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GENE TRANSFER TECHNOLOGIES IN PLANTS: ROLES IN IMPROVING CROPS

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Abstract
Gene is a segment of nucleic acid that encodes a functional protein or RNA and is the unit of inheritance. The principle objective of plant biotechnology is to create new varieties of cultivated plants by manipulating DNA molecule. Plant transformation technology has become a versatile platform for cultivar improvement as well as for analysis of gene function in plants. This article discuses and summarizes important work in the literature regarding the gene transfer technologies in plants. The main techniques focused in this article are gene transfer by *Agrobacterium tumefaciens*, microprojectile bombardment, electroporation of protoplast, polyethylene glycol method, microinjection, silicon carbide mediated transformation, liposome mediated gene transfer and sonication assisted *Agrobacterium*-mediated transformation. Moreover the application of gene transfer technologies related to the improvement of crops was also focused. This article will help the reader to have an idea on gene transfer technologies and also to the researcher working on plant genetic engineering.

Key Words: Gene; Gene transfer; Transformation technology; Crops improvement.

1. Introduction

Genetic manipulation of plants has been done by plant breeders for years with great success. Elegant schemes have been developed by plant breeders for crossing plants in order to transmit and maintain the required and desirable traits in inbred lines. However, the process of classical plant breeding are uncertain and slow. To transmit a required gene of interest by classical methods requires a sexual cross between two lines and then repeated back crossing between the hybrid offspring and one of the parents until a plant with the desired characteristic is obtained. Plant breeding is a lengthy process, taking ten to fifteen years to produce and to release a new variety. This process, however, is limited to those plants which can sexually hybridize, and genes in addition to the desired gene will be transferred.

Recombinant DNA technologies circumvent these limitations by enabling plant geneticts to identify and clone specific genes for desirable traits. Plants have a number of unique biological features that can be explored with recombinant DNA technologies. In this communication basic techniques used to manipulate plants genetically are discussed. The progresses in developing agriculturally important plants by recombinant DNA technologies are reviewed. Plant genetic engineering not only elevated the process of plant breeding, but also helps the introduction of new genes, either by overcoming the barriers of sexual incompatibility through somatic hybridisation or by the insertion of required genes into plant cells using various transformation methodologies. This article will be helpful for those researchers who are working in the field of plant genetic engineering.

2. Gene transfer technologies in plants

Presently, a number of methods exist for the genetic manipulation of plant cells. These procedures range from exploitation of the natural gene transfer system of *Agrobacterium* to the chemical treatment of isolated protoplasts by polyethylene glycol. It also includes physical procedures of DNA introduction, including electroporation of protoplasts and tissues, microinjection and silicon carbide fibre-mediated transformation. Moreover microprojectile bombardment has also received much attention as a physical method of DNA transfer and in many laboratories, is now a routine and reliable technique for the production of transgenic plants. The importance of gene transfer technologies to plants are listed in Table 1. A number of gene transfer technologies are discussed below.

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Table 1: Benefits of gene transfer technologies.

S.No	Importance of gene transfer technologies to plants
1.	Provide resistance against viruses.
2.	Acquire insecticidal resistance.
3.	To strengthen the plant to grow against bacterial diseases.
4.	Develop the plants to grow in draught.
5.	Engineering plants for nutritional quality.
6.	Make the plants to grow in various seasons.
7.	Herbicide resistant plant can be made.
8.	Resistance against fungal pathogens.
9.	Engineering of plants for abiotic stress tolerance.
10.	Delayed ripening can be done.

2.1. Gene transfer by Agrobacterium tumefaciens

Agrobacterium tumefaciens has been extensively used to introduce gene into plant cells. This bacterium is responsible for crawn gall disease in a variety of dicotyledonous plants. A plsmid carried within this bacterium cause crown gall disease [1][2]. This plasmid is called tumor inducing plasmid (T_i). T_i plasmid is upto 200 bp large and carries genes that are required for infection. This plasmid has T-DNA that becomes integrated into plant genome at an apparently random position through non homologous recombination. The size of T-DNA is approximately 23 kbp and is responsible for the cancerous properties of the transformed cells. It also synthesizes opines. In the T plasmid, T-DNA is flanked by two 25 bp imperfect direct repeats. These sequences play roles in the integration of T-DNA into the plant genome [3]. *Agrobacterium* has proved to be an incredible useful tool for the integration of genes into plants [4].

The most widely used technique for plant transformation is based on *Agrobacterium*, in which novel genes, linked to the Ti or Ri plasmid T-DNAs, are inserted into the host plant cells during T DNA transfer [5]. This approach has been used to transform numerous plants, but is almost exclusively restricted to dicotyledons [6]. Despite recent reports using a strain of *A. tumefaciens* carrying a vector with the *vir B* and *vir G* genes from the supervirulent Ti plasmid pTiBo542 to transform rice [7], attempts to infect monocotyledonous plants, which constitute some of the world's most important food crops, with *Agrobacteria* have been rarely successful [8].

2.2. Gene transfer by microprojectile bombardment

The cocept of transfering DNA-coated particles directly into cells was first conceived by Sanford and co-workers in 1984 [9]. The first results using a gunpowder-driven device to deliver tungsten microprojectiles coated with viral RNA into onion epidermal cells were published three years later [10]. In the same year, microprojectile-mediated delivery of plasmid DNA resulted in the introduction of a foreign gene, also in onion cells [11].

Microprojectile bombardment has got much attention and attraction as a physical procedure of DNA transfer in many research laboratories during the past years. This method is routine and reliable way of producing transgenic plants. The method relies on a device which utilizes a propelling force, such as compressed gas or gunpowder, to accelerate inert (usually metal) particles (the microprojectiles), coated with DNA, into target cells. This technique is also referred to as particle bombardment, particle gun method, particle acceleration and Biolistics (Biological ballistics). A number of applications of this method in plant science have been listed in Table 2.

Table 2: List of applications of microprojectiles.

S.No	Applications of microprojectile bombardment
1.	Highly versatile and adaptable technique which can be applied to a wide range of cells and tissues.
2.	Method is simple and efficient.
3.	The process of microprojectile bombardment has also led to an increased understanding of the mechanisms of gene expression and regulation.
4.	Microprojectile bombardment can even be used to wound plant tissues, allowing more efficient transformation via *Agrobacterium*.
5.	This method permits the transformation of cells from a wide range of sources including cell suspensions, callus, meristematic tissues, immature embryos, protocorms, coleoptiles and pollen.
6.	Microprojectile technique significantly reduces the time required for the production of genetically modified plants.
7.	This method help in the transformation of several major cereals, including barley, maize, wheat, rice, pearl millet, together with other monocotyledons such as tulip and orchids.

2.3. Electroporation of protoplast

High concentration of plasmid DNA containing the gene of interest is added to a suspension of protoplast and the mixture is given a shock with an electric field of 200-600 V/cm. The protoplasts are then grown in tissue culture for a period of one or two weeks. The selection pressure is then applied to select the transformed one. Both maize and rice protoplast have been successfully transformed with efficiencies of between 0.1 and 1%. Introduction and expression of transgenes in plant protoplasts were also reported [12]. Moreover transient

expression of fluorescent fusion proteins in protoplasts of suspension cultured cells were also explained [13].

2.4. Gene transfer by polyethylene glycol

This technology is applicable for protoplast only. The chemical used is polyethylene glycol. It stimulates endocytosis and thereby causing the uptake of DNA. In this method protoplast are kept in polyethylene glycol (PEG) solution. The concentration of PEG used is 15% having 8000 dalton molecular weight. After exposure of protoplasts to exogenous DNA in presence of PEG and other chemicals, PEG is removed and intact protoplast are then cultured to form cells with walls and colonies in turn [14]. Selection pressure is then applied to get the transformants. The transfer of gene across the protoplast membrane can be initiated by a number of chemicals of which polyethylene glycol is the most important. It has become the most widely used due to the availability of simple transformation protocol. Method was developed using calcium alginate micro beads to immobilize DNA molecules in combination with polyethylene glycol treatment also [15].

2.5. Gene transfer through microinjection

Transformation through microinjection is based on introducing DNA into the cytoplasm or nucleus by using a glass micro capillary-injection pipette [16]-[17]. This operation requires a micromanipulator. During the introduction of DNA into the nucleus, cells are immobilized with a holding pipette and gentle suction. Microinjection is mainly used for the transformation of large animal cells. Its importance for plant transformation is rather limited due to the characteristics of plant cell walls, which contain a thick layer of lignins and cellulose. The plant cell wall is a barrier for glass micro tools. The method allowed the incorporation not only of DNA plasmids but also of whole chromosomes into plant cells [18]-[19].

Although it has a fairly high transformation frequency (20–50%), microinjection is a time consuming process that requires specific equipment and considerable training. This technique was used to study the cellular functions of plant cells and plastid physiology, e.g. in tobacco and *Vicia faba* [20].

2.6. Silicon carbide mediated transformation

Silicon carbide mediated method is also one of the transformation method used to transform plants. This method is least complicated. In this technique fibres are used which are single crystals of silica organic minerals like silicon carbide which possess an elongated shape, having a diameter of 0.6 mm and a length of 10–80 mm. Moreover it also exhibits a high resistance to expandability. In this method silicon carbide fibers' are added to a suspension containing plasmid DNA and plant tissue (immature embryos, callus, cell cluster). It is then mixed in commercial shakers or in vortex. Fibres coated with DNA penetrate the plant cell wall in the presence of small holes produced at the time of collision between fibres and plant cells [21]-[23].

The factors on which the efficiency of transformation depends are the plant material, fiber size, parameters of vortexing, shape of the vessels used, and the characteristics of the plant cells, especially the thickness of the cell wall. This process is easy and quick. It is not so expensive and useful for various plant materials. The main drawback of this technique is low transformation efficiency, damage to cells negatively influencing their further regeneration capability, and the need of following extraordinarily rigorous precaution protocols during laboratory work, as breathing the fibers in, especially asbestos ones, can lead to serious sicknesses [24]. Silicon carbide whisker-mediated embryogenic callus transformation of cotton (*Gossypium hirsutum* L.) and regeneration of salt tolerant plants were also reported [25].

2.7. Liposome mediated gene transfer

Liposomes are circular lipid molecules with an aqueous interior that can carry nucleic acids. It encapsulates the DNA fragments and then adheres to the cell membranes and fuse with them to transfer DNA fragments. Thus, the DNA enters the cell and then to the nucleus. It is a very efficient technique used to transfer genes in bacterial, animal and plant cells. Various reports on the integration of genes introduced by means of liposomes followed by transgenic plant regeneration for tobacco [26] and wheat [27] have been published so far.

2.8. Pollen tube pathway method

The transformation method via pollen-tube pathway has great significance in agriculture molecular breeding [28]. After pollination the styles were cut. The DNA was then applied. The DNA reaches the ovule by flowing down the pollen-tube. This procedure, the so-called pollen-tube pathway (PTP), was applied first time for the transformation of rice [29]. Here the transgenic plants were obtained at remarkably high frequency. Afterward PTP was used for other species e.g. wheat [30], soybean [31], *Petunia hybrida* [32] and watermelon [33].

2.9 Sonication assisted Agrobacterium mediated transformation

Sonication-assisted *Agrobacterium*-mediated transformation (SAAT) is an efficient transformation technology, reported by Trick and Finer [34]. It is *Agrobacterium* based

technology. This method consists of subjecting the target plant tissue to brief periods of ultrasound while immersed in an *Agrobacterium* suspension. SAAT overcomes certain barriers such as the host specificity and the inability of *Agrobacterium* to reach proper cells in the target tissues. This method also enhances DNA integration in many plant groups including dicots, monocots, and gymnosperms. It is likely that the enhanced transformation rates using SAAT result from micro-wounding both on the surface and deep within the target tissue. Therefore, unlike other transformation methods, this system also has the potential to transform meristematic tissue buried under several cell layers [30]. Cotton transformation based on cavitations caused by sonication which results in thousands of micro wounds on and below the surface of plant tissue and allows *Agrobacterium* to travel deeper and completely throughout the tissue. This wounding fashion increases the probability of infecting plant cells lying deeper in tissue.

3. Roles in improving crops

Biotechnological strategies for crop improvement demand efficient procedures for routine introduction of defined foreign genes into plant genome. Successful genetic manipulation requires the ability to deliver biologically active and functional DNA into plant cells followed by recovery of transgenic plants expressing a foreign gene. Gene transfer technology is playing significant role in improving plants and their yields.

3.1. Transformation of rice

Rice is the staple food for more than one third of world's population. To feed the growing world population it is the requirement to increase the total food production. Although the world food supply has more than doubled since the onset of the green revolution but still there is a need to improve the quantity as well as quality. Biolistic was successfully used for transformation of immature embryos of rice [35]. Reports were also maderegarding the transformation of indica and javanica rice in addition to other japonica rice [36]-[48].

Fujimoto et al.[49] were the first to engineer japonica rice through electroporation with modified d-endotoxin gene (*cry*) from *Bacillus thuringiensis*. It was found that the R2 generation of transgenic rice was more resistant to insects than wild type plants. Later, Wu¨nn et al. [50] obtained transgenic indica rice cultivar IR58 expressing a synthetic *cryIA(b)* gene driven by 35S promoter through particle bombardment. The rice *Xa21* gene which confers resistance to blight pathogen, *Xanthomonas oryzae* was cloned by Song et al. [51]. Transgenic rice plants harbouring the cloned gene displayed high levels of resistance. The gene has been found to be effective against several isolates [52].

Shimada et al. [53] produced transgenic rice plants with antisense construct of rice waxy gene coding for granule-bound starch synthase under the control of 35S promoter. A significant reduction in amylose content of grain starch was observed in the seeds of these plants. Most interestingly, to confer the capability of producing precursor (β-carotene) of vitamin A in rice endosperm, Burkhardt et al. [40] engineered rice with the cDNA coding for phytoene synthase from daffodil, the first of the four specific enzymes involved in β-carotene (provitamin A) biosynthesis in plants. In the endosperm of these transgenic plants, phytoene synthase accumulation was observed indicating that engineering of provitamin A biosynthesis pathway is possible in non-photosynthetic, carotenoid lacking tissue. Recently, the same group reported *Agrobacterium*-mediated transformation of rice with all the genes necessary for the accumulation of provitamin A in transgenic rice seeds [54]. Hayakawa et al. [55] engineered the coat protein (Cp) gene of rice stripe virus into two japonica rice varieties by electroporation of protoplasts resulting in significant levels of resistance against the virus in the transgenic plants.

3.2. Genetically engineered maize

A coat protein-mediated resistance to viruses, introduced in rice via protoplast transformation [55], was transferred to maize and barley via particle gun bombardment [56]-[57]. The resistance to sulfonylurea (herbicide) conferred by the *als* gene of *Arabidopsis thaliana* was also transferred to maize [58] by particle gun technology. Maize has been reported to get transformed by Silicon carbide fiber-mediated DNA delivery system [59]-[62]. Whiskers-mediated maize transformation has also been reported [63].

3.3. Genetically engineered wheat

Wheat is a member of the *Triticeae* group of cereals. It is indisputably one of the major food crops of the world and a foundation of human nutrition. Genetic improvement of wheat has received considerable attention worldwide over the years with the purpose of increasing the grain yield to minimize crop loss due to unfavorable environmental conditions and development of resistance against various pests and pathogens.

The first transgenic wheat plants were produced by Vasil et al. [64], followed by Vasil et al. [65], Weeks et al. [66], Nehra et al. [67], and Altpeter et al. [68] employing microprojectile bombardment as a method of DNA

delivery. Subsequently, the development of methodology for the delivery of genes into intact plant tissues by bombardment of DNA-coated gold or tungsten particles has revolutionized the field of wheat transformation. In recent years, sincere efforts are being made to transform wheat genetically with different alien genes of agronomically importance [69]-[75]. However, in majority of reports, genetic transformation with a single target gene has been used for the production of transgenic wheat expressing tolerance to herbicide, resistance to fungal and viral diseases [76].

3.4. Tobacco plant

Tobacco has been found to be the key plant model for the development of transformation technology. This probably reflects the fact that tobacco was the first plant species to be regenerated *in vitro* [77] and was used to develop standardized tissue culture conditions[78]. Reports on the integration of genes introduced by means of liposomes followed by transgenic plant regeneration for tobacco have been made [26]. Tobacco has been reported to be transformed by Silicon carbide fiber-mediated DNA delivery system also [22].

3.5. Transformation in other plants

Transgenic plants, however, were only recovered in several studies using microinjection in petunia [79], rape [80], and barley [81] at very low frequency. Sonication assisted *Agrobacterium*-mediated transformation could be a promising tool for enhancing transformation efficiency in flax [82]. Direct gene transfer study and transgenic plant regeneration after electroporation into mesophyll protoplasts of *Pelargonium x hortorum* was reported [83].

Functional transient genetic transformation of *Arabidopsis* leaves by biolistic bombardment were done [84]. *Agrobacterium tumefaciens*-mediated transformation of *Allium porrum* and *Allium sativum* were also reported [85]. The possibility of using a biolistic procedure of transmitting soybean dwarf virus to soybean plants without relying on aphid vectors was investigated [86].

4. Conclusion

Plant genetic transformation provides benefit to molecular genetic studies and crop improvement. These transformation technologies are at present the scientific tools for basic research. Since their development, plant transformation technologies have also been used for the genetic modification of agronomically important plant species. In the case of monocotyledons, the agronomic potential of genetic engineering was probably the main incentive for their development. Many difficulties are still there with regards to the transformation technology it self. In future, a combination of improved transgene engineering, a reliable genetic recombination systems and efficient DNA delivery procedures should give rise to a new generation of transformation technologies.

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