Transgenic proteins in maize in the Soil Conservation area of Federal District, Mexico

Article *in* Frontiers in Ecology and the Environment · June 2007

DOI: 10.1890/1540-9295(2007)5[247:TPIMIT]2.0.CO;2

6 authors, including:

José-Antonio Serratos-Hernández
Autonomous University of Mexico City
56 PUBLICATIONS 1,013 CITATIONS

Jose Luis Gomez
Metropolitan Autonomous University
65 PUBLICATIONS 441 CITATIONS

Noe Salinas
Metropolitan Autonomous University
6 PUBLICATIONS 140 CITATIONS

Enrique Buendía-Rodríguez
INIFAP Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias
41 PUBLICATIONS 237 CITATIONS

Transgenic proteins in maize in the Soil Conservation area of Federal District, Mexico

José-Antonio Serratos-Hernández[1*], José-Luis Gómez-Olivares[2], Noé Salinas-Arreortua[2], Enrique Buendía-Rodríguez[3], Fabián Islas-Gutiérrez[3], and Ana de-Ita[4]

In 2003, the environmental authorities of the Federal District of Mexico declared that genetically modified organisms were incompatible with ecological agriculture practices established in rural areas south of Mexico City. To ensure compliance with official standards and organic agriculture policies, steps were taken to implement an early warning system for the detection of genetically modified maize in farmers' fields. In our sampling efforts, which were conducted in 2003, transgenic proteins expressed in maize were found in two (0.96%) of 208 samples from farmers' fields, located in two (8%) of 25 sampled communities. Mexico imports a substantial amount of maize from the US, and due to formal and informal seed networks among rural farmers, there are many potential routes of entrance for transgenic maize into food and feed webs. To sustain agroecological practices, preserve organic agriculture, and conserve maize landraces in the Soil Conservation area of the Mexican Federal District, environmental authorities will need to maintain and update ecological policies such as the "green seal" for organic agriculture, apply alternative technologies such as biofertilizers to enhance plant nutrition, and develop sustainable maize agriculture with the implementation of profitable intercropping systems.

Front Ecol Environ 2007; 5(5): 247–252

In 2000, in an effort to slow the rapid growth of Mexico City, all lands dedicated to agricultural activities and other green areas within the Federal District were declared "Soil Conservation" lands, to be protected from urban expansion by the Environmental Law (GODF 2000). In 2003, environmental authorities also issued an official standard which defined ecological agriculture in the Soil Conservation area as those agricultural practices that are based on biodiversity conservation and social responsibility and that reduce environmental impact. Thus, the standard set guidelines for promoting sustainable crop production, soil conservation practices, and reduction of water pollution, as well as for achieving the long-term objective of developing markets for organic agriculture (GODF 2003).

The official standard was established as compulsory for farmers or enterprises practicing ecological agriculture, and was a precondition for certification by the Environment Secretariat of the Government of the Federal District. According to the standard, ecological agriculture must not involve the use of genetically modified organisms (GMOs) and should promote the restoration and conservation of native plant genetic resources. GMOs are considered incompatible with ecological agriculture, as practiced in the rural areas south of Mexico City. Therefore, to protect the remaining maize agriculture, the authorities planned to promote in situ conservation and improvement of maize landraces under agroecological practices already in place in the Soil Conservation area. At the federal level, there were no authorizations for the field release of genetically modified (GM) maize, so the Law on Biosafety and Genetically Modified Organisms (Congreso de la Unión 2005), approved in 2005, made provisions for the establishment of GMO-free zones and a special program for protection of maize.

Although levels of maize production have decreased within the Federal District, one-third of its arable land is currently sown with maize and farmers produce it as a minor crop. Conservation of maize landraces was considered feasible because farmers are increasingly adopting organic agricultural practices and because alternative ecological technologies are being implemented in these rural areas. The environmental authorities therefore initiated steps to detect and monitor the use of transgenic maize to ensure compliance with the official standards and organic agricultural policies, and to avoid the legal consequences of proprietary transgene introduction into native germplasm .

The objective of this study was to explore the status of transgenic maize in the Soil Conservation area of Federal

[1]*Programa sobre Ciencia, Tecnología y Desarrollo, El Colegio de México, Mexico City, Mexico *(aserratos@colmex.mx); previous address: Genetic Resources Program, Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), El Batán, México;* [2]*Departa-mento de Ciencias de la Salud, Universidad Autónoma Metropolitana, Mexico City, Mexico;* [3]*Campo Experimental Valle de México, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Chapingo, Mexico;* [4]*Centro de Estudios para el Cambio en el Campo Mexicano, Mexico City, Mexico*

District and to assess the feasibility of monitoring for its detection, as an early warning system to control GM maize in farmers' fields.

Methods

Maize sampling

A random sample of 75 maize fields within the Soil Conservation area was drawn from a database of farmers who received cash subsidies for maize production in 2003 (ASERCA Procampo 2003). We limited our samples to specific communities that customarily plant maize.

After interviewing the selected farmers, only 42 out of a total of 1115 farmers (3.72%) agreed to participate in the study. The participating farmers represented 25 communities and the average field size of these farmers was 1.72 hectares. Within each field, five sampling sites were chosen; these formed a circle with a diameter of 100 m, and corresponded to the north, south, center, east, and west of the fields. At each sampling site, five plants were randomly chosen and leaves were collected 60 to 65 days after planting. Thus, 20–25 plants were sampled at each of 42 field plots, for a total of 208 samples representing 1040 maize plants (Table 1).

At the end of the harvest season, we conducted in-depth interviews with farmers to gather data on production, management, and use of maize in the region, and on seed origin and grain destination. In 2004, we organized two meetings with farmers and environmental authorities, and administered questionnaires to assess socioeconomic aspects of maize production, technologies used, and the type and source of seed used in the surveyed area. Geographical coordinates were recorded for most of the sampled fields and used to generate a database managed by ArcInfo after Serratos-Hernández *et al.* (2004).

Leaf sample preparation

At the time of collection, leaves were cleaned and stored in plastic bags on ice for transport. Once in the laboratory, collected material was flash frozen in liquid nitrogen (N_2) and stored at –80°C until freeze dried at –50°C. Before the immunoassay, leaves were ground with a cyclon grinder, poured into plastic containers, and stored at –20°C. To prevent contamination, the cyclon grinder container was cleaned with pressurized air under a fume-hood between samples.

Leaves from positive and negative controls were ground at different times than the field samples, to avoid cross-contamination. Maize hybrid (CML72XCML216), biolistically transformed by firing a microscopic pellet coated with a vector containing the *Cry1Ab* gene into the sample (Bohorova *et al.* 1999), as well as the non-transformed parental lines, were used as positive and negative controls, respectively. A different herbicide- (glufosinate ammonium) resistant maize line (posphinotricin acetyl transferase, *pat*), generated at the Applied Biotechnology Center of the Centro Internacional de Mejoramiento de Maíz y Trigo, was used as an additional negative control in all tests, because no reaction to either protein immunoassay was expected.

Enzyme-linked immunosorbent assay (ELISA) for transgenic proteins

The most common GM maize varieties commercially available are herbicide-tolerant maize and two types of insect-resistant maize. Herbicide-tolerant maize contains the transgene *EPSPS*, from the bacterium *Agrobacterium tumefaciens* strain CP4, which confers field tolerance to glyphosate herbicide. One type of insect-resistant maize expresses the transgene *Cry1Ab* delta-endotoxin, from *Bacillus thuringiensis* var *kurstaki*, which affects lepidopteran insects. The other type is known as StarLink maize and contains the transgene *Cry9C* delta-endotoxin, from *B thuringiensis* var *tolworthi*, which also protects against lepidopterans.

To detect transgenic proteins from maize leaf tissues, AGDIA kits for *C4EPSPS, Cry1Ab/Ac* and *Cry9C* were used as indicated in their protocols, with the following modification: 100 mg freeze-dried leaf sample per 1 ml extraction buffer was used. Briefly, 100 μl of each sample and of positive and negative controls were added into separate wells of the ELISA plate and incubated for 1 hour at room temperature. Plates were then washed with phosphate buffer solution containing Tween (PBST). Conjugated peroxidase enzyme (100 μl), diluted 1:100 before the test run, was added to each well and plates were again incubated for 1 hour at room temperature. Plates were then washed with PBST. Finally, 100 μl substrate solution was added to each well, and plates were placed in a plastic box for 20 minutes of incubation. Optical density (OD) was read at 650 nm wavelength in a spectrophotometer to determine light absorbance for each well. There were at least two readings for each sample in different plates, for a total of 480 wells for each protein. Plates were photographed after spectrophotometer readings. Optical density readings > 0.3 OD were considered positive.

Results

Detection of transgenic protein in maize samples

All negative controls were consistently non-reactive to the immunosorbed enzyme for the particular protein assayed. The non-transformed maize lines (CML 72 and CML 216) and the glufosinate ammonium-resistant maize line (all negative controls) did not show any blue staining. These results show that the assays for each protein were operating properly.

Leaves from our *Cry1Ab* maize were used both as nega-

Table 1. Number of samples from the Soil Conservation area of four Federal District delegations testing positive for transgenic proteins, as detected by immunoassay

Delegation	*Community ID*	*Farmers' field ID*	*Plants sampled (N)*	*Sampling sites (N)*	*Positive sampling sites (OD 650 nm)* CP4 EPSPS	CryIAb/Ac
Milpa Alta	1	34	25	5		
6 communities; 11 farmers'	2	39	25	5		
fields tested out of 296; 275 plants;	3	40	25	5		1 (0.516)
55 sites (pooled leaves from	4	32	25	5		
5 plants per site)	4	33	25	5		
	4	35	25	5		
	5	36	25	5		
	6	37	25	5		
	6	38	25	5		
	6	41	25	5		
	6	42	25	5		
Tlahuac	7	3	25	5		
2 communities; 4 farmers'	7	5	25	5		
fields tested out of 369; 95 plants;	8	2	25	5		
19 sites (pooled leaves from	8	4	20	4		
5 plants per site)						
Tlalpan	9	6	25	5		
11 communities; 16 farmers'	9	14	25	5		
fields tested out of 297; 395 plants;	10	17	25	5		
79 sites (pooled leaves from	11	21	25	5		
5 plants per site)	11	27	25	5		
	12	10	25	5		
	13	11	25	5		
	13	15	25	5		
	13	19	25	5		
	14	9	25	5		
	14	20	25	5		
	15	12	20	4		
	16	13	25	5		
	17	22	25	5		
	18	18	25	5		
	19	8	25	5		
Magdalena Contreras	20	30	25	5		
6 communities; 11 farmers'	21	23	25	5		
fields tested out of 153; 275 plants;	21	25	25	5	1 (0.381)	1 (0.322)
55 sites (pooled leaves from	21	26	25	5		
5 plants per site)	22	28	25	5		
	23	24	25	5		
	23	29	25	5		
	23	31	25	5		
	24	7	25	5		
	24	16	25	5		
	25	1	25	5		
Total	25	42	1040	208		

tive controls in the *CP4EPSPS* and *Cry9C* plates and as positive controls in the *Cry1Ab* plates. Again, the spectrophotometric values for our *Cry1Ab* maize line control were close to the negative controls in the plates with *Cry9C* and *CP4*, whereas, in the *Cry1Ab* plates, optical density values were as high as the positive material provided with the commercial immunoassay kit. After repeating a number of assays for indeterminate optical density spectrophotometer readings at 650 nm ($0.1 \leq OD \leq 0.3$) and confirming samples classified as positives in preliminary runs, final results were obtained as shown in Table 1.

There was no detection of *Cry9C* protein in maize samples from the 42 fields sampled within the four communities of the Soil Conservation area of Federal District. However, one sample from the Milpa-Alta region was positive for *Cry1Ab/Ac* protein (Table 1), and one sample from the Magdalena-Contreras region was found to contain both *Cry1Ab/Ac* and *CP4 EPSPS* proteins (Table 1; Figure 1).

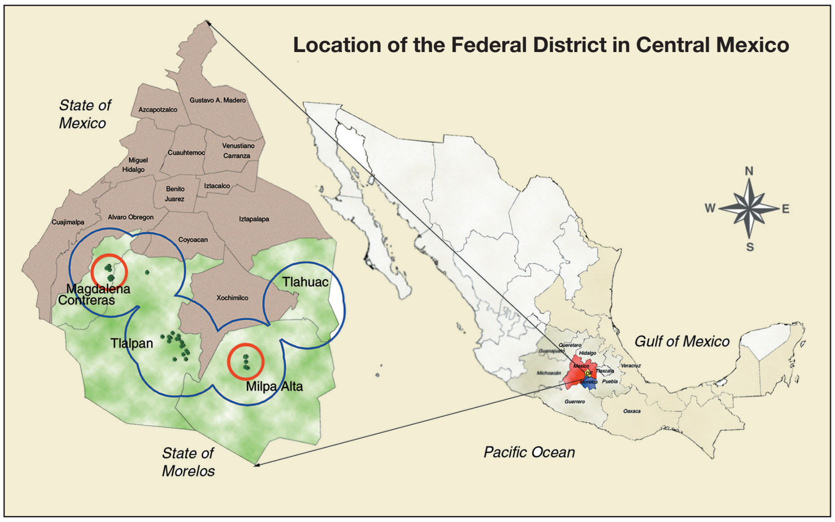

Figure 1. *Location of the four district delegations surveyed for local maize grown within the Soil Conservation area south of the Federal District. The blue line surrounds the study area. Light green areas are delegations studied. Dark green circles are farmers' fields sampled. Red circles indicate communities where transgenic protein in maize samples was found.*

Maize agriculture within the Soil Conservation area of the Federal District

Agricultural land area in the Federal District has diminished over the past 20 years. Today, the area sown to maize in the Soil Conservation area averages 7200 ha – half of what was used in the 1980s – while maize production is only 10 500 metric tons (SAGARPA nd). Maize agriculture in the Soil Conservation area is not highly profitable and is becoming less competitive compared to other activities. In the communities considered here, maize production was a secondary activity for the majority of the farmers we interviewed. However, ecological agriculture policies and practices promoted by environmental authorities are increasingly being adopted by farmers, under incentives such as the "green seal" that promotes certification of organic agriculture applied to the land in the Soil Conservation area.

Within regions of the Soil Conservation area (Figure 1), more than 60% of farmers have maize plots of less than one ha, up to 90% have less than two ha, and only a handful of farmers (0.6%) have more than 10 ha of land each. In the four regions analyzed, most maize production is destined for the *elote* (corn-on-the-cob) market; less is used as food or feed within households. Corn-on-the-cob is more profitable because, depending on the quality, it can be sold for up to 15 cents (US) per ear in a number of town markets near Mexico City. Farmers prefer seed from the landrace Cacahuacintle for *elote* production, as this type of maize has a big, round, soft, floury type of grain (Figure 2), but there are other landraces, improved varieties, and mixtures that are also used for this purpose.

Farmers in these four regions buy seed mainly from different localities in the state of Mexico, where, as in other states, there is a long tradition of seed recycling and conservation (Louette *et al.* 1997). Also, labor for shelling and selecting "good seed" is considered better in these surrounding localities. In Tlalpan, farmers cultivate maize as a monoculture, whereas the community of San Nicolas Totolapan in Magdalena-Contreras uses intercropping practices, combining maize with cucurbits, fava beans, or fruit trees, alongside horticulture. Within this community, different types of maize and landraces are sown together.

Farmers from communities in Magdalena-Contreras, Tlalpan, and Milpa-Alta have tested maize hybrids developed by scientists at the Mexican Federal Institute for Agricultural Research (INIFAP). Although there was a good response from farmers regarding the improved maize, there is still a high preference for the *criollos* (landrace) seed obtained from surrounding states. At present, most of the surveyed farmers buy seed from small-scale businesses specializing in seed selection or from rural markets in villages in surrounding states.

One of the long-term objectives of environmental authorities in the Soil Conservation area is to promote the conservation of maize landraces linked to agroecological practices that, at the same time, could improve household economies. To comply with ecological standards and policies, and to increase farmers' income, we recommend a more intensive program of information and participatory research, focusing on landraces and food specialties, to generate economic benefits for the farmer. Among the many factors that affect maize diversity, in particular within the Mexican traditional agricultural systems, lack of support for the farmer is one of the most pressing (Soleri *et al.* 2006). One way to alleviate this situation will be through public policies that enhance sustainable agriculture by means of new technologies, even alternative biotechnology, focusing on the small-scale farmer and agroecology strategies.

Discussion

According to the Commission of the European Communities, immunoassay is an appropriate analytical procedure for detecting the presence of substance residues by immunochemical reaction and is designed to avoid false positives (OJEC 1993). ELISA is a method widely used for screening purposes where a high throughput capacity is required. It allows rapid preliminary testing and is easy to handle. The test is based on the high specificity of antibodies and the high activity of enzymes (peroxidase) that allow a range of detection between 0.25 and 2.0 ng/mL from *Cry1A* expressed in GM plants. The ELISA test, which is highly sensitive, makes use of a monoclonal antibody to bind to a particular protein, in this case either the *Bt Cry* protein or the bacterial enzyme used to make herbicide-resistant crops.

ELISA is not suitable for drawing definitive conclusions, as it does not provide information on chemical structure. However, the technique has been successfully used to screen for the *Cry1Ab/1Ac* protein, to determine the expression levels of *Cry* protein, and to track the persistence and amount of insecticidal *Cry* protein in different environments (Palm *et al.* 1994; Theunis *et al.* 1998; Olsen and Daly 2000; Zwahlen *et al.* 2003). Therefore, this technique was considered sufficiently robust to identify areas in which agroecological measures to deal with transgenic maize should be implemented. Complementary tests to detect transgenic maize varieties should be carried out using molecular techniques, such as PCR, since gene silencing can occur under certain environmental conditions and can result in false negatives. However, in accordance with the precautionary principle referenced in the Mexican Law on Biosafety (Congreso de la Unión 2005), authorities need not wait for confirmation of the presence of transgenic material to act.

The objective of this investigation was not to estimate frequencies of transgenes in maize populations. At this stage, the goal was to detect the presence of transgenes in particular areas of the Federal District. In that sense, since our sampling is directed only to transgenic detection, it is less demanding and also less prone to misinterpretation than sampling intended to estimate frequencies, as highlighted by Cleveland *et al.* (2005), who compared the studies of Quist and Chapela (2001) and Ortíz-García *et al.* (2005) on transgenic maize detection in landraces.

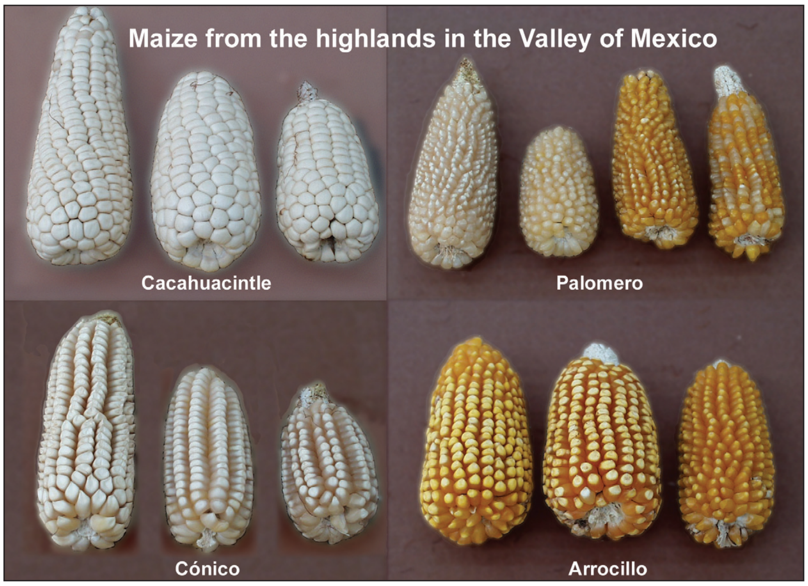

Figure 2. *Native races of maize grown in the central highland plateau of Mexico.*

Presence of transgenic proteins in maize grown within Soil Conservation areas

At this point, it is difficult to explain the presence of transgenic protein in maize cultivated within the Soil Conservation area of the Federal District. It is possible that GM seed entered as a result of informal seed trade with localities outside the Federal District. It is important to note that farmers were not intentionally planting GM maize, and that there is no way for farmers to know whether the seed they are acquiring is transgenic, especially when it is obtained through these informal seed networks.

Farmers in the Federal District do not usually select or save seed for grain production. They simply buy the "best" seed to grow maize for corn-on-the-cob, since this is more profitable in the local markets. This is because maize agriculture is declining in these communities and is becoming a secondary economic activity; farmers therefore prefer to obtain fresh seed for each cycle, to avoid the burden of selecting and preserving their own seed. In this regard, the Soil Conservation area of the Federal District is functioning as a sink for seed of many origins.

Although early reports of transgenic maize in the states of Oaxaca and Puebla (Quist and Chapela 2001; Alvarez-Morales 2002; Ezcurra *et al.* 2002) have not been confirmed (Ortíz-García *et al.* 2005), recent analyses suggest that a reexamination of the whole issue of transgene flow in Mexico may be necessary (Cleveland *et al.* 2005; Soleri *et al.* 2006). Concerns about the introduction of GM maize have not been put to rest, as indicated by the report of NAFTA's Commission for Environmental Cooperation on the impacts of transgenic maize in Mexico (NACEC 2004). These studies indicate that there could potentially be a substantial presence of transgenic maize in different parts of Mexico, and that this could serve as the source of transgenic seed in the Federal District. It has been suggested that landrace maize genetic diversity is threatened by hybridization with all types of commercial maize, since the latter contain less genetic variability. The incorporation of transgenic maize varieties therefore has the potential to affect crop diversity when acting together with socioeconomic factors that could impact in situ and traditional agricultural systems conservation (Soleri *et al.* 2006).

This study illustrates how the dispersion of transgenic maize may take place in Mexico. Production of transgenic crop varieties is growing rapidly in industrialized countries. Trade is a very efficient mechanism for transporting commodities and seed when an incentive is generated or when, due to production deficit, there is a need for grain to meet demand for food and feed. Once the transgenic seed arrives at one location, it is just a matter of time and opportunity before a proportion of the seed will spread to other communities.

It is necessary to test other hypotheses regarding the presence of GM maize, to carry out additional sampling of the surveyed communities, and to increase the surveyed area to include municipalities in other states of the Valley of Mexico. These efforts should be implemented in the near future, given that policies and standards for organic agriculture and agroecology for the conservation of soil are already in place. Secondly, molecular methods for detection of transgenes should be carried on in parallel with immunoassays to provide more information to environmental authorities that must design policies to contend with this issue.

Since the official standard at the Federal District is driven by a policy of zero tolerance for GMOs, local authorities should approach communities where positive samples have been found to exchange information. Participation by maize farmers in activities leading to conservation and utilization of preferred maize landraces, as well as increased education on agroecology practices, could, in a short time, establish a renovated economic activity, linked to specialty maize production. Farmers could also play a key role in controlling the entry of GM maize.

Conclusions

There are many routes by which GM maize may have entered the Soil Conservation area of the Federal District. The increasing production and availability of transgenic maize from the US has been identified as one of a number of possible sources of transgenic maize found in Mexico (Serratos-Hernández *et al.* 2004; Soleri *et al.* 2006).

Establishing a monitoring system based on ELISA tests to detect commercially available transgenic proteins is feasible, but only as a first step in developing a biosafety system to control the introduction of GMOs into the Soil Conservation area of the Federal District. The ELISA technique is reliable, fast, and affordable, but to comply with international standards, molecular techniques must be incorporated into the monitoring system. Nevertheless, results obtained with ELISA should stimulate action in accordance with the precautionary principle established in the Mexican legislation.

Acknowledgements

We thank J Thor Arnason for his advice, suggestions, and critical reading of the manuscript. J-AS-H wishes to thank D Hoisington for his support and facilities provided at CIMMYT during the time of this research.

References

Alvarez-Morales A. 2002. Transgenes in maize landraces in Oaxaca: official report on the extent and implications. www.bba.de/gentech/isbgmo.pdf. Viewed 13 April 2007.

ASERCA (Support and Services to Agricultural and Livestock Commercialization) Procampo (Program of Direct Subsidies to Farmers). 2003. Padrón de productores apoyados (List of beneficiaries of the program). Mexico City, Mexico: Secretaría de Agricultura, Ganadería, Desarrollo Rural y Pesca. www.procampo.gob.mx/artman/publish/article_183.asp. Viewed 1 May 2007.

Bohorova N, Zhang W, Julstrum P, *et al.* 1999. Production of transgenic tropical maize with *CryIAb* and *CryIAc* genes via microprojectile bombardment of immature embryos. *Theor Appl Genet* **99**: 437–44.

Cleveland DA, Soleri D, Aragon-Cuevas F, *et al.* 2005. Detecting (trans)gene flow to landraces in centers of crop origin: lessons from the case of maize in Mexico. *Environ Biosafety Res* **4**: 197–208.

Congreso de la Unión (Mexican Congress). 2005. Ley de Bioseguridad de Organismos Genéticamente Modificados. Diario oficial de la federación, February. Mexico City, Mexico: Cámara de Diputados.

Ezcurra E, Ortiz S, and Soberón-Mainero J. 2002. Evidence of gene flow from transgenic maize to local varieties in Mexico. In: Roseland CR (Ed). LMOs and the environment. Proceedings of an International Conference organized by OECD, USDA, and EPA; 2001 Nov 27-30; Raleigh, NC. Paris, France: Organization for Economic Cooperation and Development.

GODF (Government of Federal District, Mexico). 2000. Environmental law of the Federal District; 2000 Jan 13. Mexico City, Mexico: Government of Federal District.

GODF (Government of Federal District, Mexico). 2003. Official ecological standard NADF-002-RNAT-2002. 2003 Dec 18. Mexico City, Mexico: Secretaria del Medio Ambiente.

Louette D, Charrier A, and Berthaud J. 1997. In situ conservation of maize in Mexico: genetic diversity and maize seed management in a traditional community. *Econ Bot* **51**: 20–38.

NACEC (North American Commission for Environmental Cooperation). 2004. Maize and biodiversity: the effects of Transgenic Maize in Mexico. www.cec.org/maize/. Viewed 13 April 2007.

OJEC (Official Journal of European Communities). 1993. Commission decision of 14 April 1993 (93/256/EEC). Luxembourg City, Luxembourg: Official Journal of European Communities.

Olsen KM and Daly JC. 2000. Plant–toxin interactions in transgenic Bt cotton and their effect on mortality of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Entomol Soc Am* **93**: 1293–99.

Ortíz-García S, Ezcurra E, Schoel B, *et al.* 2005. Absence of detectable transgenes in local landraces of maize in Oaxaca, Mexico (2003–2004). *P Natl Acad Sci USA* **102**: 12338–43.

Palm CJ, Donegan KK, Harris D, and Seidler RJ. 1994. Quantification in soil of *Bacillus thuringiensis* var *kurstaki* δ-endotoxin from transgenic plants. *Mol Ecol* **3**: 145–51.

Quist D and Chapela I. 2001. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* **414**: 541–43.

SAGARPA (Secretary of Agriculture, Livestock, Rural Development, Fisheries and Agrifood). Sistema de Información Agropecuaria y Pesquera (Information System on Agriculture and Fisheries). www.siap.sagarpa.gob.mx/ar_comanuar.html. Viewed 13 April 2007.

Serratos-Hernández J-A, Islas-Gutiérrez F, Buendía-Rodríguez E, and Berthaud J. 2004. Gene flow scenarios with transgenic maize in Mexico. *Environ Biosafety Res* **3**: 149–57.

Soleri D, Cleveland DA, and Aragón Cuevas F. 2006. Transgenic crops and crop varietal diversity: the case of maize in Mexico. *BioScience* **56**: 503–13.

Theunis W, Aguda RM, Cruz WT, *et al.* 1998. *Bacillus thuringiensis* isolates from the Philippines: habitat distribution, ‰-endotoxin diversity, and toxicity to rice stem borers (Lepidoptera: Pyralidae). *Bull Entomol Res* **88**: 335–42.

Zwahlen C, Hilbeck A, Gugerli P, and Nentwig W. 2003. Degradation of the *Cry1Ab* protein within transgenic *Bacillus thuringiensis* corn tissue in the field. *Mol Ecol* **12**: 765–75.

