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ENGINEERING THE CHLOROPLAST GENOME

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Abstract

Creation of genetically modified Organisms (GMOs) to confer novel traits is an important milestone in biotechnology. However, public acceptance of GMOs has been slow due to several environmental concerns. In order to address environmental concerns, the chloroplast genetic engineering approach is being looked for. One of the biological containment strategies to reduce or prevent the flow of transgenes through pollen of most flowering crops is to incorporate the transgenes into the plant chloroplast instead of incorporating them into the plant nuclear genome. Chloroplast genome are inherited maternally through the egg and pollen does not contain plastids. Therefore chloroplast genes are only transmitted through the egg to the embryo and there will be no risk of dispersal of transgene through the pollen. Recently, much progress in plastid engineering has been made. In addition to model plant tobacco, many transplastomic crop plants have been generated which possess higher resistance to biotic and abiotic stresses and molecular pharming. In this review, we will discuss the features of the plastid DNA and advantages of plastid transformation. We will also present some examples of transplastomic plants developed so far through plastid engineering, and the various applications of plastid transformation.

Keywords: Genetically modified Organisms (GMOs), chloroplast genetic engeenering, transgenes, genome, pollen, tansplastomic.

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Introduction

Chloroplasts are the site of photosynthesis in plants mostly seen in leaves. Plastids of higher plants are generally semiautonomous. The plastid genome is a circular molecule of double stranded DNA. Despite the small size of plastid genome compared to the nuclear genome, chloroplast DNA makes up as much as 10-20% of the total cellular DNA and contains about 130 genes. With the introduction of transgenic crops, many fears have arisen regarding harmful side effects. The chloroplast transformation system has many attractive advantages over nuclear transformation, which is very much in vogue currently for generating transgenic plants. Plastids have retained many eubacterial features including gene organization in operons and prokaryotic mechanism of gene expression. Chloroplast genes are inherited maternally, so gene pollution caused by transgene escape *via* pollen can be controlled and also the risk of development of weeds resistant to toxin can be reduced. The plant cell has 10000 copies of the plastid genome. Hence many fold expression of the transgene can be expected. It is useful in evolving vaccine producing plants as it enables high level of protein production. Also there is the possibility of producing multiple proteins using polycistronic mRNAs. Transgene expression is more stable in transplastomic plants than in nuclear transformants because transgenes are integrated into chloroplast genomes by homologous recombination and not affected by gene silencing. Chloroplast transformation permits to insert several transgenes under the control of one promoter. This enables engineering of complex traits.

Advantages of plastid engineering

Production of transgenic plants, at laboratory level or commercially, has traditionally been mainly through expression of transgenes in the nucleus (Wani and Gosal, 2010). Among the ecological concerns raised about genetically engineered organismsis that transgenes could move ("transgene flow"), the process of transgene movement by recurrent hybridization *via*

pollen from the crop and into relatives growing in natural or semi-natural communities (Wilkinson *et al.*, 2003) Such concerns have led to a new field of transgene containment (Daniell, 2007). Since plastids are inherited maternally in the majority of angiosperm species, they would therefore not be found in pollen grains of corps. Insertion of transgenes, therefore, into the plastid genome has the potential of preventing gene flow *via* pollen. Hence, genes expressed in the plastome will not be transferred through pollination to weedy or wild relatives of the transgenic crop. Bansal and Sharma[5], believes that there is little risk of any transgene flow *via* pollen from transplastomic plants to the neighboring weedy or wild relatives since plastids are almost always maternally transferred to the next progeny. The authors suggested that plastid transformation could be a method of choice for generating improved transgenics in crops that grow along with their weedy or wild relatives in the same geographical region, such as rice, sorghum, cucurbits, solanaceous crops, *Vigna* and *Cajanus* species, and various *Brassica* crops.

Therefore, the focus of many researchers has shifted to plastid engineering[6], rather than nuclear transformation. Singh *et al*[6] reported that engineering of the plastid genome is gaining momentum as an attractive alternative to nuclear transformation. Ruf *et al*[7] believe that plastid transformation is considered as a superb tool for ensuring transgene containment and improving the biosafety of transgenic plants. However, they pointed out that plastid transformation would only be effective as a biocontainment measure, when applied on a landscape scale if it were combined additional mechanisms such as mitigating genes, genetic use restriction technology, and/or male sterility[7]. In a recent study, it has been demonstrated that the use of plastid transformation would provide an imperfect biocontainment for GM oilseed rape (*Brassica napus* L.) in the United Kingdom[8]. In another study, Allainguillaume *et al*[9] revealed that chloroplast transformation may slow transgene recruitment in two settings, but actually accelerate transgene spread in a third. Plastid transformation has become an attractive alternative to nuclear gene transformation due to several other advantages[10]. The high ploidy number of the plastid genome allows high levels (up to 1-40% of total protein) of protein expression or expression of the transgene[11]. Daniell *et al*[12] and Hou *et al*[13] reported that while nuclear transgenes typically result in 0.5 - 3% of total proteins, concentration of proteins expressed by plastid transgenes is much higher; up to 18%.

The greater production of the expressed protein is possible because plastid transgenes are present as multiple copies per plant cell, and they are little affected by phenomena like pre- or post-transcriptional silencing. Other advantages of plastid engineering are the capacityto express multiple genes from polycistronic messenger RNA (mRNA)[14], and the absence of epigenetic effects and gene silencing[15]. Wang *et al*[10] believe that transgene stacking in operons and a lack of epigenetic interference allowing stable transgene expression. Added to that, plastid transformation is more environmental friendly than transformation of the nuclear DNA for plant engineering because it eliminates the possibility of toxic transgenic pollen to non-target insects[16]. Adverse effects of toxic proteins might be minimized by plastid compartmentalization, but in case of nuclear transformation, toxic proteins accumulating within the cytosol might result in serious pleiotropic effects. Further, the expression of the transgene in case of plastid transformation is more uniform compared to that of trangenes inserted into the nuclear genome. Although there is a major drawback in the engineering of plastid gene expression, which is the lack of tissue-specific developmentally regulated control mechanisms[10], the many advantages of plastid engineering stated above attracted researchers to engineer the plastid genome to confer several useful agronomic traits, and hence the number of species whose plastome can be transformed continues to expand[17].

Selectable markers for plastid transformation

Primary Positive Selection

Primary markers are suitable for selectively amplifying a small number of transformed ptDNA copies. Currently known primary markers are resistance to spectinomycin, streptomycin, and kanamycin, which inhibit protein synthesis on prokaryotic-type plastid ribosomes. These antibiotics inhibit greening, cell division, and shoot formation in tobacco culture. Therefore, greening, faster proliferation, and shoot formation were used to identify transplastomic clones on a selective medium. The first transplastomic clones were obtained by spectinomycin selection.
More efficient primary plastid markers are chimeric genes in which the coding segment of a bacterial antibiotic detoxifying enzyme is expressed from plastid signals. The *aadA* gene encodes the enzyme aminoglycoside 3 adenylyltransferase that inactivates spectinomycin and streptomycin by adenylation and prevents binding to chloroplast ribosomes. Transformation events increased to 100-fold more than the mutant 16S rRNA genes[18].

Spectinomycin offers nonlethal selection[18] by not inhibiting cell division and growth at high concentrations (approximately 500 mg mL21), it was observed to be lethal in all other plant species. The neo gene is another alternative marker for plastid transformation that confers kanamycin resistance[19]. A different kanamycin resistance gene (aphA6) with relatively high transformation efficiency was reported later[20].The bacterial bar gene, encoding phosphinothricin acetyltransferase (PAT) and conferring herbicide resistance, has also been tested as a plastid-selectable marker. PAT served as an excellent marker in nuclear transformants and conferred resistance to the herbicide phosphinothricin. Expression of the bar gene in plastid conferred phosphinothricin resistance only when introduced by selection for a linked *aadA* gene. However, the bar gene was not found to be suitable for the direct selection of transplastomic lines, even when expressed at a higher level.

Secondary Positive Selection

Use of secondary selective markers is dose dependent; they are not suitable to select transplastomic clones when only a few ptDNA copies are transformed, but will confer a selective advantage when most genome copies are transformed. Examples for secondary markers are genes that confer resistance to the herbicides phosphinothricin (PPT)[21, 22] or glyphosate[22] or to the antibiotic hygromycin [based on expression of the bacterial hygromycin phosphotransferase gene.

Negative Selection

The ability to identify loss of function of a conditionally toxic gene forms the basis of negative selection. A negative selection scheme in plastids utilizes the bacterial cytosine deaminase (CD) enzyme encoded in the *codA* gene[23]. CD catalyzes

deamination of cytosine to uracil, enabling use of cytosine as the sole nitrogen and pyrimidine source. CD is present in prokaryotes and in many eukaryotic microorganisms, but is absent in higher plants. 5 fluorocytosine is converted to 5-fluorouracil, which is toxic to cells. This negative selection scheme was utilized to identify seedlings on 5-fluorocytosine-medium from which *codA* was removed by the CRE-loxP site-specific recombinase[24].

Reporter Genes

The *E. coli* GUS (3-glucuronidase gene) and the *Aequorea victoria* GFP (green fluorescent protein) are reporter enzymes that allow tracking gene expression, but do not confer a selective advantage or disadvantage to plastids. GUS enzymatic activity expressed in chloroplasts has been measured using fluorogenic assays[25-28] and visualized by histochemical staining[26]. GFP is a visual marker, allowing direct imaging of the fluorescent gene product in living cells. Its chromophore forms auto catalytically in the presence of oxygen and fluoresces green when absorbing blue or UV light. GFP has been used to detect transient gene expression[29] and stable transformation events in chloroplasts. GFP was fused with the *aadA* gene product (AAD) to be used as a bifunctional visual and selective (spectinomycin resistance) marker gene[30].

Marker Gene Elimination

The interest in developing marker elimination systems for plastids was driven by regulatory concerns to avoid releasing antibiotic resistance genes in transplastomic crops, the desire to reuse the relatively few available plastid marker genes, and the metabolic burden imposed by expressing marker genes. Three systems are available for marker gene elimination. The first system relies on the loop-out of the marker gene through directly repeated sequences. This system is practical only in exceptional cases, when introducing secondary markers, such as herbicide resistance genes, is the desired objective. A second approach involves cotransforming two independently targeted plastid transgenes and segregating out the ptDNA with the marker gene at the heteroplastomic stage[22]. The third and most efficient approach uses vectors with floxed marker genes, which can be removed with the CRE site-specific recombinase. Although convenient vectors with floxed marker genes have not yet been reported for the introduction of passenger genes, the feasibility of the approach was shown by excising *aadA*, *codA* and *clpP1* genes. Important for the application of CRE in plastids is that no detrimental ptDNA rearrangements persist once CRE is removed.

Controlled expression of plastid trans-gene in plants

A major drawback in the engineering of plastid gene expression is the lack of tissue-specific developmentally regulated control mechanisms. There is a clear need to develop tightly controllable systems for transgene expression in the chloroplast genome. It is highly desirable to

limit transgene expression to certain tissues, organs and/or developmental stages for following reasons. First, high level expression of foreign proteins may have deleterious phenotypic effects[31] and impose a significant metabolic burden on the plant. Deleterious effects of constitutive transgene expression can occur if gene products are harmful to the transformed plant. And if plastid transformation carried out in food crops, inadvertent contamination of the food chain with the plant produced chemical or pharmaceutical must be prevented. This could be accomplished by making transgene expression dependent on an inducer. To establish such a system, a β-glucuronidase (GUS) reporter gene under the control of phage T7 gene 10 promoter was introduced into the plastid genome of plants. GUS expression was dependent on nuclear-encoded plastid targeted T7 RNA polymerase (T7 RNAP) activity. First, a T7 RNAP chimeric gene containing a cauliflower mosaic virus 35S promoter and a tobacco chloroplast transit peptide sequence was introduced into tobacco by nuclear transformation. Then plastid transformation was carried out by using the plastid vector contained a β-glucuronidase (GUS) reporter gene under the control of T7 promoter.

The crossing between the nuclear transformed plants and plastid transformed plants demonstrated that a silent T7/GUS reporter gene could be activated in the F_1 generation by transmission of an active nuclear T7 RNAP gene from the male parent. GUS expression was dependent on nuclear- encoded plastid targeted T7 RNAP activity[32]. In order to induce polyester polyhydroxybutyric acid (PHB) synthesis in tobacco in a well-timed manner, a trans-activation system to regulate transcription of the phb operon in plastids was constructed. It was an ethanol-inducible T7 RNAP system. This system consists of a nuclear-located, ethanol-inducible T7 RNAP which is targeted to plastids harboring the phb operon under control of T7 regulatory elements. Following treatment with 5% ethanol, moderate induction of PHB synthesis was found. Without ethanol induction, development of flowers and fertile seeds was possible. Thus, the main problem of inhibitory transgene expression was solved[33]. More recently, a Lac repressor-based IPTG-inducible expression system for plastids has been reported, although transgene repression in the uninduced state was incomplete[34]. This is a system for external control of plastid gene expression which is based entirely on plastid components and can therefore be established in a single transformation step. It uses modified promoters containing binding sites for the bacterial lac repressor. So chemical induction can be made with intact plants or after harvesting[34]. This will be one of the major ground breaking developments in plastid biotechnology.

Metabolic pathway engineering

Plastid genome engineering represents an attractive alternative to conventional nuclear transgene expression for metabolic engineering, mainly because of the greatly increased transgene containment and the possibility to stack several transgenes by linking them in operons. The plastid harbors a large number of metabolic pathways and, for this reason, is also commonly referred to as the 'biosynthetic centre of the plant cell'. In view of its outstanding importance and the fact that many of its components are plastid encoded, photosynthesis is an obvious candidate pathway for metabolic engineering. The most complex metabolic pathway to be introduced into the plastid genome so far is that for the synthesis of the bioplastic polyhydroxybutyrate (PHB). Three enzymes of PHB biosynthesis are co-transcribed into the tobacco plastid genome[35]. Significant accumulation of PHB in chloroplasts appeared to cause male sterility and severe growth

retardation. A recent study has provided evidence that b-ketothiolase expression is responsible for the male sterility of the transplastomic plants[36]. An improved inducible PHB production system placing the operon under the control of a nuclear-encoded ethanol-inducible T7 RNA polymerase was targeted to plastids to control the *phb* operon in plastids[33].

Up to now, the application of plastid transformation to metabolic pathway engineering was restricted to the model species tobacco. To investigate the possibility of engineering a nutritionally important metabolic pathway of non-green plastids, a recent study used plastid transformation in tomato to alter carotenoid biosynthesis towards producing fruits with elevated contents of provitamin A (β-carotene), an important antioxidant and essential vitamin for human nutrition[37,38]. This study demonstrated the feasibility of metabolic pathway engineering through plastid transformation in non-green plant organs. This is the first successful example of engineering a nutritionally important biochemical pathway in non-green plastids by transforming the chloroplast genome. It is an encouraging step toward the application of plastid transformation technologies in food crops.

MOLECULAR FARMING: cheaper biopharmacuiticals for the masses of world

A therapeutic protein, human serum albumin (HSA) was firstly expressed in transgenic chloroplasts of tobacco at an expression level up to 11.1% of TSP, which is 500-fold greater than the nuclear expression[39]. So far, most efforts have been focused on the high-level production of antigens for use as vaccines and their tests for immunological efficacy in animal studies. Cholera toxin B sub-unit (CTB) of *Vibrio cholerae*, a candidate vaccine antigen, has been expressed in chloroplasts resulting in an accumulation of up to 31.1% of TSP as functional oligomers[40]. An animal vaccine epitope, 2L21 peptide that confers protection to dogs against virulent canine parvovirus (CPV), was expressed in tobacco chloroplasts as a fusion protein with CTB and with green fluorescent protein (GFP)[41]. LecA, a potential target for blocking amoebiasis, was expressed in chloroplasts to yield up to 6.3% of TSP or 2.3 mg LecA/g leaf tissue[42]. Recently, chloroplast transformation of the high-biomass tobacco variety Maryland Mammoth has been assessed as a production platform for the human immunodeficiency virus type 1 (HIV-1) p24 antigen[43].

Chloroplast system is most suitable for high-level expression and economical production of therapeutic proteins in an environmentally friendly manner. However, the cost for purification of these proteins can be eliminated if they are orally delivered or minimized by the use of novel purification strategies. Oral delivery of therapeutic proteins is emerging as a new alternative for medical treatment and will benefit those who cannot afford the high cost of current treatments. As described above, transgenic chloroplasts have been used for the production of many therapeutic proteins, It suggests that the chloroplast should contain the mechanism that allows correct folding and disulfide bond formation, resulting in fully functional proteins. Despite such rapid progress in the use of this organelle for plant molecular pharming, no glycoprotein has been expressed in transgenic chloroplasts, because N- or O-glycosylation is required for stability and functionality of many proteins[44]. If glycoproteins could be expressed, and expressed proteins have normal glycosylation sites and functions, engineering chloroplasts would be used in more aspects. Further studies will be needed to study the glycoproteins expression and the mechanism of glycosylation in the chloroplasts.

Agronomic traits engineered *via* the chloroplast genome

Several useful transgenes have conferred valuable agronomic traits, including insect and pathogen resistance, drought tolerance, phytoremediation, salt tolerance, and CMS through chloroplast genetic engineering .Genetically engineered tobacco plants expressing an insecticidal protein Cry2Aa2 have shown resistance against target insects and insects that developed resistance against insecticidal protein[45]. Expression of the Cry2Aa2 resulted in the utmost expression levels on record (approximately 46.1% of total leaf protein) and resulted in the detection of cuboidal crystals using transmission electron microscopy[46]. In addition, soybean plastid transformants expressing Cry1Ab also conferred insecticidal activity against velvetbean caterpillar[47]. The antimicrobial peptide MSI-99, an analog of magainin 2, was expressed *via* the chloroplast genome to obtain high levels of expression in transgenic tobacco plants. In planta assays with the bacterial pathogen *Pseudomonas syringae* pv. *tabaci* and the fungal pathogen *Colletotrichum destructivum* showed necrotic lesions in untransformed control leaves, whereas transformed leaves showed no lesions[48]. Environmental stress factors such as drought, salinity, and freezing are perilous to plants generally because of their sessile means of existence. Attempts to confer resistance to drought by expressing trehalose phosphate synthase 1 (tps1) gene *via* nuclear transformation have proven futile because of undesirable pleiotropic effects even at very low levels of trehalose accumulation.

However, hyperexpression of tps1 in the chloroplasts has no phenotypic variation from the untransformed control plants, and transgenic seeds sprouted, grew, and remained green and healthy in drought tolerance bioassays with 3% to 6% PEG and dehydration/rehydration assays[49]. High-level expression of BADH in cultured cells, roots, and leaves of carrot *via* plastid genetic engineering exhibited high levels of salt tolerance. Transgenic carrot plants expressing BADH grew in the presence of high concentrations of NaCl (up to 400 mM), the uppermost level of salt tolerance reported so far among genetically modified crop plants[50]. Chloroplast genetic engineering has also been used for the first time to our knowledge to enhance the capacity of plants for phytoremediation. This was accomplished by incorporating a native operon containing the merA and merB genes, which code for mercuric ion reductase (MerA) and organomercurial lyase (merB), respectively, into the chloroplast genome in a single transformation event. Stable integration of the merAB operon into the chloroplast genome resulted in high levels of tolerance to the organomercurial compound phenylmercuric acetate when grown in soil containing up to 400 mMphenylmercuric acetate[51].

Chloroplast transgenic lines absorbed mercury exceeding the levels in soil and translocated 100-fold more to shoots than untransformed plants[52]. Tobacco is ideal for phytoremediation of contaminated soil because it is a non-food non-feed crop. Naturally occurring CMS has been documented for over 100 years for oilseed rape, maize (*Zea mays*), and rice. However, such systems are not available for the majority of crops used in agriculture. In presently available CMS lines, various loci in the nuclear genome direct a range of restoration factors that are not

fully understood. Moreover, risk of sterility trait dilution through segregation and the production of transgenic seeds that spread transgenic traits to non-transgenic plants cannot be ruled out because of the possibility of cross-pollination of the male-sterile line with a restorer line or wild relative. To address some of these concerns, CMS has been engineered *via* introduction of phaA gene coding for b-ketothiolase into chloroplast genome. The transgenic lines were normal except for the male sterility phenotype lacking pollen[36]. Further restoration of male fertility was reported by changing conditions of illumination. Continuous illumination increases acetyl-CoA carboxylase activity, thereby increasing the levels of plastidic fatty acid biosynthesis, which is especially needed for the formation of the exine pollen wall.

Engineering chloroplast-based photorespiration in C3 plants

A pathway for the catabolism of a photorespiratory substrate, glycolate, has been reported for *Escherichia coli*[53] and plants[54]. In *E. coli*, glycolate dehydrogenase (GDH) uses NAD^+ as an electron acceptor to oxidize glycolate to glyoxylate. However, in plants, glycolate is oxidized to glyoxylate by glycolate oxidases that use molecular oxygen in peroxisomes. Nevertheless, a glycolate pathway similar to the recently engineered photorespiratory bypassin *A. thaliana* was reported for *Synechocystis* sp. Strain PCC 6803[55]. The role of the normal photorespiration pathway in cyanobacteria is not yet clear. Taking advantage of a fundamental difference between bacterial and plant glycolate metabolic enzymes, Kebeish *et al*[56] established the pathway in chloroplasts by stepwise nuclear transformation and post-translational targeting of proteins to plastids of the novel biochemical pathway in C3 plants.

Multigene engineering *via* chloroplast transformation

The majority of the aforementioned examples demonstrated the applicability of chloroplast transformation for the expression of simple traits of agricultural and commercial value requiring the integration of a single gene for the expression of the new trait. The vast majority of agronomic traits are quantitative and controlled polygenetically. Plant genetic engineering is shifting gears for the production of more complex traits, often requiring the use of multigenes for their completion and proper functioning. A suitable approach to engineering multigene pathways is to do it *via* the chloroplast genome. Chloroplast genetic engineering possesses many advantages that make it a suitable place to engineer multigene pathways. Among the most important advantages of chloroplast engineering is the ability of chloroplast to effectively transcribe operons and translate polycistronic mRNA. Integration of multigene operons into the chloroplast genome occurs by homologous recombination, which allows the targeting of several genes in a single transformation event, thus removing the possibility of detrimental position effect. Additionally, chloroplast genetic engineering offers increased transgene containment due to the maternal inheritance of chloroplasts. So far, no size limitation for foreign operons has been reported in chloroplast. Because the chloroplast is the site of photosynthesis, carbohydrate and fatty acid biosynthesis, it is the logical place to genetically engineer different metabolic pathways.

One example of carbohydrate engineering by chloroplast genetic engineering was the production of the osmoprotectant trehalose in tobacco chloroplast[50]. This osmoprotective sugar has been proposed as a safe preservative for foods and therapeutics, and was recently approved by the Food and Drug Administration (FDA) and the European Union regulation system[57]. The chloroplast has also been engineered with the *acc*D gene, the expression of this gene increased the levels of the plastid encoded acetyl-CoA carboxylase subunit and consequently increase the levels of fatty acid biosynthesis in chloroplast of the transgenic plants[58]. Zhang *et al*[59] demonstrated the possibility of increasing amino acid content in plants by introducing a feedback insensitive mutant version of the anthranilate synthase α-subunits into the chloroplast genome. The expression of this deregulated enzyme increased the levels of tryptophan.

Multigenes have been only recently engineered *via* the chloroplast genome to produce new metabolic traits. The *Bt cry*2Aa2 operon was used as a model system to test the feasibility of multigene operon expression in genetically engineered chloroplast[47]. In this report, the expression of the operon containing selectable marker *aad*A, *orf*1, *orf*2 (chaperone) and *cry*2Aa2 gene lead to the formation and accumulation of CRY2AaA crystals in the chloroplast to 46.1% total soluble protein. Later, targeting of an operon containing the three bacterial genes for the biosynthetic pathway of poly hydroxyl butyrate resulted in the expression of the biopolymer in plastids[35]. More recently, a multigene operon comprising a selectable marker and the *mer*A and *mer*B genes coding for the phytoremediation pathway of organic-mercury lead to the detoxification of phenylmercuric acetate to the far less toxic elemental mercury, and conferred resistant to high concentration of organomercurial to the transgenic plants[52]. These examples support the applicability of multigene engineering for the expression of metabolic pathways *via* the chloroplast genome. Chloroplast transformation has also conferred resistance to herbicides[60], conferred drought tolerance, and phytoremediation capabilities[52].

Focus for future

Although the concept is more than 10 years old, plastid transformation has been accomplished in relatively few species. There are numerous factors that have hampered the expansion of chloroplast transformation technology to different plant species. Some of the factors include insufficient regeneration protocols, not enough selectable markers, not enough promoters, etc. so the research should focus on the production of designer promoters and also to generate adequate regeneration protocols for economically important crops. Plastid genetic engineering possesses a great potential to be developed into a big platform for the production of cheaper biopharmaceuticals and oral vaccines. A higher public awareness is needed to be developed for the general acceptance of the transgenics.

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