

Chapter 16

The Importance of *Bacillus thuringiensis* in the Context of Genetically Modified Plants in Brazil

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Abstract Sustainable agriculture requires alternative interventions for pest control and management. In this context the use of microorganisms pathogenic to pests has become even more studied and widespread, especially in the successful case of bioinsecticides based on *Bacillus thuringiensis*. With advances in recombinant DNA biotechnology, *B. thuringiensis* has continued to show its potential with the insertion of its insecticide-encoding genes into plants, which thus become resistant to a varied range of pest insects. These Bt plants, often containing multiple Bt genes, are commercially available. And today, after assessing biosafety in several countries around the world, they are adequate to control pests without significant harm to humans or to the environment. If the required safety conditions are maintained, a greater use of these plants is anticipated, guaranteeing an effective tool for an environmentally friendly agriculture.

Keywords Biosafety • Bt plants • Agriculture

Insect pest control in the field has been done with chemical insecticides since the early 1940s. These insecticides controlled many pests but were associated with environmental problems, toxicity to humans and animals, and were not very specific, affecting “nontarget” organisms. Twenty-first-century sustainable agriculture increasingly requires alternative interventions for pest control and management, ones that are safe and, if possible, reduce human contact with pesticides. As an option, the use of microorganisms pathogenic to pests (insects or invasive plants) has become more studied and disseminated, proving to be safer and increasing the activity of other natural enemies. Such was the successful case using bioinsecticides based on *Bacillus thuringiensis* (Bt).

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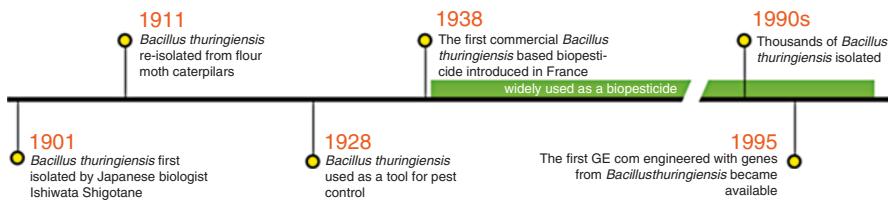


Fig. 16.1 Advances in the use of *Bacillus thuringiensis* for control of agricultural pests in the twentieth century as presented by Niederhuber (2015)

To achieve successful biological control, it was necessary, over time, to search for, among many aspects, a greater number of insect pathogenic strains, a wider range of action respect to target organisms, a higher yield in toxin production, and better characteristics for fermentation process. In addition, several Bt strains have been improved using recombinant DNA technologies.

Regarding the use of biotechnology, it has been systematically applied in the improvement of agriculture since the beginning of the twentieth century, with the development of cell and plant tissue culture, followed by the rescue of immature embryos in vitro (1930s), mutagenesis and selection (1940s), anther culture (1950s), somaclonal variation (1960s), recombinant DNA technology or genetic engineering (1970s), selection assisted by molecular markers (1980s), and genomics, proteomics, metabolomics, and bioinformatics (1990s).

Figure 16.1 summarizes the advances in the use of Bt and the development of GM plants throughout the twentieth century (Niederhuber 2015).

In the twenty-first century, innovations in biotechnology have continued to leverage advances in agriculture, including RNA interference (RNAi) and transgenic technology and, much more recently, genome editing. Several of these utilize new methodologies for genetic improvement, and a summary of them can be found in Whelan and Lema (2015).

With so many new techniques since the advent of recombinant DNA technology, it has become possible to alter parts of the genome of any organism in order to favor desired traits (Arantes 2003). The said modified organism is called a genetically modified organism – GMO – according to Art. 3, section V, of the Brazilian Federal Law No. 11,105/2005 – “available in <<http://www.ctnbiobr.gov.br/index.php/content/view/11992.html>>”. It is important to mention that “transgenesis” is a particular case of genetic modification in which a DNA sequence, total or partial, of an (exogenous) organism is transferred to another organism of a species other than its own, thus sexually incompatible. “Cysgenesis” uses the same transgenic techniques, but DNA transfer occurs in the same species or between species that cross in the wild. Although not synonyms, the terms have been grouped and referred to in this chapter under the designation of GMOs. Other designations may also be cited, such as LMOs (for living modified organisms, adopted by the Cartagena Protocol), GMF for GM food, GMA for GM animal, GMV for GM vegetable, and GMM for GM microorganism”.

This has led to a significant change in the technological standards of various sectors, including agriculture, and, as a consequence, the entire production chain related

to it. Thus, the main contribution of modern biotechnology to agriculture was the possibility of creating new varieties from the transfer of genes, including between two distinct species, aiming to aggregate an attribute of economic interest, such as:

- (a) Plants resistant to biotic (pathogens, insect pests, and weeds) and abiotic stresses (salinity, dryness, cold, flood, heat)
- (b) Improving the quality of agricultural products such as extended shelf or storage life; differential or biofortified nutritional content (proteins, fibers, oils, carbohydrates, vitamins/minerals, and phytochemicals); removal of antinutrients, allergens, and/or toxins; and better quality of wood fiber
- (c) Plants used as bioreactors to obtain drugs and/or industrial products
- (d) Faster-growing plants
- (e) Plants with greater efficiency in the conversion of biomass, in the use of water and/or nitrogen fixation of the soil
- (f) Plants with greater potential for biofuel production and phytoremediation

According to James (2015), the global cultivated area between 1996 and 2015 for GM plants was approximately 2 billion hectares. Of this total, 1 billion hectares were planted with soybean, 0.6 billion hectares with maize, 0.3 billion hectares with cotton, and 0.1 billion hectares with canola. The United States continues to lead adoption with 70.9 million hectares (39% of the global). Brazil is the second largest producer, with 44.2 million hectares planted (representing 25% of the global). Another interesting fact is that about 20 million farmers are involved in this production, wherein ~90% of them are small farmers distributed in 28 countries, of which 20 are developing countries and only 8 are industrialized countries.

16.1 Bt Plants

Since the late 1990s it has been possible to insert *cry* ("the *cry* gene is the gene that codes for the formation of the toxic protein Cry responsible for the insecticidal activity of Bt") genes into plants, forming the so-called Bt plants. These plants produce their own Cry proteins, protecting them from the attack of insects that are susceptible to such protein. The production of this plant occurs initially in the laboratory and passes through monitored processes of release in greenhouse and field and later by processes of commercial liberation denominated biosafety processes (Capalbo et al. 2014).

Prior to being introduced into a plant, the *cry* genes must be altered in their DNA sequence by site-directed mutagenesis. This is necessary so that the differences in the mechanisms of expression between prokaryotic and eukaryotic organisms do not block or decrease gene expression (Capalbo et al. 2005).

The advances are continuous, and the perspectives expand with each new technique or added knowledge. Thus many Bt plants have emerged safer and focused not only on insect control but also on the conservation of established ecological conditions in the growing areas. Researchers have developed plants in which proteins can be expressed only where and when necessary through the use of specific

tissue promoters, specific time, or inducible promoter genes. These and other precautions are taken to minimize the development of insect resistance to Cry proteins and the gene flow to wild varieties, since in the Bt plant the toxin is available in its active form rather than in the form of protein crystal.

When the first Bt plants were commercialized, the use of a refuge area was suggested or required for greater resistance control. For the use of Bt maize in Brazil, the producer must comply with two rules: the coexistence, required by law, and the Insect Resistance Management (IRM) rule recommended by the National Technical Biosafety Commission (CTNBio). This means that a 100 m border should be used to isolate transgenic corn crops from other crops. Some alternatives are offered with respect to border size if rows of non-transgenic maize of equal size and in the same cycle of transgenic maize are sown. Obviously, monitoring of plant infestation is also important because, depending on the non-GM hybrid and infestation intensity, the producer may need to adopt complementary control measures. For fall army-worm the refuge area should not be more than 800 m away from transgenic plants. This is the maximum verified distance for the dispersal of adults in the field. Also, according to the CTNBio recommendation, in the area of refuge the use of other control methods is allowed, so long as Bt-based bioinsecticides are not used. Further details on minimum distances between GM and non-GM maize commercial crops, aiming at coexistence between production systems, can be obtained in Mendes et al. (2009) and CTNBio (2007).

The non-Bt refuge area generally means a loss in production since it will be intensely available to insect attack. Everything becomes even more difficult when crop rotation is considered throughout the year in planted areas, and additional care must be taken to ensure that the same area is not cultivated with the same Bt gene inserted in different crops, which would intensify exposure to the toxin and the likelihood of developing resistance, endangering this important ally.

In the search for alternative solutions to resistance development, new technologies were developed expressing more than one Bt protein. These technologies are called pyramiding, stacking, or second generation (ISAAA 2013), referring to the process of combining two or more genes of interest in the same plant. An example of such plants is a soybean or maize that expresses two or more Bt genes whose proteins have different modes of action. But they may also be plants expressing one gene for insect resistance and another for herbicide tolerance. A list of stacked products that are available on the world market can be found in the Center for Environmental Risk Assessment (CERA) database ([Center for Environmental Risk Assessment](#)).

From the point of view of science and plant breeding, it is desirable to be able to insert several characteristics simultaneously at the same *locus*; this action is known as “molecular stacking.” In these new events, the inserted characteristics will behave essentially as a single gene, making the introgression of the new characteristic (or set of them) much simpler (Que 2010).

From the standpoint of the rural producer, the “staked” plant, when compared to the single-trait variety, offers a wider opportunity to overcome various problems in the field, such as pest insects, invasive plants, plant diseases, and environmental stresses, which favors the increase of productivity at the field level (ISAAA 2013).

16.2 Security of Bt Plants and Their Adoption

Brazil is a country with great potential for the development of agricultural biotechnology, it has great biological diversity, and it is a large potential source of natural molecules for different purposes. Among the developing countries, Brazil stands out because it has a strong national agricultural research system, consolidated after many years of scientific research aimed at making better use of its natural diversity: tropical and subtropical climates, ecosystems with agricultural aptitude, and germplasm selected and adapted for great variability. The diffusion of GMOs into Brazilian agriculture is only inferior to the United States, due to the robust and workable regulatory process in place since 2005 (in Brazil, authorization for experimental and commercial planting is obtained from the National Biosafety Commission – CTNBio), which ensures the safe use and practice of GMOs.

In order to make biotechnology products safely available to society, a biosafety analysis of the products generated must be rigorously structured and executed. This analysis measures potential risks and their probability of occurrence. As the potential risks include both environmental aspects and effects on human and animal health, the biosafety assessment of transgenic plants considers the two aspects together. The term “biosafety” in its broader application refers to the actions of prevention, minimization, or elimination of risks inherent to the activities of research, production, teaching, technological development, and provision of services that may compromise the health of humans, animals, and plants or the quality of the work carried out (Teixeira and Valle 1996).

In its narrower sense, linked to the legal framework that supports the use of recombinant DNA technology in Brazil, the term biosafety has been applied to the care of the protection of humans and the environment when GMOs and their derivatives are involved. The Brazilian biosafety legislation in force – Law No. 11,105 of March 24, 2005, which replaced Law No. 8974, of January 5, 1995 – establishes safety standards and mechanisms to supervise activities involving GMOs and their derivatives, in addition to regulating items II, IV, and V of paragraph 10 of article 225 of the Federal Constitution, which deals with the protection of the environment.

It is not the intention in this chapter to detail these regulations nor the way in which risk analysis of GMOs is conducted for biosafety purposes, but it is very important to point out that there are specific regulations for such an analysis and there are also bodies responsible for standards and their monitoring, both with regard to human and animal health and with regard to environmental issues (Capalbo et al. 2005, 2009, 2015; Hagler et al. 2010).

CTNBio is a multidisciplinary collegiate body, created through the same Law No. 11,105, whose purpose is to provide technical advisory support and advice to the Federal Government in the formulation, updating, and implementation of the National Biosafety Policy on GMOs, as well as in establishing technical safety standards and technical advice concerning the protection of human health, living organisms, and the environment for activities involving the construction, testing, cultivation, handling, transportation, commercialization, consumption, storage, release, and disposal of GMOs and derivatives.

16.3 Bt Plants in Brazil

The Bt plants approved by CTNBio for commercialization in Brazil are indicated in Table 16.1, along with pyramid (or stacked) Bt plants. More information on other events that are not listed in the Table can be found in the CTNBio database (

16.4 Bt Plants in the Rest of the World

Table 16.2 lists the countries that commercially produced Bt plants between 1996 and 2015, according to the ISAAA GM Approval Database.

16.5 Final Considerations

Bacillus thuringiensis presents more than 100 years of action in the control of agricultural pests. Even with the relatively recent development of genetically engineered modifications, Bt remains one of the most effective and safe control agents. Bt is a highly effective control agent as a biopesticide and meets the demands for use in biotechnological applications (as in the generation of Bt plants). The action of Bt toxins expressed in Bt plants is specifically directed toward the target insect, which has shown less environmental impact than methods that do not use Bt.

Bt plants possess advantages when used correctly in compliance with regulations and technical guidelines. In these cases, they have been very advantageous by virtue of:

- Facilitating pest management by allowing the farmer a longer protection during plant growth and reducing the handling and application of toxic insecticides.

Table 16.1 Summary table of genetically modified plants containing Bt gene, authorized by CTNBio for commercialization in Brazil, until August 15, 2016

Product	Commercial name	Donor organism	Characteristic ^a	Protein inserted	Applicant	Approval year
Soybean	Intacta RR2 PRO	Agrobacterium tumefaciens/Bacillus thuringiensis	TH and RI	CP4-EPSPS Cry1Ac	Monsanto	2010
	b	Bacillus thuringiensis/Streptomyces viridochromogenes	TH and RI	Cry1Ac Cry1F PAT	Dow AgroSciences	2016
Maize	YieldGard TL	<i>Bacillus thuringiensis</i> Bacillus thuringiensis/Streptomyces viridochromogenes	RI TH and RI	Cry1Ab Cry1Ab PAT	Monsanto Syngenta	2007
	Herculex	Bacillus thuringiensis/Streptomyces viridochromogenes	TH and RI	Cry1F PAT	DuPont and Dow AgroSciences	2008
YR YieldGard/RR2 TL/TG	YR YieldGard/RR2	Agrobacterium tumefaciens/Bacillus thuringiensis	TH and RI	CP4-EPSPS Cry1Ab	Monsanto	2009
		Bacillus thuringiensis/Streptomyces viridochromogenes/Zea mays thuringiensis	TH and RI	Cry1Ab PAT	Syngenta	2009
Viptera-MIR162 HR Herculex/RR2	Viptera-MIR162	<i>Bacillus thuringiensis</i>	RI	mEPSPS	Syngenta	2009
	HR Herculex/RR2	Bacillus thuringiensis/Streptomyces viridochromogenes/Agrobacterium tumefaciens	TH and RI	VIP3Aa20 Cry1F PAT	DuPont	2009
Pro Bt				CP4-EPSPS		
				Cry1A.105 Cry2Ab2	Monsanto	2009
TL TG Viptera				Cry1Ab VIP3Aa20 mEPSPS	Syngenta	2010

(continued)

Table 16.1 (continued)

Product	Commercial name	Donor organism	Characteristic ^a	Protein inserted	Applicant	Approval year
Maize Pro2	Bacillus thuringiensis/Agrobacterium tumefaciens	TH and RI	Cry1A.105 Cry2Ab2	Monsanto	2010	
YieldGard VT	Agrobacterium tumefaciens/Bacillus thuringiensis	TH and RI	CP4-EPPSPS Cry3Bb1	Monsanto	2010	
Power Core PW/Dow	Bacillus thuringiensis/Streptomyces viridochromogenes/Agrobacterium tumefaciens	TH and RI	Cry1A.105 Cry2Ab2 Cry1F PAT	Monsanto and Dow AgroSciences	2010	
HX YG RR2	Bacillus thuringiensis/Streptomyces viridochromogenes/Agrobacterium tumefaciens	TH and RI	CP4-EPPSPS Cry1Ab Cry1F PAT	DuPont	2011	
TC1507xMON810	Bacillus thuringiensis/Streptomyces viridochromogenes	TH and RI	CP4EPPSPS Cry1F Cry1Ab PAT	DuPont	2011	
MON89034 × MON88017	Bacillus thuringiensis/Agrobacterium tumefaciens	TH and RI	Cry1A.105 Cry2Ab2 Cry3Bb1 CP4-EPPSPS	Monsanto	2011	
Herculex XTRA™ maize	Bacillus thuringiensis/Streptomyces viridochromogenes	TH and RI	Cry1F PAT Cry34Ab1 Cry35Ab1	DuPont and Dow AgroSciences	2013	
Viptera4	Bacillus thuringiensis/Streptomyces viridochromogenes/Zea mays	TH and RI	Cry1Ab PAT VIP3Aa20 mCry3A mEPSPS	Syngenta	2014	

Maize	MIR 604	<i>Bt</i>	RI	mCry3A	Syngenta	2014
				Cry1F		
b		Bacillus thuringiensis/Streptomyces viridochromogenes/Agrobacterium tumefaciens	TH and RI	Cry1F	DuPont (RN 15)	2015
				Cry1Ab		
				PAT		
				VIP3Aa20		
				CP4-EPSPS		
				Cry1F	DuPont (RN 15)	2015
b		Bacillus thuringiensis/Streptomyces viridochromogenes/Agrobacterium tumefaciens	TH and RI	PAT		
				VIP3Aa20		
				CP4-EPSPS		
				Cry1F	DuPont (RN 15)	2015
				PAT		
				VIP3Aa20		
b		Bacillus thuringiensis/Streptomyces viridochromogenes/Bacillus thuringiensis	TH and RI	Cry1F	DuPont (RN 15)	2015
				PAT		
				VIP3Aa20		
				Cry1F	DuPont (RN 15)	2015
				PAT		
				VIP3Aa20		
b		Bacillus thuringiensis/Agrobacterium tumefaciens	TH and RI	VIP3Aa20	DuPont (RN 15)	2015
				CP4-EPSPS		
				Cry1Ab VIP3Aa20	DuPont (RN 15)	2015
				Cry1F	DuPont	2015
				PAT		
				VIP3Aa20		
b		Bacillus thuringiensis/Streptomyces viridochromogenes	TH and RI	Cry1Ab		
				eCry3.1Ab Cry1Ab	Syngenta	2015
				Vip3Aa20		
				Cry3A		
				Cry1F		
				PAT		
b		Bacillus thuringiensis/Streptomyces viridochromogenes	TH and RI	mEPSPS		

(continued)

Table 16.1 (continued)

Product	Commercial name	Donor organism	Characteristic ^a	Protein inserted	Applicant	Approval year
Maize	b	Bacillus thuringiensis/Streptomyces viridochromogenes/Agrobacterium tumefaciens/Sphingobium herbicidovans	TH and RI	Cry1A.105 Cry2Ab2 Cry1F PAT CP4-EPPSPS AAD-1	Dow AgroSciences	2016
		Bacillus thuringiensis/Streptomyces viridochromogenes/Agrobacterium tumefaciens	TH and RI	Cry2Ab2 Cry1A.105 Cry3Bb1/CP4 EPPSPS Cry1F PAT	Dow AgroSciences	2016
				Cry34Ab1 Cry35Ab1		
Cotton	Bolgard I	<i>Bacillus thuringiensis</i>	RI	Cry1Ac	Monsanto	2005
	Bolgard I Roundup Ready	Bacillus thuringiensis/Agrobacterium tumefaciens	TH and RI	Cry1Ac CP4-EPPSPS	Monsanto	2009
	WideStrike	Bacillus thuringiensis/Streptomyces viridochromogenes	TH and RI	Cry1Ac Cry1F PAT	Dow AgroSciences	2009
	Bolgard II	<i>Br</i>	RI	Cry2Ab2 Cry1Ac	Monsanto	2009

			Bayer	2011
TwinLink	Bacillus thuringiensis/Streptomyces hygroscopicus	TH and RI	Cry1Ab Cry2Ae PAT	
Glytrol x TwinLink	Zea mays/Bacillus thuringiensis/ Streptomyces hygroscopicus	TH and RI	Cry1Ab Cry2Ae 2mEPSPS	Bayer 2012
Bolgard II Roundup Ready Flex	Bacillus thuringiensis/Agrobacterium tumefaciens	TH and RI	Cry1Ac Cry2Ab2 CP4-EPSPS	Monsanto 2012

The pyramid (or stacked) Bt plants are in bold

^aTH tolerant to herbicide. RI resistant to insect

² Awaiting denomination

Table 16.2 Bt plants and the countries where they were commercially produced between 1996 and 2015 (As presented by [ISAAA GM Approval Database](#))

Bt crop	Country
Cotton	Argentina, Australia, Brazil, Burkina Faso, Canada, China, Colombia, Costa Rica, the European Union (EU), India, Japan, Mexico, Myanmar, New Zealand, Pakistan, Paraguay, the Philippines, Singapore, South Africa, South Korea, Sudan, Taiwan, the United States (USA)
Eggplant	Bangladesh
Maize	Argentina, Australia, Brazil, Canada, Chile, China, Colombia, Egypt, the EU, Honduras, Indonesia, Japan, Malaysia, Mexico, New Zealand, Panama, Paraguay, the Philippines, the Russian Federation, Singapore, South Africa, South Korea, Switzerland, Taiwan, Thailand, Turkey, the United States, Uruguay, Vietnam
Poplar	China
Potato	Australia, Canada, Japan, Mexico, New Zealand, the Philippines, the Russian Federation, South Korea, the United States
Rice	China, Iran
Soybean	Argentina, Australia, Brazil, Canada, China, Colombia, the EU, Japan, Mexico, New Zealand, Paraguay, the Philippines, the Russian Federation, South Africa, South Korea, Taiwan, Thailand, Turkey, the United States, Uruguay, Vietnam
Tomato	Canada, the United States

- Since Bt plants do not require the application of broad spectrum pesticides, the beneficial organisms (nontarget) are not affected and can thus proliferate, which indirectly allows the control of secondary pests.
- Another advantage already detected was the lower incidence of mycotoxins in maize, since there are no physical damages caused by pests that allow the entry of opportunistic pathogenic microorganisms. The fungi that produce these mycotoxins can be lethal to humans and animals.
- James (2015) points out that in the last 19 years of commercialization, profits obtained with Bt plants reached US\$ 86.9 billion and that for the year 2014 profits alone was US\$ 9.8 billion.
- In addition, Bt plants are another tool in the arsenal required for the control of pests that are more difficult to control with traditional pesticides.

With the increase in the world's population and the decrease in available arable land, it is more and more necessary to explore options that allow greater productivity in the same area. When used side by side with other agricultural practices, insect resistance technology (Bt plants) can bring many positive gains in productivity, benefiting the farmer, the productive chain, and, especially, the final consumer.

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