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Chloroplast transformation in plants

During the process of evolution, chloroplasts and mitochondria have shown endosymbiotic origin and evolution from the prokaryotes. Both these organelles have their own circular genome, simpler than nuclear genome. It has been found that most of the chloroplastic and mitochondrial proteins are encoded by nuclear genome and it inherited maternally.

Chloroplast Genome (Ct-genome): cells of the higher plants carrying approximately 100 chloroplasts per leaf cell. Each cell of chloroplast having minimum 98-100 copies of chloroplastic-genome or plastome. This chloroplastic genome is a circular double-stranded DNA molecule, located in the stroma. Majority of chloroplast genomes are in the size of 120-160 kbp and contain about 120-140 genes. About 100 chloroplast genes are known to code for proteins. It inherited independently of the nuclear genes. Ct genome is comparatively larger than mitochondrial genome. It is near about 140kb and 200kb in higher plants and lower eukaryotes respectively. Such variation in length is due to the presence of inverted repeats (IR).

Chloroplast Engineering: Genetic engineering of chloroplast that leads to chloroplast (plastid) transformation is an important and exciting field in modern biotechnology as it offers the following advantages:

1. Chloroplasts are maternally inherited; hence there is no danger of gene transfer through pollen to related weeds.
2. Multi-gene transfer can be conveniently carried out in chloroplasts which is rather difficult with nuclear genome.
3. Chloroplasts genome is functionally comparable to prokaryotic genome. A single promoter can control the expression of group of genes (transgenes). It is therefore possible to introduce desirable multiple genes which can be expressed under the control of a single promoter.
4. High level of transgene expression is possible with chloroplasts. There are about 100 chloroplasts per cell, each containing about 100 copies of genome. Thus, there is possibility of 10,000 copies of transgenes per cell! This is a tremendous number of transgenes carried by transformed chloroplasts,
showing very high level expression of genes and also related with the large scale production of active proteins.

5. Chloroplast transformation is not associated with gene silencing which is a major problem with nuclear genome transformation.

6. Antibiotic resistance genes need not be used as selectable markers. Even if used, they can be easily excised.

7. Toxicity associated with foreign protein production in chloroplasts is much less when compared to nuclear-controlled foreign proteins.

**Design of Vectors for Chloroplast Transformation:** A diagrammatic representation of two vector constructs for chloroplast transformation is depicted in Fig. 1

1. **A construct for expression of a single gene:** The vector for chloroplast transformation is based on the selectable marker gene *aadA* that provides resistance to antibiotic spectinomycin. The single foreign (desirable) gene is fused to regulatory sequences (promoter and terminator) which in turn are flanked on either side by chloroplast DNA (Cp DNA) (Fig. 1A).

2. **A construct for expression of multiple genes:** In this case, the selectable marker is the betaine-aldehyde dehydrogenase (badh) gene. It is flanked by a promoter and the multiple transgenes are flanked by a terminator. At both ends chloroplast DNA sequences are present. In between the transgenes, these are ribosome-binding sites (one between two transgenes) to ensure efficient translation (Fig. 1B)

![Diagram](image)

*Vector designing for chloroplastic transformation: (A) vector having insertion of single foreign gene; (B) vector carry multiple foreign genes*

**Vector designing for chloroplast transformation:**
Initially pointed mutational chloroplastic transformation vector had been designed, carried 16SrRNA (rrn16), which show resistance to streptomycin and spectinomycin. Now these days *aadA* gene carried vector has been designed which is 100 time more efficient than the rrn16-marker gene carry vector.
aadA gene have a tremendous capacity to encodes aminoglycoside 30-adenylyltransferase enzyme, which inactivate the activity of spectinomycin and streptomycin via the process of adenylation.

Introduction of Foreign Genes into Chloroplast Genome:
Most of the methods used for introducing the foreign genes into nuclear genome are not useful for chloroplast transformation. The most successful method for inserting foreign genes into chloroplasts is particle gun bombardment.

Transformation methods in chloroplast: following are methods use for the chloroplastic transformation:

(a) Particle gun method; (b) Polyethylene glycol method or PEG method; (c) microinjection method

(a) **Particle gun method:** Most effective and widely use method of chloroplast transformation. Through this method transformation rate efficiency is very high but researcher get success only in very limited number of plant species. In 1989, first experiment of chloroplast transformation done on tobacco plant (development of spectinomycin resistance tobacco plant via chloroplastic transformation). In this method, DNA taken by the protoplasts in the presence of PEG, which transported into the chloroplast genome, where it integrated.

(b) **Polyethylene glycol method or PEG method:** in this method first of all cell wall removed with the enzymatic treatment and isolate the fresh protoplast. Such protoplast treated with PEG, which increase the permeability of plasma membrane and it further helps in uptake of DNA, which later
on integrated in the chloroplastic genome. The plant so derive using this method called as transplastomic plants.

(c) **Microinjection method:** in this method, DNA with the plastid-vector coated with gold/ tungsten. Such coated micro-particles (0.6- 1 μM in diameter), will transfer into the chloroplastic genome, under the influence of high velocity.

![Basics of Chloroplast Transformation](image)

**Genetic engineering of chloroplastic transformation:** for the preparation of chloroplastic-recombinat DNA have been constructed via the following steps:

→ Coating of chloroplastic-recombinat DNA plasmid molecule with gold/ tungsten particles and then injected into the chloroplast via particle gun method. Such plasmid carry two flanking sequences that ensure that plasmid is not inserted randomly in the chloroplastic genome. Along with this it also carries some important genes like: therapeutic genes, antibiotic resistance gene, gene related expression.

→ After the successful transformation, leaf will grow on a medium containing antibiotic, which ensures that only transform cell having special gene of interest will survive. Latter on survive plant cell grow in regenerative medium, which helps in sprouting of root and shoot, and it finally develop in full transgenic plant carrying desirable gene.

**Advantages of chloroplasts transformation**

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**Biotechnology**

Plant biotechnology and crop improvement

Chloroplast Transformation II
Chloroplastic transformation shows better expression as compare to the bacterial genes in the nuclear genome. Introduction of some selected transgenes in the recombinant plamid, could improve photosynthesis and it improve the crop yields; also shows resistance to herbicides/ insects, tolerance to drought and salt. Through this type of transformation (i.e through plastid genome) agronomically traits of crops express in best way. Further there is no risk of the transgenes transmission through pollen to non transgenic plants either in the same species, or to the related species.

Using the chloroplast biotechnology scientist has develop low cost therapeutic proteins. Problem of gene silencing both at transcription and translation level has been overcome by using this method. Through the chloroplastic transgenic, growth factors (IGF 1), human serum albumin (HSA), human interferon (IFN2b) etc, having good therputic value in diabetes and other diseases, has been synthesis at commercial level. Transgenic chloroplasts increase their expression rate 500 times if compare with the nuclear expression.

**Drawback of chloroplastic engineering:** Results limited to green plants only; Quite expensive technology etc.
The Future of Chloroplast Transformation: The technology of chloroplast transformation is in the developing stages. In fact, it has not become as routine as transformation of nuclear genomes of plants. Chloroplast engineering, however, holds a great promise in plant biotechnology being an efficient, clean and environmental-friendly approach for the production of transgenic plants.

Conclusion

Chloroplast genome engineering having numerous advantages over the nuclear genome engineering but till date success of chloroplastic transformation has been achieved in very limited number of plants (e.g. Arabidopsis, egg plant, lettuce, cotton, tomato, potato, cabbage, soyabean, sugarcane, sugar beet, cauliflower, carrot etc.) Beyond this such engineering become the target of many plant genetic transformation efforts, due to its enormous advantages over nuclear genome of the plant. Through the chloroplast transformation it has been clear that this technology gives best results of high level of trans-gene expression’ a point where nuclear genome engineering approach is incapable and fails to develop expression of transgene at the higher level.
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Chloroplast Transformation II