**Title: Recent advances in organelle engineering**

**Abstract**

Chloroplasts are photosynthetic organelles having circular double-stranded DNA molecules of 120–180 kb size that encodes approximately 120 genes which are essential genetic information for the organelle’s function in photosynthesis and other cellular processes in plants. Chloroplast transformation provides several distinct advantages over nuclear transformation however, it is still limited to a few plant species. Advantages includes, high level protein expression, gene containment, avoiding gene silencing, homologous recombination, maternal inheritance, multigene expression, no positional effect and maternal inheritance. The genetic manipulation of chloroplast has been used to improve photosynthesis, carbon assimilation and to provide resistance to insects, herbicides, and abiotic stresses and also it is a promising strategy for using plants as sustainable bioreactors to produce pharmaceuticals, antibodies, and vaccines due to its high levels of transgene expression, as each plant cell contains numerous chloroplasts with a high copy number of the chloroplast genome (An *et al*., 2022).

Chloroplast transformation provides a valuable alternative platform to generate transgenic plants and it involves modification of the genome or introduction of a new foreign gene into the chloroplast. The last obstacle to reach the chloroplast is its double lipid bilayers which are formed by galactolipids and are highly dynamic. Similar to mitochondria, the outer membrane of the chloroplast envelope is permeable to small molecules but, the inner membrane is impervious to ions and metabolites, which can only enter the chloroplasts through specific membrane transporters such as the TIC/TOC transporter for chloroplast-targeted proteins. Chloroplast transformation commonly carried out by classical gene delivery methods like biolistic approach, polyethylene glycol (PEG)-mediated delivery, and Agrobacterium-mediated delivery. Currently, the major challenges limiting these technologies include lengthy transformation and regeneration steps involved in the recovery of homoplasmic-transplastomic plant lines, few selectable markers, and the recalcitrance of many plant species to existing chloroplast transformation protocols (Liu *et al*., 2023).

Recently, successful genetic material delivery methods have taken advantage of peptides, which target the receptors as a way to selectively target organelles within plants. By formulating peptide/plasmid DNA complexes that combine the functions of both cell-penetrating peptide and chloroplast-targeting peptide, DNA molecules are translocated across the plant cell membrane and delivered to the plastid efficiently. Odahara *et al.,* 2022 have demonstrated successful delivery and integration of construct DNA into plastid by fusion peptide (KH-AtOEP34) method. Another advanced technique which is gaining importance is “Nanotechnology” that enables plant biology researchers for a better understanding of chloroplast molecular biology and genetics. Nanoparticles can be coated or loaded with biomolecules for delivery of cargo that can be targeted to plant cells and organelles, such as chloroplasts, by modifying their size, charge and biorecognition coatings. Kwak *et al*., 2019 have designed and demonstrated chloroplast-targeted transgene delivery and transient expression in mature *Eruca sativa, Nasturtium officinale, Nicotiana tabacum* and *Spinacia oleracea* by designing chitosan-complexed single-walled carbon nanotubes.

Photosynthesis in major C3 crops is limited by the inefficiency of the key CO2-fixing enzyme Rubisco, owing to its low carboxylation rate and poor ability to discriminate between CO2 and O2. In cyanobacteria and proteobacteria, carboxysomes function as the central CO2-fixing organelles that elevate CO2 levels around encapsulated Rubisco to enhance carboxylation. Heterologous synthesis of a biophysical CO2-concentrating mechanism (CCM) in plant chloroplasts offers significant potential to improve the photosynthetic efficiency of C3 plants and could translate into substantial increases in crop yield (Rottet *et al*., 2021). Study by Chen *et al.,* 2023 provides proof-of-concept for a route to engineering fully functional CO2- fixing modules and entire CO2-concentrating mechanisms into chloroplasts to improve crop photosynthesis and productivity. Chloroplast engineering holds immense promise for revolutionizing agriculture, energy production, and various industries. While challenges remain, ongoing research and technological advancements are likely to unlock new opportunities and applications, leading to a more sustainable and technologically advanced future.

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