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Transgenic plants: Risks, Concerns and Effects on Ecosystem and Human Health

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Abstract

The review portrays the current status of transgenic crops in the backdrop of sharply divided opinion of anti-GM group vis-à-vis those who advocate that genetically engineered crops do not pose any threat to human or animal health nor does it disrupt the environment through introduction of superweeds. While acknowledging the concern of anti-GM activists, the article supports the view of NE Borlaug who sees no difference between the birth of a genetically complex crop such as hexaploid bread wheat and a bioengineered crop except that the former occurs in mother nature and the latter in the laboratory through genetic manipulation by molecular breeders. The article brings into focus that the methods now being employed to bring about genetic change in crops are safer as the use of antibiotic markers have been replaced by biolistic mode of gene delivery or use of chemicals such as mannose 6-phosphate marker. The article also points out that the chances of birth of 'superweeds' destroying the environment will be minimized, if genetic transformation is brought about using chloroplasts. Another important role of biotechnology that is expected to be realized soon is its use in commercial production of oral vaccines for both humans and animals. The article mentions of some of the recent gene discovery such as genes for submergence tolerance in rice or genes that minimizes allergenicity in peanuts. Production of rice varieties capable of withstanding flood will dramatically increase rice yield in flood-prone countries and peanut lines with minimum or no allergen will be welcome in the consumer market where a sizeable portion of people suffer from peanut allergy. The article emphasizes the fact that every GM crop must be subject to rigorous scrutiny to ensure that it is free from any allergen, or hazardous toxic substance and that it is environmentally safe. The article supports the idea that in Europe GM crops with built-in terminator gene and traditional crops may coexist; i.e., may be planted in the same area as the European farmers buy their seeds every year unlike their counterparts in Asia and Africa. Finally the article recommends that

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the selected GM crops should be grown in the developing countries where the option before the resource-poor population lies between going without food or fall prey to the misinformation of anti-GM activists.

Introduction

Improvement of crops through accumulation of desirable traits in the progeny has been an age-old practice. Conventionally, it is achieved through hybridization and most food plants have thus been changed to an extent that they have very little resemblance to their wild relatives. This practice is fraught with problems, when it comes to crossing two species or even subspecies because of various degrees of sterility and introduction of undesirable characters along with the ones of interest. Recurrent backcrossing sometimes helps in the elimination of undesirable traits by restoring fertility but this technique has mainly two drawbacks: The linkage between traits of interest and undesirable ones, is difficult to overcome. To obviate this problem, breeders were prompted to go in for recombinant DNA technology, in which only the gene(s) of choice is inserted into the recipient host plant. Here, a specific DNA sequence constituting a gene or a part of a gene, copied from microbes, plants or animals, is inserted, thereby reducing the chance of transmission of tightly linked undesirable traits from donor parents to enter the recipient host.

Vasil and associates (1990) inserted genes of interest into cereal suspension culture cells derived from protoplasts. They showed that protoplasts isolated from young embryos of cereals like wheat develop into embryogenic callus and such material was later found to be suitable to produce regenerants containing transgenes. Procedures such as transformation of plant tissues by means of *Agrobacterium* using antibiotic marker genes are too well-known and have been omitted in this review. The authors start with the contribution by Vasil and his associates, which group was one of the first to show that there is an alternative way to stably transform callus lines and that is by means of microprojectile bombardment of wheat cell suspension cultures (Vasil et al. 1991).

Some commercially important transgenic crops: Genetic engineering has been successfully employed in the production of herbicide tolerant, insect resistant or virus resistant crops, namely canola, corn, cotton, flax, potato, tobacco, tomato, rice, soybean, strawberry. Biotechnology Industry Organization (BIO 2005, McHughen and Holm 1995) has published an excellent review listing and briefly describing the onward march this emerging technology has done since its emergence nearly two decades ago (Table 1). It has listed a large number of GM field crops, vegetables, fruits, oil, beverages that have been released and marketed. Name any important item and it is in this list. Some value added-traits mentioned in this account are fruits with higher nutritional status, longer shelf life, drought resistance, better weed tolerance of certain cereal crops, the use of GMOs in diagnostics. Of interest in this context is the review by Pribylova et al. (2006), who describes transgenic potatoes as a unique plant for nutrition and their use in the prevention of diseases of humans and animals.

Transgenic crops for some other value-added traits: Apart from increasing the crop value, genetic engineering is also being applied to enhance the production of (a) lauric acid for soap, (b) plant-derived pharmaceutical proteins (PDP) such as production of oral rabies vaccine, vaccines against hepatitis B, (c) therapeutic proteins such as antibodies for treatment of tumour and cystic fibrosis, (d) blood-clotting factors, (e) cytokinins, (f) growth factors such as hormones, (g) recombinant enzymes and (h) human and veterinary vaccines (Table 1). Some other recent value-added traits of GM crops are that they can be used for the treatment of contaminated land and water rich in arsenic deposits (Gelernter 1997, Shantharam 1999). In this context, the review of Julian et al. (2005) deserves special mention.

According to them, low tech and inexpensive transgenic plants grown under field conditions, can provide better opportunities for the synthesis of peptides, polypeptides and complex proteins than microbes. The development of GM plants with multiple transgenes of interest and using plastids for synthesis of PDPs are some of the steps that will help in the production of GMs with value-added traits. Since transgenes are not transmitted though pollen, use of plastids to produce GMO's ensures a safer environment by eliminating the chances of formation of superweeds through this novel method.

Some important considerations about GM crops: The GM plants are also selected in segregating populations to follow pollen dispersal and outcrossing to wild species. This procedure helps determine competitiveness and survival of transgenic plants in unmanaged habitats.

Before going into the assessment study to determine gene flow, molecular breeders must ensure that the current range of markers do not code for any toxin and therefore not considered dangerous (Malik 1999). It has been suggested that engineering crops for herbicide and pest resistance could reduce use of unsafe chemicals that pollute the environment. Traits for resistance to different insect pests and diseases already exist in many cultured crops such as apple, barley, corn, grapes, pears, potato, soybean, tobacco and wheat. Introducing genes from these crops into susceptible varieties would not change their genetic make-up significantly, nor would it introduce a gene that is not already consumed by people. Even if the plants with a marker gene for antibiotic resistance develop resistance against herbicides, it should not pose any threat. In the absence of any selection pressure, chances of formation of weeds are negligible.

Table 1. List of some important transgenic crops.

Crops	Trait	Transgene introduced	Reference
Canola	High lauric acid	For soap manufacture	Ohlrogge (1999)
Canola			
Corn	Monoclonal antibody production	For lung, breast and colon tumours	Daniell et al. (2001a)
Corn	Insect resistance	*Bacillus thuringiensis* toxin gene inserted	Saxena and Stotzky (2000)
Cotton	Insect resistance	Bt gene inserted	Tabashnik et al. (2003)
Cotton	Permapress fibre		James (2003)
Cucumber	Resistance to biotic and abiotic stress	Tobacco promoter is induced	Yin et al. (2004)
Potato	Resistance to nine viruses	Mostly coat protein gene inserted	Savenkov and Valkonen (2001)
Potato	Colorado beetle larvae resistance	*CryIII Bt* gene inserted	Raps et al. (2001)
Rapeseed (canola)	Herbicide resistance	Glyphosate-, imidazolinone and glufosinate genes were inserted separately	Messeguer (2003)
Rice	Insect resistance	Genes from wild rice inserted	Khush and Brar (2002)
Rice	Golden rice	β-carotene gene inserted	Paine et al. (2005)
Soybean	Insect resistance	Bt gene inserted	James et al. (2003)
Squash	Resistance to five viruses	Mostly using coat protein	Pang et al. (2000)
Strawberry	Fungal resistance		Itoh et al. (2003)
Tobacco	Pharmaceuticals		Ma et al. (2003)
Tobacco	Resistance to six viruses	Mostly coat protein gene	Mason et al. (1996)
Tomato	High pectin and delayed ripening		Mishra et al. (1998)
Wheat	Resistance to two viruses	Using coat protein	Sivamani (2002)

In this connection the comments made by Nobel Prize winner, N. E. Borlaug (Borlaug 2001) is pertinent. Production of GM crops is the same as transgenic hybridization between distantly related species that took place in nature to give birth to important cereal crops such as bread wheat, *Triticum aestivum.* The distantly related species involved in its origin were: *Triticum turgidum* var. *dicoccoides,* and *Triticum tauschii* (Talbert et al. 1998). Furthermore, according to Borlaug and Dowswell (2001), the presence of transformed DNA *per se* in food products poses no health risk, since DNA from all living organisms is structurally similar.

Views regarding GM crops: One of the firm advocates of GM crops is CS Prakash. Conko and Prakash (2004) showed that there was nothing inherently wrong with the molecular tools that are now being used to produce transgenic crops. They mention that GM crops are produced under strict supervision and have received approval from many scientific bodies, such as FAO, WHO, Royal Society of UK, the American Medical Association and the French Academies of Medicine and Science. In support of their statement, they have cited their own work, where they did neither use *Bt* gene nor any toxic marker; instead they used mannose 6-phosphate to produce high-protein rich transgenic sweet potato.

GM and non-GM crops may coexist with no detrimental effect to the environment: According to Wager (2006) GM and non-GM crops may coexist together without any detrimental effect to the environment. He supports the production of transgenics with built-in terminator gene although individuals containing this gene die without leaving any seeds for next year's planting. Anti-terminator group vehemently opposes the terminator gene lines on the ground that resource-poor farmers are forced to buy seeds every year. To counteract the criticism, Wagner (l.c.) points out that in European countries, farmers buy seeds every year unlike their counterparts in developing countries where it is farmers' practice to save their seeds for the next year's planting. From his argument, it follows that in Europe, farmers can grow both standard varieties and transgenics containing terminator gene side by side without concern because such GM crops do not produce any viable seeds.

The probable risks: The risk of developing and introducing plants with genes inserted through GE-technology, may be manifold. The type and degree of risk will depend on the specific plant, its biology, genetic makeup, source of the transgene, its characteristic, quality, stability and the encoded trait, and the ecological condition of the country(s), where it may be released both within and outside the country of origin. The situation will be of more concern, if the territory of the released transgenic is populated by a multitude of wild relatives and there is lack of strict enforcement of regulatory laws. In the absence of rigorous vigilance by law enforcing agencies, the inserted gene may escape and contaminate other plants of the same-, related- or weedy species. The newly introduced gene within its new genetic environment in the host plant may have an indirect or unpredictable pleiotropic- or epistatic suppressive effects on the host plants or it may interact with other gene(s) (Yarrow 2001).

(1) It is a common concern that the resistance transgenes or the GM genes may escape into the surrounding populations where they may hybridize with their semi-domesticated or wild relatives. Because of high rates of gene flow such an event will result in the formation of 'superweeds', thereby creating new demands for more toxic herbicides (Savenkov and Valkonen 2001).

Daniell et al. (1998) reported the presence of 28 - 30% marker genes in wild sunflower and 50% gene flow in strawberry. They mentioned that even in the first backcross, *Brassica campestris* received herbicide gene(s) from canola. When repeatedly used, a population immune to the herbicide may also develop. Canola has many wild relatives. Based on their study of Australian population, Rieger et al. (1999) reported that there has been an introgression of transgenes from rye grass *(Lolium perenne)* into eight related species, giving rise to a significant number of herbicide resistant wild races.

The origin of some of these aggressive weedy taxa could also be due to mutation. Estham and Sweet (2002) grouped the transgenic crops into three categories based on the risk they carry in terms of flow of transgenes (a) oilseed rapes: high, (b) sugar beet: medium - high, (c) potato: low, (d) maize: medium - high, (e) wheat and barley : low, (f) fruits: medium - high.

A few cases of transgenic contamination have been reported in corn in Mexico and Canada (Ching 2004). Pollen drift by wind or other means may help transmission of transgenes to contaminate other cultivars, related wild or semi-wild species. The extent of gene transfer depends on several factors, namely: (a) the transgenic crop variety and the wild species must be sexually compatible, (b) they must be growing in the same location, (c) they should flower at the same time and (d) they should have a means for transport of pollen from the donor to the recipient (McPartlan and Dale 1994). These factors may often accidentally operate in crop fields in the same locality, thereby enhancing chances of production of aggressive weeds. In addition, the transgene may enhance the ability of new hosts to survive in adverse conditions, enabling them to turn to weeds.

(2) The chemicals such as glufosinate ammonium and glyphosate, applied to produce herbicide tolerant (HT) transgenic crops, are known to be systemic metabolic poison and may have a wide range of harmful effects on humans including neurological, respiratory or gastro-intestinal problems and are also toxic to beneficial soil fauna and flora (The Institute of Science in Society 2006). However, recent studies reveal that glufosinate (Liberty) does not pose any

significant threat to the consumers. GM corn plants can convert glufosinate into non-reactive N-acetyl glufosinate (NAG). But the fear still haunts the consumer that NAG, ingested with corn may be reactivated to glufosinate by gut bacteria and may prove to be a health hazard. The fact that very little glufosinate or NAG accumulates-into the edible grains and most of it stays either in leaves or get washed by rain should allay their apprehension. This view is in great contrast with those who are of the opinion that transgenic plants containing *Bt* gene pose a great threat to nature. In its recent report (July 13, 2006) the above institute reports that Indian Maharashtra Hybrid Seed Co. bio-engineered stem-borer resistant *Bt* eggplants. According to Ho and Cummins (2006) the above GM crop, containing Cry1Ac toxin, is linked with hundreds of allergy cases and thousands of sheep deaths.

(3) *Bacillus thuringiensis* (*Bt*) is a gram-positive soil bacterium capable of producing insecticidal crystal protein from more than 100 *Cry* genes. *Bt* toxin strains have different delta endotoxins effective against different *Lepidopteran* or other insect species. Some *Bt* toxin strains may even produce beta-exotoxins, toxic to vertebrates and invertebrates. So, if a transgenic plant is produced without proper identification of the *Bt* strain, different kinds of problems may arise. It may be mentioned here that *Bt* genes have been introduced into more than 50 different kinds of crops. In some cases, the larvae of insects killed by feeding on a transgenic *Bt* crop may kill other predators feeding on *Bt* plants (Schoenly et al. 2003). Moreover, residues of *Bt* incorporated crops may be toxic to soil organic recyclers such as *Collembola* (Donegan and Seidler 1999).

It has been reported that *Bt* toxin requires the assistance of other gut bacteria to kill the insects (Devitt 2006). Transgenic crops with *Bt* resistance may escape causing out-crossing and may increase in fitness and weediness in their capacity to colonize unsuspected areas. Such a situation arises, if the insects susceptible to the concerned endotoxin were an important hindrance to the distribution and abundance of the plants in question.

Meanwhile, several *Lepidopteran* species have been reported to develop resistance to *Bt* toxin in both field and laboratory tests. But these pockets of insect resistance to *Bt* have been reported to occur on rare occasions under frequent and prolonged use Widespread use of insecticidal gene may cause or accelerate the development of pest resistance to the insecticide involved. Already ten species of insects have evolved resistance to *Bt* spore crystal mixture or purified endotoxins (Cohen 2005) The better performance of GM cotton varieties in the USA seems to suggest that this technology is likely to play an increasingly important role in the genetic improvement of certain crops such as cotton. (Wilkins et al. 2000).

(4) In case of virus resistance transgenes also, it may be possible that outcrossing may help gene flow. Moreover, recombination with another virus may create new viruses (Pappu 1999). But the good thing about such recombinants is that a single chimeric transgene derived from two distinct viruses may confer multi-virus resistance to transgenic plants through homology-dependent gene silencing (Fagoaga et al. 2000) such as in Spotted Wilt Virus resistant tomatoes.

(5) Monoculture of a transgenic crop for a long time in a particular area may pose a threat to bio-diversity and encourage genetic erosion.

(6) Genetically engineered insecticidal organisms like *baculovirus* may attack non-target species of insects destroying the beneficial ones. Since some farmers suspect that GM crops with Bt toxin protein may also affect non-target beneficial insects such as honey bees or monarch butterflies, it is recommended that it should be made mandatory for farmers to grow border crops that do not contain *Bt* gene or toxin but will be a host plant for supposedly vulnerable beneficial insects.

(7) The ethical issues such as infringement of the intrinsic values of natural organisms, tampering with nature by recombining genes among species, introducing animal genes into plants and *vice versa*, and creating stress for the wild life should not be ignored (US Dept. of Energy, Office of Biological and Environmental Research 2006).

Risk assessment: The risks or concerns need to be assessed properly before any remedial methods can be adopted to minimize the anticipated risks or avoid them altogether. Proper estimation of the extent and rate of gene escape is very important. Success of the transgenic crop depends on proper assessment of the possibility of adoption of adequate measures to mitigate the amount of risks involved. The severity and the degree of the risks will determine the kind of measures to be undertaken to keep the risks at a minimum level. Assessment should be conducted at producer, farmer and consumer levels.

(1) A thorough analysis of the biology and ecology of the plant species in question is needed. It is also necessary to find out details of the life forms of the interacting species, its center of origin, the multitude of species with which it is related together with its potential for introgression into its near-relatives. It is essential to characterize the introduced novel traits whether or not it may directly confer weediness. The analysis of the alien gene's capacity to cause direct or indirect pleiotropic, epistatic and/or suppressive effect is also necessary. Careful monitoring should be conducted to trace escape of individuals from the population of transgenic crops under trial in order to prevent creation of a pool of herbicide resistant plants outside the areas of cultivation. Individual plants escaping unnoticed may become invasive in the long run (Daniell et al. 1998). Madsen et al. (2004) proposed that the risk

assessment study should encompass the concept of familiarity data of the species of interest including its ecosystem. Such an assessment will shed light on the kind of uncertainties inherent in the risk assessment of a GMP taxon. They also suggested that GMP characteristics should be compared to those already introduced in the same crop by conventional breeding techniques.

(2) In case of herbicide resistance, the salient features of risk assessment should include: (a) potential of the transgenic crop to become a weed or to be invasive of the natural habitat, (b) potential for gene flow to wild relatives whose hybrid offspring may be more weedy or more invasive and the rate of such an escape, (c) potential for the transgenic crop to become a plant pest; i.e., capability to produce growth inhibiting substances or be adapted to stresses, (d) potential impact of the plant with novel traits or their gene products on non-target species including humans, and (e) its potential impact on biodiversity. The important point is to find out the nature of the genetic changes and its impact on the environment into which it is introduced (Shantharam 1999). Several oilseed rape lines resistant to different herbicides may lead to the development of a weedy population with multiple herbicide tolerance through natural gene flow within the species and turn to be a difficult-to-control 'superweed'. So any new or volunteer weed should be monitored and reported immediately. Crawley and his associates (Crawley et al. 2001) report that charlock, a weed as a superweed had a very low population and the plants were not viable.

A report prepared on a decade long study with rapes, soybean, corn and potato by Crawley et al. (2001) at the Imperial College, London, indicated that the transgenic crops did not spread their altered genes to other species or survived longer than their natural counterparts. They observed the spread of a weed of the mustard family (*Brassica kaber)* and concluded that it had a low population and that hybrids were not viable.

(3) If more than one gene is transferred, the interactive situation in transgenic plant may be complicated. Before release of such a transgenic, the consequence of such transformation should be analysed on a trial-run basis to find out the effect of such a transformant on farming system and sustainable agriculture (Chopra 1999).

(4) Before releasing a *Bt* crop, the following assessment need to be made through an in-depth study, such as the probability of (a) transfer of transgene(s), (b) the transgenic crop supporting another polyphagous insect attacking nearby plants where *Bt* bio-insecticide has been used, (c) escape of target insects to non-*Bt* refuge area, (d) finding other inexpensive but effective control measures, (e) discovering the degree of susceptibility of the key pest to *Bt* plants and (f) destroying non-target insects. Only the genes for proper δ-endotoxin specific for the target insect should be inserted (Wharton and Norris 1997). The

concentration of the toxin in the transgenic plant should be high enough to kill all the insects leaving none to develop a resistant stock. Unlike *Bt* bio-insecticide, *Bt* toxin in transgenic plants is already solubilized and activated. So, its impact on health and environment should be assessed using oral toxicity to rodents, birds, honeybees, insect predators and parasites and earthworms. Researches to determine the lethal dose for each of these should be conducted immediately. Some work on the LD_{50} has already been done. Because insect fauna associated with the wild and weedy crop relatives are generally poorly known and impact of insect herbivory even less studied, faunistic surveys and possible ecological experiments may be necessary to evaluate risks of weediness, where fertile hybrids can form between *Bt* crops, wild and weedy relatives (Cohen 2005).

(5) The number of antibiotic resistance marker genes required to produce transgenics should also be ascertained beforehand, so that the critical level is not exceeded (Malik 1999). In tomato, less than 10 kanamycin resistance genes have been incorporated (Redenbaugh et al. 1992). Gay and Gillespe (2005) stated that antibiotic resistance marker genes in GM plants are not a risk to human health. Plants derived with these marker genes neither contain nor produce antibiotics. They concluded that whereas there was no evidence that antibiotic resistance from GM crops was transferred to bacteria; their statement needs to be viewed with reservation, because the possibility still exists that it might occur in the future. However, the evidence suggests that if it occurs at all, the contribution from GM plants to the burden of antibiotic resistance is low; and is dwarfed by inappropriate prescription of antibiotics and their use as animal growth promoters in agriculture. In a press release, Gay and Gillespe (2005) stated: Antibiotic resistance markers do not pose a substantial risk to human health, because the contribution that recombinant bacteria might make - should the enormous barriers to gene transfer are overcome - is so small that any contribution to antibiotic resistance by GM plants is negligible compared to the contribution made by antibiotic prescription in clinical practice.

(6) Assessment of risks involved in the use of marker genes should include determination: (a) toxicity or adverse effects of the selectable marker gene protein products or metabolites exposed to humans, wild life, animals, beneficial insects, marine life and endangered or threatened species; (b) potential of the marker genes to transfer from transgenic plants to pathogens and clinical importance of the antibiotics which the marker gene inactivates, availability of alternative therapy or other antibiotics besides the one inactivated by the marker gene, possibility of expression of the gene in plant and the stage and level of expression, stability of the gene product, whether the consumption of the transgenic plant products compromise therapeutic use of the selecting antibiotics and the extent of spread of the marker gene among the current population of

the human and animal pathogens, in soil microbes, their plasmids, integrons and transposons; (c) the possibility of transfer of herbicide resistance marker genes from transgenic crops to weedy relatives or other cultivated plants or cultivars, its impact on the use of herbicides, weed management, current agricultural practices and the consequence of the use of the transgenic crop for the environment and the possibility of the undesirable selectable marker gene flow from the transgenic crop to wild plants changing their position in the ecosystem and (d) impact on the present food web and ecosystem relations.

The marker genes from allergenic sources should be tested in the transformants. If the marker gene inactivates antibiotics used in human therapy, the potential for inactivation of an oral dose of the corresponding antibiotic marker needs to be tested.

(7) Effects of long-term monoculture of the transgenic crop on a large scale on bio-diversity should also be assessed.

Avoidance and alleviation of the risks: Proper assessment will be helpful in designing economic strategies to avoid or alleviate the risks of designing, developing and releasing transgenic crops at different levels. Biological, physical and temporal safeguards should be built-in into the test to closely monitor the field tests as well as to assess the field level performance of the engineered crop. This is the pre-requisite for successful development of strategy before the release of a transgenic crop.

The following deserve serious consideration:

(1) Creation of an international gene register to track the alien genes and constructs that have been introduced into the crop gene pool. The precautionary measures will improve the ability of researchers to predict interactions between existing transgenic plants and those designed to be produced by them (Butler and Reichherdt 1999).

(2) Introduction of a legally binding international bio-safety requirements preventing the testing and use of genetically modified organisms (GMOs) in developing countries with ineffective or no regulations; and help them formulate national bio-safety regulations flexible enough to keep the pace of biotechnology development uninterrupted.

(3) Sustainability of agriculture periodic assessment ensuring that no potential negative impacts of GM organisms are brought to bear on the environment, human health and agronomic practices. In Canada, developers of HT plants require regulatory approval, only if the new crop line is either familiar or unfamiliar but not substantially equivalent (Yarrow 2001).

(4) Discouraging repeated use of the same herbicide resistant plants in controlled setting to prevent introgression of novel tolerance that may result in the creation of herbicide ineffective weedy population. Use of a variety of

herbicides containing different chemicals would prevent formation of such herbicide-tolerant crop varieties.

There has been a novel approach in containing the escape of transgenes from the engineered crops (Chamberlain and Stewart 1999, Daniell et al. 1998 and Scott and Wilkinson 1999). Since escaping of transgenes occurs mainly through pollen outflow and plastids are not transmitted through the pollen, efforts have been made to incorporate the desired genes, particularly those for resistance to herbicides, insects and viruses into the genomes of the cytoplasmic components, namely, the plastids and thus creating transplastomic crops. Daniell et al. (ibid)) successfully introduced herbicide resistance genes from petunia into the chloroplasts of tobacco and reported no gene escape through pollen. According to Scott and Wilkinson (1999), there was only negligible escape of transgenes in *Brassica napus* from transplastomic crops to wild relatives of canola, when the recurrent parent was not a transgenic plant. Bilang and Potrykus (1998) also advocated the same procedure to bioengineer crops for avoiding transgene escape. Chamberlain and Stewart (1999) suggested that since paternal chloroplast inheritance is rare, transplastomic plants may prove highly useful for transgenic crop control. For this procedure to be effective, a strict management programme for tracing the movement of the transgenes and transgenic plants should be followed. Markers, like green fluorescent protein (GFP) gene may be inserted into the engineered plant to detect the presence of transgene in its seed coat. For the purpose of biosafety, the whole history of transformation, gene flow and ecology information for specific transgenic events per crop should be available and the Government agency responsible for law enforcement must police the import of GM crops rigorously.

Pardo (2003) suggested many practical ways, including biological and physical barriers and methods to stop gene flow.

(5) In the case of *Bt* transgenic plants, Johnson (1997) suggested the use of low dose *Bt* toxin-producing genes, so that natural enemies and insect resistant plants can interact synergistically to reduce pest population. When a high dose *Bt* transgenic plant is cultivated, it is better to have 'refuge' areas planted with non-*Bt* crops as a border crop in the field. This practice will stop the build-up of *Bt* resistant insect generations. Refuge area will involve some space with non-*Bt* crops in the field, adding some extra cost of cultivation that can be met by local farmers. Some companies claim that their crops produce very high dose of *Bt*, enough to kill all the insects and leaving none to produce a resistant stock. In addition, they also claim to maintain a refuge area. Recently the U. S. government has enforced a law, requiring a farmer to plant 20% refuge area beside each *Bt* crop.

(6) In case of transgenic virus resistant plants, phenotypic mixing and synergism do not pose any risk. The risks of recombination between virus strains may be minimized by avoiding 5′ → 3′ ends of RNAs as part of the transgene, not using transposable viral sequences as transgene sequences; introducing more stop codons in their sequence, selecting transgenic lines expressing minimal level of transgenes, using sequences coding for part of the replicase gene and avoiding use of satellite RNAs with potential to cause more severe diseases (Yarrow 2001).

(7) Although RoundUp Resistant GM crops are quite common, herbicide resistance genes should not be used as selectable markers, if an alternative method is available as an option. Recent studies have shown that in the absence of a selection pressure, the herbicide resistance gene may be lost from the offspring during subsequent cycles of cultivation without having any effect on the engineered plant phenotype. The use of antibiotic resistance gene as a selectable marker can be avoided by alternative methods as used in the case of amino acid biosynthesis. The antibiotic resistance marker may also be cleaved out of the plasmid before introduction to plants (Malik 1999). Use of vectors other than viral vectors may be safer (Butler and Reichherdt 1999).

Caddock et al. (1998) found out that transgene expression may be enhanced by a simple molecule such as ethanol and may be useful for replacing promoters.

(8) An FAO report (2004) the application of new techniques in which genetic transformation are carried out by methods other than the traditional ones by avoiding antibiotic marker genes and promoter genes such as CaMV35S that are of concern to some people. Varieties including two different *Bt* genes are reducing the likelihood that pest resistance will develop. Management strategies and genetic techniques are devolving to prevent gene flow (Julian et al. 2005).

Opinions in favour and against GMOs: There have been many arguments and opinions for, as well as against GE technology and GM products, including GM based food items. For example, Professor Mae-Wan Ho proposed a moratorium both on environmental releases of transgenic organisms and marketing of GM products in 1996, as a precautionary principle, until possibility of vector-mediated horizontal gene transfer and its consequence on bio-diversity, agriculture and human health could be assessed and appropriate legally binding bio-safety regulations firmly established (Ho and Cummins 2006). A report published in August, 2003 Issue of The Ecologist, presented five reasons to keep Britain GM-free. The reasons are: (a) the presence of GM foods in the market would minimize consumer choice, (b) exposure to health risk due to possible allergenic reaction, antibiotic resistance and probable harmful effects of industrial and pharmaceutical GM products, (c) reluctance of many EU countries, Japan, and South Korea to accept GM foods, (d) decision of some industrial concerns like Heinz, Garber and Frito, Lay using GM products, thus throwing farmers gradually out of their business, (e) generation of stronger weed population creating environmental problems and (f) inability of GM crops to feed the poor. The Weston A. Price Foundation, published a news under the heading “Action Alerts” that a ban was proposed by scientists from different disciplines of different countries.

Again, a report from the Institute of Science in Society of the U.K. under the title “The case for GM-free sustainable world” published in the Independent Science panel (ISP) in 2006, expressed concerns over GM products. They stated that due to loss in sales of GM products, the US Govt. had to give 12 billion dollars subsidy to farmers. Many countries like Zambia rejected GM corn as food aid. There were also reports of crop failure due to silencing of transgenes. According to a recent report, more toxic herbicides such as Atrazine had to be used to control HT volunteer crops and weeds. There were concerns about human health also; because, growth-factor-like substances, were detected in the test animals. *Bt* proteins have been found to be harmful to many non-target beneficial insects. Transgenes intended to prevent gene flow are spreading male sterility. It is also feared that by transfer and recombination, superior types of pathogens may evolve which might be difficult to control. Transgenic DNA of antibiotic resistance markers taken up by gut bacteria may also be difficult to control causing mammalian cells to develop cancer.

In spite of all these, there are also signs of developments and achievements:

(1) Donaldson and May (1999) concluded that evidence collected so far did not suggest that GM foods had been inherently harmful and precautionary measures that were taken at that time, were reassuring. But they suggested that high standard of regulation, continuing research strategy and instituting population health surveillance are needed.

(2) In 2003, another U.K. Govt. GM Science Review Panel stated that (a) the risk of allergy posed by GM plants was not greater than those posed by conventionally grown plants or plants from other areas, (b) the risk to human health associated with the use of viral DNA sequences in GM plants are negligible, and (c) considering the long history of DNA consumption from a wide variety of sources like food and microbes, it was concluded that viral DNA consumption poses no risk to human health and additional ingestion of GM DNA has no effect.

(3) Bhattacharya (2003) presented a major U. K. report from an independent review of 600 published scientific papers and 17 areas of concern and concluded that (a) there had been no verifiable toxic or untoward deleterious effect from world wide consumption of GM foods by human or livestock over the previous seven years, (b) GM plants were very unlikely to invade British countryside or become problematic plants, (c) there would be very little gene flow from GM

crops like beet and oilseeds to wild relatives, (d) there was no compelling evidence for gene transfer from GM food eaten by human to bacteria in the gut and (e) gene flow between GM plants and soil bacteria or viruses is theoretically possible but extremely unlikely and without precedence.

(4) In a report entitled 'The environmental effects of transgenic plants', a panel of experts assembled by the National Academy of Science, USA concluded that the genetically engineering process *per se* presents no new categories of risk compared to conventional breeding (Pew Institute 2003).

(5) According to recent reports published recently by FAO (Corey 2004) so far, (a) countries that are growing GM crops in the fields did not report of any significant health damage or environmental harm, (b) monarch butterflies, previously feared to be fatally depopulated by *Bt* were not significantly affected, (c) pests had not developed resistance to *Bt*, and though some evidence of HT weeds had emerged, (d) no superweeds had in reality invaded agricultural or natural ecosystems. On the contrary, important social and environmental benefits have been emerging. Farmers have been using smaller quantity of pesticides or using less toxic ones, thus reducing contamination to water supplies, protecting workers' health from being exposed to its poisonous effects , (e) return of beneficial insects and birds to fields and (f) replacement of antibiotic marker genes and promoter genes by recently developed new techniques of genetic transformation has opened up a safe environment for production of GM crops.

(6) Another recently published FAO report by Cohen (2005) supports the idea that a science-based evaluation system be introduced to objectively determine the benefits and risks of each individual GMO case-by-case in order to address legitimate concerns for bio-safety of each product. They assert that the extent of benefit of the product would normally outweigh the risks.

(7) Sasson (2005) has discussed the merits and demerits of GMOs and come to the conclusion that biotechnology has provided powerful molecular tools to the scientists to produce pharmaceuticals and varieties of food, medicinal and cash crops that were beyond the reach of conventional methods. Any items produced using the advanced technology should not be dismissed as environment unfriendly in the absence of any concrete proofs. Furthermore, according to this report developing countries where food shortage is a chronic problem, Government should not outright reject a food crop simply because it is a GMO crop as recent times have witnessed in some African countries (Cf. http://www.africabiotech.com). The policy makers of a country should consider merits and demerits of such a crop before it takes a decision either in favor or against it.

(8) Recently developed Genetic Use of Restriction Technology (GURT) should be applied to avoid turning-on of certain undesired transgenes (Wager 2006).

(9) The consensus expressed recently by 12 researchers from China, Egypt, France, Germany, India, Switzerland and the USA at the end of an international meeting of the Science Academy held in Berlin, Germany, on 30th May, 2006, was that food obtained from GMO is at least as safe as other foods. According to them GM plants pose no danger to the environment and they do not conflict with the so-called bio-agriculture. Professor Klaus Ammann of the University of Berne accused Green Peace of spreading lies in this respect. On the other hand, Professor Walker Heldt, Chairman of the Academic Union Commission for Green Genetic Technology called for an objective rather than an ideological discussion on the subject.

GM crops are being gradually accepted all over the world: In spite of vigorous campaign against GM products substantiated by some research findings, accomplishments so far achieved reveal that GM technology will play a vital role in shaping the world economy and contribute considerably to the relief of the hungry millions of the developing world. Pardo (2003) stated that due to population pressure and depleting land and water resources, world agriculture needs a technical jump and transgenic plants can play the role. But there are also risks in achieving these goals.

Case-by-case analysis of a GM crop before its release: Most of the articles dealing with including reviews suggest that the technology must be very cautiously adopted and case-by-case analysis and monitoring should be conducted before a transgenic product is released in the market for human and animal consumption. Assessment of the risk, benefit-cost ratio and, future effects on human health, environment, ecosystem, need to be worked out in details. Scientific development is a dynamic process. So whenever any risk is detected in regard to the adoption of a GM product, the problem should be tackled until a solution is found.

Future outlook: With the continuous improvement of molecular tools being used by breeders, the outlook of transgenic crops has become brighter.

Improved version of GM rice: The new improved variety of Golden Rice (Golden 2), fortified with five times more vitamin A, is now available (Paine et al. 2005). In 2001, when Ingo Potrykus and his team (Beyer et al. 2002) developed vitamin-rich "Golden Rice" without the use of antibiotic marker genes, the opponents of GM crops did not accept the variety. Their plea this time was not the use of antibiotic marker genes by the molecular breeders. They argued that the vitamin A content in the Golden Rice is so low that rice consumers needed to consume five times more compared to what they normally eat in order to obtain sufficient amounts of vitamin 'A' for preventing blindness.

In order to meet this opposition, Paine et al. (2005) developed an improved variety of Golden Rice. They inserted a maize gene called phytoene synthase (*psy*) in the rice genome in combination with the carotene desaturase *(crtI)* gene from *Erwinia uredovora.* In the newly developed transgenic, the total carotenoids increased 23-fold (maximum 37 mg/g) compared to the original Golden Rice and a preferential accumulation of β-carotene.

Indica version of golden rice: The need was keenly felt to transfer β-carotene gene to *indica* varieties of rice. This was because the *japonica* varieties of rice are sticky and are not popular with 200 billion people of certain parts of Asia, specially in the Indian subcontinent. Using the biolistic system of transformation, Scientists at IRRI succeeded in developing several *indica* rice cultivars (adapted to diverse ecosystems of different countries) by inserting a number of transgenes responsible for biosynthesis of provitamin A through biolistic bombardment of genes of interest. They used rice seed-specific glutelin promoter (Gt-1 P) to drive the expression of phytoene synthase (*psy*) gene. One of the popular *indica* varieties from this region. in their list was BRRIDHAN29. One Bangladeshi scientist, working with Datta in this project was Sayda Rehana. The nutritional status of the *indica* rice-eating population will radically change, when this vit-A rich BRRIDHAN29 will be released following their field trial and clearance by the biosafety enforcing agency in Bangladesh.

Prospect of developing submergence tolerance and Hopper resistant rice varieties: Xu et al. (2006) reported in rice three genes in addition to *Sub1A-1* and *Sub1A-2* that confer flood tolerance to deepwater rice varieties. Production of transgenics with submergence tolerant genes will contribute substantially toward attainment of food security in flood-prone countries.

Another development in the field of molecular breeding is the production of transgenic rice lines through insertion of *ASAL* gene initially cloned from garlic, *Allium sativum (*Saha et al. 2006). The transformants proved resistant against both Brown Plant hopper (BPH) and Green leafhopper (GLH). Furthermore, the GMO rice lines showed drastic reduction in the incidence of Tungro-virus. When released the resistant rice varieties will boost up considerably rice production in the Indian subcontinent where loss from BPH and GLH is immensely high.

A 20-year period data show no build-up of resistance against cotton bollworm: Tabashnik et al. (2003) of entomologists at the University of Arizona- and Cornell conducted a six-year study of *Bt* cotton crops covering > 62 million ha worldwide. Their objective was to monitor the development of resistance against cotton bollworm. Contrary to the expectation, pests did not evolve resistance to

Bt crops to a level that was expected following their exposure to *Bt* crops in the field. This is indeed a good indication for those countries such as Bangladesh where there is often a massive cotton crop failure due to bollworm attack (*Lepidopteran* insects). The field results, based on worldwide coverage would certainly encourage policy makers of the affected countries to take steps for growing transgenic *Bt* cotton to narrow the gap of trade deficit resulting from cotton import.

Allergen-free peanut in the pipeline: Kang and Gallo (2006) at the University of Florida, Gainesville have identified in peanut a new gene, *ara h 3-im* that codes for a protein with no apparent allergic effects. The three other genes designated *Ara h 1, Ara h 2* and *Ara h 3* cause allergy symptoms. It is well-known that peanut causes one of the most serious food allergies. From that point of view, it is a welcome breakthrough as this discovery will make it possible to produce GM nuts free from allergens, which affect millions of people and even cause death in extreme cases.

USAID-funded projects to motivate policy makers about the importance of GM crops and not to be swayed away by anti-GM activists: Although the GM crop supporters have been trying to allay the fears of anti-GM activists by NOT using the markers genes which they suspect to be toxic and harmful to human health, anti-GM crusade goes unabated. To counteract this unscientific propaganda of activists, the USAID in conjunction with Canadian Government have set up a number of Agencies such as ABSP II (Agricultural Biotechnology Support Project II) ISAAA (The International Service for the Acquisition of Agri-Biotech Applications (ISAAA), AGBIOS. They hold periodic workshops training Government field officials, policy makers, farmers and also publish Newsletters highlighting the achievements of GM crops.

ISAAA has three centers, Africenter in Nairobi (Kenya), Americenter at Cornell University and SEAsiaCenter in Los Baños, Philippines. One of the projects of ABSPII is helping resource-limited eggplant farmers in Bangladesh, India and the Philippines (estimated to be 7000,000) to grow fruit and shoot borer resistant (FSBR) transgenic lines containing the Bt Cry 1Ac gene, initially developed by Monsanto in collaboration with Maharashtra Hybrid Seed Company (Mahyco). The company has transferred the *Bt* gene into 12 standard varieties of eggplants and has signed an MoU to transfer technology that would provide capacity building and regulatory compliance support to public institutions in India, Bangladesh, and the Philippines.

PRSV resistant papaya line developed in jointly by Hawaiian and Cornell scientists: Another project of importance for Bangladesh is the introduction of Ring Spot Virus resistant (PRSV) papaya lines developed jointly by Professor Dennis Gonsalves at Cornell University and Professor Steve Ferreira at the University of

Hawaii (Gonsalves 1998, Gonslaves et al. 2004)). Under a five-year USAID funded project, the virus resistant papaya variety has been under field trials at various locations in Bangladesh.

Worldwide activity of the USAID agencies under MTA: Financed by USAID: virus resistant sweet potato, squash, cucumber, tropical pumpkin and melon have been produced in Egypt with collaboration of Cornell University. Seeds were distributed to Egypt, Jordan, South Africa, the Philippines, Indonesia and Brazil using simple licensing and Materials Transfer Agreement (MTA).

Liberal grants by multinational companies to laboratories to carry out field trials: Donor agencies are awarding grants to research institutes engaged in the field of GM crops to bridge the gap between laboratory results and running of field tests under strict supervision. This gesture by multinational companies has accelerated field testing of GM crops that require inspection under rigorous biosafety process.

One recent case in point is Monsanto's generous grant made available to the Donald Danforth Plant Science Center (http://www.danforthcenter.org/) (DC - a non-profit organization) to carry out field testing of virus resistant cassava varieties which they bioengineered in their African lab. This funding enabled them to do field testing in specially constructed greenhouses conforming to strict biosafety standards. Unfortunately, after seven years of trial, the DC scientists discovered that the bioengineered virus resistant cassava variety, lost resistance to the African cassava mosaic virus (CMVD). This is a clear example to show that there is no room for complacency. The effort should continue uninterrupted to evolve new virus resistant varieties of crops because at any time without warning the old recommended ones need to be replaced with the newly evolved resistant varieties. It is like coming up with new flu vaccines almost every year as the old ones lose their efficacy to fight the newly acquired virulence of the mutated strains.

Manufacture of oral vaccines is now a reality: There is an immense potential for the commercialization of oral plant vaccines to immunize resource-poor people in developing countries against hepatitis B, malaria, Norwalk disease causing diarrhea. One of the ways to commercialize such food products is through private and public partnership. Once the vaccines are delivered through food, it will come out excessively cheap compared to costly difficult-to-execute current vaccination program. Besides, such oral vaccines delivered through edible fruits would not require refrigeration as in the case of standard vaccines which lose efficacy in the absence of proper storage conditions. For instance, in rural Indian subcontinents, there are many villages without electricity and in those with power, the outage occurs frequently almost daily - a condition not conducive to keep the vaccines in their full potency.

The oral vaccine development by Dow Agro-Sciences: One of the recent exciting news published in the Phyto-Pharma Online Community (http://www.plantpharma.org) is about the oral vaccine development by Dow Agro-Sciences, a subsidiary of Dow Chemical. The USDA has given approval to the company to go ahead to commercialize and market a plant-made vaccine called, 'Concert'. This is an oral vaccine that will protect the poultry birds from the deadly avian flu and a human vaccine to prevent anthrax. This product is also under test for four more different vaccination projects against: West Nile Virus for horses, Avian Influenza, Bovine Pneumonia and K-9 Diabetes. The companies involved in the animal projects believe that soon some of these oral vaccines will prove effective to immunize humans.

Transgenic apples containing "reservertrol": Many consumer-oriented benefits such as improved nutritional and health-related benefits are likely to be more popular over the next ten to 20 years. One recent example is the field trial of "reservertrol"-containing popular apple varieties. The gene responsible for the synthesis of "reservertrol", a chemical well known for its strong anti-oxidant properties and its therapeutic use to prevent cardiac arrest, has been inserted in standard European apple varieties (Szankowski et al. 2003). Such an apple variety is soon expected to be available in the European market (oral communication, H. Jacobsen, Hanover University, Germany).

Labeling of GM food items: There is a lot of talk whether or nor all GMOs should be labeled. The criterion should be applied when there is a significant difference between a non-GM and a GM plant with regard to its toxicity, nutritional status and allergen content. Labeling would carry no meaning if GMOs are indistinguishable from non-GMOs with regard to their safety, apart from creating confusion among the consumers.

Lessons learnt from the deliberations of the last IAPTC&B Conference: The deliberations at the 11th IAPTC&B Congress held in Beijing, China (http://www.gnobb.org/11th_IAPTCB_congr_report_r.pdf) ended with a positive note that the fruits of biotechnological research would soon be available to public in the form of commercial production of oral vaccines, novel drugs and proteins to humans and animals as well as phyto-remediation to croplands for arsenic, other heavy metal clean-up.

Conclusions

The foregoing discussion clearly shows that molecular tools have given a new dimension to agricultural research. Programs tailored to the needs of a developing country and carried out under stringent biosafety laws will boost up its economy by adding extra income to farmers to improve their living standard, besides generating value-added products and cleaner environment through phyto-remediation and less use of pesticides.

One of the vehement arguments that Anti-GM activists put forward is that transgenics will turn into superweeds, little realizing that obnoxious and aggressive weeds may originate without the intervention of genetic manipulation. For instance, according to Global Invasive Species Database, there are 100 weedy species that have been described as the "World's Worst Invaders", and none of these weeds originated from the act of gene manipulation. The Lead Author is familiar with two weeds, *Chromolaena odorata* (L.) King & Robinson (= *Eupatoium odoratum*) and *Eichhornia crassipes* (Mart.) Solms, popularly known as water hyacinth. The first-mentioned species invade most of the vegetation in the wasteland, rendering those habitats unfit for cultivation. Water hyacinth has become a serious pest in waterways and paddy fields reducing drastically the grain yield. None of these two invasive weeds had its origin through genetic transformation.

In the resource-limited world, where there is not enough food for an estimated number of 740 million people, the scientific community responsible for producing value-added crops cannot sit idle without counteracting the unfounded accusations leveled against GM food. It is true that bioengineered crops alone cannot provide solution to banish hunger from the world, but they can provide a useful tool for addressing the many agricultural problems confronting Africa, Asia, Latin America, and other poor tropical regions. Therefore, it is no longer sufficient simply to do good science but also to embark upon a serious program for popularizing the new technology in every developing country where there is a chronic shortage of nutritious staple food such as wheat, rice, potato and corn. The objective of such a plan will be to bring home to policy makers that adequate funding is necessary for transforming lab results to the consumer level through large scale field tests – a prerequisite for the release of a high yielding, nutritious or a value-added GM crop for the countries where they are needed most to feed the ever growing population.

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