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**Review Article**

# Cisgenesis A Sustainable Approach of Gene Introgression and Its Utilization in Horticultural Crops: A Review

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**A B S T R A C T**

Plant breeding is on-going process for which heritability of trait and variation is required. Cisgenesis and Intragenesis are biotechnological approaches utilized to create more genetic variation in existing germplasm with the purpose to improve their quality and quantity. The advancement of sequencing technologies and the availability of genome information facilitates isolation of intact Cis-genes, together with associated promoter/terminator from one species and are inserted into the genome of the same or a closely related, crossable species. While, in case of intragenesis different coding and regulatory sequences are assembled either in sense or in antisense orientation. This review describes the current status, historical overview, applications, limitations and future prospects of Cisgenesis and Intragenesis in improvement of horticultural crops. Further comparative study of transgenesis, cisgenesis and intragenesis described. This technique generally utilized to develop disease resistance cultivar such as late blight resistant potato by transferring gene *Rpi-sto1*, *Rpi-vnt1.1*; similarly scab resistance apple cultivar developed. Thus we can conclude Cisgenesis as a powerful alternative approach to transfer gene of interest without linkage drag in a single-step and Cisgenic & intragenic derived genetically modified plants (GMP) are eco-friendly as classic bred plants and therefore exempted from GMP legislation.

## Introduction

Plant breeding is a long-term process, often takes more than 10 years to develop a variety especially for a tree crop like apple. Therefore there is urgent need to employ biotechnological tools to mitigate abiotic and biotic stress by developing resistant and improved quality varieties of vegetables, fruits and ornamental crops that can survive well in future. Advances in plant molecular biology and bioinformatics furnishes the isolation of plant genes associated with economically important traits, [1] which provides alternative approaches to on genetic variation present in unrelated species. Plant diversity is the fundamental for the future horticulture industry to buffer insect, disease and abiotic stresses and develop better quality products [2]. We can get the desired gene from existing germplasm or they can be recombined to isolate novel trait. Transgenic plants (GMP) have shown promising result in developing tolerance to abiotic and biotic stress. But, commercialization and consumption of transgenic horticulture crops lagged behind by agronomic crops due to involvement of environmental and health risk. To overcome limitations associated with utilization of transgenic horticultural crops, new biotechnological plant technology (NBPT) lead to advancement of new eco-friendly and speedy approaches such as cisgenesis and intragenesis. In cisgenesis and intragenesis breeding plants must be transformed with genetic material derived from the species itself or from closely related species which are capable of sexual hybridization. This new technique is in contrast to transgenesis where genes and DNA sequences translocate between any species.

Evert Jacobsen and Henk Schouten are considered as the 'fathers' of cisgenesis and Schouten coined the term 'cisgenesis' in 1999. The principle of cisgenesis entails that the genes or gene elements should be derived from the species itself, while there were no requirements for the coding sequence to include introns or for the regulatory sequences to originate from the same gene as the coding sequences. The definition of cisgenesis introduced by Dutch Scholars Schouten, Jacobsen and Krens which was internationally accepted when published in international journals in 2006[3]. According to this concept the origin of the cisgene is extended to the gene pool of sexually compatible species and the cisgene include its promoter, introns and the terminator in the normal-sense orientation.In production of Cisgenic derived plants, isolation of gene, cloning and transformation are important steps. When the gene gun is used to insert gene cassettes into a host crop genome, the concepts are easier to follow because only the gene cassettes can be inserted into the host genome [4]. However, when Agrobacterium DNA delivery system is utilized for modification of a crop genome, then intragene must be inserted "within borders isolated from the crossable DNA pool" called P-DNA (instead of T-DNA) borders [5].Availability of genome and sequencing information facilitates all these steps in achieving Cisgenic/Intragenic plants. Erstwhile, Agrobacterium-mediated transfer of a gene from a crossable (sexually compatible) plant where T-DNA borders may remain in the resulting organism after transformation cisgenesis with T-DNA borders also called cisgene[6]. While, in case of intragenesis concept P-borders and vector-backbone sequences do not raise from the sexually compatible DNA pool [7]. Even, intragenic plants do not contain foreign genes such as selectable marker genes or insecticidal genes. Also gene silencing techniques may be utilized, e.g. through RNAi[8].

However, the definitions of cisgenesis and intragenesis should be more precise with respect to the regulatory requirements for Cisgenic and intragenic crops in the future will be subjected to less stringent regulatory procedures compared to transgenic crops. Likewise, it will be important that international definitions are harmonized to prevent difficulties for the global trade of these products. Overall, we can say thatIntragenesis and cisgenesis exploit similar genepool as traditional breeding does. Hence,cisgenic crops considered more natural/acceptable than transgenic crops [9].Furthermore, some modification may help to develop marker and vector-backbone gene free intra-genic/cisgenic transformants or their progeny. As production of marker-free transgenic crops eliminates risk ofhorizontal gene transfer and could mitigate vertical genetransfer[10].

## Need for cisgenesis

In case of conventional breeding introgression of foreign gene from related or unrelated species through backcross breeding needs almost 8-10 years. While backcrossing there is probability of transfer of undesirable alleles along with desirable one due to tight linkage between them this phenomenon called linkage drag.

Such limitations of classical breeding can be overcome by employing cisgenesis and intragenesis approach.

Vegetatively propagated crops such as potato, banana, apple etc. are heterozygous (polyploid) in nature, so introgression of desired gene or gene stacking is difficult through conventional breeding. There are two ways to improve the existing varieties with, i.e. (induced) mutation breeding and genetic modification. Mutation breeding is still followed in vegetatively propagated ornamentals that are heterozygous like rose, Chrysanthemum and in fruit trees like apple and peach[11]. But in few crops like potato, application of mutation breeding found to have adverse effect on traits like tuber skin colour and other tuber related traits. In that case, cisgene and intragene would be useful to avoid such hazard from unidentified hitch hiking genes [12].

Higher expression levelof a trait can be obtained by re-introducing the gene of the traitwith its own promoter and terminator (cisgenesis) or with apromoter and terminator isolated from the sexually compatiblegene pool (intragenesis). Lower expression levels can be obtainedthrough different silencing constructs (intragenesis).

From safety point of view, Cisgenic plants are considered non-transgenic as genes within the same gene pool transferred into novel varieties without undesirable genes and alien gene. It is random process, similar to traditional induced translocation breeding [13]. As a result such plants can be introduced into the food chain without any regulation.

One of the main requirements is the absence of selectable marker genes (such as antibiotic resistance genes) in the genome.

The sensitive issues with regard to transfer of foreign genes and antibiotic resistance are overcome by employing such advanced biotechnological tool [14].

## Comparison between cisgenesis, intragenesis, transgenesis and conventional breeding

So far, we reviewed that cisgenic plants are similar to conventional products as extra gene derived from the same gene pool of the recipient parent and also contain genes and regulatory elements in their native state. However, some differences exist that distinguish cisgenesis from conventional breeding as cisgenesis modify target gene while in classical breeding there is possibility of contamination by undesirable gene. Also, traditional breeding take decades to reach a cultivar with desirable traits while cisgenesis furnishes fast, safe and acceptable introgression of desired gene. For e.g. Introgression of apple scab resistance gene Vf from Malus floribunda 821 into marketable high quality apple cultivars took approximately 50 years through classical breeding[15].The fundamental differences between cisgenic, intragenic, transgenic from conventional breeding are listed in Table 1.

### *Application of cisgenesis in crop improvement*

There are several vegetables, fruits and ornamental plants which were modified through cis-intragenesis technique. However, it is restricted to few crop species. Few examples discussed where these new approach have been exploited to improve specific defects in crop.

Cisgenic melon plants developed by transforming genes *At1* and *At2* from wild melon to susceptible variety resulted in enhanced activity of glyoxylate **a**mino**t**ransferase and resistance against powdery mildew (*P. cubensis*) [16].

Acetolactatesynthase gene utilized as a cisgenic selectable marker for Agrobacterium-mediated transformation in Chinese cabbage (Brassica rapassppekinensis)[17].

Cisgenic apple plants resistant to scab diseasedeveloped by transferring the *Rvi6* scab resistance gene of *Malus floribunda* 821, using a new transformation vector based on the Flp/*FRT* recombinase system in cultivated cultivar[18].

A cisgenic apple line C44.4.146 developed from susceptible cultivar "Gala Galaxy" using the cisgene FB_MR5 from wild apple *Malus* × *robusta* 5 (*Mr5*), which confers resistance to fire blight[19].

Cisgenesis is an acceptable tool to make potato farming more sustainable by developing durable late blight resistant variety of potato. Late blight resistance (*R*) genesfrom crossable wild potato species cloned and transferred to susceptible cultivar by *Agrobacterium tumefaciens*-mediated transformation without non-potato genes[20]. A stack of multiple *R* genes were inserted into established varieties, thereby creating a dynamic variety in which the composition of the stacks may vary over space and time. Cisgenic plants were selected based on the expression of all inserted *R* genes and trueness-to-type.

Cis-intragenesis utilized in microalgae for commercial extraction and production of attractive and highly valued compounds, particularly ofpolyunsaturated fatty acids and carotenoids[21]. In particular, these technologies represent possible alternatives for increasing the accumulation of commercially relevant metabolites through the overexpression of native enzymes, in accordance with the needs of consumers and in line with environmental conservation [22].

These new breeding technologies can lead not only to cisgenic plants but also to marker-free transgenic plants[23].

Cisgenesis may furnish the second green revolution in India by improving traditional plant breeding [24]. Cisgenesis has great potential to overcome bottlenecks of classical breeding and transgenesis. Currently, plant breeders and researchers objective is to create genetic variability, improve quality and to breed for stress tolerance and disease and pest resistance (plant incorporated protection, PIP) in adapted cultivar by incorporating additional copies of a given gene. This gene modification implemented in several important crops like cereal crops, legumes, root plants, vegetables, turf grasses, tropic plants, woody species, medicinal and ornamental plants, as well as fruit plants [25,26].

Recently, new cisgenic/intragenic and genome editing approaches based on the advancement of sequencing technologies and the availability of genome information facilitates the exploitation of PGR in crop breeding [27].

## Limitations

The major bottleneck in cisgenesis may be identification of genes encoding for the desired traits and detailed experimental characterisation required for the efficient use of identified genes, which is cost- and time-consuming.

**Table.1** Factors that make cisgenesis and intragenesis different from transgenesis and conventional breeding

| Characters | Conventional breeding | Transgenic | Cisgenic | Intragenic |
|---|---|---|---|---|
| 1. Transfer of unique gene | Not possible | Done from unfamiliar species | Done between crossable related species | Done between crossable related species |
| 2. Nature of gene | Natural gene | Foreign gene may be natural or artificial | Natural gene (Gene of interest including its own regulatory elements and introns) | Natural gene (Gene of interest from one source and include regulatory elements and introns from other source) |
| 3. Speed | Slow | Rapid | Rapid | Rapid |
| 4. Time-period requirement | Long-term process | Single- step process | Single- step process | Single- step process |
| 5. Marker gene | Not removed | Not removed | Removed | Removed |
| 6. Eco-friendly | Yes | No | Yes | Yes |
| 7. Freefrom GMP legislations | Yes | No | Yes | Yes |
| 8. Change in gene pool | No | Yes, widen the genetic resources | No | No |
| 9. Possibility of linkage drag | Yes | Yes | No | No |

For effective expression, cisgene should get inserted at active region of gene but there is possibility to get inserted at random places of the host plant genome, which could alter the expression of the same gene or of adjacent genes, thus producing variations that are difficult to predict [28].

Transfer of polygenic trait is difficult through this technique. So this technique restricted to improve monogenic traits.

To ensure that new resistances are not broken rapidly, a combination of genes should be inserted into the recipient plant (gene stacking, multi-gene cassettes).

Plant transformation remains a tedious procedure; particularly in fruit trees[29]. The same limitations (e.g. random integration of genetic constructs) as for transgenic plants apply. The complete removal of selection markers is indispensable; the efficiency depends on the method applied and needs to be verified individually.

Native promoters may lead to constitutive expression of genes, which may be above the native expression level of a gene, as shown for the HcrVf2 gene conferring apple scab resistance[30]. The altered expression can alter the environmental behaviour of the plant and also furnish considerations concerning exposure of potential consumers necessary. Gene silencing is not possible through cisgenesis can be done by utilizing intragene. Silencing of unwanted genes like gene involved in biosynthesis of asparagine in potato attempted by Rommens *et al.*, [31] and Chawla *et al.*, [32].

### *Exemptions from GMO legislations*

So far, there is stiff public opposition to the use of genetically modified organisms (GMOs) or plants (GMPs). But, still several studies states again and again the valuable contribution of GM crops to the development of a sustainable type of agriculture. This discrepancy between public opinion and the scientific evidence requires an elucidation. The development of new technologies in genetic engineering is clouding the scientific debate and challenges the public regulation [33, 34, 35, 36].

Jacobsen and Shouten[37] propose to exclude organisms created using cisgenesis from the legislation applied to GMOs. A vast amount of safety research has been performed on GM plants and concluded that they don't possess a greater adverse impact on health and the environment than other crop developed by conventional plant-breeding technologies[38]. One should focus on the risk assessment and regulation of the trait or the product rather than the technology used to produce it[39].

Europeans gave reason for accepting new genetic engineering techniques as it reduces pesticide use in case of apple [40]. Similarly many more researchers summarized the potential acceptability of cisgenic plants by consumers and embraced the application of cisgenes for improvement of various vegetable and fruit crops like potato, grapevine, papaya and apple etc.

Cisgene micro-translocation is a potent and promising alternative to achieve sustainable crop breeding. It facilitates single-step gene transfer without linkage drag and even insertion-related side effects can be overcome by normal selection. With aid of marker assisted selection, stacking of (resistance) genes would be more feasible to furnish durable resistance. Hence, this new gene modification technique and molecular biology advances have further prospects to improve existing varieties by directly using genes from the gene pool of breeders.

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