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Cisgenesis and intragenesis a new tool for conventional plant breeding: A review

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Abstract
Heterozygous nature and linkage drag often influence stacking of desirable genes from wild sources in conventional breeding for crop improvement. Recombinant DNA technology and transgenesis have enabled transformation of alien gene into plants across the kingdom barriers. The release of genetically modified (GM) crops into agricultural production has raised considerable debate, especially among the general public, politicians and bureaucrats. To meet this concern, cisgenesis were developed as new tools in crop modification and plant breeding. The cisgenesis concept imply that plants must be transformed with genetic material derived from the species itself or from closely related species capable of sexual hybridization and also foreign sequences such as 'Selectable marker genes' and 'Vector backbone' sequences should be absent. Intragenic vector construction involves identifying functional equivalents of vector components within plant genomes and using these DNA sequences to assemble vectors for plant transformation.

Keywords: Cisgenesis, intragenesis, selectable marker, vector backbone

Introduction

Plant biotechnology refers to the development of technologies based on biological systems to improve agricultural practices. The hallmark of green biotechnology is the genetic modification of crops in order to confer new traits, by either the expression of a foreign gene or the suppression of an endogenous protein to modify a function. Such organisms are known as Genetically Modified Organisms [1]. In 2017, the global area of biotech crops was 189.8 million hectares, including 24 countries that adopted this technology (ISAAA, 2017).

Although transgenic crops are a promising tool for agriculture and have shown to improve economic development, they have been a major concern for public opinion since their introduction in the 1990s. Public acceptance is an important factor for the successful development of a technology, and both ethical concerns and risk perceptions have emerged about biotech crops, mainly due to most of the approved GMOs containing genetic elements derived from non-compatible species and containing selectable markers for antibiotics or herbicide resistance. In addition, there are several limitations for the production and commercialization processes of genetically modified crops, the costly and lengthy procedures for obtaining approval of these crops are major barriers for implementation. New strategies and approaches are therefore required in the development of the genetically engineered crops of the future.

Cisgenesis

Schouten *et al.* [2] introduced the term cisgenesis and 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. Since cisgenes shared a common gene pool available for traditional breeding the final cisgenic plant should be devoid of any kind of foreign DNA viz., selection markers and vector- backbone sequences [3].

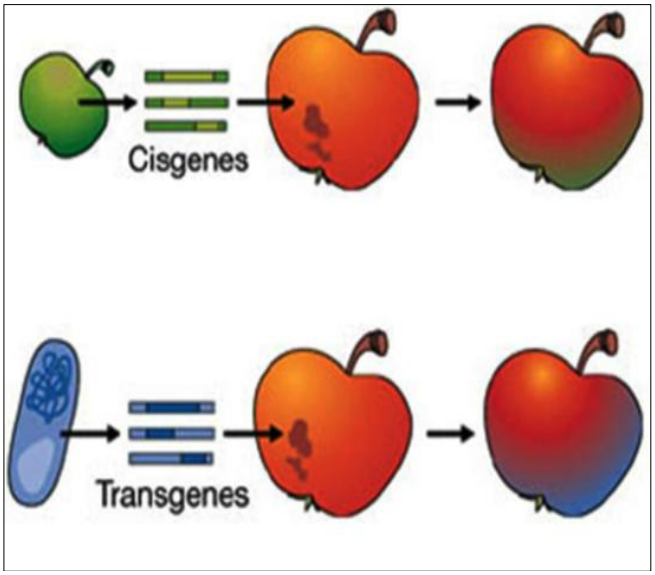

Fig 1: Comparison between Cisgene and transgene technology (*Source*: Schouten *et al.* [2])

Cisgenesis is the genetic modification of a recipient plant with a natural gene from a crossable—sexually compatible plant.

Transgenesis is the genetic modification of a recipient plant with one or more genes from any non-plant organism, or from a donor plant that is sexually incompatible with the recipient plant.

Intragenesis

The definition of the intragenic transformation concept was introduced by Rommens in 2004. Intragenesis allows for the design of cassettes combining specific genetic elements from plants belonging to the same sexually compatibility gene pool. Accordingly, coding regions of one gene (with or without introns) can be combined with promoters and terminators from different genes from the same sexually compatibility gene pool [4]. Additionally, silencing constructs can be designed by combining several different genetic elements from the same sexually compatibility group. When using Agrobacterium-mediated transformation, the T-DNA border sequences should originate from the sexually compatible DNA pool (P-DNA borders).

Considering public concerns about safety issues regarding transgenic crops and with the aim of meeting these reservations and at the same time ensuring an environmentally sound and efficient plant production, the two transformation concepts intragenesis and cisgenesis were developed as alternatives to transgenic crop development [5]. The two concepts are based on the exclusive use of genetic material from the same species or genetic material from closely related species capable of sexual hybridization. This is in contrast to transgenesis where genes and DNA sequences can be moved between any species [4, 5]. The gene pool exploited by intragenesis and cisgenesis is accordingly identical to the gene pool available for traditional breeding. Furthermore, foreign genes such as selection marker genes and vector-backbone genes should be absent or eliminated from the primary intragenic/cisgenic transform ants or their progeny.

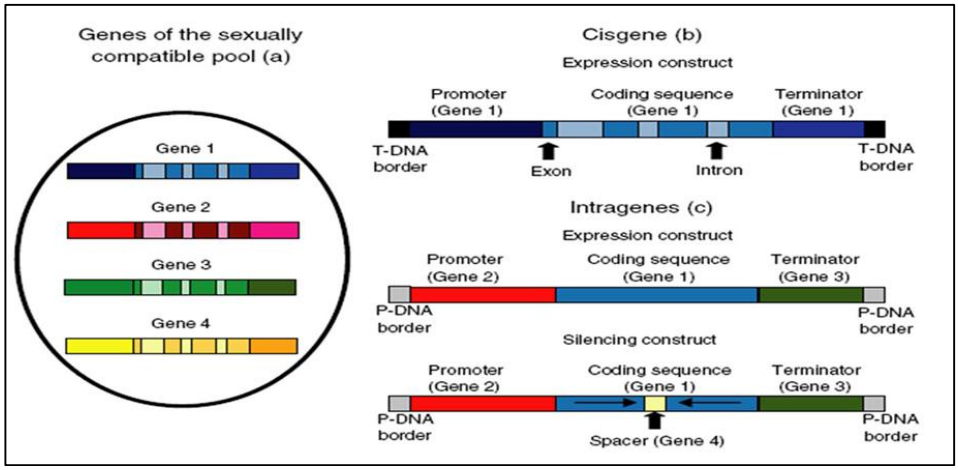

Fig 2: Illustration of cisgene and intragene constructs as defined by Schouten and coworkers in 2006 and Rommens in 2004, respectively. [Holme *et al.*, (2013)].

Advantages of cisgenesis over conventional breeding.

Faster and precise: The transfer of genes between sexually compatible plants can be speeded up. Development of new cultivars without many generations of hybridization and selection to recover the desired plant. Gene transfer from related wild species by hybridization may require upto 15-20 generations of additional plant breeding. The transfer of apple scab resistance gene Vf, which has been cloned of late, into the novel cultivars using cisgenic technique could give rise to better results within a short period of time [7]. The comparatively long period of tree breeding, which may last decades via traditional techniques, makes the genetic modification of trees a striking target [8]. Cisgenesis could be employed for the rapid introduction of desired traits into commercially successful cultivars without changing their constructive characteristics through introgression by traditional methods. In general, gene transfer technologies may successfully curtail the juvenile period of fruit trees [9].

Genetic makeup of plant variety is maintained; this techniques is a particularly efficient method for cross-fertilizing heterozygous plants that propagate vegetatively, such as potato, apple and banana [10]. It can directly improve an existing variety without disturbing the genetic make-up of the plant. Traditional introgression breeding of cross-fertilizing plants does not allow the introduction of genes from wild germplasm without mixing up the combination of alleles in the existing heterozygous elite recipient genotype.

Improving traits with limited allelic variation; higher expression level of a trait can be obtained by re-introducing the gene of the trait with its own promoter and terminator

(cisgenesis) or with a promoter and terminator isolated from the sexually compatible gene pool (intragenesis). Lower expression levels can be obtained through different silencing constructs (intragenesis).

Linkage drag is avoided; Genetic material encoding for inferior properties are sometimes so tightly linked to the gene of interest that recombination between this gene and the unwanted genetic material is almost impossible. In consequence, this 'linkage drag' may render the backcrossed line useless (Fig-3). Some of these genes affect the normal features of the crop as they may engage in the production of diverse kinds of toxins or allergens [3]. The intragenesis/cisgenesis approach avoids potential 'linkage drags' associated with classical backcross breeding. In vegetatively propagated crops like potatoes and apples, their heterozygous nature further brought impediment in successful transfer of traits of interest [11].

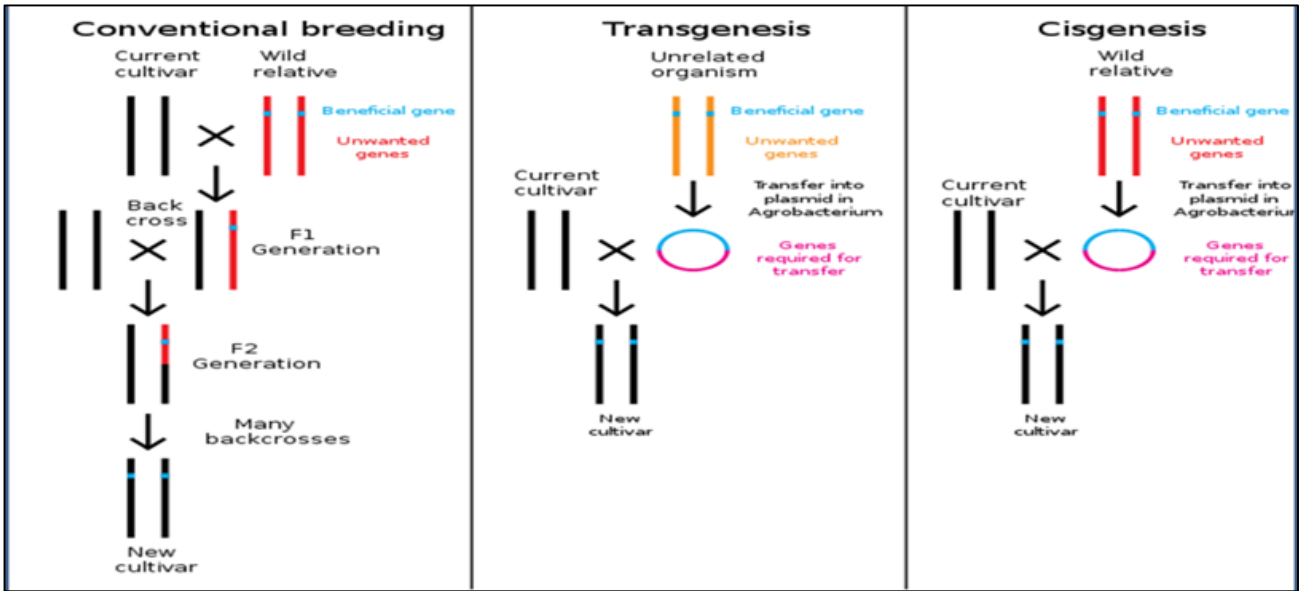

Fig 3: Genetic material encoding for inferior properties are sometimes so tightly linked to the gene of interest that recombination between this gene and the unwanted genetic material is almost impossible. In consequence, this 'linkage drag' may render the backcrossed line useless

Techniques used for the generation of cisgenic crops

Cisgenic and intragenic plants are produced by the same transformation techniques used for developing transgenic plants. Genes must be isolated, cloned or synthesized and transferred back into a recipient where stably integrated and expressed. The development of intragenic/cisgenic plants requires additional research and techniques when compared to the development of transgenic plants.

The prerequisites for developing these plants are;

- Availability of desired genes and gene elements within the sexually compatible gene pool.
- The production of plants devoid of the foreign DNA from marker genes and vector-backbone sequences.

Strategies to eliminate marker genes

(1)Marker-free transformation

The simplest way to eliminate marker genes from transgenics is to avoid their use in the transformation of plants. De Vetten *et al*., (2003) [12] first reported the transformation of potato *cv*. Kanico using *Agrobacterium tumefaciens* strain AGL0 and no selection marker genes. The best results were obtained using the *Agrobacterium tumefaciens* strain AGL0, which exhibits extremely high transformation efficiency because it contains a DNA region originating from a super virulent *A. tumefaciens* strain. In this experiment, approximately 5000 regenerated shoots were isolated and analyzed by PCR. Transgenic lines were obtained with an average frequency of 4.5%. Using supervirulent strains of *Agrobacterium*, transformation frequencies can be substantially raised with certain plant species (e.g. up to 5% in potato) which may generate a sufficient number of transform ants eliminating the need for a selection step.

The only problem observed with this system was the presence of vector backbone sequences in most of the transform ants, which is a character as undesirable as selection marker genes. In spite of several advantages, some disadvantages are associated with this method. There is no control over selective growth of the transform ants, and the researcher has to screen several putative transform ants to confirm the integration of the transgene(s), which is expensive and time consuming. Uncontrolled regeneration of a large number of chimeric plants is another drawback in the case of selection without the use of antibiotics as observed in tobacco.

(2) Co-transformation

Co-transformation is a simple and highly effective method to eliminate marker genes from the nuclear genome of transgenic plants. Co-transformation involves the transformation of plant cells with two plasmids that target insertion at two different loci in the plant genome [13] Fig (4). One plasmid carries a selection marker gene while the other carries a gene of interest. *Agrobacterium* mediated co-transformation is achieved in three ways. Though simple and effective, this method carries several unavoidable limitations. It is time consuming and compatible only for sexually propagated fertile plants. Secondly, the tight linkage between co-integrated DNAs may limit the efficiency of co-transformation. Also, this method may not be suitable for species with very low transformation efficiency. An improvement in the co-transformation procedure was introduced by Komari *et al.* [14].Three ways of Co-transformation is represented below:

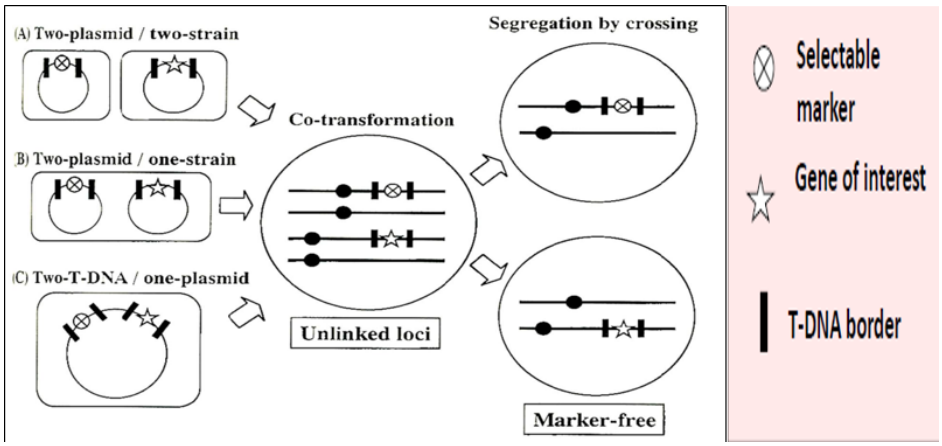

Fig 4: Co-transformation involves the transformation of plant cells with two plasmids that target insertion at two different loci in the plant genome

(3) Recombinase induced excision

Site specific recombination, DNA strand exchange takes place between segments possessing only a limited degree of sequence homology. Three site specific recombination systems are well known and described for the elimination of selection marker genes. These are the *Cre*/*lox* site specific recombination system from bacteriophage P1, the *FLP*/*FRT* recombination system from *Saccharomyces cerevisiae*, and the *R*/*RS* recombination system from *Zygosaccharomyces rouxii*. The recombination sites are typically between 30 to 200 nucleotides in length and consist of two motifs with a partial inverted repeat symmetry. The recombinase binds to these motifs, which flank a central crossover sequence at which the recombination takes place. This system is often referred to as "auto-excision" or self-excision. In spite of several advantages, these methods carry some limitations. Due to the prolonged presence of bacterial recombinase in the plants, there may be unwanted changes in the plant genome at the sites of transgene excision. An auto excision strategy has its limitations. It is, for example, successful only in flowering plants, and it is not useful for vegetatively propagated plants like grapes, potato, or banana [11].

4. Transposan based exicision

Transposons or the "jumping genes" have been used as a tool to excise the marker sequence from the gene of interest. The strategy makes use of the *Ac/Ds* transposition system and is primarily based on the fact that the DNA sequences located in the *Ds* repeats can be translocated to excise along with the *Ds* element [15]. This method involves *Agrobacterium*-mediated transformation followed by intragenomic relocation of the transgene of interest, and its subsequent segregation from the selectable marker in the progeny or direct excision of the marker gene from the genome. Both strategies were developed using the maize *Ac/Ds* transposable element, and the system could be adapted successfully to use similar autonomous transposable elements.

The major advantage of this strategy is that, the marker free transgenic plants can easily be screened at the T0 generation, avoiding the need for sexual reproduction and indicating the applicability of the strategy to the vegetatively propagated crops also. In spite of all the advantages, a few limitations are inevitable, like the very low regeneration frequency of marker-free transgenic plants and the genomic instability of transgenic plants because of the continued presence of heterologous transposons. The requirement of genetic crossing or segregation for separating the transgene and the marker gene is a time consuming process and can thus be counted as one of the drawbacks of this method.

Crops and traits currently modified by cisgenesis

Crop	Type	Gene	Trait	Author
Potato	Expression	*R-genes*	Late blight resistance	Huang *et al*., (2004)
Apple	Expression	*HcrVf2*	Scab resistance	Vanblaere *et al*., (2011)
Grapevine	Expression	*VVTL-1, NtpII*	Fungal disease resistance	Dhekney *et al*., (2011)
Poplar	Over Expression	Genes involved in growth, *PAT*	Different growth types	Han *et al*., (2011)
Barley	Over Expression	*HvPAPhya*	Improved grain phytase activity	Holme *et al*., (2012)
Durum wheat	Expression	*1Dy10*	Improved baking quality	Gadaleta *et al*., (2008)
Barley	Expression	*gTIP2 and gGS1a*	Nitrogen Use Efficiency (NUE)	Lutken *et al*., (2004)
Rye-grass	Expression	*Lpvp1*	Drought tolerance	Hanley, Z. (2008)

Conclusion

Traditional breeding provides us excellent plants with many genes working together in a concerted manner. Plant breeders may have a limited knowledge of the underlying genetic networks, but they are still able to develop superior crop cultivars. Because of the complexity of plant functions, traditional breeding has been widely used and will remain crucially important for agricultural production. cisgenic crops are acceptable to more number of people than transgenic crops. However, future developments regarding the generation and commercialization of cisgenic crops will depend on application of less stringent regulation to these crops worldwide. Development of cisgenesis into a powerful new breeding tool will depends on several factors like treatment of existing legal frameworks towards cisgenic plants, 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. Knowledge of traditional breeding remains crucial for selection of cisgenic plants in breeding by cisgenesis and intragenesis.

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