Vectors used in Plant Transgenic Studies

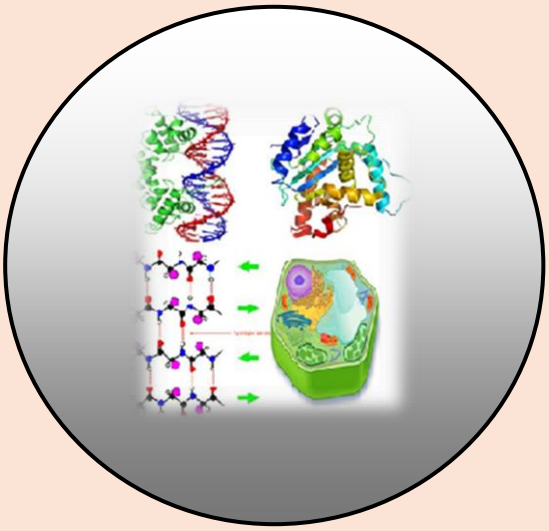
By

Hamid Kheyrodin

ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 39 (2), 2022 Pages No. 172-176



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 39, No. 2, 172-176, 2022
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 39/02/105/2022

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

Dr. Hamid Kheyrodin
http:// www.sasjournals.com
http:// www.jbcr.co.in
jbiolchemres@gmail.com

REVIEW ARTCILE

Received: 09/11/2022 Revised: 28/12/2022 Accepted: 30/12/2022

Vectors used in Plant Transgenic Studies

Hamid Kheyrodin
Faculty of Desert Science, Semnan University, Semnan, Iran

ABSTRACT

Since 1983 Genetic engineering has been applied to various types of plants to create desired characteristics. Particularly, these techniques are used to enhance specific traits of tomato, corn, soybean, banana, and tobacco, among other species of plants and crops. Since these methods involve altering the genetic data of plant species, the agriculture sector is one of the most important agricultural areas.

Since 1982, this technology has been utilized to alter the properties of various varieties of plants, from barley and corn to tomato and onion.

In this case, gene transfer is employed for many purposes that range from improving yields (crops and animal feeds, etc.) and also to increasing crop and plant tolerance to various environmental and biological aspects.

This implies that the genes of another species is introduced and then merged in the genome of the plants that alters the features of the genome originally.

Although there are many instances of plants that have been transgenic there are three major methods that are used to introduce genes into the plant cell.

Keywords: Transgenic plant, DNA and Genome.

INTRODUCTION

The term “transgenic plants” refers to plants whose DNA has been altered by genetic engineering. This implies that the genes of another species is introduced and then merged in the genome of the plants that alters the features of the genome originally. Although horizontal gene transfer is observed natural in the environment (between plants that are growing close to one another). Different artificial methods are used to add gene sequences into plants with the intention of increasing yields and making them more resilient of various environmental conditions as well as making them resilient to certain biotic stresses or other environmental stresses. This is why transgenic plants are extremely useful in agriculture as well in other industries (e.g. within the industry of pharmaceuticals) (Altpeter et al., 2016).

The first transgenic plant was created in the year 1982. It was a plant of tobacco that was resistant to antibiotics. The genetic material that is inserted into the gene of the plant could be taken by a completely different species, or from an entirely different species.

1. Vector-mediated gene transfer (also known as indirect gene transfer or vector-mediated transformation)

The name implies this method uses vectors to carry certain genes in the cells of the target to allow them to be replicated and expressed. This permits the plant to display the characteristics desired.

Although some viruses are utilized to accomplish this Two members of the family Agrobacterium are typically chosen for this process. This is because they have proved to be effective in a broad range of plant species. Particularly, this method is utilized in many monocots, including maize wheat and barley to name a few (Anjanappa and Gruissem, 2021).

GMO	Description	Picture
Golden Rice	Rice modified with daffodil genes to have more beta-carotene, which the body converts to Vitamin A	
Flavr Savr Tomatoes	Tomatoes modified by the removal of genes responsible for the softening of fruit, meaning the tomatoes spoil more slowly	
Bt Corn	Corn modified with a bacterial insecticide gene so that it produces insect toxins within its cells, protecting it from pest species	
Aqua Advantage Salmon	Salmon modified with growth hormone regulating genes in order to grow to market sizes in significantly less time	
Glow in the Dark Animals	Animals modified with genes for fluorescent proteins will glow in the dark – this novel feature serves no practical purpose	

Figure 1. Examples of Transfer Plant.

In normal conditions in normal circumstances, the bacteria Agrobacterium tumefaciens (which naturally occurs in soil) is able to sense a cut on the plant's surface and transfer certain genes of the cell of the plant, causing the disease known as crown gall.

The same mechanism is responsible for the bacteria Agrobacterium is the culprit behind hairy root diseases in a variety of plants. If the plant is injured by a bacterium, it releases specific molecules which are recognized by bacteria via a protein known in the form of VirA (located within the surface of bacterial cells).

After they have been identified that the protein is activated, it activates another protein called VirG within the cell, which triggers gene Vir in the bacteria genome.

The activation triggers genes to create endsonucleases (VirD1 as well as VirD2) which break off at 25 base pair on the plasmid, releasing only one T-DNA strand. When it is connected to the endonuclease, VirD2 the strand is then transported to the plant cell, which is then embedded into DNA of plants.

While the expression of genes in the strand leads to the creation of various products, the most significant ones are the hormones cytokinin and auxin that cause plants cells to multiply rapidly (Boch et al., 2014).

This is why an increase in the size of the gall on the crown occurs. Alongside both hormones the expression of these genes results to the creation of opines that are the carbon and nitrogen needed by bacteria to survive.

The following diagram is a representation of gene transfer via vectors:

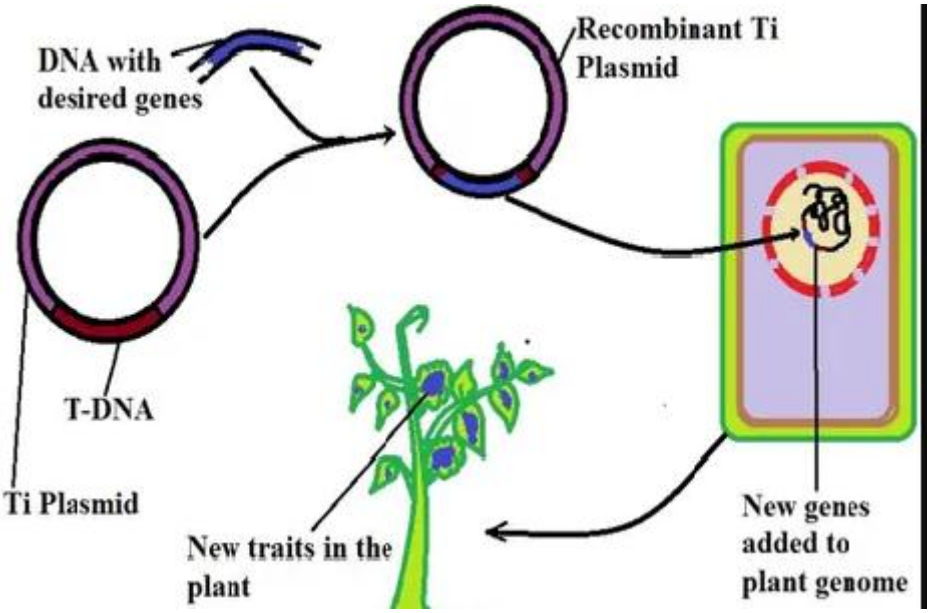


Figure 2. Diagram is a representation of gene transfer via vectors.

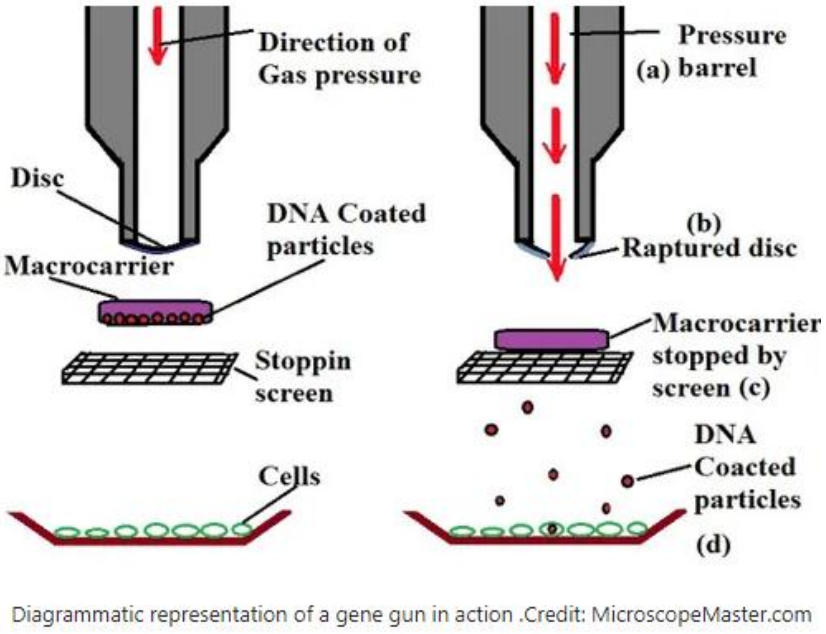
2. Particle-mediated transformation (particle bombardment or gene gun method)

In contrast to vector-mediated transformation, where it is initially placed into a plasmid prior to it is introduced to the cell of the plant particle-mediated transformation is the process of directly inserting genetic material that contains genes of interest into the cells to allow it to become part of the genome in the plant.

To accomplish this there are a variety of metal particles are able to be used to bombard the gene gun. This includes gold, rhodium and tungsten to name a few. In this method genes (DNA) is then transferred to the metal micro-particles (micro-particles could vary between 0.45 and 1.5um in size) (Chen et al., 2019).

In this instance the metal particles function as micro-carriers since they carry genetic material that is to get into the cell of plant. These carriers are then encased in plastic tubes. The tube is cut into small tubes (which serve as cartridges) approximately 1/2 inch in length by using an instrument for cutting tubes. If the tubes cannot be utilized immediately, they are kept at temperatures of 4 degrees C.

It is crucial to make sure that the cartridges are stored in a tightly sealed vial with an ingredient that keeps the vial from drying. When they are ready to use the cartridges are placed into a cartridge holder that can accommodate around 12 cartridges. The holder for the cartridge is put in the gun and then secured to the gun. Once the trigger button has been activated, an helium gas impulse is used to force the micro-carriers in a way they can be able to attack cells in the plant. This helium gas pressure provides enough force that allows the micro-particles/micro-carriers to punch holes through the cell wall and introduce the genetic material into the cells. In cells there are particles that could be found within the cytoplasm while others are located within the nucleus.



Diagrammatic representation of a gene gun in action .Credit: MicroscopeMaster.com

Figure 3. Diagrammatic of a gene in action credit.

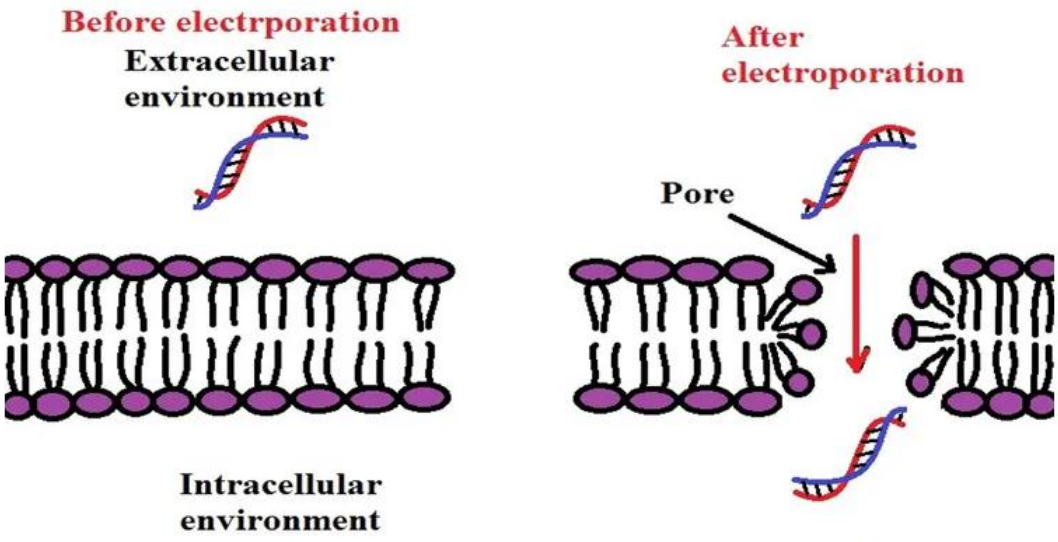


Diagram showing the impact of electroporation on the cell membrane. Credit: MicroscopeMaster.com

Figure 4. Diagram of electroporation in a cell.

However the location, the genetic material is separated from the carriers, allowing it to interact in the genome of the plant cells to create desired traits (Chen et al., 2018).
Gene guns allow you to introduce new genetic material containing desired genes into cells of plants by with force, this method is applicable to a variety of kinds of plants, including monocots as well as dicots.

3. Direct DNA absorption (gene transfer through electroporation)
As with particle-mediated transformations, direct DNA absorption via electroporation can also be a method for the transfer of genes. It is due to directly inserting genes into the cell of the plant without the need for the aid of a mediator (vector). In this case, the main goal is to make pores on the surface of the cell (also called electropores) by which genetic material can be introduced into the cell. To perform this procedure plants being used for this procedure are placed in a buffer that has some foreign DNA. This is also known as a DNA bath. It helps prepare the cell for the electroporation. The temperature range and duration of incubation are determined by the type of plant cell and also the kind of DNA that is introduced into the cells. After incubation after which the buffer is exposed to electrical impulses which create temporary pores in the cell membrane of the plant cell. Since the cells are immersed in a buffer which contains an outside DNA source that DNA will then be able to easily get into the cell through the pores, before closing.

ACKNOWLEDGEMENT

I would like to acknowledge funding from the Iran Government Research Program. Dr. M. Nassiri, Director, Semnan University was supported by grants from the Fellowship for this work.

REFERENCES

Altpeter, F., Springer, N. M., Bartley, L. E., Blechl, A. E., Brutnell, T. P., Citovsky, V., et al. (2016). Advancing Crop Transformation in the Era of Genome Editing. *Plant Cell* 28, 1510–1520. doi:10.1105/tpc.16.00196

Anjanappa, R. B., and Gruissem, W. (2021). Current Progress and Challenges in Crop Genetic Transformation. *J. Plant Physiol.* 261, 153411. doi:10.1016/j.jplph.2021.153411

Boch, J., Bonas, U., and Lahaye, T. (2014). TAL Effectors - Pathogen Strategies and Plant Resistance Engineering. *New Phytol.* 204, 823–832. doi:10.1111/nph.13015

Chen, K., Wang, Y., Zhang, R., Zhang, H., and Gao, C. (2019). CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture. *Annu. Rev. Plant Biol.* 70, 667–697. doi:10.1146/annurev-arplant-050718-100049

Chen, L., Li, W., Katin-Grazzini, L., Ding, J., Gu, X., Li, Y., et al. (2018). A Method for the Production and Expedient Screening of CRISPR/Cas9-mediated Non-transgenic Mutant Plants. *Hortic. Res.* 5, 13. doi:10.1038/s41438-018-0023-4

Corresponding author: Dr. Hamid Kheyrodin, Assistant Professor, Faculty of Desert Science, Semnan University, Semnan, Iran.
Mail: hamid.kheyrodin@semnan.ac.ir