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Marker Free Technology: an innovative approach in Crop Improvement

Naresh Thakur[1], Sunny Chaudhary[2]

[1]Dr. Y.S. Parmar University of Horticulture and Forestry, College of Horticulture and Forestry, Neri-Hamirpur

[2]Department of Agricultural Biotechnology, CSK HPKV Palampur

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The teeming populace of the world has created major problems: less land to farm and more mouths to take care of. Conventional breeding has revolutionized the agricultural sector in terms of enhanced production; still, some issues remain unrequited. Marker free technology has emerged as a revelation to the issues encountered in the classical approach. Plant transformation technologies require selectable marker genes to produce transgenic plants but such genes are of no value thereafter; in fact, marker genes in transgenic plants seem to cause potential bio-safety issues.

A selectable marker gene (SMGs) is a plant transformation technology that required to produce marker free transgenic plants. However, after selection events, SMGs are of no value thereafter. Selection marker genes in transgenic plants are apparent to pose biosafety problems. It has been contended that it is alluring to keep transgene (s) in GM crop populaces important to limit quality stream to other related species. An example of gene flow in herbicide resistance gene (bar) has been studied in Canada in canola (*Brassica napus*) fields. In all genetic transformation technologies based on direct gene transfer (electroporation of protoplasts, particle bombardment, etc) the SMGs generally co-integrate with the gene of interest(s) in one Mendelian locus in the plant genome hence, their removal is greatly desirable. This may also help in the adequacy of transgenic plants by society.

Transgenic plants that contain the desired gene of interest but lack the selectable marker gene (SMG) used in its production are termed "Marker Free" and the methods utilized in their production are referred to as "Marker Free Technology" which is a safer way to genetically modify crops. The technology can be applied to all sexually propagated plants - maize, wheat, rice, millet, cowpea, sorghum, trees, and vegetables.

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Types of Selectable Marker gene used in the production of transgenic plants:

(1.) Positive SMGs: promote the growth of transformed cells. Eg: *nptII, hpt, bar, epsp, gat4601, gat4621, manA, xylA, ipt, rol.*

(2.) Negative SMGs: death or impaired growth of transformed cells. Eg: *codA, tms2, aux2, dhlA, CYP2B6, cue, TrAP.*

(3.) Reporter Genes: Used for making GM plants visually recognized. Eg: *uidA (gusA), gfp, ipt, luc, lacZ,* transcription factors (MYB family).

Methods for eliminating Selectable Marker Genes:

(1.) Plant-derived marker gene: *Arabidopsis thaliana* (At-*WBC19*) and *DEF2* (peptide deformylase) can be used.

(2.) Non- Antibiotic resistance genes: *Escherichia coli* derived phosphomannose isomerase (PMI) was used to convert mannose-6-phosphate to fructose-6-phosphate for the positive selectable marker in plant transformation.

(3.) Excise resistant marker gene: Various approaches used to excise SMG include co-transformation, transposon-based SMG removal, site-specific recombination, intrachromosomal recombination, and genome editing enzymes.

(4.) Chloroplast transformation: The vector for chloroplast transformation is based on the selectable marker gene aadA that provides resistance to antibiotic spectinomycin. The single foreign (desirable) gene is fused to regulatory a sequence (promoter and terminator) which in turn is flanked on either side by chloroplast DNA (Cp DNA).

(5.) Markerless transformation: Pollen tube pathway and ovary dip methods are used for markerless transformation.

Conclusions

A genetic transformation is vital for biology fundamental research and engineering transgenic organisms, including plants. SMGs have been very useful to enable plant transformation, yet there are some regulatory concerns of retaining SMGs in commercialized transgenic plants, leading us to conclude that ideally, the SMG should be removed after transformation. However, development for SMG removal will proceed, and we will no uncertainty see upgrades in existing frameworks and new advancements, for example, TALENs arranged for this reason.