Development of Transgenic Crops and their Risk Assessment

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Abstract

Baum, M. and M. Madkour. 2006. Development of Transgenic Crops and their Risk Assessment. Arab J. Pl. Prot. 24: 178-181.

The International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria is exploring the possibility of using genetic engineering to achieve improved tolerance to biotic and abiotic stresses, including drought and fungal diseases resistance. Chickpea and lentil transformation is being done in cooperation with the University of Hannover, Germany. Cereal transformation is carried out jointly with the Agricultural Genetic Engineering Research Institute (AGERI) in Cairo, Egypt and the Centre for Biotechnolgy (CBS), Sfax, Tunisia. With the first products are becoming available, risk assessment has to be undertaken and risk management strategies to be developed. The countries of the Fertile Crescent are located in the Center of Diversity of many of our agricultural crops. Most economically important (barley, wheat, lentils, chickpeas) are inbreeding species and the level of out-crossing is rather low (0-2%). Nevertheless, studies that quantify the exact amount of gene-flow are being conducted to allow the development of science- based risk assessment strategies. Deployment of transgenic crops to areas outside the center of diversity (e.g. North Africa, South Asia) is another strategy to be followed. For this reason, the development of biosafety frameworks and biosafety regulations that allow the testing of transgenic crops in countries outside the Center of Diversity is encouraged and supported.

Key words: Biotechnology, Biosafety, Regulations, Genetic engineering, Legislation.

Introduction

Two major transformation systems are used in the genetic engineering of plants: the Agrobacterium-mediated transformation and the biolistic transformation system. Agrobacterium is a soil-borne, ubiquitous bacterial plant pathogen which causes tumors in infected plants. It infects plants usually through wounds. Upon wounding, plant cells produce small molecular weight components such as acetosyringone. These components have an important function to stimulate Agrobacterium transformation and are often artificially added to the culture media to enhance the infection process. Agrobacterium infects plants through the transfer of genes, the so-called T-DNA. These genes are located on the tumor-inducing (Ti)- plasmid. In natural Agrobacterium infections, tumors are induced through genes transferred with the T-DNA, the opines. Opines are carbon and nitrogen compounds, that create a chemical environment which favors the continued proliferation of the bacteria. These genes have been eliminated in the artifical transformation process to create disarmed Agrobacterium plasmids. Disarmed plasmids allow the transfer of any gene of interest to plants that are integrated between the left and right border of the T-DNA. A number of improved Agrobacterium vectors (e.g. gateway vectors) are publicly or commercially available for use in plant transformation.

In biolistic transformation, DNA is coated on small gold (or other metal) particles and these particles penetrate plant tissue cells under high velocity in the gene gun apparatus. The biolistic transformation is the typical system used for monocots and the Agrobacterium transformation for dicots. However, with more in-depth understanding of the transformation system it has become possible to transform cereals with Agrobacterium and dicots with biolistic transformation. Each of the two systems has a number of advantages and disadvantages. With an Agrobacterium-mediated transformation system it seems to be easier to reduce the copy numbers of the inserted gene. This is an important characteristic for the risk assessment and the approval process.

The Global Status of Genetically Engineered Crops

The global area of approved biotech crops in 2005 was 90 million hectares (ha), up from 81 million ha in 2004 (11). The increase was 9.0 million ha, equivalent to an annual growth rate of 11% in 2005. Twenty-one countries grew biotech crops, up significantly from 17 countries in 2004. Four new countries grew biotech crops in 2005, compared with 2004: Portugal, France, the Czech Republic and Iran.

Bt (*Bacillus thuringiensis*) rice, officially released in Iran in 2004, was grown on approximately four thousand ha in 2005 by several hundred farmers who initiated commercialization of biotech rice in Iran and produced supplies of seed for full commercialization in 2006 (11). Iran and China are the most advanced countries in the commercialization of biotech rice, which is the most important food crop in the world, grown by 250 million farmers, and the principal food of the world’s 1.3 billion people, mostly subsistence farmers. Thus, the commercialization of biotech rice has enormous implications for the alleviation of poverty, hunger, and malnutrition, not only for the rice growing and consuming countries in Asia, but for all biotech crops on a global basis. China has already field-tested transgenic rice in pre-production trials and is expected to approve biotech rice in the near-term.

In 2005, the US followed by Argentina, Brazil, Canada and China continued to be the principal adopters of biotech crops globally, with 49.8 million ha planted in the US (55% of global biotech area) of which approximately 20% were stacked products containing two or three genes. The first triple gene product made its debut in maize in the US in 2005 (11). The stacked products, currently deployed in the US, Canada, Australia, Mexico, and South Africa and approved in the Philippines, are an important and growing future trend. The largest increase in any country in 2005 was in Brazil, provisionally estimated at 4.4 million ha (9.4 million ha in 2005 compared with 5 million in 2004), followed by the US (2.2 million ha), Argentina (0.9 million ha) and India (0.8 million ha).

Biotech soybean continued to be the principal biotech crop in 2005, occupying 54.4 million ha (60% of global biotech area), followed by maize (21.2 million ha at 24%), cotton (9.8 million ha at 11%) and canola (4.6 million ha at 5% of global biotech crop area) (11). During the first decade, (1996 to 2005), herbicide tolerance has consistently been the dominant trait followed by insect resistance and stacked genes for the two traits. In 2005, herbicide tolerance, deployed in soybean, maize, canola and cotton occupied 63.7 million, 71% out of the global biotech area of 90.0 million ha, with 16.2 million ha (18%) planted to Bt crops and 10.1 million ha (11%) to the stacked genes (those that simultaneously confer more than one trait). The latter was the fastest growing trait group between 2004 and 2005 at 49% growth, compared with 9% for herbicide tolerance and 4% for insect resistance.

Genetic Engineering in Public Research Institutes in Developing Countries

Many opponents of green biotechnology claim that most of the transgenic crops grown so far are the products of multinational companies and do mainly help industrial farmers rather than small subsistence farmers. This might be partly true, however, the example of Bt rice grown in Iran, and Bt cotton grown by Chinese farmers apparently do not support this observation. Public research organisations mainly in developing countries are very much involved in developing genetic engineered products. Most of these have not yet reached the status of commercialisation, but might be a reservoir of products that will become available during the next decade.

A survey about the status of GM crops being developed in public research institutes in developing countries revealed 201 genetic transformation events were being developed for 45 different crops in 14 countries (12). Over half of the 201 transformation events involve single genes that confer resistance to either viruses or insect pests of crop plants. In 11 events, stacked genes are being tested for phenotypic combinations. Some countries are working on five or fewer crops, whereas others, such as China and South Africa, are working on 15 or more. Although most transformation events have focused on cereals, significant numbers of a diverse range of transgenic vegetables, fruits, roots and tubers have also been developed.

The largest number of transformation events were generated by the seven Asian countries [109], followed by four African countries [54], and four Latin American countries [38] (12). However, Brazil also reported 37 events contracted by the private sector working with Embrapa (Brasilia). Asian countries have products in all stages of the research pipeline, having made significant commitments to GM crops, and are already achieving significant success with insect-resistant GM cotton approvals (in China and to a lesser degree in India, and lastly, Indonesia). Despite the large number of transformation events in Asia, only the Philippines has approved a commercial feed crop for production, and China allows cultivation and use of publicly developed transgenic vegetables.

Besides the numerous agricultural crops there are a number of industrial applications where genetic engineered crops will help and are emerging as an alternative (12). One emerging area in which the power and potential of plant biotechnology is particularly relevant for health is in the development of plant-derived pharmaceuticals (PDPs) and vaccines. These are pharmaceutical proteins such as antibodies, subunit vaccines, blood products or hormones, which are expressed in plants and then extracted from the biomass. The production of PDPs is often known as molecular farming (7).

ICARDA’s Involvement in Genetic Engineering of Crops

The International Center for Agricultural Research in the Dry Areas (ICARDA) is exploring the possibility of using genetic engineering to achieve improved tolerance to fungal diseases, drought and other abiotic stresses for its mandated crops. Chickpea and lentil transformation is being done in cooperation with the University of Hannover, Germany. Cereal transformation is carried out jointly with the Agricultural Genetic Engineering Research Institute (AGERI) in Cairo, Egypt and the Centre for Biotechnolgy (CBS), Sfax, Tunisia.

Legume production in the dry areas, mainly chickpea, lentil and faba bean is limited by numerous biotic stresses such as Fusarium wilt, Ascohyta blight, Botrytis grey mold, Sitonia weevil, nematodes, weeds especially Orobanche ssp[., as well as a number of abiotic stresses such as salinity, cold and drought. Due to the narrow genetic base and non-availability of resistance gene sources, Agrobacterium mediated transformation can add additional resistant genes to improve biotic and abiotic stress resistance. In the last decade *Agrobacterium*- mediated transformation has been used successfully in grain legumes like chickpea (9), yellow lupin (2), pea (1), faba bean (4), and lentil (5). Agrobacterium-mediated transformation systems have also been adapted at ICARDA for the transformation of chickpea and lentil.

For cereals, ICARDA is developing drought and salinity-tolerant plants together with CBS in Tunisia. Wheat and barley are the main target crops.

Legislation Regulating Genetic Engineering

The Cartagena Protocol on Biosafety (CPB) came into force in September 2003 (8). It is the legally binding framework for international movement of living GMOs. The CPB does not oblige countries to create full regulatory system and it allows quite a bit of flexibility; but it provides strong incentive for harmonisation. There are number of characteristics of the CPB such as: it distinguishes between import for planting or for food, it establishes biosafety clearing house (BSC), it regulates risk assessment and risk management, it provides for capacity building, public awareness and participation, socio-economic considerations, liability and redress.

Article 8g of the Convention on Biological Diversity (CBD) and Article 2 of the CPB stipulates that: each party shall take necessary and appropriate legal, administrative and other measures to implement its obligations. The CPB provides a financial mechanism under the United Nations Environmental Programme (UNEP) – Global Environment Facilities (GEF) program for its implementation (6). Eight countries have completed their National Biosafety Framework (NBF) under UNEP-GEF projects: Algeria, Egypt, Iran, Jordan, Lebanon, Mauritania, Tunisia, and Yemen. Three countries will finish in 2006: Libya, Morocco and Syria. Oman and Saudi Arabia are developing NBFs without GEF support:. Within the West Asia North Africa region, Egypt and Iran have already developed an active biotech research and development programme. Biotech is in its early stages in Algeria, Morocco, Oman, S. Arabia, Syria, Tunisia and in Jordan, Lebanon, Libya, Mauritania, and Yemen biotech still needs to be developed.

The Syrian National Biosafety Committee (SNBC) was established by the Atomic Energy Commission of Syria in 1999 (3). The Committee is represented by members from various academic and research institutes. The Syrian biosafety guidelines have been established and were approved in the year 2001 (1). They contain guidelines for laboratories, glasshouse containment, small-scale field testing, and release into the environment. The Ministry of Agriculture is responsible for issuing certificates for the importation and release of GMO into the environment. The Ministry of Environment is responsible for implementing the Cartagena Protocol on Biosafety which Syria ratified on April 1st 2004. Syria completed its biosafety framework in cooperation and with financial assistance from UNEP – GEF in 2006. Under this framework government policies on biosafety were developed, including a regulatory regime for biosafety, a system to handle notifications or requests for authorizations, a system for monitoring environmental effects, and approaches for public information and public participation. ICARDA has received a permit to carry out transgenic research on its premises for its mandated crops.

Safety considerations of Living Modified Organisms (LMO)

Most of the controversy surrounding living modified organisms (LMOs) is the potential of gene transfer from a LMO to other related organisms. The most common example of that is the transfer of genes via pollen. One of the potential hazards of releasing LMOs into the environment is the hybridization of these plants with their wild relatives and the chance of transferring the new improved traits to weeds. However, the risks are not the only potential risks associated with genetically modified plants, also its impact on human health and the environment, and other socioeconomic impacts need to be considered.

Risk Assessment

In order to evaluate whether the introduction of an LMO into the environment poses a risks for that environment the CPB regulates the risk assessment on a case by case basis and according to scientific principles. The objective of risk assessment is to identify and evaluate the potential adverse effects of living modified organisms on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health. Risk assessment is used by competent authorities to make informed decisions regarding living modified organisms. A generally accepted methodology for biotechnology risk assessment has been outlined in several documents such as the Cartagena Protocol on Biosafety, or the EC Directive 2001/18/EEC. In the following section, we describe the stepwise methodology of risk assessment for an LMO according to the Cartagena Protocol (Table 1).

Stepwise methodology of risk assessment for an LMO, e.g. herbicide tolerance inhickpea.

1. Hazard identification: Identification of any novel genotypic and phenotypic characteristics associated with the LMO that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health;.
2. Likelihood estimation: Evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism; e.g. gene flow (outcrossing rates of 0-2% in soybean) and the presence of wild relatives.
3. Evaluation of the consequences: Evaluation of the consequences should these adverse effects be realized, e.g. fitness of the gene in the wild. Would a herbicide tolerance gene survive without the selection pressure of the herbicide application?
4. Estimation of overall risk: Estimation of the overall risk posed by the LMO based on the evaluation of the likelihood and consequences of the identified adverse effects being realized: e.g. low-moderate-high.
5. Risk management: Assessment as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks. e.g. planting dates, geographic differentiation etc.

Depending on the case, risk assessment takes into account the relevant technical and scientific details regarding the characteristics of the following subjects: (a) *Recipient organism or parental organisms.* The biological characteristics of the recipient organism or parental organisms, including information on taxonomic status, common name, origin, centres of origin and centres of genetic diversity, if known, and a description of the habitat where the organisms may persist or proliferate; (b) *Donor organism or organisms.* Taxonomic status and common name, source, and the relevant biological characteristics of the donor organisms; (c) *Vector*. Characteristics of the vector, including its identity, if any, and its source or origin, and its host range; (d) *Insert or inserts and/or characteristics of modification.* Genetic characteristics of the inserted nucleic acid and the function it specifies, and/or characteristics of the modification introduced; (e) *Living modified organism.* Identity of the living modified organism, and the differences between the biological characteristics of the living modified organism and those of the recipient organism or parental organisms; (f) *Detection and identification of the living modified organism.* Suggested detection and identification methods and their specificity, sensitivity and reliability; (g) *Information relating to the intended use.* Information relating to the intended use of the living modified organism, including new or changed use compared to the recipient organism or parental organisms; and (h) *Receiving environment.* Information on the location, geographical, climatic and ecological characteristics, including relevant information on biological diversity and centres of origin of the likely potential receiving environment.

Conclusions

In 2005, there were 90 Million ha grown to GM crops. The first generation GM crops focused on pest and disease control, but it is expected that in the next decade many more traits will be engineered. The next generation of crops involve innovations such as abiotic stress resistance (drought, heat, cold, salt), food composition (improved fatty acids, vitamins, micronutrients). Also, the number of GM crop species will expand rapidly and include other cereals, root and tuber crops, legumes etc.. In particular, public research institutes in developing countries that embrace the technology and scientists from these organisations are involved in developing alternative GM crops. ICARDA and other CGIAR centres are active in the development of drought tolerant mandated crops using transcription factors. Many of the developing countries are introducing and developing national biosafety frameworks that allows the handling and managing of LMOs. Scientific and other competent authorities will continue to apply risk assessment based on sound scientific principles to decide on the use and introduction of GM crops in their countries.

Table 1. An example of stepwise methodology of risk assessment for herbicide tolerance in soybean

Potential Adverse Effect	Estimation of Likelihood	Estimation of Consequence	Estimation of Overall Risk	Consideration of Risk Management
Weediness	Unlikely	Marginal	Low	Acceptable
Allergenicity	Not available	-	-	
Toxicity	Not available	-	-	
Unintenended effect on target organism	Unlikely	Marginal	Low	Acceptable
Effect on non target organism: Rhizobium	Unlikely	Minor	Low	Acceptable
Other	Highly likely	Major	High	
Categories	Likely	Intermediate	Moderate	
	Highly		Negligible	
Overal risk assessment for herbicide tolerance: acceptable				

الملخص

باوم، مايكل ومجدي مدكور. 2006. تطوير وتقييم مخاطر المحاصيل المحورة وراثياً. مجلة وقاية النبات العربية. 24: 178-181.

يعمل المركز الدولي للبحوث الزراعية في المناطق الجافة (إيكاردا) على استغلال إمكانية استخدام الهندسة الوراثية للحصول على نباتات محسنة متحملة للفطريات أو مقاومة للجفاف وللإجهادات غير الحيوية الأخرى. بدأ العمل بتحوير الحمص والعدس بالتعاون مع جامعة هانوفر (بألمانيا)، كما بدأ بالعمل في تحوير الحبوب (النجيليات) بالتعاون مع معهد البحوث الزراعية في الهندسة الوراثية (AGERI) ومركز التقانات الحيوية (CBS) في صفاقس بتونس. بمجرد الحصول على المنتجات الأولى لعملية التحوير الوراثي يجب أن يتم تقييم المخاطر وتحديد آلية أو استراتيجية محددة لإدارتها (لإدارة المخاطر). تتوضع دول الهلال الخصيب في مركز التنوع الوراثي للعديد من محاصيلنا الزراعية (الشعير، القمح، العدس والحمص) والتي تتميز أغلبها بأنها ذاتية الإخصاب وإن نسبة التلقيح الذاتي فيها منخفضة (من 0-2%). تجري دراسات لتحديد القيمة الدقيقة لتدفق المورثات وذلك بهدف تطوير العلم المعتمد على آلية تقييم المخاطر. تتطلب عملية نشر المحاصيل المحورة إلى مناطق خارج مركز نشوء التنوع الوراثي (مثل شمال أفريقيا وجنوب آسيا) اتباع استراتيجية مختلفة. لهذا السبب، يتم تشجيع ودعم خطة عمل للأمان الحيوي ولشروط الأمان الحيوي التي تسمح باختبار المحاصيل المحورة وراثياً في دول خارج منطقة مركز نشوء التنوع الوراثي.

كلمات مفتاحية: تقانات حيوية، الأمان الحيوي، هندسة وراثية، تشريعات.

عنوان المراسلة: مايكل باوم، المركز الدولي للبحوث الزراعية في المناطق الجافة، ص.ب. 5466، حلب، سورية، البريد الإلكتروني: m.baum@cgiar.org

References

1. **Al-Safadi, B.** 2006. The Biosafety Regulatory System in Syria. Current status, Perspective and Challenges. FAO-ICARDA Expert Meeting, Harnessing Biotechnology and Genetic Engineering for Agricultural Development in the Near East and North Africa (NENA), Helnan Shepherd Hotel, Cairo, Egypt, February 12-14, 2006.
2. **Biosafety Guidelines.** 2001. National Biosafety Committee, Atomic Energy Commission, Damascus, Syria.
3. **Böttinger, P., A. Steinmetz, O. Schieder and T. Picardt.** 2001. *Agrobacterium* mediated transformation of *Vicia faba*. Molecular Breeding, 8: 243-254.
4. **Cartagena Protocol on Biosafety**. 2005. Status of Ratification and Entry Into Force. http://www.biodiv.org/biosafety.
5. **Cohen, J.I** 2005. Poorer nations turn to publicly developed GM crops. Nature Biotechnology, 23(1): 27-33.
6. **James, C.** 2006. Global Status of Commercialized Biotech/GM Crops: ISAAA Briefs No. 34-2005.
7. **Krishnamurthy, K.V., K. Suhasini, A.P. Sagare, M. Meixner, A. de Kathen, T. Picardt and O. Shieder.** 2000. *Agrobacterium* mediated transformation of chickpea (*Cicer arietinum* L.) embryo axes. Plant Cell Reports, 19:235-240.
8. **Li, H., S.J. Wylie and M.G.K. Jones.** 2000. Transgenic yellow lupin (*Lupinus luteus* L.). Plant Cell Reports, 19:634-637.
9. **Sarker, R.H., A. Biswas, B.M. Mustafa, S. Mahbub and M.I. Hoque.** 2003. *Agrobacterium*-mediated transformation of lentil (*Lens culinaris* Medik.). Plant Tissue Culture, 13(1): 1-12.
10. **Schroeder, H.E., A.H., Schotz, T. Wardley-Richardson, D. Spencer and T.J.V. Higgins.** 1993. Transformation and Regeneration of two cultivars of pea (*Pisum sativum* L.). Plant Physiology, 101: 751-757.
11. **Twyman, R.M., S. Schillberg and R. Fischer.** 2005. Transgenic plants in the biopharmaceutical market. Expert Opin Emerg Drugs, 10: 185-218.
12. **United Nations Environment Program.** 2003. UNEP-GEF BIOSAFETY PROJECTS. UNEP-GEF Biosafety Unit. http://www.unep.ch/biosafety