Chloroplast engineering for Transplastomic

- Chloroplasts are semi-autonomous cell organelles in plant cells that have evolved by endosymbiosis of blue green algal cell during course of evolution
- It contains its own genome (Cp-DNA) but rely on some of the proteins encoded by nuclear gene.

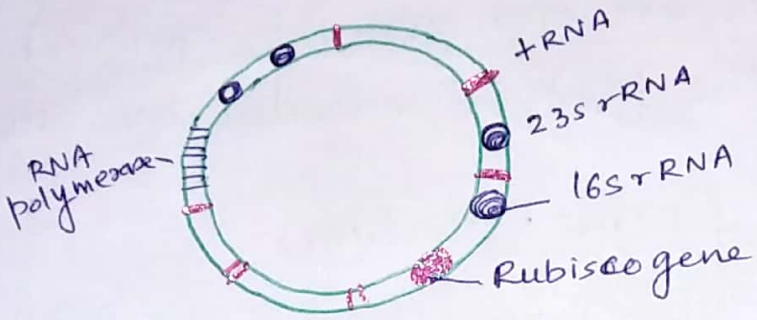
Chloroplast genome: (Cp-DNA)

- Each chloroplast contain nearly 100 copies of cpDNA called plastome, each of which is circular double stranded DNA
- Each cpDNA is about 100–160 Kb containing nearly 100 genes coding cynobacterial type proteins
- Cp DNA is maternally transmitted in majority of plants. (Hagemann, 2004)
- Though Cp. DNA resemble cyanobacterial genome but it exhibit RNA editing and gene splicing

Significance of Cp DNA in genetic transformation

- Cp DNA similar to bacterial genome allowing the control of expression of multiple gene through single promoter
- Ability of Chloroplast to transform very large number of transgene which is rather difficult through nuclear genome

- Cp DNA transformation is not associated with gene silencing which is major problem with nuclear gene transformation.

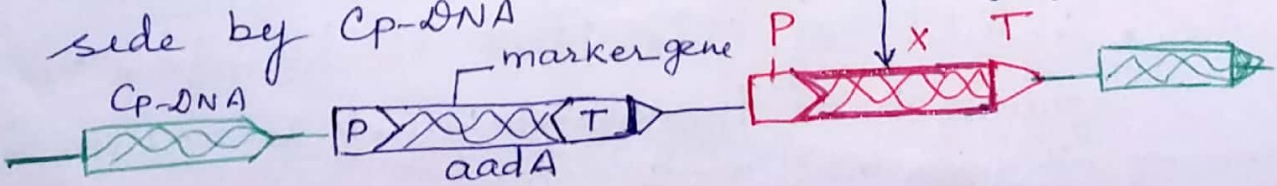


Cp. DNA of Marchantia polymorpha

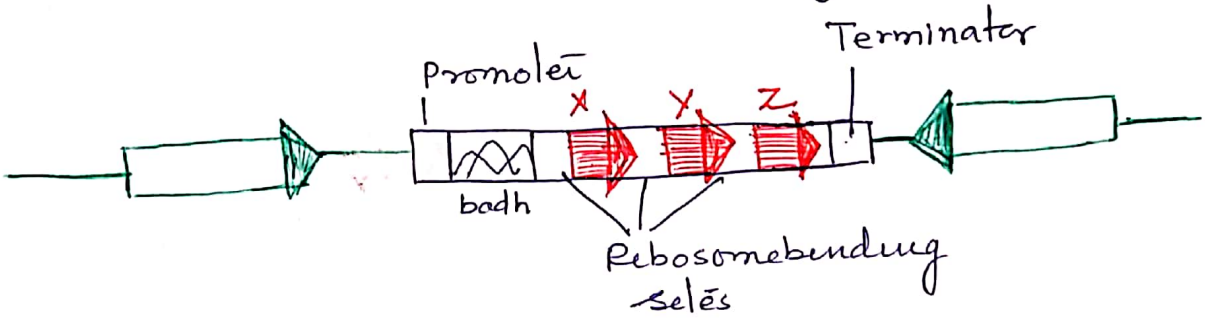
Procedure of chloroplast transformation

Design of vector construct for transformation of Cp genome.

(a) Vector construct for expression of a single gene: This vector of chloroplast transformation is based on selectable marker gene add A (resistance to spectinomycin). The desired foreign gene is associated with the promoter and terminator sequence flanked on either side by Cp-DNA



(b.) Vector construct for expression of multiple genes: The selectable marker betaine-aldehyde dehydrogenase (badh) gene is flanked by promoter and the large number of foreign genes are flanked by terminator. The promoter and terminator is flanked by cp-DNA

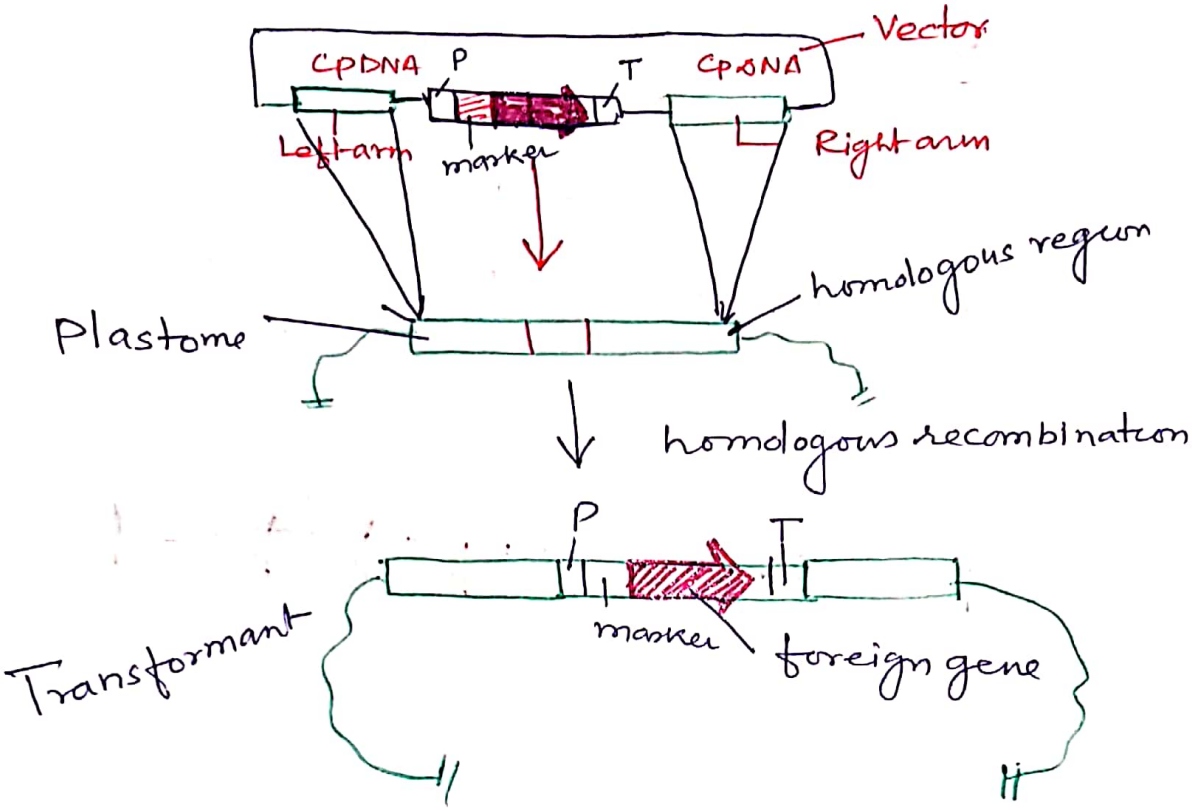


2. Introduction of foreign genes in cp-genome

The foreign genes can be inserted into chloroplasts by

(a) Beolistic or particle gun method
(b) Polyethylene glycol (PEG) treatment of protoplast

3. Homologous recombination: Chloroplast transformation involves homologous recombenation between cp DNA sequence on the vector and those on the genome. It envolve site specific integration of foreign gene into cp Genome. It is precise and predictable and minimize the insertion of unnecessary DNA that accompany nuclear genome transformation



Transplastomic plants : A genetically modified plants derived from transformation of foreign gene in the plastid genome (plastome) is called transplastomic plants.

Advantage

- Prevents the gene flow from genetically modified plant as it is located in cytoplasm
- transplastomic plants are genetically safe and can coexist with conventional crops
- Exhebit no position effect
- Not effected by transcriptional silencing
- No expression effect of toxic genes