



Review Paper

## Cisgenesis and Intragenesis: Twin Sisters for Crop Improvement

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### Abstract

*With the increase in awareness of the people about health and bio safety issue, there is reluctance for the acceptance and use of transgenic crops since it includes combination of genes between species that cannot hybridize by natural means. As an alternative way to transgenesis, two different approaches, cisgenesis and intragenesis were developed. Both these approaches use genetic transformation techniques to introduce new genes (just like transgenesis) but the donor should be from the same or sexually compatible species. In cisgenesis, the unchanged, contiguous and naturally occurring genome fragment containing the gene of interest along with its own introns and regulatory sequences are fragmented as such, and transferred into the host genome. Whereas in case of intragenesis, gene of interest is taken from other source while the regulatory elements and introns from another source and a new combination of DNA fragments are created artificially through in vitro rearrangement. But, one point to be noted here is that the source should belong to the same species or from a cross compatible species. Public research institutes based on European Union (EU) play a big role in the R&D of these techniques. These techniques will be of immense use for crop improvement if the end products are classified as non- GMOs but will have limited use if classified as GMOs. Therefore, the legal status of these techniques will decide whether to use these techniques only for crops with very high value or will use extensively for a broader field of applications.*

**Key words:** Cisgenesis, intragenesis, transgenesis, genetic transformation, GMOs.

### Introduction

The area under transgenic or genetically modified (GM) crops has been increased at a faster rate and the area being 160 million hectares<sup>1</sup>. But one of the main concerns of the public about transgenic crops is the use of artificial combination of genetic elements which are derived from different organisms that are not crossable by natural means<sup>2-4</sup>. The full potential of GM crops can be realized only with an increased acceptance by the general public. Moreover, the costly, hectic and lengthy procedures for obtaining approval of these crops and the threat for potential health risks and the spread of new genes into other unrelated crops are the major drawback in the path of implementing these techniques. Keeping in view of the above drawbacks and to ensure an eco- friendly crop improvement techniques, cisgenesis and intragenesis approaches were developed as alternatives to transgenesis<sup>1</sup>. In both the cases, a DNA fragment from the species itself or from a cross compatible species is inserted into the plant genome. In cisgenesis, the inserted gene is unchanged and contiguous and flanked by its own introns and regulatory elements whereas, in intragenesis, an artificially synthesized novel combination of DNA fragments, but from the species itself or from a cross

compatible species is used for the transformation process<sup>5</sup>. In contrast to this, transgenesis make use of foreign DNA from other species, may be microbes. The same gene pool is exploited by intragenesis and cisgenesis that are available for traditional breeding<sup>1</sup>.

### What are cisgenesis/ intragenesis?

Cisgenesis is the production of genetically modified crops/plants using donor DNA fragment from the species itself or from a cross compatible species<sup>6</sup>. The newly introduced gene is unchanged and includes its own introns and regulatory sequences<sup>5</sup> and is free of vector DNA, except T- DNA border sequences that flank the cisgene<sup>6</sup>. The resultant phenotype of the cisgenic plant can be achieved through conventional breeding also, but, it will take a much longer time<sup>7</sup>. One of the most important plus point of cisgenesis is that it introduce only the desired gene, thus avoiding linkage drag that can be resulted from conventional cross breeding and also it eliminate hectic and time consuming backcrossing to recover the recurrent parent genotype<sup>8</sup>.

Intragenesis is very much similar to cisgenesis but the difference lies in the fact that intragenesis allows creation of novel combinations of DNA fragments<sup>6</sup>. New genes can be created *in vitro* by combining functional genetic elements like promoters, coding region and terminator sequences and this new chimeric gene can be inserted into existing varieties<sup>9-13</sup>. Intragenesis also allows the use of antisense or RNA interference (RNAi) with the aim of silencing the gene(s)<sup>5,6</sup>. Unlike cisgenesis, the resultant phenotype of the intragenic plant cannot always be achieved through conventional breeding since the level and pattern of expression of the newly created gene combination may differ from the normal/natural situation<sup>6</sup>.

### Application of cisgenesis/ intragenesis in crop improvement

Cisgenic plants are enriched through the addition of one or more genes that belong to the same species or from a cross compatible species<sup>14</sup>. New traits are introduced or existing traits are modified to add value to the existing germplasm/ lines. Such modifications include improved resistance to biotic and abiotic stresses, quality enhancement and nutritional value etc<sup>5</sup>. Crops that can be commercially clone, like potato, apple, strawberry, and grapevine, were some of the crops in which cis/intragenic approaches for improvements were attempted for the first time<sup>1</sup>. Recently, cisgenesis is applied to apple and potato in order to obtain polygenic durable resistance to apple scab (*Venturia inaequalis*) and *Phytophthora infestans*, respectively. Moreover, the *MdMYB10* transcription factor from apple that upregulates the anthocyanin pathway, leading to red-fleshed apples have also been introduced<sup>15</sup>. A cisgenic approach, with the aim of enhancing fungal disease resistance in grapevine through the insertion of a grapevine pathogenesis-inhibiting protein is currently under development<sup>16</sup>. Another cisgenic approach has been used in poplar in which plants with different growth types are produced due to overexpression of growth-related poplar genes<sup>17</sup>. Till now, cisgenesis is still in research phase but in the coming 10 years, it will find its application in crop improvement<sup>6</sup>.

In USA, intragenic potatoes with improved processing qualities were developed by Rommens and coworkers. Potatoes with improved processing qualities have been obtained through silencing of polyphenol oxidase gene (*Ppo*) to reduce black spot bruise<sup>9</sup> and through silencing of three different genes to limit acrylamide formation and also reduce cold-induced sweetening<sup>10</sup>. Intragenesis is currently being used to produce non-browning apples by developing RNAi silencing constructs against the apple *polyphenol oxidase* gene ([www.okanaganbiotechnology.com](http://www.okanaganbiotechnology.com)). An intragenic strawberry with increased resistance to grey mould was developed which overexpress the polygalacturonase inhibiting protein thereby, reducing the effect of the fungal polygalacturonase<sup>18</sup>. Another intragenic approach was used in alfalfa to enhance forage quality with reduced levels of lignin in the plants through silencing of the *caffeic acid o-methyltransferase gene (Comt)*<sup>19</sup>.

Recent applications of cisgenesis/ intragenesis are given below in tabular form (table-1).

### Drawbacks of cisgenesis/ intragenesis

The gene(s) outside the sexually compatible gene pool cannot be introduced and the generation of intra-/cisgenic crops is time consuming as compared to transgenic crops. Moreover, the gene of interest or fragments of genes may not be readily available but need to be isolated from the sexually compatible gene pool<sup>1</sup>. There is also a chance that the introduction of cisgene/ intragene may influence the expression of genes that are already present in the recipient genome, if they are located around the integration site<sup>6</sup>. Position effect may lead to alteration of the gene expression<sup>20</sup> and phenotypic differences<sup>6</sup>. The production of marker-free plants often requires the implementation or development of new techniques and such techniques may not be readily available for the crop to be engineered. Thus, considerable efforts have to be given to produce high numbers of transformants, especially for crops with low transformation efficiencies<sup>1</sup>.

### Comparison of the end product of cisgenesis/ intragenesis and conventional methods:

The cisgenes already belong to the same gene pool of the recipient plant<sup>6</sup> and contain genes and regulatory elements in their natural state<sup>5</sup>. Therefore, end products could be same as produced by conventional breeding approaches<sup>14,21</sup>. However, some differences exist between end products obtained by cisgenesis and conventional breeding. In a cisgenic plant, the cisgene is present as an extra copy in the recipient genome<sup>6</sup>. The presence of such endogenous genes and regulatory elements in another plant could result in modified levels of expression of the target gene(s) and even gene silencing<sup>5</sup>. In case of intragenesis, the inserted genes are new combinations of functional genetic elements having same native origin, thus, the intragene expression may deviate from the natural situation. Hence, comparison cannot be made with the conventionally bred crops, but rather a case-by-case study need to be performed. If intragenesis is used in silencing a single endogenous gene, the end products may be compared with knock-out mutants obtained by mutation breeding<sup>6</sup>.

The random insertion of a cisgene/ intragene may result in a mutation in the recipient genome at the insertion site and such insertion may influence cisgene/ intragene expression both quantitatively and qualitatively as compared to the gene in its natural genomic context and may lead to disruption of gene function thereby, inducing phenotypic effects<sup>6</sup>. However, such effects of cisgene/ intragene integration are natural phenomena and similar to those occurring during transposon transition<sup>22</sup> and translocation breeding<sup>20</sup>.

### Safety issues regarding cisgenesis/ intragenesis

Different views regarding the safety issues of cisgenesis/ intragenesis have been given. According to Haverkort *et al.*, 2008, cisgenesis may be safer than conventional breeding since

the introduction of unwanted traits via linkage drag can be prevented<sup>23</sup>. However, the issue of any endogenous gene silencing need to be considered. Contrary to the above view, Russell and Sparrow, 2008 argued that similar safety issue as transgenic organisms should be concerned for cisgenic/intragenic organisms since they may contain new proteins or greatly altered levels of familiar proteins<sup>24</sup>.

When *Agrobacterium* mediated transformation is used for inserting the cisgene(s), fragments of the right border (RB) and left border (LB) will be integrated along with the cisgene in the plant genome and since these short sequences are non-coding, they are unlikely to have a phenotypic effect<sup>14</sup>. But in case the RB and LB sequences become part of an open reading frame of a recipient gene, they can be translated into protein and fusion protein can be formed. Such situation is undesirable and screening should be done by investigating the nature of the recipient genomic sequence that is flanking the T-DNA insert. For intragenesis, safety evaluation should be done on case-by-case basis since the expression of intragenes is expected not to have always corresponded to the expression of the native corresponding genes in their natural genomic position<sup>6</sup>.

### Regulatory issues regarding cisgenesis/intragenesis

The concept of cisgenesis was introduced by Schouten, Jacobsen and Krens in 2006 and they proposed that plants developed through cisgenesis should be exempted from regulation<sup>14,21</sup>. In 2007, the European Commission (EC) set up a working group named New Techniques Working Group (NTWG) to evaluate different novel breeding techniques and to determine whether they should be regarded as genetic modification techniques<sup>1</sup>. Moreover, to know the current status and application of novel plant breeding techniques, European Union's Joint Research Centre (JRC) make a survey and according to the survey, amongst other new techniques intragenesis/cisgenesis ranked 1<sup>st</sup> and 2<sup>nd</sup>, respectively, with respect to the number of scientific publications and filed patents<sup>8</sup>.

According to EFSA Panel on Genetically Modified Organisms (GMO), similar hazards can be obtained through cisgenic and conventionally bred plants, while novel hazards can be associated with intragenic and transgenic plants. All of these breeding techniques can produce variable frequencies and severities of unintended effects and the frequency of unintended effects may differ between breeding techniques and cannot be predicted, hence, needs to be assessed case by case<sup>25</sup>.

### Future trend

A major rationale for using these approaches in plant breeding is the issue of consumer acceptance and the argument that the use of DNA from within cross-compatible species (mimicking the potential end products of traditional breeding) is a safer option than transgenesis<sup>5</sup>. There is reasonable evidence that consumers are more comfortable with the use of genes from within the same species than transgenes originating from organisms such as bacteria<sup>14,26</sup>. However, future developments regarding the generation and commercialization of intragenic and cisgenic crops will depend on application of less stringent regulation to these crops worldwide<sup>1</sup>. Development of cisgenesis/intragenesis into a powerful new breeding tool will depend on several factors like treatment of existing legal frameworks towards cisgenic plants<sup>27</sup> consumer acceptance of end products; whether plants and end products derived from them must be considered as GMOs or non-GMOs; and intellectual property rights (IPRs) on GM genes and technologies<sup>14</sup>.

Both intragenic and cisgenic crops are acceptable to more number of people than transgenic crops<sup>18,28-31</sup>. Recently a survey was conducted in the USA and from that it came to know that consumers are willing to pay more money for intragenic vegetables with enhanced nutritional value when the vegetables are labelled as such<sup>32</sup>. On the other hand, many consumers and environmental organizations are against the acceptance of the cisgenic and intragenic concepts and oppose that the regulatory approval of these plants and its end products should be different from that of transgenic plants<sup>1</sup>.

**Table-1**  
**Different cisgenic/ intragenic crops developed or under development**

Crops/ plant	Trait	Gene(s)	Cisgenic/intragenic (cis/ intra)	References
Apple	Scab resistance ( <i>V. inaequalis</i> )	<i>HcrVf2gene</i>	cis	33
Apple	Scab resistance ( <i>V. inaequalis</i> )	<i>HcrVf2 gene</i>	cis	34, 35
Melon	DownyMildew resistance ( <i>Pseudoperonospora cubensis</i> )	<i>At1/At2- glyoxylate aminotransferase</i>	cis	36
Potato	Late blight resistance ( <i>P. infestans</i> )	<i>Rpi gene</i>	cis	37
Potato	Black spot bruise tolerance	<i>Ppo gene</i>	intra	9
Potato	Lower acrylamide level	<i>Ppo, R1 and PhL gene</i>	intra	10
Potato	Lower acrylamide level	<i>Asparagines synthetase genes (StAs1 and StAs2)</i>	intra	38
Potato	High amylopectin	<i>GBSS</i>	intra	39
Strawberry	Grey mold resistance	<i>PGIP</i>	intra	18
Grapevine	Fungal disease resistance	<i>VVTL-1</i>	cis	16
Poplar	Different growth type	Genes involved in growth	cis	17
Perennial rye-grass	Drought tolerance	<i>Lpvp1</i>	intra	40
Alfalfa	Reduced lignin level	<i>Comt</i>	intra	19
Durum wheat	Improved baking quality	<i>1Dy10</i>	cis	41
Barley	Improved grain phytase activity	<i>HvPAPhy_a</i>	cis	42

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