**GENE TRANSFER TECHNIQUES ( PLANT TRASFORMATION)**

**INTRODUCTION**

The transfer of genes from one organism to another is a natural process that creates variation in biological traits. This fact underlies all attempts to improve agriculturally important species, whether through traditional agricultural breeding or through the techniques of molecular biology. In both cases, human beings manipulate a naturally occurring process to produce varieties of organisms that display desired traits, for example, food animals with a higher proportion of muscle to fat, or disease-resistant corn.

The major differences between traditional agricultural breeding and molecular biological methods of gene transfer lie neither in aims nor in processes, but rather in speed, precision, reliability, and scope. When traditional, or classical, breeders cross two sexually reproducing plants or animals, they mix tens of thousands of genes in the hope of obtaining progeny with the desired trait or traits. Through the fusion of sperm and egg, each parent contributes half of its genome (an organism's entire repertoire of genes) to its offspring, but the composition of that half varies in each parental sex cell and hence in each cross. In addition, because the traits desired usually come from only one parent and may be controlled by one or a few genes, many crosses are necessary before the ''right" chance recombination of genes results in expression of the trait in the offspring. Even then, the progeny usually have to be crossed back to the parental variety to ensure stable adoption of the new trait. Sometimes undesired traits derived from one parent of a new, improved variety persist whereas the desired traits are lost.

Such are the difficulties and limitations of classical breeding. Molecular biological methods of gene transfer alleviate some of these problems by allowing the process to be manipulated at a more fundamental level. Instead of gambling on recombination of large numbers of genes, scientists can insert individual genes for specific traits directly into an established genome. They can also control the way in which these genes express themselves in the new variety of plant or animal. In short, by homing in on desired traits, molecular gene transfer can shorten the breeding time for new varieties and, in addition, lead to improvements not possible by traditional breeding.

Laboratory methods to move individual genes between organisms capitalize on naturally occurring mechanisms of gene transfer other than sexual reproduction. These include uptake of DNA by cells and cell-to-cell transfer of packaged genetic material such as viruses. Scientists began by studying these mechanisms in simple systems—bacteria and the viruses that infect them. Research has progressed at a remarkable rate. Now scientists can transfer genes into organisms as diverse as soybeans and sheep. Much work remains, however, to perfect gene transfer and its attendant technologies of embryo culture and plant regeneration.

Scientists have relied heavily on favorite model organisms such as the bacterium *Echerischia coli* and the fruit fly *Drosophila melanogaster* , because of their ease of manipulation and the large body of scientific knowledge accumulated about them. Model systems are critical to the progress of research. Nevertheless, molecular biologists must extend their techniques to commercially important agricultural organisms. Movement in this direction will not replace all traditional agricultural breeding with molecular gene transfer. It will, however, expand the array of methods available to improve agriculturally important species.

**METHODS OF GENE TRANSFER**

**DNA transfer by natural methods**

 1. Conjugation

 2. Bacterial transformation

 3. Retroviral transduction

4. *Agrobacterium* mediated transfer

**DNA transfer by artificial methods**

**Physical methods**

 1. Microinjection

 2. Biolistics transformation

 **Chemical methods**

 1. DNA transfer by calcium phosphate method

 2. Liposome mediated transfer

 **Electrical methods**

1. Electroporation

Electroporation

 Electroporation uses electrical pulse to produce transient pores in the plasma membrane thereby allowing DNA into the cells.

• These pores are known as electropores.

• The cells are placed in a solution containing DNA and subjected to electrical pulse to cause holes in the membrane.

• The foreign DNA fragments enter through holes into the cytoplasm and then to nucleus.

Advantages of Electroporation

 1. Method is fast.

 2. Less costly.

 3. Applied for a number of cell types.

 4. Simultaneously a large number of cell can be treated.

5. High percentage of stable transformants can be produced.



**TRANSFORMATION**

Transformation is the process by which genetic makeup of an organism is altered by the insertion of new gene(or exogenous DNA) into its genome This is usually done using vectors such as plasmids.

**The aim of producing transgenic plants is to**

a) Improve crop yields.

b) Improvement of varietal trait.

c) Give cultivated plants more protection against their pests, parasites and harsh weather conditions.

**THE Ti-PLASMIDS**

A remarkable feature of the Ti plasmid is that, after infection, part of the molecule is integrated into the plant chromosomal DNA .

• This segment, called the **T-DNA, is** between 15 and 30 kb in size, depending on the strain.

• These genes also direct synthesis of unusual compounds, called

opines, that the bacteria use as nutrient.

The **vir** (virulence) region of the **Ti- plasmid** contains the genes required for the T-DNA transfer process.

• The genes in this region encode the DNA processing enzymes required for excision, transfer and integration of the T-DNA segment.

• The T-DNA region of any Ti plasmid is defined by the presence of the right and the left border sequences.

• These border sequences are 24 bp imperfect repeats.

• Any DNA between the borders will be transferred in to the genome of the plant



**Agrobacterium-mediated Plant Transformation Process**

The Agrobacterium-mediated transformation process involves a number of steps: (a) isolation of the genes of interest from the source organism; (b) insertion of the transgene into the Ti-plasmid; (c) introduction of the T-DNA-containing-plasmid into Agrobacterium; (d) mixture of the transformed Agrobacterium with plant cells to allow transfer of T-DNA into plant chromosome; (e) regeneration of the transformed cells into genetically modified (GM) plants; (f) testing for trait performance or transgene expression at lab, greenhouse and field level.

**Advantages of Agrobacterium mediated gene Transfer**

 • Simple and comparatively less expensive

 • High transformation efficiency

• Transgenic crops obtained have better fertility percentage

• Protocols for both dicotyledons and monocotyledon are available

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