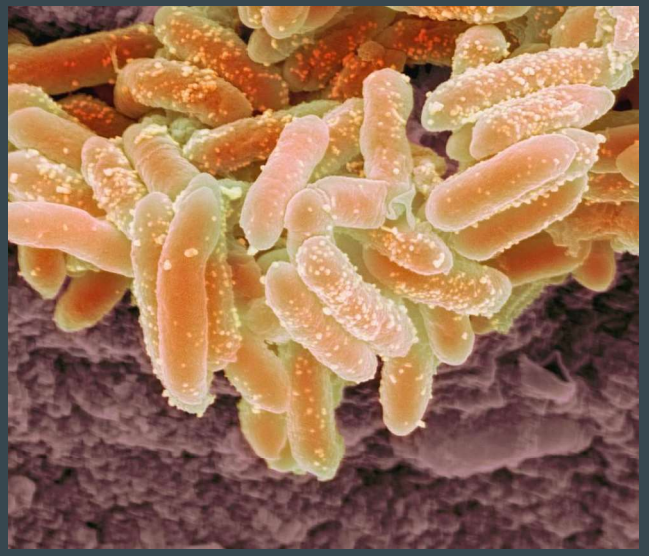


Design of Golden Rice

-Beta-carotene biosynthesis genes into rice genome.

-Donor organisms: daffodil (Narcissus pseudonarcissus) and Erwinia uredovora (a soil bacterium).

- Corn (Zea mays)



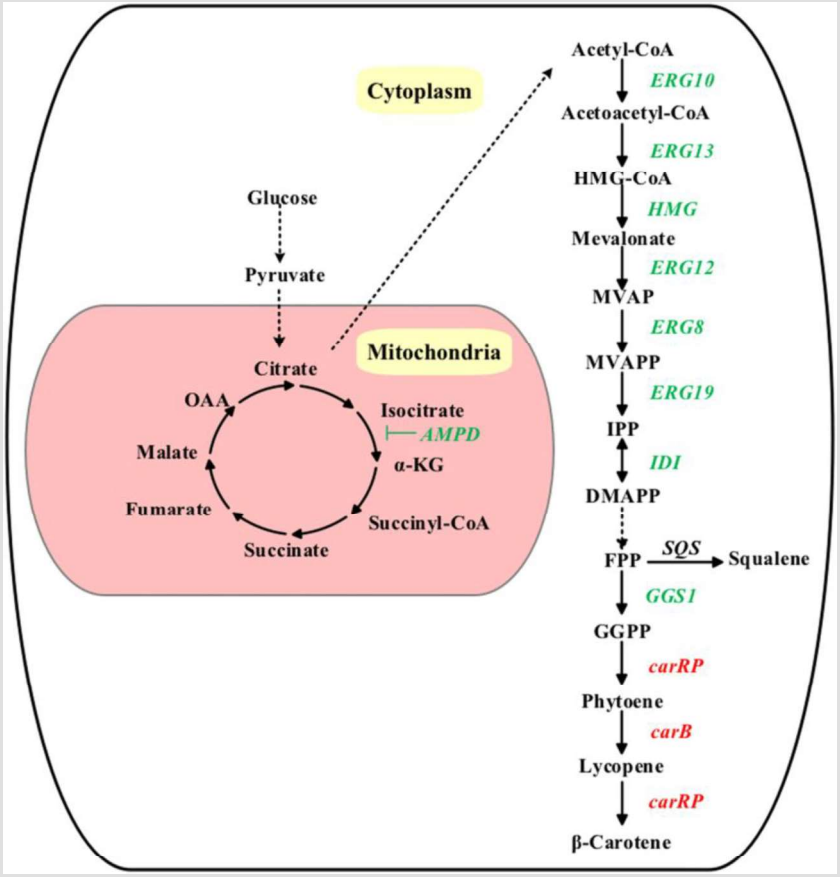
-Desired genes: phytoene synthase (psy) and phytoene desaturase (crtI)

-Method of Transformation: Agrobacterium-mediated transformation

Design of Golden Rice
Beta Carotene synthesis

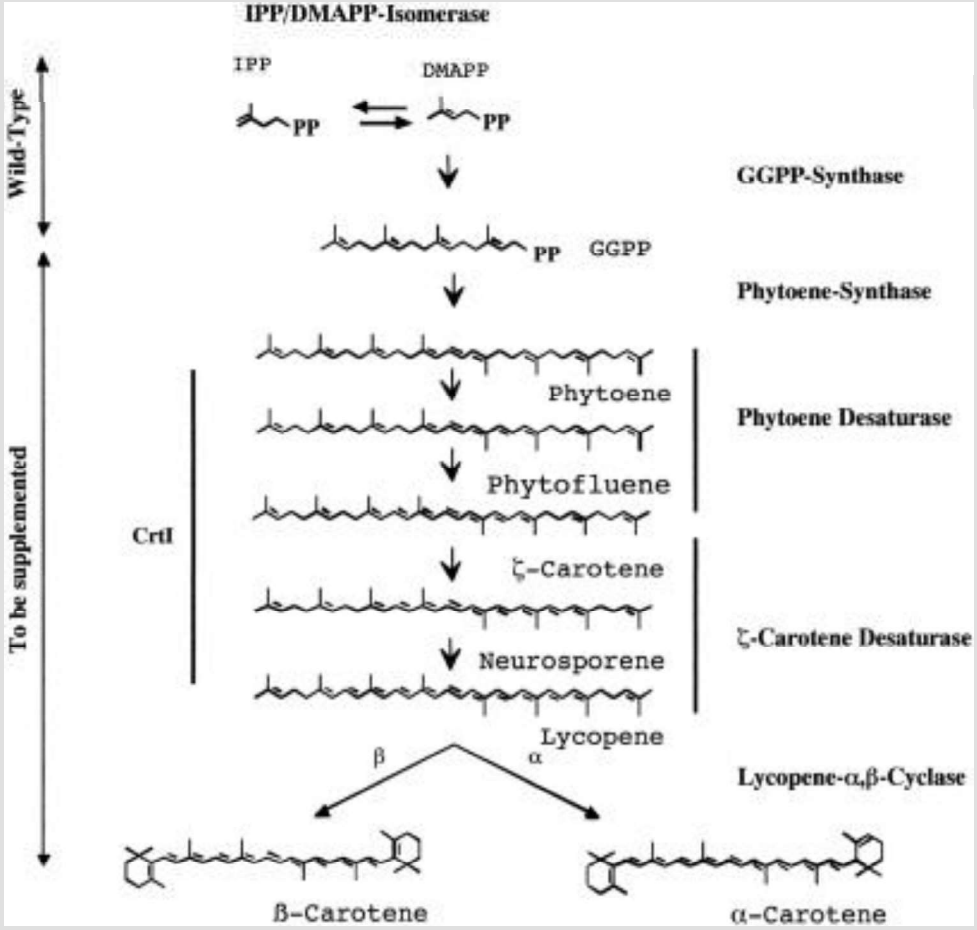
- Relies on conversion of geranylgeranyl diphosphate (GGPP) into Beta-Carotene

- GGPP previously identified in rice endosperm.



Design of Golden Rice
Beta Carotene synthesis

Two critical enzymes converts GGPP into Beta-carotene: psy and crtI



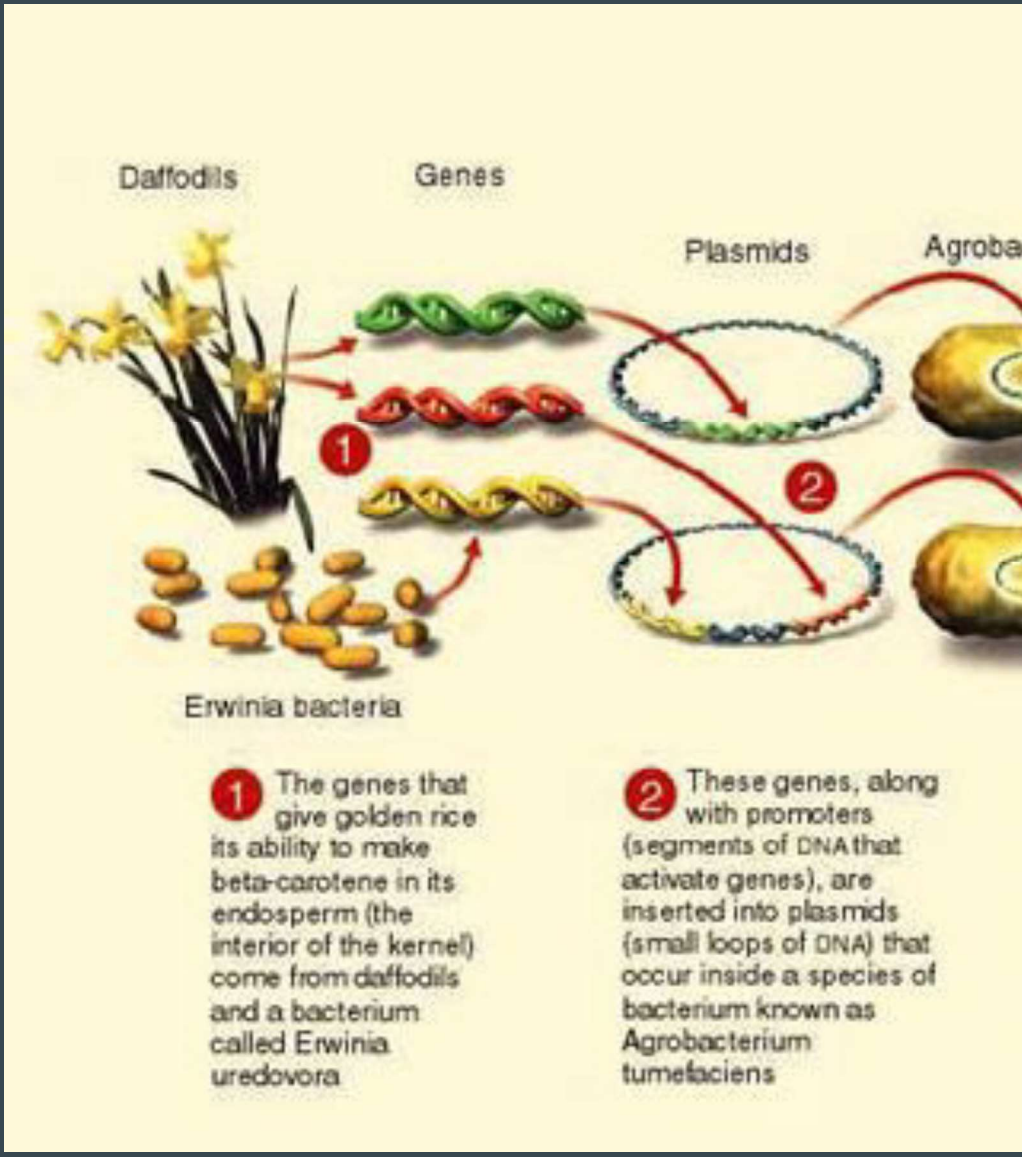
Design of Golden Rice

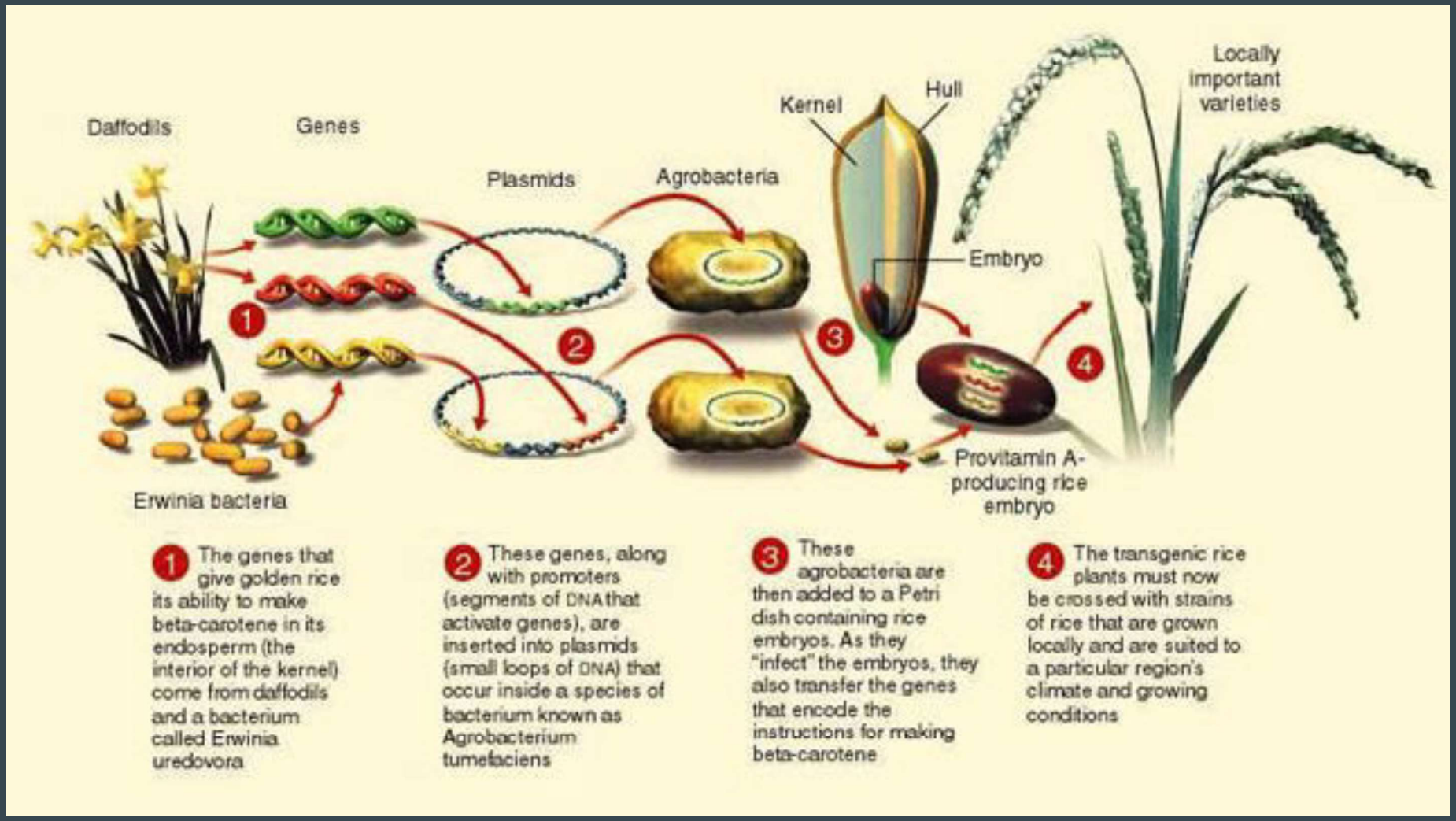
1. Gene cloning

2. Agrobacterium-mediated transformation

3. Transformed cells grown on selective media

4. Regenerated plantlets transferred to soil.
   - Transgenic rice plants.



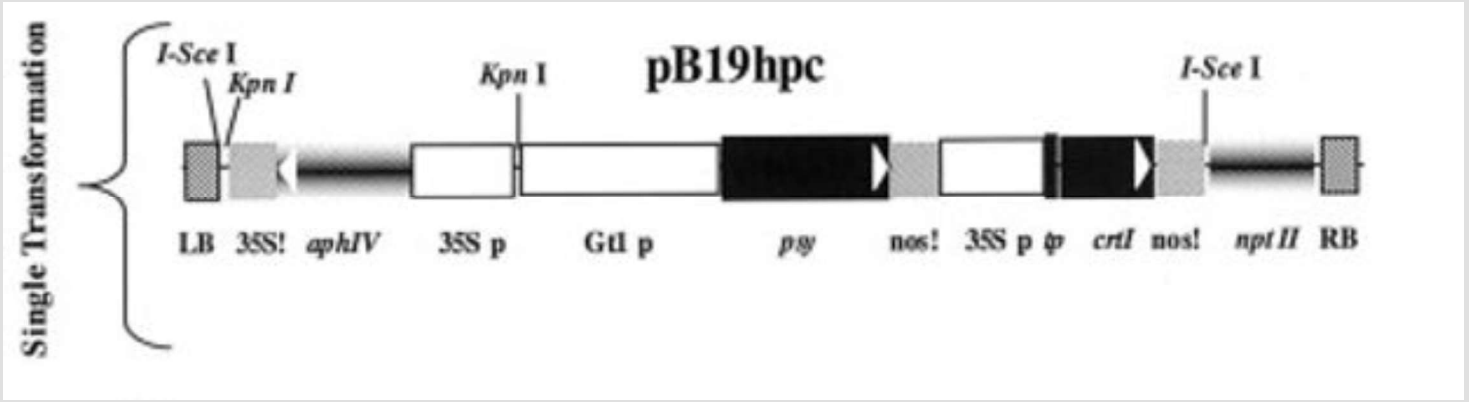

Daffodils
Genes
Plasmids
Agrobacteria
Kernel
Hull
Locally important varieties
Embryo
Erwinia bacteria
Provitamin A-producing rice embryo
1 The genes that give golden rice its ability to make beta-carotene in its endosperm (the interior of the kernel) come from daffodils and a bacterium called Erwinia uredovora
2 These genes, along with promoters (segments of DNA that activate genes), are inserted into plasmids (small loops of DNA) that occur inside a species of bacterium known as Agrobacterium tumefaciens
3 These agrobacteria are then added to a Petri dish containing rice embryos. As they "infect" the embryos, they also transfer the genes that encode the instructions for making beta-carotene
4 The transgenic rice plants must now be crossed with strains of rice that are grown locally and are suited to a particular region's climate and growing conditions

Particularities in Golden Rice development

1. Enhancing nutritional content.

2. Accumulation without adverse effects

3. Synthesis and accumulation of beta-carotene targeted to the endosperm.



"Construction of a vector that combines the purified target genes (psy and crtI) placed under the control of the **endosperm-specific glutelin** (Gt1) and the **constitutive CaMV 35S promoter**, respectively. This plasmid, thus, should direct the formation of lycopene in the endosperm, the site of GGPP formation."

Critiques

Anti GMOs movement

- Greenpeace:
  - ➢ Environmental impacts
  - ➢ Effectiveness of Golden Rice in treating Vitamin A deficiency

Counter-argument

- Comparative studies found no significant difference associated with the genetically modified variant.
- Environmental impact assessments (EIAs) and Sustainability assessments
- Bioavailability studies and clinical trials

Distribution

Ethical concerns: farmers rights, reliance on golden rice manufacturer, affordability, accessibility.

Counter-argument

- Collaborative agreement between the university-based inventors and the private sector signed guaranteeing

Current News regarding Golden Rice

Cultivation approval in the Philippines in July 2021.

➢ First commercialized GMO with direct consumer benefits.

Declared safe for consumption in four countries (Australia, New Zealand, Canada and the United States)

Expected cultivation approval in Bangladesh.

References

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Questions?