Cisgenics – Facilitating the second green revolution in India by improved traditional plant breeding

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Safe and sufficient food production is an important issue in India. Development of improved varieties by modern plant breeding is crucial, especially when global warming, population growth, environmental stresses, diminishing land resources associated with increased demand for quality food like healthy fruits and vegetables are considered as serious challenges in the future. To master these challenges, a second green revolution is needed in the country. During the first green revolution, India had taken the lead by translating new scientific developments like short straw traits[1]. Transformation of locally adapted varieties into short straw varieties by backcrossing resulted in higher yields. Nowadays, GM (genetically modified) varieties are emerging as a strong tool and promise. One of the major outcomes until now in GM technology is yield security by resistance against pathogens, pests and total herbicides. These GM crops are at the moment connected with transgenes and worldwide such GM crops were grown over 120 m ha in 2007. Between 1996 and 2006, there was about 60-fold increase in the area under GM crops, suggesting that crop biotechnology is one of the fastest adopted technologies in recent history[2].

Transgenes, cisgenes and traditional breeding

Transgenes (hybrid) are genes originating from other organisms or non-crossable species[3], which is a new source of genetic variation for plant breeding. In transgenic technology, bacterial genes coding for resistance to antibiotics or herbicides are used for the selection of transformed plant cells. Therefore, it is logical that for the introduction of these classes of genes, new GM regulations have been developed in order to master the potential risks connected with the genes.

Recent major developments in science, like genome sequencing, are being applied in crop plants like rice, maize and potato, and efficient gene isolation methods such as map-based cloning and allele mining have opened up new avenues in plant breeding using cloned indigenous genes. Natural indigenous genes, isolated from the crop plant itself or from crossable species, are now called cisgenes in order to distinguish them from transgenes[4–7]. The traits of these cisgenes represent the existing genetic variation applied in classical plant breeding. The use of genetic variation coming from the crop plant itself or from crossable species has a long history of safe use in many crops. They can be used directly in traditional breeding programmes as a source of genetic variation. If the genes are from wild species, introgression breeding and induced translocation breeding[4] combined with induced mutation breeding have been developed for transferring the desired traits into crops. The transfer of desired traits in traditional breeding is a relatively slow process and frequently connected with the problem of linkage drag (many neighbouring donor genes, of which one or a few could have negative impact). These linkage drag problems can only be solved by making more backcrosses and simultaneously looking for a recombination which is uncoupling the desired trait from the trait with negative impact in the offspring[3]. The use of isolated cisgenes coding for desired traits, could solve these problems drastically. In addition, the possibility of marker-free plant transformation, without bacterial antibiotic resistance or herbicide tolerance genes as selection marker, enables the creation of clean cisgenic plants without transgenes[8].

Second green revolution in traditional breeding by cisgenesis

Cisgenesis is better than traditional introgression and translocation breeding because of the absence of linkage drag and the reduced number of steps. Domestication of crop plants has diminished the presence of toxic components into acceptable levels, and there is increased possibility of acquiring unrelated traits/toxic compounds when wild species are used for crop improvement. In cisgenesis these problems can be eliminated easily and desired traits can be incorporated by gene stacking. For example, cisgenic apple varieties are being evolved by stacking resistance genes from crossable *Malus* plants for durable résistance to apple scab[3], and biotic interaction between potato–*Phytophthora infestans* is being studied for durable resistance by stacking several *R*-genes[9]. Similar approach can be attempted for developing cisgenic crop plants for quality traits and biotic/abiotic stress tolerance in India. Cisgenesis can be applied for all dominantly inherited traits.

A second green revolution is needed in India to overcome the challenges related to yield security, quality traits and healthy vegetables and fruits. Integrated gene management along with precision farming is suggested for evergreen agricultural revolution[1]. We believe cisgenesis can open up new options for evergreen revolution. For many crops, including vegetatively propagated ones, cisgenesis can be used directly for the improvement of existing varieties, which have already been shown to be safe for use in the market.

GM-regulations

The GM-regulations worldwide do not distinguish between transgenes and cisgenes. This means that the GM-regulations developed for transgenes (representing genes from the new gene pool for plant breeding), are also applied for situations in which only cisgenes (belonging to the existing breeding pool with a long history of safe use) are used. In the EU directive 2001/18/EC, a definition of genetic modification is given which in practice is too broad[10]. It includes aspects like *in vitro* fertilization, polyploidization, induced mutation and protoplast fusion which, except for protoplast fusion, have a long history of safe use. Therefore, *in vitro* fertilization and induced polyploidization techniques and their products are not considered as GMOs (genetically modified organisms, annex 1a; ref. 10) and plants generated from induced mutations and protoplast fusion between crossable species though considered as GMOs, are exempted from the regulation. The traits related with cisgenes are safe and being used in traditional breeding. Therefore a strong recommendation is made to add cisgenes to the list of exemptions in annex 1b (ref. 10).

In India, the framework for transgenic crops is described by the Department of Biotechnology[11], which consists of the following rules and guidelines: (1) 'Rules and Policies' with Rules 1989 under Environment Protection Act (1986) and Seed Policy (2002), and (2) 'Guidelines' for Recombinant DNA (1990) and for Research in Transgenic Crops (1998). For research, the recombinant DNA guidelines have been classified into three categories: Category I activities include those experiments involving self-cloning using strains and also inter-specific cloning belonging to organisms in the same exchanger group, which are Generally Considered As Safe (GRAS). Category I experiments need only intimation to the IBSC and are exempted from approval of 'competent higher authority'. In our opinion, cisgenes of plants belong to the same category as self-cloned genes in microorganisms. This would mean that the guidelines for research in transgenic plants, which are normally grouped under three categories (I–III), a category 0 with cisgenes, natural indigenous genes, should be added with exemption of these genes in parallel with category I of the recombinant DNA guidelines. This would mean that in the seed policy, varieties from cisgenic plants could stay out of the separate section (No. 6) on transgenic varieties. It is interesting to observe that in the Food Safety and Standards Bill of India (2005), GM food has been defined as the food which is produced through techniques in which the genetic material has been altered in a way that does not occur naturally by mating. As mentioned earlier, the cisgenic crop plants are highly comparable with traditionally bred varieties because in this approach no new class of genes from non-crossable organisms will be introduced.

Objections against exemption and clarification

A few objections against cisgenesis to exempt them from the GM-regulation are: (1) the random insertion of the gene into the genome and (2) mutation caused in the plant genome. In plant breeding such events are not new. We know from induced translocation breeding by irradiation that a piece of alien chromosome containing the desired resistance trait of the wild species, surrounded by many other donor genes, is inserted randomly in the crop genome, and such an approach is employed in wheat. In crops like maize, transposable elements can move within the genome. In practice induced mutation breeding is safe, as illustrated by safe use of more than 2500 mutant varieties in many different crops. The new GM varieties with transgenes are a good example of random insertion without problems, if selection procedures of normal breeding are applied.

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 pre-breeding 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 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 traditional plant breeding. For this goal to be achieved, exemption of the GM-regulation of cisgenes is needed.

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