Clean Gene Technology for Crop Improvement

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ABSTRACT

"Clean gene technology," also known as a gene editing technique, has revolutionised crop improvement by enabling precise modifications to plant genomes. Unlike traditional genetic modification, it allows for the targeted alteration of existing genes within a plant's genome. As our global population increases day by day and agricultural land decreases, so this process ultimately helps in the future for the development of high-yielding crops with enhanced traits, such as increased resistance to biotic and abiotic stresses, as well as improved nutritional content. The technology's precision reduces the risk of unintended changes, making it a safer and more sustainable alternative to conventional methods. As a result, this innovative approach holds the potential to address global challenges, such as food security and climate change, by producing more resilient and nutritious crops to meet the demands of a growing population.

INTRODUCTION

Global population is growing at an alarming rate and is expected to increase by one quarter in the next 30 years, to reach 10 billion. Conventional breeding has performed wonderfully to cope up with global issues like hunger threat but it requires a lot of time. To deal with these challenges, plant transformation is the one option. Plant transformation is a genetic engineering tool that involves the addition of genetic material which may be a single gene or multiple genes, integrated into a recipient plant

leading to the modification of the plant's genome. Selectable markers are those which allow the selection of transformed cells or tissue explants by their ability to grow in the presence of an antibiotic or a herbicide, such as hygromycin, kanamycin and glyphosate. SMG system are essential to plant genetic engineering, but their elimination is also very important as it is considered undesirable by many regulatory agencies. Therefore, there is a need for the development of such efficient techniques which not only allows removal of selection markers but also direct integration of transgenes at safe locations in the genome. Clean gene technology refers to a group of genetic engineering techniques that allow for the precise introduction of desirable traits into crops without leaving behind any unwanted genes or markers. This approach aims to minimize the risk of transgenic crops crossing with wild relatives or non-target species, reducing the potential for environmental impact.

METHODS OF CLEAN GENE TECHNOLOGY

There are several strategies to exclude selection gene from marker free transgenic plants.

1. **Co-transformation:** In this method, multiple copies of T-DNA can be transferred into a plant cell and integrated in the plant genome through biolistic-mediated transformation or *Agrobacterium*-mediated transformation. The principle of this strategy is the introduction of a selectable marker gene and a gene of interest from different T-DNAs. If the two genes are integrated into unlinked loci, subsequent crossing can separate the gene of interest from the selectable marker gene.

2. **Multi-auto transformation (MAT):** In this system, a chosen gene of interest (GOI) is placed adjacent to a multigenic element flanked by RS recombination sites. A copy of selectable *ipt* gene from *A. tumefaciens* is inserted between the recombinase sites together with the yeast *R recombinase* gene and this entire assembly is situated within a T-DNA element for the *Agrobacterium*-mediated transformation of plant tissues. The Ipt-type MAT vector system has been successfully used in cassava plants (Saelim *et al*. 2009) as well as disease-resistant potato plants (Khan *et al*. 2010).

3. **Site-specific recombination:** A site-specific recombinase can remove the selectable marker gene from plant's genome through enzyme-mediated site-specific recombination, if the selectable marker gene is present in flanking site of direct repeats of recognition sites for the enzyme. Basically, three site-specific recombination systems are there:

3.1 **The Cre/lox system:** This system has two components: (a) two loxP sites each consisting of 34 bp inverted repeats cloned in direct orientation flanking a DNA sequence, and (b) the *cre* gene encoding a 38 kDa recombinase protein that specifically binds to the loxP sites and excises the intervening sequence along with one of the loxP sites.

3.2 **The FLP/frt system:** In this technique, first round of transformation often results in transgenic plants that have selection marker positioned between two recognition sites that are directly orientated for corresponding recombinase. Once the single-chain recombinase is expressed, recombination reaction will start, either by crossing in plants that already express the enzyme, by transitory expression *via* a second transformation or by using an inducible promoter, resulting in marker-free transgenic plants.

3.3 **The R/rs system:** The selection of transformed plants is done on the basis of ipt shoots which exhibit a typical morphological alteration, short

internodes, reduced apical dominance and lack of rooting capacity. During sub-culturing in the tissue culture, the ipt shoots are removed by site-specific recombination mediated by recombinase of the R/rs system (Khan *et al*. 2011).

1. **Transposon-based recombination:** Two transposon-mediated strategies have been developed to generate marker -free transgenic plants. The first strategy involves *Agrobacterium*-mediated transformation followed by intragenomic relocation of transgene of interest and its subsequent segregation form the selectable marker in the progeny, and the second strategy involves excision of the marker gene from the genome (Ebinuma *et al*. 1997).

2. **Auto-excision strategy:** Auto-excision system is controlled by pollen and /or seed-specific promoters and in this system, marker is easily eliminated in the T_1 seeds of the transgenic plants, and the next generation of the transgenic plants will be marker free (Mlynarova *et al*. 2006).

3. **Gene-editing techniques:** Genome editing or genome engineering is a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism. Meganucleases are homing endonucleases, found in bacteria and eukaryotes (Chevalier and Stoddard, 2001). For removal of SMG, ZFN-overexpressing plants are crossed with transgenic plants harbouring SMG. The recognition sites for ZFNs are attached on flanking sites of SMG expression cassette in transgenic plants, as a result SMG is edited/removed. For removal of the transgene using TALENs, two identical sets of TALENs binding sequences are designed in the flanking region of SMG. After expression of TALENs, DSB occur and remove the SMG, and broken DNA is repaired by homologous recombination NHEJ repairing pathways (Chong-Perez and Angenon, 2013). Similarly, clustered regularly interspaced short pallindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) protein systems has the capability of genome editing at specified locations in the genome identified by guide RNA (gRNA). gRNA may be designed from the SMG genomic regions for its removal and production of transgene-free plants.

CONCLUSION

The improvement of agricultural production and productivity as well as the future versatility of agricultural production are dependent on the rational utilization of technologies. We stand at the convergence of an incredible array of new technologies, such as recombinant DNA technology, information technology and high-throughput genomics, to enhance our understanding of structure and function of genomes for plant improvement. Transgenic technology has contributed towards the development of various crop varieties with enhanced yield, resistance to biotic and abiotic stresses and enhanced food quality, but requires regulatory approval only after strict food/feed safety assessment due to issues raised regarding its safety to the environment and human health. Due to these major concerns associated with transgenic crops, new alternative techniques like "**clean gene technology**'' could be used to develop improved crop plants.

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