Cisgenesis – A Substantial Crop Improvement Technique

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Abstract-*The genetic modification of desired trait from wild germplasm is seriously affected by linkage drag and results in very time consuming several generations of breeding mechanisms. The disinclination for transgenic crops by consumers, as it includes combination of genes between species that cannot hybridize by natural means also paved way for finding an alternate innovative approach called "Cisgenesis". The term Cisgenesis is the genetic modification to transfer beneficial alleles from crossable species into a recipient plant. The donor genes transferred by cisgenesis are the same as those used in traditional breeding. It can avoid linkage drag, enhance the use of existing gene alleles. This approach combines traditional breeding techniques with modern biotechnology and dramatically speeds up the breeding process. This allows plant genomes to be modified while remaining plants within the gene pool. Therefore, cisgenic plants should not be assessed as transgenics for environmental impacts (1). This review covers the implications of cisgenesis towards the sustainable development in the genetic improvement of crops and considers the prospects for the technology.*

Keywords-cisgenesis, transgenic, breeding, trait, genomes.

I. INTRODUCTION

The present scenario of world population and food demands require world agricultural production be increased by 50% by 2030 (2). In the meantime, climate change and shrinking environmental resources are limiting agricultural production over the world. These challenges bring an urgent need to enhance crop productivity. To breed crops with increased yield and resistance to environment stresses, a pivotal consideration is how to effectively utilize genetic diversity. Genetic modification of plants actually involves the introduction of foreign genes into the plant genomic background. Currently, genetically modified plants give a promising impact to various crop improvement programmes. The foremost outcome is the development of varieties resistance against various biotic and abiotic stresses. Genetically engineered traits comprise priceless alternatives from the conventional breeding, but, there arise a public issue on consumption of transgenic plants. This unlocks a new vista for engineering crop plants using the DNA from a sexually compatible donor plant *i.e.,* Cisgenic **-** An Alternative of Transgenic.

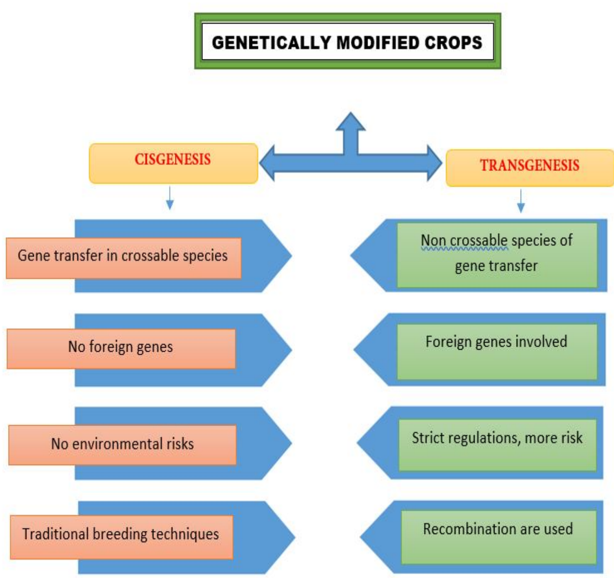
Cisgenesis is the production of genetically modified crops/plants using donor DNA fragment from the species itself or from a cross compatible species. The newly introduced gene is unchanged and includes its own introns and regulatory sequences and is free of vector DNA, except T- DNA border sequences that flank the cisgene. The resultant phenotype of the cisgenic plant can be achieved through conventional breeding also, but, it will take a much longer time. 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 [1]. To exploit the full potential of transgenic, it is very much mandate to compromise the consumers by ensuring the bio safety of the agricultural crops to the humanitarian.

II. MANDATE OF CISGENICS

Cisgenic plants are presumably considered safer than those produced through conventionally breeded plants because of the lack of linkage drag. In cisgenesis, only the desired genes are introduced without the undesirable genes. Cisgenesis furnishes no unnecessary hazard compared to induced translocation or mutation breeding. Therefore, cisgenesis prevents hazards from unidentified hitch hiking genes. 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. The primary biological advantage of cisgenesis is that it does not disrupt favorable heterozygous states, particularly in asexually propagated crops such as potato, which do not breed true to seed. One application of cisgenesis is to create blight resistant potato plants by transferring known resistance loci wild genotypes into modern, high yielding varieties. There are also legitimate public reasons that brought the obligation to clearly differentiate cisgenes from the transgenes. The notion towards transgenic technology often brought annoying circumstances to many people, followed by their firm regulation worldwide. Common people are also found to be much satisfied with cisgenic crop than transgenic crops. In Mississippi, an analysis revealed that 81% of public favored to eat cisgenic vegetables while only 14 – 23% for transgenic vegetables.

According to the Sustainability Council of New Zealand (3), interest in cisgenics also has been stimulated by:

- The idea that cisgenic GMOs will avoid the market confrontation that has overwhelmed other types of GM foods. Several researchers believe they will be able to induce consumers that the GM industry has rehabilitated, by listening cautiously to public concerns about using GM in the food chain. The argument that their GMOs will not cross the species hurdle is offered as a confirmation of that modification.
- The optimism that cisgenic GMOs will not be subject to the same regulatory examination as GMOs made by current techniques, and lobbying efforts by developers are in progress in New Zealand to secure the regulatory discount.
- The assumption that GMOs developed from this technique may prove difficult to identify and could thus be undetectable to regulators and consumers.
- However, the faith of cisgenic GM foods to be invisible to consumer is against their demand upon transparency about the use of GM foods. On the other hand, letting off cisgenic foods from the GMO regulation will facade the public's rights to know about the introduction of newly developed foods by new technologies to the food chain. Therefore, such primary tension highlights the incoherent nature of the cisgenics as a commercial approach



Advantages of Cisgenesis over Conventional Breeding:

Conquer the setback of linkage drag:

Introgression of innovative traits into the cultivated varieties by conventional methods comprises wide crosses and wide spread back crossing. However, these traits are constantly linked within a large share of unwanted chromosomes, the so called linkage drag. 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. In vegetatively propagated crops like potatoes and apples, their heterozygous nature further brought impediment in successful transfer of traits of interest (4). Hence, direct transfer of desired genes through cisgenesis into an existing variety without altering any of the properties enviable for the consumers can be accomplished. An ample amount of marker-free transformants where single TDNA was arbitrarily inserted, and produced acceptable expression of the cloned cisgene in the beneficiary species. It is followed by the selection of plants in the growth chamber then glasshouse and field. Selection of the best performing plants with realistic gene insertions and least negative side effects is made in the field where linkage drag with unwanted gene is deficient. Plant breeding techniques with the objective to introduce durable resistance to the potato-late-blight-caused by *Phytophthorainfestans* involve stacking of resistance genes from various resistant wild species including *Solanumdemissum* and *S. bulbocastanum*. Introgression of resistancegene from the new donor *S. bulbocastanum* beganin the early 1970s, but the accomplishment of the technique was hindered by linkage drag. For the time being diverse native resistance genes have been screened and isolated from the donor plants along with *S. demissum*(4) which would allow stacking of cloned resistancegenes to the susceptible elite potato cultivars by cisgenesis.

Maintains original genetic make-up of plant variety:

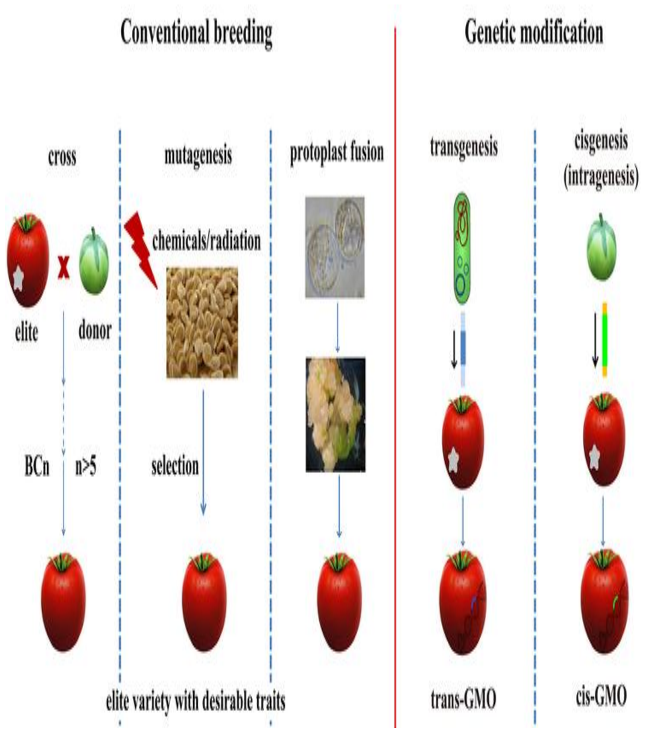
In a hybridization method, the genetic makeup of the progeny plant varies from its parents because it has been a mixture of both the parental genomes. In spite of this, there is a necessity to conserve some part of the genome which revealed certain constructive traits. Through conventional plant breeding such an approach is not possible entirely due to self incompatibility among the vegetatively propagated plants like grape, potato, apple etc. When crossing is done in a prominent grape variety Merlot or Cabernet sauvignon with a disease resistant variety the genetic constitution of the progeny plants will not at all be similar to the parent plants. Hence, traditional breeding programme will no longer confer disease and pest resistance to the notable parent cultivars (5). In a Dutch project called DURPh (Durable Resistance against *Phytophthora*), which has been going ahead since 2006 under considerable public support, cisgenic breeding tools are used in order to get up to four different resistance genes into one variety without changing other original traits of the modified variety (5). In this way, it must be probable that multiple *R* genes can supplement to a more durable resistance against late blight (6).

Reduction in pesticide application:

The key purpose of cisgenesis is to transfer disease resistance genes to susceptible varieties. The vital goal here is to lessen substantial pesticide application. As a result, there is decline in the input costs of the farmers and decreased pesticide leftovers on the plants and also in their products, which is mostly favored by the consumers. This reduced the environmental pollution by pesticides and in turn helped in sustainable agricultural development. On the other hand, if cisgenic comes under the current GMO regulation, then this novel technique will be held back (7). Potato is susceptible to different pests and diseases. Most noteworthy between them is the late blight, induced by the fungal pathogen *Phytophthora infestans*, causing maximum damage potential world-wide. As an outcome breeding efforts are massive in order to get less susceptible and resistant new varieties, respectively, and new technologies are used especially in this breeding sector. Approximately 200 wild *Solanum* species with potential resistance genes are known in Middle and South America. Only a small percentage of them has been explored for use in breeding programmes up to now (8). The availability of resistant varieties would lead to enormous reduction of pesticides input for plant protection measures as well as of the yield loss.

Time Saving:

In conventional hybridization programmes, there is linkage drag, where there is inheritance of thousands of unwanted genes to the progeny. Several backcrossed generations are required to get rid of such kind of undesired genes. Cisgenesis overcomes the problem of linkage drag and only the gene of interest is introduced into the genome of the recipient plant within a short period of time. Thus, this saves a lot of time. For example in apple-breeding, integration of a disease resistance gene takes about 40 years through traditional methods. The transfer of apple scab resistance gene Vf, which has been cloned of late (9), into the novel cultivars using cisgenic technique could give rise to better results within a short period of time. The comparatively long period of tree breeding, which may last decades via traditional techniques, makes the genetic modification of trees a striking target (10). 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.



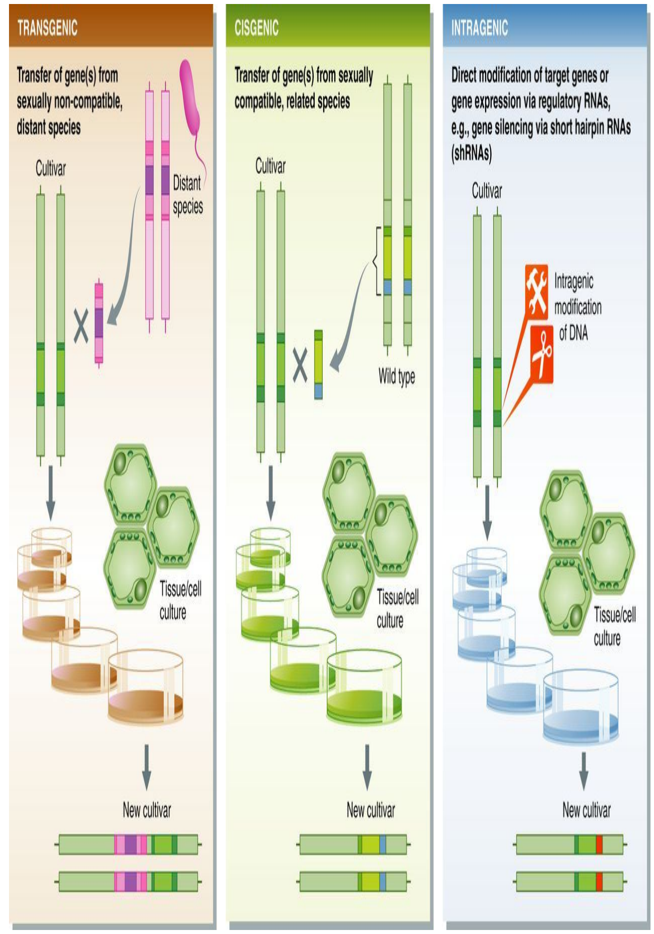
Extrication of Cisgenic and Transgenic plants:

The release of genetically modified (GM) plants is currently regulated to prevent any negative effects on the environment or human health. These regulations are based on transgenic organisms and do not distinguish transgenic plants from cisgenic plants. This means that the GM-regulations for transgenes (genes from the non-crossable species), are also applied for cisgenes (genes from crossable species). However, cisgenesis is more similar to traditional plant breeding than is transgenesis. There is a great necessity to distinguish cisgenesis from transgenesis.

Although both transgenesis and cisgenesis use the same genetic modification techniques to introduce gene(s) into a plant, cisgenesis introduce only genes of interest from the plant itself or from a crossable species, and these genes could also be transferred by traditional breeding techniques. Therefore, cisgenesis is not any different from traditional breeding or that which occurs in nature. There is no environmental risk evoked and release of cisgenic plants into the environment is as safe as that of traditionally bred plants. If the current international GMO regulations continue to fail in distinguishing cisgenic from transgenic plants, the use of cisgenesis could be seriously hindered.

Only Canada now has a product-based regulation system rather than a process-based one and this has made it legally possible to control cisgenic plants less strictly than transgenic plants. Any restrictions on cisgenesis could block or delay further research and application of improved crop varieties, especially at a time when increasing number of genes from crops and their crossable wild relatives are being isolated and are becoming amenable to cisgenesis. In Australia, cisgenic plants are treated differently under GMO regulations, as stated in Gene Technology Regulations that "a mutant organism in which the mutational event did not involve the introduction of any foreign nucleic acid" is not specified as GMO (11).

European Food Safety Authority has released a scientific assessment of the safety assessment of plants developed through cisgenesis and intragenesis. According to this report concerning the source of introduced genes, cisgenesis has similar hazards as does traditional breeding. However, the transformation techniques used in cisgenesis and transgenesis are the same, so they have similar risk linked to transfer technology. This report recommended to using the same risk assessment guides as used in transgenic plants to evaluate the cisgenic plants, but the required information might be less than that needed for transgenic plants (12).



Cisgenics in crop improvement:

Fruit trees (Rosaceae) and vegetatively propagated crops like potatoes are currently the primary target for cisgenic modification. In a first step, monogenic traits may be targeted. However, gene pyramiding is also feasible. Trees, in general, are an attractive target for cisgenic modifications. The major reason may be seen in the decreased time needed for the development of a new cultivar that will be successful in the market. For successful implementation of cisgenics technology in crop improvement, genes related with the requisite trait should be well defined. Molecular markers may assist in their identification, especially as they have become important tools of traditional plant breeding methods. The identification and isolation of these genes are to a great extent facilitated by constant achievements in plant genome sequencing. Cisgenesis denotes a next knock-favoring a new era of GM organisms. Absence of marker genes may be antibiotic or herbicide resistance genes in the final product and also the introgressed gene(s) are derived from cross compatible species to the future species will lessen environmental worries and increase the consumer‘s preferences. The first scientific statement of bringing forth a true plant obtained by cisgenic approach was reported in apple through the insertion of the internal scab resistance gene *HcrVf2* influenced by their own regulatory genes into the cultivar 'Gala' a scab susceptible cultivar (13).

"Cisgenic inhibition of the potato cold induced phosphorylase L gene expression and decrease in sugar contents" (13). However, in their approach they used an RNA silencing construct, under the influence of 35S promoter and the OCS terminator sites, as well asselected putative transgenic shoots on kanamycin containing medium. Removal of the selection marker was not reported. Kuhl *et al.* (14) presented "a partially cisgenic event" in potato, which was accomplished by introducing an 8.59-kb fragment of the *RB* gene conferring late blight resistance (including 2.5- kb upstream of the start ATG and 2.48-kb downstream of the stop codon). As the selectable marker *npt*II was retained in the transformants they referred to them, by definition correctly, as "transgenic". In strawberries, cisgenic disease resistance against *Botrytis cinerea* was investigated by Schaart (15) using the endogenous strawberry gene encoding for polygalacturonase inhibiting protein *PGIP*, observing the strict use of strawberry-own DNA sequences as target gene and as promoter and applying a selectable marker removal method for the elimination of marker genes

III. LIMITATION OF CISGENICS

Although cisgenics technology is exhibiting considerable advantages over the transgenic counterpart, but still there are a few limitations associated with this technology. Compared to transgenesis, one of the disadvantages shared by cisgenesis is that characters outside the sexually compatible gene pool cannot be introduced. Furthermore, development of cisgenic crops involves extraordinary proficiency and time compared to transgenic crops. Therefore, the required genes or fragments of genes may not be readily accessible but have to be isolated from the sexually compatible gene pool (16). The author further elaborated few issues, firstly, the production of marker free plants usually requires the development of innovative protocols, since such protocols may not be readily available for the crop in question. Secondly, since 20 – 80% of the transformants contain vector-backbone sequences, many transgenic lines have to be removed. Therefore, substantial hard work has to be done, particularly on crops with low transformation efficiencies to create large number of transformants.

IV. FUTURE PRESCRIPTIVE

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. Cisgenesis is the transfer of gene(s) from the recipient plant itself, or from a donor plant that is sexually compatible with the recipient plant. Knowledge of traditional breeding remains critical for selection of cisgenic plants in breeding by cisgenesis. New biotechnology is making cisgenesis increasingly feasible in use of gene resources and precisely obtaining new agricultural traits without insertion of foreign genes or gene fragments.

V. CONCLUSION

The generation of cisgenic crops is still very limited and has been reported in apple, in pear, in barley, and in potato. Application of cisgenic techniques enhances the possibility to introgress the preferred genes into the novel cultivars (mostly single gene in the first step), without disturbing their favorable characteristics. Therefore, the most compelling contribution of cisgenesis may be anticipated for the development of monogenic resistance traits. But, the application of gene pyramiding will also accomplish a more durable resistance. Major advantages could be expected in breeding of plants with long life spans such as trees. Traits such as abiotic stress tolerance are usually complex (*e.g.*, due to polygenic traits). It is accordingly possible that the intragenic/cisgenic route will be of major significance for future plant breeding.

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