**CISGENICS IN PLANTS – A STEP TOWARDS SECOND GREEN REVOLUTION**

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**ABSTRACT**

The classical methods of alien gene transfer by traditional breeding yielded fruitful results. However, modern varieties demand a growing number of combined traits, for which pre-breeding methods with wild species are often needed. Introgression and translocation breeding require time-consuming backcrosses and simultaneous selection steps to overcome linkage drag. Breeding of crops using the traditional sources of genetic variation by cisgenesis can speed up the whole process dramatically, along with usage of existing promising varieties. This is specifically the case with complex (allo) polyploids and with heterozygous, vegetative propagated crops. Therefore, cisgenesis is the basis of the second/ever green revolution needed in India to overcome the challenges related to yield security, quality traits and healthy vegetables and fruits.

**Keywords: Cisgenics; cisgenesis; marker-free plant; second green revolution**

1. **INTRODUCTION**
2. **Second green revolution**

Primary focus of first green revolution was higher yield of crop by using scientific developments in agriculture. After some decades of green revolution, a yield plateau was achieved. Then the focus of scientists moved toward **biotechnology**. Specially, GM varieties were developed to get various kind of stress resistances in plants. But GM varieties have fallen in the controversies regarding various kinds of environmental and human health related issues; which leads to the less acceptance of GM varieties. So, current situation itself telling us to get a solution which satisfy both the condition (increment in the crop production with minimal risk to the environment). Also, we have to keep in the mind that developing countries like India is facing problem of decreasing agricultural land and irrigation water due to increasing population.

1. **Cisgenics (a solution towards a problem)**

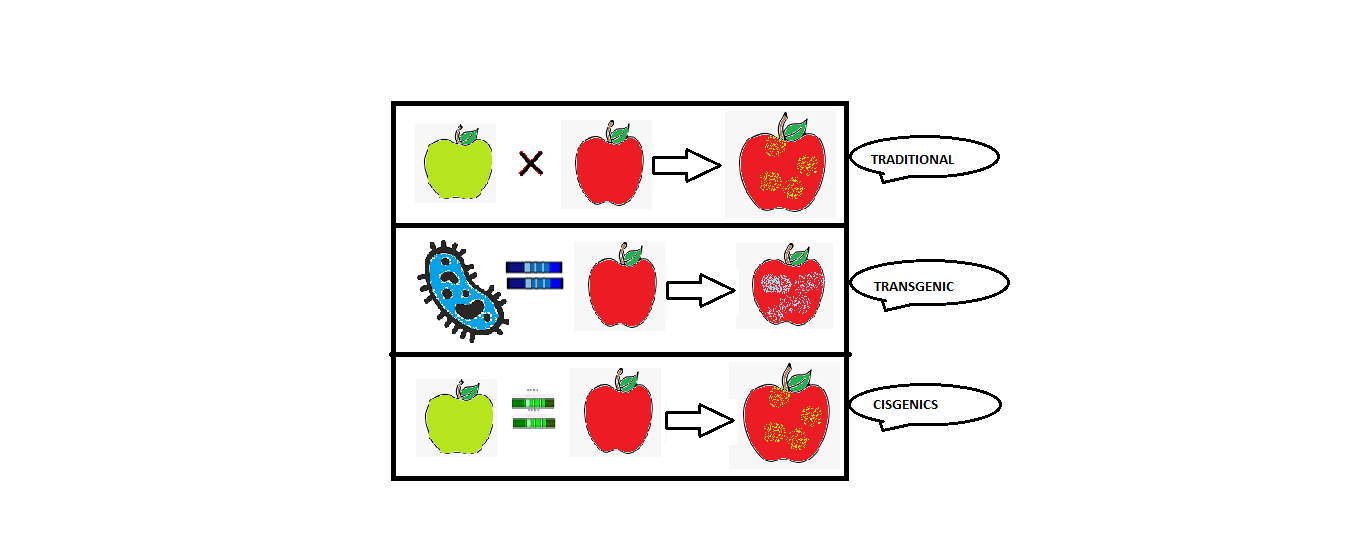
Hou *et al*. (2014) consider cisgenics as a solution which mixes the traditional breeding techniques with modern biotechnology to speed up the plant breeding. Foundation of cisgenics is based on the developments in the field of genome sequencing, map-based cloning, allele mining, etc. which helps to isolate and use indigenous genes. This natural indigenous gene, isolated from the crop plant itself or from crossable species (within the gene pool) are now called cisgenes in order to distinguish them from transgenes for environmental impacts. In a simple language, the donor genes used in the cisgenesis is the same which is used by traditional breeding, but using the modern biotechnology. So, environmental side effects of GM crops are reduced drastically while speeding up the traditional breeding work.

1. **DISSECTING THE CISGENICS**

The core concept of the initial cisgenesis was that the genes or gene elements should be obtained from the species itself. Schouten *et al.* 2006 defined cisgenesis as **the modification in the genetic background of a recipient plant by a naturally derived gene from a cross compatible species including its introns and its native promoter and terminator flanked in the normal sense orientation.**

The first scientific statement of a true cisgenic approach was reported in apple for the internal scab resistance through the insertion of gene *HcrVf2* influenced by their own regulatory genes into the cultivar Gala (a scab susceptible cultivar). Pastoral Genomics in New Zealand has registered the trademark Cisgenics and uses this trademark for their future genetically modiﬁed ryegrass [5].

**Figuratively…….**

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**Figure 1. Illustration of traditional, transgenic and cisgenic breeding in plants**

**Table 1: Difference between transgenics and cisgenics**

|  |  |  |
| --- | --- | --- |
| Comparison made for | Transgenics | Cisgenics |
| Source of the gene | Generally, a species which are neither the recipient nor a close sexually relative | Sexually compatible or the same species |
| Alteration in the gene pool | Possible | Not possible |
| Novelty generation | Recipient plant shows novel traits not present before. | No additional trait generation |
| Gene flow | Gene flow between GM crops and its wild relatives creates shift in vegetation | Gene flow does not affect fitness |
| Legal issues | Safety of deliberate release is prime issue | Release of the crop is secure and can be treated as safe as conventionally bread plant |

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

|  |  |  |
| --- | --- | --- |
| Possible threats | Cisgencs | Traditional breeding |
| DNA methylation | May be possible due to random insertion of gene | Maize shows transposons activity naturally |
| Mutation | Changes in the gene may happen due to knock out genes | Mutation is one of the natural phenomena in traditional breeding |
| Gene duplication | New sequence may insert several times in one genome | Resistance genes or other multigene families shows such duplication |
| Insertion of T-DNA borders and vector backbone [3] | These are non-coding sequences | These are identical borders to plant |
| Dispersal of pollen to wild relatives [5] | Cisgenes are from their wild relatives already | Such genes may have been employed earlier |

1. **ADVANTAGES OF CISGENESIS OVER CONVENTIONAL BREEDING**
2. **Solving the problem of linkage drag**

Linkage drag is associated with the transfer of unwanted genes from one genotype along with desired one into desired genotype. This problem is the major setback in traditional breeding. Cisgenesis transfer only desired genes and any unwanted gene segments can be avoided.

Example: Potato late blight resistance development programme was hindered due to linkage drag. But now screening and isolation of native genes from donor plant and use of cisgenesis have solve this riddle [5].

1. **Genetic makeup of the plant is maintained**

Transferring desired traits into new genome *via* conventional method leads to changes in the progeny apart from the gene of interest. These is due to hybridization between parent. While cisgenesis allow to transfer only desired gene and keeping all other genes as such in the progeny.

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

1. **Reduction in pesticide application due to development of pest resistance in the plant**
2. **Time saving**

Backcrossing program are often time consuming and when comes to forest breeding, this problem is become bigger due long plant cycle. Use of cisgenesis reduce the breeding cycle and save the time.

1. **DETAILED PROCEDURE OF CISGENIC PLANT DEVELOPMENT**

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 as point by point.

1. **Transformation**
2. **Transformation with agrobacterium**

In many crops, Agrobacterium-mediated transformation is the preferred method for gene transfer. Cells with their newly acquired traits should be preferably multiplied and regenerated into entire plants.

Selectable marker genes are usually added to track the genes-of-interest. Within the concept of cisgenesis it is imperative that such genes, when derived from other, sexually non-compatible organisms, are not present in the final product [7].

1. **Use of biolistics**

Two linear gene cassettes containing the selection marker gene and the gene of interest are used. It also avoids the integration of vector backbones.

1. **Transposable elements mediated transformation**

Marker gene is present on a transposable element in the T-DNA, called the multi-auto transformation vector (MAT). Subsequent transposon excision allows the development of plants without a selectable marker.

1. **Methods to generate marker-free transformed plants**
2. **Avoiding the use of any selectable marker**

Transformation without a selection gene and checking the presence of the gene(s) of interest by PCR will lead to the regeneration of many shoots with only gene(s) of interest. This type of work is done in many crops like apple and potato.

1. **Cotransformation**

It is used for sexually propagated crops with relatively short reproduction cycles. Use of segregation principle is associated with cotransformation in which marker gene and the gene of interest are integrated at unlinked positions which permit the subsequent segregation of the two genes into different progeny in the subsequent generations.

1. **Active marker-removal by recombination**

The plants with low transformation efﬁciencies (vegetative crops) requires the use of site-specific recombination systems for removal of the selection gene after the transformation step done.

First of all T-DNA is inserted which carries a selectable marker gene. This marker gene is flanked by two recognition sequences specific for a recombinase whose activity can be controlled. Second step requires activation of the recombinase which excises the selectable marker gene.

1. **T-dna borders or plant-derived t-dna (p-dna) borders**

In Agrobacterium-mediated transformation the DNA fragment expected to be inserted into the plant is called T-DNA (transfer-DNA with 25 base pairs). The T-DNA is flanked by the right (RB) and left (LB) border repeats. Agrobacterium-mediated transformation for cisgenesis requires generation of plants with border sequences isolated from the sexually compatible gene pool [6].

Suitable plant transfer DNAs (P-DNAs) resembling T-DNA borders have been identiﬁed in several species.

In potatoes transformed with R-genes, analysis of T-DNA border integrations revealed that 45% of the transformants containing R-genes did not show integration of vector-backbone and T-DNA border sequences [8].

1. **Vector-backbone sequences**

Integration of such sequences is due to transfer of longer T-DNAs with read-through of the left border repeat. As a result, part of the vector backbone remains attached to the border, and may be co-inserted into the plant genome. Twenty to eighty percent cisgenics plant shows such sequences. These sequences are detected through various screening techniques and get discarded after transformation [4].

1. **STATUS ON THE REGULATION OF CISGENIC CROPS**

In most countries, the release of cisgenic or intragenic crops currently falls under the same regulatory guidelines as transgenic crops (exception is Australia for small group of crops). The EU and the USA have less stringent regulations for it. European Commission believe that an unmodiﬁed gene used in cisgenesis is already present in the breeders’ gene pool which leads to similar effects as traditional breeding, while intragenesis and transgenesis may lead to novel problems [1]

1. **LIMITATIONS OF CISGENICS**

* Characters outside the sexually compatible gene pool cannot be introduced.
* Requires extraordinary proficiency and time compared to transgenic crops.
* Gene of interest may not be readily accessible but have to be isolated from the sexually compatible gene pool.
* Protocol requires for marker free plants generation.
* Many lines after transformation should be discarded for removal of vector-backbone sequences. [5]

1. **FUTURE THRUST**

Future developments regarding the generation and commercialization of cisgenic crops will depend on willingness to apply less stringent regulation to these crops worldwide. Also reducing the costs for approval of cisgenic crops, would be especially helpful to small-sized breeding and seed companies. Public perception has proven to be essential for the approval of genetically modiﬁed crops. Modiﬁcations based on the sexually compatible gene pool carries a high potential for generating plants with environmental, economic and health beneﬁts that may be essential for meeting the global need for a more efﬁcient and sustainable crop production.

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