Review Article

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Biosafety issues related to genetically engineered crops

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Introduction

Genetically engineered crops refer to alterations in the genetic makeup of the crop by introgression new traits such as herbicide tolerance, virus resistance, drought, flood and frost resistance, delay in maturation time of the crop and increased crop yield. They can be made resistant to pests and diseases which can significantly reduce the consumption of insecticide. Biodiversity is the feedstock for biotechnology industries. Although the benefits of transgenic technology are clear, the potential risks have created public concerns about the wisdom of releasing and consuming genetically modified (GM) crops. Biotechnological tool such as recombinant DNA technology has come a long way in solving the problem of food security. Genetic modification can help humankind to face new challenges as a result of high population growth, biodiversity loss and climate change. Therefore, it is imperative to have robust biosafety protocols/ procedures for India. While designing GM crops, the native species and gene in question needs to be taken into account. GM crops might become agricultural weeds or invade natural habitats if proper risk assessment (RA) is not performed prior to their release. The possible impacts of GM crops are as follows:

Weediness and invasiveness

One of the potential concerns about genetically modified organisms (GMOs) is that they will become agricultural weeds or invade natural habitats,[1] as the traits introduced by GMOs might increase the reproductive success or fitness of the crop, thereby increasing its competitive ability. One conjectural risk is that GMOs will either cause the host species to become invasive or will escape from the original host species or cause other species to become invasive. Ellstrand et al.[2] suggested that new combinations can create genotypes with different and surprising ecological behaviors. Researches have shown that the gene flow from transgenic crop is easy to escape to the weedy relative Brassica campestris. Canola is also capable of cross pollinating with several other weed species including wild radish (*Raphanus raphanistrum*) and buchan weed (*Alternanthera philoxeroides*).

Gene flow from GM crops

The transgene escape to weedy relatives through pollen is one of the potential risks of GM crops. Gene flow indicates the movement of genes or genetic materials from one population into another. There are three avenues for gene flow to occur: pollen-mediated, seed-mediated and vegetative-propagule-mediated gene flow. To minimize the possibility of transgene flow, a number of strategies have been developed or proposed, applying physical and biological approaches include confined field trial, transgenic mitigation, maternal inheritance, male sterility, cleistogamy, apomixis, incompatible genomes, temporal control via inducible primers and seed sterility. These are called "genetic use restriction technologies" or GURTs. Some of the mitigation techniques are as follows:

Confined field trial strategy

One of the ways to understand the gene flow is to conduct confined field trial (CFT). CFT is a small-scale experiment, done in the open field, with the intention of confining plant genes and plant material to trial site. CFTs are needed for breeding trials to incorporate traits into locally adapted varieties or to create populations for genetic study, to collect safety data to inform regulatory decisions on GM crops commercial release, to scale up experimental crops so that sufficient seed or other plant material is available for animal-feeding studies, or to study possible environmental impacts such as plant characteristics, potential for weediness, changes in pollen production, or gene flow.

Transgenic mitigation strategy

A transgenic mitigation (TM) strategy is also available for reducing the potential risks of escaped transgene(s) to the weedy or wild populations by co-introducing "mitigator" genes that are tandemly linked to the target transgene(s) to deliberately reduce the fitness of any hybrid and its progene i.e. the individuals carrying those traits would be eliminated in natural populations through competition with other more highly fit native individuals. Some of the deleterious traits that have been proposed are abolition of secondary dormancy, dwarfing and inhibition of shattering of seeds.[3] A mitigator dwarfing Δ*gai* (gibberelic acid-insensitive) gene, when transformed into tobacco, reduced fitness by 17% and was predicted to slow escape from a few generations to many thousands, depending on rates of gene flow and levels of recombination.[4] Thus, TM would limit transgene escape through pollen and seed flow.

Chloroplast transformation

To prevent gene flow via pollen, transgenes can be targeted to chloroplast genomes, which are generally transmitted only through ovules of the female parent. Numerous transgenes have been successfully integrated into chloroplasts in wide variety of plant species,[5] and this approach has been shown to block pollen flow of the transgene in tobacco and tomato.[6,7] Although targeting transgenes to the chloroplast will not completely limit all the gene flow, as it does not restrict transgene movement via seed dispersal.

Male sterility

Inserting transgenes into male sterile lines is another means of preventing transgene escape *via* pollen flow.[8] Either naturally derived male sterile lines can be used or male-sterility mutants can be engineered. One approach is to use tapetum-specific promoters to derive expression of a recombinant RNase gene.[8] Plant Genetics Systems (Ghent, Belgium) has engineered male-sterile and male-restorer lines of GM rapeseed utilizing two genes from *Bacillus amyloliquefaciens-barnase*, which cleaves RNA and *barstar*, a protein that binds to *barnase* and prevents its function. The central pitfall of using male-sterile lines, is it can only be used for vegetative crops.

Cleistogamy

Cleistogamy or automatic self-pollination is a process involving plant propagation by

self-pollinating flowers. A deemed *superwoman1-cleistogamy* has been isolated from rice.[9] The drawbacks of this approach are that transgenic seed movement is not restricted; the crop must be self-fertile, which may suppress the creation of genetically superior plants and characteristic must be stably expressed.[10]

Apomixis

Apomixis relates to asexual reproduction by plants without fertilization. In some of the species, seed is derived from apomictic origin and not the product of sexual reproduction. Apomictic embryos can be produced from the integument or nucellus or from megaspore mother cells or nucellar cells. An apomictic Maize/ Tripsicum hybrid has been patented.[11] Apomixis can prevent transgene escape through pollen flow but cannot restrict movement of the transgene via seeds.

Inducible promoter/gene

If transgenes could be removed before flowering by temporal control of inducible promoters, escape through both pollen and seed flow can be prevented.[12] suggested that this could be done by placing a chemically induced or fruit-specific promoter in front of a construct with a site-specific *recombinase* gene such as *Cre* that recognized lox sites flanking the transgene. When *Cre* expression is induced, the transgene would be removed. The disadvantage of this approach is that it is not applicable to traits required throughout the plants' life, the inducer must completely penetrate all the relevant plant tissues and somebody must induce the system. A related approach might be to use a chemically induced promoter that allows expression of the transgene only when a chemical is applied. If the elicitor is not found outside of the agronomic system, then the transgene would not be expressed in nature. One other mode of restricting transgene escape into relatives is to incorporate inducible genes that make seeds sterile,[13,14] developed a system where an inducible promoter is used to induce a site-specific recombinase (Cre) that removes DNA sequences flanked by lox sites, allowing for expression of a lethal gene.[12] proposed a system where *Cre* is placed adjacent to the transgene and removes both when induced. This technique requires induction of all the cells which is cumbersome. Any failure could result in some viable seeds being produced. To counter this problem approaches have been developed where the inducible seed termination mechanism is "on" until the inducer turns it "off", rather than the reverse.[15,16] In this system, a seed promoter is linked to the *barnase* gene and the *barstar* gene is linked to an inducible promoter, while this method has great potential in limiting gene flow, considerable resistance has been expressed to it because these systems would also prevent farmers from saving seed.

Impacts of GMOs on non-target organisms

The negative impact of GM crops on non-target organism is also a serious threat. Transgenic plants that produce insecticidal substances should continue to be subject to careful testing to ensure safety and minimizing the environmental risks.

A tiered approach to an ecological risk assessment is recommended, particularly in a regulatory context, as a screening tool to determine potential risks to non-target organisms from releasing a GE crop in the environment. Non-target organisms include invertebrates beneficial to maintaining a healthy ecosystem in an agricultural setting such as pollinators, biological control organisms and decomposers. Other wild lives that are not intended to be harmed by a GE crop such as birds, mammals and fishes are also considered as non-target organisms. Many direct and indirect effects could result from release of a GE crop in the environment, such as fitness costs to an organism, a reduction in the abundance or diversity of non-target organism or a reduction in functional responses. A tiered approach allows for a common testing framework and a standardized sequence of tests to be used as screening tools to evaluate and focus risk considerations.

Effects of insect resistant GM plants on non-target organisms

Insect resistance, conferred via expression of a variety of *Bacillus thuringiensis* (Bt) delta-endotoxins, is the second most commonly used trait, after herbicide resistance, in commercial GM crops. *Bt* is a ubiquitous gram positive, spore forming bacteria found in soil, insects, stored-product dust, and deciduous and coniferous leaves. The insecticidal activity starts by the formation of parasporal crystal of the bacterium during its stationary phase. The crystals are made of protoxins, referred to as *cry* toxins or endotoxins, which, when ingested by an insect, are activated by proteases in the insect mid-gut.[17,18] *Cry* toxins readily bind to receptors on the apical brush border of the midgut microvillae of susceptible insects and insert into the membrane. This insertion leads to the formation of pores causing lysis of cells, leading to starvation, paralysis, septicemia and death of the insect.[17] The potential hazard of transgenic crops engineered with plant-incorporated-protectant (PIP) trait is toxicity to non-target beneficial organism. The exposure assessment predicts the likelihood that non-target organism will have the dietary exposure to the expressed PIP at or above the hazard threshold level. An evaluation requires studies that address acute and chronic mortality effects, changes in reproductive processes and trophic level disruptions of the ecosystem. A reduction in host or feeding directly plant parts may have adverse effects on natural enemies. Beneficial insects if allowed to persist in Bt fields may aid in controlling secondary pests.[19] GM crops can affect non-target organisms such as avian including bobwhite quail, mallard duck, broiler chicken and migratory birds; wild mammals such as rodents, deer, and cats; aquatic animals like catfish, freshwater invertebrate such as *Daphnia magna*; aquatic insects like caddis flies. One of the major concerns of PIP GM crops is the toxic effect on the pollinators, (bees, flies) and parasitoids (*Nasonia vitripennis)*. The adult and larval predators feed primarily on arthropods. Indirect exposure may occur when predatory species feed on herbivores that have fed on Bt crops e.g. lady beetles, green

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lays wing etc. Another consent is the potential impact of PIP GM crops on beneficial non-target invertebrates, therefore the monarch butterfly has been used as bio indicators species to test the relative risk of Bt corn pollen. Ecological risk assessment for PIP GM crops should have a tiered approach. Lower tier test which includes dietary laboratory test, potential toxicity or fitness cost as well as trophic level effect that include function or changes in the structure and species diversity of the invertebrate community. Since pest resistant plant varieties utilizes significantly lesser amount of insecticides and because of their compatibility with biological and cultural practices, such as evaluation of impact on non-target organism, would go along with it. Therefore, scientific analysis of risks and benefits should be conducted before commercial use.

Ecological risk assessment (RA)

RA is a process that uses scientific data to determine potential hazards, exposure and the likelihood of adverse impacts on populations of organisms in the environment. This is usually implemented through the integration of hazard identification and characterization of all of the elements of risk associated with a new GM crop or derived product. Typical categories of hazards arising from the introduction of transgenic crops include: possible unintended negative health effects in a susceptible subgroup of the target population; the evolution of resistance in the targeted pest/ pathogen population when the transgene confers resistance to a pest or pathogen; non-target hazards associated directly or indirectly with the transgenic plant or transgene product outside the plant; and those associated with the integration and subsequent expression of the transgene in a different organism or species following gene flow.

The hazardous effects can either have a direct or indirect, immediate or delayed impact on the environment or human and animal health (European Parliament 2001). An RA is also conducted with a view to determine if there is a need for risk management and if so, the most appropriate methods to be used. The results of RA can then be used by the relevant competent authority to make an informed decision regarding whether or not to give approval for import or cultivation.

The potential risk of GE crop could be of following types:

Transgenic organism persists without cultivation

The trait expressed in transgenic crop might release it from constraints, thus allowing it to persist, become invasive in non-agricultural situation. This would lead to decreased biodiversity.

Transgenic organisms interbreed with related taxa

If a GM crop crosses out with a wild relative then transgene might get transferred to progene or population of wild relative. That introgressed trait then might cause the recipient population to release from a natural constraint on population size.

Evolution of resistance by the pest to the insect or disease resistance

The transgene can shorten the useful life of GM product. This is of concern if the GM product is designed to replace chemical pesticides. The evolution of resistance is also a concern to the developer of the GM crop because it needs a short period of time during which the developer can market the crop.

Development of pest resistance to pesticides and GM crops

Pesticide resistance is a genetically based phenomenon. Resistance occurs when a pest population is exposed to a pesticide. When this happens, not all insects are killed. Those individuals that survive frequently have done so because they are genetically predisposed to be resistant to that pesticide. Repeated applications and higher rates of the insecticide will kill increasing numbers of individuals but some resistant insects will survive. The offspring of these survivors will carry the genetic makeup of their parents. These offspring, many of which will inherit the ability to survive the exposure to the insecticide, will become a greater proportion with each succeeding generation of the population. Insects developing resistance to GM crops is also a major concern. All of the current commercially available insect-resistant crops use genes derived from *Bacillus thuringiensis* (Bt). Diamondback moth (*Plutela xylostella*), Indian meal moth (*Plodia interpunctella*), Colorado potato beetle (*Leptinotarsa decemlineata*) and several other insects have shown the ability to adapt to resist BT foliar sprays in the field or laboratory.[20]

Pesticide resistance management

Resistance is genetically based, controlled by one or more genes and passed from parents to offspring. Resistance is preadaptive i.e. the application of a pesticide does not "create" resistant individuals; resistant individuals are already present in the pest population, although usually at very low frequency. Application of the pesticide introduces selection pressure that results in increased frequency of the resistant genotypes. Resistance involves genetic variation, differential survival and reproduction by survivors. For example, in a field experiment,[21] potatoes containing Colorado potato beetle larvae were sprayed with carbofuran at the commercially labeled rate. Control was outstanding - 99.98% of the larvae were killed and only 96 larvae survived. These larvae carried a dominant gene for acetylcholinesterase that made them insensitive to carbofuran (Table 1).

Table 1 Considerations for designing a refuge[21]

Pest biology/ behavior	Cropping system
Larval movement	Farm/ field size
Adult dispersal	Rotational crops
Mating time	Neighboring host and non-host crops (including others with Bt)
Mating location	Other pests in the crop
Mating frequency	Type, frequency, timing of pesticide applications in the Bt and refuge crops
Generations/ year	
Alternate hosts	

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Insecticide resistance management

The key to managing insecticide resistance is to reduce selection pressure. Under the principles of IPM, insecticide applications are made based on scouting and threshold levels of pests. Several tactics could be employed in order to reduce or minimize insecticidal resistance like:

1. Alternate or mix insecticides with different modes of action, for instance apply a carbamate followed by a pyrethroid or tank-mix a pyrethroid with an organophosphate. In both the cases, if an insect is resistant to one type of insecticide and survives its application, the second insecticide will still kill it.
2. Use a high dose of active ingredient, i.e., use the highest label rate coupled with excellent coverage.
3. Leave an unsprayed refuge. A refuge is an area that is not sprayed, leaving a population of unexposed individuals. This area provides susceptible insects to mate with resistant insects generated in sprayed fields.

Management of resistant BT crops

Bacillus thuringiensis (Bt), is a naturally occurring bacterium that produces spores and protein crystals that kill insects. Based on resistant colonies and field populations, several resistance mechanisms are proposed for Bt.[23] These include modification of the alkaline pH, changes in enzymes that deactivate toxin, enhanced repair mechanisms in the gut and most importantly, a reduction or loss of binding sites in the gut.

Management of BT crops could be done by applying "*alternate Bt toxins*". In this system, plants expressing different *Bt* toxins may be grown from field to field or from year to year. In this case, if an insect is able to feed on plants expressing some toxin say toxin1, it is still susceptible to the other toxin say toxin2 and is killed by plants expressing that *Bt* as insects carrying genes for resistance to both toxins are extremely rare. Another management system may involve using "*combine Bt toxins*" which performs "gene pyramiding". For instance, Bollgard II cotton expresses both Cry 1Ac and Cry 2Ab to control cotton bollworm and tobacco budworm (*Helicoverpa virescens*). Gene pyramiding is designed to improve efficacy of the crop and to reduce the formation of resistance.

In the absence of genes to pyramid, the main strategy for resistance management is the high-dose/ refuge strategy, implemented for BT cotton and Bt corn modified to kill pest caterpillars.

1. High dose: The high dose is expected to kill both homozygous susceptible (SS) and heterozygous resistant (RS) individuals.
2. Untreated refuge: The refuge area maintains a population of unexposed, susceptible (SS) individuals to mate with the rare resistant (RR) survivors of the high-dose Bt crop. The resulting offspring from this mating are heterozygous resistant (RS) yet still susceptible and thus killed by the high Bt crop.

Herbicide resistance and factors affecting herbicide resistance

Several biological factors affect the risk of producing herbicide-resistant weeds, these factors include: frequency of resistant plants, fitness/ competitiveness of resistant plants, seed production, dissemination potential and means of inheritance.

Frequency of resistant plants The initial frequency of resistant plants in the environment is the benchmark by which the frequency will change because of selection pressure. If the initial frequency is very low, a greater amount of selection pressure will be required for the resistant plants to become dominant. If the initial frequency is high, the resistant plants will become dominant with much less selection pressure.

Plant fitness/competitiveness Plants that are well adapted and competitive in their environment will increase in frequency more rapidly than those that are less competitive than their non-resistant cohort.

Seed production and dissemination potential The strength of selection pressure is based on how greatly seed production is reduced in non-resistant plants compared to resistant ones. If the resistant plant species have high seed production capacity, the number of resistant plants will increase rapidly. In contrast, if the resistant plant species produce low numbers of seeds, the rate of increase in the population of resistant plants will be slower.

Mode of inheritance If a resistant trait is related to a single dominant gene, resistance is likely to spread more rapidly, than with a single recessive gene, since the resistance will be expressed in the heterozygous individuals.

Management of herbicide resistance in weeds

Mechanism involved: There are primarily three mechanisms that are involved in herbicide resistance in weeds:

1. Modified site of action.
2. Metabolism.
3. Compartmentalization.

(1) It is the most common and most irresistible mechanism for herbicide resistance. Herbicide sites of action are often proteins or enzymes. The herbicide will bind to the site of action and inactivate the protein or enzyme, resulting in the disruption of a biochemical or physiological process. Therefore, the herbicide is unable to disrupt the biochemical or physiological process and the plant is not affected by exposure to the herbicide.

(2) In herbicide metabolism, herbicide-resistant plants are capable of rapidly detoxifying the herbicide. The rapid detoxification of the herbicide protects the plant from significant herbicide injury.

(3) In compartmentalization, plants make herbicide molecules in compartments of plant cell vacuoles which results in the herbicide molecules prevention from interacting with the herbicide site of action and therefore the plant is not affected.[24]

Practices to reduce risk of herbicide-resistant weeds

(1) Herbicide rotation: It is important to avoid consecutive applications of herbicides with the same site of action against the same weed species unless other effective control practices are also included in the management system.

(2) Herbicide combinations: Here, two or more herbicides are applied together that have different sites of action.

(3) Crop rotation: Rotating crops within a field can be an effective practice to minimize selection pressure for resistant weeds.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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