Enhancing Plants Safely: The Clean Gene Revolution in Agriculture

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Abstract

Clean gene technology, a cutting-edge genetic engineering approach aiming to safely introduce beneficial traits in plants. By eliminating risks associated with toxic marker genes and antibiotic resistance transfer, it enhances GMO safety and environmental interactions. The technology's significance lies in its precision, compliance with regulations, and fostering public trust. Looking forward, ongoing research and innovation in this domain are poised to advance agriculture, addressing critical challenges while ensuring food security and sustainability.

Keywords: Clean gene technology, Co transformation, Auto excision and Chloroplast marker

Introduction

The development of precise and environmentally friendly strategies for introducing advantageous traits into plant species is the goal of the cutting-edge approach to genetic engineering known as "clean vector technology" in plants. Clean gene technology is an essential advancement in genetic engineering, driven by a pressing need to circumvent inherent risks associated with traditional marker genes. Firstly, some marker genes can produce products that may be toxic or trigger allergic reactions in both humans and animals. This raises significant safety concerns regarding the consumption and exposure to genetically modified organisms (GMOs). Clean gene technology addresses this concern by eliminating these potential risks, making the resulting GMOs safer for consumption and environmental interaction. Moreover, the transfer of antibiotic resistance, a common mechanism in traditional marker genes, is a major worry. The antibiotic resistance genes used as markers could potentially be transferred to pathogenic microorganisms in the soil, aggravating the problem of antibiotic resistance. Clean gene technology, by omitting these marker genes, greatly reduces the likelihood of such unintended gene transfer, thereby contributing to a safer and more responsible approach to genetic modification (Kumar et al., 2022). Additionally, conventional genetic engineering techniques employing marker genes may inadvertently lead to the creation of superweeds that are resistant to commonly used herbicides. This poses a significant challenge in managing and controlling weed populations, impacting agricultural ecosystems and practices (Afolabi, 2009). Clean gene technology plays a crucial role in mitigating this risk by eliminating the possibility of unintended spread of herbicide resistance traits, ensuring a more sustainable and controlled approach to agriculture.

Need of clean gene technology

A vital advancement that meets many urgent needs in contemporary biotechnology and agriculture is plant clean vector technology.. To begin with, it addresses environmental concerns by removing or significantly reducing the presence of selectable marker genes in genetically modified organisms (GMOs). While these markers are necessary for identifying transformed cells, they pose environmental risks by potentially transferring antibiotic resistance genes to other organisms. Clean vectors reduce these risks, encouraging safer and more responsible genetic modification.

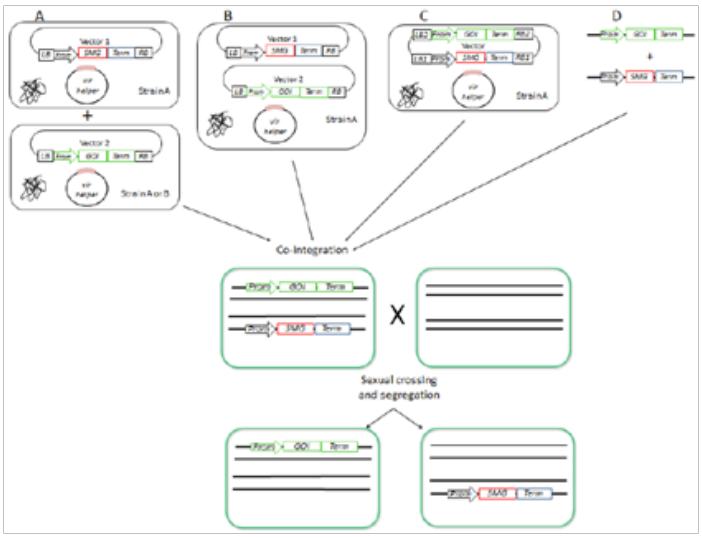
Second, clean vector technology improves compliance with stringent GMO regulatory requirements. It streamlines the regulatory approval process and ensures adherence to safety standards by producing marker-free GMOs with fewer extraneous genetic elements, facilitating the responsible deployment of genetically

modified crops.Additionally, clean vectors increase public acceptance of GMOs. The public's perception of genetically engineered plants as "natural" is critical for consumer trust. Clean vector technology accomplishes this by reducing the presence of foreign genes in GMOs, making them appear less altered, and thus increasing their market acceptability(Afolabi, 2007).

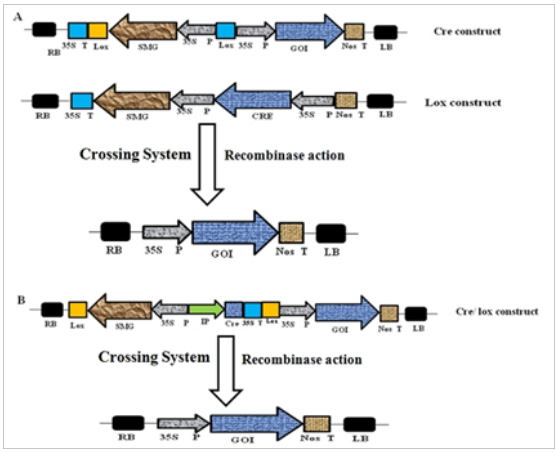
Clean vectors also allow for greater precision in genetic modifications. CRISPR-Cas9 techniques integrated into clean vector design enable the targeted insertion, deletion, or modification of specific genes in plant genomes. This precision produces crops with desired traits while also reducing off-target effects, ensuring the genetic stability and safety of the modified plants.

Methods to produce marker free lines

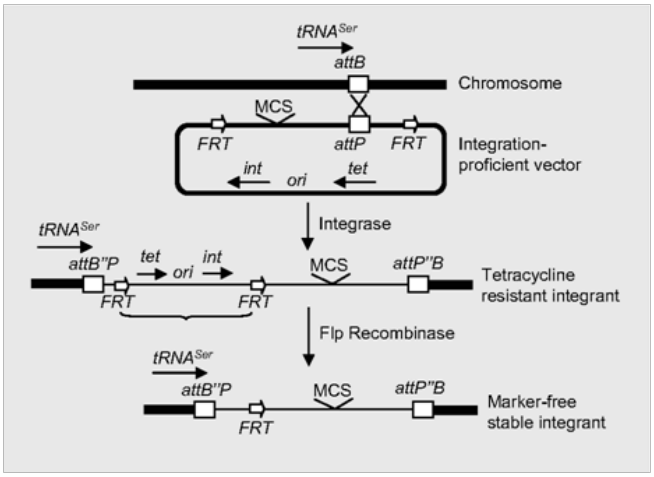
A. Co transformation- This process utilizes two plasmids for inserting genetic material into distinct plant locations. One plasmid holds a specific marker gene, while the other carries the gene of interest (GOI). In this approach, selectable marker genes (SMG) and target genes are on separate T DNAs, and they are expected to act independently following Mendelian principles. The selective marker gene (SMG) can be removed from the plant during sexual reproduction by favoring the transgene of interest over the SMG in progeny through segregation and recombination.



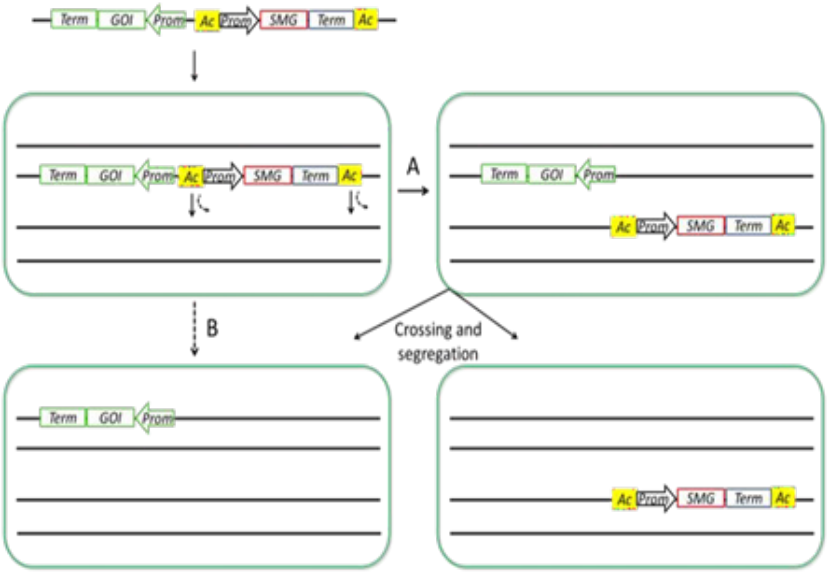
B. Cre/lox site-specific recombination system-This system comprises two key elements: (a) two lox P sites, each encompassing 34 bp inverted repeats arranged in a direct orientation, flanking a DNA sequence, and (b) the Cre gene that encodes a recombinase protein of 38 kDa, selectively binding to the lox P sites and excising the intervening sequence, including one of the lox P sites.



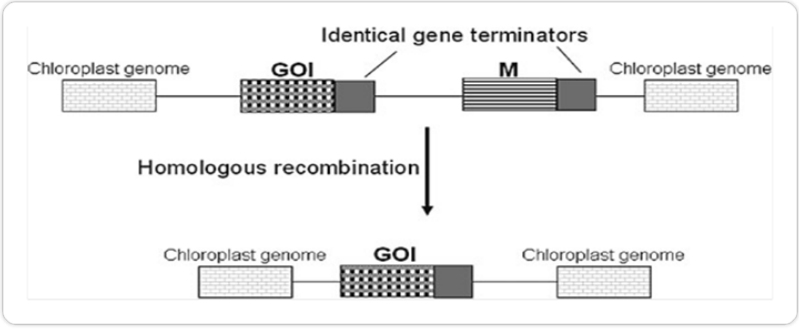
C. FLP/FRT recombination system- the FLP enzyme efficiently catalyses recombination between two directly repeated FLP recombination target (FRT) sites, eliminating the sequence between them.



D. Transposon-mediated repositioning- Ac elements are identical (4563bp) in length and Ds elements are Ac elements that have undergone deletions. Thus, all or part of transposase was eliminated. The incapacity of Ds elements to migrate in the absence of Ac is explained by this lack of transposase activity. Any Ds or Ac element can be excised by the transposase, which is encoded by Ac elements and can travel throughout the cell.



E. Chloroplast marker gene- vectors are designed with homologous flanking sequences on either side of the transgene. Because of direct repeats, co-integrates after recombination are intrinsically unstable. Therefore, recombination events create either the stable integration of a transgene of interest or loss of the



integrated vector.

F. Auto excision strategy marker is eliminated in the Tl seeds of the transgenic plants. The next generation of the transgenic plants are marker free. It is controlled by pollen- and lor seed-specific promoters and highly efficient auto excision of selective markers successfully achieved in tobacco. Autoexcisionstrategy relies on floral-specific promoters to regulate the expression of Cre recombinase to generate marker-free transgenic plants. The novel marker free approach mediated by the Cre-lox P recombination system and the Cre gene was under the control of floral specific promoter OsMADS45. The marker gene nptll was completely removed from the Tlprogeny of the rice with 37.5% efficiency.

Conclusion and Future Prospects

Clean gene technology represents a crucial advancement in genetic engineering, addressing the need for safer, more environmentally friendly, and precise methods for introducing advantageous traits into plant species. By eliminating the inherent risks associated with traditional marker genes, such as toxicity, antibiotic resistance transfer, and the creation of herbicide-resistant superweeds, clean gene technology offers a responsible approach to genetic modification. Its importance lies in its ability to reduce environmental impact, streamline regulatory compliance, enhance public acceptance, and enable precision in genetic modifications. Continued research and innovation in this field hold significant potential for advancing agriculture, ensuring food security, and promoting sustainable practices while addressing the pressing challenges of our time.

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