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Methods of development of transgenic plants

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Abstract
With increase in the population food source must be produced at a higher speed to meet the food demand. With recent advancement in the field of genetic engineering, it is now possible to insert beneficial genes from a completely different species or even kingdom into a target plant, yielding transgenic plants with multiple ideal traits. Diverse methods of plant transformation have been described including biological, chemical and physical based methods. This review present the methods of gene transfer into plants such as *Agrobacterium*-mediated transformation as well as non-biological based approaches and their applications.

Keywords: Transgenics, *Agrobacterium*, gene, crop improvement, indirect methods

Introduction
Transgenic plants are developed by modification in their genomes by insertion of useful genes and deletion of detrimental genes (Rani *et al*., 2013) [4]. A foreign gene inserted into a plant can be of a different species or even kingdom. The first transgenic plant was developed through the insertion of *nptII* bacterial antibiotic resistance gene into tobacco (Framond *et al*., 1983) [2]. Since then, with the rapid development in plant molecular biology and genetic engineering technology, a wide variety of transgenic plants with important agronomic traits such as pest resistance and drought tolerance have been developed, ranging from dicots to monocots that are amenable to genetic modifications. The main purpose in the production of transgenic plants is to produce crops, which have ideal traits, quality, and high yield. Besides being beneficial to the agriculture sector, the plants are found to be able to act as the factory for pharmaceutical protein production (Lai *et al*., 2012) [5]. The emergence of new functional genomics strategies for the identification and characterization of genes promises to provide a wealth of information with an enormous potential to enhance traditional plant breeding and to genetically engineer plants for specific purposes. This review describes some of the highlights in the development of these technologies and some of the major achievements in production and commercialization of transgenic crops (Estrella *et al*., 2005) [3].

Transformation Methods
Vector-mediated gene transfer
Agrbacterium mediated gene transfer- *Agrobacterium*, the natures' genetic engineer, has been used as a vector to create transgenic plants. *Agrobacterium*-mediated gene transfer in plants is a highly efficient transformation process which is governed by various factors including genotype of the host plant, explant, vector, plasmid, bacterial strain, composition of culture medium, tissue damage, and temperature of co-cultivation. *Agrobacterium* is the only cellular organism on Earth that is naturally capable of transferring genetic material between the kingdoms of life, from prokaryotes to eukaryotes. Thirty years ago, the concept of using *Agrobacterium tumefaciens*, a Gram-negative soil bacterium, as a vector to create transgenic plants was viewed as a prospect and a wish. Today, it has advanced from a dream to a reality for a large number of agronomically and horticulturally important species; and it has become the most essential tool for plant genetic engineering for the introduction of foreign genes into plant cells to raise transgenics. *Agrobacterium*-mediated plant transformation in cereals, legumes, and other crop plants has boosted agricultural biotechnology and has led to improved yield and quality of crops to meet the requirements of increasing world population (Mohammed *et al*., 2011) [9]. *Agrobacterium*-mediated techniques have enabled remarkable improvements in yields of crop plants. Manipulated brassinosteroid levels resulted in increased yields of rice plants under dense planting conditions (Sakamoto *et al*., 2006) [12]. Flavr savr tomatoes with prolonged shelf- and vine-life characteristics were created by manipulating the

biosynthesis of the ripening-promoting hormone ethylene (Oeller *et al*., 1991) [10], and also by increasing levels of the antiripening polyamines (Mehta *et al*., 2002) [7]. Transgenic tobacco expressing cyanobacterial enzyme showed improved photosynthetic capacity and concomitant increase in plant biomass (Miyagawa *et al*., 2001) [8]. *Agrobacterium*-mediated transformation has been successfully used in improvement of *Brassica* (Rafat *et al*., 2012) [11]. (Singh *et al*., 2010) [13] developed efficient transformation protocol in mustard using chickpea lectin gene. (Bhuiyan *et al*., 2010) [1] established an efficient *Agrobacterium*-mediated genetic transformation method for *Brassica juncea* using cotyledon explants from *in vitro*-grown seedlings by investigating the factors responsible for successful gene transfer. *Agrobacterium*-mediated transformation has also been reported in the biodiesel plant, *Jatropha* (Mazumdar *et al*., 2011, Zong *et al*., 2010) [6, 17]. Three different tissues of *O. fragrans*, including calli, floral buds, and leaves, as explants for transient transformation mediated by *Agrobacterium tumefaciens*. To obtain the optimal transient expression protocol for each explant, we evaluated the effects of *Agrobacterium* load, acetosyringone concentration, co-culture duration, bud bract removal, and explant preculture on the transformation efficiency (Zong *et al*., 2023) [17]. A pollen tube pathway-mediated *Agrobacterium tumefaciens* transformation method in peanut. It is a simple, efficient, low cost and tissue culture-independent method of peanut transformation, involving injection of a fresh suspension of transformed *A. tumefaciens* carrying *AhBI-1* encoding Bax inhibitor-1 from *Arachis hypogaea* L. into pollen tubes, integration of the gene into the host plant genome, and rapid screening for transformants using quantitative real-time PCR. The findings confirmed that this strategy provided an efficient (50% frequency of positive transformants) gene transformation technique in peanut. Overexpression of the *AhBI-1* transgene resulted in increased root and shoot growth even in the absence of exogenous abiotic stress (Zhou *et al*., 2023) [16]. Agrobacterium-tumefacien-mediated method was used to successfully obtain T2 generation *ZmBON3*-gene-overexpressing corn plants and gene-edited plants. Related phenotypes and molecular identification showed that the disease resistance of overexpression plants was significantly reduced, and the disease resistance of gene-edited plants was significantly increased, which verified that the *ZmBON3* gene was a negative regulatory gene (Zhang *et al*., 2023) [14].

Plant Viruses vector

Plant viral vectors delivered by Agrobacterium are the basis of several manufacturing processes that are currently in use for producing a wide range of proteins for multiple applications, including vaccine antigens, antibodies, protein nanoparticles such as virus-like particles (VLPs), and other protein and protein-RNA scaffolds. Viral vectors delivered by agrobacterial T-DNA transfer (magnifection) have also become important tools in research. In recent years, essential advances have been made both in the development of second-generation vectors designed using the 'deconstructed virus' approach, as well as in the development of upstream manufacturing processes that are robust and fully scalable. The strategy relies on Agrobacterium as a vector to deliver DNA copies of one or more viral RNA/DNA replicons; the bacteria are delivered into leaves by vacuum infiltration, and the viral machinery takes over from the point of T-DNA transfer to the plant cell nucleus, driving massive RNA and protein production and, if required, cell-to-cell spread of the replicons. Among the most often used viral backbones are those of the RNA viruses Tobacco mosaic virus (TMV), Potato virus X (PVX) and Cowpea mosaic virus (CPMV), and the DNA geminivirus Bean yellow dwarf virus. Prototypes of industrial processes that provide for high yield, rapid scale up and fast manufacturing cycles have been designed, and several GMP-compliant and GMP-certified manufacturing facilities are in place.

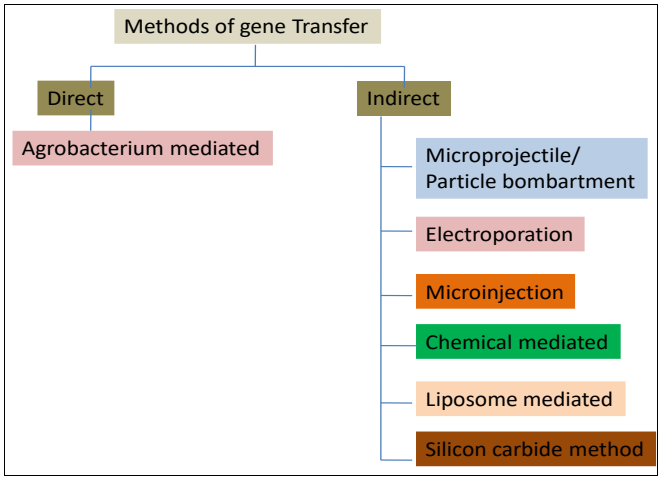

Fig 1: Methods of transformation

Direct gene transfer methods

The term direct transfer of gene is used when the foreign DNA is directly introduced into the plant genome (Figure 1). Direct DNA transfer methods rely on the delivery of naked DNA into the plant cells. The direct gene transfer can be broadly divided into two categories. A. Physical gene transfer methods—electroporation, microinjection, particle bombardment. B. Chemical gene transfer methods—Poly-ethylene glycol (PEG)-mediated, diethyl amino ethyl (DEAE) dextran-mediated, calcium phosphate precipitation.

Physical Gene Transfer Methods

1. **Electroporation:** This method involves suspension of plant protoplasts in a suitable ionic solution containing linearized recombinant plasmid DNA. This mixture is then exposed to low voltage-long pulses or high voltageshort pulses for the desired number of cycles. The electrical pulses are thought to induce transient pores in the plasma lemma through which the DNA molecules are incorporated. Treated protoplasts are then cultured to obtain cell colonies and plants. This method is called electroporation (i.e. introduction of DNA into plant cells by making minute pores in the plant cell membrane).
2. **Microinjection:** Microinjection is a direct physical method involving the mechanical insertion of the desirable DNA into a target cell. The target cell may be the one identified from intact cells, protoplasts, callus, embryos, meristems, etc. Microinjection is used for the transfer of cellular organelles and for the manipulation of chromosomes. The technique of microinjection involves the transfer of the gene through a micropipette (0.5-10.0 pm tip) into the cytoplasm/nucleus of a plant cell or protoplast. While the gene transfer is done, the recipient cells are kept immobilized in agarose embedding, and

held by a suction holding pipette. As the process of microinjection is complete, the transformed cell is cultured and grown to develop into a transgenic plant. In fact, transgenic tobacco and Brassica napus have been developed by this approach. The major limitations of microinjection are that it is slow, expensive, and has to be performed by trained and skilled personnel.

3. **Particle Bombardment Gun Method (Biolistics):** Particle bombardment is the most effective method for gene transfer, and creation of transgenic plants. This method is versatile due to the fact that it can be successfully used for the DNA transfer in mammalian cells and microorganisms. The micro projectile bombardment method was initially named as biolistics by its inventor Sanford (1987). Biolistics is a combination of biological and ballistics. In this method, 1-2 µm gold or tungsten particles coated with DNA are shot into the plant cells using a helium pressure particle gun device. Important crop plants like, wheat, rice and maize have now been transformed by this method.

Chemical Gene Transfer Methods: Polyethylene glycol (PEG)-mediated transfer: DNA molecules can be forced to enter into the host genome only in those cells which do not possess cell walls. The naked plant protoplasts are mixed with molecules of linearized plasmid DNA containing the foreign gene. The two are mixed in a transformation medium rich in Mg2+ ions in place of Ca2+ ions, following which 20% polyethylene glycol (PEG) solution is added. After the treatment, the PEG concentration is reduced and Ca2+ concentration is enhanced. It promotes the frequency of transformation. The chemical method involving transformation in presence of polyethylene glycol is convenient and simple but there are, however, some disadvantages: 1) Many cells are so sensitive that the chemical method cannot be applied whereas some cells die during the treatment. This method is not perfect because many treated cells do not contain any transfer DNA. Sometimes the foreign DNA is degraded in the cytoplasm before reaching the nucleus.

References

1. Bhuiyan MSU, Min SR, Jeong WJ, Sultana S, Choi KS, Lee Y. An improved method for *Agrobacterium*-mediated genetic transformation from cotyledon explants of *Brassica juncea*. Plant Biotechnology. 2011;28(1):17–23.
2. de Framond AJ, Bevan MW, Barton KA, Flavell R, Chilton MD. Mini-Ti plasmid and a chimeric gene construct: New approaches to plant gene vector construction. In: Downey K, Voellmy RW, Ahmad F, editors. Advances in Gene Technology: Molecular Genetics of Plants and Animals. Miami Winter Symp. 20th ed. Florida: Elsevier; 1983, p. 159-170
3. Herrera-Estrella L, Simpson J, Martínez-Trujillo M. Transgenic Plants: An Historical Perspective. In: Peña, L. (Eds) Transgenic Plants: Methods and Protocols. Methods in Molecular Biology™, Humana Press; c2005. 286. https://doi.org/10.1385/1-59259-827-7:003
4. Jhansi Rani S, Usha R. Transgenic plants: Types, benefits, public concerns and future. Journal of Pharmacy Research. 2013 Aug;6(8):879-883
5. Lai KS, Yusoff K, Mahmood M. Heterologous expression of hemagglutinin-neuraminidase protein from Newcastle disease virus strain AF2240 in Centella asiatica. Acta Biologica Cracoviensia/Series Botanica. Jan 2012;54(1):142-147.
6. Mazumdar P, Basu A, Paul A, Mahanta C, Sahoo L. Age and orientation of the cotyledonary leaf explants determine the efficiency of *de novo* plant regeneration and *Agrobacterium tumefacien*-smediated transformation in *Jatropha curcas* L. South African Journal of Botany. 2010;76:337–344.
7. Mehta RA, Cassol T, Li N, Ali N, Handa AK, Mattoo AK. Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality, and vine life. Nature Biotechnology. 2002;20:613–618.
8. Miyagawa Y, Tamoi M, Shigeoka S. Overexpression of a cyanobacterial fructose-1, 6-/sedoheptulose-1, 7-bisphosphatase in tobacco enhances photo- synthesis and growth. Nature Biotechnology. 2001;19:965–969.
9. Mohammed A, Abalaka ME. *Agrobacterium* transformation: A boost to agricultural biotechnology. Journal of Medical Genetics and Genomics. 2011;3(8):126–130.
10. Oeller PW, Min-Wong L, Taylor LP, Pike DA, Theologis A. Reversible inhibition of tomato fruit senescence by antisense RNA. Science. 1991;254:437–439.
11. Rafat A, Aziz MA, Rashid AA, Abdullah SNA, Kamaladini H, Sirchi MHT. Optimization of *Agrobacterium tumefaciens* mediated transformation and shoot regeneration after co-cultivation of cabbage (*Brassica oleracea* subsp. capitata) cv. KY cross with AtHSP101 gene. Science Horticultural. 2010;124(1):1–8.
12. Sakamoto T. Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. Nature Biotechnology. 2006;24:105–109.
13. Singh VV, Verma V, Pareek AK, Mathur M, Yadav R, Goyal P. Optimization and development of regeneration and transformation protocol in Indian mustard using lectin gene from chickpea [*Cicer arietinum* (L.)]. Journal of Plant Breeding and Crop Science. 2010;1(9):306–310.
14. Zhang C, Qi Z, Jiao P, Jiang Z, Liu S, Fan S. Cloning and Disease Resistance Analysis of the Maize *ZmBON3* Gene. Agronomy. 2023;13(1):152. https://doi.org/10.3390/agronomy13010152
15. Zhong S, Dong B, Zhou J, Miao Y, Yang L, Wang Y. Highly efficient transient gene expression of three tissues in Osmanthus fragrans mediated by Agrobacterium tumefaciens. Scientia Horticulturae. 2023;310:111725.
16. Zhou M, Luo J, Xiao D. *et al.* An efficient method for the production of transgenic peanut plants by pollen tube transformation mediated by *Agrobacterium tumefaciens*. Plant Cell Tiss Organ Cult. 2023;152:207–214. https://doi.org/10.1007/s11240-022-02388-0
17. Zong H, Wang S, Ouyang C, Deng X, Li L, Li J. *Agrobacterium*-mediated transformation of *Jatropha curcas* young leaf explants with lateral shoot-inducing factor (*LIF*). International Journal of Agriculture and Biology. 2010;12:891–896.