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Marker-Free Genetic Modification

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Marker-free transgenic plants are genetically modified organisms (GMOs) that do not contain selectable marker genes, which are often used during the initial stages of genetic transformation to identify successfully modified cells. The development of marker-free transgenic plants addresses concerns related to the potential negative effects of marker genes on human health, the environment, and regulatory acceptance.

Why marker free transgenic?

1. Biosafety and Health Concerns:
- Antibiotic Resistance: Selectable marker genes often include antibiotic resistance genes (*e.g.*, resistance to kanamycin or ampicillin). There is concern that these genes could potentially transfer to pathogenic bacteria, contributing to the growing issue of antibiotic resistance.
- Herbicide Resistance: Similarly, herbicide resistance genes (e.g., resistance to glyphosate) can raise concerns about unintended effects on non-target plants and the development of herbicide-resistant weed species.

2. Environmental Impact:
- Horizontal Gene Transfer: There is a risk of marker genes transferring to wild relatives or other non-target organisms through horizontal gene transfer. This could potentially disrupt local ecosystems and biodiversity.
- Gene Flow: The presence of marker genes in transgenic plants can lead to unintended gene flow to conventional crops, complicating co-existence and potentially affecting organic farming practices.

3. Regulatory Approval
4. Consumer acceptance

Strategies to obtain marker free transgenic plants

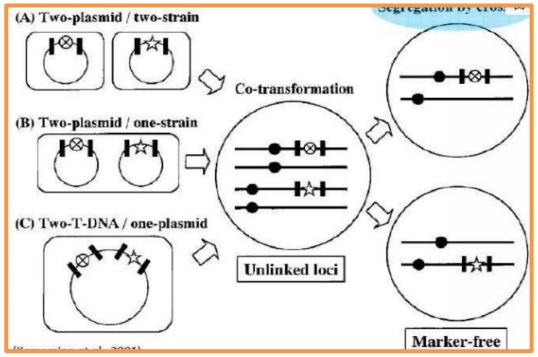
1. **Transformation without selection:** Theoretically, it should be possible to identify among a large number of cells the ones that carry a transgene directly by molecular methods particularly if transformation efficiencies can be improved. However, even in the days of auto mated analysis and polymerase chain reaction such a project is still highly demanding

2. Co-transformation

- One of the earliest methods was based on cotransformation of a transgene and a selectable marker delivered by two separate DNA molecules and thereafter, segregation of both in the progeny.

- This strategy is based on the fact that cells selected for the presence of the marker gene, often contain the non-selected gene of interest as well.
- Two different Agrobacterium strains each containing a binary plasmid carrying a single T-DNA region
- A single Agrobacterium strain, either containing one plasmid with two separate T-DNAs
- Containing two separate plasmids each containing a T-DNA

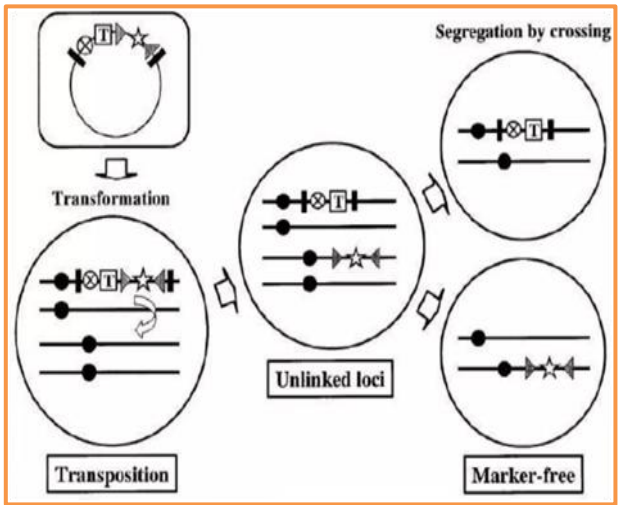
❖ **Limitation of Co-transformation**

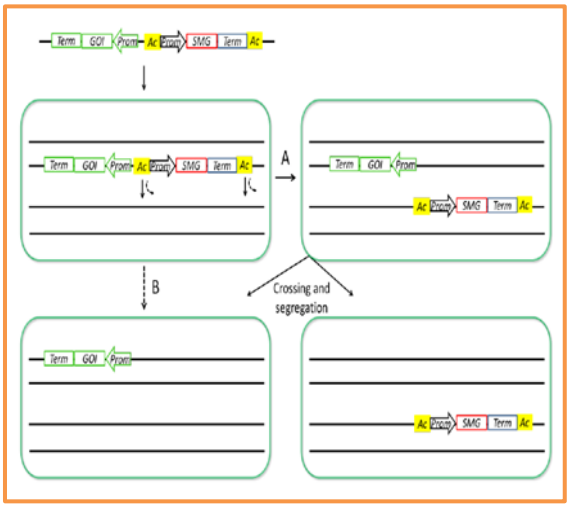


- Co-integration of both T-DNAs at the same genomic locus is frequently observed leading to linkage between the marker and the transgene, which makes their segregation impossible.
- Cannot be applied to sterile plants and vegetatively propagated species
- Requires the generation of many transformants and crossing steps

3. Selectable marker gene or the gene-of-interest on a transposable element

- Transposable elements (e.g. Ac/Ds from maize) can mediate repositioning of genetic material in the plant genome. The Ac/Ds transposable element system has been used for relocation and elimination of a selectable marker
- Re-insertion of the transposon. Here, the gene of interest (GOI) is placed on the transposable element. Thus, the GOI will be excised and can be reinserted in a locus that is not linked to the locus in which the selectable marker gene is located; they can be segregated in the next generation
- One relies on the fact that the transposon will not be re-inserted, selectable marker gene was inserted in an *Ac* derivative





- Transposon-mediated repositioning of the SMG. The SMG is cloned as part of a modified transposable element, e.g. the maize transposable element Ac, and linked to the gene of interest (GOI). Transposition may result in reinsertion of the modified element with the SMG (A); if the re-insertion occurs in an unlinked position, marker-free progeny may be obtained after crossing. Alternatively, no re-insertion occurs after excision of the modified transposable element (B), also resulting in the loss of the SMG.

❖ **Advantages:**

- This system has some advantages associated with the relocation of the gene of interest. For example, it permits to study a large range of position effects thereby generating an extensive qualitative and quantitative variation in expression levels from a single transpositionally active transformant line.
- Relocation allows elimination through recombination in the progeny of all sequences co-integrated at the original integration site. Thus the integration pattern is simplified and the

relocated transposon-borne transgene may be less susceptible to gene silencing than at the original integration.

❖ **Disadavantages:**

- The method shows low efficiency of marker gene elimination because of the tendency of transposable elements to reinsert in positions genetically linked to the original position.
- Genomic instability of transgenic plants because of the continuous presence of heterologous transposons and the generation of mutations because of insertion and excision cycles.
- Transposition can induce genome rearrangements, including deletions, inverted duplications, inversions, and translocations this system cannot be used for sterile plants and vegetatively propagated species and is impractical for plants with a long life cycle.

4. Site specific recombinase

- It is a removal of the selectable marker gene after the selection procedure via site-specific recombinases or zinc finger nucleases.
- Microbial site-specific recombinases have the ability to cleave DNA at specific sites and ligate it to the cleaved DNA at a second target sequence. The excision of foreign DNA that is placed in between recognition sites in a direct repeat orientation has been used to eliminate unwanted transgenic material from the nuclear genome of plants.
- The site-specific recombination systems can be divided in two categories according to the position of the recombinase gene.

5. Homologous recombination

The ratio of HR to NHEJ events increases if homologous sequences near the brake are available. During the repair process the gene can be converted or deleted. the method has some major disadvantages, like low efficiency, deletions of non-target genes, the recombination cannot be controlled and many transgenic events can be lost during the selection process. The mechanistic basis of the phenomenon is not yet understood and it is not yet known how the system could be applied in other crops.

6. Removal of transplastome marker gene

- In the last decade plastid genome (plastome or ptDNA) has become a popular target for engineering, as this has several advantages like potentially high level protein expression, maternal inheritance and non-dissemination of transgenes through pollen, high transgene copy number per cell and no detected gene silencing.
- However, selectable marker genes are unnecessary once transplastomic plant has been obtained. Moreover high levels of marker gene expression can cause metabolic problems.
- Four strategies to produce marker-free transplastomic plant have been developed:
- Homology- based excision via directly repeated sequences
- Excision by phage site-specific recombinanses
- Transient cointegration of the marker gene
- Co-transformation segregation approach.

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