EDUZONE: International Peer Reviewed/Refereed Multidisciplinary Journal (EIPRMJ), ISSN: 2319-5045
Volume 11, Issue 2, July-December, 2022, Available online at: www.eduzonejournal.com

"Gene Transfer Technique in Plant Callus and Selection of Transformants"

Ashok Kr. Singh

SRTC PG College Utraula, Balrampur -271604 UP India

Author Mail: ashokarsingh@gmail.com

ABSTRACT

The desired trait to a crop of a foreign gene encoding the trait must be inserted into plant cells along with a cassette of additional genetic material. The cassette includes a DNA sequence called a promoter which determines where and when the foreign gene is expressed in the host and a marker gene that allows breeders to determine which plants contain the inserted gene by screening. The marker genes render plants resistant to antibiotics that are not used medically or tolerant to certain herbicides. Two methods are used to transfer foreign genes into plants. The first method involves the use of a plant pathogen called *Agrobacterium tumefaciens* which causes crown gall disease in many species. This bacterium has a plasmid that contains tumor inducing genes (T-DNA) along with additional genes that help the T-DNA integrate into the host genome. This is done by removing most of the T-DNA while leaving the left and right border sequences which integrate a foreign gene into the genome of cultured plant cells. The second delivery method is a gene gun which fires gold particles carrying the foreign DNA into plant cells. These particles pass through the plant cell wall and enter the cell nucleus where the transgene integrates itself into the plant chromosome. The both methods of gene transfer are fairly random and must screen for the plant cells that contain the foreign gene.

1. INTRODUCTION

Transgenic plants are plants that have had their genomes modified through genetic engineering techniques either by the addition of a foreign gene or removal of a certain detrimental gene. A foreign gene inserted into a plant can be of a different species. (Singh A K 2021).The rapid development in plant molecular biology and genetic engineering technology a wide variety of transgenic plants with important agronomic traits such as pest resistance and drought tolerance have been developed ranging from dicots to monocots that are amenable to genetic modifications. The main purpose in the production of transgenic plants is to produce crops which have ideal traits, quality and high yield. Besides being beneficial to the agriculture sector the plants are found to be able to act as the factory for pharmaceutical protein production (Lai et al. 2012).

The natural transfer of genetic material from one organism to another is referred to as horizontal gene transfer**.** The foreign DNA is either randomly inserted into the host genome or recombines if there is sequence homology between the two genomes. This is different from the vertical gene transfer where the genetic material is transferred from the parents to the offs springs through sexual reproduction.

The horizontal gene transfer is facilitated by various mechanisms. In prokaryotes mainly transformation intake of genetic material from surrounding, conjugation exchange of genetic material with the physical union of two cells and transduction transmission of DNA through bacteriophages from one cell to another are responsible for the transfer of the gene within organisms. In eukaryotes the presence of the outer cell membrane and the nuclear membrane makes transfer of DNA difficult between organisms (Hotopp et al 2007).

In vertical gene transfer the transfer of genetic material is from parents to offspring. In plant vertical gene transfer is applied by crossing two plants sexually so that their genes are passed on to the next generations. Such as in the case of a cross between a transgenic crop with herbicide resistance gene and a non-transgenic crop. The novel gene may subsequently be acquired by the non-transgenic crop. This is particularly utilized in order to breed crops with desired traits.

2. Gene Constructions

A simple functional gene construct consists of a promoter region, gene coding region, and terminator/stop region. In addition, certain gene constructs may contain special sequences such as an enhancer, silencer, or reporter sequence

depending on the nature of study. Plant transformation always starts with the transgene construction. Transgene construct generally has similar elements other than the inclusion of the gene of interest and selectable markers. A proper gene construct is crucial for the success of producing ideal transgenic line.

2.1 A typical plant gene

A typical plant gene consists of the regulatory and structural genes. Regulatory genes are usually located at the 5′ upstream of a gene with its own promoter, enhancer, or silencer region. Structural genes on the other hand, begin with a catabolite activator protein (cap) site followed by a leader sequence, start codon, exons, introns, terminator, and a polyadenylation site (poly-A tail). These elements are responsible for DNA transcription. The transcribed pre-mRNAthen undergoes RNA splicing producing mature m-RNA without the introns. This mature m-RNA is delivered to the cytoplasm for translation initiated by the binding of ribosomal subunits to the promoter. Translation then begins at the start codon with the ribosome moving downstream to the next codon creating a peptide chain with the help of t-RNAs and ends once it reaches the chain terminator.

2.2 Promoters and Enhancers

The promoter region is typically located at the 5′ upstream of a gene. Promoters are known for their function in governing gene expression, likened to an onoff switch. In DNA transcription the promoter sequence is recognized by transcription factors. These transcription factors bind to the consensus region of the promoter and recruit the RNA polymerase. The formation of the RNA polymerase transcription complex marks the beginning of DNA transcription.

The promoters can be categorized into three main groups: constitutive promoters, tissue-specific promoters and inducible promoters (Hernandez & Finer, 2014).

The constitutive promoters are active at most of the developmental stages, and they directly participate in maintaining moderate and constant level of gene expression. Tissue-specific promoters provide restricted gene expression to certain tissues or gene expression involves in developmental-specific stages. Gene expressions associated with the inducible promoters are greatly affected by environmental stimuli, which allow for the regulation of genes through external factors.

The enhancers are short (50–1500 bp) regions in a gene that can be recognized and bound by activator proteins. These proteins also referred to as transcription factors, bind to the enhancer, forming an enhancer-bound transcription factor complex, which will later on interact with the mediator complex (TFIID) ultimately aiding in the recruitment of RNA polymerase II. The enhancer-bound transcription factor complex forms a loop and toward the intervening sequence and comes in contact with the promoter region, thus increasing the accessibility of the promoter to the transcription proteins (Ong & Corces, 2011). In contrast, silencers function as the direct opposite of enhancers. Silencers are binding sites for transcription factors known as the repressors. These repressors are known to down-regulate the transcription of a gene. In plant genetic engineering, suitable promoter and enhancer are chosen based on the intended regulation of gene expression. Gene expression is kept at basal level when the transgene exerts mild toxicity to the target plant. On the other hand, higher gene expression levels facilitate the detection and monitoring of a transgene which may usually be under expressed in nature.

2.3Reporter gene

Reporter genes are genes attached to the regulatory sequences or to gene of interest to allow for detection of the trans-gene expression as well as the localization of expressed proteins (Seymour & Sander, 2007). Reporter gene sequences encode proteins or products of the protein after being catalyzed for detection through instruments or simple assays. In contrast, selectable marker genes such as antibiotic genes, herbicidal-resistant genes, and anti-metabolic genes confer resistance toward certain chemical agents, which inhibit non-transgenic plant development (Ziemienowicz, 2001). The common reporter genes used to monitor plant transgene expression include green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), beta-galactosidase (Lac-Z), luciferase (Luc), and beta-glucuronidase (GUS). These reporter genes allow differentiation between transformed and non-transformed cells and enable detection of transgene localization and regulation of the expressed and tagged protein. The dual reporter systems such as LucLuc and GUSLuc are also available for better detection in distinguishing proteins (Koo et al. 2007). Ideal reporter genes should be highly sensitive, stable, and reliable for large-scale measurements within a wide range of cells and tissues.

3. TRANSFORMATION TECHNIQUES

Plant transformation refers to the process of altering the genetic constituents in a plant of interest by introducing DNA segments into the plant genome to achieve desired gene expression. Numerous types of plant transformation techniques

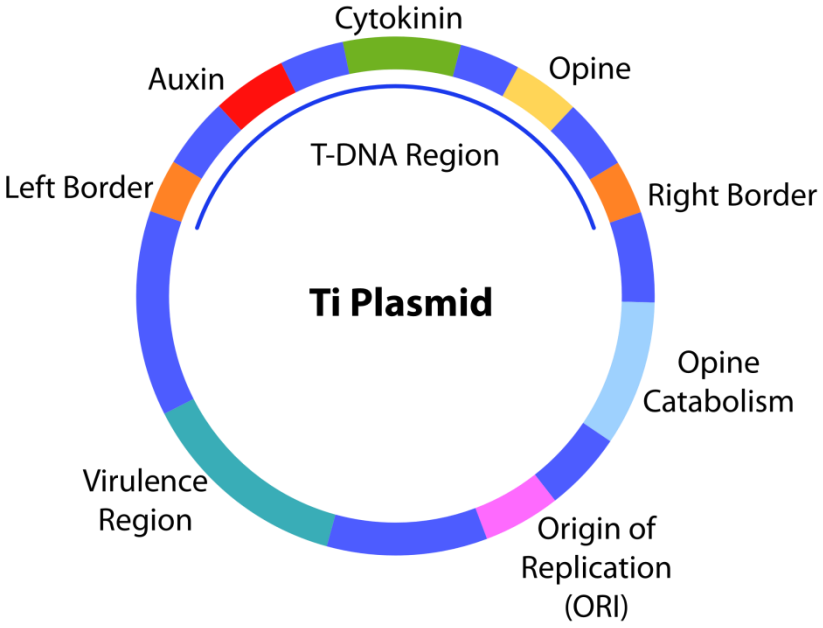
have now been made accessible to the public. These plant transformation techniques can be categorized under two groups: indirect or direct gene transfer. Indirect gene transfer also known as vector-mediated gene transfer involves the introduction of exogenous DNA into the plant genome via biological vectors, whereas direct gene transfer methods involve the introduction of exogenous DNA directly into plant genome through physical or chemical reactions.

3.1 Indirect Methods

3.1.1*Agrobacterium*-Mediated Gene Transfer

Agrobacterium-mediated transformation is the most common technique used in plant transformation as it is efficient and effective in a wide range of plants. *Agrobacteria* are indigenous to the soil ecosystem. They are pathogenic gram-negative bacteria that cause crown gall or hairy root disease in plants. The genetic information for tumor growth is encoded on a tumor-inducing plasmid (Ti- plasmid) or hairy root-inducing plasmid (Ri- plasmid) in the genome of these bacteria (Barker et al 1983).

There are generally two types of *Agrobacterium* species that are commonly used in plant transformation; *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes. Agrobacterium tumefaciens* contains the Ti-plasmid which causes crown gall disease whereas *A. rhizogenes* contains the Ri- plasmid that causes hairy root disease. The discovery of these two species provides efficient vector systems for the development of transgenic plants. This method had successfully transformed a broad variety of plants such as rice, maize, barley, and tobacco. A. *tumefaciens* used for plant transformation are modified *Agrobacteria* which has no tumor-promoting and opine-synthesis genes in their genome. These genes are removed from the bacterial plasmid and replaced with the desired foreign gene or selective markers making them useful vectors that enables the incorporation of foreign genes into plant's genome.



3.2 Direct Method

The direct gene transferinvolves the direct introduction of exogenous DNA into the plant nucleus. In order to introduce foreign DNA into the plant cell the outer membrane of the cell is first disrupted permeating it for foreign DNA to enter.

Direct gene transfer can be categorized into two main groups: physical gene transfer and chemical gene transfer. Physical gene transfer disrupts the cell wall and cell membrane via mechanical means. Among these methods, particle bombardment biolistic is the most common one used in plant transformation since it was first introduced by Sanford et al. 1990.

The DNA coated with gold or tungsten particles are shot into the target plant cell under high pressure using a "Gene Gun". The fast-moving particles allow for the penetration of coated DNA through the thick plant cell wall directing the foreign DNA into its nucleus. The coated DNA will then separate from the metal particles and integrate itself into the chromosomes within the nucleus of the plant cell (Singh AK2020).

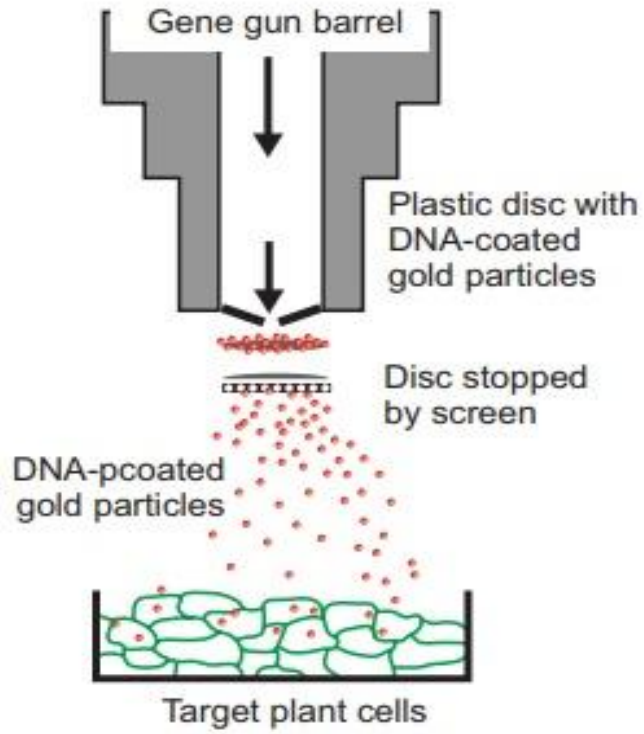
This method had been found to be effective in transforming both dicots and monocots which compensates for the less successful *Agrobacterium*-mediated transformation process. (Lai et al., 2011).

In traditional biolistic method micro projectiles gold or tungsten are normally coated with DNA in the presence of calcium chloride and spermidine. The spermidine helps to stabilize the DNA structure and enhances the binding of DNA to the micro projectiles (Singh AK 2020).

Chemical gene transfer approaches involve the use of chemical to disrupt cell membrane enabling the entry of foreign DNA. This particular method is not preferable in plant transformation as it is only effective when applied to protoplasts. One of the most prominent chemicals used in this approach is polyethylene glycol that is used for destabilizing the cell membrane in the presence of a divalent cation, thus increasing the permeability of the cell membrane, allowing for the uptake of foreign DNA. The exact mechanism for chemical gene transfer is not fully understood, but it was postulated that PEG increases the osmotic pressure and causes contraction in the protoplast; this facilitates endocytosis of the divalent cation/DNA complex (Lazzeri et al., 1991).

Besides those, liposome is another chemical method that is used in the transformation of plant's protoplast cells. Liposomes act as vehicles to encapsulate and deliver foreign genetic materials into the protoplast. The lipophilic attribute of liposomes provide easy access into the protoplast in transforming the cell (Caboche, 1990).

3.2.1 Particle Bombardment



The biolistic is a method where cells are physically impregnated with nucleic acids or other biological molecules.Abiolistic particle delivery system is a device for plant transformation where cells are bombarded with heavy metal particles coated with DNARNA. This technique was invented by John Stanford in 1984 for introduction of DNA into cells by physical means to avoid the host-range restrictions of *Agrobacterium*-mediated genetic transformation system works well for dicotyledonous plants but has low efficiency for monocots.Biolistic particle delivery system provides an effective and versatile way to transform almost all type of cells. It has been proven to be a successful alternative for creating transgenic organisms inprokaryotes, mammalian and plant species.

This process construct having gene of interest is coated on the surface oftiny particles of gold or tungsten (0.6-1 mm size). Prior to coating DNA is precipitated with calcium chloride, spermidine and polyethylene glycol. These coated micro particles are loaded on to the macrocarrier and accelerated to high speed by using pressurized helium gas.Plant cell

suspensions, callus cultures, or tissues could be used as the target of these micro projectiles. As the micro projectiles penetrate the plant cell walls and membranes to enter the cells coated DNA is released from its surface and incorporated into the plant's genome (Singh Ak 2020).

This method is especially important for monocots, for which efficiency of other transformation methods is not satisfactory. A wide range of tissues such as apical and floral meristems, embryos, seedlings, leaves, cultured cells and floral tissues could be used as target in this method.

4. SELECTION OF TRANSFORMANTS

In a genetic transformation experiment, only one in a several million to billion cells may take up the transgene depending upon the efficiency of transformation. Rather than checking every single cell/organism a selective agent that kills or gives a different phenotype to all the cells not carrying foreign DNA can be employed. These selective genes are called as marker genes. These genes also help in assessing the success rate of a genetic transformation study. Marker gene is a gene introduced into cell along with the transgene. It is used to determine if the transgene has been successfully inserted into host organism's genome as marker gene's presence can be seen or detected. There are two types of marker genes: selectable marker and screening marker.

4.1 Selectable Marker

A selectable marker is a gene that confers a trait suitable for artificial selection as it protects the organism from a selective agent that would normally kill it or prevent its growth. In most of the genetic transformation experiments only one in a several million or billion cells will take up the transgene. In order to find out transformed cellorganism a selective agent is utilized which kills all the cells without transgene, leaving only the transformed ones. Selectable marker genes can be divided into several categories depending on whether they confer positive or negative selection and whether selection is conditional or non-conditional on the presence of external substrates. Negative selectable marker genes result in death of the untransformed tissue whereas transformed cells are able to survivee.g., antibiotics, herbicidesare the most common selective agents (Hille et al 1986).

4.2 Screening Marker

A marker for screening is one which will make cells containing the gene look different and allows the researcher to distinguish between wanted and unwanted cells organisms is known as screening marker. These markers do not provide a cell with a selective advantage but are used to identify transgenic events by manually separating transgenic and non-transformed material. Reporter systems have been used to determine the intracellular localization of a gene product, efficiency of gene delivery systems, detection of protein-protein or protein-DNA interactions and activity of promoter A common example of reporter system is blue and white selection in bacteria. This assay is based upon insertional inactivation of Lac-Z gene which produces beta-galactosidase enzyme. This enzyme converts a colorless substrate into blue colored product which is responsible for blue color of colonies. Non recombinant colonies have intact lac-Zgene thus they are blue colored, whereas recombinants have insertional inactivated lac-Z geneand colorless colonies.

4.3 Histochemical Assay

GUS assay is a simple method for detection of transformed cell without needing any glucuronidase, an enzyme from the bacterium Escherichia coli is utilized in this technique. This enzyme can transform colorless or non-fluorescent substrates into colored or fluorescent products thereby giving the transformed cell a different phenotype. Histochemical GUS staining is done using X-GLU,5-bromo-4-chloro-3-indolyl glucuronide, which produces blue color. Tissue expressing the GUS gene when incubated with X-GLU produces blue color(Yin 2004).

4.4 Fluorometric Estimation

This estimation a 4-methylumbelliferyl-beta-D-glucuronide (MUG) is used. In the reaction MUG acts as a substrate, which upon hydrolysis, produces glucuronic acid and the fluorescent 4-methylumbelliferone. The 4-MU the excitation is at 365 nm and emission at 455nm.This technique is used to analyze the activity of regulatory elements by estimating expression of GUS gene either quantitatively or by studying activity in different tissues. It is very commonly used but the biggest drawback is that the cells are killed in the process.

4.4 Green Fluorescent Protein

GFPis a protein which exhibits bright green fluorescence after exposure to light in the blue to ultraviolet range. The cells containing GFP glow green under UV light and can be seen with the help of a specialized microscope. Itis made up of 238 amino acid residues and displays a major excitation peak at 395 nm and a minor one at 475 nm. It gives a green

fluorescence as its emission peak (509 nm) falls in the lower green portion invisible region of spectrum. Other versions of this protein giving yellow and red fluorescence are also available, so that multiple genes in one organism can be followed at the same time.

GFP gene is frequently used as a reporter which confirms expression of atransgene throughout the organism. It can be introduced into organisms through breeding, cell transformation or injection with a viral vector. This gene has been introduced and expressed in many bacteria, fungi, plant, fish, fly and mammalian cells.

CONCLUSION

GM crops will be a valuable alternative in solving food security problem that happens in a world of growing human population and drastic climate change. However, transgenicity remains a major controversy in the view of biosafety issues spurred by public misconceptions and perceptions to GM plants. In addition, GM crops require years of risk assessments that is time and cost consuming. On the other hand, unintended effects arise could be one of the issues in GM plant production. This is generally due to the transgene integration through illegitimate recombination in plant as the consequences of random transgene integration, gene disruptions, sequence changes, and the production of new proteins. Thus, unintended effects of gene transfer in GM crops should be examined thoroughly through metabolic profiling methods to avoid production of GM plant with significant difference in chemical composition from non-GM plant grown under the same condition.

Recently, the development of engineered site-specific endonucleases such as TFN, TALEN, and CRISPR/Cas9 allows the genetic engineering of plant to be carried out more efficiently and precisely. Problems such as heterozygosity that is commonly faced in agro and gene gun-mediated approaches can be avoided. Hence, the future of transgenic technology is shifting toward the engineered endonuclease genome editing technology. This endonucleases genome editing involves the introduction of a targeted double-stranded DNA breakage (DSB) in genome and consequently stimulating the cellular DNA repair mechanisms. In addition, different genome modification can be done dependent on the types of DSB repair pathways used: (1) non-homologous end joining (NHEJ) and (2) homologous recombination (HR). In NHEJ-mediated genome editing, the target cell self-edits its genome without the addition of foreign gene that may lead to mutation and gene knockout. Since this genome editing is performed without introducing a foreign gene, non-transgenic crops could be obtained. Hence, effort needs to be concerted toward improving the genome editing technology to genetic engineered crops with better agronomic traits and public acceptance.

REFERENCES

[1] Barker RF, Idler KB, Thompson DV, Kemp JD.1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens*.Plant Molecular Biology. 2(6):335-350.
[2] Caboche M.1990. Liposome-mediated transfer of nucleic acids in plant protoplasts. Physiologic Plantarum. 79(1):173-176.
[3] Hernandez-Garcia CM, Finer JJ.2014. Identification and validation of promoters and cis-acting regulatory elements. Plant Science. 217(1):109-119.
[4] Hille J, Verheggen F, Roelvink P, Franssen H, van Kammen A, Zabel P.1986. Bleomycin resistance: A new dominant selectable marker for plant cell transformation. Plant Molecular Biology. 7(3):171-176.
[5] Hotopp JCD, Clark ME, Oliveira DC, Foster JM, Fischer P, Torres MCM 2007. Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science* 317 :1753–1756.
[6] Koo J, Kim Y, Kim J, Yeom M, Lee IC, Nam HG.2007. A GUS luciferase fusion reporter for plant gene trapping and for assay of promoter activity with luciferin-dependent control of the reporter protein stability. Plant and Cell Physiology. 48(8):1121-1131.
[7] Lai KS, Abdullah P, Yusoff K, Mahmood M. 2011.An efficient protocol for particle bombardment-mediated transformation of Centella asiaticacallus. Acta Physiologiae Plantarum. 33(6):2547-2552.
[8] Lai KS, Yusoff K, Mahmood M. 2012. Heterologous expression of hemagglutinin-neuraminidase protein from newcastle disease virus strain AF2240 inCentella asiatica. Acta Biologica Botanica. 54(1):142-147.
[9] Lazzeri PA, Brettschneider R, Lührs R, Lörz H. 1991.Stable transformation of barley via PEG-induced direct DNA uptake into protoplasts. Theoretical and Applied Genetics. 81(4):437-444.
[10] Ong CT, Corces VG.2011. Enhancer function: New insights into the regulation of tissue-specific gene expression. Nature Reviews. Genetics. 12(4):283-293.
[11] Singh **A**K 2021.Genetically modified bacteria from natural and artificial habitats. International Journal of Recent Biotechnology.9(3),10-15.

[12] Singh AK 2020. Stable transformation of gene by direct partical bombardment method in *Leuceana leucocephala*.8(4),8-12.
[13] SanfordJC.1990. Biolistic plant transformation. Physiologia Plantarum. 79(1):206-209.
[14] Seymour PA, Sander M.2007. Immunohistochemical detection of beta-galactosidase or green fluorescent protein on tissue sections. Methods in Molecular Biology. 411(1):13-23.
[15] Yin Z, Plader W, Malepszy S.2004. Transgene inheritance in plants. Journal of Applied Genetics.45(2):127-144.
[16] ZiemienowiczA.2001. Plant selectable markers and reporter genes. Acta Physiologies Plantarum. 23(3):363-374.