Review

Transgenic crops: Current challenges and future perspectives

Shaukat Ali[1]*, Yusuf Zafar[1] Zhang Xianyin[2], Ghulam Muhammad Ali[1] and Tu Jumin[2]

[1]National Institute for Genomics and Advanced Biotechnology, National Agricultural Research Centre, Islamabad, Pakistan.
[2]College of Agriculture and Biotechnology, Zhejiang University-310029, P.R., China.

The history of transgenic crops at present can be divided in two parts. The first era deals with the development of Genetically Modified (GM) crops. As the time went on, various social, political, environmental and technical issues related to transgenic crops took their birth. The development of transgenic crops has raised some issues more especially the problem of food and environmental safety, some technical impacts like effect on non target organisms, development of cross pest resistance, use of selectable marker genes, etc. There exists a thought that the pace of research in genetic engineering of crop plants may some day lead to the development of variations that will not ensure the survival of living creatures including human beings. Most of such concerns are just psychological and are often based on fear of negative political fall out or media coverage. The genetic engineering of crop plants is now moving towards the course of correction. It is the responsibility of concerned researchers to interpret such hazards and their solutions on technical basis and, therefore, establish a based line of acceptance for transgenic crops to the consumers.

Key words: Environment, biosafety, transgenic crops.

INTRODUCTION

Food crops that are being produced or modified by the genetic engineering techniques are known by various names in literature. Such names include genetically engineered plants, bio-engineered plants, genetically modified organisms (GMO's), genetically modified crops, or biotech plants (Liu, 1999). Many important crops such as wheat, rice, cotton, potato, canola, tobacco etc. are already becoming grown from seeds with well built in resistance to herbicides, viruses, insects disease sand improvement in nutritional quality. In addition to that, several genetically modified crop plants are expected to hit the world markets in the next few years (ISAAA, 2007-08).

Gene transfer technologies, no doubt, hold promise as a means to accelerate the genetic improvement of crop plants. With the advent of *Agrobacterium tumefaciens* mediated genetic transformation; the researchers introduced new genes into plants with more accuracy in their integration and expression and overcome the previous problems faced in these aspects by direct gene transfer methods. From 1983-1989, the recombinant DNA techniques became more sophisticated for plants. However, despite the immense efforts involved in the research field of biotech crops, there has existed a controversy since the early 1990s. Some people think that the pace of research in genetic engineering of crop plants may some day lead to the development of variations that will not ensure the survival of living creatures including human beings (Masci, 1997). The development of transgenic crops has especially raised some issues more especially the problem of environmental safety (Paoletti and Pimentel, 1996). The potential benefits of trasngenic technology for improving the reliability and quality of the world food supply have been contrasted with public/ researcher concerns raised about the food safety of the resulting products, some technical impacts like effect on non target organisms (losey et al., 1999), development of cross pest resistance (de Maagd et al., 1999), use of selectable marker genes etc. In this discussion, the various mechanisms will be discussed by which such hazards may arise during genetic engineering. Furthermore, it will be assessed whether these mechanisms are

---

*Corresponding author. E-mail: shaukat_parc@yahoo.co.in.

fundamentally different from those that may arise from the well established and accepted practice of traditional plant breeding/mutation breeding (Batista et al., 2008) and the existing use of new cultivars in agriculture and which strategies can/should be adopted to popularize these versatile crops (Morris and Spillane, 2008).

Risks associated with the appearance of toxins, allergens or genetic hazards in foods derived from genetically engineered crops may arise as a consequence of the biosynthesis of specific chemical constituents in the portion of the crop that is eaten. Alternatively, hazards may arise from the elimination of metabolites that play important roles in reducing health risks e.g. antioxidants. In general terms, the mechanisms by which food hazards may arise from genetic engineering of crops fall into two categories

Inserted transgenes and their expression products

The DNA of genes and their RNA expression products are composed of nucleic acids. Since the chemical components of nucleic acids are identical in all living organisms, the physical presence of the transferred genes and their RNA expression products do not cause any new health risks over existing foods. When the transferred genes are expressed in plant cells, it is the effects of the protein expression products that need to be considered. The direct protein expression products of transgenes transferred via genetic engineering are generally known and sensitive assays for their presence are usually available (Conner and Jacobs, 1999).

Therefore, the amount and stability of these proteins in edible components of plants, before and following harvest, storage and food processing, can be determined (Kok et al., 2007). Furthermore, since the protein expression products of transgenes are known, any potential toxic, immunological, allergenic or genetic hazards can be evaluated if health concerns are associated with the presence of the specific proteins in food sources.

Secondary and pleiotropic effects of gene expression

Many transgenes transferred by genetic engineering encode the production of enzymes that catalyse biochemical reactions. These enzymes are often expressed at a high level, which may lead to altered metabolic flowthrough in biochemical pathways. In turn, this may give rise to unanticipated increases or decreases in certain other biochemicals. The biosynthesis of enzymes from transgene expression may result in the depletion of the enzymatic substrate and the concurrent accumulation of the enzymatic product. An example of such effects in transgenic crops involves the transfer and expression of a nopaline synthase gene in asparagus.

The nopaline synthase enzyme catalyses a condensation reaction between arginine and a-keto glutarate to produce nopaline. In both callus tissue and shoot cultures of transgenic asparagus, the accumulation of the product nopa- line is accompanied by a decrease in the content of arginine (Conner et al., 1990). The potential for altering flow-through of metabolites in biochemical pathways is well illustrated by the over-expression of a yeast ornithine decarboxylwase gene in tobacco (Hamill et al., 1990), provitamin A in maize (Aluru et al., 2008). Some of the derived transgenic lines had a 10–20 fold increase in ornithinedecarboxylase activity with no change in the activity of other downstream enzymes in the pathway to nicotine. A two-fold increase in the accumulation of both putrescine and nicotine was observed, with no change in the content of polyamines or other intermediate substrates. The expression of a new enzymatic activity in plant cells may also result in the diversion of metabolites from one secondary metabolic pathway to another. To promote longer shelf life of tomatoes, ethylene biosynthesis was substantially reduced by diverting metabolism of the aminocyclopropane carboxylic acid intermediate away from ethylene biosynthesis by the transfer and expression of a bacterial aminocyclopropane carboxylic acid deaminase gene (Klee et al., 1990). Similarly, the transfer and expression of tryptophan decarboxylase from the medicinal plant *Catharanthus roseus* to canola plants resulted in an accumulation of tryptamine and a corresponding lower content of tryptophan-derived indole glucosinolates considered to be antinutritional factors in crucifer crops (Chadavej et al., 1994). The development of artificial metabolic sinks to redirect metabolites away from a specific secondary pathway is usually the desired metabolic effect being targeted. However, this may result in unpredictable effects in other intermediary metabolites and the diversion of metabolic flow to other secondary pathways. Dramatic pleiotropic effects of transgene expression can result from the expression of specific transacting factors which may result in the up-regulation of a whole biochemical pathway. For example, the transfer and expression of the *R* and *C1* regulatory genes from maize to several dicotyledonous species resulted in some tissues being highly anthocyanin pigmented due to the activation of specific genes in the anthocyanin biosynthetic pathway (Lloyd et al., 1992). The *R* gene also induced the development of trichomes hairs in *Arabidopsis* (Lloyd et al., 1992).

The possibility of secondary effects of transgene expression will depend on the key regulatory points and rate limiting steps in biochemical pathways. In general terms these are poorly understood in plants, and can be expected to vary between crops as well as between cultivars and breeding lines within the same crop species (Conner, 1993). In some instances the RNA or protein expression products of the transferred genes can influence the expression of existing genes in plants. Secondary effects of gene expression may occur in plant tissues beyond those in which the transgene is expressed. It is conceivable that metabolites accumulating as a consequence of transgene-derived biochemical activity in

one tissue are translocated within the plant to other tissues and organs such as the harvested food.

ASSESSMENT OF RISKS RELATIVE TO TRADITIONAL CROP BREEDING

As outlined above, several distinct mechanisms can potentially give rise to food hazards during the genetic engineering of crops. Are these mechanisms unique to genetic engineering? Can identical situations be identified within the bounds of traditional/mutation plant breeding? The current knowledge clearly points out that chances of having food hazards in GM or non GM are potentially at par (Kuiper et al., 2001; Morris and Sipllane, 2008). However, regulations are grossly imbalanced towards GM plants.

Inserted Genes and Their Expression Products

Genetic engineering allows the nature of the DNA intended for transfer to be controlled in a very precise manner and limited to the exact minimal segment of DNA capable of conferring the desired trait. This is in marked contrast to traditional breeding where undefined genes are routinely transferred between breeding lines, species and even genera. The parental lines used in traditional plant breeding are often assumed to have large regions of uncharacterized DNA transferred from wild species. Only a small proportion of this transferred DNA is expected to carry the desired characteristics such as resistance to pests and diseases, and the vast majority of the transferred DNA has been presumed to involve linked genes of unknown function. Theoretical analyses of 'near-isogenic' lines after six generations of backcrossing have calculated that the average size of introgressed regions ranges from 24–38 cM (centi Morgan) for chromosomes ranging in size from 50–200 cM (Stam and Zeven, 1981). Two well studied examples involve the introgression of resistance genes from *Lycopersicon perulianum* to tomato *Lycopersicon esculentum*. Genetic mapping studies involving molecular markers have determined that the introgressed chromosomal region around the Tm-2 locus, conferring resistance to tobacco mosaic virus, varied from 4 to 51 cM over a wide range of cultivars (Young and Tanksley, 1989). Likewise, the *Mi* locus conferring resistance to the nematode *Meloidogyne incognita*, which was transferred to cultivated tomato over 50 years ago, has been located to an introgressed chromosomal region of 2 to 8 cM in a series of tomato cultivars (Messeguer et al., 1991). Since the genome size of tomato is approximately 950 million base pairs/1C nucleus and the total map length is about 1300 cM (Tanksley et al., 1992), an average of 730 kb per cM can be calculated for the tomato genome. The chromosomal regions derived from *L. perulianum* can, therefore, be estimated to range from 1500–40,000 kb, which is up to several orders of magnitude larger than the 5 kb usually associated with cloned and sequenced resistance genes (Song et al., 1995). The recently developed techniques of GISH, genomic *in situ* hybridisation and FISH fluorescence in situ hybridisation offer powerful tools for analysing the extent to which plant breeders have introgressed 'foreign' DNA into crop plants. These technologies enable large chromosome segments originating from wild species to be clearly visualized as distinct regions on condensed chromosome preparations in a wide range of crops, including: wheat (Schwarzacher et al., 1992), barley (Pickering et al., 1997). One remarkable example in wheat involves the identification of introgressed chromosomal segments conferring resistance to five important diseases following hybridizations with plants from three other genera: *Secale* rye, *Thinopyrum*, and *AÍena* oat (Tang et al., 1997).

Secondary effects of gene expression

The concerns about secondary effects of gene expression are not confined to genetic engineering. They can also unwittingly occur in traditional and mutation breeding programmes by the complementation of genes from different parental lines (Baudo et al., 2006; Batista et al., 2008). Complementing genes may specify enzymes with different activities along biochemical pathways and provide opportunities for unintended increases in secondary compounds, especially when the parental lines contain wild species in their pedigrees. A classic illustration of secondary effects in traditional breeding programmes involves the development of novel fruit colors in tomato following introgression of genes from some accessions of wild *Lycopersicon* species. One specific example involves a substantial increase in the intensity of the red pigmentation of tomato fruit, attributed to the concentration of lycopene, following gene transfer from *Lycopersicon hirsutum*. This was unanticipated considering the fruit of *L. hirsutum* remains green, even when fully ripe, due to the lack of an active enzyme for the final step in the pathway to lycopene (Tanskley and McCouch, 1997). The wild tomato presumably contributed a gene that enhances earlier steps in the biosynthetic pathway towards lycopene, which results in greater metabolic flow-through to higher pigment production. In a similar context, advanced potato breeding lines with novel, toxic glycoalkaloids in their tubers have been produced when wild species exist in their pedigrees (Van Gelder and Scheffer, 1991).

The risk of secondary effects of gene expression products during the development of genetically engineered crops are clearly not new, since cultivars with genes transferred from wild species have been commercialized by plant breeders for many years. When a transgene is expressed in a crop plant, the biochemistry underlying the new character is better understood than for most genes utilized in traditional breeding programmes. This increases the opportunity to predict possible secondary effects,

which can be investigated if hazardous situations are envisaged. Thus present strategy of regulatory regime solely confined to GM crops is biased and not based on scientific principles

POTENTIAL HAZARDS ASSOCIATED WITH SOME COMMERCIALLY IMPORTANT TRANSGENES

Herbicide resistance transgenes

Herbicides are chemicals used to kills plants. In several crops, weeds are closely related to those crops, preventing the use of herbicides to control them, because such herbicide will also harm the crops. The harmful effects of herbicides are circumvented when herbicide tolerant crops are created so that they are unaffected by a specific herbicide. Such a job is accomplished through either the development of transgenic plants or through traditional methods. As opposed to pest resistant crops, there are some general allegations that herbicide resistance genes have not much commercial importance because they would maintain, if they do not promote, the use of herbicides and their associated problems (Goldburg, 1992). Herbicide resistance genes might be transferred by out-crossing into weeds (Dale et al., 2002), in this way minimizing the potential deployment of herbicide tolerant transgenic varieties. However on the other hand, some of the advantages of such transgenic crops can not be denied that herbicide resistance can have some environment benefits such as facilitating reduced tillage methods to conserve soil moisture, water (CTIC, 1998), and thus promoting the use of herbicides that have low environmental impacts such as glyophosate which is less toxic (US Environmental Protection Agency) and less likely to persist in the environment than the herbicides it has replaced (Nelsen and Bullock, 2003). Increased management flexibility that comes from a combination of the ease of use associated with broad-spectrum, post-emergent herbicides like glyophosate and the increased/longer time window for spraying. Benefits of GM herbicide tolerant crops to farmers has resulted in their increased acceptability and to date GM herbicide tolerant crops contribute maximum share (67%) in globally planted GM plants (ISAAA, 2007-08).

Insect resistance transgenes

Transgenic plants with high levels of insect resistance can produce a number of benefits such as elimination/reduced use of pesticides, and consequently reduced apprehensions of environment, water and food chain pollution caused by the wide spread use of insecticides. Bt. Toxins are of particular importance in this regard as they have very low mammalian toxicity and highly effective against major insect pests in crop plants.

However, the sustainability of resistance has been raised a problem as a result of increased resistance of transgenic plants. Pest insects have shown a remarkable capacity to develop resistance to chemical pesticides with over 500 species of insects now resistant to pesticides (Moberg, 1990). With the continuous and long term use of transgenic for insect resistance, the development of cross resistance is not far away. Some insects have developed cross resistance against toxins encoded by insect resistant transgenes (Ranjekar et al., 2003) under both field and laboratory conditions. To combat with this problem, various strategies have been proposed to prevent or at least delay the development of resistance to transgenic plants (Brousseau et al., 1999). The efficacy of these strategies is difficult to prove without large scale cultivation, but simulation modeling has been used extensively to predict the results (de Maggd et al., 1999). The plausible strategies are:

a. The use of multiple toxin genes with different modes of action has been suggested so that cross resistance is unlikely to occur. Two kinds of approaches can be designed based on this hypothesis. The first one will involve the combination of multiple genes having same origin e.g. two *Cry* genes with different receptors specificity. Using such ideology, Maqbool et al. (2001) observed that although there was not significant synergism between the two genes (*Cry1Ac and Cry2A*) because the efficacy of plants (100% mortality) against rice leaf folders and yellow stem borer harboring the both *Bt* genes together and singly was almost same. It means the same level of expression by pyramiding of genes can be obtained, but to supersede such resistance developed by the plant, the insect will require several mutations in different genes or will acquire these changes through sexual cycles that may take the most probably longer time than adaptation to single gene.

b. The second approach can be that two genes of different origins with various mode of action be introduced into the same plant. A notable combination of *Bt* gene and cowpea trypsin inhibitor (*CpTi*) has been attempted earlier (Hoffman et al., 1992; Santos et al., 1997; Li et al., 2002). Nevertheless, there is conflicting information as to the behavior of these genes when combined together as some previous using mixtures of these two compounds into artificial diets have reported some synergism and even no interaction (MacIntosh et al., 1990; Li et al., 2000; Wei et al., 2000; Qian, 2003). Later, another elucidation was that some internal plant factors may be important in such type of synergism. e.g. Tannins in cotton decreases this interaction, while tannic acid in the bark of *Taxus baccata* (L.) increases it (Gibson et al.,1995).

c. The third approach can be the use of tissue specific or inducible promoters to achieve spatial or temporal variation in the expression level of toxin (Datta et al., 1998; Husnain et al., 2002; Li et al., 2002). The use of tissue specific promoters would decrease the selection pressure by allowing pests to feed unharmed on economically less important parts of plant. The use of inducible promoters would decrease selection pressure
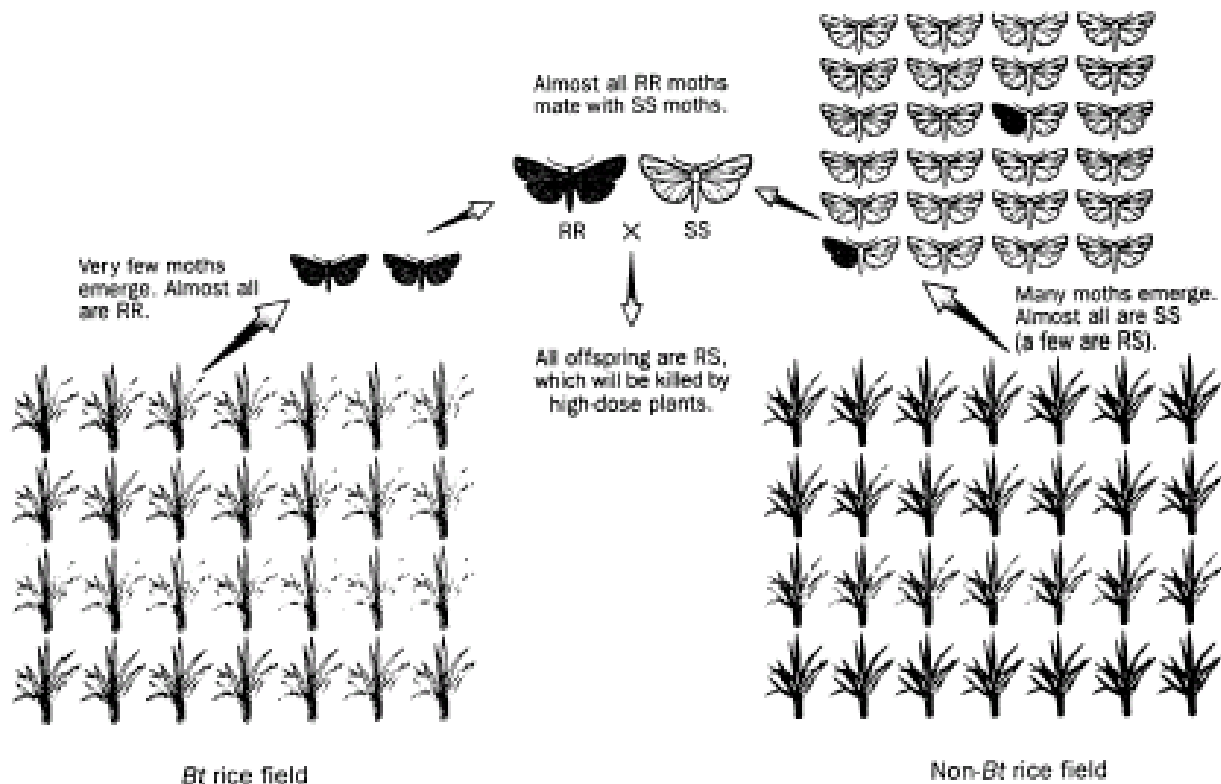
Figure 1. Mechanism of high-dose/refuge strategy to delay the increase in highly resistant (RR) insects in a pest population.

over time, as expression would only be induced when a certain economical threshold of damaged was crossed.

d. Use of temporal or spatial refuges as shown in Figure 1 (Rousch, 1997; Onstad and Gould, 1998) e.g. rotation of Bt-crops with non transgenic plants would also slow down the development of resistance, particularly if resistance is not stable in the insect population. With spatial refuges, part of field is set aside for non transgenic plants. This activity allows resistant insects that have survived on the transgenic plants to mate with the non-selected sensitive insects from the non transgenic plants and in this way, prevents the rise of a population that is homozygous for a recessive or semi-dominant resistance allele.

However in all the above cases, a thorough understanding of the biology of the crop-pest complex, the possible mechanism of resistance and the frequency of resistant alleles in the insect population would be necessary to devise an optimum and correct resistance management strategy.

USE OF SELECTABLE MARKER GENES

Plant transformation is based on the ability to integrate foreign DNA into host plant genomes and on the efficiency of regeneration of transformed cells usually into shoots or embryos. Presently, low transformation effi-

ciency of many crops necessitates the use of selectable markers to identify transgenic plants. These dominant genes confer resistance to an antibiotic or herbicide that kills non-transformed cells. Thus, single cells with an integrated transgene within a bulk of non-transformed cells can often be identified.

During recent years concerns were raised-mainly by the environmentalist and consumer organizations- that the existing of such genes as for antibiotic resistance within the environment or the food supply might be an unpredictable hazard to the ecosystem.

It has been argued that the use of antibiotics is the last line of defense against the treatment of diseases in human. So the wide spread use of antibiotic genes as selection genes may disrupt this defensive line. So consequently the antibiotic resistant microbes may result in the population and finally contribute to the growing public health problem of antibiotic resistance (Bettelheim, 1999; Hileman, 1999). Another discouraging comment came from Royal Society (1998) report. According to this: "*It is no longer acceptable to have antibiotic resistance genes present in new GM crops under development for potential use in food stuffs. It is important to encourage research into alternatives to antibiotic resistance genes. In particular, researchers in both academia and industry should not produce GM plants containing genes that confer resistance to those antibiotics that are used to treat infections in animals or humans.*"
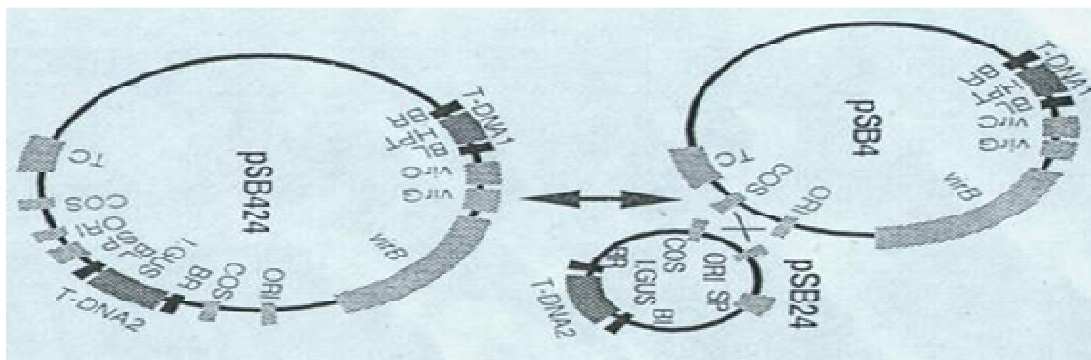
Figure 2. Co-integration vector system.

The absence of resistance genes in transgenic plants could lower the cost for developing and marketing of genetically modified products and might speed up the commercial release of new products (Kupier et al., 2001; Daniel, 2002; Smyth et al., 2002). Moreover, current transformation protocols severely limit the number of genes that can be introduced simultaneously. Therefore, re-transformation of a single line is a feasible and impor- tant approach towards selective introduction of multiple genes for complex traits such as pathogen resistance or tolerance to abiotic stress. Co-incorporation of different markers with each transgene or a set of transgenes increase safety concerns and it is expensive and time consuming. Tissue culture regimes for transformant selection would have to be optimized repeatedly, and the food safety and the environmental impact of different marker would have to be assessed on a case –by-case basis, particularly difficult for combination of resistance genes. Only a limited number of constitutive promoters are commonly used to express marker genes, and their repeated used could activate gene silencing mechanisms with negative effects on transgene expression.

Transgene elimination mechanisms would permit the re-cycling of a single marker by its removal after each transformation step. If a suitable technology becomes available in the foreseeable future, it is likely that regulatory legislation will strongly favor the absence of dispensable transgenic material in GM crops. The recent UK guidelines on "Best Practices for the Design of GM crops" recommends minimizing the foreign material in GM crops, and the European Council Directive 2001/18/EC on" the Deliberate Release into the Environ- ment of Genetically Modified Organisms" requests 'a phase out' of the use of antibiotic resistance markers that confer resistance to 'clinically' used antibiotics by 2005. Therefore, studies to avoid marker genes or eliminate them after use have been conducted and a growing num- ber of methods are under development for the elimination of these genes. The topic has increasing interest more recently (Putcha, 2000, 2003a, 2003b; Ebinuma et al., 2001; Hohn et al., 2001; Ow, 2001, 2002; Hare and Chua, 2002; Zuo et al., 2002).

In principle, there can be five ways to either avoid or get rid of 'problematic' selectable marker genes before transgenic are introduced into the field:

1. Totally avoiding the use of selectable marker genes. Theoretically, it should be possible among a large number of cells the ones that carry a transgene directly by molecular methods particularly, if the transformation efficiencies can be improved. A first report published recently indicates that such feasible techniques might be set up (Aziz and Machray, 2003).
2. Use of marker genes ('screenable markers') that have potentially no harmful biological activities. Non- toxic selective chemicals as opposed to antibiotics have been successful used e.g. phosphomannose isomerase genes (Negrotto et al., 2000), yeast 2-desoxyglucose-6-phosphate phosphatase (Kunze et al., 2001), bar gene (White et al., 1990) plant α-tubulin gene (Yemet et al., 2007) etc.
3. Co-transformation of two transgenes (Figure 2), one carrying the desired trait and the other the selection marker, followed by the segregation of the two (Komari et al.,1996)
4. Excision of selectable marker gene out of the integrated transgene after successful selection using site specific recombination or transposon (Olivier et al., 2002).
5. 5-Use of tissue specific promoters that are only active during a particular stage of development e.g. rice beta-glucanase promoter (Gns 9) that is active only during the 'callus phase' has been successfully employed in the development of marker free rice (Huang et al., 2001).

GRAIN QUALITY ALTERATIONS

There is a general pschycological effect on minds that the genes encoding toxins specially may have adverse effects on seed quality characteristics and consequently impose a hazard to human health. Foreign genes might

alter the nutritional level of foods in un-predictable ways by decreasing levels of some nutrients while increasing levels of others (Young and Lewis, 1995). So it may create a nutritional difference between the traditional strain and its transgenic counterpart. Critics consider that changes in food and diet occur at a pace far greater than the scientist's ability to predict the significance of the changes on pediatric nutrition and so the caution should be exercised regarding the use of GM food products in infant foods. In order to estimate the potential effect of transgene, characteristics related to grain quality was investigated (Wu et al., 2002). No significant difference was found between transgenic plants expressing a gene for insect resistance and control for milling quality, grain appearance and other physiochemical properties. An interesting finding was that of no Bt. insecticidal toxin found in cooked rice grains. The reasoning led to the idea that it may be de-natured at high temperature, and had become no longer a potential hazard for human health. However, further investigation is needed in this regard that whether the phenomenon of re-naturation exists or not after the heat treatment is passed. More recently, Bashir et al. (2004) conducted a comparison for physio-chemical parameters in transgenic Basmati rice. No significant difference was found for amylose content, alkali spreading value, between transgenic and non-transgenic group of plants

The development of transgenic crops using tissue specific promoter, represents an important advance in agricultural biotechnology and the development of transgenic crops that would be safe for consumers (Husnain et al., 2002; Li et al., 2002).

Despite the apparent risks associated with traditional plant breeding, the food products from new cultivars have been readily accepted as part of the human diet for many years. Similarly, new crops species have been 'domesti-cated' without any food safety assessment. A classic example is triticale, an artificial crop species developed from the inter-generic hybridisation of durum wheat and rye (Larter, 1976).

NON-TARGET RISKS

Non- target organisms are any species that are not the direct target of the transgenic crop e.g. Bt gene is targeted to control certain key pests. Any other species affected by *Bt* gene product other than target would be non target species, and consequently the list of potential non- target species by the critics is very long. These organisms can be grouped into five categories that are not mutually exclusive (Hilbeck et al., 2000).

1. Beneficial species including natural enemies of pests (e.g. lacewings, parasitic wasps etc.).
2. Non target pests
3. Soil micro-organisms
4. Biodiversity, which is the entire community of species

in a given region (Dale, 2001)
5. Species of conservation concern, including endan-gered species and popular, charismatic species (Monarch butterfly).

In fact, the first report about the effect of Bt. corn pollen on monarch butterfly really shocked the researchers about the future of transgenic crops (Losey et al., 1999). But it was a laboratory report. Later on, it was inves-tigated that the pollen concentration required to cause this type of effect in the fields is only likely to occur in a very small area of plants around the field perimeter (Dale, 2001). In a further extended study, it has been reported that Bt. protein is beneficial to Monarch butterfly instead of drastic effect and the earlier report about the effect of transgenic maize pollen on monarch was not represen-tative one (News and comments, 2001). Stanley-Horn et al. (2001) also conducted a study on if *Bt* imposed any effect on monarch. The results revealed that survivorship and weight gain were drastically reduced in non-Bt fields treated with lambda-cyhalothrin, an insecticide. The effects of Bt/ pollen on the survivorship of larvae feeding 14 to 22 days on milkweeds in fields were negligible. Similar non toxic results have been reported on the growth and development of earthworm and rats (Groot and Dicke, 2002; Wang et al., 2002). Betz et al. (2000) conclude the highly specificity of *Bt* protein in their studies as the exposed non-target organisms remained virtually un-affected. No morphological changes were observed in the mammalian hepatocytes at various concentrations of *Cry1Ab* (Shimada et al., 2003). It did not induce significant changes in the secretion of albumin even at a concentration of 2,000 ng/ml that is considered to be high enough to kill agronomically important insect pests (MacIntosh et al., 1990) shown by Figure 3. Individually, the *Bt* proteins deployed in transgenic crops show specific activity against narrow groups of pest species and usually have no direct effect on non-target species including beneficial insects (Qaim and Zilberman, 2003). Bashir et al. (2004, in their study of risk assess-ment in Basmati indica rice in Pakistan, concluded that *Bt* rice had not any effect on non-target insect pests. Apart from this, they also did not observe allelopathic effect of transgenic rice on the germination of following wheat crop. Further elucidative studies need to be formulated to have a conclusive idea regarding the impact of Bt. proteins on non-specific targets. Earlier studies have shown that larvae of the green lacewing predator *Chrysoperla carnea* are negatively affected when preying on lepidopteron larvae that had been fed with transgenic maize expressing the *Cry1Ab* gene from *Bacillus thuringiensis*. It has been reported too recently (Romeis et al., 2004) that *B. thuringiensis* toxin (*Cry1Ab*) has no direct effect on larvae of the green lacewing *C. carnea* (Stephens) (*Neuroptera: Chrysopidae*) even when the amount of toxin ingested by first instar *C. carnea* in the present study was found to be a factor of 10,000
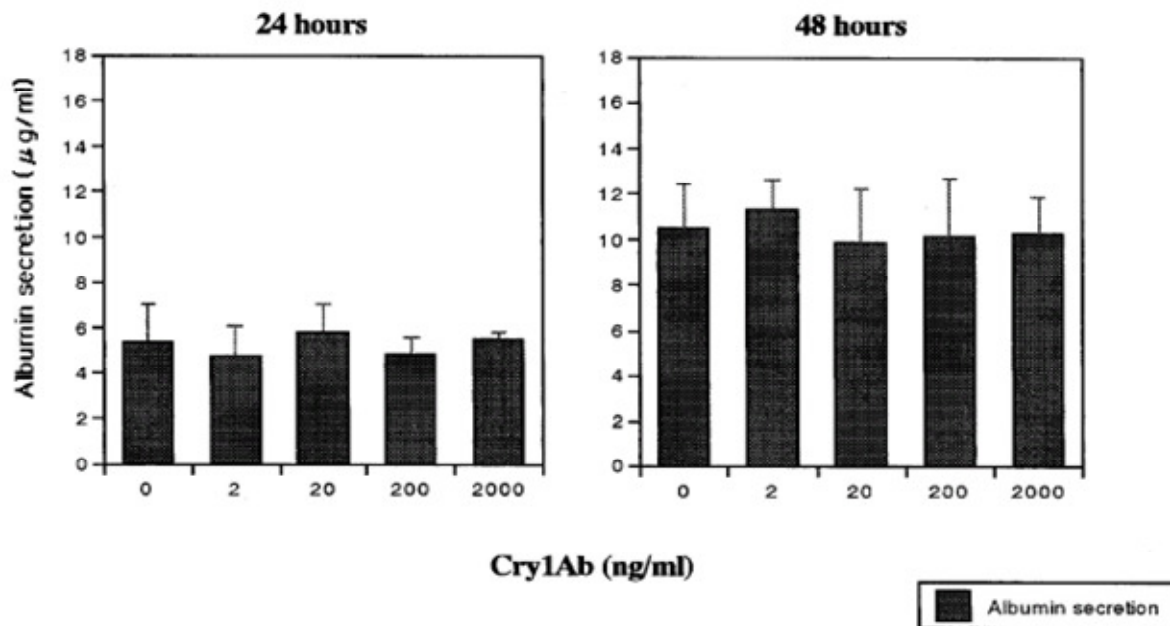
Figure 3. Effect of Cry1Ab at various concentrations on albumin secretion that was harvested after 24 and 48 h.

higher than the concentration ingested when feeding on *Bt*-reared lepidopteron larvae.

REFERENCES

Aluru M, Xu Y, Guo R, Wang Z, Li S, White W, Wang K, Rodermel S (2008). Generation of transgenic maize with enhanced provitamin A content. J. Exp. Bot. 8: 1-12.

Aziz N, Machray GC (2003). Efficient male germ line transformation for transgenic tobacco production without selection. Plant Mol. Biol. 23: 203-211.

Batista R, Saibo N, Lourenco T, Oliveira MM (2008). Micro array analyses reveal that plant mutagenesis may induce more transcript-tomic changes than transgene insertion. Proc. Acad. Nat. Sci. USA 105: 3640-3645.

Baudo MM, Lyons R, Powers S, Pastori GM, Edwards KJ, Holdsworth MJ, Shewry PR (2006). Transgene has less impact on transcriptomic of wheat grain than conventional breeding. Plant Biotechnol. J. 4: 369-380.

Bettelheim A (1999). Drug resistant bacteria: Can scientists find a way to control superbugs? CQ Researcher 9: 473-96.

Betz FS, Hammound BG, Fuchs RL (2000). Safety and benefits of *Bacillus thuringiensis* protected plants to control insect pests. Reg. Toxicol. Pharamacol. 32: 156-173.

Brousseau R, Masson L, Hegealus D (1999). Insecticidal Transgenic Plants: Are they irresistible. Agric. Biotechnol. Net. 1. ABN 022.

Bashir K, Husnain T, Fatima T, Latif Z, Mehdi SA, Riazuddin S (2004). Field evaluation and risk assessment of transgenic indica basmati rice. Mol. Breed. 13: 301-312.

Chadavej S, Brisson N, McNeil JN, De Luca V (1994). Redirection of tryptophan leads to production of low indole glucosinolate canola, Proc. Natl. Acad. Sci. USA 91: 2166-2170.

Conner AJ, Jacobs JME (1999). Genetic engineering of crop plants as a potential source of genetic hazard in human diet. Mutat. Res. 443: 223-237.

Conner AJ (1993). Food safety issues relating to genetic engineering of crop plants, Agric. Sci. 6: 36-41.

Conner AJ, Bezar HJ, Ashby JW (1990). Genetic engineering of plants for weed, disease and pest control: Science vs. politics?, Proceedings of the New Zealand Weed and Pest Control Conference 43: 200-208.

CTIC (1998). Crop residue management survey. Conservation Technology Information Centre, Indiana.

Dale PJ (2001). Environment impact of Biotech Crops. J. Anim. Sci. 79: E144-E147.

Dale PJ, Clarke B, Fontes EMG (2002). Potential for the environmental impact of transgenic crops. Nat. Biotechnol. 20: 567-574.

Daniel H (2002). Molecular strategies for gene containment in transgenic crops. Nat. Biotechnol. 20: 581-586.

Datta K, Vasquez A, Tu J, Torrizo L, Alam MF, Oliva N, Abrigo E, Khusk GS, Datta SK (1998). Constitutive and tissue specific differential expression of the *Cry1Ab* gene in the transgenic rice plants conferring resistance to rice insect pest. Theor. Appl. Genet. 27: 20-30.

de Maagd RA, Bosch D, Stiekema W (1999). *Bacillus thuringiensis* toxin–mediated insect resistance in plants. Trends Plant Sci. 4(1): 9-13.

Ebinuma H, Sugita E, Matsunaga E, Endo S, Yamada K, Komamine A (2001). Systems for the removal of a selection marker and their combination with a positive marker. Plant Cell Rep. 20: 383-392.

Gibson DM, Gallo LG, Krasnoff SB, Ketchum REB (1995). Increased efficacy of *Bacillus thuringiensis sub sp. Kurstaki* in combination with tannic acid. J. Econ. Entomol. 88: 270-277.

Goldburg RJ (1992). Environmental concerns with development of herbicide resistant plants. Weed Technol. 6: 647-652.

Groot AT, Dicke M (2002). Insect-resistant transgenic plants in a multi-trophic context. Plant J. 31: 387-406.

Hamill JD, Robins RJ, Parr AJ, Evans DM, Furze JM, Rhodes MJC (1990). Over-expressing a yeast ornithine decarboxylase gene in transgenic roots of *Nicotiana rustica* can lead to enhanced nicotine accumulation. Plant Mol. Biol. 15: 27-38.

Hare P, Chua NH (2002). Eviction of selectable marker genes from transgenic plants. Nat. Biotechnol. 20: 575-580.

Hilbeck A, Meier MS, Rapes A (2000). Review on non- target organisms and Bt plants. EcoStrat GmbH, Zurich, Switzerland.

Hileman B (1999). UK moratorium on biotech Crops. Chemical and Eng News. May 24, p. 7.

Hoffman MP, Zalom FG, Wilson LT, Smilanick JM, Malyj LD, Kiser J,

Hilder VA, Barnes W (1992). Field evaluation of transgenic tobacco containing genes encoding Bacillus thuringiensis d-endotoxin or cowpea trypsin inhibitor efficacy against *Helicoverpa zea*. J. Econ. Entomol. 85: 2516-2522.

Hohn B, Levy AA, Puchta H (2001). Elimination of selection markers from transgenic plants. Curr. Opin. Biotechnol. 12: 139-143.

Huang N, Wu L, Nandi S, Bowman E, Huang J, Sutliff T, Rodriguez RL (2001). The tissue specific activity of a rice beta-glucanase promoter (*Gns9*) is used to select rice transformants. Plant Sci. 161: 589-595.

Husnain T, Asad J, Maqbool SB, Datta SK, Riazuddin S (2002). Variability in expression of insecticidal *Cry1Ab* gene in indica Basmati rice. Euphytica 128: 121-128.

ISAAA (2007-08). Briefs. International Service for the Acquisition of Agri-biotech Applications.

Kok EJ, Keijer J, Kleter GA, Kuiper HA (2007). Comparative safety assessment of plant-derived foods. Reg. Toxicol. Pharmacol. 50: 98-113.

Komari K, Hei Y, Saito Y, Murai N, Kumashiro T (1996). Vectors carrying two separate T-DNA for co-transformation of higher plants mediated by *Agrobacterium tumefaciens* and segregation of transformants free from selection markers. Plant J.10: 165-174.

Kuiper HA, Kleter GA, Noteborn HPJM, Kok EJ (2001). Assessment of the food safety issues related to genetically modified foods. Plant J. 27: 503-528.

Kunze I, Ebneth M, Heim U, Geiger M, Sonnewald U, Herbers K (2001). 2-Deoxyglucose resistance: a novel selection marker for plant transformation. Mol. Breed. 7: 221-227.

Larter EN (1976). Triticale, in: Simmonds NW Ed., Evolution of crop plants, Longman Group, London, pp. 117-120.

Li FG, Cui JJ, Liu CL, Wu ZX, Li FL, Zhou Y, Li XL (2000). The study of insect-resistant transgenic cotton harboring double-gene and its insect-resistance. Sci. Agric. Sinic. pp. 3346-3352.

Li YC, Zhang XY, Xue QZ (2002). Obtaining a large number of *Agrobacterium*- transformed rice plants harboring two insecticidal genes. J. Agric. Biotechnol. 10: 60-63.

Liu K (1999). Biotech crops: products, properties and prospects. Food Technol. 53: 42-49.

Lloyd AM, Walbot V, Davis RW (1992). *Arabidopsis* and *Nicotiana* anthocyanin production activated by maize regulators R and C1. Science 258: 1773-1775.

Losey JK, Rayor LS, Carter ME (1999). Transgenic pollen harms monarch larvae. Nature 339: 214.

MacIntosh SC, Kishore GM, Perlak FJ, Marrone PG, Stone TB, Sims SR, Fuchs RL (1990). Potentiation of *Bacillus thuringiensis* insecticidal activity by serine protease inhibitors. J. Agric. Food Chem. 38: 1145-1152.

MacIntosh SC, Stone TB, Sims SR, Hunst PL, Greenplate JT, Marrone PG, Perlak FJ, Fischhoff DA, Fuchs RL (1990). Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invertebr. Pathol. 56: 258-266.

Maqbool SB, Riazuddin S, Thi Loc N, Angharad MR, Gatehouse JA, Christou P (2001). Expression of multiple insecticidal genes confers broad resistance against a range of different rice pests. Mol. ND, Bolkan H, Tanksley SD (1991). High resolution RFLP map around the root knot nematode resistance gene *Mi* in tomato, Theor. Appl. Genet. 82: 529-536.

Moberg WK (1990). Understanding and combating agrochemical resistance. In: Gree HM., Moberg WK. (Eds), Managing Resistance to Agrochemicals. ACS Symposium Series, Washington, pp. 3-16.

Morris SH, Sipllane E (2008). GM directive deficiencies in the European Union. EMBO Rep. 9(6): 1-5.

Negrotto D, Jolley M, Beer S, Wenck AR, Hansen G (2000). The use of phosphomannose-isomerase as a selectable marker to recover transgenic maize plants (*Zea mays* L.) via *Agrobacterium* transformation. Plant Cell Rep. 19: 798-803.

Nelsen GC, Bullock DS (2003). Simulating a relative environmental effect of glyophosate resistant soybeans. Ecol. Econ. 45: 189-200.

News and comments (2001). Trends in Genetics 19: 41.

Olivier C, Sallaud C, Bretlier JC, Meynard D, Greco R, Pereira A, Guiderdddoni E (2002). Transposan –mediated generation of T-DNA and marker free rice plants expressing a *Bt* endotoxin gene. Mol. Breed. 10: 165-180.

Onstad DW, Gould F (1998). Modeling the dynamics of adaptation to transgenic maize by European corn borer. J. Econ. Entomol. 91: 585-593.

Ow DW (2001). The right chemistry for marker gene removal? Nat. Biotechnol. 19: 115-116.

Ow DW (2002). Recombinase-directed plant transformation for the post-genomic era. Plant Mol. Biol. 48: 183-200.

Paoletti MG, Pimentel D (1996). Genetic Engineering in agriculture and environment assessing risks and benefits. Biol Sci. 46: 665-673.

Pickering RA, Hill AM, Kynast RG (1997). Characterization by RFLP analysis and genomic in situ hybridization of a recombinant and a monosomic substitution plant derived from *Hordeum Íulgare* L.=*Hordeum bulbosum* L. crosses. Genome 40: 195-200.

Putcha H (2000). Removing selectable marker genes: taking the shortcut. Trends Plant Sci. 5: 273-274.

Putcha H (2003a). Towards the ideal genetically modified plant: Homologous recombination and marker gene excision. J. Plant Physiol. 12: 743-754.

Putcha H (2003b). Marker- free transgenic plants. Plant Cell Tiss. Org. Cult. 74: 123-134.

Qaim M, Zilberman D (2003). Yield effects of genetically modified crops in developing countries. Science 299: 900-902.

Qian HF (2003). The comparison of leaf folder resistance transgenic rice harboring *CpTi* and *Cry1Ac.* Ph.D. Dissertation, Zhejiang University, P.R. China.

Romeis J, Dutton A, Bigler F (2004). *Bacillus thuringiensis* toxin (*Cry1Ab*) has no direct effect on larvae of the green lacewing *Chrysoperla carnea* (Stephens) (*Neuroptera: Chrysopidae*). J. Insect Physiol. 50: 175-183.

Rousch RT (1997). *Bt* transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? Pest. Sci. 51: 328-334.

Royal Society (1998). Report on genetically modified plants for food use. Accessed May 8, 2001.

Santos MO, Adang MJ, All JN, Boerma HR, Parrott WA (1997). Testing transgenes for insect resistance using *Arabidopsis.* Mol. Breed. 3: 183-194.

Schwarzacher T, Anamthawat JK, Harrison GE, Ilam AKMR, Jia JZ, King IP, Leitch AR, Miller TE, Reader SM, Rogers WJ, Shi M, Heslop HJS (1992). Genomic in situ hybridization to identify alien Chromosomes and chromosome segments in wheat. Theor. Appl. Genet. 84: 778-786. Shimada N, Kim YS, Miyamoto K, Yoshioka M, Murata H (2003). Effect of *Bacillus thuringiensis Cry1Ab* protein on mammalian cells. J. Vet. Med. Sci. 65: 187-191.

Stam P, Zeven AC (1981). The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing, Euphytica 30: 227-238.

Stanley-Horn DE, Dively GP, Hellmich RL, Mattila HR, Sears MK, Rose R, Jesse LC, Losey JE, Obrycki JJ, Lewis L (2001). Assessing the impact of *Cry1Ab*-expressing corn pollen on monarch butterfly larvae in field studies. Proc. Natl. Acad. Sci. USA 98: 11931-11936.

Smyth S, Khachatourians GG, Phillips PW (2002). Liabilities and economics of transgenic crops. Nat. Biotechnol. 20: 537-541.

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995). A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 270: 1804-1806.

Tang S, Zhuang J, Wen Y, Ai SA., Li H, Xu J (1997). Identification of introgressed segments conferring disease resistance in a tetra-generic hybrid of *Triticum, Secale, Thinopyrum,* and *Alena*. Genome 40: 99-103.

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992). High density molecular linkage maps of the tomato and potato genomes. Genetics 132: 1141-1160.

Tanksley SD, McCouch SR (1997). Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277: 1063-1066.

Van Gelder WMJ, Scheffer JJC (1991). Transmission of steroidal glycoalkaloids from *Solanum Íernei* to the cultivated potato. Phytochemistry 30: 165-168.

Wang ZH, Wang Y, Cui HR, Xia YW, Altosaar I, Shu QY (2002). Toxi-

cological evaluation of transgenic rice flour with a synthetic *cry1Ab* gene from *Bacillus thuringiensis.* J. Sci. Food. Agric. 82: 738-744.

Wei JW, Xu XP, Chen JT, Zhang LY, Fan YL, Li BJ (2000). Research on improving rice resistance to the pest by *Bt* and *Sbti* genes. Chin. J. Biotechnol. 16: 603-608.

White J, Chang SY, Bibb MJ, Bibb MJ (1990). A cassette containing the bar gene of *treptomyces hygroscopicus*: a selectable marker for plant transformation. Nucleic Acid Res. 18(4): 1062.

Wu DX, Shu QY, Wang ZH, Cui R, Xia YW (2002). Quality variation in transgenic rice with a synthetic cry1Ab gene *Bacillus thuringiensis*. Plant Breed. 121: 198-202.

Yemet A, Radchuk V, Bayer O, Bayer G, Pakhomov A, Baird WV, Blume B (2007). Development of transformation vectors based upon a modified plant α-tubulin gene as the selectable marker. Cell Biol. Int. 7: 1-5.

Young AL, Lewis GC (1995). Biotechnology and potential nutritional implications for children. Pediatre Clin. North Am. 42: 917-30.

Young ND, Tanksley SD (1989). RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding, Theor. Appl. Genet. 77: 353-359.

Zuo J, Niu QW, Ikeda Y, Chua NH (2002). Marker-free transformation: increasing transformation frequency by the use of regeneration-promoting genes. Curr. Opin. Biotechnol. 13: 173-180.