R-2649
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REVIEW ARTICLE Agricultural Reviews

Cisgenics Approach for Fruit Crops Amelioration: An Overview

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10.18805/ag.R-2649

ABSTRACT

Cisgenics is the change of a recipient plant's genetic make-up using a naturally derived gene from a species that is cross-compatible, along with its introns, native promoter and terminator, which are flanked at the typical sense orientation. It simply refers to genetic alteration carried out using one among the recombinant DNA technology approaches without the use of any foreign DNA; in other words, the host plant's DNA or DNA from the closely related species that is sexually compatible is used only in the manipulation. There is a public concern about eating transgenic plants, although genetically altered features offer priceless alternatives to conventional breeding. Because apples are vegetatively propagated, their heterozygous nature further hampered the successful transfer of desirable features. As a result, it is possible to directly transfer desired genes through cisgenesis into an existing variety without changing any of the desirable traits for customers. The benefits of cisgenesis over conventional breeding include its ability to overcome linkage drag, maintain the genetic diversity of the plant variety, use less pesticides and save time. The use of cisgenic approaches in various fruit crops increases the possibility of introducing the desired genes into innovative cultivars without affecting their beneficial traits.

Key words: Cisgenics, DNA, Engineered, Heterozygous, Introns, Terminator.

In reality, genetically modifying plants entails introducing foreign genes into the genomic background of the plant. Genetically Modified Plants currently have a positive impact on numerous agricultural development programmes. The creation of variations that helps in tolerance to various biotic and abiotic stress is the most important outcome. There is a public concern about eating transgenic plants, although genetically altered features offer priceless alternatives to conventional breeding (ISB News Report, 2019). This opens up new possibilities for genetically modified crops utilising the DNA of a donor plant that is sexually compatible. Development in plant molecular biology has resulted in the divergence of essential gene sources from prokaryotes to plants, which has led to the identification of numerous varieties of plant genes with agronomically desirable traits. This process will eventually be aided by ongoing genomic research. In the last decades, numerous indigenous genes from agricultural plants and their wild relatives have been extracted, described and incorporated into the genetic background of elite germplasm. These genes code for desirable traits, including diseases resistance and quality. To identify this category of native genes from transgenes, these genes have been extracted from the crop plant itself or from other species that are cross-compatible (Fig 1). As there is no introduction of new gene classes from incompatible species in the Cisgenic approach, the currently existing genetic variation represents the one employed in standard breeding programmes that have been safely used for decades. The term "cisgenesis" was first used by Schouten *et al*. (2006), who defined it as the modification of a recipient plant's genetic make-up with a naturally derived gene from a species that is cross-compatible, along with the gene's introns and native promoter and terminator flanked at the usual sense orientation. The final Cisgenic plant should be free of all foreign DNA, including selection markers and vector-backbone sequences, as Cisgenes shared a common gene pool that was available for traditional breeding. Cisgenesis is also known as *Agrobacterium*-mediated gene transfer from a sexually compatible plant, in which only the TDNA boundaries may remain in the transformed recipient organism. At the moment, there are various constraints for the development and marketing of

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How to cite this article: Haokip, S.W., Shankar, K., Yumkhaibam, T., Lian, H.N., Sheikh, KH.A., Shanker, A. and Chettri, P. (2023). Cisgenics Approach for Fruit Crops Amelioration: An Overview. Agricultural Reviews. DOI: 10.18805/ag.R-2649.

Submitted: 08-07-2023 **Accepted:** 31-10-2023 **Online:** 25-11-2023

genetically modified crops. The costly and time-consuming procedures for obtaining authorisation for these crops are significant impediments to implementation. Hence, new tactics and approaches are thus necessary in the creation of future genetically altered crops.

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

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

Why cis/intragenic crops are more acceptable?

Public concerns regarding the security of the generated food and their consequent products have sparked debate about whether GM techniques are worthwhile for establishing a highly reliable and high-quality food supply for the entire world (EFSA, 2012). The dispute has focused attention, in particular, on the likely unforeseen risks resulting from the accumulation of a few novel compounds in crop plants that pose risks to human nutrition in terms of toxicity, allergies and genetics. Due to the absence of linkage drag, Cisgenic plants are probably thought to be safer than those created through normal plant breeding. Just the desired genes, not the unwanted genes are introduced during cisgenesis but in case of transgenesis desired gene may be transferred along with unwanted genes. In contrast to forced translocation or mutant breeding, cisgenesis presents no unnecessary risk. While linkage drag is avoided through Cisgenesis, risks from unrecognized hitchhiking genes are also avoided. Since different biotic and abiotic stress resistance genes can be pyramided to generate broader and more durable forms of resistance, Cisgenesis is typically safer than traditional breeding programmes. Additionally, there are good public policy reasons for the requirement to distinguish Cisgenes from transgenes. Many individuals found the idea of transgenic technology to be aggravating, which led to its strict regulation globally. Moreover, the general public considered cis/intragenic crops to be considerably more satisfying than transgenic crops. This may have come due to the notion that Cisgenic GMOs will dodge the market backlash that has engulfed other forms of GM goods. Several experts believe that by carefully listening to public concerns about using GM in the food chain, they will be able to persuade consumers that the GM sector has been rehabilitated (Telem *et al*., 2013; Telem *et al*., 2007). As confirmation of that alteration, the notion that their GMOs will not overcome the species hurdle is advanced. According to research conducted in Mississippi, 81% of people preferred eating cisgenic vegetables to transgenic ones by a margin of 14 to 23%.

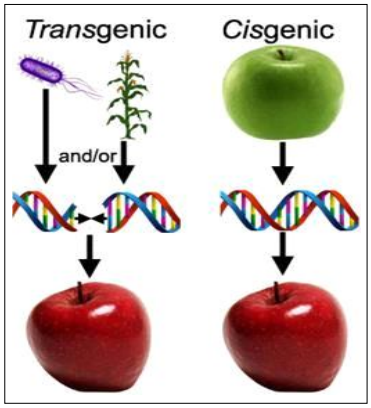

Fig 1: Transgene and Cisgene comparison (Source: Schouten *et al*., 2006).

Cisgenesis versus conventional breeding

As shown in the below Fig 2, the difference between cidgenesis and transgenesis is that elimination of marker gene occurs in cisgenic process where as in terms of transgenesis, marker gene is express along with gene of interest.

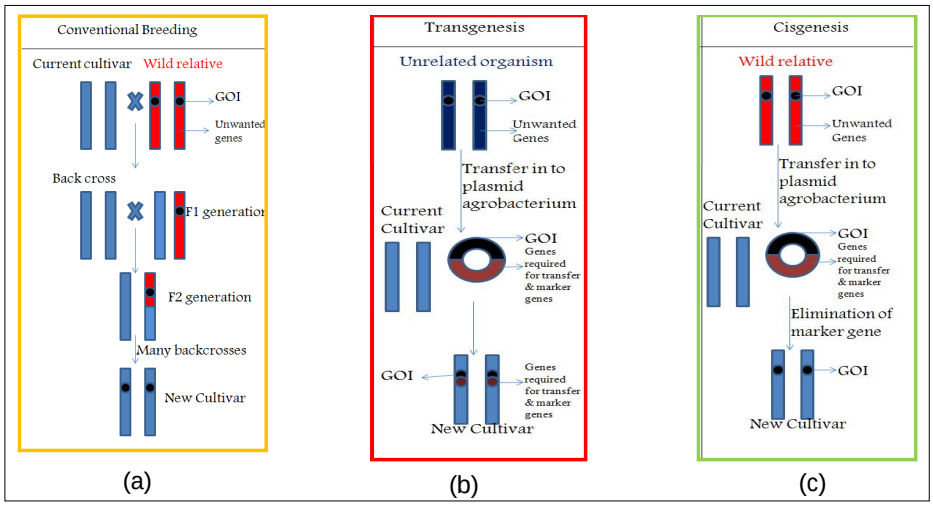

Fig 2: a) Conventional breeding b) Trangenesis c) Cisgenesis (Schouten *et al*., 2006).

Benefits of cisgenesis over conventional breeding

Conquering the hindrance of linkage drag

By using traditional techniques, novel features can be introduced into cultivated cultivars by extensive backcrossing and wide crosses. Yet, a significant portion of undesirable chromosomes are constantly linked with these features, a phenomenon known as linkage drag. The heterozygous character of crops that are propagated vegetatively, such as potatoes and apples, further hampered the successful transfer of desirable features. Thus, it is possible to directly transfer desired genes through cisgenesis into an existing variety without changing any of the desirable traits for customers (Dunwell, 2011). When a single T-DNA was arbitrarily inserted into a large number of marker-free transformants, the result was an adequate expression of the cloned cisgene in the recipient species. The selection of plants in the growth chamber, followed by the glasshouse and the field, come next. In the field where linkage drag with an undesirable gene is inadequate, the best performing plants with realistic gene insertions and least detrimental side effects are chosen. In order to add long-lasting resistance to the *Phytophthora infestans*-caused potato late blight, plant breeding techniques stack resistance genes from a variety of resistant wild species, including *Solanum demissum* and *S. bulbocastanum* (Zhu *et al*., 2011; Ewing *et al*., 2000). The introduction of the resistance gene from the new donor S. *bulbocastanum* started in the early 1970s, but linkage drag made the procedure less successful. Several native resistance genes have currently been identified and extracted from the donor plants and *S. demissum*, allowing stacking of cloned resistance genes to susceptible elite potato cultivars via cisgenesis (El-Kharbotly *et al*., 1996).

Sustaining the original genetic make-up of plant variety

Due to the mixing of the two parental genomes during the hybridization process, the progeny plant's genetic makeup differs from that of its parents (Jansky, 2006). Despite this, it is necessary to preserve a portion of the genome that showed certain beneficial features. Due to self-incompatibility among vegetatively propagated plants like grape, potato, apple, *etc*., such a method is not totally feasible through normal plant breeding. The genetic makeup of the progeny plants won't be at all identical to the parent plants when a popular grape variety, like Merlot or Cabernet sauvignon, is crossed with a disease-resistant variety. Hence, the famous parent cultivars will no longer inherit disease and pest resistance from the traditional breeding effort. Cisgenic breeding methods are employed in the Dutch project DURPh (Durable Resistance against *Phytophthora*), which has been ongoing since 2006 with strong public support, to introduce up to four distinct resistance genes into variety without altering the modified variety's other original features (Telem *et al*., 2013; Haverkort *et al*., 2009). As a result, it must be likely that several R genes will contribute to a more robust resistance against late blight (Huang *et al*., 2005; Huang *et al*., 2004).

Lessening uses of pesticide

Transferring disease resistance genes to vulnerable types is the main goal of cisgenesis. Here, reducing the use of significant pesticides is the key objective. As a result, farmers' input prices are decreasing and there are fewer pesticide residues in their products, which are largely well-liked by consumers. This promoted sustainable agricultural development by reducing the environmental harm caused by pesticides. On the other hand, this revolutionary approach will be delayed if cisgenic is subject to the current GMO legislation. A variety of illnesses and pests can affect potatoes. The one that deserves special attention is the late blight, which is brought on by the *Phytophthora infestans* fungus and has the greatest potential for global destruction (Kuhl *et al*., 2001; Kuhl *et al*., 2007). As a result, extensive breeding efforts are made to produce more resistant and less vulnerable new kinds, respectively. New technologies are also applied in this breeding field. There are 200 or so wild *Solanum* species in Middle and South America that may have resistance-related genes. Up to now, only a small portion of them have been investigated for use in breeding programmes. If resistant cultivars were readily available, both the number of pesticides used for plant protection and production loss would be drastically reduced.

Time saving

Linkage drag occurs during typical hybridization programmes and results in the progeny receiving hundreds of undesired genes. It takes several backcrossed generations to eliminate these types of undesirable genes. Linkage drag is avoided using cisgenesis, which also allows for the rapid introduction of the desired gene into the recipient plant's genome. This thus saves a lot of time. For instance, integrating a gene for disease resistance through conventional breeding takes roughly 40 years. The recently cloned apple scab resistance gene Vf could be introduced into the innovative cultivars utilising the cisgenic approach to provide better outcomes faster (Fig 3). Cisgenesis could be used to quickly introduce desired features into cultivars that are commercially successful while preserving their beneficial attributes through traditional introgression techniques (Jacobsen *et al*., 2008; Jacobsen and Schouten 2007; Jacobsen and Van der Vossen, 2009).

Disparity between transgenesis and cisgenecis

To create cisgenic plants, any relevant transgenesis approach can be applied. The main difference, which is fully covered below, is in the place where the gene of interest is obtained. Because cisgenesis makes it possible for the transfer of the desired gene and its promoter, they will last for many generations in the genome of the species or in its genetically compatible relatives. Hence, cisgenesis respects boundaries between species. Cisgenesis adds no extra characters and does not alter the target plant's gene pool in any way. The vigour that would typically change in a conventional breeding programme does not vary. As a result, cisgenesis has no negative effects on unintended species,

environmental risks, or potential allergens linked to GM food and feed. The key contrast between cisgenic and transgenic technology is found here. As a result, a careful introduction and release of cisgenic plants to reach consumers offers the same level of security as plants made using conventional techniques (Hanley *et al*., 2019). The authorities should treat cisgenic plants and conventionally bred plants equally in this matter of food security.

Role of cisgenesis in sustainable crop improvement

In the early stages of the agricultural improvement effort, traditional plant breeding techniques like somatic hybridization, induced mutation and introgressive hybridization were extremely important. These methods alter the genetic makeup of the plant in a random manner, resulting in genetic variety. Even though all of these methods have drawbacks like the connection drag issue and the lengthy time required to release a variety, the final plant can still be placed into the food chain without any restrictions as it is free from foreign genes. In general, they are regarded as safe and have been used without any issues in the past and customers didn't have any type of objection to products. Consider the possibility that when cisgenic plants are grown in fields, their pollen grains may disperse and fertilisation will occur with the nearby vegetation's wild relatives. The majority of cisgenes come from their wild relatives and have been in natural plants for a long time, which makes them an ideal solution to the current biosafety issues (Venkatesh and Steven, 2010). Moreover, these genes may have been used in conventional breeding previously. Because there is no gene invasion from the cisgenic cultivar to the non-GM cultivar, coexistence of cisgenic crops and non-GM crops won't cause issues. As the integration of the cisgene into the plant genome is a random process, much like in conventional induced translocation breeding, it is difficult to predict when this will occur. Without the transfer of unwanted genes and, more importantly, without the integration of any foreign genes, the same gene pool's genes might have been transferred into unique types in a single step. Although using plant genetic engineering and molecular biology techniques, the resulting plants are not transgenic. Due to the fact that the genes utilised for integration are predominantly of bacterial origin, current genetic engineering techniques have a choice as a result of this view. One possibility for the genetic makeup of the generated intragenic plants is a slight dislocation of endogenous genes within the species. Those alterations did not deviate from the naturally occurring revolution brought on by micro-translocations in plant genomes or by artificial mutation (Van Harten, 1998). Future plant breeding initiatives, in conjunction with transgenic strategies, will concentrate on breeding varieties with improved consumer attributes that have a direct benefit for the customer, such as functional, healthful and delicious foods. Enzymes are frequently in charge of controlling quality attributes, such as the accumulation of advantageous nutrients, which are typically regulated by a plant's metabolic network. To achieve a desired outcome, essential enzymes may be altered. Depending on the promoter's activity, this could result in significant changes to a plant's overall metabolism. The interconnections within the metabolic network are primarily responsible for the possibility of unexpected outcomes when the plant's metabolism needs to be targeted by GM. Contrary to the donor mother plant, it is obvious that the cisgene's sequence organisation will remain intact; as a result, the genotypic or phenotypic outcomes of cisgenic or intragenic plants can be taken as being equivalent to those of the donor plant (Rommens *et al*., 2007). As a result, it is reasonable to assume that sufficient knowledge about determining the safety of food and feed has been provided for the accomplishment of some distinctive tasks for the evaluation of risk factors associated with these crops.

Genes associated with the necessary trait must be clearly defined for cisgenics technology to be successfully applied in crop development. Since they are now crucial instruments in the traditional plant breeding process, molecular markers may help identify them. Constant advancements in plants genome sequencing have greatly

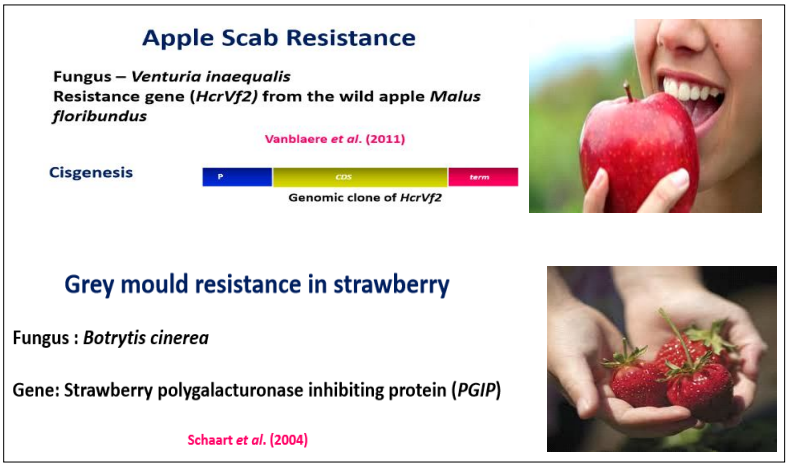

Fig 3: Disease resistance.

aided in the identification and isolation of these genes. Databases that are gradually updated are helpful resources for in silico research. So, the number of genes that are potentially accessible for cisgenic or intragenic alteration is growing, at least theoretically. Their connection to specific functions may be based on commonalities in their sequence. P-DNAs, which are employed as an alternative to traditional T-DNAs in *Agrobacterium*-mediated transformation, have been recognised in several plant genomes using the strategy to find sequences of supposedly alike role through database searches (Lusserens, 2004). However, thorough investigative characterisation of known genes, which is expensive and time-consuming, is a crucial prerequisite for their effective application. The current competent authorities' perception of cisgenic plants based on consumer preferences for the aforementioned products, the decision to label these plants and the products made from them as genetically modified (GM) and the granting of patents for GM technologies and genes will all determine whether or not cisgenesis becomes a significant technology (Lusk and Rosan, 2006; Lusser *et al*., 2011). Although it is entirely impossible for the authorities to control patents and customer preferences, it appears that assigning cisgenic and transgenic plants a distinct grade is a wise decision. On the other hand, if GM technology is used to enhance qualities other than those for food and feed, then there may not be any societal backlash against transgenic crops. The potential benefits of this technology have been overshadowed by public discussions about the potentially unpredictable results and prevailing social beliefs that GM technology is unnatural and contributes to the genetic erosion of native plant varieties. Additionally, some groups of people do not support the idea of introducing bacterial or animal genes into plant genomes. The idea of developing crops that pose no dangers to the general population and provide a product that is environmentally friendly, commercially viable and socially acceptable has become increasingly popular for all these reasons. In light of these factors, cisgenesis will be a superior option and will pave the way for sustained crop development programmes. Cisgenesis can be use in production of disease-resistant plant varieties, enhancement of fruit quality. The capacity to withstand abiotic stress could be obtained by cisgenesis. It is the best tool for improving vegetatively propagated crops. It can have a role in Gene pyramiding and producing plant varieties for organic farming.

Fruit crop improvement through cisgenesis

The development of cisgenic crops is now focused on two main objectives: stress-tolerance and resistant to disease and pest (plant incorporated protection, PIP). Moreover, adding more copies of a certain gene may enhance quality characteristics. Improvement of quality features in plants is a key objective of plant breeding programmes, as shown by trends in pipelines of biotech businesses using transgenic techniques at the moment (Varshney *et al*., 2011). Targeted characteristics include fatty acid composition (Omega-3 fatty acids, reduced saturated and improved unsaturated fatty acid levels, removal of trans fats), improved flavour, quality of fibre, shelf life as well as optimisation for usage as food, feed, biofuel, or industrial purposes.

Nowadays, the main targets for cisgenic modification are crops like potatoes and fruit trees (Rosaceae). The accessibility of the gene (or genes) accountable for the desired trait's manifestation and its monogenic or oligogenic nature determine whether it is possible to manufacture a commercially viable product. Monogenic characteristics may be targeted as a first step. Gene pyramiding is nevertheless a possibility. In general, cisgenic alterations are drawn to trees as a target. The main factor may be seen in the shorter amount of time required to produce a new varietry that will be popular in the market. The most recent scientific literature that has been subjected to peer review and claims to propose "cisgenic techniques" may not actually meet the criteria of cisgenesis in its strictest sense. Only two articles; Vanblaere *et al.* (2011) and Holme *et al*. (2013) currently exist that are likely to fulfil the Schouten *et al*. (2008) concept of cisgenesis. Vanblaere *et al.* (2011) created the "Gala" Cisgenic Apple lines. They used the ORF (Open reading frames) of the HcrVf2 genomic region, which confers scab resistance and contained 242 and 220 base pairs from the 5' and 3'UTRs (untranslated regions) of the wild cousin *Malus floribunda* (Flachowsky *et al*., 2011). Dexamethasone-induced recombination eliminated the nptII gene for kanamycin selection from the region between the recombination sites, resulting in marker-freelines. PCR was used to establish the presence of HcrVf2, absence of trfA (responsible for replication initiation), nptIII as the backbone and the fusion marker gene nptII/codA. Holme *et al.* (2013) showed "cisgenic barley with increased phytase activity". By utilising the pCleandual binary vector system, which leverages hygromycin resistance for selection, they were able to attain marker-free status of the cisgenic plants. The genomic area belongs to the 5208-bp HvPAPhy gene, which was amplified by PCR. The buildup of phytase levels in the mature barley grain will be particularly beneficial for both the bioavailability of phosphate in the grain and regarding the environmental factors with the introduction of additional copies of the HvPAPhy gene.

Protocol

Source

-NBPGR, NCBI.

Vector construction

-Plasmid, cosmid, bacteriophage, virus *etc*.

Transformations

-Microinjection (Fig 4), Gene gun (Fig 5), Electrophoresis, Agrobacterium (Fig 6), *etc*.

Selection of transformed plant

(Antibiotic containing media).

Elimination of marker and backbone

(Through DEX media).

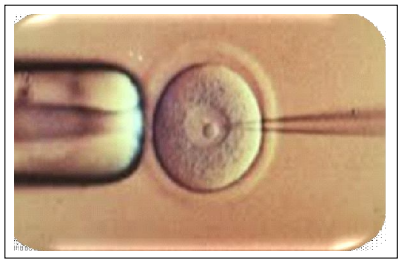

Fig 4: Microinjection.

Fig 5: Gene gun.

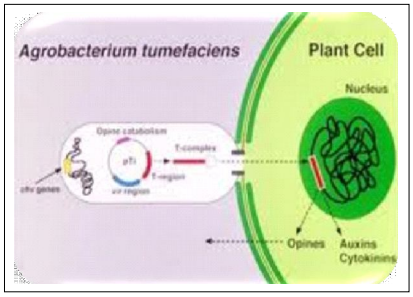

Fig 6: *Agrobacterium* mediated.

Confirmation

(Through PCR).

Expression

-Southern blotting, RT PCR, ELISA.

Drawback of cisgenesis

Although cisgenics technology is outperforming its transgenic counterpart in many ways, there are still a few drawbacks to this technology (Stein and Rodríguez-Cerezo, 2010). One drawback of cisgenesis, compared to transgenesis, is that no characteristics from the sexually uncompatible gene pool can be inserted. In contrast to transgenic crops, the development of cisgenic crops requires a great deal of expertise and effort. As a result, the necessary genes or gene fragments may not be easily available and must instead be extracted from the gene pool of individuals with the same sexual orientation. Since such procedures might not be widely accessible for the crops in question, the production of marker-free plants typically necessitates the creation of novel protocols. Second, numerous transgenic lines must be eliminated since 20-80% of the transformants include vector-backbone sequences. To produce a large number of transformants, intense labour must therefore be put out, especially on crops with low transformation efficiency.

CONCLUSION

Using cisgenic approaches increases the likelihood of introducing the desired genes into novel cultivars (Often a single gene in the first phase) without altering their beneficial traits. The evolution of monogenic resistance features may therefore be expected to benefit most from cisgenesis. Yet, the use of gene pyramiding will also produce a more robust resistance. Breeding long-lived plants, like trees, could have significant advantages. Abiotic stress tolerance is typically a complex feature (*e.g.* due to polygenic traits). To create stress-tolerant lines, frequently more than one gene or QTL must be introduced. In this regard, gene pyramiding will be required, indicating that the sequences and functions of genes have been thoroughly defined. Cisgenesis related data and information are very rare and there is only few limited statistical evidence presented in the seminars and conference proceedings till date. Therefore, it is expected that cisgenesis may eliminate the possible unwanted products and the social beliefs that the public has in their minds regarding GM technology if we broaden our area of research to include cisgenic approaches and if it has been relieved from the regulatory framework of GM technology. Briefly, we can say that there is no linkage drag because it is a one-step gene transfer. Cisgenesis is Specific in nature as only desired alleles are introduced. It is easier to stack (resistance) genes. Breeders' gene pools can be used to directly improve existing kinds. Cisgenic has been so far seen as desirable over transgenics by consumers. As a result, cisgenesis will be crucial for sustainable improvement of fruit crops.

Conflict of interest: None.

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