Maize and Biodiversity: The Effects of Transgenic Maize in Mexico

Chapter 1

Context and Background on Maize and its Wild Relatives in Mexico

for the Article 13 Initative on
Maize and Biodiversity

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Introduction

Maize is the predominant crop of the world. About 30 percent of world production is used for direct human consumption and as an industrial input, while 70 percent is used as animal feed. Mexico safeguards the main genetic diversity of maize and its wild relative teosinte, both plants native to the country. The oldest known maize ear relics were excavated in a cave in the Mexican state of Oaxaca, and were dated 6250 years before the present era. Native Mesoamericans domesticated maize, invented nixtamalization, and developed maize from a 6 cm long, popping-kernel ear to what we now recognize as modern maize with its wide variety in ear size, kernel texture, color, size, and adequacy for diverse uses. In fact, native Mesoamericans continue to develop maize on more than one million small farms in Mexico. These farmers grow their own seed and depend on maize as their main source of food. In doing this, some 84 Mexican ethnic groups are also acting as stewards of maize genetic diversity.

Transgenic food-maize has been detected—albeit in variable proportions—deep in the heartland of Mexican traditional agriculture. The Mexican Federal Government adopted a *de facto moratorium* to commercial production of transformed maize through its Ministry of Agriculture in 1998, but has allowed maize imports that may include transgenic maize.

Expected consequences of the presence of transformed maize in Mexico are analyzed in this book. Those consequences involve a number of issues: status of genetic diversity of maize and its wild relatives, natural ecosystems, agriculture, human and animal health, society and culture, risks and opportunities.

In this chapter, we will look at maize and its wild relatives in Mexico, from the time of maize domestication to the present. We will also examine some elements of the traditional approach to developing maize as a crop and conserving its genetic diversity, as well as its present day distribution in Mexico, and dispersion throughout the world. Finally, we will look into transformed maize and how it could interact with maize landraces.

Origin of maize

The hypothesis of maize descending from teosinte is the oldest of four widely considered hypotheses on the origin of maize. It was advanced by Ascherson in 1895 (Mangelsdorf and Reeves 1939). It is also the most widely accepted hypothesis at present.

Cultivated maize *Zea mays* L. subsp. *mays (*Iltis and Doebley 1980) is most likely the product of a single domestication event through human selection on annual teosinte *Zea mays* L. subsp. *parviglumis* according to Doebley *et al.* 1987, and to Matsuoka *et al.* 2002. This conclusion was reached after studying isozyme variation of maize and teosinte (Doebley *et al.* 1987) and through phylogenetic analysis based on genotyping a comprehensive sample of maize and teosinte from the American continent (Matsuoka *et al.* 2002).

Additional evidence supporting the teosinte ancestry of maize is provided by the discovery of a number of genetic loci coding for basic phenotypic differences between maize and teosinte (Doebley 1992; Wang *et al.* 1999; and Whitt *et al.* 2002).

Furthermore, Matsuoka and coworkers (2002) propose the central Balsas River Valley in Michoacán and Guerrero states of Mexico as a possible cradle of maize domestication.

McClintock's research (1959) on chromosome knob positions in maize from South America, Mexico and Central America led her to the multiple-origins proposal that was further developed by Kato (1976 and 1984), McClintock (1978), and McClintock *et al.* (1981). These authors proposed five independent centers of maize domestication, four were in Mexico—two in the Oaxaca-Chiapas region; one in the central highlands and one in the mid-highlands of Morelos-Northern Guerrero—and one in the highlands of Guatemala.

Research by Matsuoka *et al.* (2002) led them to the seemingly conflicting conclusions that the cradle of domestication lies at an altitude of 800 to 1200 meters above sea level, while the first diversification of maize occurred at a higher elevation (more than 1800 m). Three cobs of fossil maize were excavated at the Guilá Naquitz cave in Oaxaca, Mexico, (1926 m above sea level) and were directly dated by accelerator mass spectrometry 6250 calendar years before present (y.b.p.) (Piperno and Flannery 2001). The second oldest fossil maize was found at the San Marcos Cave near Tehuacán, Puebla, and was directly dated by accelerator mass spectrometry to be seven centuries younger (5500 y. b. p.) (Long *et al. 19*89).

Benz (2001) reports that Guilá Naquitz fossil maize, in contrast with its teosinte ancestor, had non-disarticulating rachis, reduced rachid length, spikelets reoriented perpendicularly to the rachis and opened cupulate fruitcases which exposed the grain. However, these characters had not yet reached fixation to the maize-like phenotype with paired spikelets. Comparing these specimens with those excavated in San Marcos cave led the author to infer that domestication efforts in the 700 year interim were focused on stabilizing the distichous, non-disarticulating, naked-grain phenotype and on increasing the number of grain-bearing spikelets per node from one to two. Maize relics from two caves bearing more recent dates are the Romero and Valenzuela caves near Ocampo, Tamaulipas (4300 years before present) (Smith, B.D. 1998) and southwestern United States (3500 years before present) (Smith 2001).

This archaeological and biological evidence shapes a northward path of dispersal of early maize. The study by Matsuoka and coworkers (2002) suggests one path that starts in Mexico's highlands and traces through western and northern Mexico into the southwestern United States and then into the eastern US and Canada. A second path goes from the Mexico highlands to the western and southern lowlands of Mexico into Guatemala, the Caribbean Islands, the lowlands of South America and the Andes mountains. In accordance with this study, maize diversified first in Mexico's highlands and dispersed to lowlands at a later stage. Some archaeological studies support this assertion (Smith 1998 and 2001). However, there is also an alternative archaeological position: diversification in the lowlands of Mexico and dispersal to the highlands at a later stage (Piperno and Pearsall 1998).

There were two driving forces at least for further domestication and diversification of early maize: (a) hybridization between maize races containing varying amounts of teosinte germplasm and various teosinte races (Wellhausen *et al. 19*52; Taba 1997), and (b) accented edaphoclimatic gradients for further human selection. Four extant, early races of maize in México are Palomero Toluqueño and Arrocillo Amarillo in the highlands, 1800 to 2600 m above sea level in the states of Mexico, Puebla and Tlaxcala; Chapalote in the lowlands of Sonora and Sinaloa and Nal-Tel in the lowlands of the

Yucatán peninsula. These early races are referred to as Ancient, Indigenous Maize Races (Wellhausen *et al. 19*52). The same authors describe these races as small-eared, early and low yielding types of plants with small, flint, and popping kernels. The two lowlandraces share a weak tunicate allele and, in stark difference with many modern tropical races, can grow almost normal ears in the highlands of Mexico.

Maize in pre-Columbian Mesoamerica

Four pre-Columbian Exotic Maize Races were introduced back to Mexico from Central and South America in prehistoric times and remain under cultivation, according to Wellhausen and coworkers (1952): Cacahuacintle (floury, large kernels, adapted at present to 2200 to 2800 m above sea level), Harinoso de Ocho (floury, large kernels, adapted at present to lowlands), Olotón (flint, large kernels, adapted at present to 2000 to 2400 m above sea level) and Maíz Dulce (large kernels, adapted at present to 1000 to 1500 m above sea level). Hybridization of pre-Columbian, exotic races with the ancient, indigenous races plus local teosintes gave rise to thirteen mestizo, prehistoric races of maize (Wellhausen *et al.* 1952). Four modern, incipient races of maize were developed through hybridization among the mestizo, prehistoric races after the Conquest. Hernández-Xolocotzi and Alanís-Flores (1970) collected and described five new maize races from the Sierra Madre Occidental as additions to the list published by Wellhausen and coworkers (1952).

The matter of consciousness and purpose of our ancestors in developing the more productive, modern types of maize from early domesticates has not been settled. There are those who believe that the role was passive and went only as far as practicing selection from opportunities created by nature, migration, geographic isolation and drive to food security. There also those (Grobman *et al.*,1961; Hernández-Xolocotzi and Alanís-Flores 1970; Hernandez-Xolocotzi 1985) who believe in an alternative role of consciously affecting the probabilities of different outcomes by (a) seed and ear selection in the granary (intervention of women), planting in proximity or mixing seeds of differing materials so as to allow interbreeding, introduction of new materials to be used *per se* or as donors of desired agronomic traits, and shared knowledge with descendants; and (b) keeping sympatric and allopatric maize genetic materials available in the farmstead so as to meet culinary needs and edaphoclimatic conditions. Certainly the hunter-gatherers that succeeded in stabilizing the maize-like phenotype out of segregating early populations during seven centuries (from fossil maize in cave Guilá Naquitz 6250 y.b.p. to fossil maize in cave San Marcos 5500 y.b.p.) were making conscious decisions (Benz 2001; Jaenicke-Després *et al. 20*03).

There is insufficient information on the chronological-geographical appearance of maize races in Mexico. We do not know for how long the ancient, indigenous races of maize were the sole maize genetic resources available in Mesoamerica. Taba (1997) tells us that soon after the early maize domestication in Mexico, a small-eared, early domesticate reached Central America and there hybridized with teosinte *Zea luxurians* (Bird 1980). Then, a variable set of more productive types, including a lineage that led to the Olotillo race, spread back into Mexico. Two thousand years ago, a new complex of precursors of races Nal-Tel and Chapalote became abundant in Mexico (Mangelsdorf 1974; Benz 1994). García-Cook (1985) relates the cultivation of the Naltel-Chapalote maize race complex in the Valley of Tehuacán, Mexico (Stanley 1977) to the initiation of construction of Maquetongo dam (Presa Barrón) by year 650 B.C. García-Cook also reports on the appearance of Palomero Toluqueño, an ancient indigenous maize race, and Cónico, a mestizo prehistoric maize race (McClung de Tapia 1977) in Teotihuacán I, period 100 B.C. to 100 A.D.

If the time of reliance on ancient, indigenous maize races in the lowlands of Mexico were the same as in the highlands, then both the Olmec (1200 B.C.–900 B.C.) and Zapotec (500 B.C.–200 B.C.) Preclassic cultures had to feed themselves on the small eared, early, and low yielding types of maize plants with small, flint, and popping kernels. It could have been the Classic cultures, Teotihuacán (300A.D.–900 A.D.) and Mayan (600 A.D.–900 A.D.) and Post-classic cultures Toltec (900 A.D.–1200 A.D.) and Aztec (1200 A.D.–1521 A.D.) that introduced and took advantage of the pre-Columbian exotic races and developed the higher yielding, mestizo, prehistoric races of maize described by Wellhausen *et al.*, (1952).

Maize was central as a source of food and in religious life throughout Mesoamerica. Its presence is recorded in many corners of this cultural region ever since the Preclassic period until contact with the Spanish conquerors (Pérez-Suárez 1997). Pérez-Suárez (1997) concludes that a common concept of a Mesoamerican Deity of Maize was developed through time. Some of the plant elements (the ear, kernel or leaves) are represented in natural, schematical or idealized styles in the head of sculptures or in murals. The same author reports that the representation of maize is frequently associated with deities of earth or rain. Splendid representations of *Cintéotl-Xochipilli* (Aztec deity of maize, Bourbon Codex, p 27, Mexican National Library of Anthropology and History (BNAH)) and of the maize plant idealized as the *axis mundi* (Bordian Codex, p. 53, BNAH) or the humanized ears in the central painting of Cacaxtla Red Temple describe a vision of centrality of maize in the Mesoamerican cultures.

Maize production technology was significantly developed in the period that lapsed between the critical yield level of 200–250 kg ha^{-1} of rain fed maize that made village life possible in Mexico and Guatemala some 3500 years ago until contact with Europeans. Some pre-Columbian achievements that led to productivity increases were: (a) disease resistant and more productive maize races (Wellhausen *et al. 19*52), (b) irrigation systems and infrastructure (García-Cook 1985), (c) improved farming systems, production and crop protection practices and hand tools (Rojas-Rabiela 1985, 1988, 1991 and 1997), (d) post harvest management (Rojas-Rabiela 1985) and (e) erosion control systems (Garcia-Cook 1985). However, the very limited or non-existent use of the wheel and lack of draft animals were insurmountable constraints to further improvements in the productivity of labor.

Social and economic relationships around maize production were based on the *calpulli* social structure. This structure would allow family groups to grow maize on communal land (Florescano 1984). Twenty out of 38 provinces within the Aztec empire would provide Moctezuma with a tribute of some 300,000 bushels (7,200 metric tons) of maize annually (Berdan 1982; Calnek 1982).

Box 1: **Nixtamalization, human nutrition, pellagra, and a balanced diet**

Nixtamalized maize and cooked common beans were the main sources of energy and protein in the Mesoamerican diet. Maize was eaten as a hot tortilla (a flattened corn cake) that functioned as an edible plate or spoon while eating beans. Small amounts of chiles and tomato prepared as a salsa would normally accompany maize and beans as flavor enhancers in every meal.

Nixtamalization, or lime-cooking, is an ancient process first described by Illescas (1943). It consists of heating a mixture of one part maize grain added to two parts of a one-percent alkaline limestone solution to 80°C for 20 to 45 minutes, and then allowing it to stand overnight. The grain pericarp or seed-coat gets hydrolyzed and separated from the grain as the cold cooking liquor (or nejayote) is decanted. The solid material now referred to as nixtamal is washed two or three times with water to remove the seed-coats, tip caps, excess limestone and any impurities in the grain. Nixtamal is ground, kneaded to dough and roasted in flattened, individual portions over a heating pan.

Nixtamalization significantly improves the bioavailability of calcium (Bressani 1990; Krause 1988 in FAO 1992), amino acids (Bressani and Scrimshaw 1958 in FAO 1992), and makes part of the bound-niacin[1] available, thus eliminating the pellagragenic property of untreated maize if eaten regularly in the absence of legumes or animal protein.

Nixtamal is also the basic ingredient for a number of other foods. For tamales, the dough, together with salsa, meat, beans, ground sauces, avocado leaves, or herbs, is wrapped in corn husks and vapor-cooked. Atole is a thick beverage meant to be consumed hot. Pozol is a refreshing drink in the lowland tropics. Pozole is a substantial soup made of nixtamalized whole kernels, meat and herbs. A number of other specialties made from nixtamal could be added to this list.

Maize protein is deficient in lysine and tryptophan but has fair amounts of sulphur-containing aminoacids (methionine and cysteine). On the other hand, the protein of food legumes is relatively rich in the essential aminoacids lysine and tryptophan but poor in sulphur-containing aminoacids. A combination of common beans and maize can fulfill the energy-protein requirements of the human diet for adults. The best combination is 30 parts beans and 70 parts maize (Bressani and Elías 1974 in FAO 1992).

The nixtamalization process for direct human consumption—well known in Mesoamerica by the time of contact with Europeans—did not accompany the dispersal of maize after Columbus introduced it to Europe in the 15th century.

Wherever it was planted, maize out-yielded the small grain cereals (wheat, oats, rye, barley) and made an inexpensive food. It became the principal source of dietary energy and protein for the poor. Unfortunately, wherever maize went in the old world, a deadly human dietary deficiency called pellagra was sure to follow. The connection between maize and pellagra was first described by Casal in Spain in 1735. Francesco Frapoli of Millan called it “pelle agra,” the disease of three d’s: dermatitis, diarrhea and dementia. If untreated, pellagra typically leads to death in four to five years (Hampl and Hampl 1997; Latham 1973). Pellagra was endemic where diets were based on non-nixtamalized maize with little meat or legumes in much of the deep south of the United States in the post-civil war period and was also endemic for Italy, southern France, Bulgaria and Rumania in the same period.

Nowadays, pellagra continues to affect the poor in Angola, Mozambique and Tanzania, where prevalence has been found to vary among locations from 0.4 to 9.4 percent of the population (Golden M. 2002).

In stark contrast to Mesoamericans, the very poor of Europe and the United States did not nixtamalize maize nor did they complement their diet with common beans, other legumes or animal protein. It was also commonly observed that while people suffered and died from strict consumption of non-nixtamalized maize, domestic animals thrived. This empirical observation marked the future use of maize as “non-fit for human consumption.” Maize became the preferred animal feed in the world because of that

[1] Niacin is essential to humans in the form of coenzymes nicotinamide adenine dinucleotide (NAD) and NAD phosphate (NADP). NAD functions as an electron carrier for intracellular respiration as well as cohydrogenase with enzymes in the oxidation of fuel molecules. NADP functions as hydrogen donor in reductive biosynthesis such as in fatty acid and steroid synthesis and, like NAD, as a code hydrogenase.

observation and belief and because of its higher yield and superior quality as a source of feed energy.

Dispersion of maize throughout the world

The southward route of maize dispersion took the Central American hybridized maize to South America some 4000 years ago, where agricultural, ceramic-using cultures had already developed (Grobman *et al.* 1961). Maize spread first into the lowlands of South America and finally into the Andes Mountains (Matsuoka *et al.* 2002).

The northward route of dispersal from Mexico probably included two parallel corridors flanking the Sierra Madre Occidental into the southwestern United States. (Carter 1945; Hernandez-Xolocotzi and Alanís-Flores 1970). The first maize race to reach the southwestern United States was Chapalote, some 3000 to 3500 years ago (Fagan 1995). Chapalote is a relic: an ancient, indigenous maize race that has a low yield potential. Maize remained as a supplement to regular foraging for a millennium or so after its initial introduction to the region. Further maize germplasm introductions from the south brought other races such as Olotón de Chiapas, Olotón and Serrano de Guatemala into the southwestern United States. (Hernandez-Xolocotzi 1985). Maize spread into the Eastern Woodlands of North America and appeared as a food in what now are New England and eastern New York during the twelfth century A.D. (Fagan 1995).

Taba (1997), quoting several authors, tells us that Columbus found maize in Cuba and introduced it into Europe (Mangelsdorf) and that maize spread into the Asian continent via three routes in the 16th century: the Mediterranean trade route, the Atlantic and Indian Ocean route and, after Magellan’s voyage, to the Philippines and eastern Indonesia (Brandolini 1970 in Taba 1997). The same author mentions that maize was introduced to Africa from Spain and Italy and that a later introduction of maize spread into Africa from the lowlands of Brazil and the Guyanas, the southern United States and northern Mexico.

Box 2. **Maize as the predominant crop in the world**

Wheat, rice and maize are the most cultivated cereals worldwide. Maize has the largest total production, wheat has the largest area harvested and rice is second to wheat in area harvested (Table 1). World maize yield per hectare is more than 60 percent higher than that of either wheat or rice at present. Wheat and rice are the main sources of human dietary energy in the world, while maize is by far the principal source of feed energy, mostly fed as grain, but also as silage. Maize is an important industrial raw material and a basic source of human dietary energy in an important part of the developing world.

Assuming trade prices of 2002/2003, world gross values of total production were US$92 billion for wheat, US$74 billion for rice, and US$65 billion for maize, while world trade was 73 million metric tons (Mmt) for wheat, 25 Mmt for rice, and 70 Mmt for maize (FAPRI 2003). As in the past, maize continues to be an inexpensive human food as compared either to wheat or rice. It also makes an inexpensive feed and industrial raw material.

Table 1. Area harvested, total production and yield of maize, wheat and rice in the world in 2002/2003.

	Maize	Wheat	Rice

Area harvested (million ha)	136.14	212.22	145.80
Total production (million metric tons)	590.52	567.51	380.27
• Food and other	176.52	455.80	380.27
• Feed use	414.00	111.71	0
Yield (ton ha^{-1})	4.34	2.67	2.61

SOURCE: FAPRI 2003. US and World Agricultural Outlook. Food and Agricultural Policy Research Institute. Iowa State University. Ames, Ia. US (http://www.fapri.iastate.edu/outlook2003/)

Maize is cultivated in the tropical, subtropical and temperate climatic regions of the world. Land cultivated to maize is divided almost equally between the tropical-subtropical areas and the temperate areas of the world. CIMMYT (2002) recognizes five mega-environments for maize in the world: lowland tropics (less than 900 meters above sea level), subtropics and mid-altitude tropical zones, tropical highlands, and temperate zones. Maize may be cultivated in spring-summer and fall-winter growing seasons in the lowland tropics and lowland subtropics whereas in the rest of the mega-environments maize may be cultivated only in the spring-summer season.

It should not be a surprise that for optimized management, our Mesoamerican domesticate out-yields both wheat and rice in non-temperate mega-environments (wheat is cultivated in the fall-winter season in the lowland subtropics and in spring-summer season in the mid-altitude and highland tropics). Maize shares the more efficient C_4 photosynthetic pathway[2] with sorghum and sugar cane, while wheat and rice and most other crops share the C_3 photosynthetic pathway.

Record maize yields are lower for irrigated, spring-summer maize than for irrigated, fall-winter maize in the lowland tropics. A record experimental yield in Mexico is about 18 ton ha^{-1} for the fall-winter lowland tropics (INIFAP 1998), the irrigated, spring-summer mid-altitude tropics and probably for the highland tropics (Diaz del Pino 1964).

The advantage of C_4 over C_3 plants disappears under the lower light intensity and lower temperatures that prevail in temperate zones where maize also thrives. Yet maize continues to out-yield wheat and all other small grain cereals in these temperate zones. Record yields in the temperate zones are 23.5 ton ha^{-1} for maize (The Maize Page, ISU), and 14.7 ton ha^{-1} for wheat (Cook and Veseth 1991).

The inseparable duo of maize and nixtamalization has yet to make its worldwide contribution to alleviating hunger in the developing world. Pellagra does not have to reappear among the world's poor as it is now happening in southern Africa (see Box 1). Furthermore, a formula that would include quality protein maize (QPM) and nixtamalization would do even better (FAO 1992). The mutant *opaque-2* allele discovered by Mertz *et al.*, (1964) almost doubled lysine and tryptophan concentrations in the kernel endosperm. However, conversion of normal maize materials into *opaque-2*

[2] The higher light intensity and temperature of the tropics and subtropics are environmental conditions in which the C_4 photosynthetic pathway excels, thus conferring C_4 plants an advantage over C_3 plants. The Rubisco photosynthetic enzyme (**ribulose bisphosphate carboxylase oxygenase**) is provided with abundant CO_2 but has limited access to atmospheric O_2 in C_4 plants, while Rubisco has access to both gases in C_3 plants. As a result, photorespiration is kept at a minimum by C_4 plants while it significantly limits net photosynthesis of C_3 plants in the tropics.

maize was linked to low yields and to other undesirable agronomic characters that limited the large-scale utilization of the mutant *opaque-2* allele. Work at CIMMYT by Vasal *et al.* (1980) led to development of QPM from *opaque-2* maize. This new improved version largely overcame the agronomic constraints of *opaque-2* maize. QPM yields approach those of commercially available genetic materials of normal maize.

Maize grain has excelled as an industrial input. The three basic types of industrial maize processing are wet-milling, dry-milling and fermentation and distillation. Starch, oil and syrups are the main products of the wet-milling industry. The dry-milling industry produces grits, meal and flour. The fermentation and distilling industry produces ethylol and whisky. Many other food and feed products and industrial derivatives are produced from maize. A fourth industry as yet very controversial, and still in an incipient stage uses genetically engineered maize as a bioreactor for the production of certain pharmaceutical products. This is referred to as plant manufactured pharmaceutical crop technology (PMP) (AgMRC 2003).

Present day distribution of cultivated maize and its wild relatives in Mexico.

(a) ***Systematics of maize landraces and wild relatives***

Wellhausen *et al.* (1952) conducted the first work on the systematics and taxonomy of Mexican maize germplasm. Maize in Mexico was classified in 4 groups: 1) Ancient Indigenous, 2) Pre-Columbian Exotic, 3) Prehistoric Mestizos; and 4) Modern Incipient. Thirty-two races were described and genealogies were suggested on the basis of morphology, physiology, genetics, and cytological characteristics for 23 of them (Figure 1). Wellhausen and coworkers also presented the first map of the distribution of maize landraces in Mexico (Figure 2).

Numerical taxonomy methods were also applied to Mexican maize landraces and relationships were found on the basis of morphological traits, genetic effects, and genotype by environment interactions (Goodman and Bird 1977; Bird and Goodman 1977; Cervantes *et al.* 1978; Sanchez and Goodman 1992a, b). These numerical studies were summarized by Goodman and Brown (1988) (Table 2) and a phenetics grouping related to distribution according to genotype by environment altitudinal parameters was established (Figure 2). Other techniques have also been utilized to

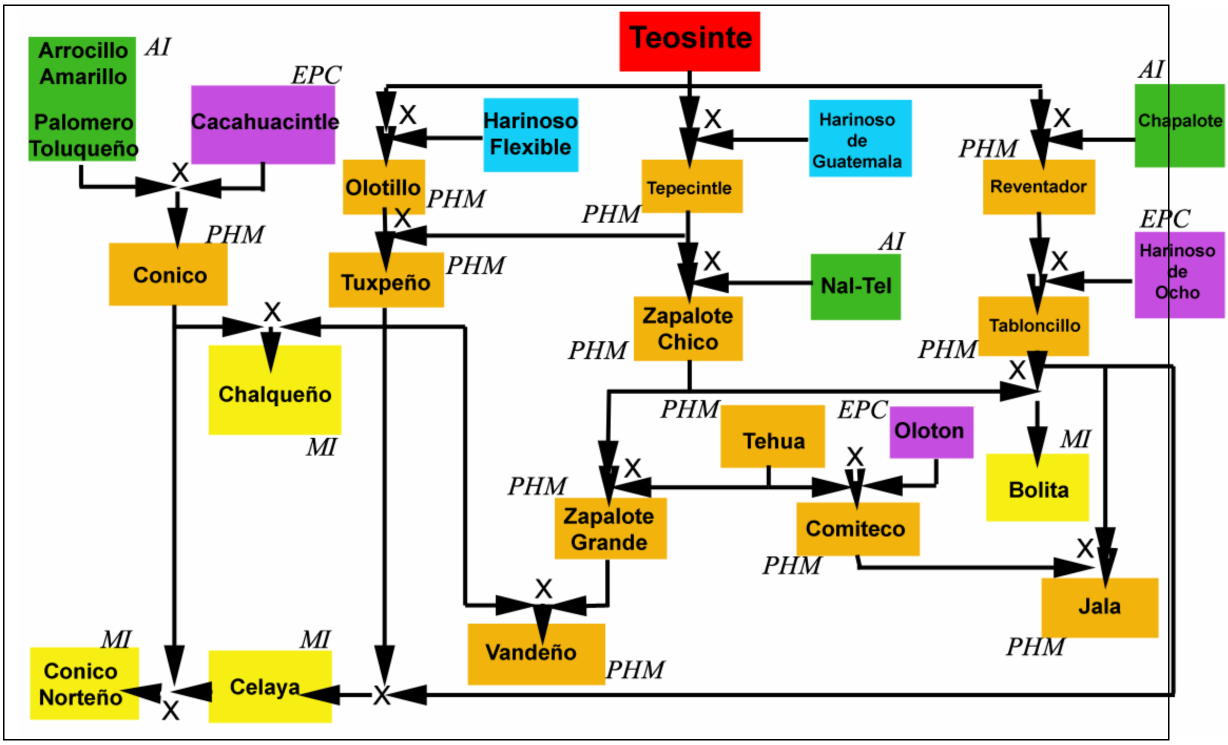


Figure 1. Assumed relationships among landraces of maize in Mexico, according to Wellhausen et al. (1952). AI = Ancient Indigenous, EPC = Exotic Pre-Columbian, PHM = Prehistoric Mestizo, and, MI = Modern Incipient. (X) indicates the assumed type of mating that could have given rise to the studied landraces (figure adapted from Serratos-Hernández 2001).

Table 2. Racial relationships of maize in Mexico (Goodman and Brown 1988).

Races within cells outlined by solid lines more closely related than races found in different cells.

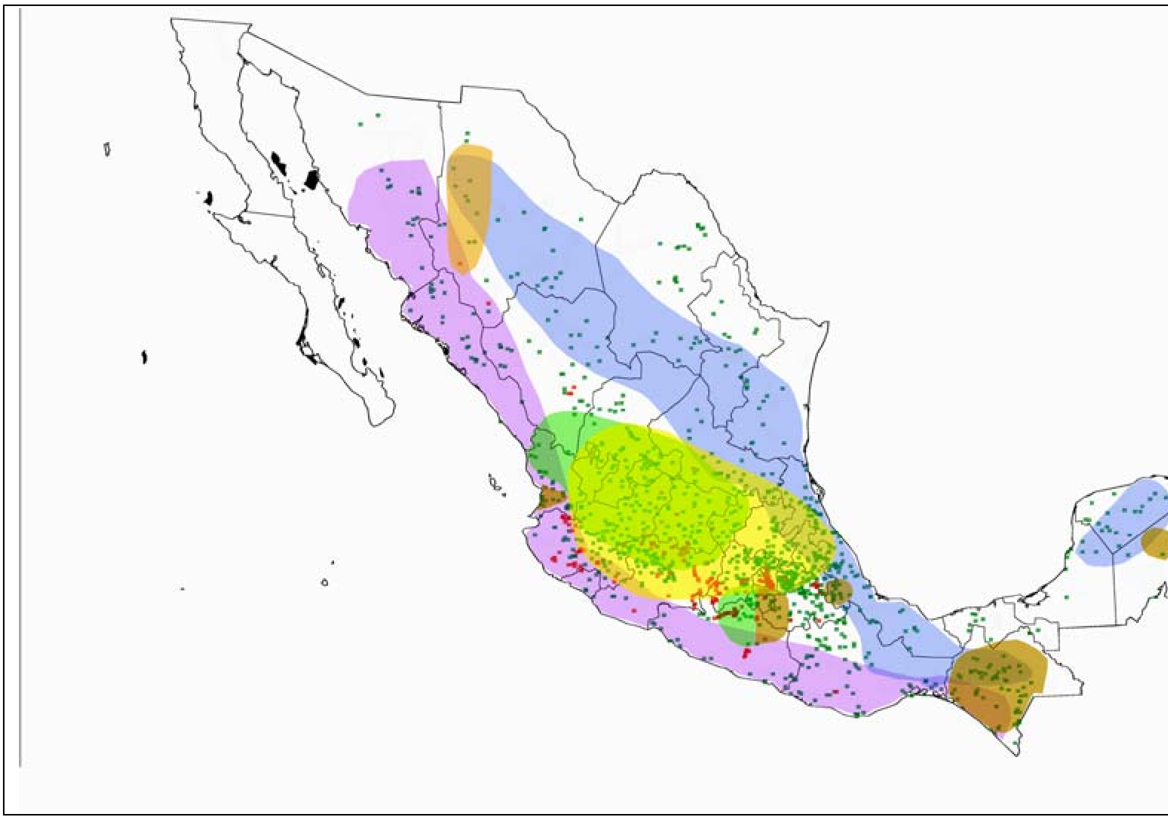
Races within gray cells are assumed to be more closely related to each other than to races found outside the subcells.

Chapalote	Reventador	Dulcillo del Noroeste
Apachito Cristalino de Chihuahua	Harinoso de ocho Blando de Sonora Onaveño Bofo Gordo Azul	Tabloncillo Tabloncillo Perla Tablilla de Ocho

Dzit-Bacal Olotillo Oloton Jala Zamorano Amarillo Comiteco	Tuxpeño Vandeño Celaya Tuxpeño Norteño Raton	Tepecintle	Zapalote Grande Zapalote Chico Bolita Ancho	Nal-Tel

Tehua				

Pepitilla	Cacahuacintle	Conico Conico Norteño Elotes Conicos	Chalqueño Arrocillo Amarillo Palomero Toluqueño Palomero de Chihuabua	Maiz Dulce



Adapted from information in Sanchez and Goodman (1992a); Taba (1995a); Serratos et al. (2001)

Figure 2. The blue and purple areas correspond to the distribution of lowland maize germplasm as grouped by Sanchez and Goodman (1992a). Mid-elevation maize is identified by the green and brown areas and, highland maize is distributed in yellow and orange areas (see Table 3). Dark green dots correspond to representative landraces, whereas red ones identify teosinte populations (Serratos *et al.* 2001).

classify the Mexican landraces: isozymes (Doebley *et al.* 1985), proteins and immunology (Yakoleff-Greenhouse *et al.* 1982), chromosome knobs (Kato 1976), grain chemistry (Hernandez-Casillas 1986), and biochemical traits such as secondary metabolites related to levels of maize resistance to pests or pathogens (Classen *et al.* 1990; Reid *et al.* 1990a, b; Reid *et al.* 1991; Arnason *et al.* 1994). These techniques cluster landraces in similar ways to that summarized by Goodman and Brown (1988) Table 2.

(b) *Distribution of maize landraces and wild relatives*

Distribution of teosinte in Mexico has been described (Wellhausen *et al.* 1952; Sanchez and Ordaz 1987; Wilkes 1967; Taba 1995b; Sanchez-Gonzalez and Ruiz-Corral 1996) and most of the information regarding several species of teosinte has been updated by Sanchez-Gonzalez *et al.* (1998). It is estimated that about 20 percent of teosinte populations remain uncollected in their potential areas of distribution (Sanchez and Ruiz 1997). Despite this lack of information, teosinte has been monitored more or less regularly and the reported distribution is considered to be precise (Sanchez and Ordaz 1987; Sánchez-González *et al.* 1998).

The genus *Tripsacum* (L.), has its center of diversity in Mexico and Guatemala and is widely distributed in Mexico (Berthaud *et al.* 1995); however, the available information is not always reliable since updated collections show discrepancies regarding botanical and taxonomic features (Berthaud *et al.* 1995). A fairly recent survey (1989–1992) by a group of scientists based at CIMMYT (Berthaud *et al.* 1995) described three groups of *Tripsacum* species in Mexico. Here we will only list the *Tripsacum* species and its range of distribution in Mexico as indicated by Berthaud *et al.* (1995). *Tripsacum lanceolatum* can be found in the state of Durango and along the northwest Sierra Madre Occidental; *T. pilosum* is found in Sinaloa. Several species *T. intermedium, T. jalapense, T. latifolium, T. laxum, T. maizar* and *T. manisuroides*n are distributed in southern states (Guerrero, Oaxaca, Veracruz and Chiapas). *T. zopilotense* is restricted to Cañon del Zopilote in the state of Guerrero. *T. bravum, T. dactyloides* var. *mexicanum, T. pilosum, T. zopilotense, T. laxum, T. maizar* and *T. dactyloides* var. *hispidum*—closely related to *T. dactyloides* from the eastern US—are found in the eastern central part of Jalisco, the Bajío and central southern part of Michoacán, northern central Guerrero, the State of Mexico and the northeastern Sierra Madre Oriental.

To know the actual distribution of maize landraces and wild relatives in Mexico is a formidable task, given there is no recent quantitative information. In order to infer the present day distribution of the genus *Zea* we rely on the information gathered by the experts in charge of germplasm banks and on the few *in-situ* conservation projects (Ortega *et al.* 2000). A principal component analysis was carried out to cluster states on the basis of state level variables maize production, productivity, use of hybrid maize and percentage of rural-peasant population (Nadal 1999, 2000). These variables were considered appropriate as indicators of the average condition of maize as a crop in each state. The information from this analysis was coupled to the data from the studies on maize systematics (Figure 2 and Table 3) to provide a broad inference on the actual distribution of landraces and wild relatives in Mexico.

The results of the principal components analysis (SAS 2003) indicate that the use of hybrids is negatively correlated with the presence of rural populations (-0.537) and is positively, but only slightly, correlated with maize production overall (0.2127). Other correlation coefficients among variables are negligible. The first three principal components explain 0.901 of the variance, with the first principal component positively associated with rural population and negatively with the use of hybrids. In contrast, the second principal component has a positive association with hybrid use and is negatively associated with rural populations, whereas production and productivity of maize both have a higher degree of positive association with this principal component. In the third principal component there is a high level of association with maize production (positive) and maize yield (negative). Using the four principal component scores as variables for a Euclidean complete linkage cluster analysis, sets of states were grouped according to these principal components (Figure 3). The map was thus developed with landraces and wild relatives accession locations and the database for maize production, as indicators of the most likely places of maize-maize and maize-relatives interactions (Serratos-Hernández *et al.* 2001). This database was managed with the INFO-ARC program installed on a UNIX system platform and processed as described in Serratos-Hernández *et al.* (2001). The purpose of this exercise is to illustrate the grouping of states, as related to overall maize production through time in Mexico, and the geographic distribution of landraces of maize. It is acknowledged that the locations for landraces and teosinte accessions in the map, in many instances, have changed through time. However, results of recent studies on diversity of maize and peasant management strongly suggest that there is a high likelihood of permanence of those types of maize (accessions) in neighboring areas of the place of collection (Aguirre *et al.* 1998; Smale *et al.* 1999; Louette 1997; Perales *et al. 20*03a,b).

The map of Mexico generated from the maize and teosinte databases (Figure 3), together with Figure 2 and data from Table 3 help develop an overview of the geographic distribution of landraces of maize and wild relatives. First, 70.61 percent (Table 3) of the catalogued landraces in Mexico are from the states in Group I (Campeche, Chiapas, Durango, Guerrero, Guanajuato, Hidalgo, Michoacán, Nayarit, Oaxaca, Puebla, Quintana Roo, Querétaro, San Luis Potosí, Tabasco, Tlaxcala, Veracruz, Yucatán, and Zacatecas). Some of these states (Michoacán, Guerrero, Oaxaca, Chiapas, and Veracruz) are good producers of maize as compared to the national average, and are

Table 3. Catalogued maize landraces in Mexico. * Between parentheses is the number of accessions of landraces in Mexico registered in the LAMP catalog (1991). ** The groupings are as described in Sanchez and Goodman (1992a). See also Figure 2.

State*	Lowland group**	Midland group**	Highland group**	Maize landraces (Cárdenas, F. en Taba 1995a)
Aguascalientes (59)				Celaya, Conico, Conico Norteño, Chalqueño, Elotes Conicos
Baja California Sur (11)				Tuxpeño, Tabloncillo Perla
Campeche (182)				Dzit-Bacal, Nal-Tel, Clavillo
Chihuahua (348)	Tuxpeño Norteño		Azul, Apachito, Cristalino de Chihuahua, Gordo, Palomero de Chihuahua	Tuxpeño, Celaya, Conico, Conico Norteño, Chalqueño, Tabloncillo, Reventador, Tabloncillo Perla, Bolita, Maiz Dulce, Harinoso de Ocho, Palomero, San Juan, Dulcillo del Noroeste, Tuxpeño Norteño, Azul, Lady Finger, Blandito, Cristalino de Chihuahua, Gordo, Tehua, Apachito, Maizon
Chiapas (795)	Zapalote Chico, Tepecintle, Zapalote Grande, Vandeño, Nal-Tel de Altura, Tuxpeño	Tehua, Comiteco, Dzit-Bacal, Motozinteco, Oloton		Tuxpeño, Celaya, Conico, Elotes Occidentales, Olotillo, Tabloncillo Perla, Dzit-Bacal, Vandeño, Nal-Tel, Tepecintle, Oloton, Zapalote Chico, Zapalote Grande, Clavillo, Comiteco
Coahuila (124)	Tuxpeño Norteño		Raton	Tuxpeño, Celaya, Conico Norteño, Elotes Occidentales, Tuxpeño Norteño, Tehua
Colima (29)				Tuxpeño, Tabloncillo, Reventador, Tabloncillo Perla, Vandeño, Jala
Durango (270)		Bofo		Tuxpeño, Celaya, Conico, Conico Norteño, Chalqueño, Elotes Occidentales, Tabloncillo, Reventador, Tabloncillo Perla, Bolita, Pepitilla, San Juan, Dulcillo del Noroeste, Bofo, Blandito de Sonora, Blandito, Cristalino de Chihuahua, Gordo, Tablilla, Tunicata
Guerrero (383)	Conejo	Ancho, Pepitilla		Tuxpeño, Elotes Conicos, Elotes Occidentales, Olotillo, Tabloncillo, Reventador, Vandeño, Nal-Tel, Pepitilla, Mushito, Tepecintle, Ancho, Conejo
Guanajuato (370)		Celaya, Elotes Occidentales	Conico Norteño	Tuxpeño, Celaya, Conico, Conico Norteño, Chalqueño, Elotes Conicos, Elotes Occidentales, Reventador, Maiz Dulce, Mushito, Fasciado
Hidalgo (236)			Chalqueño	Tuxpeño, Celaya, Conico, Conico Norteño, Chalqueño, Elotes Conicos, Elotes Occidentales, Olotillo, Bolita, Dzit-Bacal, Mushito, Cacahuacintle, Arrocillo Amarillo, Oloton, Arrocillo
Jalisco (683)		Elotes Occidentales, Bofo, Tablilla de Ocho, Tabloncillo, Zamorano Amarillo	Dulce de Jalisco, Serrano de Jalisco	Tuxpeño, Celaya, Conico, Conico Norteño, Chalqueño, Elotes Conicos, Elotes Occidentales, Tabloncillo, Reventador, Tabloncillo Perla, Bolita, Vandeño, Pepitilla, Maiz Dulce, Harinoso de Ocho, San Juan, Azul, Jala, Zamora, Complejo Serrano de Jalisco

México (724)		Ancho	Palomero Toluqueño, Conico, Cacahuacintle, Chalqueño	Tuxpeño, Celaya, Conico, Conico Norteño, Chalqueño, Elotes Conicos, Bolita, Pepitilla, Cacahuacintle, Palomero, Arrocillo Amarillo, Ancho, Azul
Michoacán (528)		Zamorano Amarillo, Tabloncillo	Mushito	Tuxpeño, Celaya, Conico, Conico Norteño, Chalqueño, Elotes Conicos, Elotes Occidentales, Olotillo, Reventador, Dzit-Bacal, Vandeño, Pepitilla, Maiz Dulce, Mushito, Cacahuacintle, Palomero, Conejo, Zamora
Morelos (165)		Pepitilla		Tuxpeño, Chalqueño, Olotillo, Tabloncillo, Vandeño, Pepitilla, Tuxpeño Norteño, Ancho
Nayarit (336)	Reventador, Harinoso de Ocho, Tabloncillo Perla	Elotes Occidentales, Bofo, Jala, Tablilla de Ocho		Tuxpeño, Celaya, Conico, Conico Norteño, Elotes Occidentales, Olotillo, Tabloncillo, Reventador, Tabloncillo Perla, Vandeño, Maiz Dulce, Harinoso de Ocho, Bofo, Jala, Tablilla de Ocho
Nuevo Leon (118)				Tuxpeño, Conico Norteño, Tabloncillo, Tablilla de Ocho
Oaxaca (562)	Zapalote Chico, Nal-Tel de Altura, Tuxpeño		Bolita	Tuxpeño, Celaya, Conico, Conico Norteño, Chalqueño, Elotes Conicos, Olotillo, Bolita, Vandeño, Nal-Tel, Mushito, Tepecintle, Oloton, Conejo, Zapalote Chico, Zapalote Grande
Puebla (943)			Conico, Elotes Conicos, Cacahuacintle, Arrocillo	Tuxpeño, Celaya, Conico, Conico Norteño, Chalqueño, Elotes Conicos, Elotes Occidentales, Olotillo, Bolita, Pepitilla, Mushito, Cacahuacintle, Palomero, Arrocillo Amarillo, Arrocillo
Quintana Roo (132)	Nal-Tel	Dzit-Bacal		Tuxpeño, Olotillo, Dzit-Bacal, Nal-Tel, Tepecintle
Querétaro (115)				Tuxpeño, Celaya, Conico, Conico Norteño, Chalqueño, Elotes Conicos, Bofo, Onaveño, Fasciado
Sinaloa (187)	Chapalote, Dulcillo del Noroeste, Reventador, Blando de Sonora			Tuxpeño, Tabloncillo, Reventador, Tabloncillo Perla, Maiz Dulce, Harinoso de Ocho, San Juan, Dulcillo del Noroeste, Blandito de Sonora, Lady Finger, Onaveño, Chapalote, Harinoso
San Luis Potosí (206)				Tuxpeño, Celaya, Conico, Conico Norteño, Chalqueño, Elotes Conicos, Elotes Occidentales, Olotillo, Tabloncillo, Dzit-Bacal, Harinoso de Ocho
Sonora (183)	Onaveño, Blando de Sonora			Tuxpeño, Tabloncillo, Reventador, Tabloncillo Perla, Nal-Tel, Harinoso de Ocho, San Juan, Dulcillo del Noroeste, Blandito de Sonora, Lady Finger, Onaveño, Chapalote
Tabasco (35)				Tuxpeño, Olotillo, Vandeño, Nal-Tel, Zapalote Grande
Tamaulipas (148)	Tuxpeño, Tuxpeño Norteño		Raton	Tuxpeño, Dzit-Bacal, Carmen
Tlaxcala (332)			Palomero Toluqueño, Elotes Conicos	Conico, Chalqueño, Elotes Conicos, Cacahuacintle, Palomero, Arrocillo Amarillo, Arrocillo
Veracruz (741)	Tuxpeño	Coscomatepec		Tuxpeño, Celaya, Conico, Conico Norteño, Chalqueño, Elotes Conicos, Elotes Occidentales, Olotillo, Bolita, Dzit-Bacal, Nal-Tel, Pepitilla, Mushito, Cacahuacintle, Palomero, Tepecintle, Arrocillo Amarillo, Oloton, Coscomatepec
Yucatán (249)	Nal-Tel			Tuxpeño, Olotillo, Dzit-Bacal, Nal-Tel, Tepecintle, Zapalote Chico, Xmenejal
Zacatecas (263)		Tablilla de Ocho, Elotes Occidentales, Bofo	Dulce de Jalisco	Celaya, Conico, Conico Norteño, Chalqueño, Elotes Conicos, Elotes Occidentales, Tabloncillo, Bolita, Maiz Dulce, San Juan, Dulcillo del Noroeste, Bofo, Tablilla

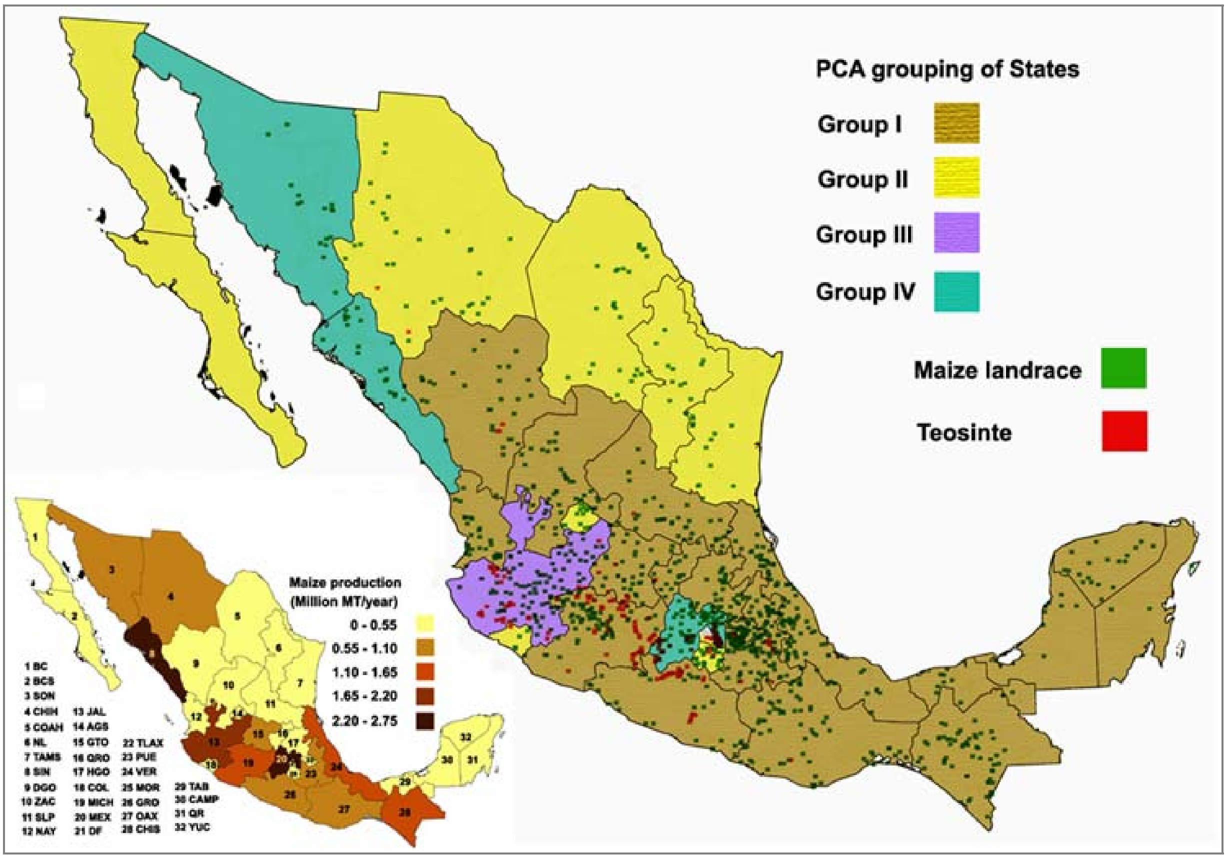


Figure 3. PCA groupings of the Mexican states, as described in the text. Maize production, productivity, use of hybrids and rural population were used as indicators of the nature of maize agriculture in each state. On this map, the locations of representative landraces (see Table 3) and teosinte populations that are catalogued in different databases are overlayed to show a probable distribution for the conservation and use of landraces.

located within the Neotropical Region with the highest biodiversity in Mexico. The region formed with the States of Group I has a high likelihood of preserving maize landrace diversity and perhaps its distribution is widely extant across these states. The exception might be Durango and Guanajuato if we consider the report of Ortega-Paczka *et al.* (2000), because of changes in land use or adoption of improved varieties. Distribution of teosinte is concentrated in Michoacán, Guanajuato and Guerrero, while *Tripsacum spp* are found in Oaxaca, Veracruz, Durango and Chiapas as well. Group I comprises states with medium to very high percentage of rural population, very low use of hybrids, medium to high production of maize with low to high yield. Group II clusters Aguascalientes, Baja California, Baja California Sur, Chihuahua, Coahuila, Colima, Distrito Federal, Morelos, Nuevo León, and Tamaulipas. It is worth noticing that Chihuahua is the state in this group—and in Mexico—that includes 23 maize landraces catalogued in 348 accessions (Table 3). States in Group II includes those with very low to medium percentage of rural population, low to medium use of hybrids and very low to medium production and productivity of maize. Group III is represented by just one state, Jalisco, and is located close to Group II as a sub-group. Jalisco is a high producer of maize with low-medium yield, has a very high use of hybrids and a medium level of rural population. Jalisco is unique because it has a high number of maize landraces and teosinte populations. Group IV comprises the States of México, Sonora and Sinaloa, these are linked because of their high and very high production and yield of maize; however, in the State of México there is a very low use of hybrids with a medium level of rural population as compared to the high use of hybrids together with medium-high rural population in Sinaloa and Sonora. It is these differences that keep the three states distinctly apart within the same group. The numbers of maize landraces reported in México, Sinaloa and Sonora states are practically the same (Table 3), but the landraces themselves are different in these three states (Figure 2 and Table 3).

It can be safely said that a significant amount of maize landraces exists widely distributed in at least three quarters of the Mexican territory. Wild relatives, specifically teosinte, are in some scattered areas closely associated to maize in general and, to cultivation of landraces in particular. Studies that relate maize producing conditions, rural-peasant population, and economic conditions to the loss of maize diversity are yet to be conducted on most maize producing areas. Clearly, a monitoring system to building a base line for future assessment, conservation, management and use of maize landraces and wild relatives is needed.

Box 3. **Genomes of maize, teosinte, and *Tripsacum*: Interbreeding and introgression**

As genome sequencing projects advance, knowledge about grass genomes in general and cereals genomes in particular has steadily increased. Although crucial questions about genome evolution remain unanswered (Gaut *et al.* 2000), major plant genome initiatives are providing the support for a leap forward in our knowledge of cereal genomics (Bennett 1998; Schlueter *et al.* 2003). Phylogeny studies with genomes of the subfamily Panicoideae, to which maize and its relatives belong, have been carried out (Bennetzen and Kellog 1997; Bennetzen *et al.* 1998; Kellogg 1998), and relevant information can be found at the Kew Gardens webpage (<www.rbgkew.org.uk/cval/>). Maize genome information is concentrated at the Maize Genetics and Genomics Database (<www.maizegdb.org/>). Relevant data on the genome of maize and its relatives is here summarized.

The maize genome contains 2.73 pg of DNA (1C value) with 2671 Mbp, and it is estimated that it contains 50,000 genes interspersed among 1/2 of retrotransposon elements comprising two thirds of the genome (Bennetzen and Kellog 1997; Walbot and Petrov 2001). *Zea diploperennis* contains 2.65 pg of DNA with 2597 Mbp; *Zea luxurians* and *Zea perennis* contains 4.58 pg (4484 Mbp), and 5.28 pg (5170 Mbp) of DNA (1C), respectively. The genome of *Tripsacum dactyloides* comprises 3798 Mbp in 3.88 pg of DNA (1C). All the 1C values were taken from <www.rbgkew.org.uk/cval/>.

Interbreeding within the genus *Zea* is very common. Maize is 100 percent open-pollinated (cross-fertilizing) therefore all maize varieties will cross-pollinate, except for certain popcorn varieties and hybrids (OECD 2003). Maize and annual teosinte produce fertile hybrids (OECD 2003), but further generations are uncommon in maize fields where teosinte is endemic. The presence of a crossing barrier gene cluster in some teosinte-incompatible stocks might explain this situation (Evans and Kermicle 2001). Analysis of the introgression of chromosome knobs from *Zea diploperennis* into maize found no evidence of natural introgression between these two subspecies (Kato and Sanchez 2002).

Inter-specific crosses between maize and *Tripsacum* have been reviewed in an OECD document (2003), which is briefly summarized here. Hybrids with a high degree of sterility and genetic instability are obtained when crossing maize and *Tripsacum* species. However, genes from *Tripsacum* have been transferred into maize by a method that utilizes a cross of *Tripsacum* and *Zea diploperennis* called tripsacorn, which is used to generate maize-tripsacorn hybrids. Attempts to transfer apomixis genes from *Tripsacum* to maize have been pursued for a number of years (Burson *et al. 19*90; Savidan and Berthaud 1994; Hanna 1995; Leblanc *et al.* 1995; Grimanelli *et al.* 1998; Grossniklaus *et al.* 1998), and consequently several patents on apomictic maize have been issued (Kindiger and Sokolov 1998; Savidan *et al.* 1998; Eubanks 2000).

Box 4. **Maize breeding strategies of Mexican farmers**

The 84 ethnic groups of Mexico have been the long-term stewards of the 59 Mexican races of maize. These ethnic groups used to occupy the whole country in pre-Columbian times. The Sierras offered refuge from the conqueror as well as geographic isolation, ecological diversity, and productive ecosystems. Today's Mexican geography-demography shows a correlation between the Sierras and many ethnic groups. The 84 ethnic groups and their ancestors domesticated maize and developed it from the small and genetically unstable type of ear found in the Guilá Naquitz cave dated 6250 years before present by Piperno and Flannery (2001) into modern maize.

Hernandez-Xolocotzi poses two questions regarding the domestication of maize. What were the purposes of maize selection under domestication? What genetic mechanisms were used in the absence of an understanding of modern genetics? One could also ask how was the nixtamalization process developed and whether people were exposed to pellagra in the early stages of maize domestication? Hernandez-Xolocotzi (1973, 1985, and 1993) shares with us some of his relevant findings based on maize, ethno-botanical exploration in Mexico, Guatemala, Cuba, Colombia, Ecuador and Peru:

(1) The native farmer would cultivate several maize types —kernel textures, color, time to maturity, etc.—and even races, either in different plots or else in the same milpa;

(2) The household woman did have a precise knowledge of the most adequate maize type for each specific use;

(3) Farmers knew the effect of pollen fertilization: "one type of maize colors another located downwind";

(4) Farmers know the different flowering and maturation times of their maize types.

(5) Native farmers are careful observers of nature, always looking for better maize materials to fit in their edaphoclimatic niches: upland and bottomland, shallow and deep soils, early and late planting, windy, drought prone, early or late frost, etc. as well as seeking resistance to biotic stresses.

(6) Farmers normally maintain early and late maturing maize, frequently flagged by color (white for late materials, yellow for the intermediate, and dark colors for short season materials) and other morphological characters;

(7) Late materials normally out-yield early materials, but are suited for only a fraction of the farms

and then only for those years of early onset of rains;

(8) Yield stability and quality of kernel are a priority over high yields;

(9) One should expect to find both accomplished as well as careless farmers.

Hernandez X. adds that these facts are very telling in terms of a farmer's procedures for selecting maize under domestication.

It has been reported that traditional farmers frequently interchange their seeds (Ortega-Paczka *et al.* 2000; Louette 1995). New maize seeds may come from neighbors, the local market, trips to distant regions or are obtained as a last resort from government aid agencies, which frequently handle imported maize. This approach would have brought maize landraces to the Sierra areas before the late 1950s and certainly different maize races would have reached all Mexican agricultural areas in the very distant past. Grain of hybrids and of open pollinated genetic materials from the National Institute of Agricultural Research—developed from some of the 59 Mexican maize races—was also introduced to the Sierras through this infiltration mechanism between the late 1950s and the late 1980s (Ortega P. 1999; Vega 1973). New commercial hybrids introduced during the 1990s that include germplasm from temperate regions and imported maize grain from the Corn Belt are now also infiltrating the Sierras. However, there are still isolated tracts of Sierra that continue to function as in the years before the 1950s.

Native farmers regularly run a two-stage test on would-be introductions of maize genetic materials. This two-stage test measures agronomic performance in the field front, and their adequacy as food on the dinner table. The material classified as acceptable to the family may either be planted as a new genetic material, or else allowed to interbreed with a target landrace. Seed mixing of materials with similar flowering time or contiguous, coordinated planting for materials of differing flowering times are two methods that allow interbreeding.

Ears and seeds are normally selected from the granary and kept aside, hanging from ear husks in a visible place. The selected place is frequently close to the cooking area so that smoke helps as a pest control. Women play an important role in the seed selection process: they pay attention to several morphological seed and ear traits that assure the maintenance of the idealized kernel and ear type for each use (Ortega-Paczka 2003).

Ethnic group and common language play a role in the long-term process of development of common genetic materials at least through: (a) consensus on the idealized type of seed and ear, (b) recirculation of genetic materials, (c) reinforcement of knowledge and maintenance of oral traditions etc., and (d) settlement in a specific-geographical area that sets limits to edaphoclimatic variability.

The central elements of this native approach to maize breeding are (a) hybridization, (b) recirculation of maize genetic materials among farms, thus sampling various edaphoclimatic niches (c) selection of ears for seed as the steering mechanism to keep population development on track and (d) multi-location repetition of this process and repetition through long periods of time.

When observed at the farm and regional levels and for a short period of time, the native approach to handling maize germplasm might seem a cluster of many independently and randomly run operations akin to stagnation. However, as judged by its startling all-time achievements, one would have to regard the collective ethnic effort as a purposeful, parallel processing method to maize breeding. Certainly, the technological prowess of native farmers did not end in modern maize by the time of contact with the Europeans.

Useful traits in areas such as human nutrition—high kernel content of Lysine and tryptophan—and in industry—high oil content—(Hernandez Casillas 1986), resistance to field pests (Waiss *et al.* 1979; Snook *et al.* 1997; Wiseman 1997; Branson *et al.* 1986; Reid *et al.* 1990a; Campos *et al.* 1989), resistance to storage pests (Serratos-Hernandez *et al.* 1987; Classen *et al.* 1990; Arnasson *et al.* 1997; García-Lara *et al.* 2003), adaptation to soil acidity and to soil alkalinity (Hernandez-Xolocotzi 1988), symbiotic associative fixation of nitrogen (Gonzalez 1994) and other traits were discovered by native farmers, integrated into maize races, and taken advantage of. Modern science became aware of these genetically controlled traits by studying the mechanisms through which those known characters were expressed—normally with high population frequencies—in certain maize races, rather than by discovering them in isolated maize plants. Lots of maize breeding must have occurred in order to concentrate the relevant alleles responsible for such traits and for integrating them smoothly into the genetic background of maize races.

Mexican ethno-botanists, maize breeders and genetic resource specialists would agree that as long as ethnic groups maintain their cultural coherence and continue to cultivate maize, the native maize breeding system will stay alive, new genetic materials will be in the making and macro in-situ conservation of maize germplasm will stay operational.

We estimate that there are as many as one million ethno-farms in Mexico caring for maize genetic resources (see section on human population dynamics in this chapter).

The native farmer approach to maize breeding has been shared with Mestizo farmers throughout the history of post-Columbian Mexico. Mestizo farmers developed what is termed “traditional farming” from the interaction between knowledge and resources of Mesoamericans and Europeans. Native farmers also adopted what they considered valuable western knowledge and resources such as the wheel, animal traction, metal implements, fertilizers, herbicides, pesticides and nowadays even maize germplasm as an input to their native maize breeding system.

A sizable fraction of Mestizo farmers is growing their own maize seed, hence participating as stewards of some of the maize landraces. However, this fraction of rural society might not be a long-term faithful steward, especially in the more productive agroecosystems. Mestizo farmers will probably join the seed market of modern hybrids in some instances, as a response to economic opportunities and government programs, as reported by Ortega P. (1999) for el Bajio region.

The farm sector and resource base of Mexico

(a) *The land resource base*

Some 31 million hectares of the Mexican territory are cultivated, 120 million hectares are pastureland, and some 38 million hectares are forested. The present ratio of farmland to population is about 0.30 hectares *per cápita*; it will become 0.28 by year 2010 and 0.24 by year 2025 (CONAPO: <http://www.conapo.gob.mx>) if no more farmland is added. About 25.49 million hectares are rain fed farmland and 5.62 million hectares are irrigated farmland. Rain fed farmland has been classified on the basis of soil depth, rainfall and evaporation into five Agronomic Provinces: (1) 5.1 million hectares of prime farmland; (2) 3.3 million hectares of good farmland; (3) 8.7 million hectares of medium-low productivity farmland; (4) 4.6 million hectares of low productivity farmland; and (5) 3.7 million hectares of marginal farmland (Turrent 1986; Gonzalez *et al.* 1990).

About 42 percent of prime and good farmland and 57 percent of the lower quality types are located on hill-slopes, largely unprotected against erosion (Turrent 1986). SAGARPA, the Ministry of Agriculture did maintain a national erosion control program in the 1946- 1982 period. Some 3.3 million hectares—27 percent of erosion-susceptible farmland—were protected with terracing infrastructure. No information is available on the fate of this infrastructure. Soil erosion continues to be the main threat to ecological sustainability.

Mexico is ranked seventh worldwide for the amount of farmland under irrigation. More than 3 million hectares of irrigated farmland are managed under large-scale irrigation districts and 2.1 million hectares in small-scale irrigation schemes. Gravity irrigation is the main method for delivering water to crops. However, secondary channels that have not been waterproofed, plus a large fraction of farmland that has not been conditioned for irrigation (e.g., it is lacking leveling and contouring) limit irrigation efficiency. It is currently estimated that about 10 percent of irrigated farmland is affected by salinity. Most of the irrigation districts were built in the northern, drier half of the country, where abnormally dry years compromise the availability of irrigation water in the dam system every five to seven years. Despite these problems, the effort to develop irrigation infrastructure has been successful in modernizing a fraction of the farm sector, bringing much-needed foreign exchange through export and fostering food security.

(b) *The fresh water resource base*

According to SARH (1988) precipitation over territorial Mexico amounts to about 1530 km^3 per year, the average national precipitation in the period 1931–1970 was 780 mm. Almost 410 km^3 of this resource flow through rivers into the sea and 147 km^3 are captured in the national dam system. About 110 km^3 of fossil water and 31 km^3 of renewable fresh water in aquifers were detected in 73 percent of territorial Mexico by 1980. About 63 percent of the national fresh water resource that flows through rivers into the sea is located in eight southern states: Veracruz, Tabasco, Campeche, Yucatán, Quintana Roo, Chiapas, Oaxaca and Guerrero. Only a small fraction of the irrigation infrastructure has been built in this water-abundant section of the country due to topographic as well as edaphologic impediments to large-scale irrigation. Food security will probably drive a future expansion of the irrigation infrastructure into the southern part of the country.

(c) *The farming sector*

There are 3.805 million farms in Mexico. Farm size distribution is 1.313 million farms with less than 2 hectares of farmland, 0.964 million with 2 to 5 hectares, 1.188 million with 5 to 20 hectares, 200,000 farms with 20 to 50 hectares and 140, 000 farms with more than 50 hectares (INEGI 1991). Only 22 percent of farms rely on tractor power, 29.7 percent use animal power, 15.6 percent use a combination of animal and tractor power and 32.5 percent rely on human power only.

Three types of farming units have been described (a) traditional, (b) subsistence, and (c) entrepreneurial. Ethno-farming in the Sierras is a fourth type that has not been formally recognized as such, and is normally included within the subsistence type. Traditional and subsistence farming account for nearly 75 percent of all farming units. Traditional farming typically produces grain surpluses in moderate amounts that go to local markets; subsistence farming would normally not produce enough food for the family and has to acquire it in the local market.

Four secular characteristics of the majority of Mexican small farming units are (a) relative abundance of labor, (b) scarcity of land, (c) scarcity of capital and (d) low productivities of land and labor. Mexican farmers use 14 man-days to produce one ton of maize while their US and Canadian counterparts require 0.14 or less man-days. Yields of farmland are also low as analyzed by Calva *et al.* (1992).

Several factors explain low yields of land and labor and a stagnant farming sector. Some of these factors are (a) socially driven cultivation of rain fed, low-productivity and marginal farm land, (b) a historic, sizable fraction of good farm land devoted to extensive cattle grazing, (c) the introduction in 1993–1994 by the government of a new per hectare subsidy called PROCAMPO which was lower in economic terms than the previous, larger subsidies to agriculture that flowed through credit, insurance, extension and support prices, and (d) a long-term decline in government investment in the farming sector (Calva *et al.* 1992; Calva 1997; Rubio 1997; Turrent y Cortés 2004). Traditional and subsistence farming (ethnic farming included) have been most affected by government structural adjustments. Migration and related germplasm resource loss for maize, common bean, squash etc. are occurring at the national level, at rates that are currently undocumented.

(d) *Human population dynamics*

The VII population census of Mexico reports some 99 million inhabitants in year 2000 and a population growth rate of 1.68 percent a year, ignoring emigration. Mexico is the 11th-most populated country in the world, with a growth rate still in excess of the world average of 1.2 percent. The population growth rate of Mexico has been dropping substantially since the climactic figure of 3.4 percent of the mid 1960s. The proportion of rural—as defined as those living in communities with less than 2500 inhabitants—to total population has also dropped from 1960 (49.3 percent) to 25.4 percent in 2000. However, absolute rural population grew in the same period from 17.2 million to 24.7 million. The indigenous population is identified in the censuses by a language category; there are 84 languages spoken in Mexico other than Spanish. The fraction that spoke one of those languages in 2000 was 7.13 percent. The indigenous population is close to 7.1 million for the same year, and would be mostly rural. Therefore, about 29 percent of the rural population is indigenous. Assuming that absolute access to land—ignoring farm size—is independent of ethnic condition, there should be close to one million ethnic-farming units—probably smaller than 2 farmland hectares each, and relying on human energy as the sole source of power—out of a total of 3.805 million. That many farming units caring for 59 races of maize should be reassuring.

However, the indigenous population of Mexico stands as the poorest of the poor. Table 4 summarizes the level of economic marginality of indigenous population in the states with higher presence. Such plight plus the ensuing emigration make the weakest flank of the ethnic cultural stability. The same threat applies to stewardship of maize landraces and to other crop germplasm co-domesticated in Mexico.

Table 4. Levels of economic marginality of indigenous population in some states of Mexico.

State	Number of counties †	Economic marginality‡			
		High	Percentage	Very high	Percentage
Oaxaca	379	185	48.8	153	40.4
Puebla	76	39	51.3	32	42.1
Chiapas	58	24	41.4	32	55.2
Guerrero	23	4	17.4	19	82.6
Veracruz	76	35	46.1	36	47.4

† Counties with more than 40 percent indigenous population.

‡ Three levels of economic marginality are included: patrimonial (*per capita* income 28-41.8 pesos of year 2000), capacity (18.9-24.7) and alimentary (15.4-20.9).

SOURCE: Poder Ejecutivo Federal. Programa Nacional para el Desarrollo de los Pueblos Indígenas 2001–2006. México, 2001.

Maize the basic crop of Mexico

The balance between national maize production and domestic consumption shows five periods within the last 55 years. The first is a period that ended by 1961 in which production equaled consumption. Production was greater than domestic consumption allowing a positive trade of about one million metric tons per year in the second period (1962–1969). The third was a 21-year-long period that ended in 1990, with deficits averaging 2.1 million metric tons (Mmt) per year. The fourth was a short-lived period of almost maize self-sufficiency. The fifth period that started in 1994 has an increasing dependency of maize imports that averaged 4.3 Mmt annually in the 1995–1999 period and 5.7 Mmt in 2000–2001 (SAGARPA 2002).

Self-sufficiency of maize and common beans was pursued as a national objective until 1994, year of the initiation of the North American Free Trade Agreement (NAFTA). The NAFTA period anticipated a two-stage period for the maize regional market: 1994–2008 and beyond 2008. In the first stage, any maize import above a historic quota—2.5 Mmt that would grow at a compounded rate of 3 percent *per annum* starting in 1995 and reaching 3.6 Mmt by 2008—should have a 206 percent *ad valorem* tariff in 1994; this tariff would decrease linearly in two stages, from 206 percent in 1994 to 172 percent in1998 and then to 0 percent in 2008 (Nadal 2000). Maize import would be tariff free after 2008. This was a negotiated agreement that Mexico sustained as a temporal protection against asymmetry among the three partner countries. Mexico expected to modernize its maize production in that period.

The Mexican government unilaterally dropped this maize protection mechanism ever since 1996 when maize import was 5.9 million metric tons and eventually adopted a negligible 3 percent *ad valorem* tariff after 2000. Maize imports reached the levels of 4.3 Mmt in period 1995–1999 and 5.7 Mmt in 2000–2001.

Several factors were involved in this Mexican government action: (1) the sector of small maize producers proved to be highly resistant to government pursued agribusiness-type modernization; (2) drive to keep maize as an inexpensive food and feed; (3) a government commitment to eliminate subsidies to agriculture in spite of changing NAFTA-partner-country policies on subsidies. However, there is increasing political pressure from the Mexican small-farmer community towards the Mexican government for an immediate return to the NAFTA-agreed protection schedule and for the eventual withdrawal of maize and common beans from NAFTA.

There is evidence that self-sufficiency in maize and common beans is technically feasible (Turrent 1986; Turrent *et al.* 1996). Some technical conditions for achieving maize self sufficiency by the year 2010 have been analyzed by Turrent and coworkers (1997) as presented in Table 5. In a recent publication, Turrent *et al.* (2004) point out an added potential of eight million tons of maize a year that could be produced as a second, irrigated crop in one million farmland hectares of the fresh water-abundant, south-southeastern region of Mexico. This addition to maize potential of Mexico brings the figure to more than 40 million metric tons per year. Current production is about 18 million metric tons a year.

Table 5. Domestic maize production and consumption in 1976–77 and 1994–95 periods and projections to year 2010.

National statistics on maize	Period of evaluation †			
	1976–77	1994–95	2010(a)	2010(b)
Consumption (million metric tons, Mmt)	10.74	18.31	23.50	25.11
Observed production (Mmt)	10.14	18.29	23.63	25.31
Potential production (Mmt) ‡	20.17	29.45	33.75	33.75
Observed yield (ton ha^{-1})	1.36	2.26	3.15	3.37
Potential yield (ton ha^{-1}) §	2.70	3.65	4.50	4.50
CTE ¶	0.504	0.621	0.700	0.750

† Total area harvested on maize was 1.6 million hectares under irrigation plus 6.48 million rain-fed hectares in 1994-95. It is assumed that area harvested to maize will be 1.5 million hectares of irrigated farmland plus 6 million hectares of rain-fed farmland in year 2010.

‡ Potential production is defined as production achieved should all farmland in maize be managed with technology recommended by the INIFAP maize program.

§ Potential yield increased at 1.8 percent annual average rate between 1976 and 1994. Potential yield is assumed to grow at 1.4 percent annually between 1994 and 2010.

¶ Coefficient of technological efficiency computed as the ratio of observed to potential yield.

SOURCE: Turrent *et al.* 1997. Plan de Investigación del Sistema Maíz-tortilla en los Estados Unidos Mexicanos. Documento de circulación interna. INIFAP. México, DF.

However, it has also been shown that small-scale production of maize—less than 5 hectares of farmland per family—cannot be made competitive under the conditions established in NAFTA (Calva *et al.* 1992; Turrent *et al. 19*97). Obviously, it is the ethnic farmer, located at the far end of the scale, who receives most of the pressure to quit cultivating maize. This is an example of an unintended effect on stewardship of maize landraces.

Box 5. **<u>Maize breeding by INIFAP and transference to farmers</u>**

The Mexican Ministry of Agriculture (Secretaría de Agricultura y Fomento, SAF) began research on maize through two programs (1) the Office of Experiment Stations (Oficina de Campos Experimentales, OCE) in the late 1930s and (2) the Office of Special Studies (Oficina de Estudios Especiales, OEE) in the early 1940s. The latter was a collaborative effort with the Rockefeller Foundation. In 1947, OCE was transformed into the Institute of Agricultural Research (Instituto de Investigaciones Agrícolas, IIA) that functioned as such until 1960. Both institutions, IIA and OEE, were fused into the National Institute of Agricultural Research (Instituto Nacional de Investigaciones Agrícolas, INIA) in 1960. INIA became the National Institute of Research on Forestry, Agriculture and Animal Husbandry (INIFAP) after fusing with two sister institutes in 1985. No state level institution either government-dependent or associated to a state university was established, except for the state of Mexico.

Research on maize has been uninterrupted and fruitful for 60 years as reviewed by Angeles (2000). Some achievements of the breeding program in the 1940–1996 period were (1) development of 189 improved varieties and hybrids (Gámez *et al.* 1996) (2) interaction with other government programs for increasing national yields by 257 percent, (3) forming a maize gene bank with 10,500 accessions and race classification, (4) training of staff that reached a cumulative 34 PhDs, 43 MSc., and an unspecified number of employees at the undergraduate level.

The breeding effort followed a two-stage process: (1) collecting landraces, developing stabilized open-pollinated varieties, developing S_1 or S_2-hybrids—one or two self pollinations on the original landrace—that would make good synthetic varieties should the grain be used repeatedly as seed; (2) development of higher-endogamy hybrids (S_5 to S_9) and more uniform, open pollinated varieties. The second stage dates from the 1980s. The races of maize most frequently used in breeding programs in Mexico were Tuxpeño, Vandeño, Celaya, Tabloncillo, Palomero, Cónico, Chalqueño, Cacahuacintle, Cónico Norteño, Zapalote, Bolita, Olotillo y Pepitilla (Angeles 2000).

The first maize-breeding stage was planned for an insufficient seed production system relative to the size of the task. The maize commission (Comisión del Maíz) a public federal entity created in the 1950s was transformed into the national seed producing company (*Productora Nacional de Semillas*—PRONASE) as a para-state entity created by federal law. A seed certification entity *(Sistema de Inspección y Certificación de Semillas*—SNICS) was also created by federal law. Participation of private seed companies was low up until the late 1980s. INIA and its successor INIFAP fed PRONASE with maize materials (mostly hybrids) free of charge. PRONASE was the only exit for INIFAP materials, but PRONASE could receive maize materials only from INIFAP. Records show that maximum seed production and sales from PRONASE reached 15 percent of area planted to maize in 1981, the first year of a significant, two-year government program known as the Mexican Alimentary System (Sistema Alimetario Mexicano, SAM). Most of the time the fractions of total area planted with certified seed were in the range 8–12 percent. Another fraction of about the same magnitude was planted with open pollinated varieties and former hybrids planted from farmer-harvested own seed.

It was established in the first maize-breeding stage that Mexican landraces of maize could not take advanced inbreeding; plant vigor would drop sharply. By the late first-breeding stage INIFAP had developed open pollinated improved materials that overcame part of the landrace susceptibility to inbreeding. CIMMYT collaborated closely with INIFAP in preparing the second breeding stage by providing genetic materials that also tolerated inbreeding better than landraces and donated excellent stem resistance to lodging, though susceptible to ear rots in some locations. New inbred lines were developed in INIFAP from own materials and from out crossings with CIMMYT materials. A new generation of INIFAP hybrids is now available for all areas under irrigation and for the more productive rain-fed agrosystems (González *et al.* 1990; Turrent 1986). The exception to this rule is the very early materials for rain-fed, late planting in the highlands. The medium to low-productivity agrosystems have hybrids from the first-breeding stage available, improved open pollinated varieties and maize landraces. Marginal lands cultivated with maize rely thoroughly on landraces.

PRONASE was liquidated by the government as a part of the lower subsidy agreement in NAFTA. Private seed companies overtook the hybrid seed market of Mexico during the1990s. Fast-growing irrigated maize areas in Sinaloa, Sonora, Chihuahua, Tamaulipas, and Guanajuato and also the more productive rain-fed agrosystems of El Bajío Region (Jalisco, Michoacán and Guanajuato states) became markets of the private seed companies. However, the high-altitude areas have not been readily penetrated by the hybrids of private seed companies due to the smallness of the farming units, the lack of materials that resist foliar diseases and ear rots, and the typical smallness of hybrid kernels, in sharp contrast with the large kernel size of landraces.

Even though resistant to ear rots and to several foliar diseases in high-altitude agrosystems, INIFAP maize materials have not been able to penetrate the Sierras as planted materials. Low light intensity (frequent fog), high rainfall and relative humidity, soil hyper-acidity (pH in the 4-5 range), foliar disease and ear rots are conditions that only landraces can thrive on so far.

Erosion of germplasm of maize and wild relatives

Ortega-Paczka (1999) lists a number of factors of genetic erosion of maize landraces in Mexico:

(1) **The hybrid seed market now covers 27–34 percent of land planted to maize. An additional 25 percent is planted with open pollinated improved varieties or progressive generations of hybrids that have been out crossed with landraces. Landraces are now grown in less than 50 percent of total area in maize.**

(2) **Several crops substituted for maize in the 1960s: sorghum in El Bajío, common beans in Llanos de Zacatecas and Durango, pastureland in much of the humid tropics.**

(3) **Illegal cultivation of enervating crops.**

(4) **Government maize modernization programs frequently seek crop substitution or selectively reject landrace grain due to kernel colors not suited for processing into flour. Yellow colored landraces are frequently rejected by the urban market.**

(5) **Some farmers will plant improved varieties aiming at the market and reduce the area devoted to landraces.**

(6) **The author quotes Brush (1995) who suggests that highly productive crops in prime agroecosystems stand at high risk of substitution by introduced elite germplasm. Ortega offers some examples of modern hybrids substituting for landraces in highly productive agroecosystems: (a) Celaya race displaced from El Bajío and Llanos de Jalisco; (b) Tuxpeño race displaced from plowed farmland of some tropical areas; (c) Tuxpeño Norteño y Raton Races displaced from irrigated farmland of central and southern Tamaulipas.**

(7) **Very early and very late maturing landraces stand at high risk of substitution. The early races are low-yield while very late maturing materials require more labor per unit of product.**

(8) **Some low-density kernel landraces—such as Pepitilla race—have disappeared from some areas now that they get sold by weight rather than by volume.**

(9) **Emigration. Even though the family stays in charge of the milpa, insufficient family labor plus funds sent from abroad may lead to buying maize rather than cultivating it. There is even a loss of traditional farming knowledge.**

(10) **Some special-use landraces are maintained by very few farmers and are at risk of disappearing. Such is the case of chaman use of a highly ramified landrace for rituals.**

Ortega-Packza (1973 and 1999) reports his results on the exploration of a region of Chiapas in 1971 and 1991, previously explored by Hernandez-Xolocotzi in 1946. The expedition in 1971 yielded 12 races of maize out of a total of 41 races for Mexico! He found all races reported by Hernandez-Xolocotzi in 1946. The 1971 collection also included samples of improved maize varieties of the Tuxpeño race introduced from other regions of Mexico and from the Caribbean. The introductions displaced some of the local Tuxpeño and Vandeño races. The 1991 field trip found a stable germplasm situation at the mid-altitude lands (1600 m or more above sea level) that permitted the author to collect the same races as in 1946 and 1971. However, Tehua, a very late maturing race was almost extinct from the tropical lands. It was displaced by more productive and earlier maturing landraces, improved varieties and hybrids of the Tuxpeño race. He also found considerable loss of early races Nal-tel and Zapalote. The upper watershed of the Grijalva River had all but lost its maize diversity in plowed farmland. In contrast, genetic diversity of maize was maintained where the slash-and-burn farming system was practiced.

Ortega also explored south-central Tamaulipas in 1992, the state of Oaxaca in 1987 and 1991, the Yucatán peninsula in 1998 and the Chalco-Amecameca area in 1995. He found that frequently improved open pollinated varieties and succeeding generations of hybrids were planted along side local races in south-central Tamaulipas. The genetic diversity of Oaxaca and Yucatán previously found by Hernandez-Xolocotzi was confirmed, but there was infiltration of improved genetic materials and some loss of early landraces in both cases. The original diversity of the Chalco-Amecameca—a region very close to Mexico City and obviously subject to intensive population mobility—was almost intact by 1995 except for yellow kernel forms of the Chalqueño race.

Aguirre (1999) tested the hypothesis of a relationship between the fate of maize genetic diversity and the level of land productivity and development of infrastructure in a region. His results show that the more isolated areas maintained greater diversity of original maize materials that included colored kernel forms. Those areas closer to markets had lost some of the original diversity and presence of colored kernel forms was infrequent.

Total farmland harvested to maize in Mexico averaged 3.05 million hectares annually in the 1925–1929 period and 3.56 million hectares in 1945–1949 (SARH 1977). Only maize landraces were planted in those years. Farmland harvested to maize grew almost linearly between periods 1945–1949 and 1965–1969 to reach an annual average of 7.68 million hectares (SARH 1977). About 10 percent of that area was planted with hybrid seed, and another 10 percent was planted with farmer-produced seeds of open-pollinated improved varieties and succeeding generations of hybrids. About 80 percent of total area planted to maize (6.1 million hectares) depended on landraces.

Maize harvested area was 7.70 million hectares in the 1995–1999 period and 7.04 million hectares in 2000 and 2001 (SAGARPA 2002). Ortega-Paczka (1999) tells us that maize landraces are planted now in less than 50 percent of the maize area which would equal 3.08 million hectares in period 1995–1999 and 2.82 million hectares in 2000–2001 for a fraction on landraces taken as 40 percent. It is highly likely though that this fraction of farmland is either a geographically isolated section of the country or else the least productive land in developed regions.

Current total area planted with landraces of maize seems similar to that planted before any substitution of improved materials for landraces took place (1945–1949), but it is lower than in the climactic 1965–1969 period. There is also evidence of selective germplasm erosion; some maize landraces have a higher risk of losing its niches: the very early and the very late maturing landraces, the low kernel density, the yellow forms, etc. (Ortega-Paczka 1999). This author also tells us that emigration and government modernization programs are factors that push maize landraces out of their former niches.

Wilkes (1996) makes a risk assessment of losing teosinte populations in Mexico and Guatemala. Sanchez-González and Ruiz-Corral (1996) present an authoritative account of the distribution of teosinte in Mexico, as well as a list of factors of genetic erosion of teosinte. The latter quote Hernandez-Xolocotzi (1993) in stating that the introduction of cattle and of mechanical forms of farming worked against teosinte populations in the last 500 years. The authors report that several recent developments work in further reducing former teosinte space: access to previously isolated areas after road opening for commercial extraction of wood, urbanization, and cultivation of export-vegetables under irrigation. There are pro-teosinte factors, and these authors quote several: (a) teosinte is good forage; (b) has plasticity for occupying unused spaces; (c) is used for breeding native maize races, and (d) it is revered by some ethnic groups as "the heart of maize."

Box 6. <u>Status of *in-situ* and *ex situ* maize and teosinte germplasm conservation</u>

The National Research Institute on Forestry, Agriculture and Animal Husbandry (INIFAP) is in charge of conserving maize germplasm in Mexico. INIFAP shares related research efforts with several Mexican universities (Rincón-Sánchez and Hernández-Casillas 2000), and the International Center for Maize and Wheat Improvement (CIMMYT). Conservation of maize germplasm in Mexico is in debt to the dedication of scientists in this field (Cuevas 1947; Bautista 1949; Hernandez-Xolocotzi and Alanís-F. 1970; Hernández-Xolocotzi 1973, 1985, 1987; Ortega-Paczka 1973; Cervantes 1976; Ortega-Paczka. and Sánchez-González 1989; Ortega-Paczka, *et al.* 1991; Kato 1996; Herrera 1999; Miranda 2000; Casas *et al.* 2001, 2003).

However, Mexico has yet to develop a National Plan for conserving and managing its plant germplasm resources (Rincón-Sánchez and Hernández-Casillas 2000). The lack of such a plan has a negative impact on both *ex situ* and *in-situ* national efforts for conserving germplasm of maize and its wild relatives. Furthermore, the work and resources spent in this activity frequently are uncoordinated and dispersed with loss of invaluable information and field materials.

INIFAP keeps an *ex situ* collection of 9881 maize accessions and 180 teosinte accessions (Sanchez-González *et al.* 1998) in its central facility, located at the Valley of Mexico Experiment Station (CEVAMEX). The materials are conserved at 0-5°C and 35-40 percent relative humidity, which allows only a short to medium term conservation (Cárdenas 1997) so that frequent regeneration efforts must be made. Most maize accessions date back to the first collection by Wellhausen and collaborators in the 1940s (1952).

CIMMYT has a program for *ex situ* conservation of maize germplasm through a specialized facility in its headquarters. CIMMYT's gene bank is housed in a two-storey, fortified-concrete building dedicated in 1996. The above ground section stores the active collection, held at -3°C and 25–30 percent relative humidity. The below-ground section stores the base collection at -18°C, primarily for long-term storage. A working maize sample is 3 kg (from 6,000 to 12,000 seeds) and a base-collection is 1 to 1.5 kg (from 2,000 to 5,000 seeds). The storage capacity of this facility was planned for 62,000 maize accessions in the long-term collection and for 390,000 wheat accessions (Pardey *et al. 19*99). The number of maize accessions was reported to be 20,411. CIMMYT gene bank duplicates 8462 maize accessions of those conserved by INIFAP gene bank under the jurisdiction of a FAO agreement, which will be replaced by the International Treaty for Plant Genetic Resources (Taba 2003).

Associated to the *ex situ* conservation of Mexican maize, the Latin American Maize Project (1985–1995) and the ongoing, Latin American Maize Regeneration Project, are two of the major projects in which INIFAP has contributed closely with CIMMYT. The main results from these projects are: 1) the agronomic characterization of landraces for breeding purposes, 2) generation of core subsets for germplasm enhancement; 3) regeneration and safety duplication of maize germplasm accessions (Taba 2003).

In-situ conservation is central to maize germplasm conservation because of its complementary nature as a strategy for conserving resources (Bellon *et al.* 1998). It has been documented that in-situ conservation is particularly important in centers of origin and diversification of crops (Perales *et al.* 1998). This *in-situ* conservation strategy is quite widespread in Mexico, and has prompted several important projects related to maize conservation, diversification and participatory research in key regions of maize diversity in Mexico. *In-situ* conservation of maize has a great potential since this strategy could represent an opportunity to improve local varieties for special traits and purposes with the participation of local communities (Eyzaguirre and Iwanaga 1996).

Presence of Transgenic Maize in Mexico[3]

Early warnings on the high probability of transgenic maize entrance to Mexico were

[3] Several acronyms are used in this section: CIBIOGEM: Interministerial Commission on Biosecurity and Genetically Modified Organisms; CONABIO: National Commission for Knowledge and Use of Biodiversity; SAGARPA: Ministry of Agriculture, Rural Development, Food and Fisheries; SEMARNAT: Ministry of Environment and Natural Resources; UNAM: National Autonomous University of Mexico; IPN: National Polytechnic Institute; CINVESTAV: IPN's Center of Advanced Research; IBT: UNAM's Institute of Biotechnology; INIFAP: National Institute of Research on Forestry, Agriculture and Animal Husbandry; CIMMYT: International Maize and Wheat Improvement Center.

given to the academic community and to government authorities since at least 1995 (Serratos-Hernández 2002). However, the first non-confirmed finding of the introduction of transgenic maize was reported to the newspapers by the environmental group Greenpeace after their analysis of maize commodities imported from the US at the port of Veracruz in 1999.

A 2001 communication by Dr. Ignacio Chapela, later published in the scientific journal *Nature* (Quist and Chapela 2001) stated that transgene sequences were found in maize from peasant's fields in Oaxaca. This ignited the concern of the government's environmental sector about the results of Quist and Chapela's report, therefore started a larger study that involved CINVESTAV's Department of Genetic Engineering and UNAM'S Institute of Ecology (Ezcurra *et al.* 2002). Besides the controversy on methodological aspects and the inferences on the impact of transgenic maize advanced by Quist and Chapela (Metz and Futterer 2002; Kaplinsky *et al.* 2002; Christou 2002; Quist and Chapela 2002), the evidence on the likelihood of transgenic maize hybridization with maize in remote farmer's field locations (Ezcurra *et al.* 2002) granted a closer analysis by the agricultural authorities (see Box 7, Table 8).

The Ministry of Agriculture (SAGARPA), CIBIOGEM and CONABIO coordinated an *ad hoc* group[4] in October 2001 to conduct a wider investigation on the presence of transgenic maize in Oaxaca and Puebla states. The aim was to confirm and if so to determine the extent and frequency of transgenic maize. The *ad hoc* group stated that the purpose of this work was to obtain reliable data for analyzing possible impacts of transgenic maize on landraces rather than publication in peer-reviewed journals. Two hundred and seventy nine samples from maize producing areas and from DICONSA rural stores in the target states were collected between November 2001 and February 2002 and sent to CINVESTAV's Genetic Engineering Department and UNAM's Institute of Biotechnology for analysis. A first report was presented at a Conference in Beijing (Alvarez-Morales 2002). However, there is no official document available as of June 2004.

Other institutions, CIMMYT and INIFAP, conducted independent studies on detecting transgenic maize in Mexico. CIMMYT studies failed to find any adventitious DNA in CIMMYT's germplasm bank (CIMMYT's web page on transgenic maize). INIFAP scientists found low proportions of positive sites (Table 8). Nongovernmental organizations, in collaboration with a number of farmer-peasant communities in several states, carried their own investigation (ETC press release 2003).

Although these studies are not comparable because differences in sampling strategies, locations and laboratory techniques, there seems to be some indication that adventitious transgenic DNA is present in Mexico. This is stated without pre-judging the quality of each one of the investigation efforts.

A *de facto moratorium* on the field release of transgenic maize in Mexico has existed since 1998. Scientists working with transformation of maize have to comply with an official standard (NOM-056-FITO-1995) set by the Ministry of Agriculture when transgenic maize is to be grown. The strict biosafety regulations permit growing maize only at the research level. Almost all reported instances of transgenic maize grown in Mexico before the *de facto moratorium* (1998) covered less than one hectare (see Table 7).

However, since the official standard did not make provisions on grain trade, imports of

[4] Jose Antonio Serratos-Hernández, coauthor of this chapter was a member of the *ad hoc* group.

maize grain remained unregulated until 2003, when Mexico ratified the Cartagena Protocol. Meanwhile, some 5 million metric tons of maize grain were imported annually from the United States in the period 1997 through 2003. The United States produces and commercializes transgenic maize since its deregulation in 1996. Maize grain imported from the United States is a mixture of transgenic (30 percent of total) and non-transgenic grain. Feed industry and other industries take a substantial part of the imported maize commodities, and the rest is distributed as food aid to poorly developed rural areas. Seventy percent of maize grain samples collected from rural DICONSA stores by the SAGARPA-CIBIOGEM-CONABIO study was imported grain. Approximately 0.6 million metric tons of maize grain, with high probability of being mixtures of transgenic and non-transgenic maize, could have reached rural areas and isolated *campesino* and indigenous communities throughout Mexico every year in the period 1997 through 2003 (Serratos *et al.* 2001; Serratos *et al.* 2004).

Agricultural systems in rural isolated areas are open systems, and it is well known that *campesinos* test different sources of improved materials from government programs or commercial seed through small-scale distribution systems. It can also be the case that maize grain from government distribution stores is the only seed available after a bad harvest season. In other words, although transgenic maize seed is regulated at the experimental level, there are alternative routes by which genetically modified maize grain can enter the maize agricultural system. Farmers can inadvertently plant transgenic maize since there is no visible way of separating transgenic from non-transgenic seed.

After deregulation of genetically modified maize in the United States in 1996, production of transgenic maize in the US Corn Belt in the ensuing year was not high. Exports of maize from the United States into Mexico in 1997 contained but a small proportion of transgenic grain. That proportion has been increasing ever since. It has increased quantitatively as well as qualitatively (see Box 7). Five biotechnology seed companies produce and sell two types—insect resistant, and herbicide resistant, with or without male-sterility—of a variety of transgenic hybrids (several genetic backgrounds). It is likely that the mixture of transgenic/non-transgenic maize grain imported from the United States and Canada is also a mixture of grain that altogether contains two or three transgene-constructs in a variety of insert locations in different chromosomes and sections of chromosomes.

Another source of transgenic maize seed could have been hybrid seed introduced by Mexican farmers from the United States (Goodman 1997), unaware of the differences between common and transgenic materials.

The probability of survival of non-adapted seed is low but not negligible (Serratos *et al.* 2001; Serratos *et al.* 2004); therefore it could be assumed that the greater the number of transgenic seeds sown the greater the probability of surviving plants. After germination and survival some proportion of the seed from non-adapted plants would be facing an environment that affects reproductive structures; however, maize tassel is likely to be successful and shed pollen, having the opportunity to fertilize viable adapted female inflorescences in the maize field. Some neighboring plants could be pollinated with different proportions of transgenic pollen competing with pollen from local maize. Transgenic maize ears would less likely be pollinated but a very low proportion of fertilization could be attained. In other words, the transgenic plant containing the transgene could reach maturity and have the opportunity to pollinate neighboring maize ears (female inflorescence). This scenario depicts a process where, purely by chance, the transgene enters a stable population escaping strong selection (Serratos *et al.* 2004).

If transgenic seed was sown by chance—there are no morphological traits to distinguish transgenic from non-transgenic seed—the transgene could have an opportunity, albeit low, to enter the stable population only by means of the heterozygote after successful crossing with the adapted maize in the field. Once the heterozygous seed is present, the dynamic cycle of spreading the transgene into the population starts. The next generation will also contain a lower frequency of transgenic homozygous plants. Without knowing the presence of the heterozygous and homozygous transgenic plants in their fields, farmers will keep transgenic seed mixed within their seed lot for planting in the next cycle. Further crop cycles would have a fluctuating frequency of the transgene due to the random nature of the conditions in small farmers' fields from one year to the next, and the variable production resulting in the highly variable quantity of seed obtained (Serratos *et al.* 2004).

Maize fields containing variable frequencies of transgenic heterozygote plants could also be envisaged in this scenario which would produce different proportions of transgenic seed, due to highly variable conditions in the farmer's fields within a region. Seed exchange in local markets and with neighbors would produce a wave of dispersion beyond particular *campesino's* plot. Transgenic grain or seed sown in isolated areas could range from one seed to hundreds in an initial planting season, which could explain the discrepancies in the results of transgenic maize frequencies obtained by different institutions. Random proportions of survival, total planted seed, fertilized eggs and reproductive growth rates, determine the general tendency of the transgene invasion into stable populations (Serratos *et al.* 2004).

One can foresee increased exposure of landraces to foreign DNA through seed and pollen-mediated mechanisms in a scenario of increased availability of transgenic maize in Mexico. Because of their breeding strategy (see Box 4) *campesinos* producing their own seed would be direct receptors of the transgenic flow. Progeny transformation and retransformation, multiple transgene copies, and further accumulation of foreign DNA in landraces of maize are irreversible processes (see box 7). There is no way those farmers growing their seed and preserving maize races in Mexico could spare them from transgenic flow. Novel transgenes and novel constructs (i.e., better promoters) would inevitably accumulate over the old generation DNA materials in maize landraces and so on. Since teosinte outcrosses with maize, both subspecies would share the fate of foreign DNA accumulation.

Two as yet unanswered questions associated to deregulation of transgenic maize are (a) is there an extent of foreign DNA accumulation (both qualitative and quantitatively) in the maize genome that will impair basic survival functions of maize landraces such as resistance to foliar, root and ear diseases, and other maize enemies, adaptation to drought, to low and high temperatures, soil hyperacidity, etc.? (b) Would there be a long-term net tendency to lower yields of maize landraces in respective niches as accumulation of foreign DNA proceeds?

Non-regulation of non-food transgenic maize is quite another story as can be inferred from the Prodigene-Nebraska incident and reaction in the US Midwest (see box 7). The stake is compounded in Mexico by the plight of *campesinos* that cultivate maize as their basic staple, grow their own seed, and are *in-situ* stewards of critical genetic diversity of maize in the world. Transgenes that transform food-maize into non-food-maize would also inevitably reach teosinte.

Critical research should be conducted in order to seek answers to questions posed above. It is necessary to expose all races of Mexican maize and all teosinte races to cross pollination with transformed maize—transgene-construct (both food and non food maize), promoters, number of transgene copies, insert location—through successive landrace generations, under strict biosafety norms; run DNA analyses; expose transformed progenies to specific biotic (foliar, stem, root and ear diseases and pests) and abiotic stresses typical of their niches (drought, heat, frost, light intensity, soil hypoxia, hyperacidity, etc.) record yields, and check performance in multiple uses of maize.

Box 7. **What is transgenic maize**

Genetic engineering is perhaps the most controversial of all biotechnologies. This technology enables the transfer of a gene, from one individual organism to another—bypassing biological barriers—regardless of the species. There are several methods for *in vitro* incorporation of a foreign gene into maize: *Agrobacterium*-mediated transformation, electroporation and direct DNA transfer or biolistic method. The last is the most efficient method found thus far, and is the strategy typically employed by biotechnology corporations. All methods of inserting foreign DNA into maize plants depend on tissue culture, which is the methodology for regenerating whole plants from single cells, and a suitable system to identify/select maize plants that had been successfully transformed through the incorporation of foreign genetic material (Hoisington *et al.* 1998).

Basic biolistic protocol consists of a bombardment of gold or tungsten micro particles coated with DNA on tissues. Maize immature embryos of non-transgenic maize are dissected from recently pollinated ear cobs and are placed in adequate media for several hours previous to particle bombardment. Tissues of the immature embryos are then bombarded with the DNA-coated particles by means of a "gene gun" which is a high speed accelerator of particles (Herrera and Martinez 2003). The foreign DNA is contained within a carrier or plasmid vector which consists of the trait gene that will serve as selection marker, which is usually an antibiotic or herbicide resistant character (Hoisington 1997). Both of these genes, trait and marker, must be assembled to DNA segments known as promoter and end signals that regulate gene expression. This molecular machinery trait and marker genes fused to promoter and end signals constitutes what is called a transgene-construct.

None to about 50 copies of the transgenic construct may be inserted into every cell genome as a result of a biolistic run. Therefore, it is necessary to locate those cells that have only one properly-inserted copy. The rest is discarded. In order to select transformed cells within embryo tissues, the bombarded embryos are placed in growth media containing herbicide or antibiotic compounds that will inhibit and suppress those cells that did not take up the foreign DNA and are not expressing resistance to the selection growth medium. Cells resistant to the selection media are kept for further development providing the necessary elements for regeneration and rooting until plantlets are obtained (Figure 4). Thus, plantlets are transferred to soil and placed in the conventional greenhouse or biosafety greenhouse, depending on the regulations of the country (Hoisington *et al*. 1998).

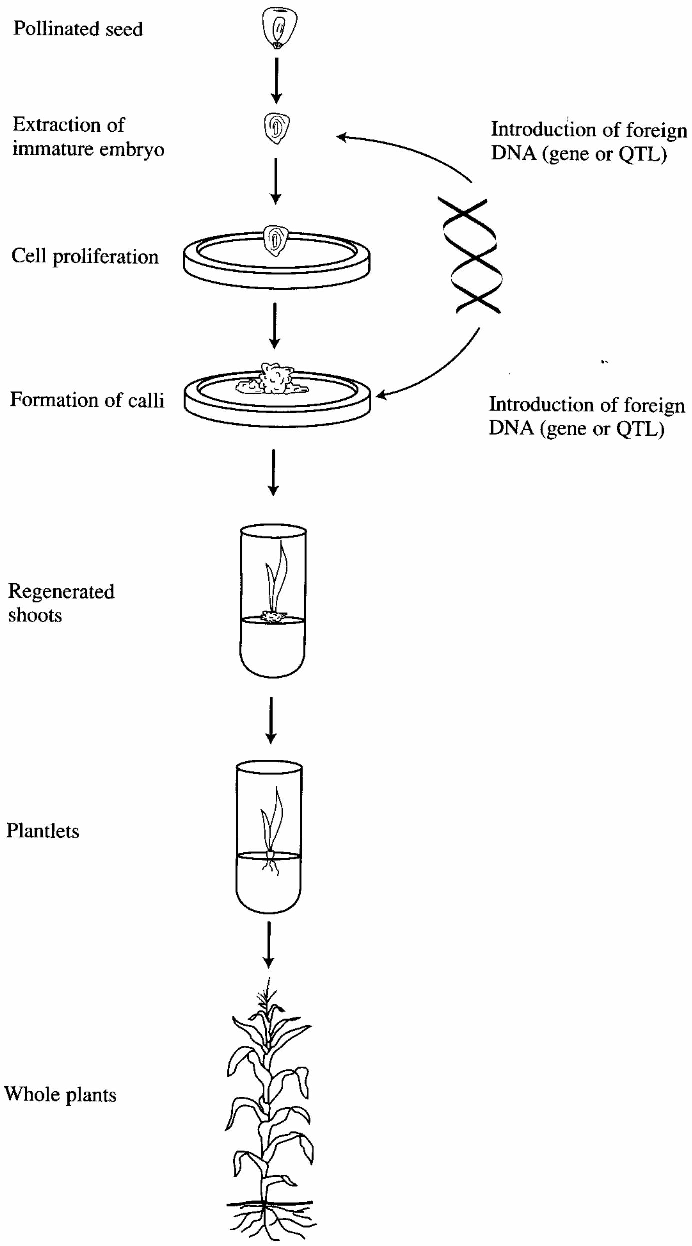


Figure 4. Schematic representation of the basic protocol for maize transformation. (Taken from Hoisington *et al.,* (1988).

The insertion of the transgene-construct by the biolistic transformation method is unpredictably inserted in any chromosome and section of a chromosome, assuming a successful single insertion event. Therefore, several independent biolistic runs of the same transgene-construct on embryos of the same genetic background would typically generate transformed maize plants that differ only in the precise location of insertion: different sections of same chromosome and/or different chromosomes. Several laboratory and greenhouse tests have to be conducted in order to select those transformed plants that express the transgene appropriately.

There are two main objections to the biolistic transformation method the incorporation of unwanted DNA segments and multiple insertions of transgene-construct copies into the maize genome (Herrera and Martinez 2003). There are contrasting reports on transgene stability/instability, silencing of transgene expression, and non-Mendelian trait inheritance in rice, barley and tobacco. These outcomes have been related to the number of transgene copies inserted, and to the rearrangement of unwanted DNA segments. Instability of transgenes is associated with multicopy, complex integration at loci and their positional effects. Kumpatla *et al*. (1997) found that silencing of transgenes due to methylation in transgenic rice is a frequent outcome of the biolistic method, because of the insertion of multiple rearranged sequences. In contrast, Gahawka *et al.* (2000) found that 29 out of 40 transgenic rice lines showed a stable, Mendelian inheritance of all the transgenes inserted over four generations. Koprek *et al.*, (2001) report stability of transgene expression by the insertion of maize transposable elements with single copy herbicide resistance (bar) gene; stable, single copy transgene expression in F3 and F4 generations was up to 81.5 percent.

Other studies with *Agrobacterium*-mediated or electroporation-transformed tobacco have suggested that vector or additional T-DNA sequences are not sufficient or necessary for transgene silencing (Meza *et al.* 2002); stability/instability in transgene expression can occur in tobacco transgenic lines whether containing simple T-DNA arrangements or multiple, incomplete T-DNA copies (Iglesias *et al.* 1997).

Insect-resistant and herbicide-tolerant maize are the two main products from the biotechnology/seed companies at present. Current status of transgenic maize events approved for field release and/or commercialization in the United States and Canada are: eight that carry insect resistance, seven are herbicide tolerant, two are male sterile; and two are a combination of insect and herbicide resistant maize. Tables 6 and 7 were taken from web sites with databases and updated information on the biosafety and regulations of genetically modified maize. These tables provide the status of transgenic food maize in the world and particularly in Mexico.

Transgenic maize cannot be distinguished from common maize once it is planted, and will enter the gene pool of surrounding populations through cross pollination. Transformed maize (*i.e*., insect resistance) cross-pollinates common maize and produces a transformed heterozygous progeny. The transgene-construct should not significantly interfere either with structure or function of resident genes of the progeny genome. Most phenotypic traits as plant architecture, adaptation to stress and reproductive capacities of the progenies—except for the transgene-coded trait—should be similar to those of non-transformed corn progenies. Farmers growing their own seed would benefit from this transformed progeny in the measure that the transgene addressed-stress affects crop yield. When this transformed progeny is exposed to pollen of second transformed maize—assuming the same transgene-construct as in first transformed maize, but different insert location—retransformed progenies would emerge with an extra copy of the transgene-construct: one copy and insert location inherited from each parent. Additional copies of transgene-construct will be accumulated in the genome in a stepwise process (gene stacking) as long as successive progenies are cross-pollinated with same transgene-construct of independently transformed maize (*i.e.*, other seed companies, different insert locations). The same process is repeated and copies accumulated in the genome of progenies through exposure to pollen of a second transgene-construct (*i.e.*, herbicide tolerance) produced independently, and sold in the same area by several seed companies.

Future generations of transformed maize will substitute for the older generations of transformed maize and/or else will add new traits. Farmers that buy their seed will benefit from the new technologies, as seed companies discontinue hybrids with old foreign DNA. However, farmers growing their own seed and conserving *in-situ* the 59 Mexican maize landraces will be stuck with the older foreign DNA and will have to continue to accumulate the new DNA.

Table 6. Modified maize with novel traits that have been released to the environment, and authorized for food and feed. Source: <http://www.agbios.com/dbase.php>.

Event	Company	Description	Country	Environ ment	Food and/or Feed	Food	Feed	Marketing
176	Syngenta Seeds, Inc.	Insect-resistant maize produced by inserting the *cry1Ab* gene from *Bacillus thuringiensis* subsp. kurstaki. The genetic modification affords resistance to attack by the European corn borer (ECB).	Argentina	1996		1998	1998	
			Australia		2001			
			Canada	1996		1995	1996	
			European Union	1997		1997	1997	
			Japan	1996		1996	1996	
			Netherlands			1997	1997	
			Switzerland			1997	1997	
			United Kingdom			1997		
			United States	1995	1995			
3751IR	Pioneer Hi-Bred International Inc.	Selection of somaclonal variants by culture of embryos on imidazolinone containing media.	Canada	1996		1994	1996	
676, 678, 680	Pioneer Hi-Bred International Inc.	Male-sterile and glufosinate ammonium herbicide tolerant maize produced by inserting genes encoding DNA adenine methylase and phosphinothricin acetyltransferase (PAT) from *Escherichia coli* and *Streptomyces viridochromogenes*, respectively.	United States	1998	1998			
B16 (DLL25)	Dekalb Genetics Corporation	Glufosinate ammonium herbicide tolerant maize produced by inserting the gene encoding phosphinothricin acetyltransferase (PAT) from *Streptomyces hygroscopicus*.	Canada	1996		1996	1996	
			Japan	1999		1999	2000	
			United States	1995	1996			
BT11 (X4334CBR X4734CBR)	Syngenta Seeds, Inc.	Insect-resistant and herbicide tolerant maize produced by inserting the *cry1Ab* gene from *Bacillus thuringiensis* subsp. kurstaki, and the phosphinothricin N-acetyltransferase (PAT) encoding gene from *S. viridochromogenes*.	Argentina	2001		2001	2001	
			Australia		2001			
			Canada	1996		1996	1996	
			European Union			1998	1998	1998
			Japan	1996		1996	1996	
			Switzerland			1998	1998	
			United Kingdom			1998	1998	
			United States	1996	1996			
CBH-351	Aventis CropScience	Insect-resistant and glufosinate ammonium herbicide tolerant maize developed by inserting genes encoding Cry9C protein from *Bacillus thuringiensis* subsp tolworthi and phosphinothricin acetyltransferase (PAT) from *Streptomyces hygroscopicus*.	United States	1998			1998	
DBT418	Dekalb Genetics Corporation	Insect-resistant and glufosinate ammonium herbicide tolerant maize developed by inserting genes encoding Cry1AC protein from *Bacillus thuringiensis* subsp kurstaki and phosphinothricin acetyltransferase (PAT) from *Streptomyces hygroscopicus*	Argentina	1998				
			Australia			2002		
			Canada	1997		1997	1997	
			Japan	1999		1999		
			United States	1997	1997			
DK404SR	BASF Inc.	Somaclonal variants with a modified acetyl-CoA-	Canada	1996		1997	1996	

		carboxylase (ACCase) were selected by culture of embryos on sethoxydim enriched medium.						
EXP1910IT	Syngenta Seeds, Inc. (formerly Zeneca Seeds)	Tolerance to the imidazolinone herbicide, imazethapyr, induced by chemical mutagenesis of the acetolactate synthase (ALS) enzyme using ethyl methanesulfonate (EMS).	Canada	1996		1997	1996	
GA21	Monsanto Company	Introduction, by particle bombardment, of a modified 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimate biochemical pathway for the production of the aromatic amino acids.	Argentina	1998				
			Australia			2000		
			Canada	1998		1999	1998	
			Japan	1998		1999	1999	
			Korea			2002		
			United States	1997	1996			
IT	Pioneer Hi-Bred International Inc.	Tolerance to the imidazolinone herbicide, imazethapyr, was obtained by in vitro selection of somaclonal variants.	Canada			1998		
MON80100	Monsanto Company	Insect-resistant maize produced by inserting the *cry1Ab* gene from *Bacillus thuringiensis* subsp. kurstaki. The genetic modification affords resistance to attack by the European corn borer (ECB).	United States	1995	1996			
MON802	Monsanto Company	Insect-resistant and glyphosate herbicide tolerant maize produced by inserting the genes encoding the Cry1Ab protein from *Bacillus thuringiensis* and the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *A. tumefaciens* strain CP4.	Canada	1997		1997	1997	
			Japan	1997				
			United States	1997	1996			
MON809	Pioneer Hi-Bred International Inc.	Resistance to European corn borer (Ostrinia nubilalis) by introduction of a synthetic cry1Ab gene. Glyphosate resistance via introduction of the bacterial version of a plant enzyme, 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS).	Canada	1996		1996	1996	
			Japan	1997			1998	
			United States	1996	1996			
MON810	Monsanto Company	Insect-resistant maize produced by inserting a truncated form of the *cry1Ab* gene from *Bacillus thuringiensis* subsp. kurstaki HD-1. The genetic modification affords resistance to attack by the European corn borer (ECB).	Argentina	1998		1998	1998	
			Australia			2000		
			Canada	1997		1997	1997	
			European Union	1998	1998			1998
			Japan	1996		1997	1997	
			Korea			2002		
			Philippines	2002		2002		
			South Africa	1997		1997	1997	
			Switzerland			2000	2000	
			United States	1995	1996			
MON832	Monsanto Company	Introduction, by particle bombardment, of glyphosate oxidase (GOX) and a modified 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimate biochemical pathway for the production of the aromatic amino acids.	Canada			1997		
MON863	Monsanto Company	Corn root worm resistant maize produced by inserting the *cry3Bb1 gene from Bacillus thuringiensis subsp. kumamotoensis.*	Australia			2003		
			Canada	2003		2003	2003	
			Japan			2002	2002	2001
			United States	2003	2001			
MS3	Bayer CropScience (Aventis	Male sterility caused by expression of the barnase ribonuclease gene from Bacillus	Canada	1996		1997	1998	

	CropScience(AgrEvo))	amyloliquefaciens; PPT resistance was via PPT-acetyltransferase (PAT).	United States	1996	1996			
MS6	Bayer CropScience (Aventis CropScience(AgrEvo))	Male sterility caused by expression of the barnase ribonuclease gene from Bacillus amyloliquefaciens; PPT resistance was via PPT-acetyltransferase (PAT).	United States	1999	2000			
NK603	Monsanto Company	Introduction, by particle bombardment, of a modified 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimate biochemical pathway for the production of the aromatic amino acids.	Australia			2002		
			Canada	2001		2001	2001	
			Japan	2001		2001	2001	
			United States	2000	2000			
T14, T25	Bayer CropScience (Aventis CropScience(AgrEvo))	Glufosinate herbicide tolerant maize produced by inserting the phosphinothricin N-acetyltransferase (PAT) encoding gene from the aerobic actinomycete *Streptomyces viridochromogenes*.	Argentina	1998		1998	1998	
			Australia		2002			
			Canada	1996		1997	1996	
			European Union		1998			1998
			Japan	1997		1997	1997	
			United States	1995	1995			
TC1507	Mycogen (c/o Dow AgroSciences); Pioneer (c/o Dupont)	Insect-resistant and glufosinate ammonium herbicide tolerant maize produced by inserting the *cry1F* gene from *Bacillus thuringiensis* var. *aizawai* and the phosphinothricin N-acetyltransferase encoding gene from *Streptomyces viridochromogenes*.	Canada	2002		2002	2002	

Table 7. Laboratory and field trials with transgenic maize authorized in Mexico until *de facto moratorium* for maize field release was in place. Source: Servicio Nacional de Sanidad Inocuidad y Calidad Agroalimentaria (SENASICA). Web site: http://web2.senasica.sagarpa.gob.mx/xportal/inocd/trser/Doc403/

Institution or Company	**Area (ha)**	**Genes/Trait**	**Location**	**Request**	**Approval**
ASGROW	0.1000	B73 and PAT genes for herbicide resistance	Los Mochis, Sinaloa State	23-Jan-96	24-Apr-96
ASGROW	0.1000	Bt gene for resistance to lepidopteran insects	Los Mochis, Sinaloa State	24-Jan-96	24-Apr-96
ASGROW	0.0350	Insect resistance gene	Los Mochis, Sinaloa State	14-May-97	18-Jul-97
ASGROW	0.1000	Insect resistance gene	Los Mochis, Sinaloa State	14-May-97	18-Jul-97
ASGROW	0.1000	Insect resistance gene	San Juan de Abajo, Nayarit State	14-May-97	18-Jul-97
ASGROW	1.0000	Insect resistance gene	San Juan de Abajo, Nayarit State	14-May-97	18-Jul-97
ASGROW	0.2500	B73 and PAT genes for herbicide resistance	Abasolo, Guanajuato State	23-Mar-98	30-Apr-98
ASGROW	0.2500	B73 and PAT genes for herbicide resistance	Celaya, Guanajuato State	23-Mar-98	30-Apr-98
ASGROW	0.2500	B73 and PAT genes for herbicide resistance	La Barca, Jalisco State	23-Mar-98	30-Apr-98
ASGROW	0.2500	B73 and PAT genes for herbicide resistance	Tlajomulco, Jalisco State	23-Mar-98	30-Apr-98
CIMMYT	Less than 0.001	GUS in tropical maize lines	El Batan, Mexico State	18-Mar-94	3-May-94

CIMMYT	Less than 0.001	Transgenic calli from tropical maize lines	El Batan, Mexico State	18-Mar-94	3-May-94
CIMMYT	Less than 0.001	CryIA(b) gene from Bt (Resistance to Lepidopterae)	El Batan, Mexico State	9-Jan-95	8-Feb-95
CIMMYT	0.0180	CryIA(b) gene from Bt (Resistance to Diatraea spp and S. frugiperda)	Tlaltizapan, Morelos State	25-Nov-95	8-Feb-96
CIMMYT	Less than 0.001	CryIA(b) gene for resistance to tropical insects	Tlaltizapan, Morelos State	3-Apr-96	7-Jun-96
CIMMYT	0.0092	CryIA(b), CryIA(c), CryIB and CryAc for resistance to lepidopteran insects	El Batan, Mexico State	23-Oct-96	22-Nov-96
CIMMYT	0.0075	CryIA(b) and BAR for lepidopteran insect and herbicide resistance	Tlaltizapan, Morelos State	1-Nov-96	22-Nov-96
CIMMYT	0.0320	CryIA(b) for resistance to lepidopteran insects under drought conditions	Tlaltizapan, Morelos State	1-Nov-96	22-Nov-96
CIMMYT	0.0195	CryIA(b) for resistance to lepidopteran insects	Tlaltizapan, Morelos State	8-May-97	19-Jun-97
CIMMYT	0.0041	CryIA(b) gene backcrosses	Tlaltizapan, Morelos State	2-Dec-97	29-Jan-98
CIMMYT	0.0041	CryIA(b) gene selfing	Tlaltizapan, Morelos State	2-Dec-97	29-Jan-98
CIMMYT	0.0195	CryIA(b) gene backcrosses	Tlaltizapan, Morelos State	21-Jul-98	10-Jan-99
CIMMYT	0.0195	CryIA(b) gene selfing	Tlaltizapan, Morelos State	21-Jul-98	10-Jan-99
CINVESTAV	Less than 0.001	BAR gene and gene from E. coli	Irapuato, Guanajuato State	10-Mar-93	1-Apr-93
MONSANTO	0.1000	Lepidopteran insect resistance gene (Yieldgard)	Los Mochis, Sinaloa State	12-Aug-97	4-Sep-97
MONSANTO	0.1000	Herbicide resistance gene (Glyphosate, RR)	Los Mochis, Sinaloa State	17-Sep-97	26-Mar-98
MONSANTO	0.2500	CryIA(b) for resistance to lepidopteran insects	Los Mochis, Sinaloa State	6-May-97	18-Jul-97
MONSANTO	0.2500	Herbicide resistance gene (Glyphosate)	Los Mochis, Sinaloa State	6-May-97	18-Jul-97
MYCOGEN	Less than 0.001	Bt gene for resistance to lepidopteran insects	Obregon, Sonora State	12-Nov-96	31-Jan-97
PIONEER	0.5000	CryIA(b) gene for resistance to European corn borer	San Jose del Valle, Nayarit State	19-Aug-97	19-Sep-97
PIONEER	0.5000	CryIA(b) gene for resistance to European corn borer	San Jose del Valle, Nayarit State	19-Aug-97	19-Sep-97
PIONEER	0.5000	CryIA(b) gene for resistance to European corn borer	Santo Domingo, Baja California Sur	19-Aug-97	19-Sep-97
PIONEER	0.0400	CryIA(b) for resistance to insects	San Jose del Valle, Nayarit State	25-Jun-98	14-Jul-98
PIONEER	0.2600	CryIA(b) gene for resistance to European corn borer	San Jose del Valle, Nayarit State	s.f.	13-Sep-96

Table 8. Investigations carried out on the presence of transgenic maize in Mexico. The work of Quist and Chapela was published in Nature (Quist and Chapela 2001; Quist and Chapela 2002). INE-CONABIO investigation was published by OECD (Ezcurra *et al.* 2002). CIMMYT's reports can be found in its web site: www.cimmyt.org. The NGOs report is in ETC group website: www.etcgroup.org. SAGARPA-CIBIOGEM report in part is found in Alvarez (2002). INIFAP's study is an internal report for SAGARPA.

Investigation (Sampling year)	State	Sampling sites (N)	Samples (N)	Sample type	Detection method	Frequency Positive localities (ELISA, Protein)[a]	Frequency Positive localities (PCR or Southern, 35S)	Frequency Positive samples (PCR or Southern, 35S)
Quist and Chapela (1). 2000	Oaxaca	3	7	Ear seed	PCR iPCR nested		1 (3/3)	0.714 (5/7)
Quist and Chapela (2). 2000	Oaxaca	3	7	Ear seed	DNA-DNA hybrid		1 (3/3)	0.714 (5/7)
INE-CONABIO. 2001	Oaxaca, Puebla	23	1876	Seedlings	PCR		0.913 (21/23)	0.069 (130/1876)
CIMMYT 16/10/2001	Gene Bank	-	840 (28 lra)	Leaves	PCR		-	0 (0/840)
CIMMYT 14/12/2001a	Gene Bank	-	750 (15 lra)	Leaves	PCR		-	0 (0/750)
CIMMYT 14/12/2001b	Oaxaca	7	840 (42 lr)	Leaves	PCR		0 (0/7)	0 (0/840)
CIMMYT 07/02/2001a	Gene Bank	-	410 (14 lra)	Leaves	PCR		-	0 (0/410)
CIMMYT 07/02/2001b	Oaxaca	1	375 (1 lra)	Leaves	PCR		0 (0/1)	0 (0/375)
SAGARPA-CIBIOGEM-CONABIO-INE. 2001	Oaxaca, Puebla	29/279	680	Leaves/ ear seed/ seedlings	ELISA/ Western blot/ Qualitative PCR/ Southern blot			-
INE-CONABIO. 2002	Jalisco	32	-	Leaves	-	0 0/32	0 0/32	-
INIFAP. 2002	Oaxaca	162	-	Ear seed	ELISA/ Western blot/ Qualitative PCR/ Southern blot	-	0.0309 5/162**	-
NGOs. 2003	Chihuahua, Morelos, Durango, Mexico, San Luis Potosí, Puebla, Oaxaca, Tlaxcala, Veracruz	138	2000	Leaves	ELISA kits	0.2391 (33/138)	-	-

A different set of transgene-constructs involving transformed maize into non-food maize is also in progress. One avenue aims at protein of the kernel endosperm and the second at endosperm starch. In both cases maize becomes a highly competitive bioreactor that is programmed for a variety of much-needed products. Some companies are developing transgenic maize to produce recombinant proteins for the pharmaceutical, animal health and industrial protein and enzyme markets. The opportunity for development of plant manufactured pharmaceutical (PMP) crop technology comes from the fact that US and European pharmaceutical companies are rapidly exhausting their available manufacturing capacity (primarily fermentation) for recombinant proteins. The number of protein-based products being developed could exceed conventional protein manufacturing capacity.

Some of these products developed for transgenic maize nowadays in final stages of product-recovery, agronomic tests and pre-commercialization trials are (Burden 2003):

- **Replacing limited, high-cost animal production systems for high protein Aprotinin, indispensable for controlling blood clotting during open-heart and other surgeries;**
- **a more affordable and readily available hepatitis-B vaccine;**
- **a novel tropical treatment for herpes viruses;**
- **a revolutionary cancer-cell growth inhibitor;**
- **human insulin from a plant source;**
- **a more affordable and readily available cervical cancer vaccine;**
- **a digestive enzyme aid to drastically improve the day-to-day lives of cystic fibrosis patients;**
- **an affordable and readily available, edible AIDS vaccine.**

The world market of recombinant therapeutic protein is worth some $17 billion—expected to double by 2010—while the market for industrial proteins is worth some $2 billion in 2004. Farmers producing this type of transgenic non-food maize are likely to increase revenue dramatically with respect to food-maize, and so are the corporative seed/processing industries.

However, far-reaching policy implications to transgenic crops followed after an incident of possible contamination of the soybean crop with PMP transgenic maize—a non-food maize by Prodigene—in Nebraska in 2001. A self-imposed moratorium for growing transgenic crops in food grain production regions was adopted by the Biotechnology Industry Organization (BIO), the Grocery Manufacturer's Association (GMA) and National Food Processors Association (NFPA) (Burden 2003). Immediately thereafter, the North American Millers' Association (NAMA) released a zero-tolerance policy for pharmaceuticals and industrial chemicals in the food-grain production stream. This was but a likely reaction to what was at stake: the commercialization and processing of 40 percent of the world's maize crop—maize production in the US Midwest amounted to 9.5 billion bushels (228 million metric tons) in 2001 according to the National Corn Growers Association (NCGA).

The incident did not end there, at least in Iowa. BIO agreed to rescind their moratorium on transgenic plantings in Iowa. This was in response to political concerns and to the official position of Iowa State University (ISU) with respect to PMP transgenic maize. ISU's position is that rules could be designed and enforced so as to prevent any PMP transgenic maize contamination. Twenty-five acres of PMP maize were grown in the farm of the Horan Brothers Specialty crops, located at Kenierm, IA, in 2001. Only one acre was grown in 2002. This maize is produced for and shipped—under very strict rules—to Meristem Therapeutics Inc., in Clermont-Ferrand, France.

It should be remarked that what is at stake in the Midwest is not compounded by the presence of farmers growing their own seed. Any escape of PMP transgenes would not have a carry on effect to the ensuing planting cycle in the Midwest, since all seed of food-maize is purchased from the seed companies every year.

<u>What is at Stake? Who are the Stakeholders?</u>

Transgenic maize has been detected—albeit in low proportions—deep in the heartland of traditional agriculture, which is also the historical, *in-situ* conservation-development ground of Mexico's maize races. Maize grain brought and currently being brought by government programs as aid in food depressed areas is probably the principal means of transgenic maize dissemination, although other means should not be discarded. Mexico currently imports mixtures of non-transgenic and transgenic maize from the United States. Paradoxically, dissemination of transgenic maize occurred while domestic production—18 million metric tons of non-transgenic maize per year—is about 50 percent larger than what a traditional maize-based diet would require for a population equal to one hundred million inhabitants in a year.

The Mexican Congress is currently examining a law initiative termed "Law on Biosafety and Genetically Modified Organisms." Whatever this law will dictate will have an impact on a number of historical, political, social, economic and ecological issues.

The objective of this book is to provide primarily the Mexican society but also international society with information that hopefully will help deal with the above issues. The objective of this first chapter is to introduce the reader to some of the complexities of maize in Mexico, a crop domesticated by Mesoamericans and a gift to mankind.

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