

Guest Editorial

Trends in biotechnology and biosafety in Brazil

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Biotechnology is a Brazilian priority, and has been recognized for its potential to promote sustainable development. The Government recently announced an ambitious program for Science and Technology, which includes strategies to develop modern biotechnology, continuing three decades of public investments on capacity building and infrastructure, aimed principally at the development of technologies applied to health, agriculture and the environment (MCT, 2008). Research initiatives have focused on genomics, proteomics, genetically modified organisms (GMOs), gene therapy, stem cells, bio-fuels and nanotechnology, among other biotechnological topics. Research projects in Brazil have been mainly developed in public universities and institutions funded by federal and state agencies, with a minor participation from the private sector (Silveira et al., 2004). Genomics, an area of considerable success in the country, was launched a decade ago by S. Paulo State Research Foundation (FAPESP), with the organization of a virtual institute, called ONSA, comprising several laboratories with the main task of sequencing the genome of the citrus pathogenic bacterium *Xylella fastidiosa* (Simpson et al., 2000).

The success of this genomic network stimulated biotechnology startup companies and projects with the focus on other genomes, such as sugarcane and coffee, including functional genomics and proteomics. Following in the footsteps of the ONSA network, the Ministry of Science and Technology created a National Genome Project Consortium involving institutions located in the major regions of the country, with the task of sequencing eight microbial and two plant genomes. Recently, they concluded the sequence of *Chromobacterium violaceum*,

a bacterium with exploitable properties, such as the ability to produce a bactericidal purple pigment (violacein) and bioplastics (Vasconcelos et al., 2003). Later on, several states launched their own genome programs. A group from Rio de Janeiro, part of the Riogene network, recently sequenced the genome of the nitrogen-fixing bacterium *Gluconoacetobacter diazotrophicus*, a sugarcane endophyte involved in enhancing growth of large crops without the addition of nitrogen fertilizer (EMBRAPA, 2008; MCT, 2008).

Agriculture plays an important role in the Brazilian economy, being responsible for ca. 40% of the exports and employing 20% of the active work force. About one third of the Brazilian GDP comes from agribusiness. Traditionally, this country has been competitive in tropical agriculture, supported by strong research programs on conventional and modern technologies. Intense capacity-building initiatives resulted in the formation of a critical mass of scientists working in molecular biology and agricultural sciences (Silveira et al., 2004). Despite these favorable factors, the adoption of GM crops has been delayed due to intense opposition organized by environmental groups and additional difficulties resulting from a conflicting regulatory framework. In this overview, we address the current status of Brazilian biosafety legislation, and discuss the perspectives for the development of molecular biotechnology in Brazil.

BRAZILIAN BIOSAFETY FRAMEWORK

A Legal Biosafety Framework has been in place since 1995, setting the standards for controlling the development, cultivation, manipulation, transportation, marketing, consumption, release and disposal of GMOs, with

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the objective of protecting human health and the environment, in compliance with the precautionary principle. Activities with GMOs are only allowed in established institutions after authorization by the National Biosafety Technical Commission (CTNBio). This regulatory commission is a multidisciplinary consulting and deliberative body, established under the Ministry of Science and Technology, providing technical and advisory support to the Federal Government for the implementation of the national biosafety policy and for elaboration of law-abiding instruments regulating all activities with GMOs and products thereof. Recently, a new Biosafety Law came into force, aiming to improve the harmonization with the legal instruments of other branches of government (Law 11.505/05, Decree 5591/05; Legislação Brasileira, 2005). Relevant modifications were introduced by this new law, including the nomination of a National Biosafety Council (CNBS), establishment of an Information System on Biosafety (SIB), regulations for the use of stem cells and the prohibition of genetic use restriction technologies (GURTs). Genetic modification of embryonic stem cells and cloning of humans remain prohibited. The provision for penalties related to non-compliance with the Biosafety law was maintained. The CNBS includes representatives of the Ministries responsible for proposing Biosafety Policy and for evaluation of GM products, taking into account socio-economic issues related to their commercialization. The CTNBio is composed of 27 members and their respective deputies, with broad representation from: the scientific community (12), government (Science and Technology, Health, Environment, Agriculture and Supply, Agrarian Development, Education, Defense, Development Industry and Foreign Trade, Foreign Relations, Secretary of Aquaculture and Fisheries) and specialists on health, occupational health, consumers, the environment, family farmers and the biotechnology sector. The CTNBio has the following main responsibilities: issue Biosafety Certificates (CQB), establish procedures for institutional Biosafety Committees (CIBio), establish GMO risk levels, request environmental studies, approve projects, provide technical support to inspection agencies, authorize GMO importations, and advise the ministerial council. The CTNBio members are designated by the Minister of Science and Technology and by Ministries for the respective representatives, based on their professional expertise. The CTNBio has been operationally impaired due to conflicts of legal interpretation between the Biosafety Law and other regulatory instruments. Additional difficulty arises from the high turnover of its members, who serve on a voluntary basis, and also endure considerable stress due to the polarized debate on transgenic crops, with constant press coverage and frequent legal actions (Fontes, 2003; Mendonça-Hagler and Aleixo, 2002). The CTNBio representatives

have been advising the government on the negotiations of international agreements, such as the Biosafety Protocol and the Codex Alimentarius (CBD, 2003; WHO, 2003).

FIELD RELEASES OF GM PLANTS

Over 1500 petitions have been approved for field releases of genetically modified (GM) plants, comprising more than one thousand hectares. Risk assessment analysis, done in a case-by-case and stepwise basis, used criteria recommended by the United Nations Environment Program Guidelines (UNEP, 1995), Edmonds Institute (1998), pertinent documents and the scientific literature. The majority of field releases of GM plants were: corn (85% of releases), soybean (7%), cotton (5%), sugarcane (2%), beans, *Eucalyptus*, potato, rice, papaya and tobacco (ca. 1%). The main traits inserted in these GM plants were herbicide tolerance (HT) 55%, insect resistance 42%, stacked genes (HT+IR) 2%, and virus resistance (VR) 1% (Mendonça-Hagler and Aleixo, 2002; Mendonça-Hagler and Oda, 2004; Mendonça-Hagler et al., 2006a). GM plants with other traits such as nutritional enrichment, lower lignin content (*Eucalyptus*), resistance to drought, tolerance to saline soils, high-sucrose sugarcane, and the expression of pharmaceuticals, are under research and development.

COMMERCIALIZATION OF GMOS

The commercialization of glyphosate-tolerant (Roundup Ready[®]) soybean was approved by CTNBio in 1998, with the requirement for post-market monitoring (Fig. 1). GM soy has been legally cultivated in Brazil for the last five years, after a long legal battle. Recent data indicate that 57% of the Brazilian soybean is transgenic, representing more than one tenth of the global transgenic crops. Insect-resistant cotton (Bollgard[®] cotton event 531) was the next crop approved (2005), with requirements of restriction zones, mandatory use of refuge areas with non-GM cotton, and additional measures for confinement (Fig. 1). The situation for GM corn has been controversial, due to the high diversity of landraces found in Brazil. Several petitions to commercialize GM corn were under evaluation during several years. Recently, three GM corn events were approved: glufosinate-tolerance event T-25 (Bayer CropScience Liberty Link[®]), insect-resistance Bt Mon 810 (Monsanto YieldGard[®]) and event Bt-11 (Syngenta Seeds) (CTNBio, 2008). Deregulation processes occurred under strong opposition by small property farmers and environmental groups. GM Liberty Link[®] and YieldGard[®] corn were submitted to further analysis by the superior council of the CNBS, in order to evaluate social and economic issues, and their

RoundUp Ready[®] Soybean (HT)

- Monitoring for five years with annual reports
- Inclusion of monitoring in representative areas of soybean production in Brazil
- Study of population dynamics of weeds and seeds in the soil
- Study of population dynamics of insects, plant pathogens, and microorganisms
- Assessment of gene transfer to compatible plants
- Assessment of gene transfer to soil microorganisms
- Assessment of glyphosate environmental impacts

***Bt* Cotton (Bollgard[®]) (IR)**

- Availability to regulators of the primer sequences for detecting the event
- Respect the exclusion zones defined by Barroso et al. (2005)
- Limitation of the crop time in cotton producing regions
- Use of refuge areas with non-GM cotton (20% of *Bt* cotton crop)
- Adoption of conservationist practices for crops (destruction of the rootstock, burn to control diseases, crop rotation, employment of trap cultures and biological control)

GM Corn: events T-25 glufosinate (HT), *Bt* Mon 810 (IR) and Bt-11 (IR+HT)

- Monitoring programs are under definition
- Co-existence measures required (CTNBio, 2008)

Figure 1. Main requirements for post-market environmental monitoring of GM crops in Brazil.

authorization was ratified, signaling a positive scenario for GM crops in Brazil.

All GM crops approved in this country have been cultivated abroad, therefore the information needed for risk assessment obtained from other countries was included in the locally submitted dossiers, and complemented by results of experiments performed in the receiving environments. These were primarily tests to assess GM cultivar agronomical features, the expression of the inserted transgenes, and their field performance in controlling targeted insects or weeds. Local environmental studies on non-target effects are scant (Faria et al., 2001; Fernandes, 2003; Fernandes et al., 2007; Frizzas, 2003; Martinelli, 2001; Teston et al., 2004). Typically, the commercialization of transgenic crops has been followed by protests from environmental authorities and NGOs, and legal actions alleging insufficient data related to impacts on regional biodiversity and ecosystem functioning. Moreover, the right of farmers to exercise their freedom of choice on the adoption of different production systems has been a strong argument in favor of the implementation of co-existence measures. Commercialization of the GM maize was approved with a requirement for post-market monitoring and compliance with co-existence regulatory norms. In Brazil, no GM crops are allowed in officially recognized preservation areas and Indian reservations (CTNBio, 2008). The development of transgenic plants, used as food and modified to produce pharmaceuticals (hormones, vaccines, etc.) increased local concerns related to GM crops (Devos et al., 2005; Fontes, 2007; Jank et al., 2006; Schiemann, 2003). Usually, transgenic seeds have been planted in Brazil before the granting of their commercial legal status. This happened with the herbicide-tolerant soybean, *Bt* cotton, and most likely the same trend is repeating with GM corn. This situation led to the export of illegally grown GM soybean, authorized by the government for two seasons, due to the economic reality represented by the extent of GM grains harvested. Also, GM cotton was found among conventional cotton seeds, forcing regulatory authorities to allow an upper limit of 1% for the adventitious presence of GM seeds, with the restriction of no cultivation of these seeds in the exclusion areas, where sexually compatible native species can be found (Barroso et al., 2005; CTNBio, 2008).

RISK ASSESSMENT OF GM PLANTS

Glyphosate-tolerant soybean

Risk analysis of GM Roundup Ready® soybean (Monsanto) was based mainly on the following elements: soybean is an exotic plant with no known wild relatives in Brazil; it is predominantly self-pollinated with low

out-crossing rate; it is a domesticated species, not expected to survive outside agro-ecosystems. Moreover, in the absence of selective pressure, the expression of the herbicide-tolerance gene was not expected to increase plant fitness, and the transgenic insertion was well characterized. No significant changes in the profile and populations of insects associated with the conventional soybean were expected for GM crops. The environmental data, presented by the company, were based on studies done in other countries. For that reason, the CTNBio required a monitoring program to be performed in representative production areas to detect possible adverse environmental effects (Fig. 1).

Bt cotton

The commercialization of Bollgard® Cotton event 531 (Monsanto), resistant to the main Lepidoptera pests affecting cotton in Brazil (cotton leafworm *Alabama argillacea*, pink bollworm *Pectinophora gossypiella* and tobacco budworm *Heliothis virescens*), expressing the transgenes *cry1Ac* and *nptII* was approved. The *Bt*-cotton risk assessment considered primarily the following criteria: the insecticidal protein Cry1Ac, produced by *Bacillus thuringiensis* is a bio-pesticide on the local market for decades; activity of the Cry1Ac protein is specific to certain species of Lepidoptera; NPTII protein is detected in several bacterial species in the environment and in human intestines; horizontal gene transfer from plant to bacteria is considered a rare event, thus representing a low risk (Kay et al., 2002; Smalla, 2000). The gene insertion was reported to have no effect on the quality of the cotton fibers. Also, a reduction in the use of insecticides promoted by the use of *Bt* cotton was expected to be significant; the safety for human and animal consumption of *Bt* cotton considered the products had similar nutrient contents as non-GM cultivars. The human consumption of cotton products is limited to cottonseed oil, and the introduced gene products are not detectable in the refined oil produced from *Bt* cotton. Bollgard® cotton was approved for commercialization under the conditions listed in Figure 1 (CTNBio, 2008).

GM corn

GM corn events (Glufosinate-tolerant event T-25 with the *pat* gene, YieldGard® *Bt* corn MON810, with the *Cry1Ab* gene, and *Bt* corn-11 with *cry1Ab* and *pat* genes) were recently approved for commercialization, after long regulatory processes, which included hundreds of previous field tests. Risk assessments were done, case by case, taking into account the scientific literature on the molecular characterizations of the events, familiarity with these

transgenic crops in different environments, and the history of safe use of the respective GM corn for human and animal consumption (Bruisma et al., 2002; Firbank, 2003; Gressel, 2000; Jesse and Obrycki, 2000; Losey et al., 1999; Saxena et al., 1999; WHO, 2003). The effects of *Bt* corn on insect natural enemies and the diversity of non-targeted organisms, reported from experiments done in Brazil, complemented the information submitted to regulatory authorities (Faria et al., 2001; Fernandes, 2003; Fernandes et al., 2007; Frizas, 2003; Marochi and Santos, 2002; Martinelli, 2001). Typically, these studies showed the efficiency of *Bt* corn in controlling the target insects, with no detected significant interference on the population dynamics of other insects. Even though Brazil is not a center of diversity for corn, there is a great deal of concern regarding the possible gene flow from GM crops to landraces, with the aim of protecting their genetic diversity.

Food safety evaluation

Production, import and marketing of GMOs and their by-products, which are intended to be used as food, feed or processing, are routinely evaluated for their safety. Basically, the substantial equivalence concept has been applied to the food safety evaluation (Tomlison, 2000), complemented by actual recommendations emanated from the Codex Alimentarius (WHO, 2003). During a local shortage of corn, the food safety of transgenic grain shipments intended for feed was evaluated under considerable opposition from environmental groups. GM cargos were transported under the jurisdiction of the Ministry of Agriculture from the port of entry directly to the milling factory. Also, several GM microbes, intended for contained use in industrial processes to produce enzymes, food ingredients, and other consumables were approved and are available on the local market.

LABELING OF GMOS

Packed food containing GMOs and their by-products, at the concentration of 1% or higher, are required to be labeled as “genetically modified (product)” or “contains genetically modified (ingredient)”, according to Decree 4680/03. A symbol for transgenic content, a triangle with a T on a yellow background, was designated. The regulation also applies to the unintended presence of GMOs in food products. In the scope of this legal instrument, labeling is perceived as a consumer’s right to have information and not being related to food safety *per se*, although the yellow triangle sign can be misleading, since it is associated with a warning for “caution”. To date, this Decree

has not been enforced by the legal authorities, and products labeled “genetically modified” usually are not found on the market (CTNBio, 2008; Mendonça-Hagler et al., 2006a).

CAPACITY BUILDING ON BIOSAFETY

The implementation of the Biosafety Law increased the awareness of general safety and risk assessment issues in Brazil. Regulators were stimulated to be updated continuously in the area by attending courses, workshops, and conferences at both the national and international levels. They also delivered training during technical visits to institutions requesting Biosafety Certificates, and organized several events on Biosafety topics, co-sponsored by universities and scientific societies. Sensing the demand for capacity-building on Biosafety and related areas, pioneer regulators founded the Brazilian Biosafety Association (ANBio). This scientific society has been involved in the development of biosafety in Latin America by organizing several events, and contributing to the implementation of the first post-graduate course focused in the area. These initiatives were followed by a larger capacity-building program on biosafety, sponsored by the Brazilian National Research Council (CNPq). This program had a broad scope, and included 15 universities offering biotechnology courses, mostly concentrated in the South and Southeast regions (Oda et al., 2008). Other initiatives are under implementation at universities and public research institutes. Stakeholders involved with GMO activities in Brazil are required to have biosafety training at the level pertinent to their work.

CONCLUDING REMARKS

Brazil has an operational Biosafety Legal Framework, compatible with the development of modern biotechnology, sustainable use of its rich biodiversity, preservation of ecosystems and human health. The country adopted a multidisciplinary Advisory Biosafety Commission (CTNBio), to handle regulatory and administrative matters. Since 2005, GM commercialization can be subjected to ratification by a superior council to evaluate social economic aspects. To date, over two hundred institutions engaged in GMO activities have been granted a Biosafety Quality Certificate (CQB). The Commission continues to face several challenges, especially related to the negative public perception associated with transgenic plants. The current status of GM technology has issues for concern: for instance, the long time to review petitions, illegal use of seeds, the prohibition of GURTs, and restrictive labeling of GM products. Despite these drawbacks, Brazil is ranked third in cultivation of GM crops,

after the USA and Argentina (James, 2007), representing 10% of the global GM cultivated areas. Although legal battles may occur before GM corn can be grown by Brazilian farmers, this will be the next transgenic crop in Brazil, since herbicide-tolerant (HT), *Bt* corn (IR) and (IR+HT) corn events have been approved recently by the regulatory authorities. After soybean, corn is the second largest crop grown in the country, with a high internal demand as the main commodity for livestock production chains. However, there are frequent objections to import of GM corn. Deregulation of GM rice is under evaluation.

Food products containing GMOs or derivatives are legally subject to labeling, nevertheless transgenic labels are rarely found on marketed GM products. In contrast to GM crops, recombinant products applied to health and industry have been generally accepted by Brazilian consumers. Apparently, there is less awareness concerning molecular biotechnology products available on the market of health and other consumer goods, such as enzymes produced by GM microbes that often used in food, detergents, etc. (Mendonça-Hagler et al., 2006b). Usually, the potential benefits of biotechnology for the improvement of crop yields in developing countries are not taken into full account by local governments (Taylor and Fouquet, 2000). The recent deregulation of GM corn is a clear tendency toward the acceptance of agro-biotechnology in Brazil. Moreover, a recent research and development program reinforces biotechnology as an area of interest to receive public investment. Locally developed transgenics are in the pipeline. A great deal of governmental incentives have been given to bio-fuel technology. In this area, Brazil disputes with the USA the first place in the production of bio-ethanol (MCT, 2008). Also, the country established legal instruments to protect intellectual property rights, becoming more attractive to foreign investments. Taking into account this overall Brazilian scenario comprising a consolidated Biosafety Legal Framework and proactive public policies fostering research, development and innovation, a substantial development of agro-biotechnology can be expected to happen in the near future.

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