IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Role of Cisgenesis and Intragenesis as New Plant Breeding Technique in Crop Improvement

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Abstract

Major concern in public today is about accepting transgenic crops whose genetic material is combined artificially as they cannot hybridize naturally. Cisgenesis and Intragenesis concepts involve plants transformation using same species or cross compatible species. There is no involvement of any foreign genes selected or any vector supported genes in their concept. In this, like transgenesis there is introduction of new genes using techniques of genetic transformation which is limited within sexually compatible species. Cisgenesis involves transferring the natural genome as it is with its own non coding and regulatory sequences. In intragenesis it is possible to construct a new genetic component by taking desired gene from the same species or cross compatible species whose introns and regulatory sequences are transferred to host genome. Potato, apple, straw berry are the crops in which intragenic and cisgenic approaches have been studied from 7 years ago. The regulation is under evaluation in the EU and in the USA. The end products if recognized as GMO's will have less use but if recognized under NON GMO's would have immense use for crop improvement. Accordingly, it is possible that cisgenic and intragenic approach will be of great significance for future plant breeding.

Key words: cisgenesis, public opinion, alternative breeding approach, intragensis, regulation.

Introduction

Plant breeding unceasingly leaned on evolvement of various disciplines to procreate and directed entry into the widespread genetic variability. (Eriksson *et al.*, 2019). Genetically modified crops or transgenic crops have been utilized ecunemically on a prodigious commercial basis for 18 years; and in the year 2012, 17.3 million farmers in 28 countries implanted 160 million hectares of crops practicing this technology (Stewart, 2016).

Despite of the fact that transgenics played a prominent role in meliorating economic advancement, they are of hefty concern among the public from the time when they were introduced in 90's (Espinoza *et al.*, 2013) because of usability of hyped up combinations of genetics elements which are derivatives of organisms cross incompatible to each other by natural mode (Holme *et al.*, 2013; Devi *et al.*, 2013). Aforesaid novelette gene might replenish the target plant with a unique character that does not exist in the beneficiary species in nature and neither it can be imported via traditional breeding (Schouten *et al.*, 2006).

Intragenesis and cisgenesis evolved as novel breeding approach substituting transgenics for attaining eco-friendly and efficient plant production (Holme *et al.*, 2013; Devi *et al.*, 2013) hinging on usability of genetic material from species having potential to hybridize sexually as they utilize the gene pool same as used in traditional breeding. Intragenic or cisgenic transformants and their progeny should be devoid of any alien gene like selection marker or vector backbone genes.

Cisgenesis

It was developed by Jochemsen and Schouten in 2000 (Holme *et al.*, 2013). The "cisgenic plant" refers to "a crop plant that has been genetically modified with one or more natural genes isolated from a crossable donor plant". Cisgenesis is protracted to the gene pool of species possessing sexual compatiblity as the cisgene is carbon copy of the natural gene inclusive of the promoter, introns and the terminator in the normal orientation along with its regulatory elements. Cisgenesis should not be assayed as a transgenic threat to the environment since it is not transforming gene pool of the recipient species (Singh *et al.*, 2015). On the basis of usability of indigenous genes, cisgenesis can be considered more proximate to traditional breeding than intragenesis.

The procedure of developing cisgenic plant is similar to transgenic development only differing in the type of gene used. In addition to this, T-DNA borders emanating from Agrobacterium at times dwell in the end product (Red colouration in Fig. 1). This innovative approach amalgamates traditional breeding with modern technology for efficaciously accelerating the breeding process resulting in miniaturized undesirable effects of linkage drag. (Rommens, 2007).

Intragenesis

Intragenic approach deals with confinement and recombination of explicit genetic elements from one plant to the other sharing same gene pool under lab conditions. (Rommens *et al.* 2004). It attributes to the genetically modified organisms in which imported intragene comes from same or other cross compatible species. As compared to the cisgenes, these genes are mergers possessing genetic elements of distinct genes (Rommens *et al.*, 2007) because of which gene expression of a particular gene can be altered using divergent terminators or promoters resulting in development of new genetically modified organisms possessing brand new expression arrangements.

Intragenesis in comparison to conventional breeding techniques is an exceptional combo of current breeding methodologies alongwith transgenics for overall enhancement of crop growth and development as it wards off unwanted and uncertain genome conversions occurring due to arbitrary gene introductions (Singh *et al.*, 2015).

Intragenesis coupled with RNAi (RNA interference) impel crop advancement through modification in the internal mechanism via gene silencing in which micro RNA (miRNA) are produced artificially(Schouten andJacobsen 2008). This technique has been extensively used in pomology (Molesini *et al.*, 2012).

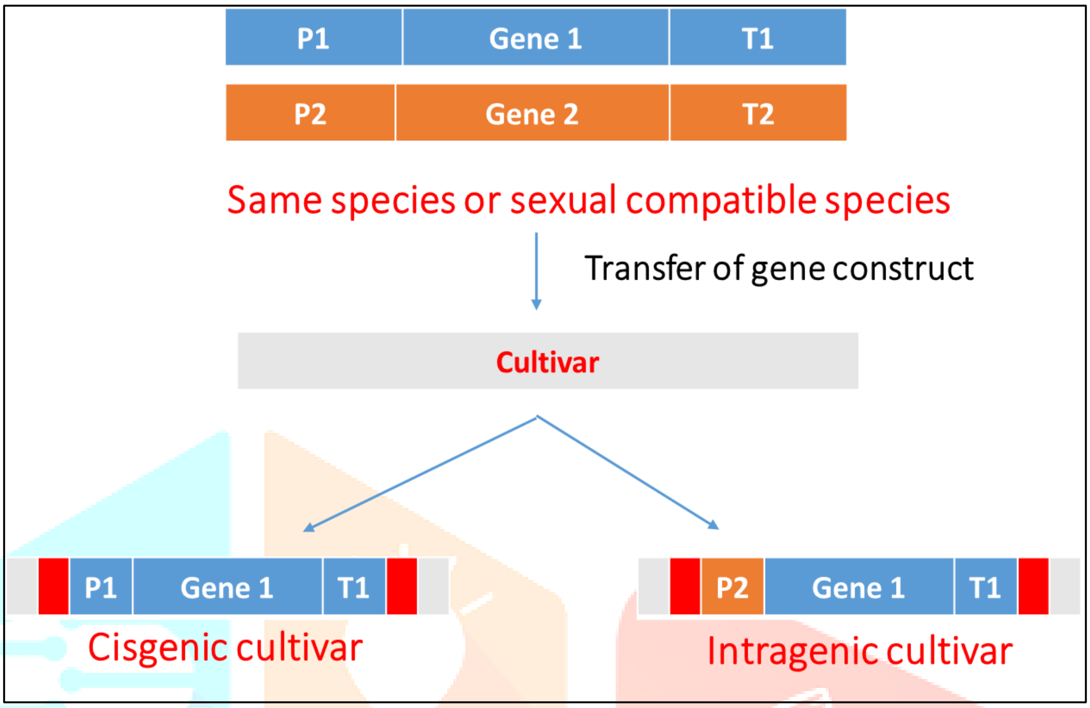

Fig. 1 procedure followed in gene transfer into cultivar through Agrobacterium transformation technique. P- Promoter and T- Terminator.

When it comes to develop characters in the cross compatible species with narrow genetic base within the same gene pool, both cisgenesis and intragenesis approach serve as the fastest means of gene transfer among such plants. In analogy to the benefits , these concepts have some constraints in comparison to conventional breeding as they can be applied only to the species showing sexual compatibility. In addition to this for conducting these approaches it requires a lot of proficiency and time. (Table 1).

Table 1. Comparison between cisgenesis, intragenesis and traditional breeding methods

Particulars	Cisgenesis	Intragenesis	Traditional breeding methods
Regulatory elements	Promoter, introns, terminator are of the gene itself.	Novel coding configuration and promoters built.	Terminators, promoters and introns are owned by the gene itself.
Linkage drag	Absent	Absent	Present
Time required	Fast and precise tool for gene transfer than back crossing	Demands selection in multiple generations and time exhausting	Time consuming and required head tic back crossing for 4-5 generations
Genes introduced	Not modified	Modified and creates new variability	Not modified
Products of gene	Same as native gene	Modified	Same as native gene

Approaches utilized in developing crops through cisgenesis or intragenesis

For the production of cisgenic or intragenic crops, genome sequencing has proved a boon as it helps in identifying and providing the required internal genetic elements from the species under cultivation and also from the originators of those species as well as the landraces which share the same gene pool (Hongwei *et al.*, 2014).

Divergence proves to be the most important tool for analyzing different plants showing variable expression for both temporal and spatial arrangement. This kind of variation among expression is advantageous for cisgenesis, whereas if promoters are to be determined then intragenesis is to be considered.

Steps involved in development of intragenesis and cisgenesis crops are:

- DNA isolation for required
- Insertion of foreign DNA into the plasmid
- Transformation
- Eradication of selective marker
- Selection for transformed cells showing recombination

Similar methodologies are used for the production of cisgenic, intragenic and transgenic crops. (Schouten *et al.*,2006). Plants which are produced through intragenesis or cisgenesis should be devoid of any other type of marker. Various methods can be used for eliminating the marker genes to make the plant eco friendly like areco-transformation, excision by homologous recombination, recombinase induced excision etc.

Different crops and traits improved by cisgenesis and intragenesis

Crop	**Character**	**Desired gene**	**Cis type or intra type**
Cisgenesis			
Apple	High anthocyanin content		
Melon	Resistant to downy mildew	*At1/At2- glyoxylate aminotransferase*	Expression
Potato	Resistant to late blight	*Rpi gene*	Expression
Tetraploid wheat	Quality of baking	*1Dy10*	Expression
Barley	Grain phytase activity improved	*HvPAPhy_a*	Expression
Grapevine	Resistant to fungal disease	*VVTL-1*	Expression
Apple	Scab resistance (*V. inaequalis*)	*HcrVf2 gene*	Expression
Intragenesis			
Perennial rye-grass	Tolerant to drought	*Lpvp1*	Expression
Alfalfa	Reduced level of lignin	*Comt*	Silencing
Potato	Tolerant to black spot	*Ppo gene*	Silencing
Potato	High amylopectin	*GBSS*	Silencing
Strawberry	Resistant to grey mold	*PGIP*	Over expression

Draw backs

Introduction and production of crops through cisgenesis and intragenesis require a lot of time and expertise. In addition to this the desirable gene which is identified should be confined from the species sharing same gene pool (Devi *et al.*, 2013). Generation of the eco friendly crops devoid of selective markers need novel methods. So, major emphasis need to be given for the development of such methods.

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