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A CASE STUDY:

Cisgenic approach for crop improvement and its biosafety issues

M. S. DUDHARE, N.E. JAYEWAR AND P.V. JADHAV

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Author for correspondence :

M. S. DUDHARE
Biotechnology Center, Vasantrao Naik College of Agricultural Biotechnology, (Dr. P.D.A.U.) YAVATMAL (M.S.) INDIA
Email : mahendra_s_d@yahoo.com

See end of the article for authors' affiliations

A new method of genetically engineered (GE) crop plants known as cisgenics. A cisgenic plant is a plant that has been genetically modified using genes and regulatory elements exclusively from plants to which it can be crossed by normal breeding (Schaart, 2004). Because of the similarity of the introduced genes to those of the host plant, such improvements may be complitted efficiently through intragenic modification, a new approach to genetic engineering that transforms plants with native genetic elements only.

Intragenic modification as a new extension to plant breeding :

The genetic complexity of most undesirable features complicates efforts to eliminate them systematically through traditional breeding. Furthermore, it is difficult to increased food quality without compromising yield. Many genes associated with the biosynthesis of toxins play an important role in the plant's physiology and cannot be simply knocked-out. The easiest route to carefully modifying the expression levels of specific genes is afforded by genetic engineering. A few large agricultural biotechnology companies established a near-monopoly position on commercial applications, which were directed toward the permanent incorporation of bacterial, viral, and synthetic DNA into crops. Although the resulting varieties displayed high levels of herbicide tolerance and insect resistance, their release into the environment triggered widespread biosafety and ethics concerns. In 2003, Kaare M. Nielsen (University of Troms, Norway) proposed to bridge the gap between agricultural biotechnology companies on one side and consumers and NGOs on the other by diversifying genetically engineered crops based on the genetic distance between DNA source and target crop (Nielsen, 2003). He defined organisms transformed with foreign DNA as transgenic, while using the term intragenic for plants containing native DNA. Intragenic modification isolates specific genetic elements from a plant, recombines them *in vitro* and inserts the resulting expression cassettes into a plant that belongs to the same sexual compatibility group using

plant-derived transfer DNAs and marker-free transformation (Rommens, 2004). This new approach to genetic engineering improves the agronomic performance or nutritional characteristics of crops but does not introduce traits that are new to the sexual compatibility group. Intragenic modification could also be applied to eliminate numerous allergens or toxins by silencing the associated genes. In contrast to traditional plant breeding, intragenic plants lack new unknown DNA that may comprise genes associated with the production of toxins, allergens, or anti-nutritional compounds. The modified plants also lack selectable marker genes, powerful insecticidal genes, or any other foreign genes that are new to agriculture or the food stream. Furthermore, the modified expression levels of one or several native genes are expected to trigger phenotypic, biochemical, or physiological variations that already evolved within the sexual compatibility group. One argument for this assertion is that any modification accomplished through all-native DNA transformation could, at least theoretically, be created by recombination. At one end of the spectrum are the knock-out or loss-of-function mutations that can be isolated for many non-essential genes in natural populations, and are obtained at higher frequency using either natural or chemical mutagens. Individuals with enhanced gene expression, at the other end of the spectrum, may be recovered during plant selection, such as those adapted to specific environmental stresses. Both classes yield rare phenotypes pursued by breeders that can often be developed using intragenics. Thus, intragenic modification provides an effective means of enhancing the value of food crops in sustaining and enhancing health, while avoiding issues associated with the traditional breeding and transgenic approaches. Although transgenesis and cisgenesis both use the same genetic modification techniques namely the introduction of one or more genes and their promoters into a plant. Cisgenesis involves only genes from the plant itself or from a close relative, and these genes could also be transferred by traditional breeding techniques.If the current international GMO regulations, which are mainly based on the process of transferring transgenes, continue to fail to differentiate between cisgenic and transgenic plants, the use of cisgenesis could be seriously hindered. In Europe, currently, this process is governed by the same laws as transgenesis but researchers at Wageningen University in the Netherlands feel that this should be changed and regulated in the same way as conventionally bred plants. However, other scientists, writing in Nature Biotechnology, have disagreed writing, "Although lowering regulatory hurdles may increase profits in the short term, it could place the long-term potential of improved agriculture through GE in jeopardy. We would prefer to see plant molecular biologists focus their attention on developing more sophisticated methodologies such as a targeted gene knock-in strategy or genomics-assisted breeding rather than on schemes to evade regulatory mechanisms with products that are still generated by relatively crude transgenic technology (Schubert and Williams, 2006). In 2012 the European Food Safety Authority (EFSA) issued a report with their risk assessment of cisgenic and intragenic plants. They compared the hazards associated with plants produced by cisgenesis and intragenesis with those obtained either by conventional plant breeding techniques or transgenesis. The EFSA concluded that "similar hazards can be associated with cisgenic and conventionally bred plants, while novel hazards can be associated with intragenic and transgenic plants." They concluded that the existing European guidelines for risk assessment of food and feed from genetically modified plants and the guidelines on the environmental risk assessment of genetically modified plants were applicable for the evaluation of food and feed products derived from cisgenic and intragenic plants and did not need to be developed further (Staff, 2012). Only Canada now has a product-based rather than a process-based regulation system, and therefore has the legal possibility to control cisgenic plants less strictly than transgenic plants.

Cisgenesis and transgenesis use artificial gene transfer, which results in less extensive change to an organism's genome than mutagenesis, which was widely used before genetic engineering was developed (Schouten *et al.*, 2006). Some people believe that cisgenesis should not face as much regulatory oversight as genetic modification created through transgenesis as it is possible, if not practical, to transfer alleles among closely related species even by traditional crossing. The primary biological advantage of cisgenesis is that it does not disrupt favourable heterozygous states, particularly in a sexually 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 (Jacobsen and Schouten, 2008). Any restrictions on cisgenesis could block or delay further research on improving crop varieties, particularly as an increasing number of functional genes from crops and their crossable wild relatives are being isolated and are becoming amenable to cisgenesis. Cisgenic plants are fundamentally different from transgenic plants, and should therefore be treated differently under GMO regulations. In the case of transgenesis, the transferred gene usually derives from an alien species that is neither the recipient species nor a close, sexually compatible relative. In other words, transgenesis can extend the gene pool of the recipient species. Such a novel gene might provide the target plant with a new trait that neither occurs in the recipient species in nature nor can be introduced through traditional breeding. This novel trait might affect the fitness of the recipient species in various ways; a change in fitness can then spread through gene flow between a GM crop and its wild relatives (Den *et al.*, 2004), potentially creating shifts in natural vegetation. Consequently, lawmakers and regulatory authorities have paid much attention to the safety of deliberate releases of transgenic crops into the environment and have put in place biosafety frameworks to control this risk. In the case of a cisgenic plant, the gene of interest, together with its promoter, has been present in the species or in a sexually compatible relative for centuries. Therefore, cisgenesis does not alter the gene pool of the recipient species and provides no additional traits. No changes in fitness occur that would not happen through either traditional breeding or natural gene flow. Similarly, cisgenesis carries no risks such as effects on non-target organisms or soil ecosystems, toxicity or a possible allergy risk for GM food or feed other than those that are also incurred by traditional breeding. This is the fundamental difference between cisgenesis and transgenesis. Consequently, the deliberate release and market introduction of cisgenic plants is as safe as the release and market introduction of traditionally bred plants. On the issue of safety, regulators could treat cisgenic plants the same as conventionally bred plants (Schouten *et al.*, 2006).

Biosafety consideration :

Many scientists suggest that cisgenic plants provide an advantage over breeding because of the problem with linkage drag of undesirable traits with the latter. However, the insertion of any transgene is very likely to cause a mutation that cannot be removed by breeding for the added trait. Therefore, cisgenic plants will be susceptible to a more deleterious form of linkage drag. Many scientists pointed out that cisgenic plants should not be regulated as transgenic plants that contain genes from noncrossable organisms. Instead, that cisgenic plants should be free of any regulation, and food derived from them should not be labeled as genetic engineering product because introduced gene is already present in a related plant, “cisgenesis does not add an extra trait and is therefore both safe for consumers and poses no environmental hazard.

Biosafety considerations for the release of cisgenic into environment:

– Transgenes expression may change when gene is placed in different genetic background through breeding due to gene silencing that involves gene into gene interaction might occur in case homologous DNA sequences.

– There is chance of genetic rearrangement or deletion in insert or flanking regions may occur when we transfer those genes in other genetic background.

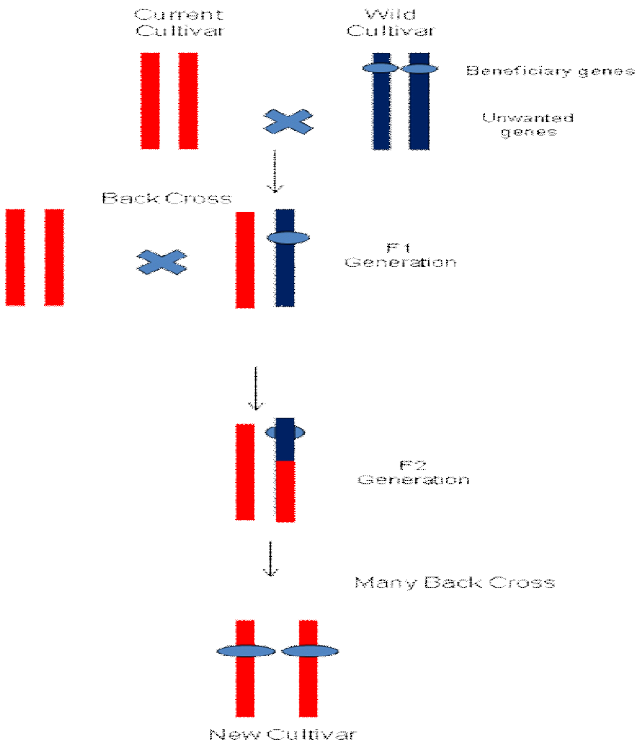

Fig. 1 : Conventional breeding

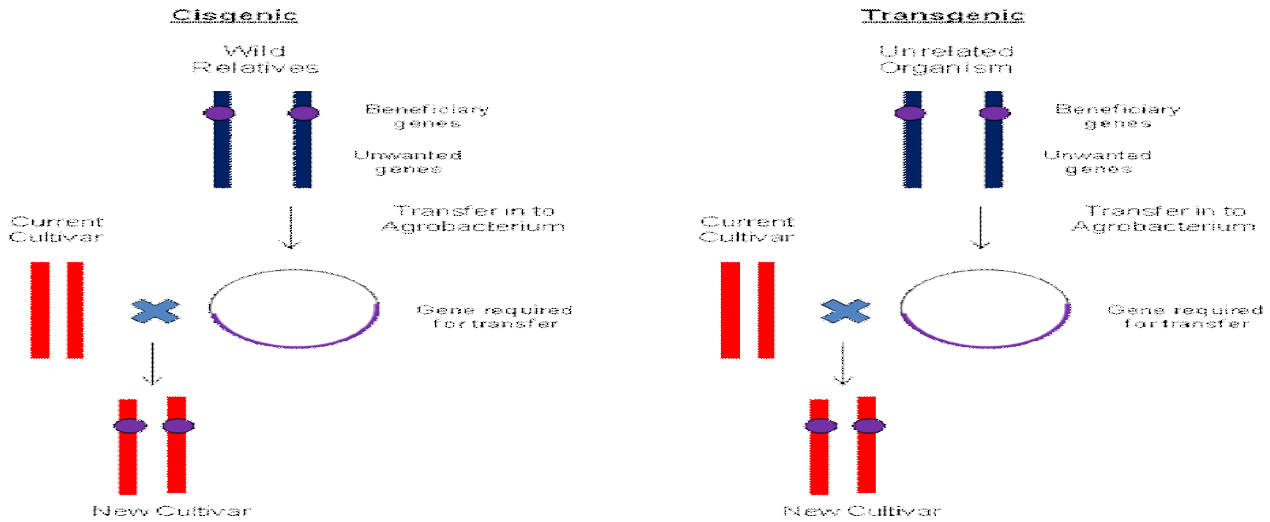

Fig. 2 : Cisgenic and transgenic

– If there is any structural modification occurred and we are going to identified or trace out by using southern or northern blotting we can not trace out but that might occurred during traditional breeding which will impose risk.

– We have to study the stability of insert over several generations because of segregation of that particular gene upto 100 per cent homozygocity, because here we are talking about at the level of protein or enzyme.

– It is scientifically well know the GE can introduced unknown allergens into food. Normally every gene transfer in crop results in some protein production and protein are what trigger allergic reactions.

– Data on the stability of the insert over several generations will be relevant, given the prolonged environmental exposure.

– The combined presence of two toxins might result in changed effect on target and non-target organisms or lead to cross-resistance.

– A hybrid obtained through the crossing of two GMOs is considered as new GMOs and therefore should be evaluated for its risks for the environment and human/ animal health.

Authors' affiliations :

N. E. JAYEWAR, Department of Agricultural Entomology, Vasantrao Naik Marathwada Krishi Vidyapeeth, PARBHANI (M.S.) INDIA

P. V. JADHAV, Department of Agriculture Botany, Biotechnology Centre, Dr. Panjabrao Deshmukh Agricultural University, AKOLA (M.S.) INDIA

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