Plant Transformation Technologies for Crop Improvement and Gene Function Studies

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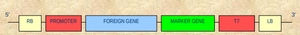
Genetic transformation is the transfer of a gene (naked DNA) from one organism to another. Transformation is one of the most important technologies to analyze the expression of genes, create plants that express new traits, and manipulate the genome of the recipient organism. Transformation is mainly accomplished in four steps: 1) Gene isolation, 2) Gene transfer, 3) Regeneration and selection of transformed plants, and 4) Analysis of transgenic plants (gene integration and expression).

Step 1. Gene isolation. Different molecular biology techniques and regulatory elements are crucial to gene isolation and expression. For instance, in order to express a gene in plant cells that has been isolated from bacterial or animal cells, a plant promoter and polyadenylation signals must be added. The gene may also need to be additionally modified to improve transcription (e.g., introduction of introns, 5', 3' UTRs), and translation (e.g. improve codon bias, use better 5' UTRs, and Kozak sequence surrounding the ATG).

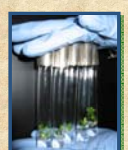


T-DNA with gene of interest and a selective marker gene

Step 2. Gene transfer. To introduce a foreign gene into a plant requires a variety of plasmid DNA vectors and specific techniques to deliver the naked DNA into cells. In biotechnology, the most common technique is *Agrobacterium*-mediated transfer that is a soil bacteria that infects plants. *Agrobacterium* has a Ti plasmid that contains a T-DNA, that is transferred from the bacteria to the host plant cell. The T-DNA is bracketed by two 25-bp direct repeats called LEFT and RIGHT borders. The plasmid also contains many virulence genes, which are required for the infection process. For plant transformation the plasmid has been highly modified into two plasmids called BINARY VECTORS. The larger one contains the virulence genes and the smaller one contains only the T-DNA region. This type of design is to facilitate the addition of any foreign gene into the T-DNA region. The smaller T-DNA plasmid also contains two origins of replication, one for *E. coli* and one for *Agrobacterium*, and an antibiotic resistance gene for bacterial selection. These modifications are important for the plasmid to be cloned and manipulated in *E. coli* and then transferred to *Agrobacterium* prior to transformation.

Plant viruses, electroporation, particle bombardment, and fibers of silicon carbide are also used for introducing DNA into the chromosomes of the plant cell. The plant cell wall is the hardest barrier to overcome in transformation. Meanwhile, introduction of DNA into bacteria and animal cells is easier because they do not have a cell wall.

Step 3. Regeneration and selection of transformed plants. The biggest hurdle in transformation of various species is regenerating the single transformed cell into the whole plant. Tissue culture techniques and manipulation of hormone balance are used to induce embryogenesis or organogenesis from the transformed cells to regenerate normal and fertile plants.



In vitro tissue culture for the regeneration of transgenic plants

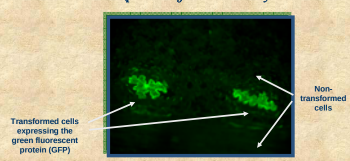
The production of transgenic plants involves the use of a selectable marker that enables the selection and recovery of transformed cells. In genetic transformation of plants only a minor fraction of the treated cells become transgenic while the majority remain untransformed. A considerable number of selectable markers have been developed that allow the survival of transformed cells and prevent the regeneration of the non-transformed cells. The most common strategy currently used is known as "**negative selection**". The term refers, in general, to the elimination of non-transformed cells in conditions where the transformed cells are allowed to thrive. The mode of action entails treatment of cells with chemicals, in conjunction with a transgene that confers resistance or tolerance through detoxification or modification of the chemical. The most widely used selectable markers in negative selection are a) **antibiotic resistance genes,** which make transformed cells resistant to antibiotics such as kanamycin and hygromycin, and b) **herbicide resistance genes,** which make transformed plant cells tolerant to herbicides such as phosphinothricin (glufosinate, BASTA) and glyphosate (ROUNDUP).

Selection of transgenic plants resistant to Kanamycin

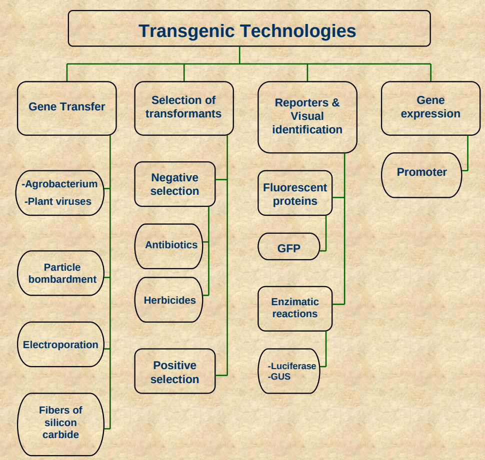
Selection of transgenic plants resistant to Basta herbicide

Another mean to select for transformants is by **"positive selection".** This strategy relies on the expression of a transgene product in transformed cells to utilize a compound that confers a growth or developmental advantage over non-transformed cells. This approach has great potential of displacing antibiotic and herbicide resistance genes because it addresses public concerns as well as technical issues. The best known positive selection systems are those based on the use of the mannose phosphate isomerase, and the reporter genes **β-glucuronidase (GUS), green fluorescent protein (GFP) and luciferase**.

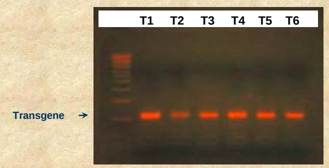
Detection of β-glucuronidase (GUS) gene expression using a histochemical assay



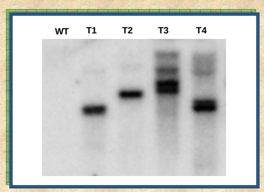
Detection of the green fluorescent (GFP) protein in transgenic tissue



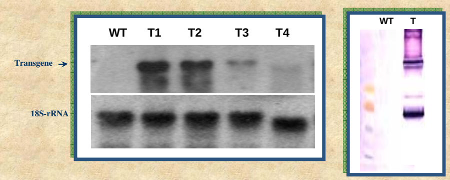
Step 4: Analysis of gene integration and expression. Molecular and biochemical analyses are used to evaluate the presence and levels of expression of the transgene.



Detection of the transgene by the polymerase chain reaction (Genomic PCR). T1-T6= transgenic plants



Detection of the transgene using DNA blot analysis of transgenic (T) plants. WT= wild type untransformed plant



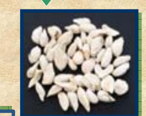
Analysis of gene expression by RNA blotting (left panel) and protein immunoblotting (right panel). Note the abscennce of RNA and protein band in the wild type (WT) untransformed plants.

Citrus transformation

Citrus is the most important fruit crop in the world, with a production of almost 100 million tons. It is grown in more than 100 countries, mainly in tropical and subtropical areas (approximately 40° latitude in each side of the equator) where favorable soil and climatic conditions occur. Citrus fruits are marketed fresh or as processed juice and canned segments. The introduction of genes into Citrus by means of genetic engineering is becoming an important technique in plant breeding.

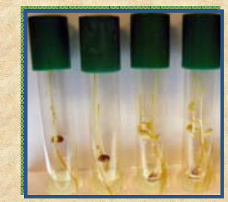
Citrus is considered a recalcitrant specie to genetic transformation due to several factors: citrus species are not natural hosts of *Agrobacterium*, and there are problems in the selection and rooting of shoots from transformed cells. The PTRC is trying to establish an efficient and reliable procedure to produce transgenic plants from the Carrizo cultivar, using epicotyl segments from *in vitro* grown seedlings. The use of an *Agrobacterium* strain carrying a super virulent vector, the optimization of infection and co-cultivation conditions, and the establishment of appropriate culture media are important factors for the production of the transgenic plants. In addition, the use of good physiological source material, the identification of competent cells for transformation in explants, and the use of appropriate marker genes, and the production of whole transgenic plants through grafting of regenerated transgenic shoots into vigorous rootstocks, have also been crucial towards the production of transgenic plants.

Steps In Citrus Transformation



Citrus (Carrizo) transformation efficiency

Total explants	Shoots growing on selection media	% potential transformants
646	287	45 %



Importance of Plant Transformation in Research and Crop Improvement

Advances in DNA technologies, *in vitro* tissue culture and transformation techniques has become a versatile platform for genetic manipulation of crops to enhance productivity through increasing resistance to diseases, pests and environmental stress.

Transgenic plants are potentially one of the most economical systems for large scale production of recombinant proteins for industrial and pharmaceutical uses.

Beyond crop improvements, the ability to engineer transgenic plants is a powerful and informative tool for studying gene function and the regulation of physiological and developmental processes.

Transgenic plants are also being used as an assay system for the modification of endogenous metabolism or gene inactivation.

Acknowledgements:

Many thanks to the following people that collaborate in different ways in the preparation of this poster: Aliona Glijin (Visiting Professor, Moldova State University); Donaji Ramirez (Graduate Student - CINVESTAV, Mexico); Xin Ran (Postdoctoral Fellow, UCR); and Javier Narvaez-Vasquez (Assistant Researcher, UCR).