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"Prospects and Consequences of Plant Genetic Engineering"

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What is genetic engineering?

- Genetic engineering is a novel technique which are often directed to introduced a specific gene to produce superior genotypes, to alter the inherent biological pathway to modify the end product to mass harvested compound from the plant or to introduce specific enzymes to cater reactions leading to alteration or generation of new phenotypes.

Why to go for genetic engineering

- Useful genes cloned from viruses, bacteria, fungi, insects, animals, human beings and even the genes synthesized in the laboratory can be introduced into plants.
- Unlike conventional plant breeding only the specific cloned gene(s) are being introduced without the co-transfer of undesirable genes from donor. No need for repeated back crossing
- Removal of certain specific defects in crops.
- Development of 'designer plants' to as serve as bioreactors / factories for molecular farming
- Novelty can be introduced
- Reduces time required in gene transfer
- Can exploit horizontal variability, thus widen option for crop improvement

Genetic Engineering is an Extension of Traditional Plant Breeding

DNA is a strand of genes, much like a strand of pearls. Traditional plant breeding combines many genes at once.

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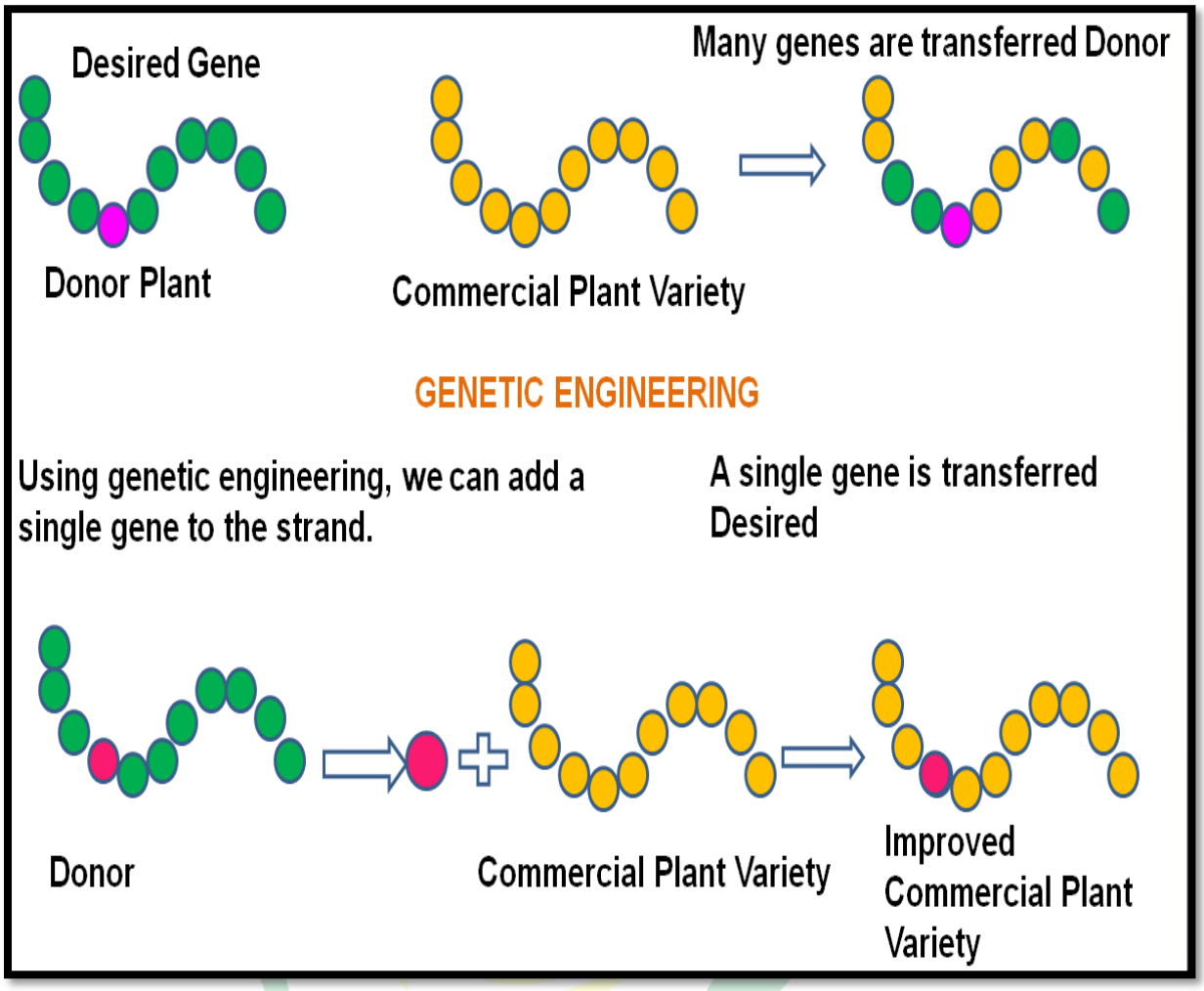

Conventional Breeding

- Limited to exchanges between the same or very closely related species
- Little or no guarantee of any particular gene combination from the million of crosses generated
- Undesirable genes can be transferred along with desirable genes
- Takes a long time to achieve desired results

Genetic Engineering

- Allows the direct transfer of one or just a few genes, between either closely or distantly related organisms
- Crop improvement can be achieved in a shorter time compared to conventional breeding
- Allows plants to be modified by removing or switching off particular gene.

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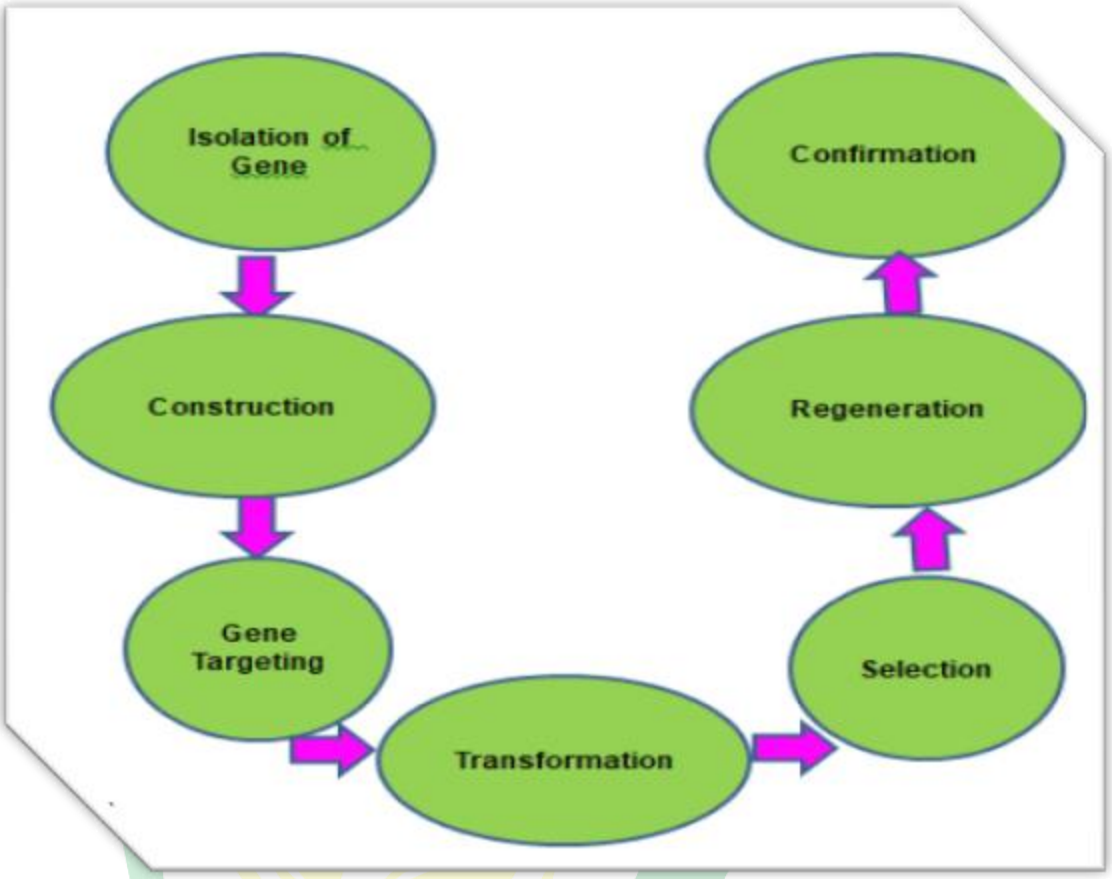
Steps followed in genetic engineering



Gene Transfer Methods

Vector mediated :- *Agrobacterium* Mediated and Viral mediated

***Agrobacterium* Mediated**

- Commonly used vector for gene transfer
- Naturally occurring gram negative soil bacterium
- With two common species viz. *A.tumifaciens* and *A.rhizogenes*
- These are known as natural genetic engineer for their ability to transform plants in its natural environment
- Wild type *A. tumifaciens* causes crown gall disease.
- *A. rhizogenes* causes hairy root disease.

Ti –Plasmid: The Ti plasmid is a mega plasmid (~200Kb) containing four regions in common:

- Region A- Comprising T-DNA, is responsible for tumour induction
- Region B- Is responsible for replication

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- Region C- Is responsible for conjugation
- Region D- Is responsible for virulence, this region is called virulence(*vir*) region and plays a crucial role in transfer of DNA into plant nuclear genome.

Transcriptional complexes present in *vir* region and their functions

Complex	Functions
❖ *Vir* A	Transmembrane protein acting as receptor for signal, phosphorylation *vir* G
❖ *Vir* B	Membrane protein , transmembrane passage of DNA
❖ *Vir* C	Bind to overdrive region, enhance vir D activity
❖ *Vir* D	Site specific endonucleases, cuts T-DNA
❖ *Vir* E	SS DNA proteins binds to T-DNA during transfer
❖ *Vir* F	Discriminates host from non - host
❖ *Vir* G	Transcriptional factor regulating expression of *vir* genes

Steps in T-DNA transfer

- ❖ Host bacterium recognition.
- ❖ Expression of *vir* gene.
- ❖ Formation of T-DNA transfer complex and T-DNA transfer.
- ❖ Integration of T-DNA into host genome

Viral vector mediated transformation

- ❖ Viruses are considered as potential gene delivery vectors because they have natural system of infection and delivery of their own nucleic acid to host cell.
- ❖ The advantages of DNA viruses are that they can integrate directly into genome avoiding the c-DNA synthesis step.
- ❖ Ex ample: TMV (SS RNA) transformed gene is chloramphenicol acetyl transferase and plant transformed is tobacco.

Direct Gene Transfer methods:

- ❖ Particle bombardment
- ❖ Physiochemical uptake of DNA
- ❖ Liposome encapsulation
- ❖ Microinjection
- ❖ DNA injection into intact plants

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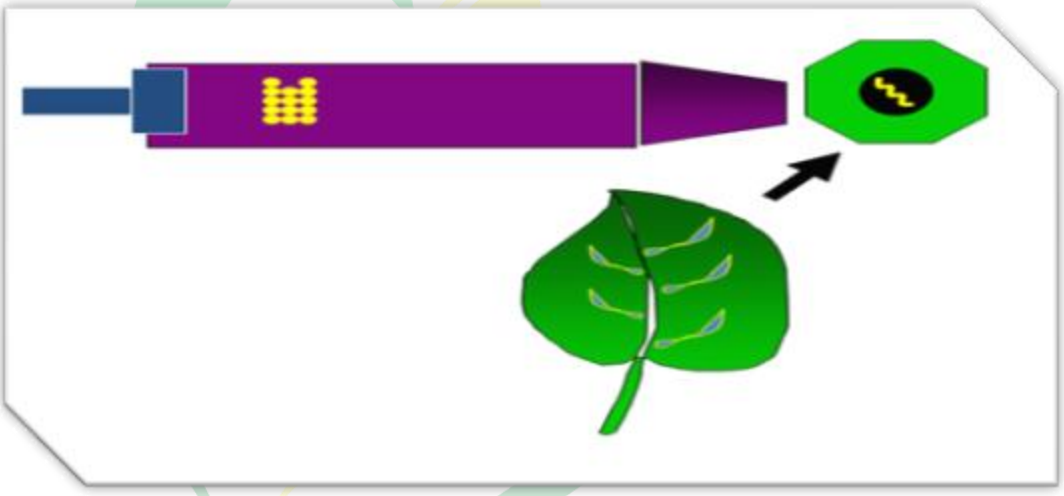
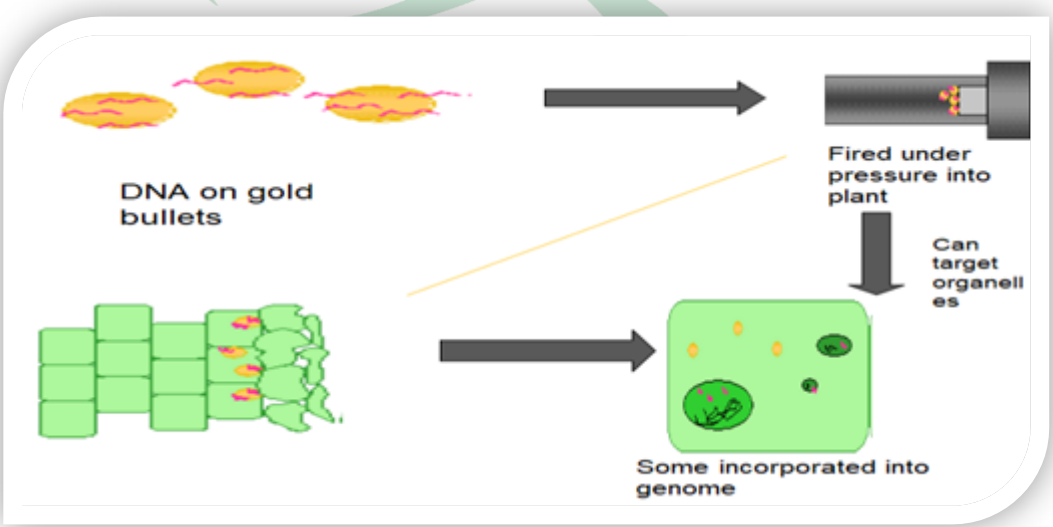
- Incubation of seeds with DNA
- Pollen tube pathway
- Laser micro beam
- Electroporation into tissues
- Silicon carbide fiber

Particle gun/ Gene gun / Biolistic / Micro projectile bombardment/ particle bombardment

- Among the vector less direct methods of gene delivery, biolistic transformation has been most successfully applied in development of transgenic plants.
- The basic concept of the process of biolistic involves acceleration of DNA into the cells with sufficient force such that a part of it get integrated into DNA of target cells.





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Microinjection:

- Crossway *et al.* 1986 developed the holding pipette technique to microinject tobacco protoplast.
- Protoplasts are held onto a 5-10µm pipette by gentle suction.
- Foreign DNA (about 2 pico liter) is injected into nucleus of protoplast using 0.2µm diameter injection pipette.

Electroporation of protoplast

- The process by which macromolecules present in extra cellular medium are internalized in living cells on exposure to brief electric pulse is termed as eletroporation. This technique allow transfer of chimeric genes into the plant cells (protoplast) by passing the short pulse of electricity which has voltage peak value of 250-350v with an RC constant in millisecond range.

Liposome encapsulation

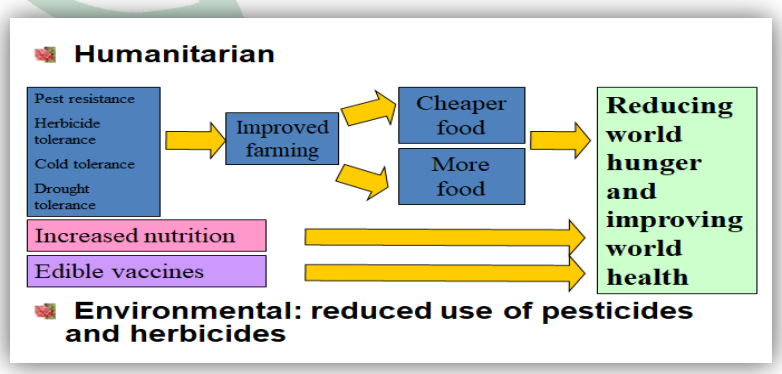
- Liposomes are small lipid bags enclosing large no. plasmids. The DNA enclosed in lipid vesicles when mixed with protoplasts under appropriate conditions penetrates into the protoplasts where lipase activity of the protoplasts dissolve the lipid vesicles and DNA get released for integration into host genome

PEG mediated transfer

- It is based on the ability of protoplast to uptake the foreign DNA from surrounding solution. An isolated plasmid DNA is mixed with the protoplast in the presence of PEG, and calcium phosphate; which enhances the uptake of DNA by protoplasts. After 15-20 min of incubation the protoplasts are cultured in the presence of appropriate selective agent.

Delivery of DNA through pores

- Natural pores
- Laser micro beam delivery
- Siliconcarbide whiskers mediated delivery
- Sonication



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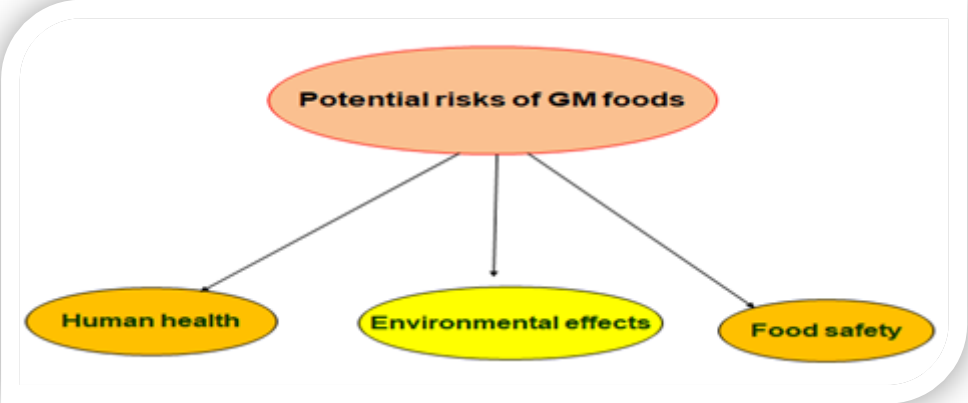

Human health risk

- Allergens
- Toxicity
- Antibiotic resistance
- Unintended side effects

Potential Environment Hazards

- Gene flow via pollen.
- Erosion of biodiversity.
- Development of insect resistance.
- Non target organisms affected

Food safety

- Allergenicity
- Toxicity
- Anti-nutritional

Who make sure GM food are safe ?

Government agencies regulate GM food

- FAO/WHO
- European Union
- Canadian legislation
- Ministry of Environment and Forest (MoEF)
- Department of Biotechnology, Government of India

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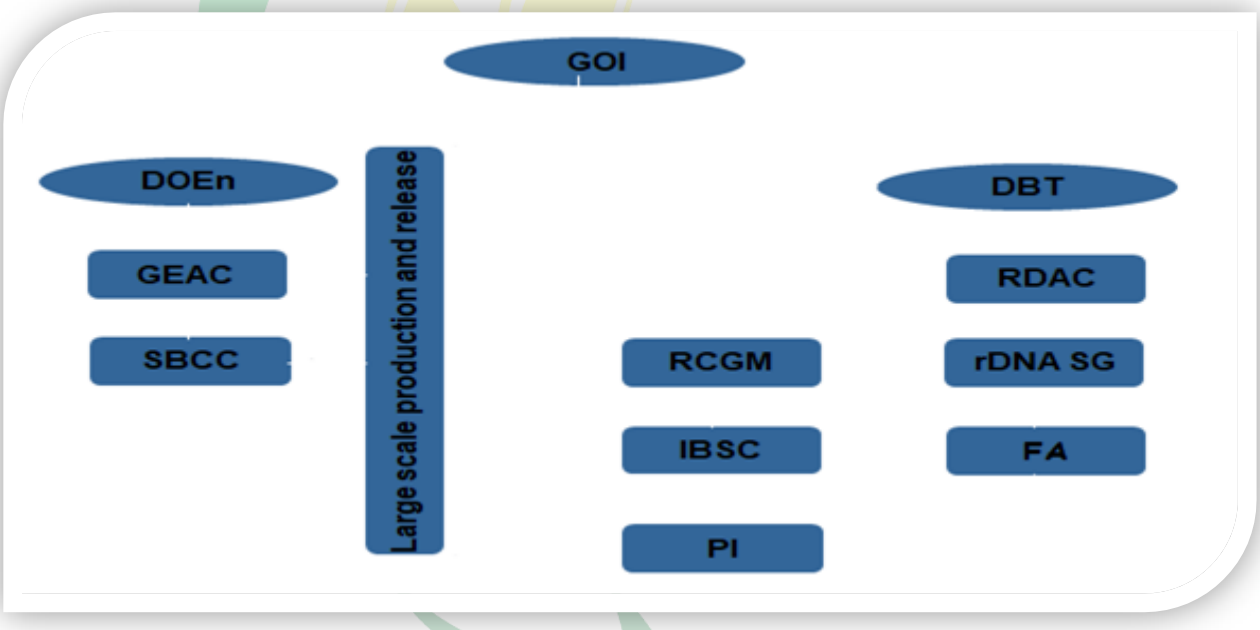
Risk Analysis Framework

Institutional mechanism



Future Prospects

- The future of biotech crops looks encouraging.
- Commercialization of drought tolerant maize is expected in 2012; Golden Rice in 2013; and Bt rice before the Millennium Development Goal (MDG) of 2015, which will potentially benefit 1 billion poor people, in Asia alone.
- Biotech crops could possibly contribute in the achievement of 2015 MDG, particularly in decreasing poverty by half, through maximizing crop productivity in a

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proposed global initiative to honor the legacy of ISAAA's founding patron, and Nobel Peace Laureate Norman Borlaug, who saved 1 billion people from hunger.