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Involvement of Cisgenics in Crop Improvement: A Hope for a Second Green Revolution

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The Need for a Second Green Revolution

During the first green revolution, India had taken the lead by translating new scientific developments like short straw traits which resulted into higher yields. Nowadays, GM varieties are emerging as a strong tool that helps in yield security by providing resistance against pathogens, pests and herbicides. But the evergreen revolution emphasises the need for enhancing productivity without ecological harm. In population rich, but land-hungry countries like India, China and Bangladesh, there is no option except to increase production under conditions of diminishing per capita availability of arable land and irrigation water and expanding biotic and abiotic stresses without being trapped in a Faustian bargain that threatens freedom and security.

What is Cisgenic?

A concept named cisgenesis was introduced by Jocobsen and Schouten in 2000 in the book 'Toetsen en begrenzen : Een ethische en politieke beoordeling van de moderne biotechnologie'. The main principle of this initial cisgenesis concept was that the genes or gene elements should be derived from the species itself. According to Schouten *et al*. (2006), cisgenesis is the modification of a recipient plant's genetic background by a naturally derived gene from a cross-compatible species, including its introns and native promoter and terminator flanked in the normal sense orientation. The first scientific statement of bringing forth a true plant obtained by a cisgenic approach was reported in apple through the insertion of the internal scab resistance gene HcrVf2 influenced by its own regulatory genes into the cultivar Gala, a scab susceptible cultivar.

Advantages of Cisgenesis over Conventional Breeding

Cisgenesis conquers the setback of linkage drag. It maintains the original genetic make-up of the plant variety. Due to cisgenesis, there is a reduction in pesticide application. Cisgenesis is a time-saving method compared to conventional breeding.

Methods for Cisgenic Development

1. **Transformation Methods**
   - **a.** Agrobacterium mediated transformation
   - **b.** Biolistic mediated transformation

2. **Methods to Generate Marker-Free Transformed Plants**
   a. Avoiding the use of any selectable markers
   b. Cotransformation
   c. Active marker-removal by recombination
   d. Use of transposable elements
3. **T-DNA Borders or Plant-Derived T-DNA Borders (P-DNA Borders)**
4. **Vector-Backbone Sequences**

Case Studies

Han *et al.* (2011) studied the effects on plant growth from insertion of five cisgenes that encode proteins involved in gibberellin metabolism. Intact genomic copies of PtGA20ox7, PtGA2ox2, PtRGL1_1, PtRGL1_2 and PtGAI1 genes from the genome sequenced Populus trichocarpa clone Nisqually-1 were transformed into *Populus tremula* × *alba* (clone INRA 717-1B4). Overexpression of gibberellic acid biosynthesis enzymes increased the growth rate, while overexpression of catabolic enzymes and negative signal regulators decreased the growth rate and reduced stature.

Vanblare T. *et al.* (2011) developed a cisgenic apple with scab resistance through the transfer of the HcrVf2 genomic clone, including its own promoter and terminator, into apple cv Gala.

Holme, I.B. *et al.* (2014) used a barley phytase gene (HvPAPhy_a) expressed during grain filling to evaluate the cisgenesis concept in barley. The marker gene elimination method was used to obtain marker-free plant lines. Seeds of one cisgenic line homozygous for a single extra cisgene copy of HvPAPhy_a showed a 2.8-fold increase in phytase activity corresponding 2200 phytase units (FTU)/kg flour.

Jo *et al.* (2014) pursued a cisgenesis approach to introduce two broad spectrum potato late blight R genes, Rpi-sto1 and Rpi-vnt1.1 from the crossable species *Solanum stoloniferum* and *Solanum venturii*, respectively, into three different potato varieties. These cisgenic events were selected because they showed broad spectrum late blight resistance due to the activity of both introduced R genes.

Kost, T.D. *et al.* (2015) generated a cisgenic apple line C44.4.146 using the cisgene FB_MR5 from wild apple Malus × robusta5 (Mr5), Significantly lower disease symptoms were detected on shoots of the cisgenic line compared to those of untransformed 'Gala Galaxy'.

Achievements

Various varieties of apple, barley, rye-grass, potato and strawberry have been developed for the various kinds of useful traits through cisgenesis.

Limitations of Cisgenics

1) The gene pool's limit
2) Extensive skill and time are required.
3) Requires the isolation of genes or gene fragments from the pool of sexually compatible genes.
4) A novel protocol for marker-free plant development is required.
5) Hard work has to be done on crops with low transformation.

Conclusion

The classical methods of alien gene transfer by traditional breeding yielded fruitful results. However, modern varieties demand a growing number of combined traits, for which prebreeding methods with wild species are often needed. Introgression and translocation breeding require time-consuming backcrosses and simultaneous selection steps to overcome linkage drag. Breeding of crops using the traditional sources of genetic variation by cisgenesis can speed up the whole process dramatically, along with the usage of existing promising varieties. This is specifically the case with complex (allo) polyploids and with heterozygous, vegetative propagated crops. Therefore, we believe that cisgenesis is the basis of the second/ever green revolution needed in India to overcome the challenges related to yield security, quality traits and healthy vegetables and fruits.

Future Thrust

1) Future developments regarding the generation and commercialization of cisgenic crops will depend on the willingness to apply less stringent regulation to these crops worldwide.
2) Reducing the cost of cisgenic crop approval would also benefit small-scale breeding and seed companies.
3) This would provide these breeders with an additional tool for crop improvement and thus increase the number of cisgenic crops developed.
4) Public perception has proven to be essential for the approval of genetically modified crops.
5) Modifications based on the sexually compatible gene pool carry a high potential for generating plants with environmental, economic and health benefits that may be essential for meeting the global need for more efficient and sustainable crop production.

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