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Chloroplast engineering: boon for third-world countries as therapeutic proteins

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Abstract: Chloroplasts are the site of photosynthesis in plants mostly seen in leaves and some eukaryotic algae that provides the primary sources of the world's food productivity. Plastids of higher plants are generally semiautonomous with a ~120–150 kb genome. Chloroplast transformation has become an attractive alternative to nuclear gene transformation due to its advantages, high protein levels, the feasibility of expressing multiple proteins from polycistronic mRNAs, and gene containment through the lack of pollen transmission. The review presents the recent trends and methods for plastid genome engineering and transgene expression and summarizes the potential of plastid transformation in various fields of biotechnology and also as a source of therapeutic proteins.

Keywords: *Chloroplast, Transformation, Therapeutic, Polyethylene glycol mediated method (PEG), Transplastomic genome*

I. Introduction

Chloroplast is an important primary cell organelle also known as plastids, mostly found in plants and in eukaryotic algae. Chloroplast helps to perform the primary synthetic process of photosynthesis. Other important activities that occur in plastids include evolution of oxygen, sequestration of carbon, production of starch, synthesis of amino acids, fatty acids, pigments, and is also a key aspects of sulphur and nitrogen metabolism[1,2].

The concept of chloroplast genetic engineering was developed in the 1980s by the work of Daniell and Mc Fadden (1987) [3], who show for the first time the uptake of genes by plant chloroplast. The plastid genome of higher plants is an attractive target for engineering because it provides readily obtainable high protein levels [4]. Chloroplast transformation generally results from homologous recombination, with a fragment of transforming DNA replacing the corresponding chloroplast DNA. Boynton et al., (1988) [5] reported successfully chloroplast transformation in *Chlamydomonas reinhardtii* through gene gun method. This method gain higher popularity due to simple operation and higher efficiency. Other method used is the polyethylene glycol (PEG) mediated transformation [6]. In 1989, stable chloroplast transformation in higher plants was achieved in Pal Maliga's Laboratory by the biolistic process, with which the *Escherichia coli* plasmids containing a marker gene and the gene of interest were introduced into chloroplasts or plastids. The foreign genes were inserted into plasmid DNA by homologous recombination *via* the flanking sequences at the insertion site [7]. This method of chloroplast recombination has proven to be more efficient than that

of nuclear gene transformation due to great potential, high protein levels, the feasibility of expressing multiple proteins from polycistronicm RNAs, gene containment through the lack of pollen transmission and pleiotropic effects due to sub-cellular compartmentalization of transgene products [4, 8, 9, 10]. Positive gene transformation in chloroplast has been carried out in plants like tobacco (*Nicotiana rustica*) and (*Nicotiana tabacum*) [11,7], Arabidopsis (*Arabidopsis thaliana*) [12], rice (*Oryza sativum*) [13] potato (*Solanum tuberosum*) [14], carrot (*Daucus carota*), and tomato(*Solanum lycopersicum*) [15, 16]. However, plastid transformation is mainly restricted to tobacco as its efficiency is much higher than in other plants [16].

Recently efficient plastid transformation using non-green tissues has been accomplished in carrot; in which the chloroplast transgenic lines were generated via somatic embryogenesis from tissues containing pro-plastids [17]. Keeping the importance of this technique an attempt is being made to highlight the recent trends in chloroplast engineering and its potential application as therapeutic proteins.

II. Transgene expression at chloroplast level

Chloroplast transformation was generally achieved by the biolistic process (bombardment) or the Polyethylene glycol mediated method (PEG). However, the bombardment method has become a favourable means for chloroplast or plastid transformation due to its higher transformation efficiency and simple operation [18] .Transformation is accomplished by integration of the transgene into a few genome copies, followed by 25 to 30 cell divisions under selection pressure to eliminate untransformed plastids, thereby achieving a homogeneous population of plastid genomes [2,19].

Chloroplast genes are basically transcribed by two RNA polymerases that recognize two different promoter regions, T7 like nuclear encoded polymerase and bacterium like plastid encoded polymerase. Both these polymerase promoters regions are found on plastid genes and encode for rRNA and mRNA. Transcription of transgenes inserted into the plastid genome is driven by plastid promoters usually the 16S rRNA promoter (P*rrn*16) or the *psbA* promoter [20, 21]. To date, the most commonly used site of integration is the transcriptionally active intergenic region between the *trn*I-*trn*A genes, within the *rrn* operon, located in the IR regions of the chloroplast genome. The foreign gene expression levels obtained from this site are among the highest ever reported [22]. It appears that this preferred site is unique and allows highly efficient transgene integration and expression. Many synthetic and hybrid promoter system have been developed that use GFP protein expression system or a specific sigma factor that bind to polymerase only at specific tissue. Still these systems are not completely adopted as it has been reported that these gene expression system affects nuclear genes as well and may cause impairment of plant growth [23].

The incorporation of suitable 5'-untranslated region (UTRs) of mRNA into chloroplast transgene along with stable 5' and 3' can also affect the protein expression level. Therefore, engineered UTR can be exploited more to enhance the expression of these transgenes in many of the higher plants to achieved desired products [24].

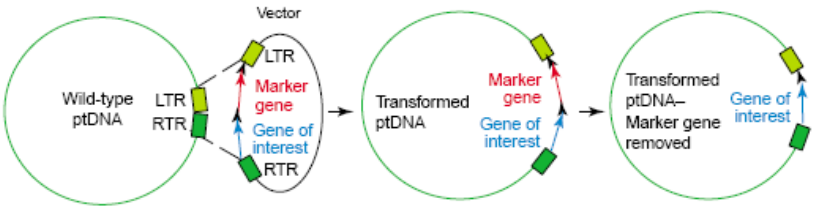

Fig1: Mechanism of transgene expression at chloroplast level (photo source: anonymous)

III. Application of Chloroplast engineering

Plastid transformation has been utilized in basic science, biotechnology and agronomy, pharmaceuticals and various other sectors of medicines and industrial production [25]. Some of the selected areas where chloroplast/ plasmid engineering has been applied are listed below.

III.1. Improvement of plant character

To improve plant traits and to produce plant capability to adapt stressful environment like biotic and abiotic stress factors, many genes have been engineered through chloroplast engineering. Characters like herbicide resistance, pest resistance and tolerance to drought and salinity stress has been improved using this technology. Transgenic chloroplasts have been reported to offer resistance to the fungal pathogen *Colletotrichum destructive* in tobacco [26]. *Cry* genes has also been expressed extremely well in the plastid genome and leaves of such plant prove to be toxic to insects feeding on such plant leaves [27]. Insect-resistant trans-plastomic soybean plants offers optimism for the transfer of the technology to important (food) crops [28] and thus is helpful for development of new stocks of resistance plants. New applications in these areas include the development of a plastid resistance gene against D-amino acids that potentially could be used as herbicides [29] and the successful expression of enzymes of the antioxidant system to provide increased tolerance to abiotic stresses [30] .Trans-plastomic carrot plants expressing BADH could be grown in the presence of high concentrations of NaCl (up to 400 m mol/L) [17].

III.2. Production of biopharmaceuticals

Chloroplast engineering is also suitable for high-level expression and economical production of therapeutic proteins in an environmentally friendly manner. Protein-based polymers derived from chloroplast transformation are affective with medical uses such as wound coverings, artificial pericardia, and programmed drug delivery [31]. This field is still at nascent stage but it emerges as an alternative for the production of medicines that is more chemical in nature. The production of therapeutic protein, human serum albumin (HSA) from transgenic chloroplasts of tobacco plant [32], high-level production of antigens for use as vaccines and their tests for immunological efficacy in animal studies, has been a revolutionary development in the pharmaceutical sector. Cholera toxin B sub-unit (CTB) of *Vibrio cholera* and virus type 1 (HIV-1) p24 antigen [33] against HIV virus has promising application in medicines development. Chloroplast-produced human IFN-g offered complete protection to human lung carcinomas against infection by the EMC virus. Chloroplast transformation can also serves as an effective expression system that can provide a clean, safe, and efficacious vaccine system.

III.3. In metabolic pathway engineering

Chloroplast represents the central organelle of a plant cell and many metabolic pathways involve the expression of genes present at chloroplast. An engineered chloroplast organelle can throw light on the way genes and their products express in plants. Particularly, chloroplast been the central house for photosynthesis and one of the key process in plant growth development that can be studied as metabolic pathway engineering [34].Further studies are carried out in different fruiting plants to increase the production of vitamins and minerals through plastid engineering. There is also growing interest in using trans-plastomic plants as factories for the production of so-called ‘green chemicals’: raw materials and building blocks for the chemical industry [35].

Many studies are carried out in the field of photosynthesis to understand the role of Rubisco protein through altered expression using chloroplast transformation. It is used for modifying the efficiency of Rubisco in favor of increasing catalytic activity or reducing the mechanism of photorespiration [36, 37].

III.4. In food

Developing protocols for important crops continues to pose a formidable challenge in plastid biotechnology and significant strides forward are likely to require conscientious efforts and long-term investments in both the academic and the industrial sectors. There is an urgent need to develop the concept of chloroplast transformation in economically important crop species such as carrot, cotton, rice, and soybean. Transformation of the plastid genome in commercial crops was achieved through somatic embryogenesis by bombarding embryogenic non-green cells or tissues [38].

Conversion of cellulosic biomass into fermentable sugars can be expressed from the plastid genome to very high levels. These include various cellulases, xylanases, glucosidases, pectatelyases and cutinases enzymes [38, 39].Stable integration of the ubiC gene into the tobacco chloroplast resulted in hyper-expression of the enzyme and accumulation of this polymer up to 25% of dry weight [38].

IV. **Conclusion**

The chloroplast engineering provide a good platform of foreign gene expression and holds great potential for the introduction of agronomic traits as well as the production of therapeutic proteins or vaccines in plants indigenous to developing countries such as India where people do not have access to these medicinal compounds. Also introducing the C_4 photosynthetic pathway and its proteins into the C_3 plant for better utilization of photosynthesis can be achieved by chloroplast engineering. Some of the major obstacles to extend this technology to major crop species include inadequate tissue culture and regeneration protocols, selectable markers and inability to express transgenes in developing plastids.

However, with recent advances in plastid engineering it has become a powerful biotechnological tool for the study of biogenesis and improvement of our understanding on crop development. Generation of trans-plastomic plants hold great promise for the commercialization of the technology and provides an efficient platform for the production of therapeutic proteins, vaccines, and biomaterials using an environmentally friendly approach.

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