CISGENICS IN PLANTS – A STEP IN THE RIGHT DIRECTION FOR THE SECOND GREEN REVOLUTION

Abstract

Conventional breeding using the old techniques of transgenesis produced successful outcomes. Modern cultivars, however, requires an increasing number of mixed features, necessitating pre-breeding techniques with wild species. To eliminate linkage drag in introgression and translocation breeding, time-consuming backcrosses and simultaneous selection procedures are needed. Use of cisgenesis with traditional sources of genetic variation can speed up the crop breeding. This is particularly true for heterozygous, vegetatively propagated crops and allopolyploids. As a result, the second/ever green revolution based on cisgenesis is what India needs to address the problems with yield security, quality characteristics, and nutritious vegetables and fruits.

Keywords: Cisgenics, marker-free plants, second green revolution

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I. INTRODUCTION

1. **The second green revolution:** The first green revolution's main goal was to increase crop productivity via the use of agricultural advancements. A yield plateau was achieved after some decades of the green revolution. Then, scientists began to concentrate on biotechnology. In particular, GM varieties were developed to get various kinds of stress resistance in plants. However, GM varieties have been embroiled in controversies over a variety of environmental and human health issues, leading to fewer acceptances of GM varieties. Therefore, the current situation itself is pushing us to find treatments that meet both conditions: increasing crop production with minimal risk to the environment. Also, we have to keep in mind that India is facing a problem of decreasing agricultural land and irrigation water due to an increasing population.

2. **Cisgenics (solution to the problem):** Hou et al. (2014) consider cisgenics as a solution which mixes modern biotechnology and conventional breeding techniques to speed up plant breeding. The foundation of cisgenics is based on the developments in the fields of genome sequencing, map-based cloning, allele mining, etc., which help to isolate and use indigenous genes. This natural gene, isolated from the crossable species, is called cisgenes in order to differentiate them from transgenes for environmental impacts. In short, cisgene is the same gene used by traditional breeding with the utilisation of modern biotechnology. So, the environmental side effects of GM crops are drastically reduced while speeding up the traditional breeding work.

II. EXPLORING THE CISGENICS

The core concept of early cisgenesis was to use genes and their elements obtained from the species itself. According to Schouten et al. (2006), cisgenesis is the change of a recipient plant's genetic make-up by a naturally produced gene from a species that is cross-compatible, together with its introns and native promoter and terminator flanked in the usual sense orientation.

The insertion of the gene HcrVf2, which was controlled by its own regulatory genes, into the cultivar Gala in apples was the first scientific description of a real cisgenic technique (a scab susceptible cultivar). For its upcoming genetically modified ryegrass, Pastoral Genomics in New Zealand has registered the trademark ‘Cisgenics’ [5].

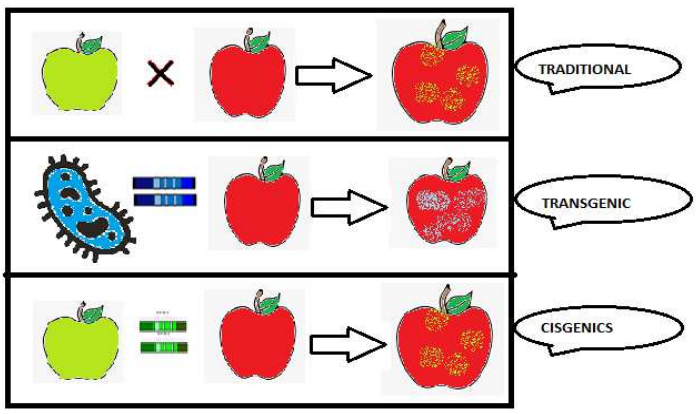

Figure1. Illustration of traditional, transgenic and cisgenic breeding in plants

Table 1: Distinction between transgenics and cisgenics

Comparison made for	Transgenics	Cisgenics
The origin of the gene	Typically, a species that is neither the receiver nor a sexually related species.	Sexually compatible or of the same species.
Alteration in the gene pool	Possible.	Not possible.
Novelty generation	The recipient plant shows novel traits not present before.	There is no additional trait generation.
Gene flow	Gene transfer among GM crops and their wild relatives creates a shift in vegetation	Gene flow does not affect fitness.
Legal issues	The safety of deliberate release is the prime issue.	The release of the crop is secure and can be treated as safely as conventionally bred plant.

Table 2: Dangerous possibilities due to the cisgenics are comparable with traditional breeding

Possible threats	Cisgenesis	Traditional breeding
DNA methylation	May be possible due to random insertion of genes.	Maize shows the transposons' activity naturally.
Mutation	Changes in the gene may happen due to the knockout of the genes.	Mutation is one of the natural phenomena in traditional breeding.
Gene duplication	A new sequence may be inserted several times in one genome.	Resistance genes or other multi-gene families shows such duplication.
Insertion of T-DNA borders and vector backbone [3]	These are non-coding sequences.	These are identical borders to plant.
Pollen dispersal to wild relatives [5]	Cisgenes are already descended from their wild relatives.	Such genes could have been used in the past.

III. CISGENESIS HAS BENEFITS OVER CONVENTIONAL BREEDING

1. **Solving the problem of linkage drag:** Linkage drag is associated with the transfer of unwanted genes from one genotype along with a desired one into the desired genotype. This problem is a major setback in traditional breeding. Cisgenesis transfers only desired genes and any unwanted gene segments can be avoided.
   For example, the potato late blight resistance development programme was hindered due to linkage drag. But now screening and isolation of native genes from donor plants and use of cisgenesis have solved this riddle [5].

2. **The genetic makeup of the plant is maintained:** Transferring desired traits into a new genome via conventional methods leads to changes in the progeny apart from the gene of interest. These are due to hybridization between parents. While cisgenesis allows only the desired gene to be transferred, all other genes remain in the progeny.

   Development of resistance with traditional breeding in the plant may face the problem of losing the yield potential of the recipient plant.

3. **Pesticide application is being reduced due to the development of pest resistance in the plant**

   Time saving: Backcrossing programmes are often time-consuming, and when it comes to forest breeding, this problem has become bigger due to the long plant cycle. Use of cisgenesis reduces the breeding cycle and saves time.

IV. A DETAILED PROCEDURE FOR THE DEVELOPMENT OF CISGENIC PLANT

The whole procedure includes steps of transformation, marker-free plant development, dealing with T-DNA borders or plant-derived T-DNA borders, as well as vector-backbone sequences. Let’s look at it point by point.

1. Transformation

- **Transformation with agrobacterium:** The predominant approach for transferring genes is Agrobacterium-mediated transformation in many crops. It is best to grow more cells with their newly acquired qualities and regenerate them into whole plants.

  To follow the target genes, selectable marker genes are often inserted. It is crucial that these genes, when taken from other, sexually incompatible species, are absent from the end result under the idea of cisgenesis [7].

  Use of biolistics: The selection marker gene and the gene of interest are included in two linear gene cassettes. Additionally, vector backbone integration is avoided.

- **Transposable elements mediated transformation**: The multi-auto transformation (MAT) vector has the transposable element in the T-DNA with a marker gene. Plants lacking selectable markers are allowed to grow after the transposon excision.

2. Methods to generate marker-free transformed plants

- **There is no selectable marker used:** Transformation without a selection gene and using the PCR to check the transformation of the gene(s) of interest will result in the regeneration of many shoots with only the gene(s) of interest. This type of work is done on many crops, like apples and potatoes.

- **Cotransformation:** It is used for crops that are sexually propagated and have relatively quick reproductive cycles. The use of the segregation principle is connected to cotransformation, in which the marker gene and the gene of interest are combined at unlinked sites, allowing the two genes to be subsequently segregated into distinct offspring in succeeding generations.

- **Active marker-removal by recombination:** The plants with low transformation efficiencies (vegetative crops) use site-specific recombination systems and remove the selection gene after the transformation.

  First of all, T-DNA with selectable markers is inserted. The markers are flanked by two recognition sequences related to the controlled recombinase. The second step requires activation of the recombinase, which excises the selectable marker gene.

3. **T-dna borders or plant-derived t-dna (p-dna) borders:** T-DNA is 25 base pairs long with flanked right (RB) and left (LB) border repeats. Cisgenesis necessitates such boundaries in the sexually compatible gene pool [6]. P-DNA borders are present in some species. R-genes transformation in potatoes showed 45% of the transformants with no integration of vector-backbone and T-DNA border sequences [8].

4. **Vector-backbone sequences:** Integration of such sequences is due to longer T-DNAs with read-through of the left border repeat. Therefore, a piece of the vector backbone stays attached to the border and is co-inserted into the genome. Such sequences can be found in 20% to 80% of cisgenic plants. These sequences are detected through various screening techniques and get discarded after transformation [4].

V. STATUS ON THE REGULATION OF CISGENIC CROPS

Most countries treat cisgenic and transgenic crops equally (the exception is Australia for some crops). The EU and the USA have less stringent regulations for it. The European commission believes that cisgenesis with genes present in the same gene pool shows similar effects to traditional breeding, while intragenesis and transgenesis may lead to novel problems [1].

VI. LIMITATIONS OF CISGENICS

1. The introduction of characters between gene pools is difficult.
2. It requires extraordinary proficiency and time compared to transgenic crops.
3. Isolation of the gene of interest is required from a compatible gene pool.

4. The protocol requires marker-free plants' generation.
5. Many lines after transformation should be discarded for removal of vector-backbone sequences [5].

VII. FUTURE THRUST

The loosening of regulation regarding cisgenic plants is fruitful for the world. Cost reduction for granting the cisgenic crops would be helpful to small-scaled industries. People should accept genetically modified crops. Cisgenic plants have potential to meet the global need for a more efficient and sustainable crop production.

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