

Transgenic crops: Priorities and strategies for India[†]

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Development of plant transformation technology is impacting crop improvement options in unprecedented ways. While the new technology promises greater efficiency and precision to plant breeding, to be effective, its application needs to be carefully planned on a case-by-case basis. Transgenic crops, in this context, have the additional dimension of biosafety consideration and hence their development and deployment demands greater care. Here we discuss the various factors that need to be considered while prioritizing crops and traits for transgenic improvement and show how intelligent use of some of the strategies can help alleviate biosafety concerns associated with commercialization of transgenics.

DEVELOPMENT of transgenic crops during the 1990s is an important landmark in the history of crop improvement. Since the first commercial release in 1994, transgenic crops have registered steady increase in area (67.7 mha, 2003) and have slowly spread across nations (18 countries, 2003)¹. The concept of dramatic alteration of crop traits through direct introduction of one or a few genes (or DNA sequences) has aroused great interest and raised hopes of radical alteration of crops to suit new environments and end-uses. Thanks to ready availability of new tools and techniques, creation of transgenic event is today within the reach of any modest laboratory. This power of plant transformation, coupled with the vast available information about genes, has attracted new recruits to plant-improvement efforts from among plant biologists. To take full benefit of these developments, plant breeding programmes will need to intelligently integrate plant molecular biology techniques. This requires active collaboration between plant breeders and plant molecular biologists for developing transgenic crops of commercial value.

Transgenic crops, however, have not found ready acceptance among public at large. The ability to transfer and express genes from any organism into plants transgressing the sexual barrier, has raised concerns about the possible hazards to human beings and the environment. Therefore, in all countries, transgenic crops are subjected to elaborate tests to assess the risks, and to ensure safety, before they are approved for commercial cultivation. These tests include assessment of hazards to humans (allergenicity and toxicity), animals, non-target organisms and the environment. In addition, overall economic benefits of trait engineering to

the farmers, consumers and the environment are also taken into consideration before approval of transgenics for commercial cultivation. Transgenic crop variety development thus involves additional time, cost and expertise. In view of these, it is imperative to prioritize the crops and traits to be engineered, and strategies to be adopted for improvement via transformation. In this article we shall consider the priorities for India.

Transgenic crops should not be viewed in isolation, but should form a part of the overall National Agricultural Policy. Thus, transgenic crops should contribute to long-term national goals, namely to (i) ensure enhanced food production, (ii) provide nutritional security, (iii) operate sustainable agricultural technologies, (iv) generate employment, (v) reduce regional imbalances in growth and (vi) minimize gender inequity. A number of factors are relevant for priority setting for commercial purposes. These can be grouped under two broad heads, namely those related to economic and social aspects, and those concerning biosafety.

Target crops for transgenic improvement

For transgenics to make significant impact, it is necessary that the crops chosen are of national importance. Thus crops that contribute the most to our food and nutritional security (e.g. rice, wheat, chickpea, pigeonpea, groundnut, mustard, tomato, peas, cauliflower, banana, etc.) should be accorded high priority. Similarly, commercial crops such as sugarcane and cotton that provide rural employment and occupy large area also deserve high priority. In view of strong opposition to transgenic produce in some major importing countries in Europe and in Japan, the international trade opportunities should be kept in mind while selecting crops for transgenic improvement. The case of rice is relevant in this context. The Basmati rice trade is a major foreign exchange earner for India (about US\$ 800 m per annum)². Therefore, it is prudent to keep on hold the cultivation of

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transgenic Basmati and to keep the Basmati-belt free from transgenic rice. This measure will be necessary to avoid inadvertent mixture of transgenic and non-transgenic seeds during cultivation, transport or trade.

Biosafety concerns are the major reasons for non-adoption of transgenic crops in most countries. These concerns include transgene movement to other varieties and wild relatives leading to possible development of super weeds, erosion of genetic diversity and ecological disturbances. Similarly, there is widespread apprehension that transgene products could be toxic or allergenic to humans and animals. Also, transgenic crops (e.g. pest-resistant varieties) could have adverse impact on non-target organisms. Emergence of more virulent forms of pests and pathogens is also an important concern. Therefore, the traits to be altered and strategies to be adopted should be carefully considered to minimize the biosafety risks.

Breeding behaviour of the crop

Biology of the crop assumes significance from the transgene movement perspective. If the crop is vegetatively propagated and does not normally produce seeds (e.g. banana, potato, sugarcane), the probability of transgene escape is limited. Similarly, in crops that are highly self-pollinated (e.g. rice, wheat, groundnut, chickpea), transgene movement will be highly restricted. On the other hand, in cross-pollinated crops (such as maize, pearl millet, mustard), preventing transgene movement poses serious challenge. Therefore, crop priority from the transgene movement angle will be vegetatively propagated > self-pollinated > cross-pollinated. Biotechnology offers novel ways to modify breeding behaviour of crops and thereby allows effective options to address concerns of transgene movement (see later).

Presence of wild relatives of the crop

There is considerable inter-breeding among crop varieties and their wild relatives in areas where they coexist. Therefore, if transgenic crops are grown in centres of crop origin/diversity, there is likelihood of transgene escape to the wild relatives. The exact consequences of such gene escape on biodiversity are difficult to predict, and will depend on the trait conferred by the transgene and the environment. Therefore, in most countries, transgenic crops are not deployed in areas where there are wild relatives growing in nature. India being one of the major centres of crop diversity, it will be prudent to take this aspect into consideration while prioritizing crops for transgenic improvement. Wherever necessary, adequate countermeasures should be incorporated in the transgenic crops to prevent transgene escape.

Consumption pattern of the crop

The way a particular crop is used is also highly relevant. If the produce is not eaten (e.g. cotton), the concerns of toxicity are minimal. Similarly, highly processed and purified

products (sugar, refined oil) should raise less concern. Products eaten raw and those used to feed children would need highest assurance. Thus priority-setting based on this factor alone will be non-edible crops > fodder crops > crops subjected to industrial processing and purification > crops eaten after cooking > crops consumed raw.

Target traits for modification

Target traits for genetic engineering would be automatically related to crop priorities and major breeding objectives. Opinion survey among plant breeders, as conducted by Grover and Pental³, is one way to prioritize traits for genetic engineering. This, however, needs to be tempered with other considerations such as technical feasibility. Currently, the transgenic approach is feasible to engineer traits that are controlled by one or a few major genes. Quantitative traits like yield are not easily amenable to improvement through transformation (see exception below). Further, traits that can be routinely modified via conventional breeding need not be targeted for transformation. Availability of molecular markers to track genes controlling complex traits has further reduced the need for transgenic approach to crop improvement. This, however, does not mean that the transgenic approach is unimportant. Since genes can be sourced from any organism for plant transformation, novel traits can be engineered with ease. From a practical viewpoint, therefore, such deficiencies that severely limit crop production and for which conventional approaches are inadequate, should be accorded high priority for transgenic improvement.

Resistance to pests is generally lacking in many crops. Chemical control measures are hazardous and environmentally non-sustainable. At present, *Bt*-transgenics have proved to be highly effective in management of lepidopteran pests of several crops⁴. Other genes such as lectins, protease inhibitors, etc. have also shown promise⁴. Similarly, viruses inflict heavy crop losses and pose severe challenge to management, as chemical control measures are not available to confine their spread. Conventional breeding has not been successful in addressing complex traits such as tolerance to various abiotic stresses, such as water stress (flood and drought), temperature stress (heat and cold), and salt stress. Knowledge of basic biochemical pathways and identification of key regulatory genes of stress-response pathways will be crucial for tackling these problems. Encouraging reports on salt and cold tolerance through genetic engineering in tomato^{5,6}, *Brassica napus*^{7,8}, etc. point to the relevance of transgenic approach for engineering these traits. Thus transgenic crop development could profit from an emphasis on engineering biotic and abiotic stress tolerance.

Addressing malnutrition is a national priority. However, it is a complex issue and a variety of strategies can be pursued to tackle this problem. Improving nutritional composition of common foodstuff can be helpful, but not suffi-

cient, towards this endeavour. Genetic engineering offers elegant ways to fortify food with vitamins and minerals, as demonstrated with rice^{9,10}, canola¹¹ and tomato¹². Therefore, although not a priority in the strict sense, nutritional enrichment via transgenics has become feasible and is receiving international support.

Shelf-life of vegetables and fruits is short in our tropical climate and a sizable part of the harvest is lost during transport and marketing. Inadequate infrastructure and services (roads, power supply and cold stores) aggravate the problem. These traits are not readily amenable to breeding improvement. The transgenic approach has shown great promise for engineering this trait^{13,14}. Since vegetable and fruit cultivation is labour-intensive and many farmers with small holding are involved in vegetable cultivation, transgenic crops will be highly relevant to improving farm employment and income. Thus genetic engineering of crops for slow ripening should receive high priority.

Heterosis breeding is an important approach to productivity enhancement of most crops. Production of commercial hybrid seeds in crop plants bearing hermaphrodite flowers requires special genetic stocks, where sexuality (male sterile and bisexual) of the plant can be controlled. Cytoplasmic male sterility, a trait under the control of mitochondrial–nuclear gene interactions, is used in most crops. However, such stocks are not readily available in all crops and creation of such stocks is difficult. Genetic engineering approach is highly relevant in this respect, as demonstrated in *Brassica*.

Engineering male sterility can also be helpful in transgene containment. Since pollen is the major route through which transgenes escape to other varieties and wild relatives, keeping transgenics in a male sterile background can significantly lower transgene spread. This is particularly feasible in crops where seed is not the commercial product. For example, in vegetable crops such as brinjal and tomato, the important commodity is the fruit. Engineering parthenocarpy (fruit set without pollination) is feasible and relatively simple¹⁵. Thus linking together genes for parthenocarpy and male sterility with genes for other traits (e.g. slow ripening, nutrient fortification), will allow development of environmentally-safe transgenic crops.

Genes and strategies

The genes used and the strategies employed assume significance from biosafety perspective. In general, if the DNA sequences utilized are derived from non-pathogenic organisms or do not code for any product, they pose less concern. Genetic engineering for virus resistance is an exception in that transgenic plants expressing viral genome sequences resist attack by the corresponding viruses. In particular, gene silencing strategies have been shown to be effective. Since produce from plants infected with viruses is widely consumed and has caused no harm to humans

and animals, transgenic plants bearing a small piece of the viral genome would not evoke serious biosafety concerns. This also applies to extension of shelf-life of fruits and vegetables through antisense expression of gene sequences derived from the same or related plants. This approach does not abolish normal plant metabolism, but lowers the rate of specific metabolic steps in the ripening process. Further, many crops contain antinutritional compounds or allergens. Antisense or gene silencing strategies are effective in eliminating such compounds. Thus transgenic crop variety development using such gene sequences and strategies should be given high priority.

Current methods of transformation require the use of selection markers (antibiotic or herbicide resistance, ability to utilize special metabolites) to eliminate untransformed cells. However, the marker genes have no utility in cultivation or in the commercial products. In fact, constitutive expression of marker gene products is a drain on plant metabolism and there is considerable opposition to their use. Fortunately, now strategies are available to eliminate the marker genes after transgenic plants have been produced. Such strategies can be incorporated in the beginning of the transgenic variety development programme to minimize biosafety concerns. Since plastids are transmitted from the maternal side in most plants, plastid transformation has been advocated for restricting transgene spread. However, there are now reports that a transgene present in the plastid genome can rapidly relocate itself into the nuclear genome^{16,17}. Moreover, transgene expression in transplastomic plants is generally high and may not be desirable in most situations. Considering the above, the generally advocated plastid transformation is not warranted in all situations.

So far we have examined priorities considering one factor at a time and have presented a broad framework for decision-making. For preparing a final list of crops for commercial development of transgenics, it is important that all relevant factors are considered together. A case-by-case approach with appropriate weights for each factor will be needed to arrive at the right decision. We illustrate this with the example from potato. We have stated earlier that the transgenic approach may not be considered where the desired improvement could be achieved through conventional breeding. In potato, a gene conferring broad spectrum resistance to late blight disease has been transferred from the wild species, *Solanum bulbocastanum* to the cultivated species through somatic hybridization¹⁸. This gene can now be incorporated into any potato variety through conventional breeding and would not attract biosafety regulation. This gene has recently been cloned and makes the transgenic approach feasible to achieve the same goal¹⁹. However, such a transgenic variety will have to undergo biosafety testing. Potato is a tetraploid and is vegetatively propagated. Most of the varieties are highly heterozygous. Transfer of genes through conventional backcross breeding into already established varieties will be a Herculean task. Similarly, the gene conferring resistance against apple

scab disease has been cloned, thereby providing an opportunity for transgenic improvement of apple²⁰. In such situations the transformation approach should be given high priority. This case also highlights the fact that new developments could suddenly alter the equation in favour of the transgenic approach over the conventional approach. Therefore, the priority-setting approach will need to be flexible and revised in a dynamic fashion. The above discussion specifically pertains to development of commercial products. For research purposes, the transgenic approach may be vigorously pursued to gain understanding of biological mechanisms, to address biosafety concerns and for developing newer, better and more efficient tools and technologies.

For transgenic technology to be successful in delivering its promises, matching public policies will need to be in place. At present, most of the transgenic technology and products are in private domain. Hence, there is a general apprehension that the transgenic crops are being promoted solely with profit motive and not for the good of the public. Thus the opposition to transgenic crops is not only on account of biosafety but also due to IPR, ethical and moral issues. One of the consequences of these debates is reduced interest in agribiotech investments from the private sector. For technology to progress and reach the farmers, both public and private investments will be essential. Therefore, in our opinion, the government should be proactive and make important policy decisions regarding transgenic crops. For instance,

- (i) A comprehensive list of crops and traits where transgenics will be considered for commercial release may be released to direct transgenic crop development by interested parties.
- (ii) A clear stand about marker genes (whether or not acceptable, and if acceptable, which ones) in commercial transgenics may be taken and made public.
- (ii) Areas/zones and crops where transgenic crop cultivation is prohibited on grounds of biodiversity or trade interest may be demarcated.
- (iv) A transparent review process for approval of transgenics for commercial cultivation and an effective executive network to ensure adherence to biosafety guidelines regarding growing of transgenic crops (for instance, compliance of refugia for pest resistance management in *Bt*-transgenic crops) may be put in place.

In conclusion, transformation is a powerful approach to plant improvement. However, it is not a panacea for all problems. Several biosafety concerns associated with transgenic crops are real. By judicious choice of crops, traits, strategies and government policies, however, engineered crops can be developed and deployed to achieve national agricultural goals.

Note added in proof: Recently, Celis *et al.* (*Nature*, 2004, **432**, 222–225) engineered nematode resistance into a male sterile line of potato for safe deployment in the Centre of Potato Diversity in Peru.

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