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Cisgenesis: An Approach for Crop Improvement

Rameshraddy[1], Khan, J. A.[2], Salimath Mahesh[1] and A. G. Shankar[1*]
[1]Department of Crop Physiology UAS, GKVK Bengaluru
[2]Department of Plant Biotechnology UAS, GKVK Bengaluru
*Corresponding Author E-mail: ambara8@hotmail.com
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ABSTRACT

Cisgenesis is a precise science where in genetic modification is by transferring beneficial alleles from crossable species into recipient plant it has additional advantages over traditional breeding / conventional breeding. Cisgenesis can avoid linkage drag, enhance the use of existing alleles. It saves the time to develop variety by combining traditional breeding techniques and modern biotechnology and hence speeds the breeding processes. There are several successful study the utilization of cisgenesis in developing disease resistant crop, quality control, value added to the economic products of many crops perhaps, this technique has its own demerits, hoping that modern techniques will overcome these demerits. Cisgenesis allows plant genome to be modified while remaining plants within the genepool therefore cisgenic plants should not be assessed as transgenic plants.

Key words: *Cisgenesis, Allels, Crop, Linkage.*

INTRODUCTION

The agricultural production has seen various revolutions from the green revolution of 1960's to the production of transgenic (genetically modified, GM) crops. This has led to unprecedented increase in the cultivation of GM crops which was introduced 15 years ago. Keeping the pace, cultivation of GM now accounts for 185 million hectares across globe covering 26 countries GM technology in crop production has been phased into two generations, while first generation GM crops mostly account for tolerance to herbicide and insect larvae. Currently, so-called second generation GM crops aims at enhancing quality for varied health benefits as well as for major abiotic stress like drought with higher nitrogen use efficiency. One of the typical examples for abiotic tolerant GM crop is '*Drought Gourd*'. However, other trait introgressions are in process of being implemented[1, 2].

The impact of GM technology was loud and clear by its success in scientific arena. It is unfortunate that the potential of GM crops is not being realized, primarily because of perception of public about transgenic crops being contaminated and mixed with DNA of other organisms that cannot be crossed by natural means.

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This had led to prejudice of GM crops to be unnatural and against the will of God[3]. This reservation is often linked to a notion of respect for nature and also appears to be interlinked with fears for potential health risks and for the spreading of new gene combinations in the environment.

With the aim of overcoming major problem of transgenic i.e., genes from foreign organism, a novel scientific intervention came to rescue known as cisgenesis. With promise of environmental safety[4], the transformation concept of cisgenesis was developed as an alternative transgenic process. The main objective of cisgenesis is based on exclusive use of genetic material from the same species or genetic material from closely related species capable of sexual hybridization[5, 6]. On contrary to transgenics, where the genes or DNA sequence from unrelated species are transferred. Hence, the gene pool exploited by cisgenesis mimics that of gene pool exhibited by traditional breeding [7]. Another key point of cisgenesis is the elimination of selection marker genes and vector backbone genes from the transformants. Similarity between transgenics, Cisgenesis and conventional breeding is integration of the gene into the genome requires chromosome breaks and DNA repair mechanism.

Concept of cisgenesis/ what are cisgenesis

A concept named cisgenesis was introduced by Jochemsen and Schouten in 2000 in the book '*Toetsen en begrenzen. Een ethische en politieke beoordeling van de moderne biotechnologie*' and made famous by Schouten and colleagues[8]. They claimed cisgenesis as a transformation process wherein, genetic modifications of recipient organism done with a gene from sexually compatible organisms. Genes from sexually compatible species which could be same species or closely related includes introns and is also flanked by its native promoter and terminator in sense orientation. Even though there has been various definitions as to how a cisgenesis would be defined,[7,9] as more complexity arises from intragensis where P-border and vector backbone sequence do not originate from the sexually compatible species. Hence, the concept and process of cisgenesis and intragenesis are different and regulatory framework of these need to be different as well.

In the process to develop a cisgenic plant, which contains one or more genes from its sexually compatible species, any suitable technique from transgenic organism can be utilized. Gene isolation, cloning and transformation are necessarily important step for cisgenesis also. Sometimes the term cisgenesis is also used to describe an 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[10].

During the last few decades, a variety of indigenous genes, coding for valuable traits like disease resistance and quality, from crop plants and their wild relatives have been isolated, characterized and introduced into the genetic background of elite germplasm. These native genes, isolated from the crop plant itself or from other cross compatible species, are currently referred as cisgenesis to distinguish such group of genes from the transgenes[7] (as in cisgenic approach as there is no introduction of new gene class from cross incompatible species). Hence, exploiting genetic variation symbolize an important application of cisgenesis which are applied in conventional breeding programme that has been safely used since decades.

The worthiness of GM for promising high quality and quantity of food has got a major setback about the concerns of safety in terms of growing and eating. The matter is further aggregated by some misconceptions of 'newspaper reports' for the probable unpredictable hazards to biodiversity and confer toxicity, allergy and genetic threat to human nutrition. These arguments are based on a misreading of the world's real problems and are driven not by rationality. People mostly tend to rely on intuitive feelings to give judgement on GMO's [11]. GMO liberalization within a larger framework and to examine how governments deal with this kind of policy through their communication is also a driving force and needs to be assessed[12]. Analysing the gravity of the situation scientists considered developing 'publicly accepted' technology which can be perceived as safe as conventional breeding preferably lacking the linkage drag. Hence, the cisgenesis was conceived with a main objective of transferring only desired genes and furnished no unnecessary hazard compared to induced translocation or mutation breeding.

Application of cisgenesis in crop improvement

Cisgenesis precludes linkage drag, and hence, prevents hazards from unidentified hitch hiking genes[13]. Due to this reason, cisgenesis is normally safe than traditional breeding programmes and various biotic and abiotic stress resistance genes can be pyramided to provide wider and long lasting forms of resistance. Common people are found to be more satisfied with cisgenesis compared to transgenic crop. In Mississippi, ananalysis revealed that 81% of public favored to eat cisgenic vegetables while only 14 – 23% for transgenic vegetables[14]. Also the notion towards transgenic has brought firm regulations worldwide which made it necessary to differentiate between these two technologies. Although in Indian scenario[15], no significantly different ($P = 0.16$), 76% and 73% of respondents stated a willingness-to-consume GM and cisgenic foods, respectively. Pertinently, a recent study in Denmark indicated that higher level of scientific knowledge generally makes people less likely to care about the technology involved (cisgenesis or transgenic)[16].

Success stories of Cisgenesis

The development of cisgenic Apple plant[17] employed the use of ORF of endogenous apple scab resistance gene *HcrVf2* from the wild relative *Malus floribunda* under the control of its own regulatory sequence into scab susceptible cultivar 'Gala'. The segment between the recombination sites that contains the *npt*II gene for kanamycin selection was removed through dexamethasone-induced recombination and thus resulted in marker-free lines. Presence of *HcrVf2*, absence of *trfA* (responsible for initiation of replication) and *npt*III as part of the backbone, and the fusion marker gene *npt*II/*codA*was demonstrated by PCR. The author considers this as the first report of generation of true cisgenic plant.

Cisgenic barley with improved phytase activity[18] was demonstrated with barley phytase gene (*HvPAPhy_a*) which is expressed during grain filling. Marker gene elimination method was employed to obtain marker free plant lines. Both the gene of interest and the selection gene were flanked by their own T-DNA to allow unlinked integration of two genes. Homozyous plants for (*HvPAPhy_a*) were slected which showed an increase of 2.6 to 2.8 fold increase in phytase activity which was evaluated till three generations. The lines with absence of truncated vector-backbone sequences linked to the borders were classified as cisgenic.

Cisgenic inhibition of the potato cold induced phosphorylase L gene expression and decrease in sugar contents[19] uses RNA silencing construct controlled by 35S promoter and the OCS terminator and selected putative transgenic shoots on kanamycin-containing medium. Here, removal of the selection marker was not reported. However, silencing of starch phosphorylase L gene reduced starch breakdown during cold storage conditions.

Expression of *KxhKN4* and *KxhKN5* genes in *Kalanchoë blossfeldiana* 'Molly' results in novel compact plant phenotypes[20]**.** This is a novel approach towards a cisgenesis alternative to growth retardants. To control the elongated habit of potted plants, growth regulators are used. It is expected that in near future these regulatory may be banned and there should be an alternative for growth regulators. Authors in this study have used two constructs with the coding sequence of the class I and class II homeobox KN genes, *KxhKN5* and *KxhKN4*, respectively, were overexpressed in the commercially important ornamental *Kalanchoë blossfeldiana* 'Molly'. Furthermore, a post-transcriptional gene silencing construct was made with a partial sequence of *KxhKN5* and also transformed into 'Molly'. Several transgenic plants exhibited compact phenotypes and some lines had a relative higher number of inflorescences. A positive correlation between gene expression levels and the degree of compactness was found. These transgenic plants show that a cisgenesis approach towards production of compact plants with improved quality as an alternative to chemical growth retardants may be feasible.

Gibberellin-associated cisgenes modify growth, stature and wood properties in Populus[21] by inserting five cisgenes that encode proteins involved in gibberellin metabolism or signalling**.** All cisgenes were isolated and transformed along with their promoter and terminator regions (in this case 1-2-kb of 5’ and 1-kb of 3’ flanking DNA), and as contiguous sequences including all exons and introns. Basta (glufosinate-ammonium) was used for selection during plant regeneration (*bar* gene with NOS promoter and terminator), no removal of the selectable marker gene was demonstrated. The genes used in the study were expressed in the xylem and phloem and identified by microarray expression data. They observed a great variation in the large number of independent events they analysed. The successful insertion of the cisgene was PCR verified using primers directed at flanking T-DNA sequence that was not present in wild type plants.

A partial cisgenic event was done for late blight resistance in potato using resistant gene *RB* from the wild species *Solanum bulbocastanum* Dun[22]. However, transformed plants contained kanamycin resistance gene *NPTII* with normal RB construct. Hence, this even is considered in partial cisgenesis.

Drawbacks of cisgenesis

Unlike transgenics, the gene(s) from sexually incompatible cannot be introduced and the generation of cisgenesis is time consuming[23]. 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[24]. Position effect may lead to alteration of the gene expression[25] and phenotypic differences[26]. Development of marker free transgenics requires implementation of new technologies, and such technologies may not be available for crop to be engineered. Thus, considerable efforts have to be given to produce high numbers of transformants, especially for crops with low transformation efficiencies

Safety and regulatory issues regarding cisgenesis

There are different views emanated regarding cisgenesis in recent past. Cisgenesis may be safer than conventional breeding, wherein linkage drag can be prevented[27]. However, the issue of any endogenous gene silencing need to be considered. The donor sequence does not replace an allelic sequence, but is added to the recipient species’ genome. Owing to the process of gene transfer, it is possible that the new sequence is inserted several times in one genome, which might affect gene expression and, therefore, phenotype. However, gene duplication is a common natural occurrence, for instance in the case of resistance genes or other multigene families[28]. Further, the cisgenic plant might contain some small, non-coding sequences from the vector such as T-DNA borders, which are 25-base-pair imperfect repeats that delimit the DNA segment transferred to plant cells when using Agrobacterium mediated gene transfer. Similarly as transgenics, similar safety issue should be concerned for cisgenesis, since they contain new gene / protein (s)[29].

CONCLUSION

By definition, cisgenesis is a form of genetic modification, as it transfers a gene and its promoter to a recipient species. However, the product is clearly different from transgenic plants, which are derived by transferring ‘foreign’ or artificial genes, or artificial combinations of genes and promoters. Cisgenesis therefore respects species barriers, and in this sense differs fundamentally from transgenesis. Cisgenic plants are similar to traditionally bred plants, because the transferred genes come from the same gene pool. Consequently, cisgenic plants are as safe as traditionally bred plants.

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