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Engineered Plastids Assure Biosafety of Transgenics

Muhammad Sarwar Khan*

Department of Plant Molecular Biology, Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad, Pakistan

Amongst the main items on wish-list of plant biotechnologists are: incorporating multiple foreign genes into the plant genome through a single transformation event, accumulation of foreign proteins to high levels, elimination of position effect and containment of transgenes. Despite being enslaved by the nucleus, plastids are capable of expressing foreign genes as polycistronic units, and their expression yields high levels of protein with *bonafide* structure. Plastid genome engineering offers a number of other unique advantages, including elimination of positional effects that are frequently observed with nuclear transformation, and transgenes in plastids are contained by stringent maternal inheritance (not transmitted by pollen) in most cultivated plant species; nevertheless, transfer of plastid genes to the nucleus has been reported with implications for transgene containment. However, significance of these transfers will depend on the likelihood that they will become functional nuclear genes, as well as on the success of strategies that can prevent the expression of transferred plastid genes in the nucleus [1]. One of the strategies developed is intein-mediated protein trans-splicing. In this strategy, the gene to be inserted is split into two halves, and the activity of the transgene product is reconstituted upon self excision of inteins and concomitant ligation of the truncated protein products called exteins [2,3].

Recently, lateral genome transfer from one species to another is witnessed through the phenomenon of 'organelle capture'. The grafts are developed to demonstrate horizontal transfer of plastid genome from the cultivated tobacco, *Nicotiana tabacum*, into two other species: *Nicotiana glauca*, the woody species; and *Nicotiana benthamiana*, the herbaceous species [4,5]. After the stock and scion fusion has occurred, the graft sites were excised to regenerate shoots and in the regenerated shoots, genome transfer is demonstrated. However, significance of these genome transfers will depend on the likelihood that such transfers occur naturally between distant plant species, because the transfer of organelles between such species may result in plastids-nucleus incompatibilities, which represent natural barriers to chloroplast capture and possibly prevent the horizontal transfer of plastid genomes. Despite such gene or genome outflow, plastid genome is an attractive target to express proteins of commercial importance.

Historically, plastid transformation was achieved in a unicellular alga, *Chlamydomonas reindhartii* [6], followed by stable transformation of chloroplasts of tobacco using *aadA* gene, which encodes aminoglycoside 3'-adenyltransferase and confers resistance to two broad spectrum antibiotics i.e. spectinomycin and streptomycin [7]. After successful demonstration of *aadA* in tobacco as a dominant selectable marker, a reporter gene *gfp* that encodes green fluorescent protein (GFP) from jellyfish was expressed in tobacco plastids [8], which facilitated the extension of plastid transformation to non-green plastids of rice [9], a long awaited goal. This was the time when chloroplast transformation was started in several academic and industrial laboratories to functionally analyze plastid genes, express genes of agronomic and biotechnological importance and extend plastid transformation to other plants, including Arabidopsis, potato and tomato [10-14]. More recently, chloroplast transformation in major crops including soybean, sugarcane, egg plant, lettuce, rape seed, cotton and cabbage is achieved, extensively reviewed elsewhere [15]. Plastid transformation in dicotyledonous plants is a routine, however, extending plastid transformation to monocotyledonous sugar and cereal crops including rice, wheat and sugarcane is still at its early stage of development due to a number of impediments related to purification of transgenome to homoplasmic state [15]. Mitochondria are other maternally inherited organelles within plant cell. Where mitochondrial genome has been sequenced for a number of plant species, engineering its genome is achieved so far in an alga, *Chlamydomonas reindhartii* using biolistic gun [16-18], providing likelihood to engineer mitochondria in plants.

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***Corresponding author:** Dr. Muhammad Sarwar Khan, Department of Plant Molecular Biology, Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, P. O. Box 38000, Faisalabad, Pakistan, E-mail: sarwarkhan_40@hotmail.com

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