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A Review on Plant Transformation Methods

Prof. Nirod Kumar Dhal[1], Mr. Bidwan Ranjan Sahu[2], Mrs. Amlan Sushree[3]
[1,2,3]Department of Agriculture, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha
Email -[1] nirodkumardhal@gmail.com

Abstract

Plant transformation is now a central plant biology research tool, and a realistic tool for the transgenic plant growth. There are many confirmed approaches for stable integration into the nuclear genomes of different plant species with novel genes. As a result, transgenic plant gene transfer and regeneration are no longer the factors that limit the development and application for many plant species of practical transformation systems. However, the desire for greater efficiency in transformation has stimulated work not only to improve different existing methods, but also to invent new methods. The most widely publicized methods for transmitting genes into plant cells have been rejected or either disproved or inefficient for use in daily transgenic plant growth. Practically all the transformation work in many laboratories depends on the particle bombardment with the Agrobacterium-mediated transformation or DNA-coated microprojectiles for the gene transfer to develop transgenic plants from a variety of plant species. The achievements in gene transfer include most of the major economic fruits, vegetables and medicinal plants. Consequently, transgenic plant gene transfer and regeneration are no longer the considerations that hinder the production and deployment of functional transformation systems for many plant species. The strategies continued to evolve in order to overcome a great variety of hurdles faced in the early stages of plant transformation growth..

Key words: Agrobacterium, Gene Transfer, Plant Transformation, Regeneration, Transgenic

Introduction

Plant genetic engineering enables the direct incorporation of agronomically valuable genes into key crops and by creating new and genetically diverse plant materials provides an important method in breeding programs. The guided beneficial transition of gene from one organism to another and the resulting secure incorporation and expression of a foreign gene in genome is called "genetic transformation"[1]. The transferred gene is known as "transgene" and as "transgenics" are considered the species that evolve after a positive gene transfer. Genetically modified plants expressing δ endotoxin genes from the *Bacillus thuringiensis (B t*), plant lectins and protease inhibitors have been successfully developed, tested and proven to be extremely viable for the pest management in various crop systems over the past decade, among the numerous r- DNA technologies[2]. One of the major successes in applying plant genetic engineering technologies to agriculture is insect-resistant crops. Many plant-derived genes cause harmful effects rather than toxic ones, and many insect species are less prone to most of these influences or not. Therefore, better solutions are expected to be provided by the genes for δ -endotoxins[3].

Biotechnology developments have created many unique opportunities including exposure to different plant regeneration technologies, innovative and efficient drugs, and ability to change gene expression levels, ability to change gene expression patterns, and transgenic production of different insecticidal genes. With the emergence of techniques of genetic transformation based on recombinant DNA technology, it is now feasible to inject foreign genes which confer insect resistance into plant genome[1], [2]. To maintain the capacity for crop yields and satisfy the growing food demand, crop productivity requires to be increased. Nevertheless, the genetic capacity is believed to have been fully exploited for yield improvement in most crops. As a result, any increase in efficiency under optimal nutrition and abiotic conditions has to be about reducing losses due to pests and diseases. Recombinant DNA technology combined with cultivation of plant tissue has helped to develop new options for economic management of various types of biotic stress including the insect pest[3]. These techniques, including insect pests, would be of immense value in reducing the losses caused by biotic stresses.

Transgenic plants possess significant potential to support both developing and developed countries. Transgenic plants that transmit insecticidal *Bt* proteins alone/in combination with herbicide-tolerant proteins revolutionize farming. Since their first appearance in the mid 1 990s, the use of such crops with input traits for pest management, especially insects and herbicide resistance, has increased dramatically[1].

India, the world's biggest cotton-growing region, has increased productivity by up to 50 percent while cutting insecticide sprays by half, with the environmental and health consequences, in addition to increased farm income since Bt cotton was launched in 20 02[4]. Performance achieved in cotton has acted as an outstanding blueprint for emulating rice, wheat, pulses and oilseeds in many other crops that have the potential to make agriculture a viable career for Indian farmers. Plant transformation is now a key plant biology research tool, and a practical tool for transgenic plant development[1]. There are many confirmed methods for stable integration into the nuclear genomes of different plant species with novel genes.

1. Transformation methods

Gene delivery systems require the use of several methods to transfer the isolated genetic materials into a functional host cell. Two types of delivery systems currently exist (Table 1)[2]:

a) Non-biological mechanisms (which include physical and chemical methods)

b) Biological systems.

The need for greater transformation efficiency has driven research not only to improve different existing methods but also to develop novel methods.

1.1. Biological requirements for transformation

The basic conditions for the production of transgenic plants in a gene transfer system are[5]:

- Availability of target tissue including cells with plant regeneration competence.
- A method for injecting DNA into these regenerable cells and
- A method for selecting and regenerating transformed plants at a sufficient frequency.

1.2. Practical requirements for transformation

In addition to the biological requirements for achieving transformation and the technical requirements for verifying reproducible transformation, desired features to be considered in evaluating alternative techniques or developing new ones for cultivar improvement include[2], [5]:

1. High efficiency, reproducibility and economy, to willingly produce numerous independent transformants for the testing.

2. Safety to operators, procedures avoiding, or objects requiring cumbersome precautions in order to avoid higher risk to operators (such as possible carcinogenicity of the Silicone carbide whiskers)[6].

3. Technical ease, involving least demanding or inherently variable manipulations, like protoplast regeneration and production.

4. Minimum tissue culture time, to decrease associated costs and preventing unwanted somaclonal variation.

5. Stable, consistent (nonchimeric) transformants for the vegetatively propagated species and fertile germ line transformants for the sexually propagated species.

6. Easy integration patterns and less copy number of the introduced genes, to decrease the probability of the undesired gene disturbance at the insertion sites, or multicopy linked transgene silencing[3].

7. Stable expression of the introduced genes in pattern expected from selected gene control sequences.

When tested against above requirements, most published strategies for moving genes into plant cells must be rejected as either disproved or inefficient for use in daily transgenic plant growth. As a result, nearly all of the transformation research in many laboratories depends on Particle bombardment with DNA-coated microprojectiles or Agrobacterium-mediated transformation for gene transfer to generate transgenic plants in a number of plant species[1], [5].

1.3. Non-Biological Based Transformation

Particle bombardment/Biolistics

Particle bombardment was first mentioned in 1987 as an alternative to protoplast transformation as a tool for the development of transgenic plants, and in particular for transformation of more recalcitrant cereals[6]. This methodology's main benefits over alternate propulsion systems are addressed elsewhere in terms of the variety of organisms and genotypes that have been engineered, and the high frequencies of transition for large agronomic crops.

The main applications of biolistics in plant science include temporary gene expression analyses, transgenic plant development and inoculation of plants with viral pathogens[5].

Biolistics gene structures can be in the form of linear or circular plasmids, or a sequence with linear expression. Embryogenic cell cultures are probably the best explants to be used for biolistic transition, because they can be distributed as standard cell targets and also have a higher capacity for recovery. Rice transformation was also successfully accomplished through the bombardment of the embryogenic calli, where the quality of transformation was improved to 50%. Particle bombardment has arisen as reproducible method of transformation of wheat, and first stable transformation in the commercially valuable conifer species (*Picea glauca*) has been accomplished using embryogenic callus tissue as an explant[3], [4], [6]. Particle bombardment, however, has some disadvantages. The efficiency of transformation may be lower than the transformation induced by Agrobacterium, and it is also more expensive. Intracellular targets are random and do not defend DNA from damage[1]. Because of the higher frequency of complex fusion patterns and numerous copy insertions which could induce gene silencing and transgene expression variability, many researchers have rejected the approach of particle bombardment.

1.4. Biological Gene Transfer

Agrobacterium Mediated Transformation

In the development of transgenic plants the natural ability of soil bacteria, *Agrobacterium rhizogenus* and *Agrobacterium tumefaciens*, to transform the host plants was exploited The possibility of using *A. tumefaciens* in the 1970s were revolutionary for the logical gene transfer of the exogenous DNA to crops[7]. Genetic alteration of plants has been considered a possibility. In comparison, *Agrobacterium* was the rational and normal transformation candidate to suggest since it spontaneously transfer DNA (T-DNA) found on the plasmid-tumor inducing (Ti) into the nucleus of the plant cells and integrates the DNA stably into the plant genome[3], [7].

Nevertheless, several obstacles remain to the individual genotype transition of many economically important crop species, and also forest species. With other nonbiological plant transformation methods emerging, *Agrobacterium* mediated transformation remained popular

and one of the most successful. This is particularly true for most dicotylledonous species, where *agrobacterium* is normally infectious[8]. It was believed that gene transfer mediated by Agrobacterium to monocotylledonous plants was not necessary.

Procedures related to targets for transformation can be divided into two categories: (a) those involving tissue culture, and (b) in planta procedures[8].

The most important requirement in tissue culture systems for plant transformation is a large number of regenerable cells that are accessible for the treatment of gene transfer and that will maintain the regeneration potential for the duration of the required target preparation, cell proliferation and selection treatments. A higher multiplication ratio from micropropagation device does not necessarily imply a huge number of gene-transferable regenerable cells. If the ability for effective regeneration is short lived, any time migration of genes into potentially regenerable cells may not permit the recovery of transgenic plants[6], [7]. Furthermore, tissue culture-based methods may cause unwanted somaclonal variations like cytosine methylation changes, point mutation induction and multiple chromosome aberrations. On the other hand, in a significant number of crop species, the realization of the whole plant transformers has been a problem, as these plants have proved to be extremely recalcitrant in vitro. As a result, other approaches are emerging in which the part of the tissue culture is obviated in the treatment and these are known as planta methods[1], [3], [8]. Plant genetic transformation is especially beneficial for the molecular genetic studies, crop development, and pharmaceutical material growth. Methods based on agrobacteria are generally superior for several species including monocots and dicots. Biolistics is the most commonly used method of direct transformation, both commercially and experimentally.

In Planta Transformation

While promising methods of plant regeneration have been developed, the technology has not produced regeneration in several other crops for use in transformation protocols, which represents a significant drawback to the full potential of the application of gene transfer technologies. Despite this significant restriction, it becomes necessary to develop strategies of transformation that do not rely on regeneration of the tissue culture or those that significantly reduce the steps of the tissue culture that interfere[9]. Methods of in planta transformation provide such an opportunity. Procedures requiring the transmission of transgenes directly into the intact plants in the form of naked DNA are called as in procedures for in planta transformation[10]. These methods restrict steps in the tissue culture, rely on simple protocols and require a short time to obtain whole transformed individuals.

In many cases, in planta procedures, meristems or other tissues have been addressed with the expectation that at fertilization, the egg cell recognizes the transfer of a full genome from the sperm cell that ultimately gives birth to zygotes and thus is the correct stage for introducing transgenes[9], [10]. Microprojectiles bombardment or Agrobacterium co-cultivation has been guided to turn cells in or around apical meristems for non-tissue culture-based approaches to

planta transformation. Naked DNA was also reported to be inserted into ovaries to create transformed progeny.

Table 1: DNA delivery methods available to produce plant transformants

Plant transformation	
Non-biological based transformation (Direct method)	**Biological gene transfer (Indirect method)**
A) DNA transfer in protoplasts 1. Chemically stimulated DNA uptake by protoplast 2. Electroporation 3. Lipofection 4. Microinjection 5. Sonication B) DNA transfer in plant tissues 1. Particle bombardment / Biolistics 2. Silicon carbide fiber mediated gene transfer 3. Laser microbeam (UV) induced gene transfer	A) Agrobacterium mediated transformation Primarily two methods 1. Co-cultivation with the explants tissue 2. *In planta* transformation B) Transformation mediated by viral vector

Using naked DNA, the cotton transformants were retrieved about a day after self-pollination after injection of DNA into the axil placenta[8]. Likewise, a combination of DNA and pollen was either added to receptive stigmatic surfaces or DNA was specifically inserted into rice floral tillers, or DNA was imbibed with soybean seeds. Such techniques, fascinating as they are, are actually inefficient due to their poor reproducibility[9]. Crop species that have been transformed effectively by injuring apical meristem of differentiated seed embryo of germinating seed and infecting with *Agrobacterium* comprises sunflower, *Arachis hypogaea L.*, peanut, *Helianthus annuus L.*, cotton, safflower, *Carthamus tinctorius L.*, *Dolichos lablab L.*, field bean and cotton[10].

Conclusion

In addition, the above achievements have provided a great leverage for the easy production of transgenic pants, since the technique is fast, cost-effective and it does not need higher infrastructural requirements even to manage recalcitrant crops like groundnut. Therefore gene transfer technology for the production of recalcitrant crops has become a realistic opportunity to experiment and generate viable transformants. Optimizing the interaction between Agrobacterium and plant is, however, essential for efficient transformation. Several factors are important, including the form of explants, and they must be sufficient for the recovery of the whole transgenic plants. While biotechnological advancements have produced many technologies for the gene transfer to plant cells, nearly all transformation research relies

solely on particle bombardment with DNA-coated microprojectiles or transformation mediated by Agrobacterium to generate transgenic plants for gene transfer. Therefore, this paper emphasizes the importance of this approach heavily.

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