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Review Article

Cisgenesis and Intragenesis in Modern Plant Breeding

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A B S T R A C T

Current varietal improvement program involve modern plant breeding approaches which are highly dependent on new molecular technologies to modify genetic composition of plant. Traditional breeding approaches are based on gene pool of crossable species, associated with linkage drag problem. In transgenic techniques, major concern related to the origin of transgene which is isolated from unrelated species such as microorganism and animal which limits its public acceptance. As an alternative, cisgenesis and intragenesis are modern plant breeding approaches that can act upon intermediate to transgenic and traditional breeding method. Both these technologies involve biotechnological tools for transfer of gene between crossable species but foreign genes such as vector backbone and marker genes should be absent from the primary cisgenic and intragenic transformants. However, unlike cisgenesis that has all the necessary regulatory elements of a natural gene, intragenes are hybrid genes that can have genetic elements from different genes and loci, thus by using different promoter or terminator regions, expression of gene can be modified. According to these concepts, a number of traits have been improved in varieties of crop plant such as potato, apple, durum wheat, barley, grapevine, melon etc. It is believed that cisgenesis and intragenesis will revolutionize traditional plant breeding because these techniques speed up the gene transfer without linkage drag. 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 is a safer option than transgenic.

Keywords

Cisgenesis, Intragenesis, Linkage drag, Modern plant breeding, transgenesis

Introduction

Conventional breeding approach mainly concentrates on transfer and introgression of desired gene/genes present in cross compatible source plant. Existing variability within a species or its sexually compatible close relatives is useful for genetic improvement through traditional breeding (Acquaah 2015).Transfers of gene require repeated back crossing from a donor with recipient plant followed by selection for the desired trait. The final product from conventional breeding is a plant containing a gene of interest from the donor plant in combination with the unwanted genes leading to linkage drag (Jacobsen and Schouten 2007). These problems can be overcome by transferring specific target genes, utilizing newly developed breeding techniques such as transgenesis and cisgenesis and intragenesis. A number of transgenic crops have been

developed using genetic engineering technique however, this originally promising method for crop improvement has been controversial since transgenic plants contain DNA sequences from incompatible or unrelated organism. The widespread application of transgenic techniques in food plants raised public issues mainly about health safety although there is no scientific evidence that transgenic crops are harmful to the human health (Fahlgren *et al.,* 2016, Kamthan *et al.,* 2016). However, its use continues to be a topic of debate due to questions concerning intellectual property and biosafety issues drawn in open field planting (Lucht, 2015; Yaqoob *et al.,* 2016). To overcome these deficiencies of transgenics and linkage drag of conventional breeding approach, another generation of Genetically Modified Organisms (GMO) is first introduced by Schouten, Jacobsen and Krens in 2006 (Schouten *et al.,* 2006). Later, the intragenesis concept was used to describe the combination of genes or intergenic fragments from the cross compatible plants. These methodologies, in contrast to transgenics, only allow combinations of DNA sequences originated from the naturally compatible species and improved final product shares genes of cross compatible source (Almeraya and Sánchez-de-Jiménez 2016, Schouten *et al.,* 2006, Schouten and Jacobsen 2008). In this context, the efforts of the present study were oriented towards basic concept, methodology and comparative analysis of the related techniques. This is followed by describing role in cop improvement, regulatory issues, current status and future prospects of these techniques.

What are Cisgenic and Intragenic?

Schouten *et al.,* (2006) defined cisgenic plant as the alteration of a receiver plant genome using native gene or genes from a closely related species. The native gene contains intron and flanking regions such as its own regulatory sequences in the sense orientation identical to that found in the donor plant. In principle, cisgenic crop is generated through transferring a native and entire copy of a natural gene complete with its own regulatory regions and maintaining its natural genetic elements. In cisgenesis, foreign genes including the vector backbone genes and the selectable marker genes are not found in the final product. The difference between cisgenesis and conventional breeding is that cisgenic crops contain only the desired gene and there are no other genes being transferred (Espinoza *et al.,* 2013). Unlike transgenesis, in the cisgenic approach, plants receive genes only from crossable species via genetic engineering and those imported genes are under the control of their own native regulatory components in their natural orientation (Schouten *et al.,* 2006).

In intragenesis, different plant genetic components are recombined in vitro to produce an expression gene construct that is introduced into a plant within the same sexually compatible gene pool (Rommens *et al.,* 2007). However, unlike cisgenes, intragenes are hybrid genes i.e. they can have genetic elements from different genes and loci. Thus, by using different promoter or terminator regions, expression of genes can be modified. Hence, there is a possibility of new gene recombination by *in vitro* rearrangements of functional genetic elements. The phenotype obtained through intragenesis is not essentially same as traditional breeding because the expression level of hybrid gene may differ from that observed naturally (Devi *et al.,* 2013). It is essential for *Agrobacterium* mediated transformation to use sequences of T-DNA border from sexually compatible DNA pool i.e. P-DNA borders. In intragenesis, antisense or RNA interference (RNAi) can be

employed for silencing the gene (Schaart and Visser 2009).

Comparison between cisgenesis, intragenesis, transgenesis and conventional breeding

Conventional breeding approach is associated with linkage drag problem in introgression breeding since many unwanted genes introgressed together with desired gene. The cisgenes already belong to the same gene pool of the recipient plant and contain genes and regulatory elements in their natural state. Therefore, end products could be same as produced by conventional breeding approaches. However, some differences exist between final products obtained by cis/intra-genesis, transgenic and conventional breeding (Figure 1 and Table 1). In a cisgenic plant, the cisgene is present as an extra copy in the recipient genome (Schaart and Visser, 2009).

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 (Lusser *et al.*, 2011). In case of intragenesis, the inserted genes are new combinations of functional genetic elements having same native origin, thus, 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 (Schaart and Visser, 2009).

Development of Cisgenic and Intragenic plants

Developmental process of cisgenic and intragenic plants are similar to the transformation techniques used for transgenic plants. Additionally, some prerequisites are required for the development of cisgenic and intragenic plants. The prerequisites for the development of cisgenic and intragenic plants are the availability of desired genes within the sexually compatible gene pool and the modified plant should be devoid of the foreign DNA of marker genes and vector-backbone sequences. The major steps involve isolation of the gene of interest, insertion into a suitable plasmid vector, transformation using an appropriate method, elimination of selectable marker genes and, at last, recombinants selection having desired gene sequences (Moradpour and Abdullah 2017). For the production of cisgenic plants *Agrobacterium*-mediated transformation has been used more frequently, while biolistic transformation techniques have been used less frequently (Lusser *et al.*, 2012). A critical requirement to produce eco-friendly cisgenic and intragenic plants are the elimination of selectable marker genes. Moreover, vector backbone and selectable marker genes should not be present in the primary cisgenic and intragenic transformants. Different approaches allowing the construction of marker free transformation, co-transformation, excision induced by recombinase or transposon have been illustrated in the literatures (Dalla Costa *et al.*, 2016, Moradpour and Abdullah 2017, Mujjassim *et al.*, 2019)

Table.1 Comparison between traditional approach, transgenic, cisgenesis and intragenesis

Characters	Traditional approach	Transgenic	Cisgenesis	Intragenesis
Unique gene transfer from	Not possible	Unrelated source	Compatible source	Compatible source
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 regulatory elements from other source)
Speed	Slow	Rapid	Rapid	Rapid
Time required	Time consuming	Very less	Less (but more than transgenic)	Less (but more than transgenic)
Markers gene	Not removed	Not removed	Removed	Removed
GM regulatory constrain	No	Yes	No (Applicable in countries where treated as transgenic)	No (Applicable in countries where treated as transgenic)
Linkage drag	Yes	No	No	No
Availability of gene of interest	Not readily available	Readily available	Not readily available	Not readily available
People acceptance	High	Low	Intermediate	Intermediate
End product	Similar to cisgenic	Different	Comparable to traditional approach	Less comparable to traditional approach

Fig.1 Comparative Illustration of traditional breeding, transgenic and cis/intragenesis as defined by Schouten *et al.*, (2006)

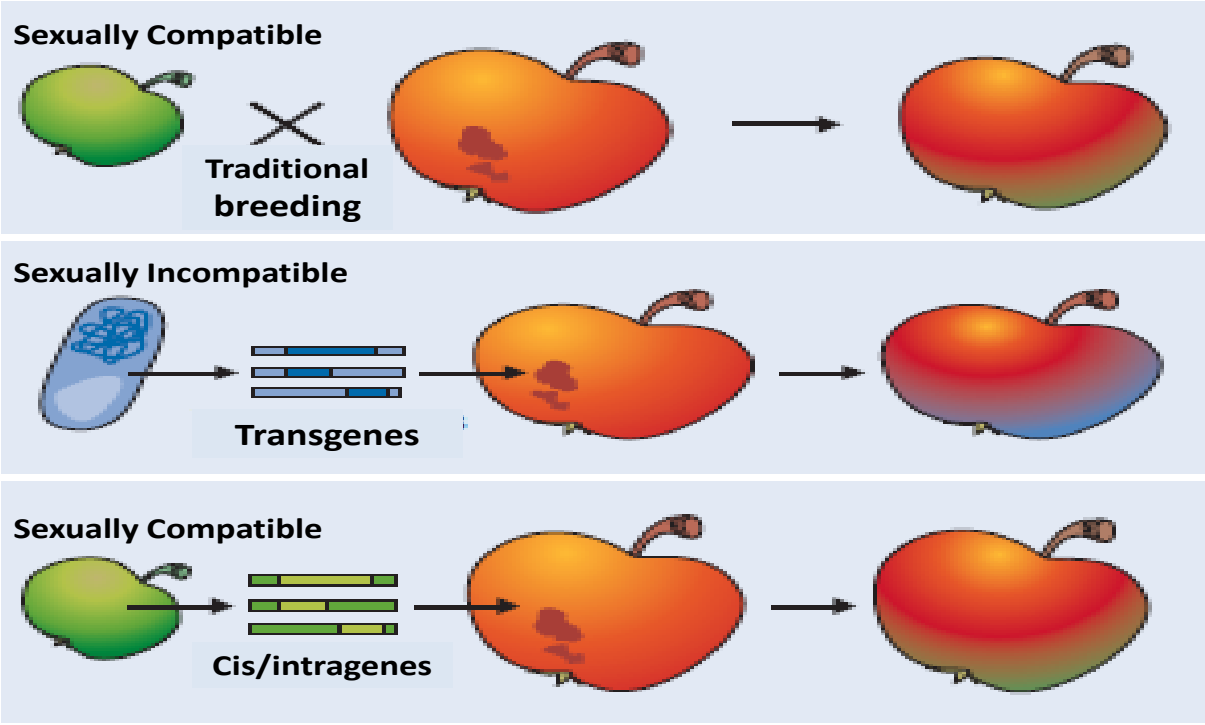

Table.2 Trait improved or varieties developed using cisgenesis and intragenesis techniques (Holme *et al.*, 2013, Moradpour and Abdullah 2017).

Crops	Type	Gene	Trait improved	References
Cisgenic				
Solanum tuberosum (Potato)	Expression	R-genes	Late blight resistance	Haverkort *et al.*, (2009)
Triticum turgidum var. durum (Durum wheat)	Expression	*1Dy10*	Improved baking quality	Gadaleta *et al.*, (2008)
Triticum aestivum (Bread wheat)	Introduction of genes only from closely related species	Wheat class I chitinase gene	Fungal pathogens resistance	Maltseva *et al.*, (2014)
Hordeum vulgare (Barley)	Over expression	*HvPAPhy_a*	Improved grain phytase activity	Holme *et al.*, (2012)
Cucumis melo L. (Melon)	Introduction of genes from related species	*At1/At2*- glyoxylate aminotransferase	Downy mildew resistance	Benjamin *et al.*, (2009)
Malus domestica (Apple)	Expression	*HcrVf2*	Scab resistance	Vanblaere *et al.*, (2011)
Vitis vinifera (Grapevine)	Expression	VVTL-1	Fungal disease resistance	Dhekney *et al.*, (2011), Dalla Costa *et al.*, (2016)
Poplar (*Populus* spp.)	Over expression	Genes involved in growth	Different growth types drought	Han *et al.*, (2011)
Intragenic				
Solanum tuberosum (Potato)	Silencing	*GBSS*	High amylopectin	de Vetten *et al.*, (2003)
Solanum tuberosum (Potato)	Silencing	*Ppo*	Preventing black spot bruise	Rommens *et al.*, (2004)
Solanum tuberosum (Potato)	Silencing	*Ppo, R1, PhL*	Limiting degradation of starch. Limiting acryl-amide formation	Rommens *et al.*, (2006)
Solanum tuberosum (Potato)	Silencing	*StAs1, StAS2*	Limiting acrylamide formation	Rommens *et al.*, (2008)
Solanum tuberosum (Potato)	Silencing	*StAs1*	Limiting acrylamide formation	Chawla *et al.*, (2012)
Lolium perenne (Perennial ryegrass)	Overexpression	Lpvp1	Drought tolerance	Bajaj *et al.*, (2008)
Medicago sativa (Alfalfa)	Silencing	Comt	Reduced levels of lignin	Weeks *et al.*, (2008)
Strawberry (*Fragaria* spp.)	Overexpression	*PGIP*	Grey mould resistance	Schaart (2004)

Role of cisgenesis and intragenesis in crop improvement

A number of plant species with commercially widespread clones, seed propagating and wood species have been developed (Table 2). Potato, apple, strawberry and grapevine are some of the crops that contain commercially widespread clones that are difficult to breed by traditional methods, and these were among the first crops in which the cisgenic and intragenic concepts were implemented. Improvements have been obtained through the silencing of undesired gene activities or through enhancement of disease resistance. The crop most widely used for intragenic gene silencing approaches is potato (Holme *et al.,* 2013). The intragenic/cisgenic approach to improve traits has also been or is currently attempted in the outcrossing forage crops alfalfa and perennial ryegrass. Both forage crops can readily cross with wild or uncultivated relatives, are invasive and are readily adaptable to marginal land. Thus, a major concern is that transgenes can rapidly spread to the environment via pollen flow. For cisgenic and intragenic crops the risk of transfer of modified gene is limited to those already present in the same sexually compatible gene pool and should therefore cause few ecological concerns beyond those faced by their classical bred counterparts (Nielsen, 2003). Even if, genes can be transferred from crossable species to cultivars through classical backcrossing without making large changes to the parental genotype, genetic transformation is still a faster and more precise tool of gene transfer in self-pollinating homozygous species, with avoiding linkage drags. For traits with limited natural allelic variation in the sexually compatible gene pool, cisgenesis and intragenesis can overcome the restriction of traditional breeding. This is confirmed by the cisgenic approach to develop barley with improved phytase activity. Furthermore, both barley and wheat belong together with rye in the Triticeae tribe. Due to the allopolyploid nature of the Triticum genus, it has been possible to make crosses resulting in fertile hybrids between rye and wheat to create the amphiploid crop triticale, now widely grown as an animal feed crop (McGoverin *et al.*, 2011). Likewise, hybrids have been obtained between barley and wheat to create the amphiploid crop tritordeum (Martin *et al.*, 1999). This opens up two very divergent sexually compatible gene pools (Triticum-Secale and Triticum-Hordeum) with many possibilities for cisgenesis and intragenesis. Cisgenic barley with improved phytase activity has been developed while classical breeding for higher phytase levels is difficult because the natural allelic variation for phytase activity is limited in barley. Like the previously mentioned crops, woody plants are highly heterozygous, intolerant to inbreeding and have very long generation times all making conventional breeding very slow and difficult (Han *et al.,* 2011). To date, one cisgenic approach has been attempted in poplar with modified architecture.

Current status on the regulation and safety issues of cisgenic and intragenic crops

In most of the countries, the release of cisgenic or intragenic crops currently falls under the same regulatory guidelines as transgenic crops. According to Schouten *et al.,* who introduced the concept of cisgenesis in 2006 and they proposed that plants developed through cisgenesis should be exempted from regulation and separated from the category of transgenic. To evaluate different novel breeding techniques and to determine whether they should be regarded as genetic modification techniques, the European Commission (EC) set up a working group named New Techniques Working Group (NTWG) in 2007 (Holme *et al.,* 2013). Furthermore, to know the recent status and

application of modern plant breeding techniques, European Union's Joint Research Centre (JRC) carried out which revealed that amongst other new techniques intragenesis and cisgenesis occupied first and second rank, with respect to the scientific publications and filed patents (Lusser *et al.*, 2012). According to EFSA Panel (2012) on Genetically Modified Organisms (GMO), similar hazards can be obtained through cisgenic and conventionally bred plants, while novel hazards can be coupled with intragenic and transgenic plants. All of these breeding techniques can produce variable frequencies and severities of accidental effects and the frequency of unintended effects may vary between breeding methods and cannot be predicted, hence, needs to be assessed case by case.

The primary significance of the cisgenic and intragenic concept is that it may facilitate a new conversation with regards to genetic modification of plants between breeders on the one side and consumers on the other. Cisgenesis may be safer than traditional breeding since the introduction of unwanted traits via linkage drag can be prevented (Haverkort *et al.*, 2008). However, the issue of any endogenous gene silencing needs 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 and intragenic organisms since they may contain new proteins or greatly altered levels of familiar proteins. When *Agrobacterium* mediated transformation is used for inserting the cisgenes, 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 (Schouten *et al.*, 2006). 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 created. Such situation is objectionable 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 (Schaart and Visser 2009).

Future Trends

Genetic modifications based on the sexually compatible gene pool carries a high potential for generating plants with environmental and economic benefits that may be necessary for meeting the global need for a more proficient and sustainable crop production. Future developments regarding the creation and commercialization of cisgenic and intragenic crops will depend on willingness to pertain less stringent regulation. A less comprehensive regulation of cisgenic/intragenic crops, reducing the costs for approval, would be especially helpful to the developer and seed companies. This would provide an additional tool for crop improvement to the breeder and thus increase the production of cisgenic and intragenic crops.

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