COMPARATIVE REVIEW OF PLANT TRANSFORMATION TECHNIQUES

Abstract

Plant transformation is now an important biotechnological tool in plant biology and a practical tool for transgenic plant development. There are many verified methods for stable introduction of novel genes into the nuclear genomes of diverse plant species. As a result, gene transfer and regeneration of transgenic plants are no longer the factors limiting the development and application of practical transformation systems for many plant species. However, the desire for higher transformation efficiency has stimulated work on not only improving various existing methods but also in inventing novel methods. Different methods of transferring the gene into plant cells have been developed and continuous efforts have been made to increase its efficiency. Both direct and indirect methods of gene transfer have their own merits and demerits. Efforts have been made continuously to eliminate drawbacks and to develop an easy and eco-friendly method to transfer foreign genes. Many methods of genetic transformation have been proposed and tried in the laboratories, but most of them result to transient expressions. However, transformation work based on particle bombardment with DNA coated micro projectiles and *Agrobacterium* mediated transformation have proved to be promising in producing stable transgenic plants from a range of plant species.

Key words: Transgenic, *Agrobacterium*, electroporation, particle bombardment, transformation, tissue culture.

Introduction

Plant genetic transformation permits direct introduction of agronomically useful genes into important crops and offers a significant tool in breeding programs by producing novel and genetically diverse plant materials. The directed desirable gene transfer from one organism to another and the subsequent stable integration and expression of a foreign gene in the genome is referred to as 'Genetic Transformation'. The transferred gene is known as 'transgene' and the organisms that are developed after a successful gene transfer are known as 'transgenics' (Babaoglu *et al*., 2000). Advances in biotechnology have provided several unique opportunities that include access to various plant transformation techniques, novel and effective molecules, and ability to change the levels of gene expression, capability to change the expression pattern of genes, and develop transgenics with different insecticidal genes. With the advent of genetic transformation techniques based on recombinant DNA technology, it is now possible to insert foreign genes that confer resistance to insects into the plant genome. To sustain the crop yield potential and to meet the growing demand for food, crop productivity needs to be increased. However, in most crops it is believed that the genetic potential has been fully exploited for yield increase. As a result, any improvement in productivity has to revolve around the reduction of losses due to pests and diseases under optimal nutrition and abiotic factors. Recombinant DNA technology coupled with plant tissue

culture has helped develop novel options for the economic management of various kinds of biotic stresses including insect pests. These technologies would be of immense value in reducing the losses caused by biotic stresses, including insect pests.

Among the various r-DNA technologies, genetically modified plants expressing δ- endotoxin genes from *Bacillus thuringiensis* (*Bt*), protease inhibitors and plant lectins have been successfully developed, tested and demonstrated to be highly viable for pest management in different cropping systems during the last decade and a half (Gatehouse, 2008). Insect resistant crops have been one of the major successes of applying plant genetic engineering technology to agriculture. Most of the plant derived genes produce chronic rather than toxic effects and many insect pests are less or not sensitive to most of these factors. Therefore, the genes for δ-endotoxins are expected to provide better solutions.

Transgenic plants display considerable potential to benefit both developed and developing countries. Transgenic plants expressing insecticidal *Bt* proteins alone or in conjunction with proteins providing tolerance to herbicide are revolutionizing agriculture (Shelton *et al*., 2002). The use of such crops with input traits for pest management, primarily insects and herbicide resistance, has risen dramatically since their first introduction in the mid-1990s.

The continuous existing of humans is possible only by increasing food grain production and productivity. To achieve this conventional plant breeding approaches should be used in combination with biotechnological methods and develop a more reliable efficient method (Kavipriya *et al.,* 2019). Genetic engineering facilitates the easy transfer of gene which is inter-generic, inter-specific and also even inter-kingdom. Developing a systematic tool to transfer genes is important because it serves as a base for every genetic engineering research works. Both direct and indirect methods developed till date proved their efficiency yet they have their own drawbacks as shown in Table 2 and 3 respectively. *Agrobacterium*-mediated gene transfer is an indirect method and is highly efficient compared with other methods that have been discovered unexpectedly during the search for the causal organism for crown gall disease (Que *et al*., 2019). After understanding the molecular mechanism involved in transferring T-DNA into host it has been developed into a successful vehicle to transfer our gene of interest. During the last two decades, many improvements have been done in transformation techniques and it leads to rapid growth in the genetic engineering approach in plant breeding programmes. Modern biotechnological transfer of gene sequences has overcome the obstacles of incompatibility, sterility, pre-fertilization barriers, post-fertilization

barriers, and risk in transferring gene between gene pools and also between different unrelated organisms, related species, wild species (Jeu, 2000). This review gives a brief discussion about different gene transfer methods.

Biological requirements for transformation

The essential requirements in a gene transfer system for production of transgenic plants are:

- Availability of a target tissue including cells competent for plant regeneration.
- A method to introduce DNA into those re-generable cells and.
- A procedure to select and regenerate transformed cells and tissues at a satisfactory frequency.

Methods of Transformation

Gene delivery systems involve the use of several techniques for transfer of isolated genetic materials into a viable host cell. At present, there are two classes of delivery systems (Table 1) (a) Non-biological systems and (b) Biological systems. The desire for higher transformation efficiency has stimulated work on not only improving various existing methods but also in inventing novel methods.

- **Table 1: Showing DNA delivery methods available to produce transgenics (Plant transformation).**

(A): (Direct) or non-biological methods	(B): (Indirect) or biological methods
1) Chemically stimulated DNA uptake by protoplast Polyethylene glycol (PEG) – induced DNA uptake. 2) Electroporation 3) Particle bombardment / Biolistics 4) Silicon carbide fiber mediated gene transfer 5) Shock-waves 6) Microinjection 7) Sonication 8) Agitation with Glass Beads 9) Laser Microbeam 10) Pollen Tube-Mediated Gene Transfer	1) *Agrobacterium* mediated transformation Primarily two methods a) Co-cultivation with the explants tissue b) *In planta* transformation 2) Transformation mediated by viral vector

(Keshavareddy *et al.*, 2018).

(A): Non-Biological Based Transformation (Direct Method)

- **Chemically Stimulated DNA Uptake by Protoplast/ Polyethylene glycol (PEG) – induced DNA uptake**

The polyethylene glycol is a polyether compound that has the capacity to attach with DNA and along with Ca^{2+} transfer the DNA into the target by penetrating the membrane. The method is simple and does not require specialized equipment (Liu and Vidali, 2011). In this method the target is protoplast and so the method starts with the preparation of protoplast. Usually, leaf mesophyll is used to prepare protoplast. The leaf disc is treated with cellulase and protease enzymes to degrade cell wall (Sahab *et al.*, 2019). The enzyme mixture contains

osmoticum because maintaining tonicity is very important while preparing protoplast and the protoplast is purified with a mannitol solution. The protoplast prepared are made to suspend in the solution of PEG/Ca^{2+} and the DNA solution of concentration 60µm. The gentle shake to the solution of DNA and protoplast are incubated to a brief period of time. Finally, the protoplast treated with DNA is spread over the suitable medium and the protoplast containing the transferred DNA is isolated by using marker and regeneration of the whole plant from the transferred protoplast that results in the transgenic plant (Sahab *et al*., 2019). Though the methodology is simple and devoid of costly equipment, it suffers difficulty in the regeneration of plant from a protoplast. The concentration of various chemicals and DNA are standardized based on the target. The multiple copies of
target DNA are produced either by *E. coli* or PCR

- **Electroporation**

Plant cell electroporation generally utilizes the protoplast because thick plant cell walls restrict macromolecule movement (Bates, 1999). Electrical pulses are applied to a suspension of protoplasts with DNA placed between electrodes in an electroporation cuvette. Short high-voltage electrical pulses induce the formation of transient micropores in cell membranes allowing DNA to enter the cell and then the nucleus (Yoshihiro *et al.,* 2012), described in Figure 1 below.

It is the most popular physical genetic transformation method. This is due to its quickness, low cost, and simplicity even when it has a low efficiency, requires laborious protocols for regeneration after genetic transformation, and can only be applied to protoplasts (Bahi *et al.,* 2011). Electroporation is based on the application of a strong electrical field to enhance the formation of pores on the cell membrane due to a polarity alteration, caused by the electrical field (alternated or pulsed) that induces a dipolar moment inside the cells, and a potential difference through the plasmatic membrane (Escoffre *et al.,* 2009). If the cell is exposed to a high frequency field, its cellular membrane suffers a short circuit and its dipolar moment grows and rotates towards the direction of the field, producing a cellular stretching along this direction, leading to a temporal permeabilization of the membrane (Bates *et al*., 1983). When using protoplasts of plants, fungi or animals, the uptake of DNA can be achieved by electrofusion, i.e., two membranes located very close to each other can be fused by application of an electric field and the DNA present in the cell suspension is trapped in the cytoplasm of the joined cells (Kotnik *et al,*. 2013).

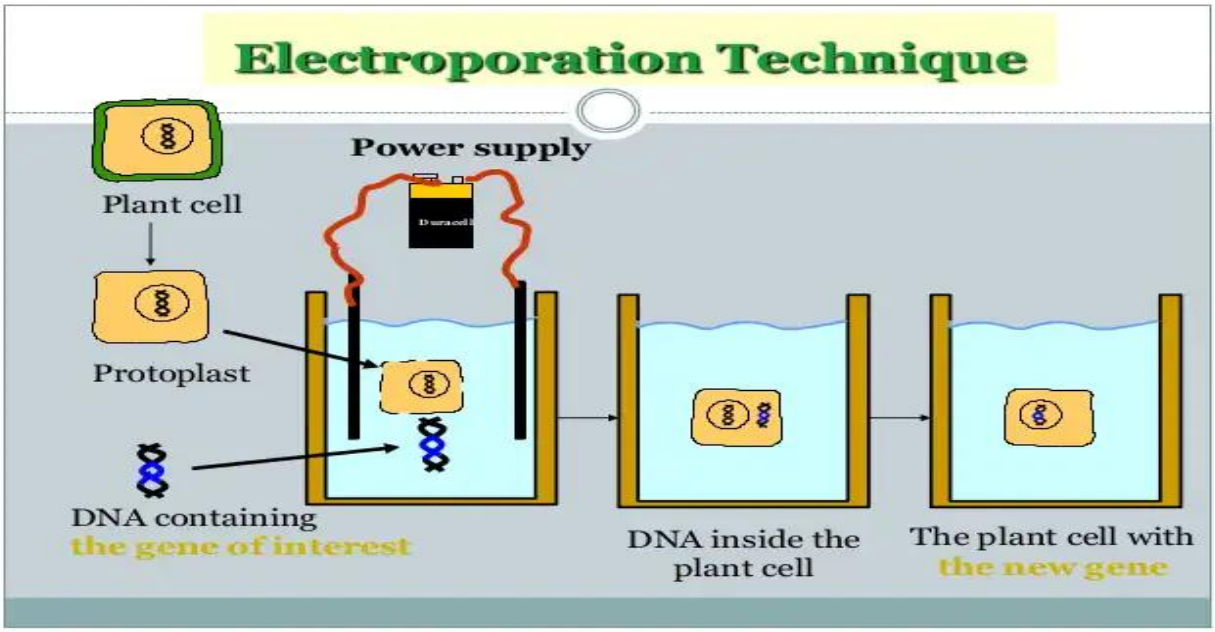

Figure 1: Showing instrumentation of electroporation (https://slidetodoc.com/direct-dna-transfer-introduce-dna-into-cells-assay/).

- **Particle bombardment/Biolistics**

Particle bombardment is a technique used to introduce foreign DNA into plant cells (Birch and Franks, 1991, Yao *et al*., 2006). Gold or tungsten particles (1–2 μm) are coated with the DNA to be used for transformation. The coated particles are loaded into a particle gun and accelerated to high speed either by the electrostatic energy released from a droplet of water exposed to high voltage or using pressurized helium gas; the target could be plant cell suspensions, callus cultures, or tissues. The projectiles penetrate the plant cell walls and membranes. As the microprojectiles enter the cells, transgenes are released from the particle surface for subsequent incorporation into the plant's chromosomal DNA (Yoshihiro *et al.*, 2012). The instrumentation of the biolistic technique is shown in Figure 2 below.

Figure 2: Showing the instrumentation of particle bombardment method of plant transformation technique (https://slidetodoc.com/direct-dna-transfer-introduce-dna-into-cells-assay/).

- **Silicon carbide whisker**

Silicon carbide fibers are capable of puncturing cells without killing them. Using this property, the silicon carbide (SiC) mediated transformation (SCMT) method was proposed to transform maize and tobacco (Svensson *et al.,* 1997). SCMT is an easy, cheap, and quick procedure that can be effectively implemented for various cells (Rivera *et al*., 2014), however, it has low transformation efficiency, and may damage the cells influencing their regeneration capability. It could also cause injury to the laboratory staff (Svensson *et al.,* 1997). Silicon carbide fibers are added to a suspension of tissue (cell clusters, immature embryos, or callus) and plasmid DNA using a vortex, shaker or blender. DNA coated fibers penetrate the cell membrane through small holes created by collisions between the plant cells and the fibers. The exact transformation mechanism by SCMT is unknown, but it has been proposed that the strong and sharp edges of the silicon carbide fibers cut the cellular wall when they collide, acting as needles allowing the delivery of DNA into the target cells (Yamagishi *et al.,* 2006). SCMT efficiency depends on the fiber size, vortex parameters (type, duration and speed of agitation), vessel shape, and the thickness of the cell wall (Rakoczy *et al.,* 2002). The diagrammatical application of the SCMT is represented in Figure

3 below.

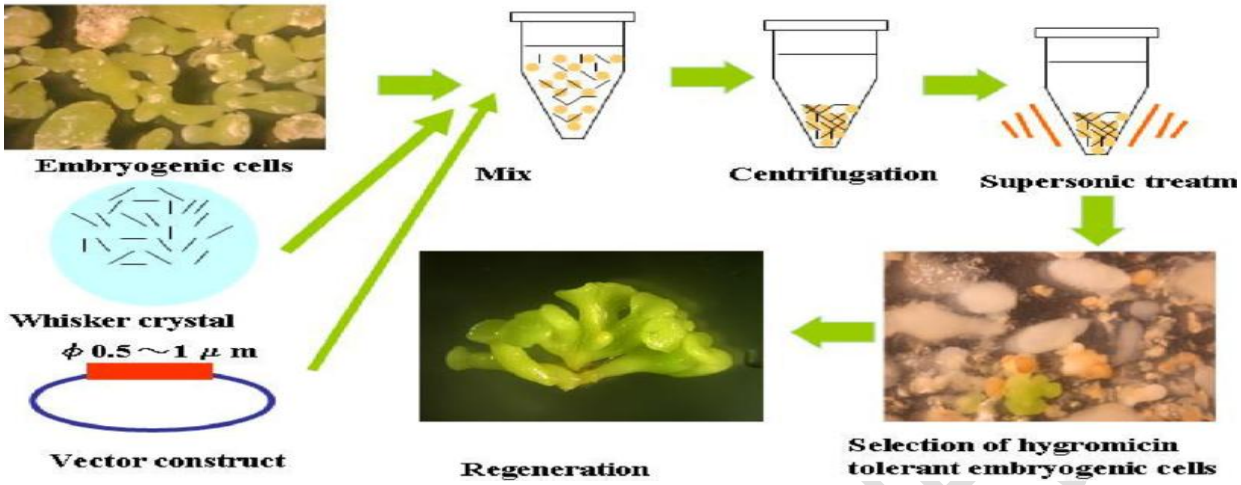

Figure 3: Showing efficient production of transgenic soybean (*Glycine max* (L) Merill plant mediated via Whisker-supersonic (WSS) method (https:www.researchgate.net/figure/scheme-of-whisker-mediated-transformation-of-soybean_fig2_241753945).

- **Shock-waves**

Shock waves are pressure pulses with a peak positive pressure in the range of 30 to 150 MPa, lasting between 0.5 and 3 µs, followed by a tensile pulse of up to -20 MPa with duration of 2 to 20 µs. They are produced by electrohydraulic, electromagnetic or piezoelectric shock wave generators (Loske, 2011), as shown in the instrumentation in Figure 4 below. The exact mechanism responsible for shock wave-assisted cell permeabilization is still not clear, but there is evidence that it is due to shock wave-induced cavitation (Rivera *et al*., 2014).

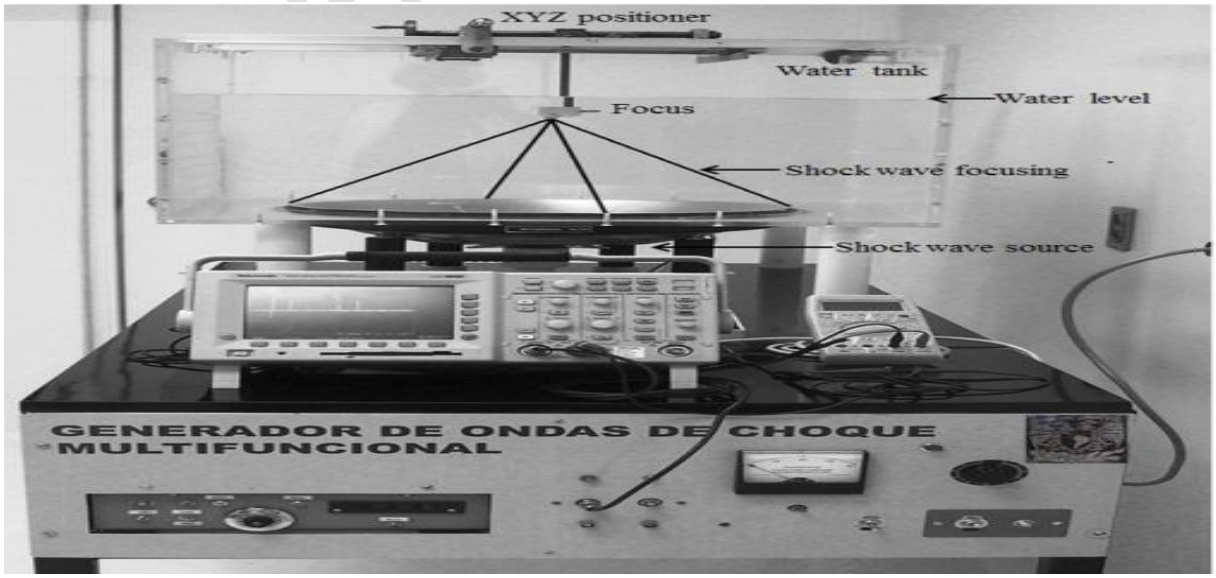

Figure 4: Showing instrumentation of the shock-wave generator method of plant transformation technique (Loske, 2011).

- **Microinjection**

Microinjection is a surgical technique that uses the micropipettes to directly deliver the DNA into the nucleus or cytoplasm. The microinjector connected to a micromanipulator deliver the DNA into the target (Baskaran and Dasgupta, 2011). The holding pipette holds the cell and the injector pipette injects the DNA and all these processes happen under a microscope (Hosokawa *et al*., 2009) as shown in Figure 5 below. It is an easy direct method of DNA delivery and now in order to increase its efficiency capillary microinjection has been developed. The size of the micropipette is usually 0.5- 5µm (Saito, 2005).

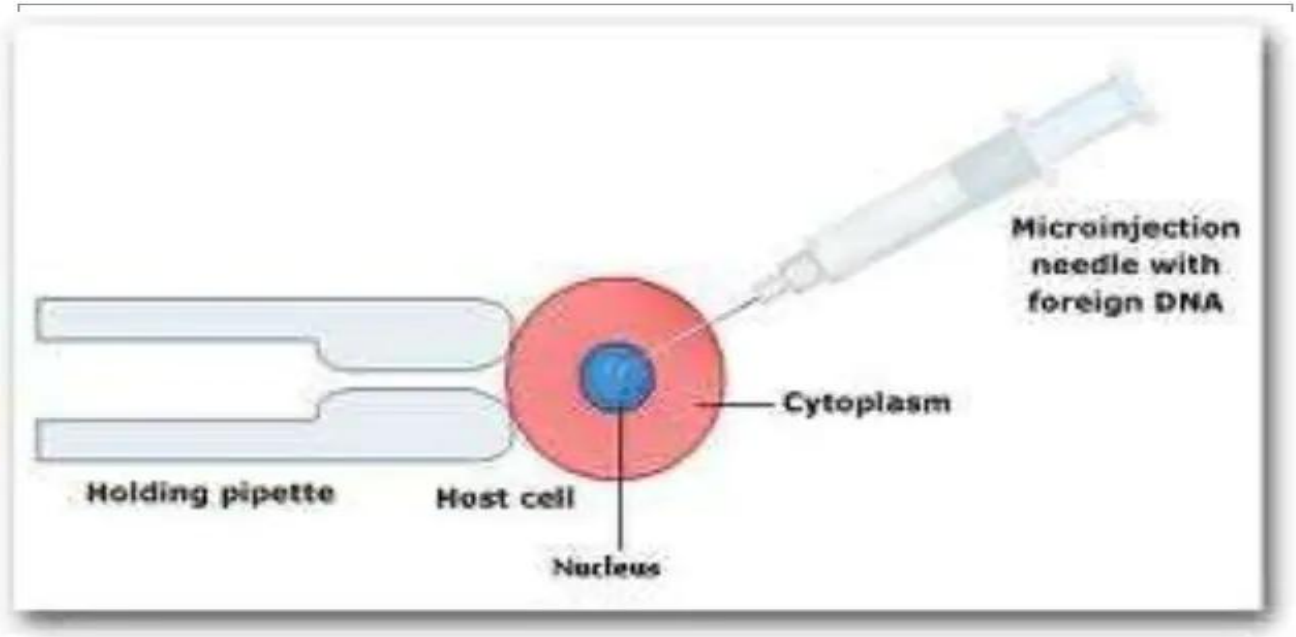

Figure 5: Showing DNA delivery by microinjection (https://slidetodoc.com/direct-dna-transfer-introduce-dna-into-cells-assay/).

- **Ultrasound/Sonication**

Ultrasonic wave-mediated transformation, also known as sonication, is based on sonoporation (the rupture of cellular membranes by acoustic waves). It is a non-invasive way to introduce DNA molecules into cells via acoustic cavitation that temporarily changes the permeability of the cell membrane (Rivera *et al*., 2014). Ultrasound increases the transfection efficiency of animal cells, *in vitro* tissues and protoplasts with spatial and temporal specificity. However, it has been reported that ultrasound can damage the cell, completely breaking its membrane (Liu *et al.*, 2006). Crucial parameters are the intensity, exposure time, central frequency, the type of application (continuous or pulsed), the pulse repetition frequency, and the duty cycle

(Miller *et al.*, 2002). Figure 6 elucidates the instrumentation of sonication method of gene transfer techniques.

Figure 6: Showing diagrammatical instrumentation of the Sonication bath (https//www.sciencedirect.com/topic/chemistry/sonication).

- **Agitation with Glass Beads**

Cells can be genetically transformed by rapid agitation with glass beads in the presence of carrier and plasmid DNA. It can be performed without sophisticated devices and does not require chemical treatments or enzymatic cocktails. This technique is easy, cheap, and rivals electroporation for being the least time consuming methodology, but it is also one of the least efficient, because high quantities of DNA are damaged in the process and the viability of the cells is drastically reduced (Gurpilhares, 2006).This methodology was first used with the yeast *Saccharomyces cerevisiae* (Costanzo and Fox, 1988), and later with Rhizobacteria (Kloepper, 1991), other bacteria (Rattanachaikunsopon, 2009), and also algae (Fawley and Fawley, 2004). Cytogenetic studies have also been performed through agitation with glass beads (Virtue and Cole, 1999).

- **Laser microbeams**

This is a technique of DNA delivery into cell organelles like chloroplasts, this method is strenuous and difficult to achieve (Weber *et al*., 1988). To avoid these difficulties it is possible to use laser microbeams to introduce genetic materials into cells (Berns *et al*., 1983). Laser-mediated transformation works by a focused laser microbeam to puncture self-healing holes (≈0.5 μm) into the cell wall. These holes close again in less than five seconds. Through the temporary opening in the membrane, the buffer together with DNA enter the cell.

Membrane perforation can also be performed using laser pulses (laserporation) and can be combined with laser-facilitated partial removal of the cell wall (Broglia, 1988). Therefore, exogenous DNA could simply be taken up by cells. Complete manipulation by laser light allows precise and gentle treatment of plant cells, subcellular structures, and even individual DNA molecules. For this it is necessary to have an adequate laser system (like nitrogen lasers, excimer pumped dye lasers, or titanium–sapphire lasers) that can be used as an optical tweezer with the appropriate microscope (Greulich *et al*., 2000). UV laser microbeam cell fusion has been induced selectively and DNA was introduced into isolated chloroplasts (Weber *et al*., 1988).

This method is not popular because it requires expensive equipment to allow focusing a laser beam on dimensions of the order of 100 nm (Lin and Ruddle, 1981)., even when a large number of cells can be irradiated and the cells recover completely after the DNA incorporation. It also has to be conducted with a lot of care because laser radiation can damage biological material, so it is necessary to restrict the beam through a channel and control the energy and pulse duration with high precision and reproducibility Hoffmann, 1996).

- **Pollen Tube-Mediated Gene Transfer (PTT)**

The PTT method, which is the transfer of exogenous DNA into the plant embryo, it was first reported in cotton (*Gossypium hirsutum L*.) (Zhou *et al*., 1983). Pollen tube transformation commonly involves the removal of the recipient plant's stigma shortly after pollination and fertilization with subsequent application of an exogenous DNA solution onto the severed style of the recipient plant (Luo and Wu, 1988) (Fig. 7). The exogenous donor DNA is transported via pollen tube growth to the ovary of the recipient plant, where it then integrates with the undivided but fertilized recipient zygote(s). Thus, in successful PTT, foreign genes are incorporated into the recipient's genome at the stage of embryo formation and are therefore present in transformed seeds. The PTT approach, therefore, does not involve protoplast manipulation, cell culture, or plant regeneration procedures (Luo and Wu, 1988). The PTT approach reduces the lengthy DNA transfer time required by other procedures [e.g., regeneration time using Agrobacterium transfer DNA (T-DNA) (Liu *et al*., 2009a) or biolistic bombardment (Klein *et al*., 1988).

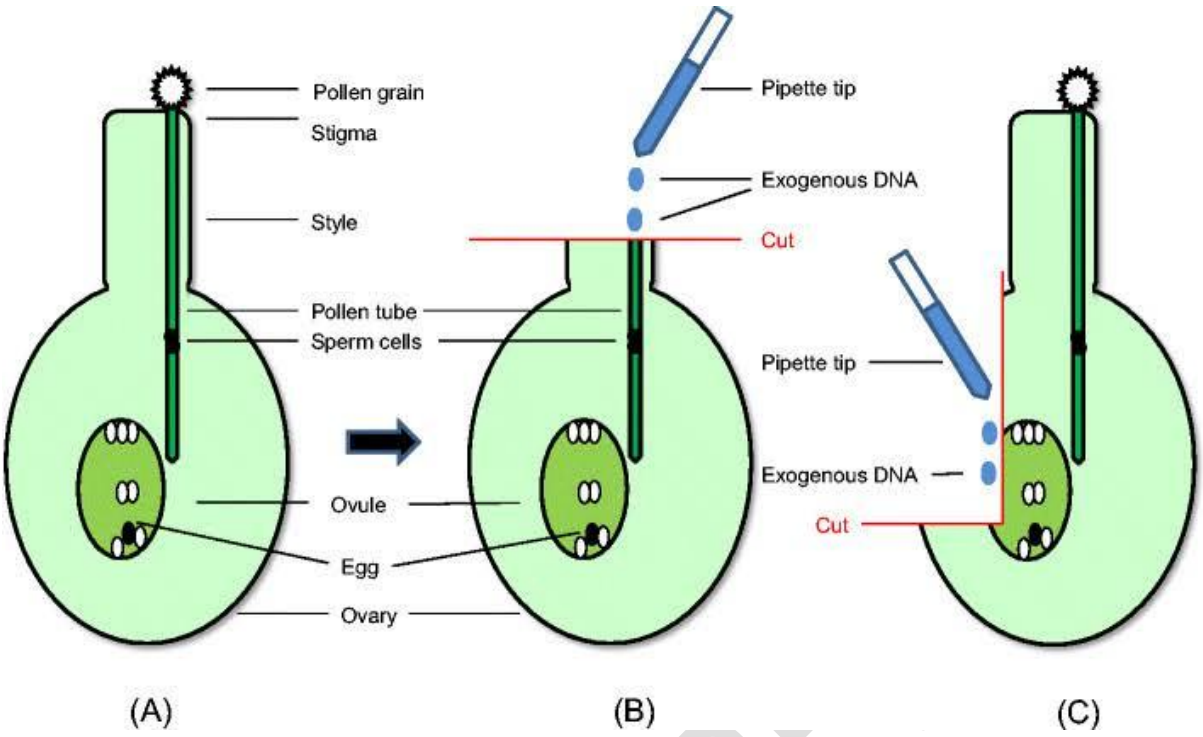

Figure 7: Diagrammatic representation of pollen tube-mediated gene transfer (PTT) to induce genetic transformation. Image adapted from (www.researchgate.net)
(A) Standard fertilization showing the pollen grain (consisting of a vegetative and a generative cell) adhering to the stigma. The pollen grain germinates and the vegetative cell produces a pollen tube that grows through the style eventually penetrating the ovule. A generative cell divides into two sperm cells which travel through the pollen tube to allow for fertilization (not shown),
(B) Application of exogenous DNA on mechanically cut style to facilitate PTT.
(C) Ovary drip method that exogenous DNA is directly delivered into the ovule by cutting and opening the ovary.

- **Transfection**

Transfection is a process by which foreign nucleic acids are delivered into a eukaryotic cell to modify the host cells genetic makeup (Chow *et al*., 2016). Understanding the molecular pathway of disease allows the discovery of specific biomarkers that may be applied to diagnose and prognose diseases (Ye *et al*., 2017). Besides, transfection can be employed as one of the strategies in gene therapy to treat incurable, inherited genetic diseases (Yao *et al*., 2008; Tomizawa *et al*., 2013). Today, the advancement in life-sciences technology allows different types of nucleic acids to be transfected into mammalian cells, and these include Deoxyribonucleic acids (DNAs), Ribonucleic acids (RNAs) as well as small, non-coding RNAs such as siRNA, shRNA and miRNA (Sork *et al*., 2016; Shi *et al*., 2018). Generally, transfection can be classified into two types, namely stable and transient transfection (Kim & Eberwine, 2010; Stepanenko and Heng, 2017). Stable transfection refers to sustaining long-term expression of a transgene by integrating foreign DNA into the host nuclear genome or maintaining an episomal vector in the host nucleus as an extra-chromosomal element (Lufino,

et al., 2008). The transgene may then be constitutively expressed even with the replication of cells (Kim and Eberwine, 2010).

In contrast, transient transfection does not require integrating nucleic acids into the host cell genome (Riedl *et al*., 2018). Nucleic acids may be transfected in the form of a plasmid (Nejepinska *et al*., 2012). Therefore, transgene expression will eventually be lost as host cells replicate (Kim and Eberwine, 2010). Transient transfection is usually applied in short-term studies to investigate the effects of knock-in/-down of a particular gene (Kim & Eberwine, 2010). In contrast, stable transfection is useful in long-term genetic and pharmacology studies in which large-scale protein production is needed (Elgundi *et al*., 2017). A vector construct that carries the specific nucleic acids to be transfected can be further divided into either viral or plasmid vector. Viruses and plasmids facilitate the expression of a foreign transgene via the presence of a suitable eukaryotic promoter (Colosimo *et al*., 2000). A viral vector may trigger an immunogenic response in the host cell while a non-viral vector is comparatively less immunogenic (Hardee *et al*., 2017). A delivery mechanism is needed to facilitate the transfer of targeted nucleic acids or vector construct into the host cell (Kim and Eberwine, 2010). Some of these entail physical methods while others involve the use of a delivery vehicle, which may be lipid-based (Balazs and Godbey, 2011) or non-lipid based (Jin *et al*., 2014), to help enhance the contact between vector-vehicle complex with the host cell membrane, thereby facilitating the entrance of the complex into cells (Balazs and Godbey, 2011). The limitation of this technique is in designing and initiating a transfection assay which can be challenging, especially with the vast variety of transfection approaches or strategies to choose from Tan *et al*. (2019).

(B): Biological Gene Transfer (Indirect method)

1) *Agrobacterium* mediated transformation; Primarily there are two methods that is applied using this method.

a) Co-cultivation with the explants tissue

b) *In planta* transformation

2) Transformation mediated by viral vector

Agrobacterium mediated transformation

The natural ability of the soil bacteria, *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*, to transform host plants has been exploited in the development of transgenic plants. In 1970s the prospect of using *A. tumefaciens* for the gene transfer of exogenous DNA into crops was revolutionary. Genetic transformation of plants was viewed as a prospect. In retrospect, *Agrobacterium* was the logical and natural transformation candidate to consider since it naturally transfers DNA (T-DNA) located on the tumor inducing (Ti) plasmid into the nucleus of plant cells and stably incorporates the DNA into the plant genome (Chilton *et al*., 1977). Now forty five years later, this method has been the most widely used and powerful technique for the production of transgenic plants. However, there still remain many challenges for genotype independent transformation of many economically important crop species, as well as forest species. Despite the development of other non-biological methods of plant transformation, (Arenchibia *et al.,* 1992), *Agrobacterium* mediated transformation remains popular and is among the most effective. This is especially true among most dicotyledonous plants, where *Agrobacterium* is naturally infectious. *Agrobacterium* mediated gene transfer into monocotyledonous plants was thought to be not possible. However, reproducible and efficient methodologies have been established for rice (Hiei *et al.,* 1994), banana (May *et al*., 1995), corn (Ishida *et al.,* 1996), wheat (Cheng *et al.,* 1997), sugarcane (Arencibia *et al.,* 1998), forage grasses such as Italian ryegrass (*Lolium multiflorum)* and tall fescue *(Festuca arundinacea*) (Bettany *et al.,* 2003). Among the commercially important conifers, hybrid larch was the first to be stably transformed via co-cultivation of embryogenic tissue with *A. tumefaciens* (Levee *et al*., 1997). Subsequently, this method was successfully applied to several species of spruce (Klimaszewska *et al*., 2001).

Methods relative to transformation targets can be classified into two categories: (a) those requiring tissue culture and (b) *in planta* methods.

In Tissue Culture

In tissue culture systems for plant transformation, the most important requirement is a large number of regenerable cells that are accessible to the gene transfer treatment and that will retain the capacity for regeneration for the duration of the necessary target preparation, cell proliferation and selection treatments. A high multiplication ratio from a micropropagation system does not necessarily indicate a large number of regenerable cells accessible to gene transfer (Livingstone and Birch, 1995). Sometime gene transfer into potentially regenerable cells may not allow recovery of transgenic plants if the capacity for efficient regeneration is short lived (Ross *et al*., 1995). Further, tissue culture based methods can lead to unwanted somaclonal variations such as alterations in cytosine methylation, induction of point mutations and various chromosomal aberrations (Clough, 2004). On the other hand, realization of whole plant transformants has been a problem in a large number of crop species as these plants have proven to be highly recalcitrant *in vitro*. As a result, other strategies are being evolved wherein the tissue culture component is excluded in the procedure and these are known as *in planta* methods. The technique is illustrated diagrammatically in Fig. 8 below.

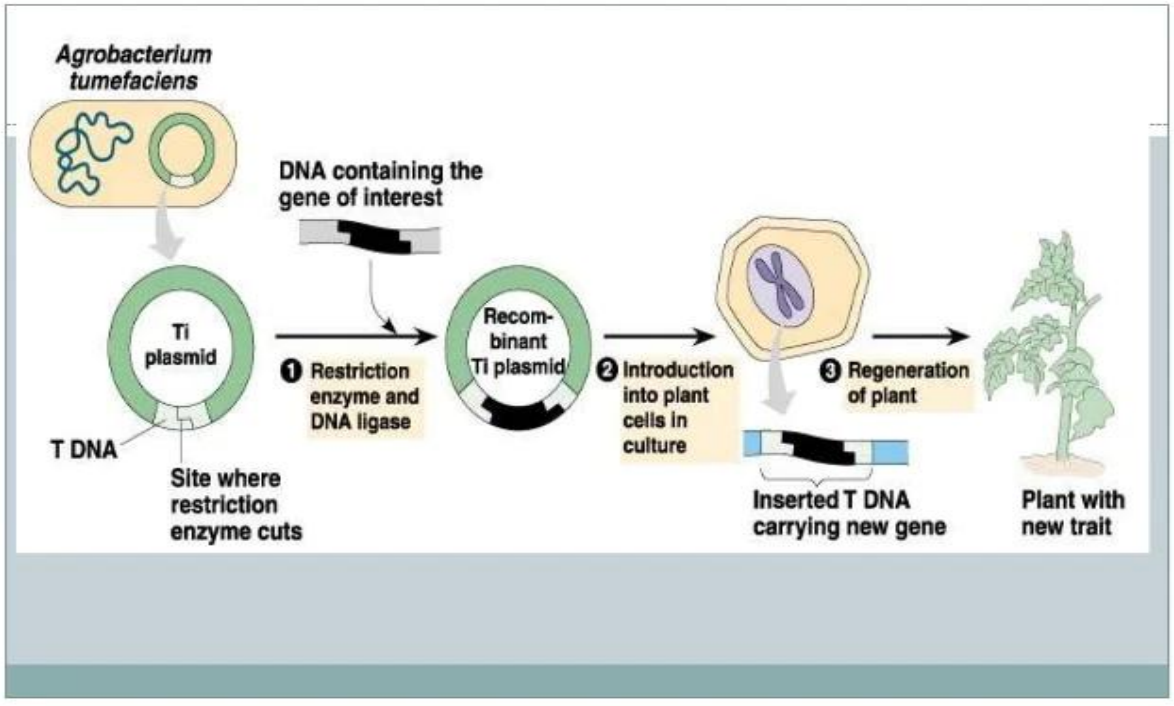

Figure 8: Showing steps involved in co-culture of *Agrobacterium tumefaciens* mediated transformation (https://www.researchgate.net/figure/process-of-A-Tumefaciens-mediated-plant-transformation_fig2_299552428).

In planta transformation

Although successful plant regeneration has been achieved through the application of co-culture method, the technique has not provided regeneration in several other crops transformation protocols, which is a serious limitation to the exploitation of gene transfer technology to its full potential. In the light of this major constraint, it becomes necessary to evolve transformation strategies that do not depend on tissue culture regeneration or those that substantially eliminate the intervening tissue culture steps. *In planta* transformation methods provide such an opportunity. Methods that involve delivery of transgenes in the form of naked DNA directly into the intact plants are called *in planta* transformation methods. These methods exclude tissue culture steps, rely on simple protocols and required short time in order to obtain entire transformed individuals. In many cases, *in planta* methods have targeted meristems or other tissues with the assumption that at fertilization, the egg cell accepts the donation of an entire genome from the sperm cell that will ultimately give rise to zygotes (Chee and Slighton, 1995), and therefore is the right stage to integrate transgenes.

Transformation rates greatly improved when Bechtold *et al.* (1993) inoculated plants that were at the flowering stage. At present, there are very few species that can be routinely transformed in the absence of a tissue culture based regeneration system.

Recent studies with *Agrobacterium* inoculation of germinating seeds of rice has provided transformation efficiencies higher than 40% (Supartana *et al*., 2005), while providing 4.7 to 76% efficiency for the flower infiltration method and from 2.9 to 27.6% efficiency for the seedling infiltration method (Trieu *et al.*, 2000).

Crop species that were successfully transformed by injuring the apical meristem of the differentiated embryo of the germinating seeds and then infecting with *Agrobacterium* include peanut, (*Arachis hypogaea* L.) (Rohini and Rao, 2000), safflower (*Carthamus tinctorius* L.) (Rohini and Rao, 2000).

The above successes have in fact provided a great leverage for easy development of transgenic plants, as the methodology is simple, cost effective, does not call for high infrastructural requirement even to handle recalcitrant crops such as groundnut. Thus the

technology of gene transfer for the development of recalcitrant crops has become a practical possibility for experimenting and producing viable transformants. However, the optimization of *Agrobacterium*-plant interaction is crucial for efficient transformation.

Non- Agrobacterium-Base Method

Four decades before it was identified that some members (*Rhizobia* spp) of *Rhizobiaceae* family also have the capacity to transfer the gene to the host. *Ensifer adhaerens, Ochrobactrum haywardense and Rhizobium etli* are some of the species related to *Agrobacterium* used in gene transfer yet they have the disadvantage of limited host range (Mullins, 2018).

Viral-mediated gene transfer

Plant-infecting RNA and DNA viruses can be used as a vector to transfer genes to the target. The gene to be transferred is made integrated into the viral genome and now the virus acts as a vector to transfer the gene. The virus with the transferred gene is made to infect the target cell and its results in successful transformation. The main disadvantage is the high copy number per cell and viral- mediated gene transfer can only produce transient transfer and not the stable transformation that is they are unable to transfer to the progeny. Some of the viral vectors used are a Retrovirus, Adenovirus (Chailertvanitkul and Pouton, 2010), Adeno-associated virus, Herpes virus, Pox virus, Human Foamy Virus (HFV) and Lentivirus (Fiandaca and Federoff, 2014).

Table 2: Shows the various gene delivery methods with their rate of efficacy.

Gene delivery method	Transformation efficiency	Range of transformable plant species	Tissue culture phase	Type of explant	Remarks
PEG – induced DNA uptake.	Low to high	Recoverable species from protoplast	With and without tissue culture phase	Protoplast	It suffers difficulty in the regeneration of plant from a protoplast.
Electroporation	Low to high	Unrestricted	With and without tissue culture phase	Protoplasts, meristems or pollen grains	Fast, simple and inexpensive in contrast with biolistics.

Microinjection	High	Recoverable species from protoplast	With tissue culture phase	Protoplast.	Very slow, precise, single cell targeting possibility, requires high skill, the chimeric nature of transgenic plants and ability of whole chromosome transformation
Sonication	Low	Unrestricted	With and without tissue culture phase	Protoplast cells, tissues and seedlings	Effective to transfect by virus particles and able to increase the *Agrobacterium* based transformation efficiency
Particle bombardment	High	Unrestricted	With and without tissue culture phase	Intact tissue or microspores	Efficient for viral infection, complex integration patterns, without specialized vectors and backbone free integration
Shock waves	Low	Unrestricted	With tissue culture	Variety of cell types	Rapid, inexpensive and easy to set up
Agitation with glass beads	Low	Unrestricted	With tissue culture	Variety of cell types	Rapid, inexpensive and easy to set up
Silicon carbide mediated transformation	Low to High	Unrestricted	With tissue culture	Variety of cell types	Rapid, inexpensive and easy to set up
Laser microbeam	Low	Unrestricted	With tissue culture	Cells and protoplast	Effective for chloroplast transformation.
Pollen Tube-Mediated Gene Transfer	High	Restricted to flowering structures.	Without tissue Culture phase	Intact Pollens	Rapid, efficient and inexpensive to set up.
Transfection	Low to High	Unrestricted	With and without tissue culture method Phase.	Variety of cell types,	Efficient for viral infection, complex integration patterns, with or without specialized vectors for gene integration.

***Agrobacterium* mediated method**	High and stable	Many species, specially dicotyledonous plants	With and without tissue culture method	Different intact cells, tissues or whole plant	Possibility of *Agroinfection*, combination with sonication and biolistic methods and transgene size up to 150 kb
Virus based method	High and transient	Virus host specific limitation	With tissue culture	In planta inoculation	Rapid, inducible expression and with mosaic status

(Rivera *et al*., 2014).

Various DNA Transfer Techniques with their Advantages and Limitations

The various DNA transfer techniques are enumerated in Table 3 detailing the procedure, parameter used as well as their advantages and disadvantages.

Table 3: Shows the advantages and disadvantages of the various plant transformation techniques

Techniques	Procedure	Most Important Parameter Involved	Advantages	Drawbacks
PEG – induced DNA uptake.	The polyethylene glycol is a polyether compound that has the capacity to attach with DNA and along with Ca2+ transfer the DNA	The protoplast prepared are made to suspend in the solution of PEG/Ca^{2+} and the DNA.	The method is simple and does not require specialized equipment.	It suffers difficulty in the regeneration of plant from a protoplast.

	into the target by penetrating the membrane.			
Electroporation	DNA is inserted through pores due to permeability of the cell membrane induced by strong electrical pulses.	Pulse length, energy and duration of the electrical field, extent and duration of membrane permeation, mode and duration of molecular flow, DNA concentration, tolerance of cells to membrane permeation.	Simple, fast, low cost.	Low efficiency, requires laborious protocols, and transforms mainly protoplasts.
Biolistics	High density carrier particles covered with genes are accelerated through the cells leaving the DNA inside by an adsorption mechanism.	Kinetic energy of the bombarding particles, temperature, the amount of cells, their ability to regenerate, susceptibility of the tissue, the number of DNA-coated particles, as well as the amount of DNA that covers each particle.	Simple, no need to treat the cell wall, allows transformation of different cells, independent of the physiological properties of the cell, allows the use of multiple transgenes.	High cost, low efficiency Transformation parameters must be optimized to each biological target employed, there is a risk of multiple copies of the introduced genes, DNA and cells can be damage.
Agitation with glass beads	Rapid agitation with glass beads allows the penetration of the plasmid DNA.	DNA and concentration, sensitivity of cells to membrane permeation, amount of cells and their ability to regenerate.	Fast, simple, low cost. Does not need sophisticated devices, chemical treatments or enzymatic cocktails.	Low efficiency because DNA get damaged
Silicon carbide whisker	Silicon carbide fibers are mixed in a vortex with a	Fiber size, vortex parameters (type, duration and speed of	Simple, fast, low cost and can be used in different cell	Very low efficiency. Cells can be damaged

	suspension of tissue and DNA allowing introduction by abrasion.	agitation), vessel shape, thickness of the cell wall and cell's ability to regenerate	types.	affecting regeneration capabilities. Could be hazardous to technicians due to fibers' inhalation.
Ultrasound	Introduces DNA molecules into cells via acoustic cavitation that temporarily changes the permeability of the cell membrane.	Intensity, exposure time, central frequency, type of application (continuous or pulsed), pulse repetition frequency, and duty cycle.	High efficiency, medium cost and can be used in different cell's types.	May damage the cells by breaking their membrane.
Microinjection	Microinjection is a surgical technique that uses the micropipettes to directly deliver the DNA into the nucleus or cytoplasm	The microinjector connected to a micromanipulator deliver the DNA into the target	Technique is precise, single cell targeting possibility.	Very slow (time consuming), requires high skill,
Shock waves	Cell permeabilization occurs due to shock wave-induced cavitation.	Frequency, energy, voltage, shock wave profile and number of shock waves.	Fast, easy to perform, reproducible with high efficiency, no need of enzymatic cocktails, can be used to transform several cell types.	Shock wave generators for this purpose are not on the market yet and experimental devices are relatively expensive.
Laser microbeams	A laser microbeam punctures self-healing holes into the cell wall allowing DNA penetration.	Laser characteristics to be used as optical tweezers coupled to the appropriate microscope.	Allows precise and gentle treatment of cells, subcellular structures, and even individual DNA molecules.	High cost (expensive equipment required) and laborious.
Pollen Tube-Mediated Gene	Transfer of exogenous DNA into the plant	Recipient plant's stigma shortly after	Relatively low cost and	Limited to flower Structure

Transfer (PTT)	embryo.	pollination and fertilization with of an exogenous DNA solution onto the style of the recipient plant.	expensive equipment is not required	
Transfection	Transfer of foreign nucleic acids into a eukaryotic cells.	stable and transient transfection of eukaryotic cells.	Flexibility in approach of application.	May result to transient transformation.
***Agrobacterium* mediated method**	*Agrobacterium* is a natural transformation technique that transfers DNA (T-DNA) into the host cells.	T-DNA (transfer DNA), located on the plasmid inducing Ti or Ri on the host cells	Most reliable regenerable method of gene transfer technique with excellent stability rate.	Has limitation of host rate.
Virus based method	Plant-infecting RNA and DNA viruses can be used as a vector to transfer genes to the target.	Virus infecting RNA and DNA of host plants.	Wide host range	Only transient transformation

(Source : Rivera *et al*., 2014).

Conclusion

Plant genetic engineering has become one of the most important genetic tools in modern molecular breeding of crops. However, transformation of some plant species and elite germplasm still remain a challenge to breeders. The following salient findings are drawn on the basis of this investigation. The different methods of gene transfer techniques into plant cells have been developed and continuous efforts have been made to increase its efficiency. Both the direct and indirect methods of gene transfer techniques have their own merits and drawbacks, example is the Silicon carbide whisker, laser microbeams and Ultrasound, which can be detrimental if in contact with the human skin as illustrated in (Table 3) above. However researchers are working tirelessly in attempt to eliminate these limitations. Of the many methods of genetic transformation techniques that have been proposed and tried in the laboratories, transformation work based on particle bombardment with DNA coated micro projectiles and *Agrobacterium* mediated transformation have proved to be promising in producing stable transgenic plants from a range of plant species.

References

Arencibia, A. P., Molina, C., Gutierrez, A., Fuentes, V., Greenidge, E., Menendez, G., De la Riva, E. and Housein, G. S. (1992). Regeneration of transgenic sugarcane (*Saccharum officinarum* L.) plants from intact meristematic tissue transformed by electroporation. *Biotechnologia Aplicada*, 9: 156-165.

Arencibia, A. D., Carmona, E. R., Tellez, P., Chan, M. T., Yu, S. M., Trujillo, L. E. and Oram, A. P. (1998). An efficient protocol for sugarcane (*Saccharum* sp. L.) transformation mediated by *Agrobacterium tumefaciens. Transgenic Research*, 7(3): 213-222.

Babaoglu, M., Davey, M. R. and Power, J. B. (2000). Genetic engineering of grain legumes: key transformation events. *Agricultural Biotechnology Network*, 2:1–12.

Bechtold, N., Elli, S. and Pelletier, G. (1993). *In-planta Agrobacterium*-mediated gene -transfer by infiltration of adult *Arabidopsis thaliana* plants. CR *Academic Sciences III*--VI E Paris, 316: 1194-1199.

Bahi, M. M., Tsaloglou, M. N., Mowlem, M. and Morgan, H. (2011). Electroporation and lysis of marine microalga *Karenia brevis* for RNA extraction and amplification. *Journal of the Royal Society Interface*, 8: 601-608.

Balazs, D. A. and Godbey, W. (2011). Liposomes for use in gene delivery. *Journal of Drug Delivery*, 2011:326497 DOI 10.1155/2011/326497.

Baskaran, P. and Dasgupta, I. (2011). Gene delivery using microinjection of *Agrobacterium* to embryonic shoot apical meristem of elite indica rice cultivars. *Journal of Plant Biochemistry and Biotechnology*, 21(2): 268-274. doi:10.1007/s13562- 011-0078-x.

Bates, G. W. (1999). Plant transformation via protoplast electroporation. *Methods in Molecular Biology*, Vol.111: 359-366, ISSN 1064-3745.

Bates, G. W., Gaynor, J. J. and Shekhawat, N. S. (1983). Fusion of plant protoplasts by Electric fields. *Plant Physiology*, 72: 1110-1113.

Berns, M. W., Aist, J., Edward, J., Strahs, K., Girton, J. and McNeill, P. (1983). Laser microsurgery in cell and developmental biology. *Science*, 213(4507):505–13.

Bettany, A. J., Dalton, S. J., Timms, E. J., Manderyck, B., Dhanoa, M. S. and Morris, P. (2003). *Agrobacterium tumefaciens* mediated transformation of *Festuca arundinacea* (Sahreb.) and *Lolium multiflorum* (Lam.). *Plant Cell Reports*, 21(5): 437-444.

Birch, R. G. (1997). Plant transformation: problems and strategies for practical application. *Annual Review of Plant Physiology and Plant Molecular Biology*, 48: 297-326.

Broglia, M. (1988). Lasers in plant genetic engineering. *Basic Application of Histochemistry*, 32:342.

Chailertvanitkul, V. A. and Pouton, C. W. (2010). Adenovirus: a blueprint for non-viral gene delivery. *Current Opinion in Biotechnology*, 21(5): 627-632. doi:10.1016/j.copbio.2010.06.011.

Chee, P. P. and Slighton, J. L. (1995). Transformation of soybean (*Glycine max*) via *Agrobacterium tumefaciens* and analysis of transformed plants. In: *Agrobacterium* protocols: *Methods in Molecular Biology*, 44: 101-109.

Cheng, M., Fry, J. E., Pang, S. Z., Zhou, H. P., Hironaka, C. M., Duncan, D. R., Conner, W. and Wan, Y. C. (1997). Genetic transformation of wheat mediated by *Agrobacterium tumefaciens. Plant Physiology*, 115(3): 971-980.

Chilton, M. D., Drummond, M. H., Merio, D. J., Sciaky, D., Montoya, A. L., Gordon, M. P. and Nester, E. W. (1977). Stable incorporation of plasmid DNA into higher plant cells: The molecular basis of crown gall tumorigenesis. *Cell*, 11(2): 263-271.

Chow, Y. T., Chen, S., Wang, R., Liu, C., Kong, C-W., Li R. A., Cheng, S. H. and Sun, D. (2016). Single cell transfection through precise microinjection with quantitatively controlled injection volumes. *Scientific Reports*, 6(1):24127 DOI 10.1038/srep24127.

Clough, S. J. (2004). Floral Dip: *Agrobacterium*-mediated germ line transformation. In: Methods in molecular biology. Transgenic plants: methods and protocols, Pena, L. *Humana Press Incoporated: Totowa*, 286: 91-102.

Colosimo, A., Goncz, K. K., Holmes, A. R., Kunzelmann, K., Novelli, G., Malones, R.W., Bennett, M. J. and Gruenert, D. C. (2000). Transfer and expression of foreign genes in mammalian cells. *BioTechniques*, 29(2):314–331 DOI 10.2144/00292rv01.

Costanzo, M. C. and Fox, T. D. (1988). Transformation of yeast by agitation with glass beads. *Genetics*, 120: 667-670.

Elgundi, Z., Sifniotis, V., Reslan, M., Cruz, E. and Kayser, V. (2017). Laboratory scale production and purification of a therapeutic antibody. *Journal of Visualized Experiments*, 119:e55153 DOI 10.3791/55153.

Escoffre, J. M., Portet, T., Wasungu, L., Teissié, J. and Dean, D. (2009). What is still not known of the mechanism by which electroporation mediates gene transfer and expression in cells and tissues. *Molecular Biotechnology*, 41: 286-295.

Fawley, M. W. and Fawley, K. P. (2004) A simple and rapid technique for the isolation of DNA from microalgae. *Journal of Phycology*, 40: 223-225.

Fiandaca, M. S. and Federoff, H. J. (2014). Using viral-mediated gene delivery to model Parkinson's disease: done on human primate investigations expand our understanding? *Experimental Neurology Journal*, 256: 117-125. doi:10.1016/j.expneurol.2013.03. 014.

Gatehouse, J. A. (2008). Biotechnological prospects for engineering insect-resistant plants. *Plant Physiology*, 146: 881-887.

Greulich, K. O., Pilarczyk, G., Hoffmann, A., Meyer, Z., Hörste, G. and Schäfer, B. (2000). Micromanipulation by laser microbeam and optical tweezers: from plant cells to single molecules. *Journal of Microscopy Oxford*, 198(3):182–7.

Gurpilhares, D. B. (2006). Optimization of glucose-6-phos phate dehydrogenase releasing From Candida guilliermondii by disruption with glass beads. *Enzyme Microbial Technology*, 39: 591-595.

Hardee, C. L., Arévalo-Soliz, L. M., Hornstein, B. D. and Zechiedrich, L. (2017). Advances in non-viral DNA vectors for gene therapy. *Genes*, 8(2):65 DOI 10.3390/genes8020065.

Hiei, Y., Ohta, S., Komari, T. and Kumashiro, T. (1994). Efficient transformation of rice (*Oryza sativa*) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *Plant Journal*, 6(2): 271-282.

Hoffmann, F. (1996). Laser microbeams for the manipulation of plant cells and subcellular structures. *Plant Science*, 113(1):1–11.

Hosokawa, Y., Iguchi, S., Yasukuni, R., Hiraki, Y., Shukunami, C. and Masuhara, H. (2009). Gene delivery process in a single animal cell after femtosecond laser microinjection. *Applied Surface Science*, 255(24): 9880-9884. doi:10. 1016/j.apsusc.2009.04.111.

Ishida, Y., Saito, H., Ohta, S., Hiei, Y., Komari, T. and Kumashiro, T. (1996). High Efficiency transformation of maize (*Zea mays* L.) mediated by *Agrobacterium tumefaciens. Natural Biotechnology*, 4: 745-750.

Jeu, M. J. D. (2000). Invitro techniques for ornamental breeding. *Acta Horticulturae.* 508: 55 -60.

Jin, L., Zeng, X., Liu, M., Deng, Y. and He, N. (2014). Current progress in gene delivery technology based on chemical methods and nano-carriers. *Theranostics*, 4(3):240–255 DOI 10.7150/thno.6914.

Jinturkar, K. A., Rathi, M. N. and Misra, A. (2011). Gene Delivery Using Physical Methods. *In Challenges in Delivery of Therapeutic Genomics and Proteomics*, 1: 83-126.

Kavipriya, C., Yuvaraja, A., Senthil, K. and Menaka, C. (2019). Genetic Transformation Methods for Crop Improvement: A Brief Review. *Agricultural Reviews.* 4: 281-288.

Keshavareddy, G., Kumar, A., Vemanna, R. and Ramu, S. (2018). Methods of Plant Transformation- A Review. *International Journal of Current Microbiology and Applied Sciences*, 7(7): 2656-2668.

Kim, T. K. and Eberwine, J. H. (2010). Mammalian cell transfection: the present and the future. *Analytical and Bioanalytical Chemistry*, 397(8):3173–3178 DOI 10.1007/s00216-010-3821-6.

Klein, T. M., Formm, M., Weissinger, A., Tomes, D., Schaaf, S., Sletten, M. and Sanford, J. C. (1988). Transfer of foreign genes into intact maize cells with high-velocity microinjection. *Proceedings of the National Academy of Sciences USA*, 85: 4305–4309.

Klimaszewska, K., Lachance, D., Pelletier, G., Lelu, M. A. and Seguin, A. (2001). Regeneration of transgenic *Picea glauca,* P. mariana and P. abies after co-cultivation of embryogenic tissue with *Agrobacterium tumefaciens*. *In Vitro Cell Development Biology of Plant*, 37(6): 748-755.

Kloepper, J. W. (1991). Comparative analysis of 5 methods for recovering Rhizobacteria From cotton roots. *Canadian Journal of Microbiol*ogy, 37: 953-957.

Kotnik, T. (2013). Lightning-triggered electroporation and electrofusion as possible contributors to natural horizontal gene transfer. *Physics of Life Review*, 10: 351-370.

Levee, V., M. A. Lelu, M. A., Jouanin, L., Comu, D. and Pilate, G. (1997). *Agrobacterium tumefaciens* mediated transformation of hybrid larch (*Larix kaempferi* L. deciduas) and transgenic plant regeneration. *Plant Cell Report*, 16(10): 680-685.

Lin, P. F. and Ruddle, F. (1981). Photoengraving of coverslips and slides to facilitate monitoring of micromanipulated cells or chromosome spreads. *Experimental Cell Research*, 134:485–8.

Liu, J., Su, Q., An, L. and Yang, A. (2009). Transfer of a minimal linear markerfree and vector-free smGFP cassette into soybean via ovary-drip transformation. *Biotechnology Letters*, 31:295–303.

Liu, Y. C. and Vidali, L. (2011). Efficient polyethylene glycol (PEG) mediated transformation of the moss *Physcomitrella patens. Journal of Visualized Experiments*, (50). doi:10.3791/2560.

Liu, Y., Yang, H. and Sakanishi, A. (2006). Ultrasound: mechanical gene transfer into plant cells by sonoporation. *Biotechnology Advances.* 24: 1-16.

Loske, A. M. (2011). New Trends in Shock Wave Applications to Medicine and Biotechnology. *Research Signpost*.

Lufino, M. M. P., Edser, P. A. H. and Wade-Martins, R. (2008). Advances in high-capacity extrachromosomal vector technology: episomal maintenance, vector delivery, and transgene expression. Molecular Therapy 16(9):1525–1538 DOI 10.1038/mt.2008.156.

Luo, Z. X. and Wu, R. A. (1988). Simple method for the transformation of rice via the pollen-tube pathway. *Plant Molecular Biology Repository*, 6:165–174.

May, G. D., Afza, R., Mason, S. H., Wiecko, A., Novak, F. J. and Amtzen, C. J. (1995). Generations of transgenic banana (*Musa acuminata*) plants via *Agrobacterium* mediated transformation. *Biotechnology*, 13(5): 486-492.

Miller, D. L., Pislaru, S. V. and Greenleaf, J. E. (2002). Sonoporation: mechanical DNA delivery by ultrasonic cavitation. *Somatic Cell and Molecular Genetics*, 27: 115-134.

Mullins, D. S. R. (2018). Alternative non-*agrobacterium* based methods for plant Transformation. *Annual Plant Reviews.* 1: 1-17. doi:10.1002/9781119312994.apra0659.

Nejepinska, J., Malik, R., Moravec, M. and Svoboda, P. (2012). Deep sequencing reveals complex spurious transcription from transiently transfected plasmids. *PLOS ONE* 7(8):e43283 DOI 10.1371/journal.pone.0043283.

Que, Q., Chilton, M. M., Elumalai, S., Zhong, H., Dong, S. and Shi, L. (2019). Repurposing Macromolecule Delivery Tools for Plant Genetic Modification in the Era of Precision Genome Engineering. *Methods in Molecular Biology.* 1864: 3-18. doi:10.1007/978-1-4939-8778-8_1.

Rattanachaikunsopon, P. and Phumkhachorn, P. (2009). Glass bead transformation method For gram-positive bacteria. *Brazilian Journal of Microbiology*, 40: 923-926.

Rakoczy-Trojanowska, M. (2002). Alternative methods of plant transformation--a short review. *Cell Molecular Biology Letters*, 7: 849-858.

Riedl, S., Kaiser, P., Raup, A., Synatschke, C., Jérôme, V. and Freitag, R. (2018). Non-viral transfection of human T lymphocytes. *Processes*, 6(188):1–17 DOI 10.3390/pr6100188.

Rivera, A. L., Magaña-Ortíz, D., Gómez-Lim, M., Fernández, F. and Loske, A. M. (2014). Physical methods for genetic transformation of fungi and yeast. *Physics of Life Review*, 10: 184-203.

Rohini, V. K. and Rao, K. S. (2000a). Embryo transformation, a practical approach for realizing transgenic plants of safflower (*Carthamus tinctorius* L) *Annals of Botany*, 86: 1043-1049.

Rohini, V. K. and Rao, K. S. (2000b). Transformation of peanut (*Arachis hypogaea* L.): a non–tissue culture based approach for generating transgenic plants. *Plant Sciences*, 150: 41-49.

Ross, A. H., Manners, J. M. and Birch, R. G. (1995). Embryogenic callus production, plant regeneration and transient gene expression following particle bombardment, in the pasture grass *Cenchrus ciliarus* (Gramineae). *Australian Journal of Botany*, 43:193–199.

Sahab, S., Hayden, M. J., Mason, J. and Spangenberg, G. (2019). Mesophyll Protoplasts and PEG-Mediated Transfections: Transient Assays and Generation of Stable Transgenic Canola Plants. *Methods in Molecular Biology*, 1864: 131-152. doi:10.1007/978-1-4939-8778-8_10.

Shelton, A. M., Zhao, J. Z. and Roush, R. T. (2002). Economic, ecological, food safety and Social consequences of the deployment of *Bt* transgenic plants. *Annual Review of Entomology*, 47: 845-881.

Shi, B., Xue, M., Wang, Y., Wang, Y., Li, D., Zhao, X. and Li, X. (2018). An improved method for increasing the efficiency of gene transfection and transduction. International *Journal of Physiology, Pathophysiology and Pharmacology*, 10(2):95–104.

Sork, H., Nordin, J. Z., Turunen, J. J., Wiklander, O. P., Bestas, B., Zaghloul, E. M., Margus, H., Padari, K., Duru, A. D., Corso, G., Bost, J., Vader, P., Pooga, M., Smith, C. E., Wood, M. J., Schiffelers, R. M., Hällbrink, M. and Andaloussi, S. E. L. (2016). Lipid-based transfection reagents exhibit cryo-induced increase in transfection efficiency. *Molecular Therapy—Nucleic Acids*, 5(3):e290 DOI 10.1038/mtna.2016.8.

Supartana, P., Shimizu, T., Shioiri, H., Nogawa, M., Nozue, M. and Kojima, M. (2005). Development of simple and efficient in planta transformation method for rice (*Oryza sativa* L.) using *Agrobacterium tumefaciens*. *Journal of Bioscience and Bioengineering*, 100 (4): 391-397.

Stepanenko, A. A. and Heng, H. H. (2017). Transient and stable vector transfection: pitfalls, off-target effects, artifacts. *Mutation Research/Reviews in Mutation Research*, 773:91–103 DOI 10.1016/j.mrrev.2017.05.002.

Svensson, I., Artursson, E., Leanderson, P., Berglind, R. and Lindgren, F. (1997). Toxicity *in vitro* of some silicon carbides and silicon nitrides: whiskers and powders. *American Journal of Industrial Medicine*, 31: 335-343.

Tan, S., Tao, Z., Loo, S., Su, L., Chen, X. and Ye, L. (2019). Non-viral vector based gene transfection with human induced pluripotent stem cells derived cardiomyocytes. *Scientific Reports*, 9(1):14404 DOI 10.1038/s41598-019-50980-w.

Trieu, A. T., Burleigh, S. H., Kardailsky, I. V., Mendoza, . M., Versaw, W. K., Blaylock, L. A., Shin, H., Chiou, T. J., Katagi, H., Dewbre, G. R., Weigel, D. and Harrison, M. J. (2000). Transformation of *Medicago truncatula* via infiltration of seedlings of flowering plants with *Agrobacterium. Plant Journal*, 22(6): 531-541.

Tomizawa, M., Shinozaki, F., Motoyoshi, Y., Sugiyama, T., Yamamoto, S. and Sueishi, M. (2013). Sonoporation: gene transfer using ultrasound. *World Journal of Methodology* 3(4):39–44 DOI 10.5662/wjm.v3.i4.39.

Virtue, M. A. and Cole, E. S. (1999). A cytogenetic study of development in mechanically Disrupted pairs of Tetrahymena thermophila. *Journal of Eukaryotic Microbiology*, 46: 597-605.

Weber, G., Monajembashi, S. and Greulich, K .O. (1988). Genetic manipulation of plant cells and organelles with a laser microbeam. *Plant Cell, Tissue and Organ Culture*,12(2):219–22.

Yamagishi, N., Terauchi, H., Kanematsu, S. and Hidaka, S. (2006). Biolistic inoculation of Soybean plants with soybean dwarf virus. *Journal Virology Methods*, 137: 164-167.

Yao, C. P., Zhang, Z. X., Rahmanzadeh, R. and Huettmann G. (2008). Laser-based gene transfection and gene therapy. *IEEE Transactions on Nanobioscience,* 7(2):111–119 DOI 10.1109/TNB.2008.2000742.

Ye, Q., Tian, G-P., Cheng, H-P., Zhang, X., Ou, X., Yu, X-H., Tan, R-Q., Yang, F-Y., Gong, D., Huang, C., Pan, Y-J., Zhang, J., Chen, L-Y., Zhao, Z-W., Xie, W., Li, L., Zhang, M., Xia, X-D., Zheng, X-L. and Tang, C-K. (2017). MicroRNA-134 promotes the development of atherosclerosis via the ANGPTL4/LPL pathway in Apolipoprotein E knockout mice. *Journal of Atherosclerosis and Thrombosis*, 25(3):244–253 DOI 10.5551/jat.40212.

Yoshihiro Narusaka, Mari Narusaka, Satoshi Yamasaki. and Masaki Iwabuchi. (2012). Methods to Transfer Foreign Genes to Plants, *Transgenic Plants*, ISBN: 978-953-51-0181-9.

Zhou, G. Y., Weng, J., Zeng, Y., Huang, J., Qian, S. and Liu, G. (1983). Introduction of exogenous DNA into cotton embryos. *Methods in Enzymology*, 101:433–481.