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METHODS OF GENETIC TRANSFORMATION: MAJOR EMPHASIS TO CROP PLANTS

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ABSTRACT

Advancements in gene transfer technology have indeed opened up exciting possibilities for more effectively manipulating the genetic makeup of live organisms, ranging from microorganisms to plants and animals. Direct and indirect transformations are the two basic types of gene transfer techniques. Indirect method comprises *Agrobacterium* mediated method as it involves intermediate host between gene of interest and target and this method is most opted one out of all present. Direct gene transformation methods, on the other hand, do not involve the use of an intermediate host organism. Instead, they rely on physical means to transfer genes between cells. Biolistic transformation uses high-velocity particles to deliver DNA into target cells, while microinjection and macroinjection involve the direct injection of DNA into cells. Protoplast fusion combines the genetic material of two different cells by fusing their protoplasts. Natural methods for gene transfer encompass mechanisms that occur naturally in various organism, includes transposition, conjugation, phage and retroviral transductions and bacterial transformation. Chemical techniques utilize chemical agents to facilitate gene transfer, such as calcium phosphate-mediated transformation, polyethylene glycol (PEG)-mediated transformation, DEAE (Diethylethanolamine)-Dextran-mediated transformation. Genes can be also being transferred using electrical techniques such as electroporation and electrofusion.

Crop improvement and trait improvement are now being hastened by the fast-rising number of sequenced plant genomes, information from functional genomics data to understand gene function, innovative gene cloning, and tissue culture techniques. Despite being indispensable, its progress is still hindered by the fact that many plant species and agricultural genotypes exhibit low transformability or are resistant to established tissue culture and regeneration conditions. Here, we review the techniques employed in plant transformation and provide a concise overview of their evolution in agricultural crops, from their first inception to present time.

Keywords: Direct and In-direct gene transfer methods, Electrofusion, *Agrobacterium* mediate transformation, Transgenics

INTRODUCTION

Genetic transformation - the technique by which DNA molecules are introduced into plant cells – is gaining popularity as a means of improving agriculture, acquiring sustainability, and preserving the future **(Rakoczy -Trijanowska 2002; Rivera *et al.* 2012)**. It took a long time, but the first attempts at genetic plant transformation were made on maize about half a century ago **Coe and Sarkar (1966)**. Restriction enzymes were used to make recombinant DNA molecules in the early 1970s **(Meselson, Yuan 1968; Smith *et al.* 1970)**. This was followed by the generation of genetically stable transformed plants **(Chilton *et al.* 1977)** like tobacco **(Bevan 1983 ; Herrera-Estrella 1983)**, potato **Chakravarty *et al.* (2007)**, tomato **Horsch *et al.* (1985)**, petunia **Fraley *et al.* (1983)**, rice **(Yang *et al.* 1988; Zhang *et al.* 1996)**, grape **Perl *et al.* (1996)**, cassava **Zhang *et al.* (2003)**, millets **Antony *et al.* (2009)**, and chrysanthemum **Chávez *et al.* (2002)** in the 1980s. In 1994, tomato was the first crop to be approved as the first transgenic to enter markets of the United States by the Food and Drug Administration (FDA). Genetically modified (GMo) crops now account for 7% of global agricultural land and are a key source of income for many countries **Darbani *et al.* (2008)**. More than half of the kinds generated through traditional breeding **Godfray *et al.* (2010)** have been supplanted by genetically modified (GM) plants with specific traits. Furthermore, breakthroughs in the usage of genetically modified plants to manufacture novel recombinant proteins with pathogen-free status and cheaper manufacturing costs have given the pharmaceutical industry a fresh lease on life, allowing corporations to scale up production **(Ma JKC *et al.* 2003; Fischer *et al.* 2004)**. Furthermore, as evidenced by the numerous patents linked with the subject, current plant genetics has already begun to play a critical role in the generation of biofuels and has had a significant biotechnological impact **Chen *et al.* (2010)**.

Various techniques are employed to introduce DNA into the cellular structures of several organisms, including plants, fungi, bacteria, mammals, and other species. The revolutionary finding by (**Griffith, 1928)** regarding genetic transformation had a profound impact on the field of molecular biology. However, it was not until the groundbreaking work of Meselson and Yuan (1968) that the first instance of generating recombinant DNA from *Escherichia coli* using restriction enzymes, known as biochemical scissors, initiated the process of cell genetic transformation. For genetic transformation of cells, recombinant DNA fragments must be synthesized, transferred into the cell *via* membrane permeabilization, integrated into a chromosome, and then maintained and replicated (fig.2). It usually entails clone multiplication in vitro, selecting appropriate promoters for a specific gene, over-expressing activator genes, removing epigenetic silencing, introducing heterologous genes, generating strains with novel properties, improving bioinformatic random mutagenesis programs, identifying sequences that confer antibiotic resistance (selective markers), and producing enzymes that produce a unique characteristic not found in the wild type of strain **Rivera *et al.* (2012)**.

New ways for an efficient, repeatable, and safe delivery system require more attention to increase levels of output in terms of obtaining transformants. Transgene integration has also been used to boost the metabolic process' capabilities by promoting the expression of specified proteins. Furthermore, genetic engineering has attempted not only the introduction of exogenous genes, but also the deletion of genes to reduce metabolic fluxes in alternative pathways and shift fluxes to the product-forming route **(Fischer, *et al.* 2000; Meyers *et al.* 2010)**. Antibodies, which are part of our immune system, can be synthesized by plants by altering them with the proper genes Vaccines against a variety of diseases; including germs that cause diarrhea **(Moeller and Wang 2008)** can also be created.

Transformation can occur in a variety of ways, depending on the species **(Sawahel and Fukui K 1995)**. A major obstacle in the transformation is: the highly protective hydrophobic layer of the cell membrane that acts as a barrier to any transformation in cell genetic makeup along with DNA being highly charged macromolecule, its diffusion over the cell membrane is quite a stumbling problem.

The two most popular methods for genetic transformation are indirect and direct transformation **Niazian *et al.* (2017)**. The term "indirect" refers to bacteria-based biological techniques (fig.1). These are based on *Agrobacterium tumefaciens*-mediated transformations and protoplast transformations using cell wall-destroying enzymes. Direct techniques use physical and chemical ways to penetrate the cellular wall. Artificial or non-biological methods that alters and transfer genes from one organism cells to another via physical mode includes biolistic transformation, micro- injection and macro-injection, electroporation, agitation using glass beads, ultrasound waves and shockwave mediated, vacuum infiltration, silicon carbide whisker, laser microbeams. (Table 1) compares all physical methods those are also discussed briefly in this review report. Chemical-

based techniques include calcium phosphate co-precipitation and lipofection, PEG mediated, and Diethyl amino ethyl (DEAE) dextran mediated (Table 2).

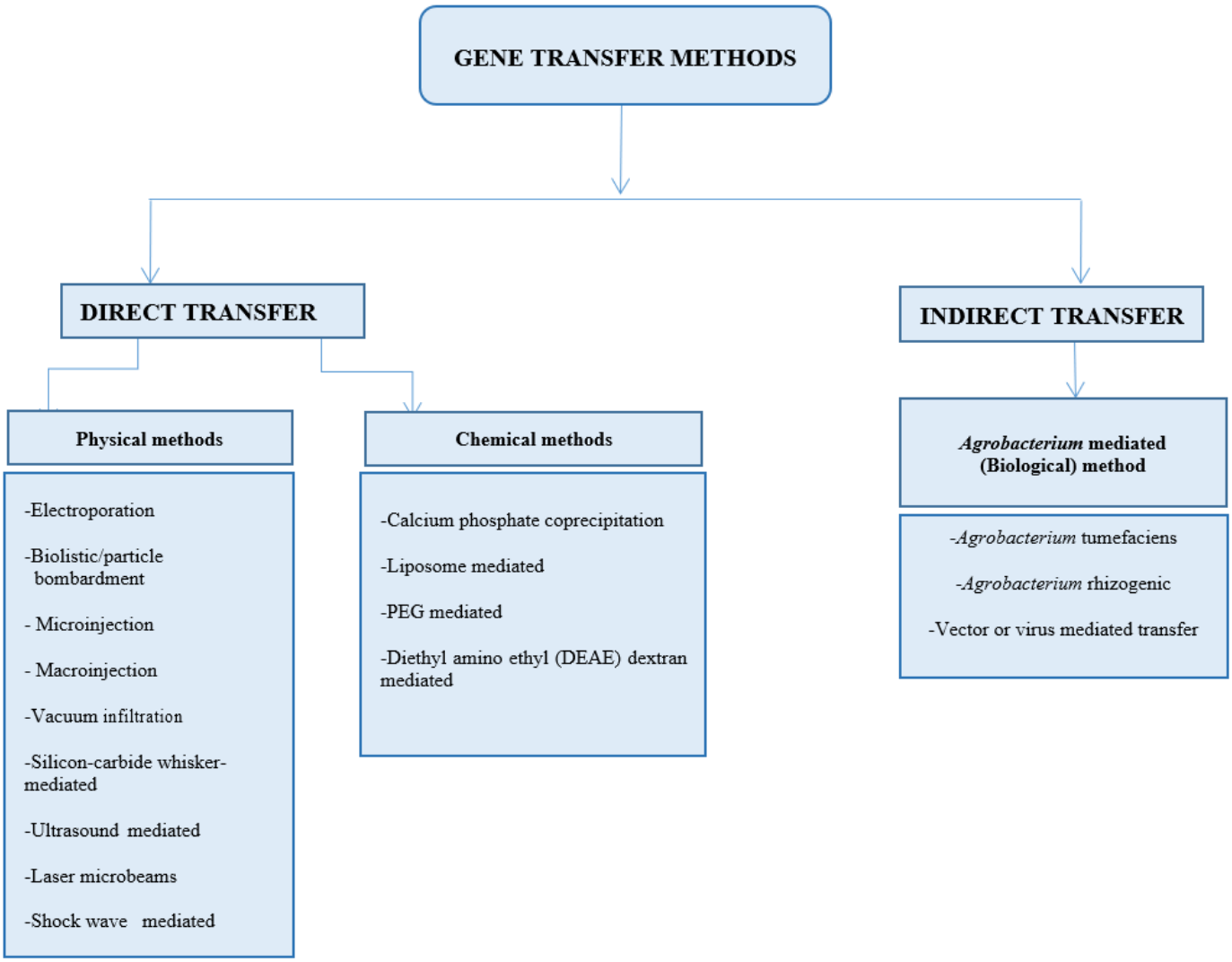

Figure 1 Various methods of gene transformation in plants

Identification/ characterization of novel genes to be transferred

(Desirable genes located in wild species, unrelated plant species)

Gene construct preparation

(Gene isolation, molecular cloning by selecting appropriate binary vector, promoter, marker and designing them together to obtain competency of transfer)

Transfer of gene into target plant
(Insertion of the cloned construct into host cells of the target plant)

Identification of transformant/ Molecular analysis
(Marker genes aid in identifying transformed cells and further regenerating them into whole plantlet, gene specific PCR, qRT-PCR, and hybridization techniques)

Obtained plantlets compared with wild type (non-transformed)
(Trait specific tests

Figure 2 Generalized steps of genetic transformation

Table 1 Comparison of the direct physical methods of genetic transformation of plants (Rivera et al. 2012)

METHOD	PROCEDURE	ADVANTAGES	DISADVANTAGES
Electroporation	An electrical impulse induces membrane permeability and provides a local driving force for ionic and molecular transport through the pores.	All types of plant protoplasts and cell types can be used, simple, fast, and cheap.	Lengthy protocol. It depends on the electrophysiological properties of the plant. Low transformation efficiency.
Biolistic	Small particles coated with genes are accelerated to penetrate the cell wall.	Easy to perform, no cell wall pretreatment is required. Independent of cell physiology. Transformation with multiple transgenes is possible.	Expensive. A stable supply of consumables is required. DNA can be damaged. It can generate multiple copies of the introduced gene, leading to various disadvantages. Low transformation efficiency.
Microinjection	Direct DNA transfer into plant cells by injection pipette.	Very high transformation efficiency. Allows introduction of plasmids and whole chromosomes.	Costly, tedious, and slow.
Macroinjection	Injection of genetic material with a hypodermic syringe.	High stability and reproducibility.	Only some parts of the plant are transformed. very low efficiency.
Vacuum infiltration	The vacuum creates a negative pressure that contracts the spaces between the cells of plant tissue, allowing the invasion of bacteria such as *Agrobacteria*.	Easy and fast. medium efficiency. In vitro plant regeneration has transformed many independent plants.	Some *Agrobacterium* strains are unable to infect certain cell types and risk multiple copies of the introduced gene.
Silicon carbide whisker-mediated	Silicon carbide fibers are mixed with tissue and DNA suspensions in a vortex and allowed for insertion by abrasion.	Easy, fast, and cheap. Can be used for various plants without limitations.	Damage to cells impairs their ability to regenerate. Potential for injury from inhaling fibers. Very low efficiency (lower than biolistic).
Laser microbeams	A laser microbeam opens self-repairing holes in the cell wall that allow DNA delivery.	Laser properties for use as optical tweezers coupled to a suitable microscope.	High cost (expensive equipment required), and laborious.
Ultrasound	Acoustic cavitation introduces DNA molecules into cells, temporarily altering the permeability of the cell membrane.	It is highly efficient, moderately costly, and can be used with a wide variety of cell types.	May damage the cells by breaking their membrane.
Shock waves	Cell permeabilization occurs through shock wave-induced cavitation.	It is rapid, easy to perform, highly efficient and reproducible, requires no enzyme cocktails, and can be used to transform multiple cell types.	Shock waves generators for this purpose are not in the market and experimental equipment is relatively expensive.

Table 2 Comparison of direct chemical methods of genetic transformation of plants

METHOD	PROCEDURE	ADVANTAGE	DISADVANTAGE
Calcium-phosphate coprecipitation	Foreign DNA can also be carried with Ca^{++} ions released inside the cell in the form of calcium phosphate because of calcium precipitation.	Comparatively easy to optimize for variety of plasmids. Cost effective.	Low transfection efficiency, typically ranging between 1% and 10%.
Liposome- mediated	Gene-carrying liposomes can be used to fuse with protoplasts to transfer genes.	DNA is protected from environmental influences and damage. Moreover, it is stable and can be stored in liposomes for some time before transfer.	Difficulty associated with the regeneration of plants from transformed protoplasts.
PEG Mediated	Plant protoplasts can be formed by treatment with PEG in the presence of divalent cations.	It is simple, efficient and can process many samples simultaneously. Achieve transformed cell populations with high viability and division rates.	Regeneration of fertile plants from protoplasts is problematic for some species. The DNA used is also susceptible to degradation and rearrangement.
Diethyl amino ethyl (DEAE)dextran mediated	Transformation of cells with DNA complexed with the high molecular weight polymer diethylaminomethyl (DEAE)-dextran is used to obtain efficient transient expression.	Simple and inexpensive, more sensitive, and applicable to different cell types	Does not produce stable transformants. Toxic to cells at high concentrations. Transfection efficiency varies by cell type

Indirect gene transfer or vector Mediated

Agrobacterium tumefaciens, a naturally occurring organism with genetic engineering capabilities, possesses a plasmid referred to as a Ti-plasmid, which is accountable for the initiation of tumorigenesis. The transfer of a specific segment known as T-DNA (transferred DNA) from this plasmid to the genome of infected plant cells has been observed to induce the formation of tumours in plants **Zaenen et al. (1974)**. The utilisation of Ti-plasmid as vectors for the delivery of advantageous new genes into target cells is facilitated by its distinctive property. In order to replace undesirable DNA sequences, the T-DNA borders of the Ti-plasmid are utilised to clone both a plant selection marker gene, such as *nptII* which provides resistance to kanamycin, and the gene of interest, such as the *Cry* genes

for lepidopteron insect resistance **(Gelvin and S.B 2017).** The success of transformation is significantly influenced by the choice of explants and their capacity for totipotency. In order to proceed with the experiment, it is customary to obtain leaf discs for dicots or embryogenic calluses for monocots, such as cereals. These plant tissues are subsequently inoculated with an *Agrobacterium* containing a recombinant disarmed Ti-plasmid vector. Subsequently, the afflicted tissue can undergo co-cultivation for a duration of 2-3 days on a medium conducive to shoot regeneration. This period facilitates the transfer of T-DNA and foreign genes. Subsequently, in order to specifically eliminate non-transformed tissues, the explants, which are plant tissues such as leaf discs or calli, are transferred onto a selection medium containing a lethal dosage of an antibiotic (e.g., kanamycin or hygromycin). The inclusion of a bacteriostatic agent, such as the antibiotic cefotaxime, in the selection medium is an essential element in the process of *Agrobacterium* mediated transformation. The process of hardening or acclimatisation of regenerated plants involves the transplantation of mature plants into soil over a span of roughly 3 to 4 weeks. Following a duration of 3-5 weeks, the shoots that have undergone regeneration from leaf discs are subsequently relocated to a medium that promotes root formation.

Molecular techniques, such as polymerase chain reaction (PCR) and hybridization methods (dot blot, southern blot), can be employed to validate the existence of transgenes within the presumed transgenic plants. A roadmap has been shown in (fig. 3) illustrating stepwise *Agrobacterium* mediated transformation protocol for transgenic development. Till now the process of plant genetic transformation is largely dependent on the utilization of the bacterial pathogen *Agrobacterium tumefaciens* as an effective mechanism for introducing desired genes into a recipient plant. Within the cellular organelle known as the plant nucleus, the DNA that has been transferred possesses the ability to integrate itself into the genetic material of the plant, so ensuring its inheritance by subsequent generations **(Gelvin and S.B 2003)**. This process is widely known for stable transformation. In an alternative scenario, the exogenous DNA has the capacity to temporarily persist within the nucleus without undergoing integration into the host genome. However, it can still undergo transcriptional processes to generate gene products that are deemed advantageous (referred to as transitory transformation). This technique offers several advantages in the context of genetic modification. These advantages encompass the precise transfer of DNA fragments with well-defined ends, limited rearrangement of genetic material, the ability to transfer relatively large DNA segments, the integration of a small number of gene copies into the chromosomes of plants, and the production of transgenic plants that exhibit high quality and fertility. Nevertheless, the process of transformation does not consistently result in occurrences that are characterized by a high degree of clarity or simplicity. The utilization of *Agrobacterium*-mediated gene transfer is experiencing continued growth and expansion. It has emerged as a valuable technique in the investigation of gene function and promoter analysis. Novel vectors engineered for the purpose of transporting significantly large DNA segments have been successfully created and subjected to thorough experimentation. The utilization of techniques aimed at the specific integration of transgenes into the genomes of higher plants has garnered significant interest in the scientific community. Several technologies have been developed to produce transgenic plants without the use of selection markers, aiming to enhance public acceptance of biotechnology goods.

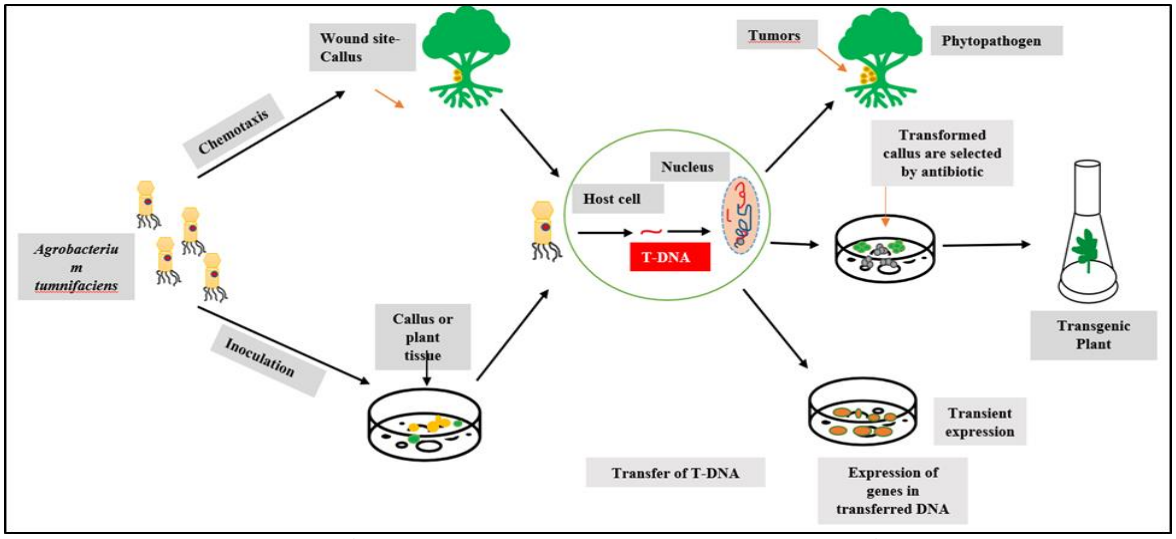

Figure 3 Plant transformation process using *Agrobacterium* method includes the following steps

Due to the widespread interest in altering genomes of nearly all crops which was not possible with *the Agrobacterium* mediated method alone and complications such as formation of repetitive sequences of transgenes, specific rearrangements, integration of DNA segments unrelated to the intended target, and the potential for unstable expression of transgenes. While a considerable number of transgenic plants often exhibit positive traits during initial testing, the buildup of non-desirable plants becomes notable after repeated rounds of characterization and screening, therefore consequently additional improvements in each of the aforementioned processes are highly desirable. Hence direct methods of gene transfer were also studied in parallel, starting since 1980s **(Neumann *et al.* 1982; Potter 1988)**. It involves several method for incorporating multiple copies of a chosen gene into the genome at random locations with a low level of cellular damage. Their drawbacks include issues with plant regeneration and a low level of transgene transient expression. These are electroporation **(Murray *et al.* 1991)**, biolistic, vacuum infiltration, ultrasound, silicon carbide fibers, microinjection, macroinjection, and laser microbeams are examples of direct methods now in use or being studied **(Danilova *et al.* 2007; Bajaj and Mohanty, 2005; Spörlein *et al.* 1991)**. This paper places significant focus on doing a comprehensive examination of direct approaches.

Direct gene transfer methods

Physical methods

Electroporation

Electroporation is an electrical method of transformation in which the electric pulse is applied, and transient pores are produced in the plasma membranes of prokaryotic and eukaryotic cells **Rolong *et al.* (2018)**. The protoplasts of plant in suspended in an appropriate ionic buffer which contains a recombinant linearized plasmid DNA. Then this mixture is exposed to either a low voltage for long pulses or high- voltage for short pulses for the standardized number of cycles. This results in the inducement of transient pores in the plasma membrane through which the DNA molecules get inserted. The transformed protoplasts now regenerated complete plantlets.

Biolistic: particle bombardment

Biolistic (biological ballistics) also known as particle gun -mediated gene transfer, is the method of directly bombarding DNA fragments onto cells using an instrument called a gene gun. Particle bombardment is the most effective method out of all other methods of this category for gene transfer, and formation of transgenic plants **Klein *et al.* (1987)**. This method is versatile as it is successfully being used for DNA transfer in animal and microbial cells. The microprojectile bombardment method was initially named as biolistic by its inventor Sanford (1987) **Boynton *et al.* (1988)**. The gene gun works by accelerating high-density carrier particles with a diameter of around two microns, coated with desired genes, able to penetrate through cells to insert the DNA inside. It was created in 1987 at Cornell University to exhibit the genetic modification of cereals, but it can be applied to all plant species irrespective of being dicot or monocot as restricted with vector mediated protocol. The process can be used to transform chloroplasts and nuclei also includes cells, protoplasts, structured tissues like meristems (a collection of undifferentiated cells in active mitosis), embryos, and calluses (vegetable tissue growing in an erratic pattern).

The biolistic approach was initially created with the intention of transforming monocotyledons, a class of flowering plants that are resistant to transformation by *Agrobacterium.* According to a comparison between *Agrobacterium* and biolistic employing fluorescent in-situ hybridization, *Agrobacterium* offers considerable benefits over biolistic **Synder *et al.* (1999)** in terms of transformation efficiency, transgene copy number, expression, inheritance, and physical structure of the transgenic locus. However, biolistic is the most widely used direct method for genetically altering plants because it can be applied to a wide range of species,

subcellular organelles, microbes, and even animal cells, has a quicker processing time, incurs little expense in the creation of transgenic plants, and is straightforward for the introduction of multiple genes or chimeric DNA (DNA from two different species). Additionally, it is independent of the electrophysiological characteristics of the cell, such as the electrical potential and the structural elements of the cellular membrane **Sanford *et al.* (1993)** and does not require a vector with a specific sequence. However, each biological target used requires a different set of transformation parameters to be optimized **Sanford *et al.* (1993)**. The main biolistic applications in plant research are transient gene expression investigations, the development of transgenic plants, to improvement in terms of resistance/tolerance against various insect-pest or diseases, nutrition enhancement etc. The technique has a transformation efficiency of 0.002, with a genetic translational degree ranging from 17 to 36% of relative activity during events in a single bombardment, and up to 70% of activity in the genetic expression during events in several bombardments **Oard *et al.* (1990)**. Several plants have been genetically altered by particle bombardment. (Table 3).

Comparing transgenic rice produced using *Agrobacterium* and biolistic techniques **Dai *et al.* (2001)** reveals that *Agrobacterium*-mediated transformation results in a higher percentage of transgenic plants with intact copies of foreign genes, particularly non-selection genes, more stable transformation, and better fertility, whereas biolistic has a higher efficiency with a wide range of gene expression. The presence of multiple copies of introduced genes, which can result in a variety of unfavorable side effects like gene silencing or altered gene expression, is the limiting factor for the use of the gene gun **(Rakoczy, 2002)**. It's important to consider the high cost of gene gun accessories.

Microinjection

Microinjection, a technology also used for plants, is the most efficient way to genetically alter animal cells **(Davey *et al.* 1989)**. Microinjection has also been used for several plant species. The procedure entails injecting DNA precisely and directly into the plant cell using a glass microcapillary injection pipette **(Morikawa and Yamada 1985; Crossway *et al.* 1986)**. The method is laborious, sluggish, and necessitates a pricey micromanipulator to immobilize the cells using a holding pipette and moderate suction. However, it is incredibly effective, exceedingly exact in terms of delivery, and permits the introduction of complete chromosomes in addition to plasmids into plant cells **(Korzh and Strahle 2002)**. The efficiency of the final transformation was around ten times lower than that of biolistic **Holm *et al.* (2000)**. However, this strategy has been proposed as a potential approach for stable plant transformation used in various types **(Jones-Villeneuve *et al.* 1995)**. In many animal systems, DNA microinjection is the preferred technique for stable transformation. Plant cell microinjection is technically more challenging than animal cell microinjection for several reasons **Crossway *et al.* (1986)**:

- **Cell Wall Barrier:** The cellulose-based stiff cell walls that surround plant cells serve as a barrier to microinjection. The cell wall must be pierced by the needle without the cell being harmed.
- **Cell Size Variability:** Plant cells come in a wide range of sizes, making it difficult to choose the right ones for microinjection.
- **Low Throughput:** Because microinjection requires a lot of manual labour and has a low throughput, it is less suitable for applications that call for the delivery of molecules to a lot of cells. **Cunha *et al.* (2018)**.
- **Cell Viability:** Cell viability and the success of the transformation might be impacted by cell damage caused by the microinjection technique.

There are some efforts made to optimize plant cell microinjection:
Design of the Needle: Scientists have created specialized microneedles that are sharp enough to pierce the cell wall while causing the least amount of harm to the plant cell. These needles are frequently constructed of silicon or glass **Cao et al. (2015).**

Optimal Injection Pressure: To successfully distribute molecules without endangering cell viability, injection pressure must be carefully controlled. The pressure settings must be optimized.

Microinjection Systems: By enhancing throughput and reproducibility, microinjection systems, such as automation and robots, have made it possible to carry out injections on a larger scale.

Cell Preparation: To make plant cells more receptive to microinjection, a portion of the cell wall is frequently removed using an enzyme treatment. To increase the success of injections, cell preparation procedures have been improved **Shou et al. (2004).**

Co-injection Methods: To track the effectiveness of injection and minimize cell harm, some researchers have looked into co-injecting molecules with markers (such as fluorescent dyes) **Torres *et al.* (2005).**

While DNA microinjection has been successful in achieving transformation in certain algal species, these techniques are mostly utilized for plant transformation. Therefore, there is a need for more robust, less challenging, and highly efficient approaches that can generate a higher number of transformed cells within a given timeframe. The utilization of isolated protoplasts possessing partially regenerated cell walls has served as a model system for the development of novel techniques in microinjection into plant cells **(Khan and Kishwar, 2009)**. Protoplast immobilization techniques have been employed, including the use of holding capillaries, adhesive compounds such as polylysine, or embedding in a media containing either agarose or alginate **Kost et al. (1995)**. Various fluorescent dyes, including Lucifer yellow, have frequently been employed to facilitate the visualization of the injection procedure throughout the injection process. Novel single cell culture techniques have been developed to facilitate the proliferation of individual protoplasts that have been introduced by injection. According to **Kost et al. (1995)** successful stable transformation has been achieved using DNA microinjection into protoplasts. By employing a proficient protoplast embedding and culture system, researchers were able to generate stably transformed tobacco lines. Recent advancements have been made in the cultivation of isolated plant zygotes, as per the latest findings. The technology of gene transformation using microinjection in separated zygotes holds significant potential as a crucial method in plant systems. Nevertheless, the achievement of efficient microinjection into plant cells remains limited to a few numbers of systems and necessitates the expertise of highly skilled individuals **(Neuhaus and Spagenberg, 1990)**. In order to fully harness its potential, it is imperative that the process undergoes technological refinement.

Macroinjection

Macroinjection is the term for the use of a hypodermic syringe to inject inheritable substances like immature embryos, meristems, immature pollen, germinating pollen, etc. **Zhou *et al.* (1983)**. The risk of producing chimera plants with only a portion of the original plant altered is this technique's principal drawback. However, it is possible to later produce transformed plants with single cell origins from this chimera plant. Different species have been treated using this method. **Touraev el al. (1997)** Additionally, compared to biolistic, the ultimate transformation efficiency was nearly ten times lower. This strategy has been put out as a potential strategy for stable plant transformation, nevertheless.

Vacuum infiltration

Applying a vacuum for a predetermined period is another method to mediate the incorporation of *Agrobacterium* for plant transformation. Physically, a vacuum creates a negative atmospheric pressure that causes the air spaces between plant tissue cells to narrow, allowing harmful bacteria to infiltrate the intercellular spaces **Tague *et al.* (2006)**. There is less air space within the plant tissue the longer the vacuum is in place and the lower the vacuum pressure. The infiltration medium, which contains the infectious transformation vector, might migrate into the plant tissue because of an increase in pressure. It is important to monitor how long a plant part or tissue is exposed to vacuum because prolonged exposure can lead to hyperhydricity **Subramanyam *et al.* (2011)**. The first report on the transformation of *Arabidopsis* using *Agrobacterium*-mediated transformation and vacuum infiltration was published in 1993. Since then, numerous advancements have been made to create protocols and implement changes in various plants **Oliveria *et al.* (2009)**. Recent successful production of a plant-derived vaccine under the current Good Manufacturing Practice (cGMP) regulations for human clinical trials has shown the effectiveness of this method **Charity *et al.* (2002)**. One of the *Agrobacterium*-based transformation technologies, vacuum infiltration has been utilized successfully to create transgenic bean, *Arabidopsis*, coffee, cotton, and wheat plants. By enhancing *Agrobacterium* cells' ability to penetrate deeper into the layers of plant tissue, this procedure improves the efficacy of gene transfer. Through vacuum infiltration, *Medicago truncatula* (Barrel medic) can achieve up to 76% transformation efficiency **Tague *et al.* (2006)**. Vacuum infiltration-facilitated transformation has several benefits, including:
a) the ability to produce numerous independently transformed plants from a single plant,
b) reduction in somaclonal variation due to the absence of tissue culture,
c) the potential for high throughput testing due to the speed of the procedure, d) the technique may also be helpful for transforming plants that are resistant to plant tissue culture and regeneration.

Silicon carbide whisker-mediated transformation (SCMT)

It is one of the latest developed methods for the purpose of delivering DNA into plants. Fibers made of silicon carbide have unique physical and chemical properties that allow them to pierce cells without damaging them. This approach has the benefits of being quick, affordable, simple to set up, and efficient on a range of cell types. Low transformation efficiency, cell damage, and potential health risks from inhaling the fibers are some of its drawbacks **(Komatsu *et al.* 2006; Kaeppler *et al.* 1990, 1992; Wang *et al.* 1995)**. The efficiency of SCMT depends on the fiber size, parameters of vortexing, shape of the vessels used, plant species and explant and characteristics of the plant cells and especially the thickness of the cell wall **(Racoczy-Trijanowska, 2002)**. There are a number of practical applications of SCMT which have gained success including rice (Komatsu *et al.*,

2006), tobacco **Kaeppler *et al.* (1990)** maize **(Kaeppler *et al.* 1992; Wang *et al.* 1995; Petolino *et al.* 2009)**, wheat **Brisibe *et al.* (2006)**. In some cases, SCMT has been shown to be as effective as biolistic, so it represents another choice for soybean embryogenic tissue transformation **Terakawa *et al.* (2005)**. Additionally, wounding with silicon carbide fibers can increase the frequency of Agrobacterium-based transformation **(Singh and Chawla, 1999)**. Limitations of the Silicon Carbide Whisker-Mediated Transformation at the Present Time **(Frame et al. 2000; Dhakarey et al. 2019; Zhang et al. 2006):**

- **Safety Issues:** Safety is a key drawback of silicon carbide whisker-mediated transformation. Researchers and personnel involved in the procedure may be exposed to health risks due to silicon carbide whiskers, which are known to be dangerous when inhaled. Experiments may become more expensive and complex because of the strict safety precautions and protective gear that must be used.
- **Low Transformation Efficiency:** The comparatively low transformation efficiency of silicon carbide whisker-mediated transformation is one of its main drawbacks. This technique can result in a low percentage of successfully converted cells and ineffective introduction of foreign DNA into plant cells. When trying to create transgenic plants for study or commerce, this might be a considerable disadvantage.
- **Tissue Specificity:** This technique's tissue specificity is yet another drawback. Certain plant tissues and species may respond better to silicon carbide whiskers than others. This limits the number of possible uses and the variety of plant species that can be effectively modified using this technique.
- **Plant Cell Damage:** During the transformation process, plant cells may sustain mechanical damage due to the physical properties of silicon carbide whiskers. This may result in cell death or other unforeseen consequences in the changed cells, which could be harmful to the transformation's overall success.
- **Limited DNA Cargo:** The delivery of comparatively little amounts of DNA is frequently accomplished using silicon carbide whisker-mediated transformation. It might not be appropriate for complicated genetic engineering projects that frequently call for introducing huge DNA constructions or many genes into plant cells.

The Prospects and Improvements that can be made to overcome limitation in SCMT (**Hiei et al. 1994; Jones& H. D 2005):**

- **Process Optimization:** To increase its effectiveness and lessen the possibility of cell injury, scientists are still working to optimize the silicon carbide whisker-mediated transformation process. This entails studying different whisker length and shape variations and improving the transformation conditions.
- **Combination with Other approaches:** Silicon carbide whisker-mediated transformation may be improved and some of its drawbacks may be solved by combining it with other genetic engineering approaches like CRISPR-Cas9. This might make plant genome editing more accurate and effective.
- **Species-Specific Protocols:** By adapting the procedure to plant species or tissues, one may be able to get around problems with tissue specificity and increase transformation efficiency. This topic is still being researched.
- **Advances in nanotechnology:** Advances in nanotechnology could result in the creation of more precise and effective delivery methods that could increase the efficacy and accuracy of genetic transformation in plants, potentially making silicon carbide whiskers obsolete.

Ultrasound-mediated transformation

One of these potential methods is sonication, or the use of ultrasonic waves. Because sonoporation increases membrane permeability **Wyber *et al.* (1997)** and makes it easier for macromolecules to enter cells, it opens the possibility of non-invasively introducing molecules like DNA to the interior of cells for therapeutic applications. Explants are suspended in a few milliliters of sonication media in a microcentrifuge tube while using this procedure. Following the addition of plasmid DNA (and optionally carrier DNA), the samples are quickly mixed and prepared for sonication. Finally, the cells are moved to brand-new growth medium. The effectiveness of uptake in this instance was determined by sound frequency and exposure time **Liu *et al.* (2005)**. When compared to protoplast sonication, stable transformation of tobacco by sonication of leaf fragments required an ultrasound treatment that lasted 1500–2000 times longer **Zhang *et al.* (1991)**. Potato tuber disc ultrasonication is another illustration of intact tissue sonication-based transformation **(Sawahel, 1996).** The above-mentioned Sonication-Assisted *Agrobacterium*-mediated Transformation (SAAT) in plant cells or tissues is the primary goal of the ultrasonic technology **(Trick and Finer 1997; Horsch *et al.* 1985; Weber *et al.* 2003)**.

SAAT may be helpful for changing woody trees, especially Eucalyptus species **Monica *et al.* (2004)**. Germinating seeds and seedlings were treated to in-planta based transformation by SAAT, which improved the efficiency of transformation **González *et al.* (2002)**. Recently, genetic materials were introduced using a laser beam. Laser-mediated transformation works by creating brief, self-healing holes (0.5 m) in the cell wall and membrane using a focused laser microbeam. Therefore, cells might easily take up foreign DNA **Badr *et al.* (2005)**. The method must be further evaluated for both the various experimental settings and plant species since it was just recently established.

Laser microbeams

Laser-mediated transformation works by creating self-healing holes (about 0.5 m) in the cell wall using a focused laser microbeam. In less than five seconds, these holes seal once more. The buffer and DNA enter the cell through the membrane's brief opening. Laser pulses can also be used to perform membrane perforation (laser poration), which can be paired with laser-facilitated partial cell wall removal. Therefore, cells might easily take up foreign DNA. Plant cells, subcellular structures, and even individual DNA molecules can all be precisely and gently treated with laser light. For this, a suitable laser system that can function as an optical tweezer with the right microscope is required, such as nitrogen lasers, excimer pumped dye lasers, or titanium-sapphire lasers **Greulich *et al.* (2000)**. A continuous IR laser, such as a diode or diode-pumped Nd-YAG laser, makes up an optical tweezer. Cell fusion using a UV laser microbeam has been selectively induced, and DNA has been added to isolated chloroplasts. The fragility of many species' protoplasts, which are unable to regenerate into plants, is a drawback of the previously mentioned direct approaches for plant transformation. Even more challenging is the introduction of DNA into organelles like chloroplasts **Weber *et al.* (1988)**. Laser microbeams can be used to transfer genetic material into cells to get around these issues.

Even though many cells can be irradiated using this technique, it is not widely used since it requires expensive equipment to focus a laser beam on diameters of the order of 100 nm **(Lin and Ruddle 1981)**. However, after DNA insertion, the cells fully recover. It also needs to be done carefully because laser radiation can harm biological material. For this reason, it's important to channel the beam and precisely and consistently control its energy and pulse duration. The strategy must be further evaluated for various experimental setups and plant types **(Hoffmann 1996)**.

Shock wave-mediated transformation

Shock wave generators designed for extracorporeal shock wave lithotripsy (SWL), orthopedics and other fields of medicine **(Loske, 2011)**, have been used successfully for cell transfection and transformation **Jagadeesh *et al.* (2004)**. In this system microsecond pulses with a peak positive pressure within the range of 30 to 150 MPa, of 0.5 and 3 μs, followed by a tensile pulse of up to −20 MPa and duration of 2 to 20 μs has maintained. To produce underwater shock waves for biomedical applications, electrohydraulic, piezoelectric, or electromagnetic devices have been designed **(Cleveland and McAteer, 2007)**.

Chemical methods

Cells or protoplasts can be manipulated to take up DNA using chemicals such as Polyethylene glycol (PEG), also the most used chemical for DNA insertion. It aids in precipitation of DNA, which can then be engulfed by the cells through the process of endocytosis.

Calcium phosphate precipitation method for gene transformation

This method is based on the precipitation of plasmid DNA and calcium ions by their interaction. In this method, the precipitates of calcium phosphate and DNA being small and insoluble can be easily adsorbed on the surface of cell. This precipitate is engulfed by cells through endocytosis and the DNA gets integrated into the cell genome resulting in stable or permanent transfection **(Kwon and Firestein 2013)**.

Liposome mediated gene transformation or Lipofection

Liposomes are like small lipid bags full of plasmids. Induction of these liposomes can be exhibited by PEG in order to fuse with protoplasts for gene transfer. Liposomes can be preloaded with DNA by two methods- membrane fusion and endocytosis thus forming DNA- liposome complex. This complex fuses with the protoplasts to release the contents into the cell. Animal cells, plant cells, bacteria, yeast protoplasts are susceptible to lipofection method. Liposomes can be classified as either cationic liposome and anionic or pH sensitive **(Deshyes *et al.* 1985)**.

(i) Positively charged liposomes

Cationic liposomes or positively charged liposomes are associated by electrostatic interactions to the negatively charged DNA molecules forming a stable complex. Neutral liposomes are generally used as DNA carriers and helpers of cationic liposomes due to their non-toxic nature and high stability in serum.

(ii) Negatively charged liposomes

Generally, pH-sensitive, or negatively charged liposomes are not efficient for gene transfer. They do not form a complex with it due to repulsive electrostatic interactions between the phosphate backbone of DNA and negatively charged groups of the lipids. Some of the DNA molecules get entrapped within the aqueous interior of these liposomes.
In this technique, protoplasts transform *via* endocytosis of liposomes in these serial steps:

Step-1 adhesion of the liposomes to the protoplast surface,
Step-2 fusion of liposomes at the site of adhesion and
Step-3 release of plasmids inside the cell.
Successful transformation based on this system was reported for tobacco **Dekeyser *et al.* (1990)**, wheat **Zhu *et al.* (1993)** and potato **(Sawahel, 2002)**. In the recent case frequency of stable transformation was 7% of Calli regenerating from protoplasts. This method is relatively non-toxic **Antonelli and Stadler (1990)**, is simple to perform with readily available chemical reagents, is highly reproducible and efficient also requires no sophisticated equipment **(Antonelli and Stadler 1990; Felgner *et al.* 1987)**. Transformation of intact YACs into plant cells was achieved via lipofection-like particle bombardment. A lipofection-PEG combination method was more efficient than each one of them separately **Wordragen *et al.* (1997)**. It is determined lipoplex size is a major factor determining lipofection efficiency which large lipoplex particles showed, in general, higher lipofection efficiency than small particles **Almofti *et al.* (2003)**.

Polyethylene glycol (PEG)-mediated transfer

Only protoplast is suitable for this technology. Polyethylene glycol serves as a chemical. It promotes endocytosis, which results in DNA absorption. Protoplasts are maintained in polyethylene glycol (PEG) solution using this technique. PEG is removed after protoplasts have been exposed to exogenous DNA while still containing other chemicals, and the intact protoplasts are then cultivated to create cells with walls and colonies one at a time **(Jogdand 2006)**. The transformants are then obtained under selection pressure. Polyethylene glycol is the most significant molecule that can start the transfer of a gene through the protoplast membrane. Because there is a straightforward transformation technique available, it has grown to be the most used. A technique was created to immobilize DNA molecules using calcium alginate micro beads in addition to polyethylene glycol treatment **Liu et al. (2004)**.
Only cells without cell walls can drive DNA molecules into the host genome. The linearized plasmid DNA containing the foreign gene is combined with molecules of the bare plant protoplasts. In place of Ca2+ ions, the two are combined in a transformation medium that is rich in Mg2+ ions. Next, 20% polyethylene glycol (PEG) solution is added. PEG concentration is lower and Ca2+ concentration is higher following the treatment. It encourages transformation frequency.
Although the PEG mediated transformation is very generalized and straightforward, but it has these drawbacks:
1) Delicate cells cannot be used while some cells can be so swiftly pass through the procedure.
2) Because many treated cells lack transfer DNA, this procedure is not ideal.
3). Usually, foreign DNA is broken down in the cytoplasm before it enters the nucleus.

Diethyl amino ethyl (DEAE) dextran mediated transformation

This method was initially reported by **Vaheri and Pagano (1965)** for enhancing the viral infectivity of cells but later adapted as a method for plasmid DNA transfer **Lalani and Misra (2011)**. A soluble polycationic carbohydrate called diethyl aminoethyl dextran (DEAE-dextran) encourages connections between DNA and the cell's endocytosis machinery. According to this approach, the positively charged DEAE-dextran and negatively charged DNA interact electrostatically to create aggregates that eventually take the form of a polyplex. The DEAE - dextran/DNA complex is generated when there is a little excess of DEAE - dextran in the mixture. When introduced to the cells, these complexes attach to the negatively charged plasma membrane and are ingested by the process of endocytosis. Osmotic shock with DMSO or glycerol can enhance complex DNA transport with DEAE-dextran. For a particular cell line, several variables such as cell count, polymer concentration, transfected DNA concentration, and transfection time should be tuned. Stable transformants cannot be produced using this method.

Agricultural crops improvement by genetic transformation

Rice – A monocot crop

Moreover, one third of the world's population relies mostly on rice for dietary requirements. The need to boost global food production is necessary to feed the expanding global population. Even though the global food supply has more than doubled since the green revolution, but still, we need to work on increasing both the quantity and quality. The modification of young rice embryos using Biolistic proved successful **(Christou *et al.* 1991)**. Reports were also made regarding the transformation of indica and javanica rice in addition to other japonica rice **(Burkhardt *et al.* 1997; Zhang *et al.* 1996)**. Fujimoto **Patnaik and Khurana (2001)** were the first to engineer japonica rice through electroporation with modified ∂-endotoxin gene (cry) from *Bacillus thuringiensis*. Transgenic rice shows enhanced resistance to insects than its wild counterpart. Later, by particle bombardment **(Wu¨nn *et al.* 1996)** was able to create the transgenic indica rice cultivar IR58 that expresses a synthetic *cry IA(b)* gene driven by the 35S promoter. Song *et al.* successfully cloned the rice Xa21 gene, which provides resistance to the blight disease *Xanthomonas oryzae* **(Song *et al.* 1995)**. The cloned gene was used to create transgenic rice plants that exhibited high levels of resistance. The gene has been discovered to work against several isolates **(Wang *et al.* 1996)**. Shimada *et al.* created transgenic rice plants that contained a 35S promoter-controlled antisense construct of the rice waxy gene, which codes for granule-bound starch synthase **(Shimada *et al.* 1993)**. The amylose content of grain starch was significantly reduced in the seeds of these plants. Most intriguingly, rice was modified with the cDNA coding for daffodil's phytoene synthase, specialized enzymes involved in carotene (provitamin A) biosynthesis in plants, to give rice endosperm the potential to produce precursor (-carotene) of vitamin A.
The increase of phytoene synthase in the endosperm of these transgenic plants suggests that the provitamin A biosynthesis pathway can be engineered in non-photosynthetic, carotenoid-deficient tissue. *Agrobacterium*-mediated transformation of rice with all the genes required for provitamin A accumulation in transgenic rice seeds was recently described by the same group.
By electroporating protoplasts, Hayakawa *et al.* inserted the coat protein (Cp) gene of the rice stripe virus into two kinds of japonica rice, leading to notable levels of virus resistance in the transgenic plants **(Hayakawa *et al.* 1992)**. By these studies it has comprehended that particle bombardment of rice offers a superior alternative to *Agrobacterium* for transgenic development also it can be expected that bacteria or virus-based rice transformation methods will become obsolete in future due to its associated drawbacks **Bajaj and Mohanty (2005)**.

Maize

The world's most important grain crop is maize. It is also the crop for which extensive use of genetic engineering has been made to enhance its numerous features. Particle bombardment, protoplast transformation, *Agrobacterium*-mediated, in planta transformation, and other techniques for gene transfer have all been explored and refined throughout time. The very first successful transformation in maize was performed by uptake of naked DNA through electrochemical method in Black Mexican Sweet maize protoplast **(Fromm *et al.* 1986)**. Firstly, a robust biolistic transformation protocol was developed by **Songstad *et al.* (1996)** in Hi-II genotype. Since then, various studies have reported successful use of biolistic mode of transformation in maize **(Klein *et al.* 1989; Gordon-Kamm *et al.* 1990; Genovesi *et al.* 1992; Frame *et al.* 1994; Wan *et al.* 1995; Pareddy *et al.* 1997)**. A silicon carbide fiber-mediated DNA delivery device has been shown to alter maize **Bullock *et al.* (2001)**. Initially, it was supposed that *Agrobacterium* mediated technique could not work for monocot plants. The major breakthrough in maize transformation came from **Ishida *et al.* (1996)**, modifies the traditional protocol such as heat pre-treatment, addition of copper and silver ions to co-cultivation media, increase in co-cultivation period etc. Now these have been used for developing commercial transgenic events in maize. Although, maximum commercial transgenic events are developed by particle bombardment, followed by *Agrobacterium*-mediated transformation also including with genome editing methods **Kang M *et al.* (2022)**.

Wheat

Without a doubt, it is a staple crop of the human diet and one of the world's primary food crops. Over the years, there has been a lot of focus on improving the genetics of wheat to increase grain output, reduce crop loss due to unfavorable weather circumstances, and create resistance to numerous pests and viruses. More recently several studies have reported efficient *Agrobacterium* transformation of many wheat cultivars **(Ishida *et al.* 2015; Richardson *et al.* 2014; Wang *et al.* 2019; Ye X *et al.* 2023)**. The cultivar Fielder, a model variety in *Agrobacterium* transformation, declared by the group of researchers from Japan Tobacco Company, where the detailed protocol "Pure Wheat" was developed with transformation efficiency of 40–90%. *In vitro* methods such as in planta approach have shown its potential in overcoming the problem of genotype dependency and other associated hurdles of bacteria-based transformation system. In contrast to vector-based protocols of gene transfer, microprojectile bombardment mode to deliver DNA is also to be mentioned as the field of wheat transformation. It was subsequently transformed by the invention of a technology for delivering genes into intact plant tissues by bombarding them with DNA-coated gold or tungsten particles. Sincere attempts have been made in recent years to genetically alter wheat using various foreign genes that are significant from an agronomic viewpoint **(Altpeter *et al.* 1999; Bieri *et al.* 2000)**. Since the first successful genetic transformation of wheat by Monsanto using biolistic protocol was conducted at Florida University, USA **(Vasil, 1992)**, several reports of transgenic

event in wheat has been reported **(Bieri *et al.* 2003.; Wiley *et al.* 2007; Fahmy *et al.* 2013)** .

Tobacco

In transgenic research, tobacco has emerged as a model plant system because its molecular genetics are well understood, its genomic mapping is almost complete, genetic transformation is simple to accomplish, tobacco plants thrive *in vitro* and in greenhouse environments, and tobacco yields a lot of biomasses. Proteins and enzymes can be extracted, processed, and employed in the production of pharmaceuticals and other valuable industrial compounds, such as biopolymers, by using tobacco plants as living factories to create the necessary quantities of these substances **Jube and Borthakur (2007)**. Reports on the incorporation of genes introduced in tobacco plant are plenty via liposomes mediated **Deshayes *et al.* (1985)**, Silicon carbide fiber-mediated DNA delivery systems **(Kaeppler *et al.* 1992)** *Agrobacterium* mediated **(Niedbala *et al.* 2021; Leng *et al.* 2020)**.

Potential applications of GMOs

Genetic engineering in agriculture indeed offers a wide range of benefits that have the potential to transform our food production systems and address critical global challenges. Genetic engineering allows for the development of crop varieties that are tailored to specific environmental conditions, such as high salinity or drought. This means that crops can be grown in regions where they were previously unsuitable, expanding agricultural possibilities and increasing food production. GMOs can also aid in preserving biodiversity, reduce soil and water pollution, and promote ecosystem health by reducing the usage of chemical pesticides and herbicides.

Genetic engineering can be used to enhance the nutritional content of crops, addressing malnutrition and dietary deficiencies. It is also being applied to the production of biofuels and bioplastics, providing renewable and environmentally friendly alternatives to fossil fuels and traditional plastics. In summary, genetic engineering in agriculture offers a multifaceted approach to addressing global challenges related to food security, environmental sustainability, and human health. While it presents numerous opportunities, it is essential to carefully consider ethical, social, and regulatory aspects to ensure responsible and sustainable deployment of these technologies. The ongoing research and development in this field hold great promise for meeting the needs of a growing global population and mitigating the impact of environmental stressors on our food supply.

For analysing multiple independent transgenic lines to ensure the stability and reproducibility of the introduced traits is detrimental as several genetic factors that influence the expression of newly inserted genes in transgenic plants. These are epigenetic effects, gene silencing, including transgene silencing, and elucidates the underlying genetic signals associated with these phenomena. Based on existing knowledge, many approaches can be employed to establish stable transgenic lines. While preliminary laboratory and field experiments conducted over a few generations provide valuable insights into the long-term stability of individual transgenic lines, the introduction of many genetic events through crossbreeding gives rise to novel inquiries. Due to the presence of identical or comparable promoter elements in many transgenic lines and the potential for sequence homologies in coding areas, novel epigenetic interactions can emerge. Consequently, it is essential to conduct comprehensive expression testing of transgenic events resulting from genetic transformation.

Table 3 Comparison of transformation efficiency by direct methods and indirect (Biological method) of major crop plants.

Plant Name	*Agrobacterium*-mediated (Indirect Method)	Electroporation	PEG –Mediated	Biolistic: particle bombardment	Silicon carbide whisker-mediated
Rice	46% Saho *et al.* (2011)	26% Yang *et al.* (1988)	8.5% Yang *et al.* (1988)	50% Dai *et al.* (2001)	< 10% Matsushita *et al.* (1999)
Tobacco	75% (Silva *et al.* 2018)	2.2×10^{-4} Riggs *et al.* (1986)	10^{-6} (Díaz and koop, 2021)	4.8×10^{-2} Klein *et al.* (1988)	50% Kaeppler *et al.* (1992)
Cotton	85% Firoozabady *et al.* (1987)	6.25% Rao *et al.* (2016)	1% QANDEEL-E-ARSH *et al.* (2021)	3% Lee *et al.* (2013)	94% Asad S *et al.* (2008)
Maize	45% Que *et al.* (2014)	9% Lyznik *et al.* (1989)	85% Lyznik *et al.* (1989)	60% Kaeppler *et al.* (1992)	4×10^{-6} Kaeppler *et al.* (1992)
Soyabean	10% Li *et al.* (2017)	50% Christou, Swain *et al.* (1990)	50% Wu F and Hanzawa (2018)	12% Khalafalla *et al.* (2006)	26% Khalafalla *et al.* (2006)
Wheat	25% Hayta *et al.* (2019)	1×10^{-5} Liang *et al.* (2005)	2.25×10^{-6} Ajmone *et al.* (1993)	1-5% Ismagul *et al.*, (2018)	61.5% Serik *et al.* (1996)

CONCLUSION

The process of genetically transforming crops has introduced a novel avenue for enhancing productivity, hence yielding advantages for both agricultural producers and consumers. Since their inception, plant transformation methods have been employed for the purpose of genetic modification of agriculturally important plant species. The optimal utilisation of its impact can be observed in underdeveloped or emerging nations, where the productivity of crops is significantly hindered by both biotic and abiotic stressors. The growing reliance on biotechnological research calls for the advancement of novel approaches to manipulate and integrate genetic sequences into plants, aiming to improve their characteristics in alignment with societal goals, while ensuring simplicity, reliability, and reproducibility. Conclusively the efficacy of physical or other genetic transformation methods is relatively limited in comparison to the *Agrobacterium* mediated method, which has been the predominant approach utilized thus far. However, considering the aforementioned limitations, direct physical methods offer a compelling alternative for overcoming certain barriers. The most popular direct transformation technique, both commercially and experimentally, is by far biolistic. To make the most of the techniques ultimately improved for penetration of the cellular wall and integration of the transgene, it is crucial to understand the physics underlying several of these strategies for a correct application. There is still much study to be done in order to fully utilize the approaches that have already been successfully developed for a small number of plant species and to improve the effectiveness and reproducibility of genetic alterations. More stringent protocols will be easier to create with a greater understanding of the physics involved, and new approaches to genetic plant transformation may become possible.

In contrast to the prevailing resistance towards genetically modified crops in Eastern Europe, numerous countries in Asia and North America have embraced the cultivation and adoption of transgenic crops. The main obstacle resides in comprehensively grasping the intricate workings of the fundamental process underpinning gene expression, and there exists an urgent imperative to investigate gene expression, particularly its regulatory mechanisms.

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