In Vitro Cell. Dev. Biol.—Plant 38:106–115, March–April 2002
DOI: 10.1079/IVP2001268

1054-5476/02 $10.00+0.00

DEVELOPMENT AND DEPLOYMENT OF TRANSGENIC PLANTS: BIOSAFETY CONSIDERATIONS

KIRAN K. SHARMA*, H. C. SHARMA, N. SEETHARAMA, AND RODOMIRO ORTIZ

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India

(Received 9 May 2001; accepted 13 September 2001; editor T. A. Thorpe)

Summary

Recombinant DNA technology has great potential to enhance and extend the advantages of conventional plant breeding, and increase the production and productivity of crops to meet the increasing demand for food and food products in the future. Judicious application of this technology provides opportunities for alleviating some of the major constraints to crop productivity under subsistence farming conditions in the developing countries. Considerable progress has been made in developing strategies for the production and deployment of transgenic crops. However, biosafety concerns have been raised regarding the deployment and release of genetically engineered plants. This debate has divided the farming and consumer communities over acceptability of genetically modified foods. There is a need for a thorough investigation regarding the fate of transgenic plants in the environment, and their interaction with wild relatives and non-target organisms. The production and release of transgenic plants should be based on experience and sound scientific reasoning. The regulatory requirements for deployment of transgenic crops should be streamlined and harmonized, in order to achieve sustainable food production, poverty reduction, and environmental protection in resource-poor countries in the semi-arid tropics.

Key words: biosafety; environmental protection; genetic transformation; recombinant DNA; transgenic plants.

Introduction

Impressive gains in crop productivity were realized in the 20th century through conventional plant breeding, combined with improved agricultural practices (Borlaug, 1983). However, with ever-increasing human and livestock population pressure, conventional plant breeding is constrained either due to the limited gene pool or because of the restricted range of organisms between which genes can be transferred due to interspecific barriers. There is limited scope for increasing the amount of land available for agriculture without having a serious impact on the environment. New biotechniques, in addition to conventional plant breeding, are needed to boost yields of crops that feed the world (Borlaug, 1997). Genetic transformation provides a complementary means for the betterment of field crops. Application of biotechnological tools holds great promise for alleviating some of the major constraints to crop productivity in developing countries (Sharma and Ortiz, 2000). Research on transgenic crops, as is the case with conventional plant breeding, aims to alter selectively by adding or removing a character of choice in a crop plant, bearing in mind the regional needs and opportunities. However, the promise of biotechnology for increasing the production and productivity of crops for sustainability has been dimmed by the perceived safety of the transgenic organisms (Williamson et al., 1990). There is also a concern regarding evolution of resistant strains of insects (Miller and Flamm, 1993). In developed countries, social and environmental groups have raised concerns about the real or conjectural effects on non-target organisms, while in developing countries, the caution has given rise to fear because of lack of information. In response to these concerns, a biosafety working group has been formed by the Food and Agricultural Organization (FAO), the United Nations Environment Program (UNEP), the United Nations Industrial Development Organization (UNIDO), and the World Health Organization (WHO); and guidelines for handling and release of genetically modified organisms (GMOs) have been published (Tzotzos, 1995). In developed countries, biotechnology is seen to be of strategic importance for increasing the share of world market. However, there are serious concerns about the introduction of this technology in developing countries. Investment in research is linked to innovation, timely product development, and commercialization. Delays and controls arising because of biosafety concerns may become a major disincentive for investment in biotechnology.

GMOs have a better predictability of gene expression than conventional breeding methods, and transgenes are not conceptually different from the use of native genes or organisms modified by conventional technologies. The focus of biosafety regulations needs to be on safety, quality, and efficacy (Levin, 1988; Wyngaarden, 1990). The need and extent of safety evaluation may be based on the comparison of the new food and the analogous food, if any. Further, the interaction of the transgene with the environment needs to be investigated. The potential of recombinant technologies that allow a greater modification than is possible with the conventional technologies has a greater bearing on the environment. In several developing countries, there is no system in place to regulate the production and use of GMOs. The management, interpretation, and utilization of information will be an important component of risk assessment, and will determine the effectiveness and reliability of

*Author to whom correspondence should be addressed: Email k.sharma@cgiar.org

this technology. This needs to be transparent with standardized databases and protocols.

Recent Developments in Transgenic Research

Significant progress has been made over the past decades in introducing foreign genes into plants, and has provided opportunities to modify crops to increase yields, impart resistance to biotic and abiotic stresses, and improve nutritional quality. Several procedures have been successfully employed to insert foreign genes into crop plants (Potrykus, 1990; Hooykaas and Schilperoort, 1992; Kung and Wu, 1993; Zupan and Zambryski, 1995; Sharma and Ortiz, 2000). Although transgenic approaches have considerably broadened the range of gene pools, which are now accessible for crop improvement purposes (Flavell, 1999), there are several problems in the development of plant transformation systems that need to be solved. The key issues to be resolved in the practical application of these systems, and strategies by which these limitations may be overcome, have been discussed extensively by Birch (1997). The application of useful gene transfer from microorganisms through genetic engineering techniques ranges from introduction of vaccine antigen genes (Mason et al., 1996; Arakawa et al., 1998) to aluminum tolerance genes (de la Fuente et al., 1997) into food plants. Isolated plant genes such as those conferring resistance against insect pests and pathogens can now be transferred between sexually incompatible species (Whitham et al., 1996; Molvig et al., 1997; Wilkinson et al., 1997).

Despite significant advances over the past decade, the development of efficient transformation methods to introduce foreign DNA can be a substantial barrier to the application of recombinant methods in some crop plants (Sharma and Ortiz, 2000). For genetic transformation to be successful for routine generation of transgenic plants, several key factors play an important role. These include the development of reliable tissue culture and plant regeneration systems, preparation of gene constructs and transformation with suitable vectors, efficient techniques of transformation for the introduction of genes into the crop plants, recovery and multiplication of transgenic plants, molecular and genetic characterization of transgenic plants for stable and efficient gene expression, transfer of genes into elite cultivars by conventional plant breeding methods if required, and evaluation of transgenic plants for their effectiveness in alleviating biotic and abiotic stresses without causing environmental risks. The flow diagram presented in Fig. 1 describes the integration of transgenic technology with conventional plant breeding to accomplish transgenic genetic enhancement in crop plants. While traditional plant breeding is generally considered safe, the inclusion of transgenes needs to be assessed for its biosafety. The various components of biosafety and means to accomplish this in an environment friendly manner are discussed in the following sections.

Application of Transgenic Technology for Crop Improvement in Developing Countries

Demand for food is influenced by a number of factors, including population growth and movement, income levels and economic growth, human resource development, lifestyles, and food preferences. Almost 80 million people are likely to be added to the world's population each year over the next quarter-century,

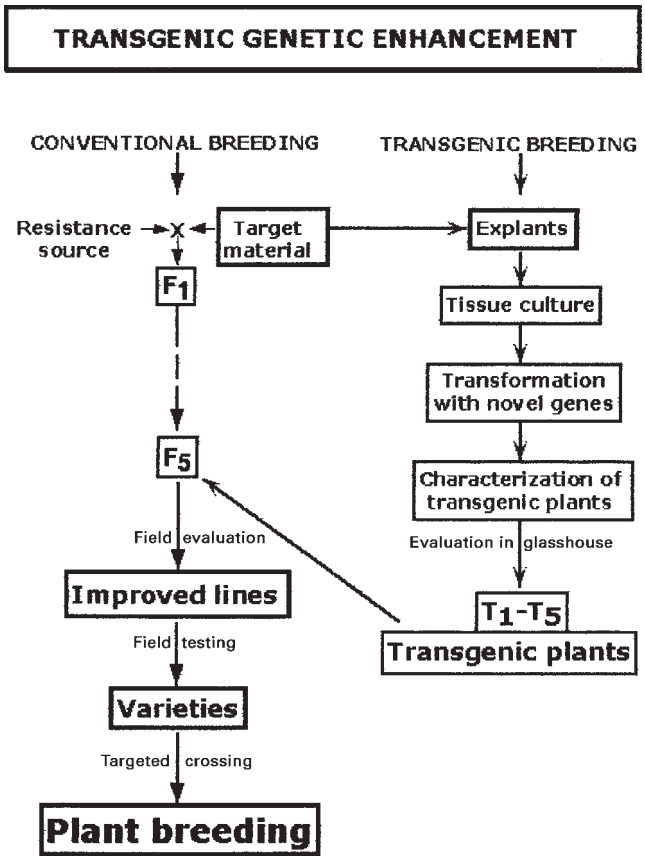

Fig. 1. Flow diagram showing the integration of transformation technology with conventional plant breeding.

increasing the world population by 35% from 5.69 billion in 1995 to 7.67 billion by 2020 (UN, 1996). Most of this increase will occur in developing countries, and there will be many more mouths to feed under complex social and economical circumstances. Agricultural transformation will be essential to meet the global challenges for reducing poverty, feeding the world's burgeoning population, and protecting the environment (Serageldin, 2000). Nobel laureate Norman Borlaug, father of the Green Revolution, has indicated that 'to meet projected food demands by 2025 the average yield of all cereals must be 80 percent higher than the average yield in 1990' (Borlaug, 1997). These increases must come primarily from increasing biological yield, and not from area expansion and more irrigation. Over-consumption and waste in rich countries and population pressure in poor countries have already placed a dangerous burden on the ecosystems on which we all depend. It is estimated that some 40 000 people die every day worldwide from hunger-related causes (Serageldin, 2000).

Resource-poor farmers are unlikely to have easy access to finance and agricultural inputs such as pesticides, fertilizers or irrigation. The low productivity under subsistence farming tends to perpetuate rural poverty to the extent that approximately 1 billion people live below the poverty line in developing countries; this includes 633 million in Asia, 204 million in Africa, 27 million in the Near East and North Africa, and 76 million in Latin America (Jazairy et al., 1992). However, the small-scale resource-poor farming sector is

responsible for 80% of agricultural production in developing countries and therefore is the key to future food security. There are many socioeconomic factors underlying rural poverty, such as lack of access to land and other resources, low purchasing power, political powerlessness, fragile environments, and peripherality from markets (Spillane, 2000). Research in agricultural biotechnology could have a major impact on rural poverty. Over the long term, there is little doubt that some biotechnological approaches to improvements in agriculture will generate social, economic, and environmental benefits targeted at specific needs, especially those of poorer groups (Spillane, 2000). The revolution in the biological sciences such as molecular genetics, informatics, genomics, and transgenics has opened up a host of possibilities. The promise of biotechnology as an instrument of development lies in its capacity to improve the quantity and quality of plants quickly and effectively. In the past few years, there have been continuing increases in the area planted to transgenic crops. In 1998, the global area planted to transgenic crops more than doubled over that of 1997. However, the revolution in the biological sciences has both promise and problems. Human beings are confronted by profound ethical and safety issues, complicated by the issues of proprietary science. Many protests have been made by civil society institutions on ethical or ecological grounds. These concerns cannot and must not be ignored.

According to the Consultative Group on Agricultural Research (CGIAR), the critical issue is that every instrument of agricultural transformation should be mobilized in our efforts to feed the hungry, help the poor, and protect the environment (Serageldin, 2000). The ethical dimension of depriving the poor and hungry of the advantages that biotechnology with adequate safeguards can bring must be weighed against the economic returns. Both sets of issues need to be confronted boldly by finding ways of realizing the promise of biotechnology while avoiding the possible pitfalls. If agricultural biotechnology is to help address problems of rural poverty and malnutrition, it will have to intentionally shift its focus from crops that feed chickens, to the staple crops that feed poorer people; from meeting the needs of large, low-employment farms to the needs of smallholders and farm laborers (Lipton, 1999). Hence, it will be necessary for relevant public sector institutions to clearly identify which of the farmers' or consumers' needs are of concern to their research or funding agenda. Poor people should be included directly in the debate and decision-making about the technological change, the possible risks of the change, and the consequences of no change or the available alternatives.

Biosafety Considerations

For genetically improved organisms, the risks classified as inherent in the technology are frequently summarized as biosafety risks. Biosafety assessment requires that risks, benefits, and needs be given a balanced assessment in relation to the transgenic organisms. In 1998, over 40 million acres of transgenic crops were grown around the globe. As these products are traded and pass from one country to another, it is important to ensure that domestic regulatory regimes are in place to ensure the safe use of these products. Currently, the biosafety regulations do exist in several countries, but there is a need to make them consistent across the globe. The primary principle of biological safety (i.e., biosafety) is containment. The term containment refers to a series of safe methods for managing infectious agents in the laboratory. The purpose of containment is to reduce or eliminate human and environmental exposure to potentially harmful or undesired agents. Much controversy and public scaremongering has been generated by the anti-biotechnology lobby over the safety of transgenic plants in relation to their perceived negative impact on human health or the environment.

Various approaches addressing the risks are concerned with establishing good standards of laboratory practice, efficiency and security of the containment facilities, and effects of modified organisms on human health and the environment (Levin and Strauss, 1993). The risk is assessed in the form of access: as a measure of the probability that a modified organism (or the DNA inserted in it) will be able to enter the human body and survive there; and the anticipated or known level of expression of the inserted DNA. Risk also measures damage in the form of harm likely to be caused to a person by exposure to the modified organism.

Environmental hazards posed by a modified organism may include tendency of a self-pollinated line to outcross because of self-incompatibility or any other factors. A plant may have a tendency to become a weed, produce toxic substances in the product or there may be changes in the toxins produced by the plant. Any of these attributes may pose a risk to people consuming the product, working directly with it or to the environment. In the case of pesticidal toxin genes inserted into the plants, the range of the expressed toxin may be much wider than expected, with adverse consequences for the environment. Plants may also display a change in morphology, reaction to other biotic and abiotic stress factors, or the end-use characteristics. However, it is not easy to measure weediness, as such characteristics are not easily defined. The ecological consequences in most cases are only qualitative. Therefore, risk assessment for genetically modified plants requires a detailed assessment of the modified plant in comparison to the plant from which it has been derived. The assessment should include complete information about the donor and the receiving species. The receiving plant species forms the baseline with which the transgenic plant should be compared. Information is also needed about the gene donor species, the vector used in transformation, and the antibiotic or herbicide resistance genes used as a marker. Finally, there should be complete information about the transgenic plant, molecular data on the genes inserted, stability of expression, changes in allergenicity, toxicity, persistence in particular environmental conditions, and ability to invade new habitats. The changes in the transformant should be measured against the unmodified control genotype. The procedures adopted should take cognizance of the environment where the plant is to be released.

Some of the major considerations for managing and minimizing (Anonymous, 1998) the perceived risks are as follows:

Containment laboratory facilities. There are two levels of biological containment, namely primary and secondary. Primary containment protects people and the immediate laboratory environment from exposure to infectious agents. Good microbial techniques and safety equipment can provide sufficient levels of primary containment. Examples of primary barriers include safety equipment such as biological safety cabinets, enclosed containers, and safety centrifuge cups. When it is impractical to work in biological safety cabinets, personal protective equipment such as laboratory coats and gloves can act as the primary barrier between personnel and infectious materials. Secondary containment protects the environment external to the laboratory from exposure to

infectious materials. Good facility design and operational practices provide secondary containment. Examples of secondary barriers include work areas that are separate from public areas, decontamination facilities, hand-washing facilities, special ventilation systems, and airlocks.

The three key elements of biological containment include laboratory practices, safety equipment, and facility design. To ensure minimal exposure, the workers must assess the hazards associated with their work and determine how to apply the biosafety principles appropriately. The basic laboratory encompasses all laboratories working with Risk Group I and Risk Group II agents, including those that present low or moderate risk to the laboratory worker and low or limited risk to the community (Anonymous, 1998). Besides following good laboratory practices (GLP), specific practices need to be followed for handling recombinant DNA materials and it is the institutional responsibility to adhere strictly to the code of practice. Emergency procedures to deal with any eventuality that may arise at the institution engaged in recombinant DNA work must be in place.

Containment glasshouse facilities. Care needs to be taken that pollen and seed of transgenic plants from the containment glasshouse facilities do not escape to the outside. The plants should be labeled properly. There should be no mixing between the transgenic plants. There is a need for high levels of quality control over the DNA sequences, gene constructs, transgenic plants, and the experimental results. Growing the plants in the greenhouse involves the same level of controls as in the laboratory. The greenhouse should be properly designed to keep out insects and pollen. The facilities should be run under the control of a biosafety committee, and the level of containment should depend on the type of transgenic plants. The greenhouses should have a controlled and filtered airflow system, control of water outlets and sterilization. Autoclaving of plant and soil material coming out of the greenhouse is very important. Accordingly, the guidelines for microbiological and biomedical laboratories suggests four biosafety levels in an incremental order depending on the nature of the work. These biosafety levels for work with recombinant DNA techniques take into consideration the source of the donor DNA and its disease-producing potential. These four levels correspond to P1 $<$ P2 $<$ P3 $<$ P4 facilities that approximate to four risk groups assigned for etiological agents (Anonymous, 1998). Unless determined otherwise by the Institute Biosafety Committee (IBSC), for most of the experiments dealing with transgenic plants, a P2 level of containment is recommended.

Contained field trials. A comprehensive risk assessment is necessary once a plant has to be released for small-scale experiments, and eventually commercial production. At this stage, the scientists concerned, the biosafety committee, and the national or international regulatory authorities should determine whether it is acceptable to release the specific transgenic plants, and if needed, the restrictions to be imposed. Field containment should be in place to limit the possible environmental impact of the release experiment. This may include (according to the species and plant characteristic) isolation from the sexually compatible species, prevention of flowering, use of male-sterile lines, and subsequent monitoring protocols. Data required for risk assessment includes: (i) general information, (ii) DNA donor, the receiving species, and the transgenic plant, (iii) environment and the conditions of release, (iv) transgenic plant–environment interactions, and (v) control, monitoring, and waste treatment.

Before going into open field trials, it is necessary to generate safety information in a contained environment. Such an environment could be created in a glasshouse, polyhouse, or screenhouse where conditions can be created to regulate light, humidity, air flow, temperature, and effective barriers for preventing the entry of microbial organisms or insects, and efficient trapping of the pollen grains and biological materials. In order to enable the regulatory authorities to take a view about the proper assessment of risks and hazards from the use of transgenic plants before permitting their large-scale release, information on the above-mentioned aspects is required to be compiled in a standard format.

Human health. Since some of the transgenes code for proteins that ordinarily may not be present in the particular host plants, there is concern about the potential allergenicity or toxicity of these new varieties to both human and livestock health (Kessler et al., 1992; Lehrer et al., 1996). An adverse reaction to a food is viewed as a clinically abnormal response attributed to exposure to that food or food additive, and includes both immunological and non-immunological reactions (Sampson and Metcalfe, 1991). Theoretically, all types of foods can cause allergic reactions. However, it is difficult to estimate precisely the prevalence of food allergies. In general, food proteins that maintain their immunogenicity following processing, cooking, and digestion, while remaining soluble and absorbable through the intestinal tract, are more likely to elicit an allergic response rather than those that are not as resistant to such processes (Lehrer et al., 1996; Taylor and Lehrer, 1996). When evaluating the potential effects of newly developed food products as a result of biotechnology, two issues must be considered: first, effects of the transfer of known protein allergens into new foods, and second, effects of transfer of recombinant proteins of unknown allergenic activity into new foods (Kessler et al., 1992; Lehrer et al., 1996).

Several traditional detection methods have been proposed to detect recombinant allergens in transgenic foods (Lehrer and Reese, 1997). These include *in vitro* assays such as Western blotting, radioallergosorbent test (RAST) inhibition, and enzyme-linked immunosorbent assay (ELISA). These methods are well established, specific, sensitive, and reproducible, and have been used effectively to investigate recombinant food proteins in the assessment of transgenic food products for their potential allergenicity. Burks and Fuchs (1995) investigated transgenic soybeans in which a gene was introduced to confer tolerance to glyphosate, the active ingredient in the herbicide Round-up™. The extracts of different wild-type and transgenic varieties were analyzed by Western blot for allergenic proteins through immunoglobulin E (IgE) binding. There appeared to be no increased binding activity of IgE antibodies from a serum pool of soy-allergic individuals to the transgenic soybean extracts as compared to the wild type. In a separate study, an allergen was detected in a transgenic soybean. The donor gene originated from Brazil nuts and was expressed in soybeans to increase their methionine content (Nordlee et al., 1996). It bound IgE from Brazil nut-sensitive individuals, and was identified as a major Brazil nut allergen. This demonstrated the possibility of testing new products for allergens when proteins are transferred from sources that contain known allergenic material.

Environmental considerations. Modern agriculture is intrinsically destructive of the environment, particularly of biological diversity when practiced in a very resource-inefficient way or when it applies technologies that are not adapted to the ecosystem in a particular region. The widespread application of conventional

agricultural technologies such as herbicides, pesticides, fertilizers, and tillage has resulted in severe environmental damage in many parts of the world. Thus, according to a working group consisting of members from the National Academy of Sciences, USA (NAS) of both the industrialized and developing world, the environmental risks of new genetically modified technologies need to be considered in the light of the risks of continuing to use conventional technologies and other commonly used farming techniques (Anonymous, 2000). Most of the environmental concerns about biotechnology in plants have derived from the possibility of gene flow to the close relatives of transgenic plants, the possible undesirable effects of the exotic genes or traits (e.g., insect resistance or herbicide tolerance), and effects on the non-target organisms.

It is important to describe the invasiveness of transgenic plants in the wild habitat, their ability to propagate sexually or asexually, the possibility of transferring transgenes to the same or related species and to microorganisms, and the consequences of gene transfer. The risk to the target environment requires qualitative judgement, and should be based on a case by case study, depending on the accumulated experience. Information about the purpose of the release, size, design, and agronomic requirements is important for risk assessment at the national and international level. Ecological information about the release site, survey of plant species growing in the target region, and the nature of pollen dissemination are important. The anticipated target and non-target organisms with which the transgenic plant will interact need to be noted. Information should also be recorded whether the transgenic plant would become a better or worse host for receiving genes from related species. The risk to the environment includes harmful effects on the beneficial non-target organisms.

Once the transgenic plants are released for commercial cultivation, measures such as prevention of flower production, and destroying all plant parts is not possible. Therefore, the risk assessment should also take into account pollen transfer between the non-transgenic crop and wild relatives. There may also be possibilities for taking the transgenic plants into areas where the sexually compatible wild relatives of the crop are present in large numbers. Transgene instability may also be another cause of concern when the transgenic crops are grown on a large scale. Strategies for introducing herbicide resistance into several crops or different toxins against the prevalent pests and diseases have to be properly devised. Similarly, options for containment after large-scale cultivation of a transgenic plant are limited. Therefore, risk assessment must take these factors into account, and consider all factors available from small-scale experiments. To overcome such problems, it may be useful to use tissue-specific expression (target site for insect feeding or infection by the pathogen, or use of male-sterile lines to limit the dispersal of pollen, as is the case with hybrids produced by conventional plant breeding). Another possibility is growing the transgenic crops where there are no wild relatives of the crop, and in areas free from non-transgenic crop. The license for cultivation may be canceled if the results of growing a transgenic crop are unsatisfactory or there is a risk to human health and the environment.

The recommendations from a working group that included policy makers and scientists from six countries, under the auspices of the NAS (Anonymous, 2000), have suggested that, as with the development of any new technology, a careful approach is warranted before the development and deployment of a commercial product. Further, it must be shown that the potential impact of a transgenic plant has been carefully analyzed and that, if it is neutral or innocuous, it is preferable to the impact of the conventional agricultural technologies that it is designed to replace (May, 1999).

Solutions to Manage the Potential Risks Associated with Genetically Modified Plants

The behavior of a transgenic plant in the open environment cannot be predicted in a generalized way. The main concerns are therefore the right assessment of the magnitude of the consequences from the use of genetically modified plants to the habitat including humans and other animals, flora and fauna, and the environment. At present, there does not seem to be any mechanism for formal or informal consultations with the different interest groups of the society for arriving at a consensus on the introduction of transgenics in agriculture. More social interaction involving stakeholders can be a step in the right direction for quicker acceptance of GMOs. Promoting greater participation of organizations who actually represent the needs of farmers and consumers should be an integral part of biosafety risk assessment procedures (Spillane, 2000). Society must have a large number of knowledgeable people, who are able to understand and appreciate different aspects of this technology, thus necessitating incorporation of appropriate public awareness programs into the educational system for developing the needed manpower. Some of the areas for special attention are as follows:

Public perceptions. Public perception is likely to have a great impact on innovation, introduction, and diffusion of products of biotechnology. A negative public perception is likely to keep the technology or product away from reaching the market place of its utilization. Public perception is influenced by a broad range of issues, including human and environmental safety, ethics, legal repercussions, economic gains, and socioeconomic impact. The impact of public perception on biotechnology cannot be gauged purely on scientific grounds, as public opinion can be influenced by non-scientific considerations based on impressions created by the media and pressure groups. Concerns about recombinant technology were raised in the early 1970s (Leopold, 1993). This led to protests over the setting up of biotechnology laboratories in developed countries. Subsequently, more and more organizations entered the public debate. As a result, regulations and guidelines for handling DNA-based technologies have been formulated in several developed and developing countries.

In the late 1980s, the research in biotechnology underwent several changes, and the main thrust of the technology shifted from laboratories to the commercial market, and biotechnology was seen as a critical technology for international competition. Applications included biomedicine and agriculture, and the potential risks related to accidentally engineered organisms to health and safety were raised. With increasing stakes for economic gains and potential risks, public debate over the need to develop regulatory mechanisms became more important. This also led to intense public debate amongst scientists, particularly between molecular geneticists and ecologists. As a result, the politicians, the industry, and environmental groups began to take a more active stance. Much of this debate is likely to play itself out in the regulatory arena, with the shift of focus from the laboratory to commercial applications. This

role needs to be taken up by the international and national agriculture, forest, health, food, and environmental regulatory authorities.

In a study on public perceptions of agricultural and environmental biotechnology applications, including commercialization of transgenic crops, two different subsets of issues have emerged (Hagedorn and Allender-Hagedorn, 1997, 1998). Twenty-four issues were identified under eight categories. However, the frequency scores from scientific and public sources were different for 14 of the 24 issues. Those appearing most frequently in public sources (popular press, newspapers, surveys) included: health and ethical issues associated with transgenic animals, nutrition, value and labeling of transgenic foods, ethical considerations in creating biotechnology products, public safety and input into the regulatory process, decisions on the use and availability of biotechnology products, and the impact of biotechnology education (e.g., television and the press). In contrast, those issues appearing most frequently in scientific sources (technical/regulatory) included: risks and environmental impact of transgenic microbes, gene transfer in transgenic plants, patenting and freedom of information, regulatory structure and risk assessment process, and safety and product availability in foreign countries. This study identified five issues as being problematic and involving potential risks: herbicide resistance in target plants, pest resistance to transgenic plants, weediness and gene transfer, environmental concerns, and impact on agriculture and farming. The authors concluded that these five issues represent the best candidates for developing educational materials directed at improving public understanding of transgenics.

In general, the scientific community and the public agree that the risks of genetic engineering are largely exaggerated, but there is a need for a strict regulatory mechanism. Generally speaking, the farmers and the public believe that biotechnology will lead to increased food production and improved nutrition. The level of education, religion, socioeconomic factors, pressure by the non-governmental organization (NGO) environmental groups, and governmental policy are likely to shape public opinion about biotechnology. Scientific literacy, scientific proof of the non-sustainance of presumed risks, informal dissemination of information through the public media, clear standards, food labeling, reducing the extent of exaggerated expectations, allowing the public to be part of the decision-making process, and reliability of information are important to have a clear picture of the benefits and risks of biotechnology. Public learning is influenced by economic conditions, education, social and institutional means of participation, traditions, cultural and religious values, and historical background.

In developing countries, there is a need for an environment that is institutionally, socially, culturally, politically, and educationally favorable. Developing countries that are adopting biotechnology tools also have a large proportion of the population capable of making rational decisions. If large sections of the population are under the poverty line and illiterate, the NGOs often become the advocates of public opinion, which at times may be guided by several extraneous factors. In such a situation, the UN (through FAO, WHO, and UNIDO) or international agriculture research centers such as those of the CGIAR are expected to play an important role in enhancing the public perception of the usefulness and the risks associated with the introduction of GMOs into the agricultural systems, the food chain, human health and the environment. According to Spillane (2000), assessment of the immediate needs of different groups of farmers and consumers should become an integral component of biosafety risk assessment procedures, where costs and benefits could be seen on social rather than solely on environmental terms exported from countries where food surpluses are a common phenomenon.

Potential risks of genetically modified plants. Of the 'risks' that have been associated with plant-based agriculture, virtually all are the consequence of the management practices needed to grow crop plants and keep them healthy (Cook, 2000). Some of the important environmental risks associated with the growing of crops include soil erosion because of tillage used to form the seedbed and weed control, nitrates left unused in the soil because of over-fertilization (or under-utilization because of disease), non-target effects of pesticides on beneficial insects, and smoke from burning the crop residue. Genetic modification of crop plants has been suggested to be the best route to mitigate some of these risks, but must be accomplished without introducing new risks (Cook, 2000). In spite of the safety record, there is public concern worldwide that plants with genes introduced from outside their normal range of sexual compatibility (genetically improved plants) might present new risks to the environment and human health. Hence, besides exploring the potential benefits of genetic modification for sustainable agriculture, the potential and perceived risks associated with growing transgenic plants must be examined carefully.

The Ecological Society of America (ESA) produced a document with the objective of providing rigorous support for the development of a biosafety policy to encourage innovation without compromising the adequate and safe management of the environment. In this document, six types of evolutionary and environmental concerns related to the potential risks of the new biotechnology methods to the environment and to biodiversity have been listed (Tiedje et al., 1989). These potential risks include: the creation of new weeds, the amplification of existing weeds, damage to non-target species, the perturbation of biotic communities, adverse effects on ecosystem processes, and waste of precious biological resources. While the vertical movement of genes within the species or genera need to be adequately addressed in risk assessment studies, the lateral or horizontal gene transfer, i.e., non-sexual transfer of genetic information between genomes, although rare, is possible. Numerous claims for the lateral transfer of genomic sequences have been made during the past two decades (Kidwell, 1993). Almost all of the well-documented cases of gene transfer within eucaryotes seem to involve mobile elements in chromosomes or other parasitic sequences. Although it is very difficult, and often impossible, to prove conclusively that lateral transfer has occurred in any particular instance, improved methods for detecting such phenomenon are forthcoming. For example, rapid methods of DNA sequencing at low cost can reveal sequence variations that are not consistent with species phylogenies. Hence, there is an urgent need not only to generate reliable information on natural vertical gene transfer, but also horizontal exchange of genomes in all plants including transgenics to address the biodiversity concerns.

Considerations for risk management. Studies conducted by the NAS (1987, 1989) on the safety of GMOs have concluded that 'crops modified by molecular and cellular methods should pose risks no different from those created by classical genetic methods for similar traits'. Although claims such as those mentioned above are not supported by science, some of them might turn out to be true in

certain circumstances. Hence, governments, research organizations, and companies must respond to these concerns, and must have in place the means to scientifically assess the report on real risks presented by the crop plants. The focus should be on the product and not the process, and hence, the steps used to conduct a risk assessment should be the same for all crop plants, regardless of the source of genes or methods used to transfer these genes (Cook, 2000). Furthermore, whether the risk assessment is done by a government regulatory agency, an institutional biosafety committee, or private organization, the assessment process as well as the conclusions on safety should be in the public domain.

Genetically improved foods are not intrinsically good or bad for human health. Their health effects depend on their specific content. Hence, the risks and opportunities associated with genetically improved foods should be integrated into the general food safety regulations of a country. The regulatory systems of a country are needed to govern food safety and assess any environmental risks, monitor compliance, and enforce such regulations. The regulatory arrangements should be country-specific and reflect relevant risk factors. As a result of such intervention, the possible commercialization of soybeans with a Brazil nut gene that also carried with it a major allergenic domain was avoided. Another intervention may be the need to label the content for cultural and religious reasons or simply because the consumers may want to know what their food contains. While the public sector must design and enforce safety standards as well as any labeling required to protect the public from health risks, other labeling might best be left to the private sector in accordance with consumer demands for knowledge (Pinstrup-Anderson and Cohen, 2000).

Biosafety Regulations on Genetically Modified Plants

Since biotechnology products are being adopted rapidly around the world, there is an increasing need to ensure that consistent safety standards are put in place to protect human health and the environment from any potentially adverse effects of these products. There were 864 field trials of genetically modified plants until 1992, of which 316 had occurred in the USA, 302 in Canada, and 217 in the European Community (Dale et al., 1993). Genetically modified plants have been released in over 22 countries. The regulations governing the use of transgenic plants vary considerably in different countries. Existing regulations have been applied to the production and release of genetically modified plants, but may not be adequate to cover the potential environmental effects.

In the USA, health and safety aspects of genetically engineered organisms are covered by the Environment Protection Agency, USA (EPA), the United States Department of Agriculture (USDA), and the Food and Drug Administration, USA (FDA) (Levin and Strauss, 1993). The laws related to products of gene technology have been published in the Federal Register in 1986. Guidelines related to the use of GMOs have also been issued under the Commission of the European Communities (CEC, 1990a, b). Latin American countries have plant quarantine and regulation systems to deal with plant introduction, and these may have to be altered to deal with GMOs (IAICA, 1991). Permission for the release of a modified organism is given after risk assessment by the individual countries. Risk assessment is carried out by the competent authority, based on the data supplied by the applicant. The competent authority has to give the decision within a specified period. Legislation in the the USA is product specific, while in the European Union, it is process specific. Different countries in the world are adopting approaches related to these models, depending upon the type of legislation already in place. In future, when the use of transgenic plants becomes widespread, the need for harmonizing the standards and procedures will become very important. In this regard, the initiative of some countries (Canada, Argentina, and Chile) is noteworthy, and a Canadian–Latin American Network (CamBioTec) has been developed to promote safe and effective use of agricultural and environmental biotechnology (Flint et al., 2000). A meeting of Kenyan scientists in October 1999 concluded that attempts by anti-biotechnology lobby groups from the Organization for Economic Cooperation and Development (OECD) countries to limit the application of biotechnologies to food surplus-prone OECD agriculture are having negative spill-over effects regarding any possibilities that modern agricultural biotechnologies might be applied to helping the African continent to achieve its long-term food security objectives (Spillane, 2000).

To date, there are only three international organizations (OECD, UNIDO, and UNEP) that have, or plan to invest, significant resources in biosafety information systems. Recent pressures, due to increasing global trade in these products and the UN Biosafety protocol, have encouraged cooperation between these organizations. Some initiatives have already been taken in this direction by OECD (1992). The OECD Programme on the Harmonization of Regulatory Oversight in Biotechnology is an initiative designed to ensure that environmental health and safety aspects are properly evaluated, while avoiding non-tariff trade barriers to products of biotechnology. The majority of OECD member countries have (or are developing) a system of regulatory oversight for the products that are intended for release into the environment. The program is expected to play a coordinating role for regulatory departments in member countries. To encourage information dissemination, an online database (BioTrack) has been developed and is used to track regular developments and field trials of transgenic plant products in OECD member countries.

United Nations organizations such as UNIDO, UNEP, FAO, and WHO have published a 'Voluntary code of conduct' for the release of organisms into the environment (UNIDO, 1991). UNIDO has also supported an international Biosafety Information Network and Advisory Service (BINAS), which helps developing countries in the setting up of national authorities that are qualified to handle the release of GMOs. A similar regional initiative, involving Association of South East Asian Nations (ASEAN) countries, is currently being considered. There was cooperative development of BioTrack and BINAS in 1996 that resulted in the construction of a joint BioTrack/BINAS page (BIOBIN) on the World Wide Web. This is expected to contribute towards a global information system related to regulatory issues and harmonization of biosafety regulations.

The UN Convention on Biological Diversity is the first UNEP initiative to focus on issues of biotechnology and more specifically biosafety. The convention, signed in 1992, laid out provision for the development of a biosafety protocol. In November 1997, the council of the Global Environmental Facility (GEF) approved a UNEP/GEF pilot biosafety enabling project that is aimed to provide assistance to developing countries, and countries with economies in transition, in formulating national biosafety frameworks for the implementation of the UNEP International Technical Guidelines for Safety in

Biotechnology, and the future implementation of any agreements on biosafety.

Recognizing the need for regulating the use of modern biotechnology, biosafety issues have become of utmost importance in India. The Department of Biotechnology (DBT), Ministry of Science and Technology of the Government of India, formulated and released Recombinant DNA Guidelines in 1989 under the Environmental Protection Act in 1986 (Anonymous, 1998; Ghosh and Ramanaiah, 2000). These guidelines include: (1) genetically engineered organisms, (2) genetic transformation of green plants and animals, (3) recombinant DNA (rDNA) technology in vaccine development, and (4) large-scale production and deliberate/accidental release of organisms, plants, animals, and products derived by rDNA technology. After the signing of the Convention on Biodiversity by the world community in 1992, the DBT revised its earlier guidelines of 1990 to accommodate the safe handling of GMOs in research applications and technology transfer in 1994. This includes the large-scale production and deliberate release of GMOs, plants, animals, and products into the environment. In accordance, DBT has framed general safety measures to be practiced in all kinds of experiments involving modern biotechnology.

To implement these guidelines, the Government of India issued Rules and Procedures (Rules) for handling GMOs and hazardous organisms through a Gazette Notification No. GSR 1037(E) dated December 5, 1989 from the Union Ministry of Environment and Forests that directs the creation of various committees. Currently, these guidelines are being implemented through three-tier mechanisms (Ghosh and Ramanaiah, 2000). These include: (1) Institutional Biosafety Committees (IBSC) to monitor the research activities at the institutional level. (2) Review Committee on Genetic Manipulation (RCGM) functioning in the DBT, which presents research activities in laboratory-based controlled field experiments. The RCGM also reviews these activities from safety considerations. (3) The Genetic Engineering Approval Committee (GEAC) of the Ministry of Environment and Forests that has the authority to permit large-scale use of GMOs at the commercial level, and open-field trials of transgenic materials including agricultural crops, industrial products, or healthcare products.

Realizing the fact that biotechnology safety guidelines would never be a one-time exercise as the knowledge is ever expanding, the DBT has set up the recombinant DNA Committee to prepare a modified draft of guidelines from time to time on the basis of current scientific information and from the experience gained locally and outside the country on the use of the new biotechniques in the area of research, or possible manufacture and applications. Hence, the reader is advised to refer to the most current guidelines of the DBT in practice (Anonymous, 1998). In general, the Biosafety Guidelines deal with the definition of recombinant DNA, classification of pathogenic microorganisms, containment facilities and their types, biosafety levels and appropriate conditions, guidelines for recombinant DNA research activities, large-scale experiments, release of GMOs to the environment, import and shipment of rDNA and its products, and quality of biologicals produced by recombinant DNA technology.

ICRISAT's Strategy for Biosafety

ICRISAT, which is part of the CGIAR, has recognized the importance of the application of plant biotechnology techniques in the genetic enhancement of its mandate crops that feed the poorest of the poor. It is fully aware of, and recognizes, the importance of biosafety of food and the environment (Ortiz, 1999). ICRISAT is committed to the highest international standards of biosafety to honor the regulatory policies of the host countries. In accordance with the requirements of the Indian Government, ICRISAT's biotechnology research is carried out under the supervision of the Institute Biosafety Committe (IBSC) that is represented by its own scientists and nominees of the Department of Biotechnology, Government of India. In the pursuit of maintaining high standards of biosafety, ICRISAT has recently commissioned a P2-level containment facility for the pre-field screening of transgenic plants (Ortiz, 2000) that has been approved by the IBSC. This facility was built to conform to the highest international standards prescribed for the P2 level of containment. This facility is pollen proof, insect proof, shatter proof, and has negative pressure conditions. The effluent treatment plant stops soil-borne and water-borne dispersal and has a provision to prevent contamination from personnel by decontaminating clothes and equipment. Excess water after watering is collected into a sump and then pumped by using level sensors into another sump located outside the building, to stay for one day before it is discharged into the drainage system in an automated manner. All the discarded material leaving the facility is sterilized by autoclaving. Moreover, the plants grown in the vicinity of the facility are constantly monitored. The application of transgenic genetic enhancement would provide new opportunities to help improve the lives of the poor while conserving the environment, which will show the 'Human Face of Science', of ICRISAT's research for development agenda.

Sources of Information on Biosafety on the World Wide Web

For more information about biosafety and associated issues, the reader may check the following URLs and the links therein: http://www.icgeb.trieste.it/biosafety/; http://binas.unido.org/binas/index.php3; http://www.oecd.org/ehs; http://www.oecd.org/ehs/biobin; http://www.oehs.upenn.edu/bio/bsm/; http://who.enep.ch/biodiv/; http://binas.unido.org//binas/binas.html; http://www.nal.usda.gov/bic/federal_biotech/news/; http://www.cgiar.org; http://www.oehs.upenn.edu/bio/bsm; http://www.biodiv.org/biosafety; http://www.absa.org; http://www.biotech.co.in; http://www.unep.org/program/matres/biodiv/irb/; http://agbio.cabweb.org/ABTAGBIO.htm; http://food.jrc.it/gmo.

Outlook

Modern agricultural biotechnology is one of the most promising developments in modern science. Discussions on transgenic crops have placed undue stress on risk assessment, while the potential advantages are relegated to the background. Recombinant DNA technology provides a powerful tool to transfer genes across wide taxonomic groups. Used in collaboration with traditional or conventional breeding methods, it can raise crop productivity, increase resistance to pests and diseases, develop tolerance to adverse weather conditions, improve the nutritional value of some foods, and enhance the durability of products during harvesting or shipping. With reasonable biosafety regulations, this can be done with little or no risk to human health and the environment.

Therefore, it may be important to go beyond the considerations of

immediate impact on the environment, since we all should share the responsibility for increasing crop production, and conservation of the environment. The production and release of transgenic plants should be based on experience. A fast-track process can be adopted for transgenes and promoters, which are known to give satisfactory results in other crops and environments. There is a need for harmonization of release criteria, and a move towards simplified regulatory mechanisms in the future. The rapid escalation of increasingly stringent biosafety regulations regarding transgenic plants or food, in the absence of any scientifically proven generic risk, is most likely to limit any application of transgenic research to meeting either sustainable staple food production or poverty alleviation needs.

It is essential that agricultural biotechnology research be relevant to the needs of farmers in developing countries, and that the benefits of that research are transmitted to small-scale farmers and consumers in those countries at affordable prices. Condemning biotechnology for its potential risks without considering the alternative risks of prolonging the human misery caused by hunger, malnutrition, and infant mortality is unwise and unethical. The global community must keep its sights set on the goal of assuring food for all and cannot afford to be philosophical and elitist about any part of a possible solution, including agricultural biotechnology.

Acknowledgments

We greatfully acknowledge financial support from the Directorate General International Cooperation (DGIC), Belgium.

References

Anonymous. Background document for the Workshop on biosafety issues emanating from use of genetically modified organisms (GMOs). New Delhi: Biotech Consortium India Ltd.; 1998:289 pp. [http://www. biotech.co.in].

Anonymous. Transgenic plants and world agriculture. Washington, DC: National Academy Press; 2000:1–40. [http://www.nap.edu/html/transgenic].

Arakawa, T.; Chong, D. K. X.; Langridge, W. H. R. Efficiency of a food plant-based oral cholera toxin B subunit vaccine. Nature Biotechnol. 16:292–297; 1998.

Birch, R. G. Plant transformation: problems and strategies for practical applications. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:297–326; 1997.

Borlaug, N. E. Contributions of conventional plant breeding to food production. Science 219:689–693; 1983.

Borlaug, N. E. Feeding a world of 10 million people: the miracle ahead. Plant Tiss. Cult. Biotechnol. 3:119–127; 1997.

Burks, A. W.; Fuchs, R. L. J. Allergy Clinic. Immunol. 96:1008–1010; 1995.

CEC (Commission of the European Communities). Council Directive of 23rd April 1990 on the Contained use of Genetically Modified Microorganisms. Reference no. 90/219/EEC. Official Journal L117 vol. 33. Brussels: Commission of the European Communities; May 1990a.

CEC. Council Directive of 23rd April 1990 on the Deliberate Release into the Environment of Genetically Modified Organisms. Reference no. 90/220/EEC. Official Journal L117, vol. 33. Brussels: Commission of the European Communities; May 1990b.

Cook, R. J. Science-based risk assessment for the approval and use of plants in agricultural and other environments. In: Persley, G. J., Lantin, M. M., eds. Agricultural biotechnology and the poor. Washington, DC: CGIAR; 2000:123–130. [http://www.cgiar.org/biotechc/bioconf.htm].

Dale, P. J.; Irwin, J. A.; Schefler, J. A. The experimental and commercial release of transgenic crop plants. Plant Breed. 111:1–22; 1993.

de la Fuente, J. M.; Ramirez-Rodriguez, V.; Cabrera-Ponce, J. L.; Herrera-Estrella, L. Aluminium tolerance in transgenic plants by alteration of citrate synthesis. Science 276:1566–1568; 1997.

Flavell, R.B. Nature Biotechnol. Suppl. 17:BV7; 1999.

Flint, J.; Gil, L.; Verastegui, J.; Irarrazabal, C.; Dellacha, J. Biosafety information management systems. A comparative analysis of the regulatory systems in Canada, Argentina, and Chile. Elect. J. Biotechnol. 3:9–29; 2000. [http://www.ejb.org/content/vol3/issue1/full/2].

Ghosh, P. K.; Ramanaiah, T. V. Indian rules, regulations and procedures for handling transgenic plants. J. Sci. Ind. Res. 59:114–120; 2000.

Hagedorn, C.; Allender-Hagedorn, S. Issues in agricultural and environmental biotechnology: identifying and comparing issues from public opinion surveys, the popular press, and technical/regulatory sources. Public Understand. Sci. 16:233–245; 1997.

Hagedorn, C.; Allender-Hagedorn, S. Public perceptions of transgenics in biocontrol. Agbiotech. Bull. 10:217N–222N; 1998.

Hooykaas, P. J. J.; Schilperoort, R. A. Agrobacterium and plant genetic engineering. Plant Mol. Biol. 19:15–38; 1992.

IAICA (Inter-American Institute for Cooperation on Agriculture). In: Guidelines for release into the environment of genetically modified organisms. San Jose, Costa Rica: IAICA; 1991.

Jazairy, I.; Alamgir, M.; Panuccio, T. The state of world rural poverty: an inquiry into its causes and consequences. New York: IFAD/New York University Press; 1992.

Kessler, D. A.; Taylor, M. R.; Maryanski, J. H.; Flamm, E. L.; Kahl, L. S. The safety of foods developed by biotechnology. Science 256:1747–1749; 1992.

Kidwell, M. G. Lateral transfer in natural populations of eukaryotes. Annu. Rev. Genet. 27:235–256; 1993.

Kung, S., Wu, R., eds. Transgenic plants, vol. 1–2. San Diego: Academic Press; 1993.

Lehrer, S. B.; Horner, W. E.; Resse, G. Why are some proteins allergenic? Implications for biotechnology. Crit. Rev. Food Sci. Nut. 36:553–564; 1996.

Lehrer, S. B.; Reese, G. Recombinant proteins in newly developed foods: identification of allergenic activity. Int. Arch. Allergy Immun. 113:122–124; 1997.

Leopold, M. The commercialization of biotechnology, the shifting frontier. In: Tzotzos, G. T., ed. Biotechnology R & D trends. Science policy for development. New York: New York Academy of Sciences; 1993:214–231.

Levin, M.; Strauss, H. S. Overview of risk assessment and regulation of environmental biotechnology. In: Levin, M., Strauss, H. S., eds. Risk assessment in genetic engineering. New York: McGraw Hill Inc.; 1993:1–17.

Levin, S. A. Safety standards for the release of genetically engineered organisms. Trends Biotechnol. 6:547–549; 1988.

Lipton, M. Crawford Memorial Lecture, 28/10/99, CGIAR International Centres' Week, Washington, DC; 1999.

Mason, H. S.; Ball, J. M.; Shi, J. J. Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its oral immunogenicity in mice. Proc. Natl Acad. Sci. USA 93:5335–5340; 1996.

May, R. Genetically modified foods: facts, worries, policies and public confidence. London: Office of Science and Technology; 1999.

Miller, H. I.; Flamm, E. L. Biotechnology and food regulation. Curr. Opin. Biotechnol. 4:265–268; 1993.

Molvig, L.; Tabe, L. M.; Eggum, B. O.; Moore, A. E.; Craig, S.; Spencer, D.; Higgins, T. J. Enhanced methionine levels and increased nutritive value of seeds of transgenic lupins (*Lupinus angustifolius* L.) expressing a sunflower seed albumin gene. Proc. Natl Acad. Sci. USA 94:8393–8398; 1997.

NAS (National Academy of Sciences). Introduction of recombinant DNA-engineered organisms into the environment: key issues. Washington, DC: NAS; 1987:24.

NAS. Field testing genetically modified organisms. Washington, DC: NAS; 1989:170.

Nordlee, J. A.; Taylor, S. L.; Townsend, J. A.; Thomas, L. A.; Bush, R. K. Identification of a Brazil nut allergen in transgenic soybeans. N. Engl. J. Med. 334:688–692; 1996.

OECD (Organization for Economic Cooperation and Development). Workshop on the Monitoring of Organisms Introduced into the Environment. Paris: OECD; 1992.

Ortiz, R. Transgenic crops at ICRISAT; 1999. [http://grep.icrisat.cgiar.org/grep/ro_trans.htm]

Ortiz, R. ICRISAT containment facility for transgenic plants; 2000 [http://grep.icrisat.cgiar.org/grep/P2Facility.htm].

Pinstrup-Andersen, P.; Cohen, M. J. Modern biotechnology for food and agriculture: risks and opportunities for the poor. In: Persley, G. J., Lantin, M. M., eds. Agricultural biotechnology and the poor. Washington, DC: CGIAR; 2000:159–169 [http://www.cgiar.org/biotechc/bioconf.htm].

Potrykus, I. Gene transfer to plants: assessment and perspectives. Physiol. Plant. 79:125–134; 1990.

Sampson, H. A.; Metcalfe, D. D. Immediate reactions to foods. In: Metcalfe, D. D., Sampson, H. A., Simon, R. A., eds. Food allergy: adverse reactions to foods and food additives. Oxford: Blackwell Scientific Publishers; 1991:99–112.

Serageldin, I. The challenge of poverty in the 21st century: the role of science. In: Persley, G. J., Lantin, M. M., eds. Agricultural biotechnology and the poor. Washington, DC: CGIAR; 2000:25–31 [http://www.cgiar.org/biotechc/bioconf.htm].

Sharma, K. K.; Ortiz, R. Program for the application of genetic transformation for crop improvement in the semi-arid tropics. In Vitro Cell. Dev. Biol. Plant 36:83–92; 2000.

Spillane, C. Could agricultural biotechnology contribute to poverty alleviation? AgBiotechNet 2: ABN041, 1–40; 2000.

Taylor, S.; Lehrer, S. B. Principles and characteristics of food allergans. Crit. Rev. Food Sci. Nutr. 36:S91–S118; 1996.

Tiedje, J. M.; Colwell, R. K.; Grossman, W. L.; Hodson, R. E.; Lenski, R. E.; Mack, R. N.; Regal, P. J. The planned introduction of genetically modified organisms: ecological considerations and recommendations. Ecology 70:298–315; 1989.

Tzotzos, T. Genetically modified microorganisms: a guide to biosafety. Wallingford: CAB; 1995.

UN (United Nations). World population prospects: the 1996 revisions. New York: UN; 1996.

UNIDO (United Nations Industrial Development Organization). Voluntary code of conduct for the release of organisms into the environment. Vienna: UNIDO; 1991.

Whitham, S.; McCormick, S.; Baker, B. The *N* gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato. Proc. Natl Acad. Sci. USA 93:8776–8781; 1996.

Wilkinson, J. Q.; Lanahan, M. B.; Clark, D. G.; Bleecker, A. B.; Chang, C.; Meyerowitz, E. M.; Klee, H. J. A dominant mutant receptor from *Arabidopsis* confers ethylene insensitivity in heterologous plants. Nature Biotechnol. 15:444–447; 1997.

Williamson, M.; Perrins, J.; Fitter, A. Releasing genetically engineered plants. Trends Ecol. Evol. 5:417–419; 1990.

Wyngaarden, J. B. The future role of biotechnology in society. Advances in biotechnology. Stockholm: Swedish Council for Forestry and Agricultural Research; 1990:260.

Zupan, J. R.; Zambryski, P. Transfer of T-DNA from Agrobacterium to the plant cell. Plant Physiol. 107:1041–1047; 1995.