Plant Archives Vol. 14 No. 1, 2014 pp. 605-610 ***ISSN 0972-5210***

Review Article

BIOSAFETY ISSUES RELATED TO TRANSGENICS AND ENVIRONMENTAL CONCERNS

Prabharani Chaudhari, Suchi Gangwar[1], Megha Dubey[1] and Nidhi Verma[1]

Department of Genetics and Plant Breeding, College of Agriculture, I.G.K.V., Raipur (C.G.), India.
[1]Jawaharlal Nehru Krishi Vishwavidhalaya, Jabalpur (Madhya Pradesh), India.

Introduction

In the past, plant breeders, in their efforts to improve the crop plants were restricted to using the genetic variability available in the primary gene pool of the reproductively compatible species. In some cases such as tomatoes, tobacco, potato, brassica species, wheat and rice, gene from the secondary gene pool of the wild and weed relatives of the cultivated species were utilized through distinct hybridization and in the recent past through protoplast fusion. The recombinant- DNA techniques have made it possible to transfer, integrate and express the desired gene from the global biodiversity of microbes, reproductively incompatible plant species, animals including humans and synthesized genes into the plant genomes. Such plants with alien genes transferred using the recombinant DNA techniques are referred as transgenic or genetically modified organisms or genetically engineered plants (GEPs). They have also been referred as plants with novel traits (PNTs).

The developments in genetic engineering of plants around 1983 promised that this new biotechnology would benefit and even revolutionize agriculture. The transfer of desirable genetic traits across species barriers has the potential for solving problems in the management of agricultural crops (Anonymous, 2000).

Transgenic crop plants need

In order to remove hunger malnutrition and to satisfy the demands of economically ascendant population in the next century, the world food production should be doubled by 2025-30. Moreover, this increase must come by enhancing the productivity per unit area, water nutrient and energy to meet the demands of the growing and economically ascendant population looking for quality, convenient foods, free from pesticide residues this can be archived by development transgenic crop plant, however transgenic crop plant enhance stability of production by incorporating resistance to various biotic and abiotic stresses, development of sustainable production systems by minimizing the use of chemical pesticides, prevention of post harvest losses in fruit and vegetables by enhancing the shelf life, improving the end use qualities, reducing the adverse environmental impact of the production systems and reducing production costs. In India, where the productivity level per ha are much lower due to various biotic and abiotic stresses that crops face, lack or improper use of pesticides, the possible benefits could be much larger. The emerging challenges in the field of Agriculture can be met only through appropriate integration of recombinant technology.

Area of transgenic crops

The development of transgenic crops using recombinant DNA techniques is relatively recent, but their applications are increasing rapidly because of advantage over the conventional crops. The global area of approved biotech crops in 2005 was 90 million hectares, equivalent to 222 million acres, up from 81 million hectares or 200 million acres in 2004. The increase was 9.0 million hectares or 22 million acres equivalent to an annual growth rate of 11% in 2005. A historic milestone was reached in 2005 when 21 countries grow biotech crops, up significantly from 17 countries in 2004.

The largest increase in any country in 2005 was Brazil, provisionally estimated at 4.4 million hectares (9.4 million hectares in 2005 compared with 5 million hectare in 2004) followed by the US (2.2 million hectares), Argentina (0.9 million hectares) and India (0.8 million hectares). India had by far the largest year -on – year proportional increase with almost a threefold increase from 500,00 hectares in 2004 to 1.3 million hectares in 2005 (Anonymous, 2005). In 2009, the global area of Bt cotton was increase many fold (27 million hectare). The largest area coverage of Bt cotton in any country was India (9.6 million hectare) followed by China (3.7 million hectare).

Global area of transgenic crops in 2008 : by country (million hectares).

Rank	Country	Area (million hectares)	Transgenic crops
1	USA	62.5	Soybean, maize, cotton, canola, squash, papaya, alfalfa, sugar beat
2	Argentina	21.0	Soybean, maize, cotton
3	Brazil	15.8	Soybean, maize, cotton
4	Canada	7.6	Canola, maize, soybean sugar beat
5	China	3.8	Cotton, tomato, papaya, sweet peeper
6	Paraguay	2.7	Soybean
7	India	9.6	Cotton
8	South Africa	1.8	Maize, soybean, cotton
9	Uruguay	0.7	Soybean, maize
10	Australia	0.2	Cotton
11	Mexico	0.1	Cotton, soybean
12	Romania	<0.1	Soybean
13	Philippines	0.4	Maize
14	Spain	0.1	Maize

Source: ASAAA Publication2008

Global coverage of Bt cotton (2009)

S. no.	Country	Area (mha)
1	India	9.6
2	China	3.7
3	World	27.42

Commercially four genetically modified crops dominate global biotech agriculture with soybeans accounting for 60% of GM crop area, maize accounting for 23% of GM crop area, cotton accounting for 11% of GM crop area and canola accounting for 6% of GM crop area.

Risks associated with transgenic crops

As more and more transgenic crops are released for field testing and commercialization concerns have been expressed regarding potential risks to both human health and environment.

These apprehensions arise because transgenic technology crosses the species barrier as compared to classical selection techniques, there by permitting the gene transfer among microorganisms, plants and animals. There is no evidence that any unique hazards exists in the development of transgenic crops, because of novel combinations of genes. Transgenic crops are not toxic nor are likely to proliferate in the environment. However, specific crops may be harmful by virtue of novel combination of traits they possess. This means that the concerns associated with use of GMO's can differ greatly depending on the particular gene-organism combination and therefore a case – by – case approach is required for risk assessment and management potential risks from the use of transgenic crops are human health and environment.

Risks to human health

Risks to human health are related mainly to toxicity, allergenicity and antibiotic resistance. The risk of toxicity may be directly related to the nature of the product whose synthesis is controlled by the transgene or the changes in the metabolism and the composition of the organisms resulting from gene transfer. Most of the toxicity risks can be assessed using scientific methods both qualitative and quantitively.

The introduction of newer protein in transgenic crops from the organisms which have not been consumed as food, sometimes has the risk of these proteins becoming allergens. However, it may be noted that there is no evidence that transgenic crops pose more risks than conventional products in triggering allergies. Further, the new transgenic crops can be tested for allergens prior to their commercial use.

The uses of genes for antibiotic resistance as selectable markers have also raised concerns regarding the transfer of such genes to microorganisms and thereby aggravate the healthy problems due to antibiotic resistance in the disease causing organisms. Although, the probability of such transfer is extremely rare, steps are being taken to reduce this risk by phasing out their use. For Bt gene the vertebrates have been found to be lacking receptor binding site for Cry protein, hence, their health hazards are out of Cry toxin purview and Cry proteins are not toxic to vertebrates

Risk to environment

Assessing the environmental impact of GM crop is often difficult as many factors are considered. Scientists focus on the potential risks of GM crops and also emphasize their potential benefits. Risks to environment due to release of transgenic crops include introduced genes to outcross to weedy relatives as well as potential to create weedy species, direct effect on non-target organisms, effect on biodiversity and development of insect resistance.

Ecological scientists have little doubt that *gene flow* from transgenic fields into conventional crops and related wild plants will occur. Gene flow from transgenic to conventional crops is of concern to farmers because of its potential to cause herbicide resistance in related conventional crops. For example, in western Canada, three different herbicide-resistant canola varieties have cross-pollinated to create canola plants that are resistant to all three types of herbicide (Ellstrand and Norman, 2001).

Gene flow from transgenic crops to wild relatives creates a potential for wild plants or weeds to acquire traits that improve their fitness, turning them into “super weeds.” There is already evidence of such outcrossing from herbicide-resistant wheat to jointed goatgrass. Other traits that wild plants could acquire from transgenic plants that would increase their weediness are insect and virus resistance (Erwin *et al*., 2001).

Research conducted before the commercial application of the gene, and experience during five years in USA show that Cry1Ac binds to a specific receptor [170 kDaAPN Binding site in *H. virescense* (Luo *et al*., 1997) in the midgut of the sensitive insects, but does not affect mammals or insects that do not have the receptors,. The presence of the specific toxin receptor binding sites makes the Cry1Ac specifically toxic to a particular group of insects affecting mainly the *lepidopteran* insects and particularly, the cotton bollworm *H. virescense*. All other insects, wild life, marine life are not affected. The vertebrates have been found to be lacking receptor binding site for Cry protein, hence, out crossing is unintentional breeding of a domestic crop with a related plant. A major environmental concern associated with GM crops is their potential to create new weed through outcrossing with wild relatives, or simply by persisting in the wild themselves. The potential for the above to happen can and is associated prior to introduction and is monitored after the crop is planted as well. Study initiated in 1990 demonstrated that there is no increased risk of invasiveness or persistence in wild habitats for GM crops and traits tested when compared to their unmodified counter parts. A recent study shows that only very limited effects on the environment have been detected in relation to out crossing. It is therefore important, however, as regulations require evaluating individual GM crops both prior to release and after commercialization.

The major environmental consequence resulting from the massive use of Bt toxin in cotton or other crops occupying a large area of the agricultural landscape is that neighbouring farmers, who grow crops other than cotton, but sharing similar pest complexes, may end up with resistant insect populations colonizing their fields. This is because the lepidopteran pests that develop resistance to BT cotton, moves to adjacent fields where farmers use BT as a microbial insecticide (Gould, 1994). So emphasis has to be laid on studying the impact of transgenic crops on birds, mammals and soil biota.

It is a known fact that due to application of broad-spectrum insecticides many non-target, beneficial insects are also eliminated. Hence, their population is greatly reduced. It is also evident that BT cotton is toxic to only some lepidopteran insects and do not possess adverse effect on non-lepidopteran, beneficial fauna.

By keeping pest population at extremely low levels, Bt crops can starve natural enemies as these beneficial insects need a small amount of prey to survive in the agro-ecosystem. Parasites would be most affected because they are dependent on live hosts for survival and development, where as some predators thrive on dead or dying prey. Aphids were capable of sequestering the toxin from Bt crops and transferring it to its Coccinellid predators, thus, in turn affecting reproduction and longevity of the beneficial beetles. The potential of Bt toxins moving through food chain poses serious implication of natural bio control in agro ecosystem.

A report from the US environmental Protection Agency (EPA) indicated that data provide a weight of evidence indicating no unreasonable adverse effects of Bt proteins expressed in plants to target wild life. Lab experiments confirms that transgenic crops with insect resistance may have negative impact on beneficial insects, predators including lacewings (Hilback *et al*., 1998), monarch butter fly larvae (Losey *et al.*, 1999) and soil biota (Watrud and Seidler, 1998).

Transgenic crops engineered with Bt genes (derived from *Bacillus thuringiensis*) offer protection against lepidopteran pest complex and may reduce the usage of synthetic insecticides that are used for control of insect pests. Since most of the crops have a diversity of insect pests than lepidopterans, insecticides will still have to be applied to control these pests, which are most susceptible to the endotoxin proteins expressed by the Bt crops. Most Bt toxins have a similar mode of action and once resistance develops to one toxin it can confer resistance to other related toxins, which is known as cross- resistance (Gouldburg *et al*., 1992). The management of resistance in transgenic crops is in the experimental stage. However, based on previous experience some conceptual strategies have been advocated for effective management of resistance in transgenic crops. For resistance management, the important strategies are protein pyramiding, planting refuge row and Cry gene crop rotation is important. The field level tactic is one, which appears to be practicable to the farmers and can be readily practiced while the other strategies require high scientific intervention.

The scientist recognizes that insects will develop resistance to Bt toxin and use of refuge was always considered an interim solution. Now multiple gene constructs with another insertion of Cry gene apart from Cry1Ac (Voth, 2001 and Penn *et al.*, 2001) is available. The new Cry gene in Bollgard II is Cry2Ab. The genotype of Bollgard II has shown promise for improved bio-efficacy and increased spectrum of control (Voth *et al*., 2001). Cry2Ab expression has been found to be more than ten times as compared to the level of Cry1Ac in Bollgard II plants. The high plant expression of Cry2Ab has contributed to higher efficacy against important lepidopteran insects in cotton. In China, Wang *et al.* (1998) have also transformed cotton using construct consisting of P-lec and cowpea trypsin inhibitor genes for the control of bollworms, Hence, in future cotton varieties with multiple gene constructs will be a common feature and Schular *et al.* (1998) have given wide range of genes of bacterial, plant and animal origin which are effective against lepidoptera and some against coleoptera.

Biosafety

Biosafety is associated with the safe use of GMOs and more generally, with the introduction of non-indigenous species into natural or managed ecosystems.

Biosafety legislation and regulatory institutions to implement them have been put in place by many countries including India, engaged in transgenic research and commercialization. India has well defined regulatory mechanism for development and evaluation of GMO's including transgenic crops and products thereof.

The need for biosafety evaluation of the transgenic plant

Crop cultivars developed using traditional methods of plant breeding such as selection, hybridization, induced mutation, distant hybridization, protopast fusion etc. Have never been subjected to biosafety evaluation as required for the transgenic (some of these methods are already) time- tested fo ill effects. The reasons for biosafety evaluation of the transgenic plants are the following:

- The recombinant _DNA methods are considered more powerful tools for genetic manipulation than the conventional methods used by the plant Breeders so far.
- Prior to the r-DNA methods, genetic manipulations were confined to the use of primary or at best, the secondary genepool of the species.
- With the new techniques, genes of a large variety from any limiting organism or a synthesized gene can transferred and expressed in plants, which could have much larger adverse effects, such genes from widely different organisms were outside of the gene pool of the crop species during evolution. The conventional methods of breeding were limited to replacing the alleles available in the gene pool or their deletions
- This presents unique risks to the environment as prediction of the long term effects is not possible due to insufficient data, speculations and misconceptions.
- Poor reliability of the predictions.

Safety assessment of transgenic crops in India

Safety assessment of a transgenic crop is the most important step in this development process. Extensive testing and a long approval process includes comprehensive analysis of the risks and their scientific management to ensure food, feed and environmental safety before introduction into the market.

Safety assessments of a transgenic crop start with determining whether the product is substantially equivalent (except for defined differences) to conventional varieties. Further analysis then focuses on the evaluation of the defined differences by assessing potential safety risks of the host plant, gene donor (s) and the protein introduced.

Experiments are designed to identify the hazards, to assess risks and to take steps to manage the risk by applying logical strategies. Information on the following aspects needs to be generated on a case - to – case basis.

1. Characteristics of the donor organisms providing the target gene such as identification, pathogenicity, toxicity and allergenicty, the geographical origin, distribution pattern and survival mechanisms and the method of transfer of its genetic material to other organisms.

2. Characteristics of the vectors used such as the origin, identity and habitat, sequence and frequency of mobilization.
3. Characteristics of the transgenic inserts such as the specific functions including the marker gene inserts the expression levels, and the toxicity of the expressed products on the host plant, humans or animals.
4. Characteristics of the transgenic plant including methods of detection of the transgenic traits in the environment, toxicity and pathogenicity of the transgenic plants and their seeds to other plant, human and animals, possibility of the extent of transgenic pollen escape and pollen transfer to wild relatives and the impact on the environment.
5. Toxicity and allergenicity data are generated using standard protocol devised by national and international agencies.

Important precautions may be taken for minimizing the risks at release of transgenic plants.

1. Special separation for isolation to prevent reproduction/fertilization and seed setting.
2. Biological prevention of flowering by making use of sterility properties.
3. Human intervention for reproductive structures of flowers.
4. Controlling and destroying volunteer plants from experimental field.
5. Controlling the reproductive structures of transgenic plants like seeds and the plant prop gules from unaccounted spread.
6. Appropriate training of field personnel while handling the transgenic plants.
7. To take into account the proximity to human activity in case the transgenic plants have allergenic properties to human and animals.

All the data generated by the developing organization is then submitted in detailed formats to the government for seeking permission for commercial release. The initial risk assessment in India begins at the institutional level itself. The Institutional Bio safety Committee evaluates the proposal following which it has passed on to Review Committee on Genetic Manipulation and then Genetic Engineering Approval Committee. Another round of assessment with respect to agronomic benefits is undertaken under the ICAR system. There is provision for continuous monitoring by Monitoring and Evaluation Committee even after release of the variety at the center and state levels.

Conclusion

In conclude we can say that biosafety issues are complex. There are no straight forward yes or no anwers for the environmental risk/safety of a crop having a single transgene. The risks, except the gene flow, are manageable and the benefits for outweigh the possible risks. With small holdings, it would not be possible to prevent intercultivar gene flow. In future, it is reasonable to except that many of the desired agronomic, quality and resistance to biotic and abiotic stresses transgenes so far transferred in independent rice plant, will be combined by hybridization in a single “super transgenic rice“ cultivar. The same scenario is likely to emerge for the other major crops. It the risk of single transgene (s) is acceptable, presence of several transgenes in one cultivar should also be acceptable through it would enhance exposure. Conventionally bred cultivars have multiple resistance is to various stresses, besides the other yield and quality traits. How long, it would take to resolve the biosafety of the rice and other crops? Will it be possible to obtain approval for its commercial release from the regulatory authorities in a reasonable time? If not, the benefits to the society will be delayed, at the same time breeders intending to incorporate transgenes into their cultivars should initiate parallel experiments to establish their biosafety to satisfaction of the regulatory authorities.

References

Anonymous (2000). Experiment Station and Extension Committees on Organization and Policy (ESCOP and ECOP). 2000. Agricultural Biotechnology: Critical Issues and Recommended Responses from the Land-Grant Universities. pp 20.

ASAAA (2008). Global status of commercialized biotech / GM Crops. 39-2008.

Ellstrand, Norman C. (2001). When transgenes wander, should we worry? *Plant Physiology*, **125** : 1543-1545.

Ervin, David E., Sandra S. Batie, Chantal Line Carpentier and Rick Welsh (2001). Transgenic Crops and the Environment: The Economics of Precaution. Invited paper, Western Agricultural Economics Association. July 10. p. 1-12. Logan, Utah.

Gouldburg, R. J. (1992). Environmental concerns with the development of herbicide tolerant plants. *Weed Technology*, **6** : 647-652.

Gould, F. (1994). Potential and problems with high dose strategies for pesticidal engineered crops. *Biocontrol Science and Technology*, **4** : 451-461.

Hilbeck, A. M., P. M. Baumgartner and F. Bigler (1998). Effect of transgenic bacillus Thuringiensis corn-fed prey.

Losey, J. E., L.S. Raynor and M. E. Carter (1999). Transgenic pollen harms monarch larvae [scientific correspondence]. *Nature*, 399-414.

Penn, S. R., R. Barb, O. Janson, E. Kris and J. E. Greenplate (2001). Quantification of lepidopteran activity in a gene product. A two year summary of Bollgard II. Proceedings of the Beltwide Coton Conference. National Cotton Council of America, Memphis USA.

Schuler, T. H., G. M. Poppy, B. R. Kerry and I. Desholm (1998). Insect resistant transgenic plants, *Tibtech*., **91**: 168-173.

Voth, R. D., T. John, J. E. Greenplate and J. W. Mullions (2001). Bollgard II cotton technical review. Proceedings of the Beltwide Cotton Conference , National Cotton Council of America, Memphis USA.

Wang, W., W. Chen, Y. Gao and Z. Zhu (1998). Obtaining of insect resistant cotton by transformation with Agrobacterium. Proceedings of World Cotton Res. Conference-2, held at Athens, Greece: 350-354.

Watrud, L. S. and R. J. Seidler (1998). Non-target ecological effects of plant, microbial and chemical introductions to terrestrial systems. Soil Chemistry and Ecosystem Health. Special Publication. 52. Soil Science Society Of America, Madison, Wisconsin, p. 313-340.