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**Series on Harmonisation of Regulatory Oversight in Biotechnology, No. 27**

**CONSENSUS DOCUMENT ON THE BIOLOGY OF ZEA MAYS SUBSP. MAYS (MAIZE)**

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Series on Harmonisation of Regulatory Oversight in Biotechnology

**No. 27**

**Consensus Document on the Biology  
of *Zea mays* subsp. *mays* (Maize)**

**Environment Directorate**

**Organisation for Economic Co-operation and Development**

**Paris 2003**

## **ABOUT THE OECD**

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 30 industrialised countries in North America, Europe and the Pacific, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised Committees and subsidiary groups composed of Member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's Workshops and other meetings. Committees and subsidiary groups are served by the OECD Secretariat, located in Paris, France, which is organised into Directorates and Divisions.

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## FOREWORD

The OECD's Working<sup>1</sup> Group on Harmonisation of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on the development of *consensus documents* which are mutually acceptable among Member countries. These consensus documents contain information for use during the regulatory assessment of a particular product. In the area of plant biosafety, consensus documents are being published on the biology of certain plant species, on selected traits that may be introduced into plant species, and on biosafety issues arising from certain general types of modifications made to plants.

This document addresses the biology of *Zea mays* subsp. *mays* (Maize). It contains general information as well as more specific information on taxonomy, identification methods, centre of origin/diversity, reproductive biology, crosses and agro-ecology. It is intended for use by regulatory authorities and others who have responsibility for assessments of transgenic plants proposed for commercialisation, and by those who are actively involved with genetic improvement and intensive management of the genus.

Mexico served as lead country (see Appendix E) in the preparation of this document. The document has undergone several rounds of revision based on the input from other member countries.

The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology has recommended that this document be made available to the public. It is published on the authority of the Secretary-General of the OECD.

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<sup>1</sup> In August 1998, following a decision by OECD Council to rationalise the names of Committees and Working Groups across the OECD, the name of the "Expert Group on Harmonisation of Regulatory Oversight in Biotechnology" became the "Working Group on Harmonisation of Regulatory Oversight in Biotechnology."



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## PREAMBLE

OECD Member countries are now approving the commercialisation and marketing of agricultural and industrial products of modern biotechnology. They had previously therefore identified the need for harmonisation of regulatory approaches to the biosafety assessment of these products, in order to avoid unnecessary trade barriers.

In 1993, **Commercialisation of Agricultural Products Derived through Modern Biotechnology** was instituted as a joint project of the OECD's Environmental Policy Committee and Committee on Agriculture. The objective of this project is to assist countries in their regulatory oversight of agricultural products derived through modern biotechnology - specifically in their efforts to ensure safety, to make oversight policies more transparent and efficient, and to facilitate trade. The project is focused on the review of national policies, with respect to regulatory oversight that will affect the movement of these products into the marketplace.

The first step in this project was to carry out a survey concentrating on national policies with regard to regulatory oversight of these products. Data requirements for products produced through modern biotechnology, and mechanisms for data assessment, were also surveyed. The results were published in *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results* (OECD, 1995a).

Subsequently, an OECD Workshop was held in June 1994 in Washington, D.C, with the aims of improving awareness and understanding of the various systems of regulatory oversight developed for agricultural products of biotechnology; identifying similarities and differences in various approaches; and identifying the most appropriate role for the OECD in further work towards harmonisation of these approaches. Approximately 80 experts in the areas of environmental biosafety, food safety and varietal seed certification, representing 16 OECD countries, eight non-member countries, the European Commission and several international organisations, participated in the Workshop. *The Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology* was also published by the OECD in 1995 (OECD, 1995b).

As a next step towards harmonisation, the Working Group on Harmonisation of Regulatory Oversight in Biotechnology instituted the development of **consensus documents**, which are **mutually acceptable** among Member countries. The goal is to identify common elements in the safety assessment of a new plant variety developed through modern biotechnology, to encourage information sharing and prevent duplication of effort among countries. These common elements fall into two general categories: the first being the biology of the host species, or crop: and the second, the gene product. This document, Biology of *Zea mays* (maize), is the eighth crop plant chosen for review; the first being *Brassica napus* L. (Oilseed Rape), the second being *Solanum tuberosum* subsp. *tuberosum* (Potato), the third being *Triticum aestivum* (Wheat), the fourth being *Oryza sativa* (Rice), the fifth being *Glycine max* (L.) Merr. (Soybean), the sixth being *Beta vulgaris* L. (Sugar Beet) and the seventh being *Prunus* sp. (Stone Fruits).

Safety issues that could give rise to a safety concern are identified in the consensus documents on the biology of a specific crop and include the potential for gene transfer, weediness, trait effects, genetic and phenotypic variability, biological vector effects and genetic material from pathogens (OECD, 1993a). They make no attempt to be definitive in this respect, however, as the many different environments in which the crop species may be grown are not considered individually.

This document is a "snap-shot" of current information that may be relevant in a regulatory risk assessment. It is meant to be useful not only to regulatory officials, as a general guide and reference source, but also to industry, scientists and others carrying out research.

In using this document and others related to the biology of crop plants, reference to two OECD publications which have appeared in recent years will prove particularly useful. *Traditional Crop Breeding Practices: An Historical Review to Serve as a Baseline for Assessing the Role of Modern Biotechnology* (OECD, 1993b) presents information concerning 17 different crop plants. It includes sections on phytosanitary considerations in the movement of germplasm and current end uses of the crop plant. There is also a detailed section on current breeding practices. *Safety Considerations for Biotechnology: Scale Up of Crop Plants* (OECD, 1993a) provides a background on plant breeding, discusses scale dependency effects, and identifies various safety issues related to the release of plants with "novel traits".<sup>2</sup>

To ensure that scientific and technical developments are taken into account, OECD countries have agreed that consensus documents will be updated regularly. Additional areas relevant to the subject of each consensus document will be considered at the time of updating.

Users of this document are therefore invited to provide the OECD with relevant new scientific and technical information, and to make proposals concerning additional areas that might be considered in the future. ***A short, pre-addressed questionnaire is included at the end of this document. The information requested should be sent to the OECD at one of the addresses shown.***

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<sup>2</sup> For more information on these and other OECD publications, contact the OECD publications Service, 2 rue André-Pascal, 75775 Paris Cedex 16, France, Fax: (33) 01.49.10.42.76; E-mail: [PUBSINQ@oecd.org](mailto:PUBSINQ@oecd.org); or consult <http://www.oecd.org>

## SECTION I - GENERAL INFORMATION

1. Maize, or corn, is a member of the *Maydeae* tribe of the grass family, *Poaceae*. It is a robust monoecious annual plant, which requires the help of man to disperse its seeds for propagation and survival. Corn is the most efficient plant for capturing the energy of the sun and converting it into food, it has a great plasticity adapting to extreme and different conditions of humidity, sunlight, altitude, and temperature. It can only be crossed experimentally with the genus *Tripsacum*, however member species of its own genus (teosinte) easily hybridise with it under natural conditions.

2. This document describes the particular condition of maize and its wild relatives, and the interactions between open-pollinated varieties and teosinte. It refers to the importance of preservation of native germplasm and it focuses on the singular conditions in its centre of origin and diversity. Several biological and socio-economic factors are considered important in the cultivation of maize and its diversity; therefore these are described as well.

### A. Use as a crop plant

3. In industrialised countries maize is used for two purposes: 1) to feed animals, directly in the form of grain and forage or sold to the feed industry; and 2) as raw material for extractive industries. "In most industrialised countries, maize has little significance as human food" (Morris, 1998; Galinat, 1988; Shaw, 1988). In the European Union (EU) maize is used as feed as well as raw material for industrial products (Tsafaris, 1995). Thus, maize breeders in the United States and the EU focus on agronomic traits for its use in the animal feed industry, and on a number of industrial traits such as: high fructose corn syrup, fuel alcohol, starch, glucose, and dextrose (Tsafaris, 1995). It is also noteworthy to understand how corn is used in the rising consumption of sweet corn and popcorn in developed countries (White and Pollak, 1995; Benson and Pearce, 1987).

4. In developing countries use of maize is variable; in countries such as Mexico, one of the main uses of maize is for food. In Africa as in Latin America, the people in the sub-Saharan region consume maize as food, and in Asia it is generally used to feed animals (Morris, 1998).

5. Maize is the basic staple food for the population in many countries of Latin America and an important ingredient in the diet of these people. All parts of the maize plant are used for different purposes: processed grain (dough) to make "tortillas", "tamales" and "tostadas"; grain for "pozole", "pinole" and "pozol"; dry stalks to build fences; a special type of ear cob fungi can be used as food (that is, "corn smut", or *Ustilago maydis*). In general, there are many specific uses of the maize plant depending on the region. Globally, just 21 % of total grain production is consumed as food.

6. The countries, which have the highest annual maize consumption per capita in the world, are listed in Table 1.

**Table 1: Consumption of maize *per capita* by country.**

Country	Annual consumption of maize per capita (Kg)
Malawi	137
Mexico	127
Zambia	113
Guatemala	103
Honduras	98
South Africa	94
El Salvador	93
Kenya	93
Zimbabwe	89
Lesotho	87
Venezuela	68
Nicaragua	56

Source: (Morris, 1998).

**Figure 1. Maize production worldwide.**



Source: Morris, 1998.

7. According to Morris (1998), "maize is the world's most widely grown cereal, reflecting its ability to adapt to a wide range of production environments" (Fig. 1).
8. Transgenic maize is already being used as a crop not only with agricultural purposes in several industrialized countries. Industrialised countries have dominant production of maize, because they possess advantageous factors that contribute to generate maize surplus. First, "maize production is generally concentrated in zones of abundant rainfall and fertile soils" (Morris, 1998), and, second, the use of many inputs and technology is extensive (Pollak and White, 1995; Rooney and Serna-Saldivar, 1987; Shaw, 1988; White and Pollak, 1995). By contrast, in developing countries the situation is highly variable. From Mexico to the Northern Andean region in South America, maize is a very important staple food in rural areas and the use of technology together with improved varieties is limited. However, Brazil, Argentina and Chile resemble industrialised countries because in these countries maize is a "cash crop grown by large scale commercial producers using extensive mechanisation" (Morris, 1998).
9. In many countries of Latin America maize is produced on small units of land. For example, in Mexico most of the land planted with maize (77 %) is less than 5 hectares in size, which contributes 67 % of total production (Calva, 1992 in Turrent-Fernández *et al.*, 1997). Only 5 % of the units of land dedicated to the production of maize averaged 12.2 hectares. More recently (Turrent-Fernández *et al.*, 1997), land units of maize production have increased in size but the technology inputs are below average: only 40 % of producers utilised improved seed; 64 % used nitrogen and phosphorous to fertilise the soil; and only 42 % received technical assistance.
10. In Africa, maize is an important crop mainly in the eastern and southern regions where it is "the dominant food crop and the mainstay of rural diets" (Morris, 1998). Also, maize production in Africa is similar to the production in some Latin American countries because the peasants of less developed rural areas grow maize in small plots, using negligible amounts of inputs or technology and no improved varieties.
11. In Asia, China dominates maize production. China is the second largest producer of maize closely behind the United States (Morris, 1998). Asian countries produce maize for livestock feed and likewise Meso-America and most African countries; "farms are small, use of improved germplasm and purchased inputs is modest, and yields are generally low" (Morris, 1998).

## SECTION II - TAXONOMIC STATUS OF ZEA

12. The Western Hemisphere genera *Zea* and *Tripsacum* are included in the tribe Maydeae (Table 2). The Asian genera of Maydeae are *Coix* ( $2n = 10, 20$ ), *Polytoxa* ( $2n = 20$ ), *Chionachne* ( $2n = 20$ ), *Schlerachne* ( $2n = 20$ ) and *Trilobachne* ( $2n = 20$ ).

13. Based on the morphology of the glumes of the male spikelets, Iltis and Doebley (1980) and Doebley and Iltis (1980) proposed a new classification system of the genus *Zea*. First, *Zea* was separated into two sections: LUXURIANTES and ZEA. The section LUXURIANTES grouped three species: *Z. luxurians*, *Z. diploperennis* and *Z. perennis*, and very recently it has included *Z. nicaraguensis* (Iltis and Benz, 2000). The section ZEA comprises only one species, *Z. mays*, which in turn is sub-divided into three subspecies: ssp. *mays*, for maize, ssp. *mexicana* for the races Nobogame, Central Plateau, Durango and Chalco (Wilkes, 1967; 1977) and ssp. *parviglumis*. This latter in turn is separated into two varieties, var. *parviglumis* for the race Balsas of Wilkes (1967) and var. *huehuetenangensis* for the race Huehuetenango of Wilkes (1967). Later on Doebley (1984, 1990) suggested that the var. *huehuetenangensis* should be elevated to a subspecies level.

14. Regarding the separation of the genus into sections LUXURIANTES and ZEA there is no controversy since morphological (Doebley, 1983; Smith *et al.*, 1981), isoenzymatic (Doebley *et al.*, 1984; Smith *et al.*, 1984), cytoplasm organelle DNA (Doebley *et al.*, 1987a, b; Sederoff *et al.*, 1981; Timothy *et al.*, 1979), and cytological (Kato, 1984; Kato and Lopez, 1990) evidence supports it.

15. The main controversy resides on the classification system within the section ZEA, particularly the grouping of the annual teosintes and maize into a single species, *Z. mays*. There is evidence showing that annual teosintes and maize are completely isolated from each other based on chromosome knob data (Kato, 1984; Kato and Lopez, 1990), and morphological-ecological data (Doebley, 1984). Although the isoenzymatic data suggest a low level of introgression between populations of these two plant types (Doebley, 1984; 1990), they have mainly the same isozyme alleles and the frequencies of these are distinct between most of the races of teosinte and most of the races of maize (Goodman, 1988). If it is accepted that the annual teosintes and maize are genetically isolated, then according to the biological species concept, the classification of the section ZEA made by Iltis and Doebley (1980) and Doebley and Iltis (1980) would not be acceptable, and would support the one proposed by Wilkes (1967).

16. Wilkes (1967) classified the annual teosintes within six races: Nobogame; Central Plateau; Chalco; Balsas; Huehuetenango; and Guatemala. Bird (1978) raised the race Guatemala into species rank, *Z. luxurians*.

17. The perennial teosintes from Jalisco in Mexico are separated into two more species (Iltis *et al.*, 1979) that have a ploidy difference, *Z. perennis* ( $2n=40$ ) and *Z. diploperennis* ( $2n=20$ ).

18. Doebley and Iltis (1980) and Iltis and Doebley (1980) classified teosinte as two subspecies of *Z. mays*: *mexicana* (Chalco, Central Plateau, and Nobogame) and *parviglumis* (var. *parviglumis*=Balsas and var. *huehuetenangensis*=Huehuetenango).

**Table 2. Classification of the genus *Zea* within the tribe Maydeae of the Western Hemisphere, and the genus *Tripsacum*.**

Family: Poaceae

Subfamily: Panicoideae

Tribe: Maydeae

Western Hemisphere:

Genus *Zea*<sup>1</sup>

Section *ZEA*

*Zea mays* L. (maize)

*Zea mays* subsp. *mays* (L.) Iltis (maize,  $2n^2 = 20$ )

*Zea mays* subsp. *mexicana* (Schrader) Iltis (teosinte,  $2n = 20$ )

    race Nobogame<sup>3</sup>

    race Central Plateau<sup>3</sup>

    race Durango<sup>4</sup>

    race Chalco<sup>3</sup>

*Zea mays* subsp. *parviglumis* Iltis and Doebley (teosinte,  $2n = 20$ )

    var. *parviglumis* Iltis and Doebley (=race Balsas)

    var. *huehuetenangensis* Doebley (=race Huehuetenango)

Section *LUXURIANTES* Doebley and Iltis

*Zea diploperennis* Iltis, Doebley and Guzman (perennial teosinte,  $2n = 20$ )

*Zea luxurians* (Durieu) Bird (teosinte,  $2n = 20$ )

*Zea nicaraguensis*<sup>5</sup> ( $2n = 20?$ )

*Zea perennis* (Hitcch.) Reeves and Mangelsdorf ( $2n = 40$ )

Genus *Tripsacum*

*T. andersonii* ( $2n = 64$ )

*T. australe* ( $2n = 36$ )

*T. bravum* ( $2n = 36, 72$ )

*T. cundinamarce* ( $2n = 36$ )

*T. dactyloides* ( $2n = 72$ )

*T. floridanum* ( $2n = 36$ )

*T. intermedium* ( $2n = 72$ )

*T. manisuroides* ( $2n = 72$ )

*T. latifolium* ( $2n = 36$ )

*T. peruvianum* ( $2n = 72, 90, 108$ )

*T. zopilotense* ( $2n = 36, 72$ )

*T. jalapense* ( $2n = 72$ )

*T. lanceolatum* ( $2n = 72$ )

*T. laxum* ( $2n = 36?$ )

*T. maizar* ( $2n = 36, 72$ )

*T. pilosum* ( $2n = 72$ )

<sup>1</sup> Iltis and Doebley, 1980; Doebley, 1990. <sup>2</sup> diploidy number. <sup>3</sup> Wilkes, 1967. <sup>4</sup> Sánchez-González et al., 1998. <sup>5</sup> Iltis and Benz, 2000.

### SECTION III - IDENTIFICATION METHODS

#### A. General description of *Zea mays*

19. *Zea mays* is a tall, monoecious annual grass with overlapping sheaths and broad conspicuously distichous blades. Plants have pistillate inflorescences enclosed in numerous large foliaceous bracts (ears), from 7 to 40 cm long, with spikelets in 8 to 16 rows on a thickened axis (cob) in the leaf axils and staminate spikelets in long spike-like racemes that form large spreading terminal panicles (tassels).

#### B. Identification among races of *Zea mays*

20. To study and classify this huge variation, a system of racial classification was established (Wellhausen *et al.*, 1952; Wellhausen *et al.*, 1957; Brown, 1953; Sato and Yoshida, 1956; Hateway, 1957; Roberts *et al.*, 1957; Briger *et al.*, 1958; Timothy *et al.*, 1961, 1963; Grobman *et al.*, 1961; Grant *et al.*, 1963; Brandolini, 1968; Mochizuki, 1968; Costa-Rodriguez, 1971; Paterniani and Goodman, 1977; Wellhausen, 1988; Avila and Brandolini 1990). Latin American countries, specifically Mexico, possess a great wealth of maize genetic diversity. There have been more than 40 land races of maize in Mexico (Wellhausen *et al.*, 1952; Hernández-Xolocotzi and Alanís, 1970; Ortega-Pazcka, 1980; Benz, 1986; Sánchez-González, 1989), and almost 250 land races in the Americas (Goodman and Brown, 1988).

#### C. Identification among *Zea mays* and wild species

21. The closest known relative of *Zea* is *Tripsacum*. The genus *Tripsacum* comprises two sections: section FASCICULATA with five species; and section TRIPSACUM with twelve species. The chromosome number varies from  $2n=36$  to  $2n=108$ . All species are perennials (deWet *et al.*, 1982, 1983). Twelve of these are native to Mexico and Guatemala with an extension of *T. dactyloides* throughout the eastern half of the United States, the tetraploids being near the East coast and the diploid in the central region. *T. lanceolatum* occurs in the southwest of the United States and *T. floridanum* is native to South Florida and Cuba. Three species of *Tripsacum* are known in South America.

22. Species of the section FASCICULATA are mostly and widely distributed in Meso-America, however, *T. lanceolatum* is found along the North of Sierra Madre Occidental, Mexico, up to Arizona. On the other hand, species of the section TRIPSACUM are distributed more extensively than the section FASCICULATA, although different species are found in relatively restricted territories; for example, *T. dactyloides* is found from a latitude about  $42^{\circ}$  North and  $24^{\circ}$  South. *T. dactyloides* tetraploid forms are also found in Kansas and Illinois in the United States. *T. manisuroides* is known only from Tuxtla Gutierrez, Chiapas, Mexico (deWet *et al.*, 1981, 1982, 1983). *T. andersonii* is of uncertain origin and is mostly sterile, it is an unusual species in that there is cytological (deWet *et al.*, 1983) and molecular evidence showing that its  $2n=64$  chromosomes comprise 54 *Tripsacum* chromosomes and ten *Zea* chromosomes (Talbert *et al.*, 1990).

#### D. Genetics and molecular identification

23. Maize has been one of the best studied plants in disciplines ranging from classical genetics to molecular biology. The study of maize has contributed to major breakthroughs in science such as the discovery of transposable elements (McClintock, 1929, 1934, 1944a, 1944b, 1944c, 1945; Fedoroff and



Botstein, 1992). McClintock first characterised the ten chromosomes of maize using mitotic studies. Presently cytological research is being conducted on chromosome staining techniques, meiotic mutants, examination of the B chromosomes and better understanding of the events involved during synapsis. Transposable elements are very important in maize genetics. Many different transposable element systems have been described for maize, the best characterised has been the Activator (Ac) and Dissociation (Ds) system. Ac/Ds comprises a family of maize transposable elements. Ac is the autonomous member of the family, capable of producing a transposable factor needed for mobility. Ds elements are not autonomous and capable of transposition only when trans-activated by Ac. Both genes have now been cloned and their mode of action is well characterised (Tsiftaris, 1995). A recent review of transposable elements is found in Federoff (2000).

24. The genetics of mitochondria and chloroplast in maize are of special importance. The mitochondrial genomes (mtDNAs) of higher plants are larger than those of mammalian or fungal mitochondrial genomes. The higher plant mitochondrial genomes are also more variable in their organization and have a larger coding capacity than mitochondrial genomes in mammals and fungi. Five types of mitochondrial genomes have been identified. Their designations are NA and Nb for the normal male fertile phenotypes, and T, S and C for the three different cytoplasmic male sterile (cms) phenotypes. Physical maps for three of the maize cytotypes have been completed. Mitochondrial genomes of higher plants have integrated DNA sequences that originate from other cell compartments (Tsiftaris, 1995). In contrast to plant mitochondria genomes, the chloroplastic genome is smaller and simpler; thus many chloroplastic genomes have been completely sequenced. The similarities between the genomes of chloroplasts and bacteria are striking. The basic regulatory sequences, such as transcription promoters and terminators, are virtually identical in both cases. Protein sequences encoded in chloroplasts are clearly recognisable as bacterial, and several clusters of genes with related functions are organised in the same way in the genomes of chloroplasts, *E. coli*, and cyanobacteria. In about two-thirds of higher plants, including maize, the chloroplast as well as mitochondrial DNA, is maternally inherited (Tsiftaris, 1995).

25. There is an abundant literature on the genetics, physiology, cytogenetics and molecular biology of maize and concise, thorough reviews are available (Coe *et al.*, 1988; Carlson, 1988; Walbot and Messing, 1988; Hageman and Lambert, 1988; Freeling and Walbot, 1994).

## **E. Maize Genome Maps**

26. The first RFLP map of corn was developed by Helentjaris *et al.* (1985, 1986a, 1986b). The corn linkage map encompasses approximately 1200 map units. The RFLP markers are not randomly distributed. The corn genome is about  $5 \times 10^6$  kb, then there would be approximately  $4 \times 10^3$  kb per map unit. It includes highly repeated sequences that constitute about 20% of the genome; these sequences are present in about ten superabundant sequence types. There are more than 1000 different moderately repetitive sequence families collectively representing 40% of the genome, this leaves approximately 40% single copy sequences, or more than  $10^6$  approximately gene size pieces.

27. Maize has one of the most well saturated genetic maps of any cultivated plant of this genome size. In principle this offers the possibility of easily locating any transgene and/or identifying any specific genotype (Tsiftaris, 1995). Recent maize genome maps and most of the information on the maize genome can be found in the following web addresses: <http://www.agron.missouri.edu>; <http://www.zmdb.iastate.edu>; <http://w3.aces.uiuc.edu/maize-coop/>. An expressed sequence tag (EST) database can also be found at <http://www.zmdb.iastate.edu>.

#### SECTION IV - CENTRE OF ORIGIN / DIVERSITY, MAIZE DIVERSITY

28. There are four main hypotheses on the origin of maize.
1. **The descent from teosinte hypothesis.** This is the oldest proposal and was advanced by Ascherson in 1895 (Mangelsdorf and Reeves, 1939) and proposes that maize was domesticated from teosinte by human selection. This is the most widely accepted hypothesis at present (Beadle, 1986; deWet and Harlan, 1972; Doebley and Stec, 1991; Doebley, 1990; Galinat, 1977; Iltis and Doebley, 1980; Goodman, 1988; Kato, 1984; Kato and López, 1990; Timothy *et al.*, 1979). The main problem with this hypothesis was how the distichous small female spike could have been transformed into the polistichous gigantic maize spike (ear) by human selective domestication. However, Doebley *et al.* (1990) have found five major genes controlling 'key' traits distinguishing maize and teosinte, and more recently Wang *et al.*, (1999) have discussed a gene controlling the inflorescence character in teosinte and maize.
  2. **The tripartite hypothesis.** The main assumption of this hypothesis is that there existed a wild maize in the past, which is considered extinct at present. This wild maize gave origin to the annual teosintes by crossing with *Tripsacum*. Further crossing of teosinte with wild maize gave rise to the modern races of maize (Mangelsdorf and Reeves, 1939; and Mangelsdorf, 1974). Later on Mangelsdorf *et al.*, (1981) based on experimental crossing between *Z. diploperennis* and the race Palomero Toluqueño of maize and further observations of its progenies, proposed that the annual teosintes are the products of this crossing. The fact that until now no evidence at all has been found about the existence, in the past or at present, of a wild maize, this hypothesis has lost much credence with time (although see Eubanks, 1995).
  3. **The common origin hypothesis.** This hypothesis proposes that maize, teosinte and *Tripsacum* originated by "ordinary divergent evolution" from a common ancestor. Consequently, it is conceived that there existed a wild maize plant that further was transformed into a cultivated plant by the selection and care of man (Weatherwax, 1955; Randolph, 1955; Randolph, 1959). The postulation that wild maize existed in the past makes this hypothesis not acceptable, as in the case of the tripartite hypothesis.
  4. **The catastrophic sexual transmutation hypothesis.** This hypothesis proposes that the maize ear evolved from the terminal male inflorescence of teosinte lateral branch by a "... sudden epigenetic sexual transmutation involving condensation of primary branches [and further] genetic assimilation under human selection of an abnormality, perhaps environmentally triggered" (Iltis, 1983). The finding of five mutant genes controlling key characters separating maize from teosinte (Doebley and Stec, 1991; Doebley *et al.*, 1990) seems to make the catastrophic sexual transmutation hypothesis untenable.

### *Centre of maize domestication*

29. The Meso-American region located within middle South Mexico and Central America is recognised as one of the main centres of origin and development of agriculture as well as centre of origin and diversification of more than one hundred crops (Vavilov, 1951; Smith, 1995; Harlan, 1992). At the present time, there is no agreement about where exactly maize was domesticated and there are several proposals in this regard. Based on the findings of archaeological materials from the maize plant (pollen, cobs, husks, and other remnants) in the United States and Mexico, which are older than those found in South America, Randolph (1959) proposed that maize was domesticated, independently, in the southwestern United States, Mexico, and Central America.

30. Mangelsdorf (1974) proposed that "corn had not one origin but several in both Mexico and South America", because the archaeological evidences are found in Mexico and several morphological characteristics in extant population found in the maize races of South America (Andes region) in comparison to those races of Meso-America.

31. The preliminary studies of McClintock (1959, 1960) on the chromosome knob constitutions of several races of maize from South America, Mexico and Central America, led her to conclude "that present-day maize may have derived from several different centres". These chromosome studies were further exploited (Kato, 1976, 1984; McClintock, 1978; McClintock *et al.*, 1981). They confirmed McClintock's previous conclusion and led to the proposal that maize was domesticated, independently, in four centres located in Mexico (two in 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. "This conclusion is based on the fact that chromosome knobs are not geographically and racially distributed at random, and that some knobs show restricted distributions following clear-cut pathways through specific territories, dispersion that clearly indicate that they were started in specific regions or centres of distribution. These centres are then considered as the places where original maize germplasm was domesticated from teosinte populations that were already cytogenetically well diversified" (Kato, 1984).

32. Contrary to the above multicentres origin of maize proposals, the isoenzymatic variation studies of maize and teosinte suggested to Doebley *et al.* (1987a) that maize was domesticated once in the Balsas basin region because "... all maize races of Mexico are isoenzymatically closer to var. *parviglumis* than to other teosintes...". Supporting this hypothesis, further molecular genotyping studies also suggest that maize originated from a single domestication in southern Mexico 9000 years ago (Matsuoka *et al.*, 2002).

### *Maize Diversity*

33. From the time of the discovery of America, Columbus noted the presence of corn on the North coast of Cuba and introduced it to Europe through Spain. At that time, corn was grown from Chile to southeastern Canada. Within two generations, after its introduction in Europe, corn became a cultivated crop throughout the world (Goodman, 1988). Germplasm resources are preserved *ex-situ* in many parts of the world, however, only in the Meso-American region there still exists, *in situ*, the original ancient maize that gave rise to improved varieties that are grown in all regions of the world. Most of the maize variation can be found in the Meso-American region and the northern part of South America. The great diversity of environments and conditions have created the basis for the development of maize varieties well adapted to harsh conditions of soil and climate as well as to biotic stresses. There is a close correlation among community culture, production system and the type of consumption of maize, with the diversification and variation of maize (Aguirre *et al.*, 1998; Louette and Smale, 1998).

34. Maize germplasm diversity is threatened by several factors: improved seed adoption; shift to cultivation of cash crops; and change in land use (Aguirre *et al.*, 1998; Bellon *et al.*, 2000; Louette, 1997). In some areas the adoption of hybrids and improved seed has increased dramatically, which has reduced the production of maize for traditional uses and, consequently, the increase of genetic erosion. Although these factors play an important role in reducing maize germplasm diversity, the persistence of maize land races in the Central American region is evident. Small farmers, peasants and indigenous ethnic groups and communities in many Latin American countries still preserve and select traditional maize.

35. Some arguments to explain the maize land race survival have been advanced (Ortega-Pazcka, 1973). The paramount importance of native maize for small communities, ethnic groups, small farmers and peasants, resides in the fact that land races of maize have very specific qualities for food and special uses as mentioned in Section I, rather than maize yield itself; therefore, many land races of maize have not been displaced by more productive maize types promoted by governmental agencies. For example, in Mexico after 50 years of maize genetic improvement programs, the adoption of hybrids and improved varieties is low. The research of Hernández-Xolocotzi (1972), Ortega-Pazcka (1973), Benz (1986), and Ortega-Pazcka *et al.* (1988), on maize diversity and peasant communities, demonstrates that local maize has been preserved by peasants, using traditional methods, basically intact for decades. As the result of a poll carried out in 1992 (CIMMYT, 1994), it was concluded that open pollinated land races of maize cover 42% of arable land dedicated to maize in less developed countries.

36. The approach for conservation of Latin American maize land races relies on two main criteria: the adaptation to a particular ecological niches and special forms of consumption of specific land races. Native germplasm utilisation has varied depending on the country and the needs of development. In general, the strategy is to identify sources of elite germplasm by means of characterizing and evaluating samples from land race collections, consisting of composite groups, populations and pools. National programs, international institutions, private seed industries and universities use these germplasm materials. Native maize land races have not been widely used for improvement programs and in Mexico, for example, only 10% of Mexican maize land races have been incorporated in specific breeding programs. There are a couple of examples in Mexico where native races of maize were characterised and evaluated for selection to generate improved populations, which were released as new open pollinated varieties: variety V520 (from land race San Luis Potosí-20); and variety Rocamex V7 (from land race Hidalgo-7). However, there is still germplasm in farmers' fields that have not been evaluated for their improvement and utilisation (Márquez-Sánchez, 1993).

37. Examples of maize land races specifically adapted to special conditions are (Hernández-Xolocotzi, 1988): Gaspé, short growing season (early maturity); Guatemalan Big Butt, long growing season (late maturity); Tuxpeño, Celaya, Chalqueño, Cuban Yellow Flint and Cuzco Gigante, high efficiency and productivity under good rainfed conditions; Chococeño, Enano and Piricinco, tolerance to high temperature and humidity; Cónico norteño, tolerance to semi-dry environments; Palomero Toluqueño, Cónico, Cacahuacintle and Sabanero, well adapted to high elevations, low temperature; Nal-tel, adapted to calcareous soil.

## SECTION V - REPRODUCTIVE BIOLOGY

### A. Sexual reproduction

38. *Zea mays* is an allogamous plant that propagates through seed produced predominantly by cross-pollination and depends mainly on wind borne cross-fertilisation. *Z. mays* is a plant with a protandrous inflorescence; however, decades of conventional selection and improvement have produced varieties of maize with protogynous traits. *Z. mays* has staminate flowers in the tassels and pistillate flowers on the ear shoots.

39. **The tassel.** The structure and development of the stamens are similar to other grasses. The anther develops four chambers or loculi each one containing a central row of archeosporial cells that gives rise to sporogenous tissue. After seven weeks the microspore mother cells are in the meiosis stage. Microspores are organised around four nuclei and become mature pollen grains. The amount of pollen produced by a tassel is estimated at 18 million pollen grains (Kiesselbach, 1980). Probably the best-improved varieties would produce more than this. On average 21,000 pollen grains could be produced for each kernel on an ordinary ear with 1000 kernels. Kiesselbach (1980) calculated that: "With a stand of three stalks in hills 42 inches apart, an area of 588 square inches is available in the field for each stalk. Thus an average of 42,500 pollen grains are provided for each square inch of the field. If the silks of an ear display a total surface of 4 square inches they will intercept about 170,000 pollen grains. Estimating 1,000 silks per ear, this amounts to 170 pollen grains per silk. Considering that corn in the field sheds pollen for 13 days, each silk receives an average of 13 pollen grains per day."

40. **The ear shoot.** At each node of the stem there is an axillary bud enclosed in the prophyllum. Only one or two of these axillary buds will develop as ear shoot and reach the fertilisation stage. At first the ear is smooth but protuberances soon form in rows. The basal protuberances are formed first and development advances towards the tip of the ears. Each one becomes two lobed, each lobe developing into a spikelet with two flowers, only one of which commonly persists. The growing point of the upper flower is differentiated to form the functional pistil. The part above the attachment of the carpels develops a single sessile ovule, which consists of a nucellus with two integuments or rudimentary seed coats. The united carpels, which will form the ovary wall or pericarp of the mature kernel, grow upward until they completely enclose the ovule. Where they meet, the functionless so-called stylar canal is formed. The two anterior carpels, which face the ear tip, form outgrowths, which develop into the style or silk. The surface of the silk becomes covered with numerous hairs, which are developed from cells of the epidermis. At the base of the silk is a growth zone where new cells develop, causing continuous elongation of the silk until it is pollinated and fertilisation takes place. The development of the embryo sac is characteristic of the grass family. One of the three nuclei at the micropylar end enlarges and becomes the nucleus of the egg, while the others become the nuclei of the synergids. At this stage the embryo sac is ready for fertilisation but if pollination is prevented it may remain in this condition for some time, perhaps two weeks, after which the embryo sac and nucellus disorganise and fertilisation is no longer possible.

41. Fertilization occurs after the pollen grain is caught by the silk and germinates to create the pollen tube which penetrates up to the micropyle and enters the embryo sac. The pollen is carried mainly by wind, thus it is highlighted that pollination can occur even, although rarely, over long distances measured in kilometers.

**B. Asexual reproduction**

42. There is no asexually reproductive maize. Cell/tissue culture techniques can be used to propagate calli and reproduce tissues or plants asexually; however, with maize cells and tissues these techniques are difficult.

## SECTION VI - CROSSES

### A. Intra-specific crosses

43 Maize is essentially 100% open-pollinated (cross-fertilising) crop species. Until the 20<sup>th</sup> century, corn evolved through open pollinated varieties, which are a collection of heterozygous and heterogeneous individuals developed by mass selection of the people from the different civilizations existing in the Americas (Hallauer, 2000). Corn pollen is very promiscuous, lands on any silk, germinates almost immediately after pollination, and within 24 h completes fertilisation. Thus all corns will interpollinate, except for certain popcorn varieties and hybrids that have one of the gametophyte factors of the allelic series *Ga* and *ga* on chromosome four (Kermicle, 1997).

44 There is a great sexual compatibility between maize and annual teosinte and it is known that they produce fertile hybrids (Wilkes, 1977). In areas of Mexico and Guatemala maize and teosinte freely hybridise when in proximity of each other. Wilkes (1977) reported a frequency of one F1 hybrid (corn x teosinte) for every 500 corn plants or 3 to 5 % of the teosinte population for the Chalco region of the Valley of Mexico. Kermicle and Allen (1990) have shown that maize can introgress to teosinte; however, there is incompatibility between some maize populations and certain types of teosinte resulting in low fitness of some hybrids that prevents a high rate of introgression (Evans and Kermicle, 2001).

### B. Inter-specific crosses

45 Although it is extremely difficult, *Tripsacum* species (*T. dactyloides*, *T. floridanum*, *T. lanceolatum*, and *T. pilosum*) can be crossed with corn; however, hybrids have a high degree of sterility and are genetically unstable (Mangelsdorf, 1974). Galinat (1988) advanced that since *Tripsacum* and *Zea* have different chromosome numbers, the addition of an extra *Tripsacum* chromosome into the maize genome would occur with a low frequency and consequently the rate of crossing-over would be extremely reduced. Despite these arguments, Eubanks (1995, 1998) developed a method for transferring *Tripsacum* genes into maize. In this method two wild relatives of maize, *Tripsacum* and diploid perennial teosinte (*Zea diploperennis*), are crossed to produce a hybrid, which is called tripsacorn, used to generate maize-tripsacorn hybrids. The use of tripsacorn is intended to confer resistance to pests and disease, drought tolerance and improved uniformity. Recently it has been claimed (Eubanks, 2000) that traits such as apomixis, totipotency, perennialism, adaptation to adverse soil conditions and to carbon dioxide enriched atmosphere can be transmitted to maize via maize x *Tripsacum*-perennial teosinte (and/or its reciprocal).

46 The cross between maize and *Tripsacum* has been studied since long ago (deWet *et al.*, 1973; Bernard and Jewell, 1985), and recently efforts have been made to transfer genes related to traits like apomixis from *Tripsacum* to maize (Burson *et al.*, 1990; Savidan and Berthaud, 1994; Hanna, 1995; Leblanc *et al.*, 1995; Grimanelli *et al.*, 1998; Grossniklaus *et al.*, 1998). Maize x *Tripsacum* hybrids have been produced and consequently several patents on apomictic maize have been published (Kindiger and Sokolov, 1998; Savidan *et al.*, 1998; Eubanks, 2000).

### C. Gene flow

47 The interaction between domesticated plants and their wild relatives can lead to hybridisation and in many cases to gene flow of new alleles from a novel crop into the wild population (Ellstrand *et al.*, 1999). While gene flow *per se* is not a concern, theoretically, it can lead to the potential for the evolution of aggressive weeds or the extinction of rare species. There has been preliminary documentation of this in some cases although not for maize (Ellstrand *et al.*, 1999).

48 As mentioned in Section VI-A some teosinte species can produce fertile hybrids with maize. All teosintes, members of the Section LUXURIANTES and subspecies *mexicana* and *parviglumis*, occur only in Mexico and Guatemala (Sánchez-González and Ruiz-Corral, 1997). It has been documented that maize and teosinte often interact, particularly with *Zea mays* ssp. *mexicana* (Wilkes, 1977). Also, the known distribution of teosintes, together with high likelihood of the presence of land races in the maize production areas of Mexico indicates, as shown in Appendix B, that there exist high probabilities of genetic exchange between conventional maize, land races and teosinte (Sánchez-González and Ruiz-Corral, 1997; Serratos-Hernández *et al.*, 1997; Serratos-Hernández *et al.*, 2001). However, there is some evidence of restricted gene flow between *Zea* spp. that occurs predominantly from teosinte into maize (Doebley *et al.*, 1987a). To date, there is no genetic analysis of morphologically intermediate plants that could identify “whether the maize-teosinte intermediates are true hybrids, introgressants or crop mimics” (Ellstrand *et al.*, 1999). Out-crossing of maize with *Tripsacum* species is not known to occur in the wild.

49 Another factor to take into account regarding gene flow is the exchange of seed and traditional maize improvement practised by peasant communities and small farmers. As observed by Louette (1997), rural communities are open systems where “...there is a constant flow of genetic material among communities over large areas.” therefore, as in the case of Mexico, “...a land race variety, an improved variety, or a transgenic variety of maize, can reach any zone of the country, even the most isolated ones, such as those where teosinte grows.” The human factor together with the changes in policy and strategies in maize production (Nadal, 1999) may increase several fold the chance of gene flow between improved maize, teosinte and landraces.



## SECTION VII - AGRO-ECOLOGY

### A. Cultivation

50 Although maize was domesticated and diversified mostly in the Meso-American region, at present it is cultivated mainly in warm temperate regions where the conditions are best suited for this crop (Norman *et al.*, 1995).

51 Maize is an annual plant and the duration of the life cycle depends on the variety and on the environments in which the variety is grown (Hanway, 1966). Maize cannot survive temperatures below 0° C for more than 6 to 8 hours after the growing point is above ground (5 to 7 leaf stage); damage from freezing temperatures, however, depends on the extent of temperatures below 0° C, soil condition, residue, length of freezing temperatures, wind movement, relative humidity, and stage of plant development. Light frosts in the late spring in temperate areas can cause leaf burning, but the extent of the injury usually is not great enough to cause permanent damage, although the corn crop will have a ragged appearance because the leaf areas damaged by frost persist until maturity. Maize is typically grown in temperate regions due to the moisture level and number of frost-free days required to reach maturity. The number of frost-free days dictates the latitude at which corn varieties with different life cycle lengths can be grown. Maize having a relative maturity of 100 to 115 days is typically grown in the U.S. corn belt. Maize varieties with different relative maturities do not occur in parallel east-to-west zones because they are also dependent on prevailing weather patterns, topography, large bodies of water, and soil types (Troyer, 1994 in Hallauer, 2000).

52 In tropical regions, maize maturity increases due to altitude effects. Tropical land races of maize in the tropics characteristically show three to five ears and axillary tillering, as opposed to modern cultivars that suppress lower ears and tillers (Norman *et al.*, 1995). In the tropics Oxisols, Ultisols, Alfisols and Inceptisols are best suited for maize production; however, maize is adapted to a wide variety of soils in the tropics, from sands to heavy clay. Of particular importance is aluminium toxicity for maize on acid tropical soils. Liming can solve this problem, "Deep lime incorporation in the subsoil of some Oxisols has overcome aluminium toxicity, thereby improving rooting depth in maize and tolerance to dry periods" (Norman *et al.*, 1995).

53 The farmland of Mexico covers a wide range of ecological conditions: from sea level to 2800 meters, from very dry to wet climates, well drained to poorly drained soils, flat to severe slopes, shallow to deep soils, low to high solar radiation; drought, wind and frost damage are common.

54 The poorest farmers are typically Indian farmers that inhabit the Sierras. Dry beans, squash, grain amaranth and several other species were also domesticated by the inhabitants of the region, as complements to their diet. They also developed the typical "milpa cropping system" as a cultivated field that may involve the association, inter-cropping, or relay-cropping of maize, beans, squash, grain amaranth, tree species and several tolerated herbal species. The isolation of these farming communities has caused the development of a great resource of maize germplasm diversity, which is conserved using *in situ* and *ex situ* (germplasm banks) means. Inter-cropping of maize with other crops is practiced in many areas

of less developed countries (Norman *et al.*, 1995). These systems imply changes at the level of cultivation and management of maize production which are important in terms of ecological relationships.

## **B. Volunteers and weediness**

55 Maize has lost the ability to survive in the wild due to its long process of domestication, and needs human intervention to disseminate its seed. Although corn from the previous crop year can overwinter and germinate the following year, it cannot persist as a weed. The presence of corn in soybean fields following the corn crop from the previous year is a common occurrence. Measures are often taken to either eliminate the plants with the hoe or use of herbicides to kill the plants in soybean fields, but the plants that remain and produce seed usually do not persist during the following years. Volunteers are common in many agronomic systems, but they are easily controlled; however, maize is incapable of sustained reproduction outside of domestic cultivation. Maize plants are non-invasive in natural habitats (Gould, 1968). In contrast to weedy plants, maize has a pistillate inflorescence (ear) with a cob enclosed with husks. Consequently seed dispersal of individual kernels does not occur naturally. Individual kernels of corn, however, are distributed in fields and main avenues of travel from the field operations of harvesting the crop and transporting the grain from the harvested fields to storage facilities (Hallauer, 2000).

## **C. Soil ecology (Microbiology of Maize Rhizosphere)**

56 Maize root system acts as a soil modifier due to its association with several microbial groups such as bacteria, fungi, actinomycetes (Vega-Segovia and Ferrera-Cerrato, 1996a), protozoa and mites. The highest microbial population usually is bacteria, followed by fungi and actinomycetes. All these microbial groups play a particular role in the soil ecology, such as nutrimental cycling and the availability of nutrients for plant growth. In addition, these microbial organisms contribute to the protection of the root system against soil pathogens.

57 Some research has been oriented to understand more on microbial activity and its physiology. For instance, the physiology of free nitrogen-fixing bacteria such as *Azotobacter*, *Beijerinckia* and *Azospirillum* which have been found in the rhizosphere of several maize cultivars and teosinte (González-Chávez *et al.*, 1990; González-Chávez and Ferrera-Cerrato, 1995; Vega-Segovia and Ferrera-Cerrato, 1996b).

58 There is information related to symbiosis with arbuscular mycorrhizal fungi (AMF) which shows that these endophytes associate with specific maize genotypes (González-Chávez, and Ferrera-Cerrato, 1989; González-Chávez and Ferrera-Cerrato, 1996). There are reports related to the capability of a single AMF to establish symbiosis with a wide range of maize land races and teosinte (Santamaría and Ferrera-Cerrato, 1996; Benítez *et al.*; unpublished data). All these materials are used in Mexican agriculture. The role of these symbiosis relationships is to increase root metabolism in order to improve phosphorus uptake.

59 A great deal of life diversity is associated with maize grown in the milpa system of the Sierras. One example is the adaptation developed by a type of maize race in the Mixe Sierra of Oaxaca. The brace roots are overdeveloped and covered by a mucilaginous material that harbours species of nitrogen fixing free bacteria (R. Ferrera-Cerrato, personal comm.).

60 Soil ecology studies are undertaken to identify micro-organisms with agricultural value in places where maize is cultivated (Pérez-Moreno and Ferrera-Cerrato, 1997). Nowadays, these micro-organisms are being studied for the potential to augment corn cultivation. Selective breeding and nutrient management are also being evaluated for enhancing maize production.

**D. Maize-insect interactions**

61 In Appendix C, a list of common insect pests and pathogens of maize is presented.

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**APPENDIX A: MAIZE BIOTECHNOLOGY**

- A) For practical purposes maize biotechnology could be divided into two fields: genetic engineering and molecular genetics.
- B) Molecular genetics refers to the identification and location (genome mapping) of genes within the genome of organisms by means of molecular techniques that make use of the chemical properties of DNA (Hoisington *et al.*, 1998). The marker technologies presently available for genomics work are: 1) Restriction Fragment Length Polymorphisms (RFLPs); 2) Random Amplified Polymorphic DNAs (RAPDs); 3) Sequence Tagged Sites (STSs); 4) Simple Sequence Repeats (SSRs); 5) Amplified Fragment Length Polymorphisms (AFLPs); and 6) Single Nucleotide Polymorphisms (SNPs). These technologies have been applied in maize breeding through fingerprinting for identification of genotypes, monitoring genetic diversity and for the efficient management of genetic resources (Hoisington *et al.*, 1998). Other applications of molecular genetics and molecular markers are 1) Comparative Mapping, and 2) Marker Assisted Selection.
- C) Genetic engineering methodologies can make possible the insertion of foreign DNA, from organisms of different species, into another individual organism. In maize, at the commercial level, the introduction of foreign DNA has been successfully accomplished through a technique known as biolistics. In this technique, DNA coated microparticles are shot by means of an air compression device, to cells in plant tissue or callus. In the case of maize, embryogenic callus is used for bombardment with foreign DNA. To identify the cells that have taken up the foreign DNA in maize, a herbicide resistant selectable gene has been used. Fertile transgenic maize plants have also been produced using 1) PEG-mediated protoplast transformation; 2) electroporation of intact or partly degraded cells of immature embryos, callus or embryonic suspensions; 3) 'whiskers' technology; and 4) *Agrobacterium*-mediated transformation.
- D) At present there are two types of commercially released transgenic maize produced by means of genetic engineering: 1) Insect pest resistant maize or Bt-maize; and 2) Herbicide resistant maize. However, more research and development in this area is underway. Transgenic maize with elevated (10 KD) zein and methionine has been obtained (Anthony *et al.*, 1997). Antifungal proteins, such as chitinases and beta-1,3-glucanases, have been genetically engineered to attempt expression in the maize kernels with the aim to prevent the growth of *Aspergillus flavus* and the production of aflatoxins (Duncan *et al.*, 1985; Wu *et al.*, 1994; Wan *et al.*, 1995). Transgenic maize will serve as bioreactors for producing various biomolecules with applications in food, feed and the pharmaceutical industry (Nikolov, 1999).
- E) The complicated and plastic nature of organellar genomes especially those of maize mitochondria, requires special consideration for the stability of the cytoplasmic male sterility genes if they are used for preventing pollen formation. Equally these features of organelle genomes would also apply to any genes cloned into them (since recent developments indicate that organelles could be a better target for generating transgenic plants). Therefore, stable incorporation of a transgene into the plastid genome guarantees amplification of the transgene, potentially resulting in a very high level of foreign gene expression. Since chloroplast (and mitochondrial) genomes resemble the genomes of other organisms and are most probably evolutionarily related, the possible transfer of genes from

these organelles to microorganisms should be studied in the future if more and more transgenes are targeted to these organelles

- F) The great similarity between the chloroplastic genome and microbial genomes was one of the reasons for choosing the chloroplast as a target for transferring native microbial genes to plants. For instance since the transcriptional machinery of the plastid is prokaryotic in origin and its genome is relatively A-T rich, it was possible that native Bt toxin genes from *B. thuringiensis* might be efficiently expressed in this organelle without nuclear modification. In addition, plant cells may contain up to 50,000 copies of the circular plastid genome.
- G) Transposable elements are not expected to affect transgenes differently from their reported effects on non-modified genes of maize, unless sequences of the transposable element are contained in the inserted genetic material (Tsaftaris, 1995).
- H) The potential crossing of landrace maize germplasm with transgenic improved maize, hybrids or inbreds should be considered carefully since, for example in Mexico, it is well known the high incidence of transposable elements in landraces of maize (Gutiérrez-Nava *et al.*, 1998).
- I) Several investigations conducted by national and international research institutions have demonstrated that gene exchange between improved maize and landraces is a continuing process taking place in small farmers' corn fields. The report on the presence of transgenes in peasants' maize fields of Oaxaca (Quist and Chapela, 2001), have been further demonstrated by the Mexican government (INE-CONABIO, 2001), confirming that gene movement in traditional agriculture is an open system.

#### *Weediness of transformed corn varieties*

- J) Gene transformation is the acquisition by a cell of new gene(s) by the uptake of naked DNA, which in the case of maize can be by direct introduction of DNA. As stated before, the more common applications of gene transfer in corn are insect resistance or tolerance to herbicides. Herbicide tolerance is usually conferred by single genes that interact with key enzymes in important metabolic pathways. Insect resistance is conferred by the expression of an insecticidal protein from *B. thuringiensis*. The overall phenotype of transformed plants with these two types of genes is similar to the original phenotype: the reproductive organs (tassels and ears), duration of plant development, methods of propagation, ability to survive as a weed, will not change with these two types of genes.
- K) Gene exchange between cultivated corn and transformed corn would be similar to that which naturally occurs at the present time. Wind-blown pollen would move about among plants within the same field and among plants in nearby fields. Free flow of genes would be similar to that which occurs in cultivated corn. The transformed plants include individual genes, and depending on the relative expression of the transformed genes (relative levels of dominance for gene expression), plant architecture and reproductive capacities of the inter-crossed plants will be similar to non-transformed corn. With the transgenic maize that is available at this moment in the world, the chance that a weedy type of corn will result from inter-crossing of transgenic maize with cultivated conventional maize is remote.
- L) Out-crossing of transformed corn plants with wild relatives of corn will be the same as for non-transformed corn plants. Out-crossing with teosinte species will only occur where teosinte is present in Mexico, Guatemala and probably in some other places of Central America. Out-crossing with *Tripsacum* species is not known to occur in the wild.



*Unintended effects*

- M) The commercial release of transgenic maize expressing delta-endotoxin from *Bacillus thuringiensis* has driven the interest of ecologists concerned with the evolution of pest resistance to pesticide plants (Bergvinson *et al.*, 1997; Willcox and Bergvinson, 1997; Marvier, 2001; Obrycki *et al.*, 2001). The evolution of pest resistance is commonly known in any system where negative selection occurs from the use of traditional chemical pesticides, including plants bred traditionally for pest resistance. Recently, an effect of pollen from transgenic maize on the monarch butterfly larvae, a non-target insect, has preliminarily been described (Losey *et al.*, 1999). However, recent studies in the field have shown a less dramatic effect on non-target organisms (Wraight *et al.*, 2000; Hellmich *et al.*, 2001; Sears *et al.*, 2001; Zangerl *et al.*, 2001).

**APPENDIX B: DISTRIBUTION OF MAIZE LANDRACES AND TEOSINTE IN MEXICO**



Source: Serratos-Hernández *et al.*, 2001.

**APPENDIX C: COMMON DISEASES AND INSECT PESTS OF MAIZE  
(CIMMYT AND DGSV GUIDES)**

Maize	Insect pests	Diseases
Stalk	<p><b>Termites</b> (<i>Coptotermes formosanus</i>), <b>Sugarcane borer</b> (<i>Diatraea saccharalis</i>), <b>Southwestern corn borer</b> (<i>Diatraea grandiosella</i>), <b>Neotropical corn borer</b> (<i>Diatraea lineolata</i>), <b>Asian maize borer</b> (<i>Ostrinia furnicalis</i>), <b>Spotted sorghum stem borer</b> (<i>Chilo partellus</i>), <b>African maize stem borer</b> (<i>Busseola fusca</i>), <b>African pink borer</b> (<i>Sesamia calamistis</i>), <b>African sugarcane borer</b> (<i>Eldona saccharina</i>), <b>Maize stem weevils</b> (<i>Cilindrocopturus adpersus</i>), <b>European corn borer</b> (<i>Ostrinia nubilalis</i>).</p>	<p><b>Charcoal rot</b> (<i>Macrophomina phaseoli</i>), <b>Diplodia stalk rot</b> (<i>Diplodia maydis</i>), <b>Gibberella stalk rot</b> and <b>Fusarium stalk rot</b> (<i>Fusarium</i> spp), <b>Brown spot</b> (<i>Physoderma maydis</i>), <b>Black bundle disease</b> (<i>Cephalosporium acremonium</i>), <b>Late wilt</b> (<i>Cephalosporium maydis</i>), <b>Maize bushy stunt disease</b> (MBSD), <b>Botryodiplodia stalk rot</b> (<i>Botryodiplodia theobromae</i>), <b>Maize lethal necrosis</b> (simultaneous infection of maize chlorotic mottle virus and either maize dwarf mosaic virus or wheat streak mosaic virus), <b>Maize chlorotic mottle virus</b> (MCMV), <b>Corn stunt disease</b> (<i>Spiroplasma</i>), <b>Pythium stalk rot</b> (<i>Pythium aphanidermatum</i>, <i>Pythium</i> spp.), <b>Erwinia stalk rot</b> (<i>Erwinia carotovora</i> f. sp. <i>zetae</i>)</p>
Leaf	<p><b>Corn stunt leafhoppers</b> (<i>Dalbulus maidis</i>), <b>Maize streak virus leafhoppers</b> (<i>Dalbulus maidis</i>, <i>D. elimatus</i>), <b>Fall armyworm</b> (<i>Spodoptera frugiperda</i>), <b>Armyworm</b> (<i>Mythimna unipuncta</i>), <b>Spider mites</b> (<i>Oligonychus mexicanus</i>), <b>Corn leaf aphid</b> (<i>Rhopalosiphum maidis</i>, <i>R. padi</i>), <b>Maize Whorl Maggots</b> (<i>Euxesta</i> spp.), <b>Sugarcane Froghoppers</b> (<i>Aeneolamia postica</i>, <i>Prosapia simulans</i>), <b>Chafers</b>, <b>Grasshoppers</b> (<i>Sphenarium</i> spp., <i>Melanoplus</i> spp.).</p>	<p><b>Downy mildew</b> (<i>Sclerospora</i> spp., <i>Sclerophthora</i> spp), <b>Curvularia leaf spot</b> (<i>Curvularia lunata</i> and <i>Curvularia pallescens</i>), <b>Cercospora leaf spot</b> (<i>Cercospora zetae-maydis</i>), <b>Septoria leaf blotch</b> (<i>Septoria maydis</i>), <b>Turcicum leaf blight</b> (<i>Helminthosporium turcicum</i>), <b>Diplodia macrospora leaf stripe</b> (<i>Diplodia macrospora</i>), <b>Phyllosticta leaf spot</b> (<i>Phyllosticta maydis</i>), <b>Helminthosporium carbonum leaf spot</b> (<i>Helminthosporium carbonum</i>), <b>Bacterial leaf stripe</b> (<i>Pseudomonas rubrilineans</i>), <b>Eyespot of maize</b> (<i>Kabatiella zetae</i>), <b>Leptosphaeria leaf spot</b> (<i>Leptosphaeria michotii</i>), <b>Maydis leaf blight</b> (<i>Helminthosporium maydis</i>), <b>Stewart's wilt</b> (<i>Erwinia stewartii</i>), <b>Maize dwarf mosaic</b> (MDMV), <b>Southern rust</b> (<i>Puccinia polysora</i>), <b>Common rust</b> (<i>Puccinia sorghi</i>), <b>Tropical rust</b> (<i>Physopella zetae</i>), <b>Zonate leaf spot</b> (<i>Gloeocercospora sorghi</i>), <b>Banded leaf and sheath spot</b> (<i>Rhizoctonia solani</i> f. sp. <i>sasakii</i>), <b>Tar spot</b> (<i>Phyllachora maydis</i>), <b>Brown spot</b> (<i>Physoderma maydis</i>) <b>leaf anthracnose</b> (<i>Colletotrichum graminicola</i>), <b>Phaeosphaeria leaf spot</b>, <b>Fine stripe virus</b>, <b>Corn streak virus</b>, <b>Bacterial leaf stripe</b>, <b>Maize chlorotic mottle virus</b>, <b>Fine stripe</b></p>

		virus, Fine mosaic virus I, Corn stunt disease, Black bundle disease.
Ear	<b>Ear maggots, Corn earworms</b> ( <i>Helicoverpa zea</i> ), <b>Stink bugs</b> ( <i>Euschistus servus</i> , <i>Nezara viridula</i> ), <b>Angoumois grain moth</b> ( <i>Sitotroga cerealella</i> ), <b>Indian meal moth</b> ( <i>Plodia interpunctella</i> ), <b>Grain weevils</b> ( <i>Sitophilus granarius</i> , <i>S. zeamais</i> ), <b>Grain borers</b> ( <i>Prostephanus truncatus</i> ).	Corn stunt disease, Botrydiplodia, Penicillium ear rot, Cladosporium ear rot, Giberella ear rot, Maydis leaf blight (T strain), Nigrospora ear rot, Tar spot, Black bundle disease, Maize dwarf mosaic, Downy mildew, Giberella ear rot, Helminthosporium carbonum ear rot, Banded leaf and sheath spot, Ergot of maize, Head smut, Aspergillus ear rots, Banded leaf and sheath spot, Maize stripe virus, Comon smut, Gray ear rot, Diploidia ear rot, Charcoal ear rot.
Tassel	<b>Corn stunt leafhoppers</b> ( <i>Dalbulus maidis</i> ), <b>Maize streak virus leafhoppers</b> ( <i>Dalbulus maidis</i> , <i>D. elimatus</i> ), <b>Fall armyworm</b> ( <i>Spodoptera frugiperda</i> ), <b>Armyworm</b> ( <i>Mythimna unipuncta</i> ), <b>Spider mites</b> ( <i>Oligonychus mexicanus</i> ), <b>Corn leaf aphid</b> ( <i>Rhopalosiphum maidis</i> , <i>R. padi</i> ), <b>Maize Whorl Maggots, Sugarcane Froghoppers</b> ( <i>Aeneolamia postica</i> , <i>Prosapia simulans</i> ), <b>Chafers, Grasshoppers</b> ( <i>Sphenarium</i> spp., <i>Melanoplus</i> spp.).	Head smut, Downy mildew, Maize chlorotic mottle virus, Bacterial leaf stripe, False head smut, Corn stunt disease, Maize stripe virus.
Seed, Root, and Seedling	<b>Seedcorn maggots</b> ( <i>Hylemya platura</i> ), <b>Wireworms</b> ( <i>Agriotes lineatus</i> ), <b>Flea beetles</b> ( <i>Phyllotreta</i> spp.), <b>Diabrotica beetles</b> ( <i>Diabrotica</i> spp.), <b>Maize billbugs</b> ( <i>Sphenophorus maidis</i> ), <b>White grubs</b> ( <i>Phyllophaga</i> spp., <i>Anomala</i> spp.), <b>Cutworms</b> ( <i>Agrotis</i> spp.), <b>Thrips</b> ( <i>Frankliniella</i> spp.), <b>Lesser cornstalk borer</b> ( <i>Elasmopalpus lignosellus</i> ).	

## APPENDIX D: MAIZE WORLD PRODUCTION

<i>World</i> <i>Maize</i>	Element			
	Seed (Mt)	Area Harvested (Ha)	Yield (Hg/Ha)	Production (Mt)
1961	6,223,099	105,484,151	19,435	205,004,683
1962	6,370,267	103,418,906	19,808	204,856,937
1963	6,193,721	108,384,382	20,319	220,228,333
1964	5,785,022	107,790,032	19,961	215,162,627
1965	5,988,088	106,591,240	21,252	226,524,256
1966	5,944,346	111,157,704	22,096	245,609,160
1967	5,872,917	112,313,038	24,266	272,538,473
1968	5,981,586	111,494,042	22,927	255,620,551
1969	5,838,480	111,242,302	24,226	269,491,068
1970	6,013,828	113,027,431	23,519	265,831,145
1971	6,185,867	118,150,571	26,544	313,622,622
1972	6,137,730	114,910,552	26,875	308,826,290
1973	6,132,362	116,856,034	27,238	318,290,469
1974	6,074,833	119,772,684	25,572	306,287,347
1975	6,429,594	121,442,141	28,133	341,656,971
1976	6,170,127	124,154,181	28,382	352,370,866
1977	6,181,283	125,192,168	29,679	371,561,355
1978	6,235,069	124,664,903	31,570	393,562,091
1979	6,281,256	123,598,634	33,866	418,577,993
1980	6,373,981	125,694,717	31,551	396,573,388
1981	6,440,288	127,816,716	34,950	446,722,107
1982	6,300,922	124,310,829	36,109	448,875,780
1983	6,605,234	117,763,540	29,468	347,024,034
1984	6,711,131	127,703,340	35,269	450,399,992
1985	6,646,135	130,454,042	37,214	485,474,301
1986	6,806,025	131,754,681	36,293	478,178,515
1987	6,623,584	129,888,090	34,880	453,054,894
1988	7,013,976	129,902,556	31,019	402,940,593
1989	7,158,041	131,711,470	36,203	476,833,660
1990	7,090,222	131,315,568	36,801	483,248,513
1991	7,379,181	134,125,220	36,851	494,267,664
1992	5,487,753	136,974,563	38,945	533,443,038
1993	5,497,737	131,500,199	36,242	476,576,466
1994	5,360,864	138,334,591	41,139	569,095,143
1995	5,474,640	136,271,016	37,914	516,655,836
1996	5,691,964	139,856,300	42,127	589,171,299
1997	5,588,723	141,270,173	41,407	584,954,064
1998	5,788,484	138,816,826	44,308	615,063,554
1999	5,765,380	138,460,288	43,786	606,261,782
2000	5,722,092	138,738,942	42,742	592,999,083
2001	5,912,420	137,596,759	44,273	609,181,620

Source: FAOSAT <http://apps.fao.org>

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