Review Paper:

Cisgenics as New Horizon in Crop Improvement

Barman Mainak*, Singh Satish Kumar, Kumar Ashutosh and Parveen Rabiya
Department of Plant Breeding and Genetics, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, INDIA
*mainakbarman4@gmail.com

Abstract

Crop improvement with the execution of molecular biology started more than three decades ago. There are many relevant difficulties that affect crops which could be alleviated by the use of Genetically Modified Organisms (GMO). On the other hand, improving crops utilizing the GMO techniques is also associated often with different environmental risks, safety hazards, and health concerns as a result of the presence of foreign DNA. These constraints have encouraged the development of substitute technologies. Cisgenesis has been developed as a novel tool intended to modify diverse crops. Cisgenesis is a specific science in which the genetic modification is done by relocating beneficial alleles into the recipient plant from species that are compatible sexually. There are additional advantages of this technology over conventional plant breeding. In this technology, cis-genes from the crossable plants are employed whereby the hitch of linkage drag of other undesirable genes is surmounted. The idea of cisgenic crops implies that the transformation of the plants must be done with the help of genetic material derived either from the species itself or from any closely related species that is/are able to hybridize sexually.

One another criteria for cisgenic crop production is foreign sequences such as 'Vector backbone' and 'Selectable marker genes' sequences should not be present. Cisgenic plants should be treated in the same way as the classically-bred plants and differently from the plants bred by transgenic means. It is suggested to let the crops off from the regulation GM-plants that contain cis-genes only. This current review discusses the implications of cisgenesis in the direction of sustainable development through genetic improvement of crops and also considers the anticipations for the technology.

Keywords: Cisgenesis, GMO, Linkage Drag, Genetic Improvement, Selectable marker genes.

Introduction

Plant breeding is a progressive motion of mankind that instigated thousands of years back when the shifting of human being came about from the activities of hunting and gathering of food materials towards the sowing of seeds and nurturing them to raise crops under a stable settlement and keeping the seeds for raising crops in the forthcoming season. Plant breeding turned out to progressively be more science-driven following the staggering invention of Mendel's Law.

Nowadays, it is progressing in an incredibly swift manner because of the advancement of many innovative technologies and scientific disciplines with their suitable functional ability. The major goal of the discipline of plant breeding coupled with several other scientific disciplines like Genetics, Botany, Biotechnology, Biochemistry, Plant physiology etc. is to feed the globe with proper nourishment.

In the last two hundred years, the global population has reached about 7 billion whereas to reach 1 billion, it spent more than 2 lakh years of human-being history. In this ever-increasing population, the key challenge is to keep on providing proper nutrient-rich food crops to all living beings to eradicate hunger and malnutrition from the globe.[66] As per the information provided by the Food and Agriculture Organization (FAO), the present population growth rate will require a boost of 70% of the food production by the year 2050[14]. In this circumstance, environmental impact of the agricultural sector comes forward as a significant concern to be addressed with the intention of controlling and containing the negative impact on the land depletion, natural resources, and global warming, and also maintaining the levels of yield[38,65].

But unfortunately, the breeders are facing several constraints to come up with potential plant varieties to serve the sphere in time with their two traditional weapons- crossing and mutation. Then in the eighties and nineties of the very last century, approaches were developed for genetic modification of living plant cells to overcome numerous constraints faced by breeders. The term 'biotechnology' in general refers to the recently-developed scientific means accustomed to construct products by the alteration of the genetic makeup of various organisms and generating exclusive traits or individuals that by far are not achieved through the techniques of conventional breeding.

These creations are habitually referred to as the bioengineered, transgenic, or genetically modified since they include genetic material of foreign origin[45]. Characteristic of the green biotechnology is the modification of crops through genetic means with the intention of conferring novel traits. It can be achieved either by the appearance of one gene of foreign source or by suppressing a protein of endogenous origin to transform any function. These types of organisms are recognized as Genetically Modified Organisms[11].

In the year of 1983, genetic engineering of crop plants set off[15] with the expression of a gene of bacterial origin in tobacco. Flavr Savr™ tomato introduced by the Calgene Company in 1994 was the first commercialized genetically-engineered crop in the world[4]. These genetically-engineered tomatoes had the capacity of shelf life longer than the conventional others. Regulating the level of a polygalacturonase enzyme involved in fruit ripening through the expression of an antisense RNA was the key to success in this case[32].

Although, genetic modification of crops is the first technology in the world to date that has not extensively been accepted by the end-users i.e. the consumers. The "Golden Rice" (and currently "Golden Rice 2"), a transgenic crop, was produced to enhance the β-carotene in an exploitable form for the human-beings. No production yet of this crop has taken place in a commercial mode[21]. The bursting prospective of genetically modified crops can be appreciated merely with an augmented recognition by the common people. The public debate concerning compulsory labeling of the GM foods, and facts from an immense list of researches, reveal unease for GM foods among the consumers[6,39].

Furthermore, the expensive, tedious and prolonged procedures for achieving endorsement of these GM crops and the hazards for probable health menaces together with the spread of novel genes into another unrelated crop species are the most important drawbacks in the lane of executing these practices. Keeping insight into these discussed drawbacks, scientists searched for a sustainable and effective way out for all these dilemmas with a motto to ensure eco-friendly crop enhancement techniques.

Thus, with a swear of the safety of the environment[1], the cisgenic approach was bloomed as an alternative to the transgenic strategy. The central idea of cisgenesis is based upon the special employment of genetic material from the alike species or by collecting from the closely associated species which is/are sexually hybridizable. The gene pool subjugated by cisgenic crops apes the same gene pool which is revealed by the traditional plant breeding[53]. This is a more modern form of genetic modification that began around the year 2015[52].

What are cisgenic crops?

Jochemsen and Schouten[30] introduced the unique conception of cisgenesis in the year of 2000 in their book entitled as "Toetsen en begrenzen. Een ethische en politieke beoordeling van de moderne biotechnologie". The concept was made legendary in the year 2006 by Schouten et al[53,54] by their publications in the EMBO Reports and Nature Biotechnology. They indicated cisgenic crops as the crop plants that have been modified genetically with one or more than one genes that are isolated from a donor plant that falls within the crossable limit. Their claim was that the genes in cisgenic crops are flanked by its promoter and terminator of native origin in the orientation of sense manner. The method of producing a cisgenic crop is termed as cisgenesis.

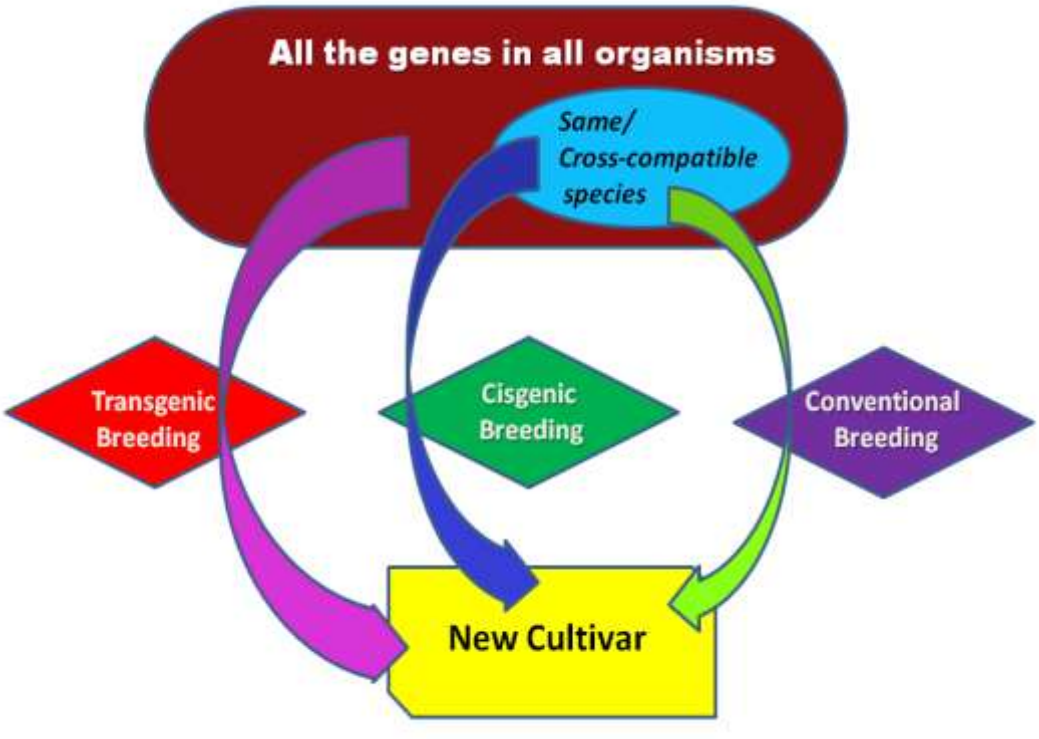

Fig. 1; Major concepts of crop improvement

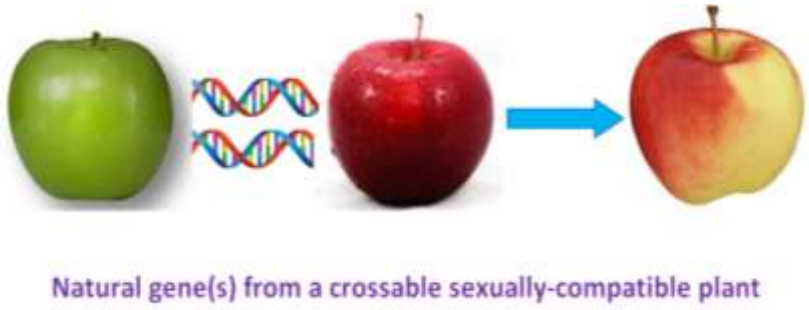

Fig. 2: Overview of cisgenic breeding technique

Cisgenesis vs. conventional plant breeding: One major target of plant breeding is to advance a cultivar for a definite trait establishing one gene from any suitable donor plant. Any wild germplasm, for instance, could be employed as one disease resistance source. From that wild germplasm, the breeder never desires to transmit any of the other undesirable genes that might trim down the agronomic performance of that particular cultivar. The method of backcrossing is one such approach through which a definite gene introduction is accomplished in conventional plant breeding. In these circumstances, one vital drawback is observed often called a linkage drag. This phenomenon is denoted as the fall of a cultivar's fitness due to detrimental genes established along with the favorable gene during the process of backcrossing. To rise above the dilemma of linkage drag, cisgenesis is a perfect solution as it includes the transfer of the particular gene(s) which are required with the help of biotechnological tools.

As compared with the plants which are conventionally bred, cisgenic crops are as harmless as those[9]. The genetic make-up of the novel cultivar is conserved in the cisgenic crops like the conventional breeding approach and one or few genes are added with that only which keeps the cisgenic crops away from hampering the ecosystem by any means. Traditional crop breeding methods are very time-consuming whereas the time required for the production of the cisgenic crop is very less as the gene(s) of interest is integrated inside the genome of the beneficiary plant within a petite period which makes the strategy far ahead from the conventional procedures. Conventional methods of plant breeding in general cause modification in vigor which do not turn out for the case of cisgenic crops and it maintains the novel genetic make-up of the plant variety.

Moreover, cisgenic crops do not alter the gene pool. The foremost intention of cisgenesis is to relocate genes of disease resistance in the varieties which are susceptible. The crucial ambition here is to minimize the substantial exploitation of pesticides. This in turns results in lowering the input expenses of the farmers and also drops off in the pesticide snippets on the plants as well in products of them, which is the utmost desire of the consumers.

Cisgenics vs. transgenics: In the case of transgenics, the transferred gene is generally derived from an alien species. Such a gene in the target plant might endow with a novel trait. It opens up scope for raising various environmental and social issues by the anti-GM-activists. But the cisgenic crops do not allow space for any alien species' gene. The gene(s) exploited here, in this case, are taken from the plant itself or from another plant that is sexually compatible in crossing.

In many countries, transgenic researches are limited before the stage of flowering. There it is not possible to observe the segregation and finalize the heritability of the transferred trait ultimately in case of transgenic crops. The flow of genes towards the wild relatives from the crops produced by the transgenesis approach results in the wild plants to attain traits that advance their "fitness", switching them to "superweeds"[52]. In cisgenic crops, there is no risk on the non-target crop or the ecosystem itself as the gene is from the same or related species. The cisgenic crops do not bring any change in the fitness of the crop in question as no new alien gene is being introduced.

Pre-requisites for cisgenic crop production: The cisgenic crop development or the cisgenesis process requires mainly two pre-requisites as follows:

a) The sequence information of the plant genome in question.
b) From crossable relatives, isolation and the characterization of a gene(s) of interest are required.

Techniques to breed cisgenic plants eliminating marker genes

Four broad approaches are there to achieve a cisgenic crop as follows-

Marker-free transformation: The most trouble-free approach to eliminate the marker genes from transgenics is to evade their utilization in the transformation process of plants. First reported case was the potato cv. *Kanico* transformation without utilizing any selection marker genes and only by the use of AGL0 strain of A*grobacterium tumefaciens.* Regardless of a number of advantages, a few disadvantages are also coupled with this technique. Over the transformants' selective growth, the control is nil, and to validate the incorporation of the transgene(s), the researcher personnel have to screen a number of putative transformants. This phenomenon is costly as well as time-consuming. The regeneration of a huge quantity of chimeric plants in an

uncontrolled manner is an additional negative aspect of carrying the selection process without employing the antibiotics as detected in the tobacco.

Co-transformation: Co-transformation is an uncomplicated and exceedingly efficient process to eradicate marker genes from the nuclear genome of those plants that are made transgenic. There are four approaches for the co-transformation process as described in fig. 3[5]. This co-transformation method holds quite a lot of limitations besides being a simple and effective approach which is unavoidable. First, it is a time-taking and well-suited only for those fertile plants which are propagated sexually. Secondly, the efficiency of the co-transformation process may be limited by a tight linkage between the DNAs which are co-integrated. This technique may not be appropriate for those species which have extremely low effectiveness of transformation[60].

A progress in the co-transformation method was put forward by Komari et al[31] in 1996. Cisgenic rice plants (expressing blast resistance) were produced in 2018 using this technology that were free from selectable marker[58].

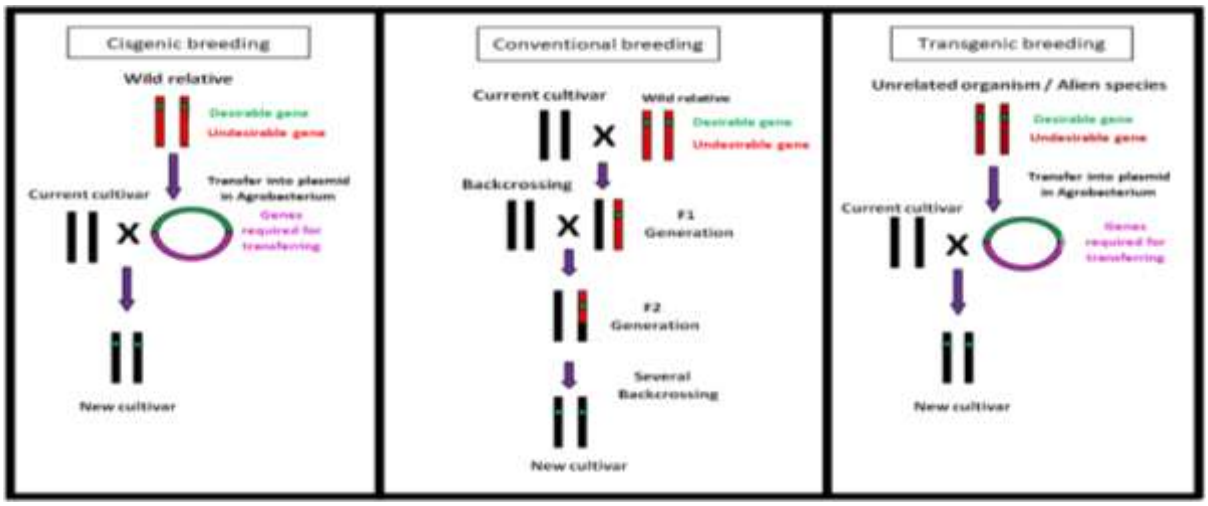

Fig. 3: A comparative diagram of the methods of Cisgenic breeding, Conventional breeding and Transgenic breeding[37]

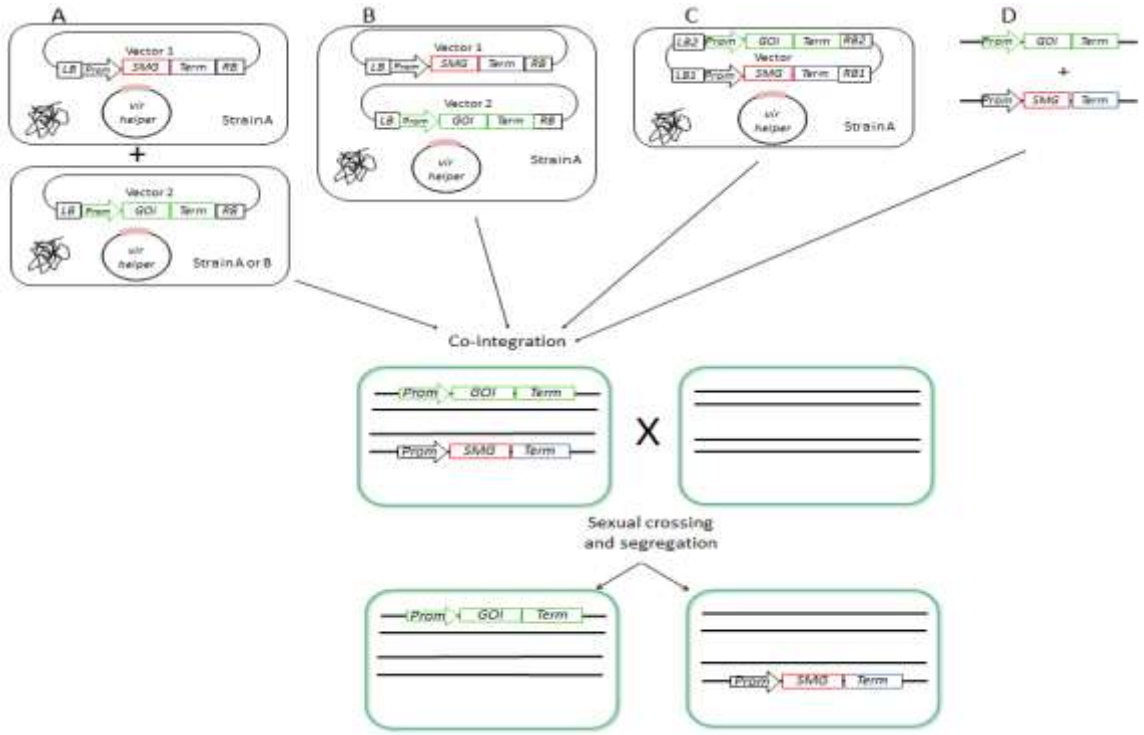

Fig. 4: Co-transformation / segregation approach to achieve marker-free transgenic plants. (A) The Selectable Marker Gene (SMG) and the gene of interest (GOI) are established on different T-DNAs present inside two dissimilar A*grobacterium* strains, (B) in the same A*grobacterium* strain but on separate vectors (C) the vector is same (D) by using a direct gene transfer method, for example, particle bombardment, delivery of the two genes can also be done.

Recombinase induced excision: The process of recombination can be begun involving two DNA molecules that have merely extremely short sequences in common without having the region of wide homology. This is called site-specific recombination. There are three well known site-specific recombination systems illustrated for the purpose of selection marker genes' elimination. These three are the Cre/lox site-specific recombination system from the P1 bacteriophage, the FLP/FRT recombination system from *Saccharomyces cerevisiae*, and the R/RS recombination system from *Zygosaccharomyces rouxii*. Marker-Free apple plants were generated using this technique that epressed the gene for supersweet protein[61].

The three systems are similar in terms of its recombination mechanism. The sites of recombination are in general between nucleotide lengths of 30 to 200, consisting of two motifs along with a fractional symmetry of inverted repeat. Upon combining of recombinase to these motifs, a central crossover sequence is flanked by it at which point the recombination comes to pass. This scheme is frequently referred to as "self-excision" or "auto-excision"[44].

As a result of the stretched existence of bacterial recombinase inside the plants, unwanted changes in the plant genome can occur at the spots where transgene excision takes place. The strategy of auto-excision has its inadequacies or limitations also. It is, for instance, victorious only in the flowering plants as done in the case of strawberry[50] and it is not practical for the plants that are vegetatively propagated like potato, grapes, or banana[27].

Transposon based excision: Transposons or the so-called "jumping genes" have been widely exploited as a means to eliminate the sequence of a marker from the gene(s) of interest. The tactic makes the utilization of the system of Ac/Ds transposition. The approach is principally based upon the fact that the DNA sequences positioned in the Ds (Dissociator) repeats can be translocated for the purpose of excision together with the Ds element[35]. This method engages the Agrobacterium-mediated transformation schemes followed by the intra-genomic rearrangement of the transgene of interest (TOI) and its successive segregation from the selectable marker in the offsprings. Marker gene's direct excision from the genome can also take place.

Ac/Ds transposable element of maize was used to develop both the discussed strategies and the scheme effectively could be settled in to exercise autonomous transposable elements that are identical. The marker-free transgenic plants can effortlessly be screened at the generation T_0, shunning the necessity for sexual reproduction and also signifying the relevance of the tactic to the crops that are propagated vegetatively. It makes the chief benefit of this approach.

Regardless of many advantages, a small number of limitations are unavoidable. The awfully low regeneration frequency of the marker-free transgenic plants and the genomic precariousness of transgenic plants as a consequence of the constant occurrence of heterologous transposons are the major among them. The necessity of genetic crossing and segregation for the purpose of separating the marker gene and the transgene is a time-consuming procedure which can be considered as one of the negative aspects of this technique. precariousness

Genome Editing: 'Genome editing' takes account of a set of practices that permit us to edit, delete, insert or replace definite genomic sequence(s) of interest in a targeted site. In diverse organisms, counting plants also, the methods are anchored in the induction of cuts in the double-stranded DNA (double-strand breaks), which subsequently go through repairing with two dissimilar practices: (i) the NHEJ i.e. Non-homologous End Joining (ii) HDR i.e. Homology-directed Repair.[17,48,49,64]

The breaks in the double-stranded DNA can be tempted by four mechanisms based on definite enzymes: (a) Meganucleases, (b) Zinc finger nucleases (ZFN), (c) Transcription activator-like effector nucleases (TALEN) and (d) Clustered regular interspaced short palindromic repeats/CRISPR-associated nucleases (CRISPR/Cas). These techniques allow us to make modifications in our gene of interest.

Regulation of Cisgenics: To the extent that the regulation of these produces is concerned, broadly speaking, Canada, United States, and Australia are presenting a more unwrapped orientation and are aspiring to make a distinction of them from the conventional GMPs[2,30], although, in Europe and many other countries, the approach is far more guarded. In Canada, the system of regulation is product-based rather than being a system that is process-based. There it has been made possible legally in controlling cisgenic plants less stritctly than the plants that are made through transgenic technology. The conclusion of the European Food Safety Authority(EFSA) about cisgenic plants is that they cause risks alike to those attained with the conventionally bred plants[23], Additionally, cisgenic products were recorded having greater recognition as compared to the subsequent transgenic crops by the consumers authenticated by a number of recent reports[63].

The cisgenic crops are recently launched into the commercial markets[40]. Although there was a preliminary effort to grant various regulation of cisgenic crops[52], these crops will be delighted by United States Department of Agriculture (USDA)- Animal and Plant Health Inspection Service (APHIS) as alike to crops produced through the breeding practices of traditional manners. Since the most contemporary cisgenic crops are not "pesticidal" in characteristic, the regulatory authority for the engagement of EPA has until not recently been essential. The exemption is a new-fangled pesticidal corn seed called as DvSnf7 dsRNA. It acts in a way comparable to Bt corn using an NGMT (new

genetic modification technique) known as RNAi (RNA interference).

Recently cisgenic crops-releasing companies, all have endured the voluntary pre-market consultation progression with the Food and Drug Administration (FDA). One of the very first instances of a cisgenic crop to be presented commercially is a soybean generating oil that is additionally nutrition-balanced and nearer to the olive oil[43]. Cautious scrutiny of regulatory status in cisgenic assembles is compulsory. It is suggested to exempt from the regulation of GM-plants that include cis-genes only[28].

Limitations of Cisgenesis: Even though the cisgenic technology is revealing significant advantages more than the transgenic counterpart, however, there are a small number of limitations still present related to this tool. One of the major disadvantages contributed by the cisgenic approach compared to transgenic, is that characters cannot be introduced that are exterior to the sexually well-matched gene pool. Moreover, extraordinary proficiency and time are required for the development of cisgenic crops as compared to the transgenic crops. Hence, the genes or fragments of genes may not be readily accessible that are needed. Those from the sexually compatible gene pool, have to be isolated[22].

There are some more issues, first of all the creation of marker-free plants typically necessitates the innovative protocol development, may be such protocols are not readily presented for the crop under consideration. Secondly, as vector-backbone sequences are contained by 20 – 80% of the transformants, removal of a lot of transgenic lines has to be carried out. Therefore, extensive hard labor has to be performed principally on the crops with less transformation effectiveness to generate a huge number of the transformants.

Table 1
Instances of agronomic traits modification through the employment of cisgenesis in various crops: As reviewed in recent times[12,36], the approach of cisgenic has been used for betterment in quality traits and pathogen resistance in a number of crops.

Crop	Trait	Gene	Donator	Year
Potato	Late blight resistance[10,24,25,29]	R2, R3a, R3b, R5, R6, R7, R8, R9, R10, R11	*Solanum demissum*	1996,2004, 2005, 2009
Potato	Late blight resistance[13]	Rpi-ber1	*Solanum berthaultii*	2000
Potato	Nematode resistance (G. *rostochiensis*)[46]	Gro1-4	*Solanum tuberosum*	2004
Potato	Late blight resistance[28,34,47]	Rpi-blb1, Rpi-blb2, Rpi-blb3	*Solanum bulbocastanum*	2005, 2007, 2009
Strawberry	Fruit rot (*Botrytis cinerea*)[51]	PGIP	_	2007
Apple	Induces anthocyanin accumulation/red apple fruit color[12]	MdMYB10	*Malus domestica*	2007
Durum wheat	Baking quality[16]	1Dy10	_	2008
Rye-grass	Drought tolerance[19]	Lpvp1	*Lolium perenne*	2008
Potato	Late blight resistance[29]	Rpi-vnt1	*Solanum venturi*	2009
Melon	Downy Mildew resistance (*Pseudoperonospora cubensis*)[3]	At1/At2- glyoxylate aminotransferase	_	2009
Barley	Phytase activity[56]	HvPAPhy_a	_	2010
Apple	Scab resistance[63]	HcrVf2	*Malus floribunda*	2011
Barley	Nitrogen Use Efficiency (NUE)[41]	gTIP2 and gGS1a	_	2011
Grapevine	Fungal disease resistance[7]	VVTL-1	_	2011
Apple	Scab resistance[33]	Rvi6	*Malus floribunda* 821	2015
Rice	Rice Blast disease resistance gene[58]	Pi9	_	2018
Apple	Supersweet protein gene expression[61]	thaumatin II	*Thaumatococcus daniellii*	2019

The eagerness of people about cisgenic crops: 75% of German consumers wish to see the food manufacturers and retailers put together broad utilization of voluntary "GM-free" labeling proposal of Germany and would pick for manufactured goods labeled "GM-free" if offered[57]. Gaskell et al[18] accomplished a study from the sample population signifying 32 European countries on the subject of the consumer inclination about transgenic and cisgenic approaches taking up the apple scab resistance, canker and mildew. From their study, it was exposed that in the whole of the European Union (EU), 33% were towards the transgenic apples but 55% of people upheld cisgenic apples.

Mielby[42] performed a sociological investigation to expose the penchant level of bread made from the cisgenic-derived crop flour amongst the target section of people in Denmark. He documented that absolutely only about 25% was not in favor of the merchandise. Shew et al[55] conducted 300 interviews in Jaipur, India for assessing the cisgenic rice acceptance of consumers in India and they concluded that 73% of respondents stated their willingness to consume foods produced by cisgenic means. Edenbrandt et al[8] conducted their study on preferences of consumers for rye bread substitutes based on the cisgenic or transgenic rye. They concluded that the use of the cisgenic approach was clearly far more acceptable than the transgenic approach.

Conclusion

The application of cisgenic practice boosts the opportunity to introgress the ideal favored genes into the fresh cultivars (solo gene in the initial step in most of the cases) without troubling their constructive characteristics. Hence, the most persuasive role of cisgenesis may be foreseen for the advancement of resistance traits that are monogenic. The cisgenic approach has also been renowned as a budding valuable approach to boost the tree-biomass which are proper for the production of bioenergy[20]. Cisgenic alteration of the gibberellic acid pathway in the poplar is an instance of such an attempt[**26**].

The knowledge distribution on the subject of cisgenic crops and cisgenesis is very infrequent and there is merely definite frolicsome information given in the seminars and conference happenings. It is predictable that cisgenesis may annihilate the expected uncertain conclusions and the social credence that the public has kept in their mentality concerning GM technology. Therefore, it is expected that cisgenic crops will play a vital task in crop improvement in a sustainable manner if we spread our vicinity of research out towards this approach. Moreover, Cisgenic plants should always be treated likewise the classically bred plants and should receive a different viewpoint from the transgenically bred plants[**59**].

References

1. Andersson H.C., Arpaia S., Bartsch D., Casacuberta J., Davies H., Jardin P.D., Flachowsky G., Herman L., Jones H., Kärenlampi S. and Kiss J., Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis, *EFSA J*., **10(2)**, 2561 **(2012)**

2. Belfanti E., Silfverberg-Dilworth E., Tartarini S., Patocchi A., Barbieri M., Zhu J., Vinatzer B.A., Gianfranceschi L., Gessler C. and Sansavini S., The HcrVf2 gene from a wild apple confers scab resistance to a transgenic cultivated variety, Proc. Natl. Acad. Sci., USA, **101**, 886-890 **(2004)**

3. Benjamin I., Kenigsbuch D., Galperin M., Abrameto J. and Cohen Y., Cisgenic melons overexpressing glyoxylate aminotransferase are resistant to downy mildew, *European J. Pl. Patho*., **125**, 355- 365 **(2009)**

4. Bruening G. and Lyons J.M., The case of the FLAVR SAVR tomato, *California Agriculture*, **54**, 6-7 **(2000)**

5. Chong-Pérez B. and Angenon G., Strategies for Generating Marker-Free Transgenic Plants, Chapter 2, INTECH, http://dx.doi.org/10.5772/55573 **(2013)**

6. Costa-Font M., Gil J.M. and Traill W.B., Consumer acceptance, valuation of and attitudes towards genetically modified food: Review and implications for food policy, *Food Policy*, **33**, 99–111 **(2008)**

7. Dhekney S.A., Li Z.T. and Gray D.J., Grapevines engineered to express cisgenic Vitis vinifera thaumatin-like protein exhibit fungal disease resistance, *In Vitro Cell. Dev. Biol.-Plant*, **47**, 458-466 **(2011)**

8. Edenbrandt A.K., Gamborg C. and Thorsen B.J., Consumers' Preferences for Bread: Transgenic, Cisgenic, Organic or Pesticide-free?, *Journal of Agricultural Economics*, **69(1)**, 121–141, DOI: 10.1111/1477-9552.12225 **(2017)**

9. EFSA Panel on Genetically Modified Organisms (GMO), Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis, *EFSA J*. **10**, 2561, DOI: 10.2903/j.efsa.2012.2561 **(2012)**

10. El-Kharbotly A., Palomino-Sanchez C., Salamini F., Jacobsen E. and Gebhardt C., R6 and R7 alleles of potato conferring race-specific resistance to Phytophthora infestans (Mont.) de Bary identified genetic loci clustering with the R3 locus on chromosome XI, *Theor. Applied Genet*., **92**, 880-884 **(1996)**

11. Espinoza C. et al, Cisgenesis and Intragenesis: New tools For Improving Crops, *Biol Res*., **46**, 323-331 **(2013)**

12. Espley R.V., Hellens R.P., Putterill J., Stevenson D.E., Kutty Amma S. and Allan A.C., Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10, *Plant J*., **49(3)**, 414-427 **(2007)**

13. Ewing E.E., Simko I., Smart C.D., Bonierbale M.W., Misubuti E.S.G., May G.D. and Fry W.E., Genetic mapping from field of qualitative and quantitative resistance to Phytophthora infestans in a population derived from Solanum tuberosum and Solanum berthaultii, *Mol. Breed*., **6**, 25-36 **(2000)**

14. Food and Agriculture Organization, FAO's director-general on how to feed the world in 2050, *Popul. Dev. Rev*., **35**, 837–839, https://doi.org/10.1111/j.1728- 4457.2009.00312.x **(2009)**

15. Fraley R.T. et al, Expression of bacterial genes in plant cells, Proc Natl Acad Sci., **80**, 4803-4807 **(1983)**

16. Gadaleta A., Giancaspro A., Blechl A.E. and Blanco A., A transgenic durum wheat line that is free of marker genes and expresses 1Dy10, *J. Cereal Science*, **48**, 439—445 **(2008)**

17. Gaj T., Gersbach C.A. and Barbas C.F. 3rd, ZFN, TALEN and CRISPR/Cas-based methods for genome engineering, *Trends in Biotechnology*, **31**, 397—405 **(2013)**

18. Gaskell G., Allansdottir A., Allum N., Castro P., Esmer Y., Fischler C., Jackson J., Kronberger N., Hampel J., Mejlgaard N., Quintanilha A., Rammer A., Revuelta G., Stares S., Torgersen H. and Wager W., The 2010 Eurobarometer on the life sciences, *Nat. Biotechnol*., **29**, 113-114 **(2011)**

19. Hanley Z., GM approaches without GM outcomes, Pastoral Genomics Presentation, http://bit.ly/ixguSB **(2008)**

20. Harfouche A., Meilan R. and Altman A., Tree genetic engineering and applications to sustainable forestry and biomass production, *Trends in Biotechnol*., **29(1)**, 9-17 **(2011)**

21. Hilbeck A. and Herren H., Millions spent, no one served: who is to blame for the failure of GMO golden rice?, Independent Science News, www.independentsciencenews. org/health/millions -spent-who-is-to-blame-failure-GMO golden-rice **(2016)**

22. Holme I.B., Wendt T. and Holm P.B., Current developments of intragenic and cisgenic crops, ISB News Report, 1-5 **(2013)**

23. Holme I.B., Dionisio G., Brinch-Pedersen H., Wendt T., Madsen C.K., Vincze E. and Holm P.B., Cisgenic barley with improved phytase activity, *Plant Biotechnol. J*., **10(2)**, 237-247 **(2011)**

24. Huang S., The discovery and characterization of the major late blight resistance complex in potato: Genomic structure, functional diversity and implications, Ph.D. Thesis, Wageningen University, Wageningen, Netherlands **(2005)**

25. Huang S., Vleeshouwers V.G.A.A., Werij J.S., Hutten R.C.B., Van Eck H.J., Visser R.G.F. and Jacobsen E., The R3 resistance to Phytophthora infestans in potato is conferred by two closely linked R genes with distinct specificities, *Molecular Plant-Microbe Interactions*, **17(4)**, 428-435 **(2004)**

26. ISB News Report, http://www.isb.vt.edu/news/2009/nov09.pdf **(2012)**

27. Jacobsen E. and Schouten H.J., Cisgenesis strongly improves introgression breeding and induced translocation breeding of plants, *Trends Biotechnol*., **25**, 219-223 **(2007)**

28. Jacobsen E. and Van der Vossen E.A., Plant disease resistance, Cisgenesis: a new tool for traditional resistance breeding In Encyclopedia of microbiology, 3rd ed., Mesolio Schaechter Editor, 597–612 **(2009)**

29. Jacobsen E. and Van der Vossen E.A., In Plant disease resistance: breeding and transgenic approaches, Encyclopedia of microbiology, Schaechter M., eds., Oxford, Elsevier, 597-612 **(2009)**

30. Jochemsen H. and Schouten H.J., Ethische beoordeling van genetische modificatie, In Toetsen en Begrenzen. Een Ethische en Politieke Beoordeling van de Moderne Biotechnologie, Jochemsen H., ed., Amsterdam, Netherlands, Buijten and Schipperheijn, 88–95 **(2000)**

31. Komari T., Hiei Y., Saito Y., Murai N. and Kumashiro T., Vectors carrying two separate T-DNAs for cotransformation of higher plants mediated by Agrobacterium tumefaciens and segregation of transformants free from selection markers, *Plant J*., **10**, 165174 **(1996)**

32. Kramer M. and Redenbaugh K., Commercialization of tomato with an antisense polygalacturonase gene: The FLAVR SAVR™, *Euphytica*, **79**, 293-297 **(1994)**

33. Krens F.A., Schaart J.G., van der Burgh A.M., Tinnenbroek-Capel I.E.M., Groenwold R., Kodde L.P., Broggini G.A.L., Gessler C. and Schouten H.J., Cisgenic apple trees; development, characterization, and performance, *Front. Plant Sci*. **6**, 286, DOI: 10.3389/fpls.2015.00286 **(2015)**

34. Kuhl J.C., Zarka K., Coombs J., Kirk W.W. and Douches D.S., Late Blight Resistance of RB Transgenic Potato Lines, *J. American Society for Hortil. Sci*., **132(6)**, 783-789 **(2007)**

35. Kunze R., The maize transposable element Activator (Ac), In Transposable Elements, Springer, Berlin, Heidelberg, 161-194 **(1996)**

36. Lamalakshmi Devi E., Chongtham S.K., Holeyachi P., Kousar N., Singh M., Behera C., Telem R.S., Singh N.B. and Wani S.H., Cisgenesis and intragenesis: twin sisters for crop improvement, *Res. J. Agric. Forestry Science*, **1**, 22—26 **(2013)**

37. Li R., Quan S., Yan X., Biswas S., Zhang D. and Shi J., Molecular characterization of genetically-modified crops: challenges and strategies, *Biotechnol. Adv*., **35(2)**, 302–309 **(2017)**

38. Lovarelli D., Bacenetti J. and Fiala M., Water footprint of crop productions: a review, *Sci. Total Environ*., **548-549C**, 236–251 **(2016)**

39. Lusk J.L., Jamal M., Kurlander L., Roucan M. and Taulman L., A meta-analysis of genetically modified food valuation studies, *Journal of Agricultural and Resource Economics*, **30(1)**, 28–44 **(2005)**

40. Lusser M., Parisi C., Plan D. and Rodriguez-Cerezo E., New plant breeding techniques, State-of-the-art and prospects for commercial development, JRC, European Commission, 19-55 **(2011)**

41. Lutken H., Laura M., Borghi C., Orgaard M., Allavena A. and Rasmussen S.K., Expression of KxhKN4 and KxhKN5 genes in Kalanchoe blossfeldiana 'Molly' results in novel compact plant phenotypes: towards a cisgenesis alternative to growth retardants, *Plant Cell Rep*., **30(12)**, 2267-2279 **(2011)**

42. Mielby H., Public attitudes to cisgenic crops, Ph.D. Thesis, Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen, Denmark **(2011)**

43. MIT Technology Review, These are not your father's GMO's, **121(1)**, 31-40 **(2018)**

44. Mujjassim N.E., Mallik M., Rathod N.K.K. and Nitesh S.D., Cisgenesis and intragenesis a new tool for conventional plant breeding: A review, *Journal of Pharmacognosy and Phytochemistry*, **8(1)**, 2485-2489 **(2019)**

45. National Agricultural Law Center, Biotechnology: Overview, http://nationalaglawcenter.org/research-by-topic/biotechnology **(2018)**

46. Paal J., Henselewski H., Muth J., Meksem K., Menendez C.M., Salamini F., Ballvora A. and Gebhardt C., Molecular cloning of the potato Gro1-4 gene conferring resistance to pathotype Ro1 of the root cyst nematode Globodera rostochiensis, based on a candidate gene approach, *Plant J*., **38**, 285-297 **(2004)**

47. Park T.H., Gros J., Sikkema A., Vleeshouwers V.G.A.A., Muskens M., Allefs S., Jacobsen E., Visser R.G.F. and Van der Vossen E.A.G., The late blight resistance locus Rpi-blb3 from Solanum bulbocastanum belongs to a major late blight R Gene cluster on chromosome 4 of potato, *Molecular Plant-Microbe Interactions*, **18(7)**, 722-729 **(2005)**

48. Puchta H. and Fauser F., Synthetic nucleases for genome engineering in plants: prospects for a bright future, *Plant Journal*, **78**, 727—741 **(2014)**

49. Rinaldo A.R. and Ayliffe M., Gene targeting and editing in crop plants: a new era of precision opportunities in Molecular Breeding, *Molecular Breeding*, **35**, 1-15 **(2015)**

50. Schaart J.G., Krens F.A., Pelgrom K.T.B., Mendes O. and Rouwendal G.J.A., Effective production of marker-free transgenic strawberry plant using inducible site-specific recombination and a bifunctional selectable marker gene, *Plant Biotechnol. J.*, **2**, 233–240, doi: 10.1111/j.1467-7652.2004. 00067.x **(2004)**

51. Schaart J.G., Towards consumer-friendly cisgenic strawberries which are less susceptible to Botrytis cinereal, Ph.D. thesis, Wageningen University, Wageningen, Netherlands **(2004)**

52. Schahczenski J., Genetically Modified Crops: Transgenics and Cisgenics, NCAT Agricultural and Natural Resource **(2018)**

53. Schouten H.J., Krens F.A. and Jacobsen E., Cisgenic plants are similar to traditionally bred plants, *EMBO Rep.*, **7**, 750–753, DOI: 10.1038/sj.embor.7400769 **(2006a)**

54. Schouten H.J., Krens F.A. and Jacobsen E., Do cisgenic plants warrant less stringent oversight?, *Nat. Biotechnol.*, **24**, 753, DOI: 10.1038/nbt0706-753 **(2006b)**

55. Shew A.M., Nalley L.L., Danforth D.M., Dixon B.L., Nayga Jr R.M., Delwaide A. and Valent B., Are all GMOs the same? Consumer acceptance of cisgenic rice in India, *Plant Biotechnology Journal*, **14**, 4–7, DOI: 10.1111/pbi.12442 **(2016)**

56. Stein A.J. and Rodríguez-Cerezo E., International trade and the global pipeline of new GM crops, *Nat. Biotech*., **28**, 23-25 **(2010)**

57. Sustainability Market Intelligence Report, Quarterly Report, http://bit.ly/lMnwbG **(2009)**

58. Tamang T., Park J., Kakeshpour T., Valent B., Jia Y., Want G. and Park S., Development of selectable marker-free cisgenic rice plants expressing a blast resistance gene Pi9, *World Congress on In Vitro Biology*, **54**, 544 **(2018)**

59. Telem R.S., Wani Shabir H., Singh N.B., Nandini R., Sadhukhan R., Bhattacharya S. and Mandal N., Cisgenics - A Sustainable Approach for Crop Improvement, *Current Genomics*, **14**, 468-476 **(2013)**

60. Thole V., Worland B., Snape J.W. and Vain P., The pCLEAN Dual Binary Vector System for Agrobacterium-Mediated Plant Transformation, *Plant Physiol*., **145**, 1211-1219 **(2007)**

61. Vadim T., Tatiana M., Alexander P. and Sergey D. Production of Marker-Free Apple Plants Expressing the Supersweet Protein Gene Driven by Plant Promoter, *Frontiers in Plant Science*, **10**, 388, DOI=10.3389/fpls.2019.00388 **(2019)**

62. Van der Vossen E.A.G., Sikkema A., Hekkert B., Gros J., Stevens P., Muskens M., Wouters D., Pereira A., Stiekame W. and Allefs S., An ancient R gene from the wild potato species Solanum bulbocastanum confers broad-spectrum resistance to Phytophthora in cultivated potato and tomato, *Plant J*., **36**, 867-882 **(2003)**

63. Vanblaere T., Szankowski I., Schaart J., Schouten H., Flachowsky H., Broggini G.A.L. and Gessler C., The development of a cisgenic apple plant, *J. Biotech*., **154**, 304- 311 **(2011)**

64. Voytas D.F., Plant genome engineering with sequence-specific nucleases, *Annual Review in Plant Biology*, **64**, 327—350 **(2013)**

65. Zhang L., Pang J., Chen X. and Lua Z., Carbon emissions, energy consumption, and economic growth: evidence from the agricultural sector of China's main grain-producing areas, *Sci. Total Environ*., **665**, 1017–1025 **(2019)**

66. Barman M., Choudhary V.K., Singh S.K., Parveen R. and Gowda A.K., Correlation and Path Coefficient Analysis in Bread Wheat (*Triticum aestivum L.*) Genotypes for Morphophysiological Traits along with Grain Fe and Zn Content, *Current J. App. Sci and Technol.*, **39(36)**, 130-140 **(2020)**.

(Received 22nd December 2019, accepted 11th March 2020)