Release of transgenic crops in a center of genetic diversity: The case of transgenic corn in Mexico

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EMBL- Heidelberg, November 2009

Release of transgenic plants in centers of origin and genetic diversity.

Does gene transfer from GM-varieties to local varieties and landraces represent a major risk for genetic diversity preservation?

Mexico center of origin and domestication of many crop:

- Maize
- Chili peppers
- Common beans
- Peanuts
- Pumpkins
- Avocado

The origin of maize

Maize was domesticated from its wild progenitor teosinte (*Zea mays* ssp *parviglumis* or Balsas teosinte), a grass native to Mexico and Central America.

Archeobotanical studies and multilocus microsatellite genotyping indicates that a single domestication event occurred approximately 9,000 years ago in Central Mexico

Coxcatlán cave in the Tehuacán Valley

After the early domestication events a wide variety of native landraces were produced through continuous divergent selection for 8000 years by diverse Mexican native groups.

As the source of numerous maize populations with distinct environmental adaptations, Mexican landraces have been essential to generate inbred lines used for hybrid production and to harness important traits for crop improvement.

Maize genetic diversity is considered a Mexican treasure; main source of new traits!

Maize has a major spiritual and culture importance in Mexico and MesoAmerica. Mayan culture considered that men was created from maize by the gods

Different types of maize are used to prepare a wide variety of traditional dishes.

Maize production in Mexico

Between 2.0 and 2.5 million farmers grow maize as their major crop

90% are small/subsistence farmers with 2 or less hectares of land

60% of farmers are native Indians.

15 to 18 million people depends on the income of maize farmers

Mexico imports 10 million tons of maize per year, a significant Percentage is transgenic

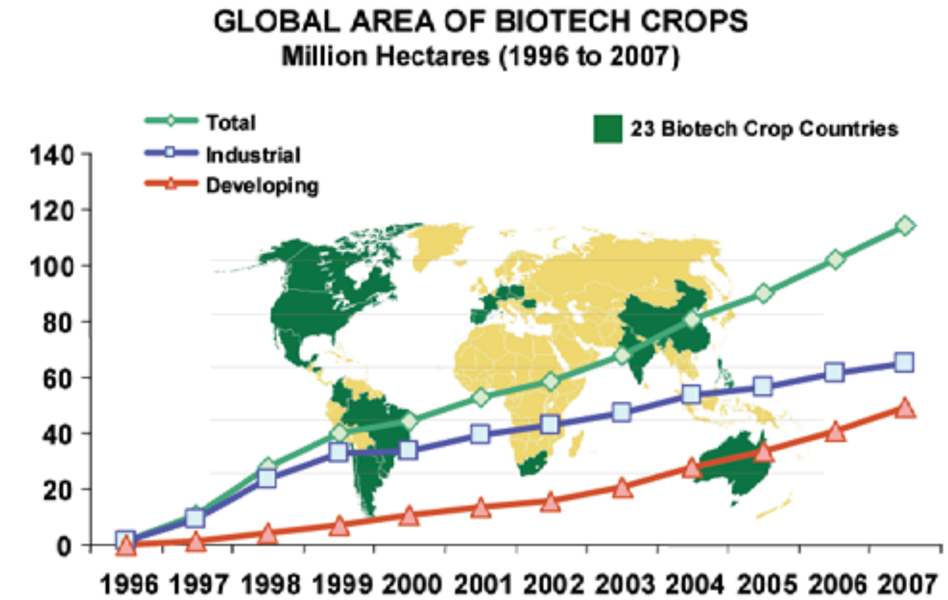
Corn production in different parts of the world

Country	Area [Million hectares]	Yield [tons/hectare]	Production [Million metric tons]
USA	28.72	8.46	242.86
Brazil	12.50	2.72	34.00
Mexico	6.10	2.40	12.50
EU	4.05	8.81	35.68

Source: FAO, 2000

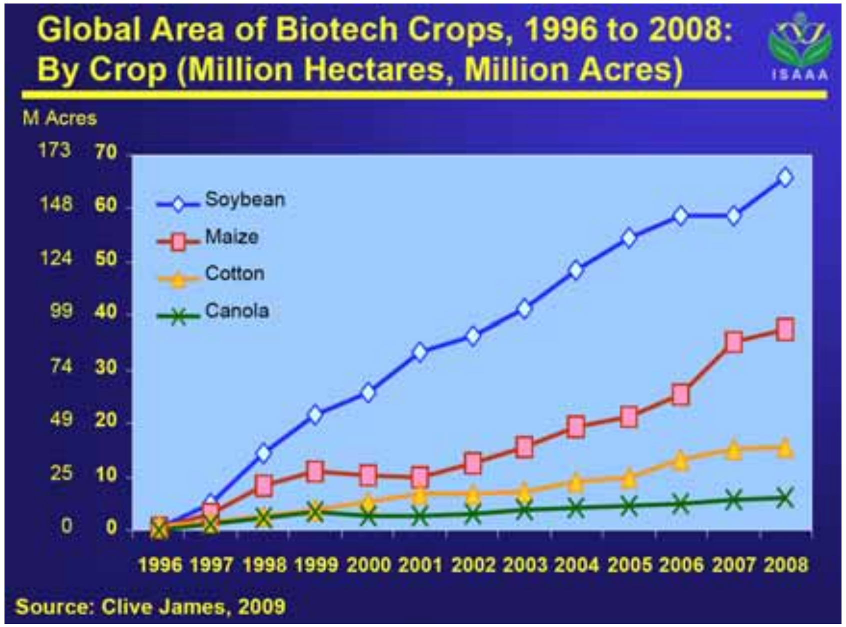
If Mexico could increase maize production from 2.4 to 3.2 tons per hectare, we would not need to import maize. Fertile soils, but Insect pests and drought major problems.

GM technology emerges as a powerful complementary technology to plant breeding.
It has produce effective insect and virus resistance for maize; Drought tolerance under way



Increase of 12%, 12.3 million hectares (30 million acres), between 2006 and 2007.

Source: Clive James, 2007.



Most of the GM technology was developed at publics institutions in Belgium, Germany, France and the US, not by companies

Potential Negative Impacts of using transgenic maize in Mexico

Human and animal health

Environment

Loss of genetic diversity of indigenous varieties and wild relatives.

The problem in Mexico

Some environmentalist and academic groups have major concerns about gene flow from transgenic maize to landraces and local varieties. The fear: Transgene “contamination” will destroy Maize biodiversity.

In contrast to Europe, this concern is not general, it is mainly expressed by groups of intellectuals, including scientists

Biosafety for GM crops in Mexico

-First field experiment in 1988 with Bt tomatos.

-In 1990 a group of experts was appointed by the Federal Government as the Biosafety committee to analyze request to carry out experimental field trails with Transgenic plants.

-Over 250 experimental field trails were carried out 1990- 1998

-In 1998 a moratorium on experimental trails was imposed by the Government

-Starting in 1998 several proposals for a biosafety law were presented to the Mexican Congress

-In March 2005 the biosafety law was approved by the Mexican Congress, requiring a especial status for maize. Case by case, step by step legislation.

-In 2008 the regulations to enforce the biosafety law were finally approved.

-2009 the first permits for experimental field trails with transgenic maize were approved by the Mexican Government.

Factors that reduce maize genetic diversity in Mexico

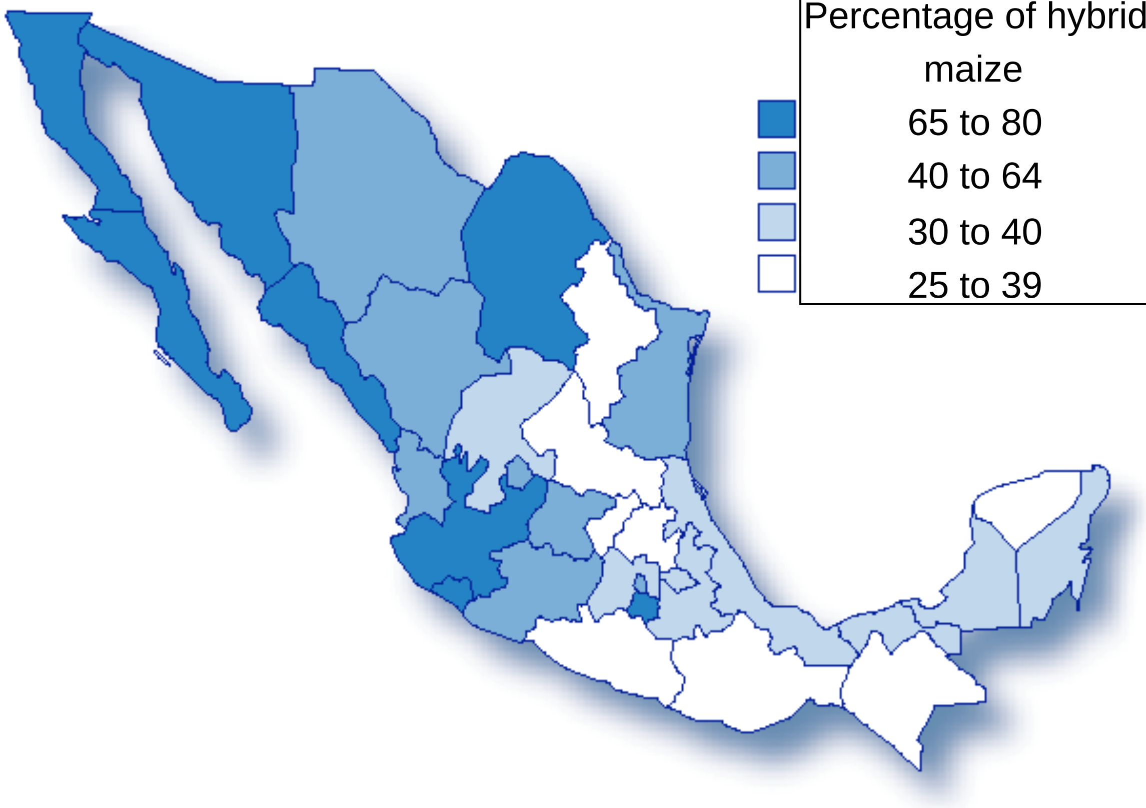
Increasing use of commercial varieties and hybrids

Migration to cities and the US and Canada

Globalization and free trade agreements. International prices determine the price of maize in Mexico and the amount we import

Transgenic maize ??????

Use of hybrid maize in Mexico has increased in the last decade and extended to all Mexican states



Percentage of hybrid maize	No States
65 to 80	8
40 to 64	8
30 to 40	8
25 to 39	8

The area planted with landraces and local varieties has decreased significantly in the past two decades.

Over 300,000 Mexicans migrate to the US, mainly from rural areas of Mexico

In many rural areas only children, women and elderly are left, maize production is lost.

The iron fence does not stop Mexican immigrants nor pollen flow

Bt Maíz in Honduras

- Field trials: 2000
- Commercial fields: 2004

Conventional maize

Bt maize

Can we contain GM maize from legally or illegally entering Mexico?

Gene flow between transgenic and Mexican Maize landraces

We have to assume that, unless technology is incorporated into GM-varieties that contain gene flow (such as incorporation of transgenes in organellar genomes or the so-called terminator gene technology), genes will eventually be transferred from GM plants to wild relatives or landraces.

Gene flow between transgenic and Mexican Maize landraces

-Gene flow between commercial varieties and landraces has occurred for several decades.

-Farmers actively select for seed with desired characteristics and only introgress traits that make a difference

-Potential effects of gene flow MUST be studied, however, are there reasons to be alarmed?

-The best way to preserve landraces is to incorporate genes that ensure competitiveness. Otherwise, sooner or later, they will be replaced by uniform, commercial varieties or farmers will migrate to the US.

Why landraces have maintained their identity?

Landraces have special culinary qualities usually associated with them.
People recognize them and will very seldom use substitutes.

She will not grow this!

If she wants this corn

THE GROWER DOES NOT SELECT AGAINST HIS COSTUMERS, NOR AGAINST HIS OWN TRADITIONS!!

Crosses between US maize commercial lines and Mexican landraces are easily identified and have low productivity under local conditions

Landraces | Hybrids

Transgene escape into Mexican maize landraces

Nature 2001

Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico

David Quist & Ignacio H. Chapela

Department of Environmental Science, Policy and Management, University of California, Berkeley, California 94720-3110, USA

Absence of detectable transgenes in local landraces of maize in Oaxaca, Mexico (2003–2004)

S. Ortiz-García*, E. Ezcurra*†, B. Schoel‡, F. Acevedo§, J. Soberón§¶, and A. A. Snow‖**

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Transgenes in Mexican maize: molecular evidence and methodological considerations for GMO detection in landrace populations

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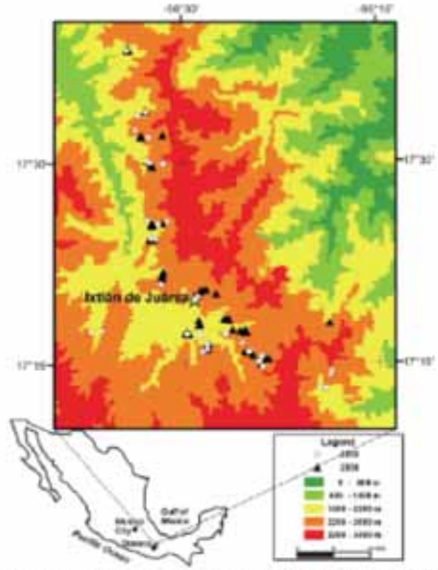

What we know: The moratorium on transgenic maize lead to illegal planting in Mexico; farmers eager to get the technlogy

Campesino comparecerá por producción del grano

Confirma Sagarpa siembra de maíz transgénico en Chihuahua

Rubén Villalpando (Corresponsal)

- Afirma Sagarpa que será destruido, pero omitió más detalles

Incautan 2 mil 500 toneladas de maíz transgénico en Chihuahua

- Dirigente de El Barzón pide al gobierno abaratar insumos agrícolas

Rubén Villalpando, Miroslava Breach y Ernesto Martínez (Corresponsales)

México aprueba la siembra de maíz transgénico

Por Maria Murillo
Posted 03/10/2009

Tamaño de Fuente:

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Ciudad de México. El pasado lunes 9 de marzo, a través de un comunicado de prensa el Secretario de Medio Ambiente y Recursos Naturales (Semarnat), el Ing. Juan Rafael

Potential Negative Impacts of using transgenic maize in Mexico

Gene flow between transgenic maize and local landraces seems unavoidable. Two neighboring countries, the US and Honduras, have commercial plantations, Mexican immigrants bring back transgenic seed.

What are the real concerns:

1) Loss of maize biodiversity due to the replacement of landraces by transgenic lines. No difference from what has happened in the past two decades with hybrids

2) Unexpected effects of the presence of transgenes on the genome of local varieties and landraces (maize genetic diversity.

GENE FLOW BETWEEN CROPS AND WILD SPECIES IS OFTEN CONSIDERED TO BE AN UNDESIRABLE CONSEQUENCE OF ADOPTING TRANSGENIC CROPS.

From a regulatory perspective, it is essential to compare the effects of transgenes to those genes from non-transgenic crop that spread to wild and/or indigenous varieties.

We have to keep in mind that certain traits developed through the introduction of transgenes (e.g. herbicide tolerance, herbivore and pathogen resistance, and resistance to harsh environmental conditions) have been produced through traditional breeding as well

Potential impact of trangenes on genome integrity

Could transgene introgression have a greater effect on
the genetic diversity of landraces or wild relatives than genes from hybrids?

Could the presence of a transgene alter the chemical composition of maize?
What is the reference to compare, which genotype, which growing
conditions?

Will transgenes cause genomic instability in a different fashion
than that naturally created by transposons or other mobile elements
that are abundant in plant genomes, and which cause mutations,
chromosomal breakage and translocations?

Could we expect to have more unexpected pleiotropic effects
from transgene insertion than those caused by transposons that
naturally activate or modulate the expression of neighboring genes?

Can gene insertion alter gene expression or chemical composition in unexpected ways?

It has been argued that the presence of transgenes in GM crops can alter gene expression patters due to the site of insertion and the presence of enhancers and that this can lead to alterations in chemical composition.

The concept of substantial equivalence was proposed, GM crops should have similar transcription and biochemical profiles than conventional products.

Which reference should we take?

Genome Size Variability in Mexican Landraces

20 landraces from Highlands and Lowlands
230 accessions INIFAP and CIMMYT
Flow cytometry

Landrace		1C value (pg)	Size (Gb)
Dzit Bacal	*YCT3499*	3.27	3.2
Tehua	*CHPS8023*	2.86	2.8
Olotillo	*CHPS2101*	2.86	2.8
Reventador	*BCN2111*	2.76	2.7
Palomero de Chih.	*CH9083*	2.61	2.55
Apachito	*CHB7649*	2.5	2.45
Arrocillo	*PBL4555*	2.45	2.4
Chalqueño	*TLX9023*	2.35	2.3
Mushito	*MCH1011*	2.35	2.3
Negrito	*OXX3423*	2.25	2.2
Palomero Toluqueño	***EMX2231***	**2.1**	**2.05**
Palomero Toluqueño	***EMX2233***	**1.9**	**1.95**

Vielle et al, Science in Press

Alterations in gene expression and metabolite content in maize as a consequence of nutritional stress

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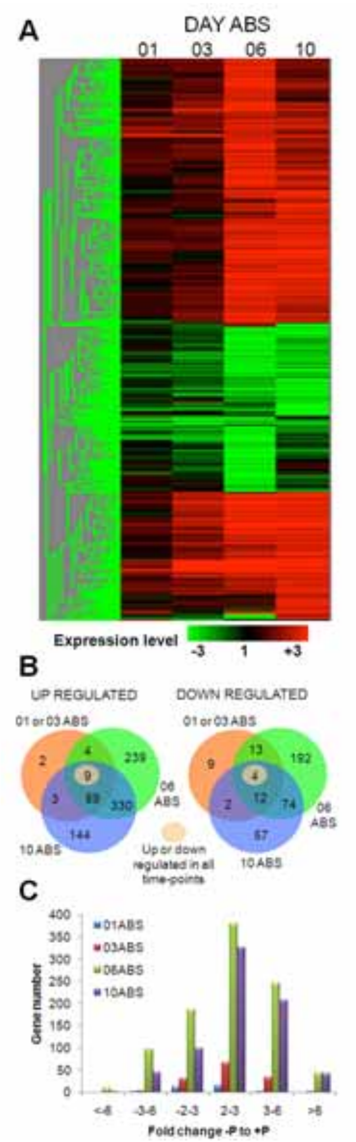
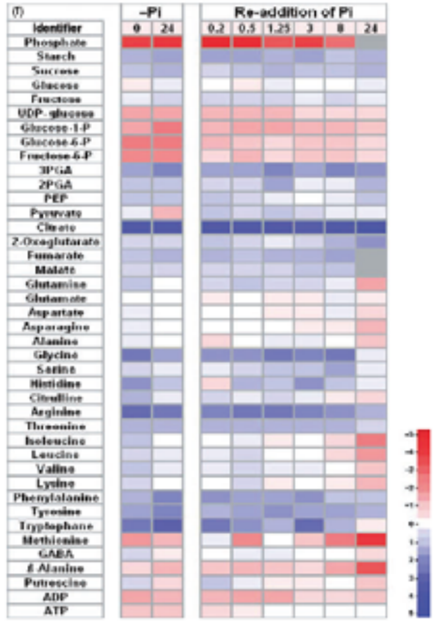


1,183 genes had alterations in their mRNA level upon transfer to low Pi conditions.



Many changes in chemical composition

Calderón et al, 2008

Basal differences in gene expression of three different land races grown under the same conditions

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◆ 85-2 vs Cajete criollo	429 GENES
◆85-2 vs Michoacan 21	409 GENES
◆Cajete criollo vs Michocana 21	154 GENES
◆85-2 vs GM85-2	41 genes
◆Cajete criollo vs GMCajete criollo	37 genes
◆Michocan 21 vs GM Michoacan 21	18 genes

LABORATORIO NACIONAL DE GENOMICA
PARA LA BIODIVERSIDAD

Alterations in gene expression due to drought stress

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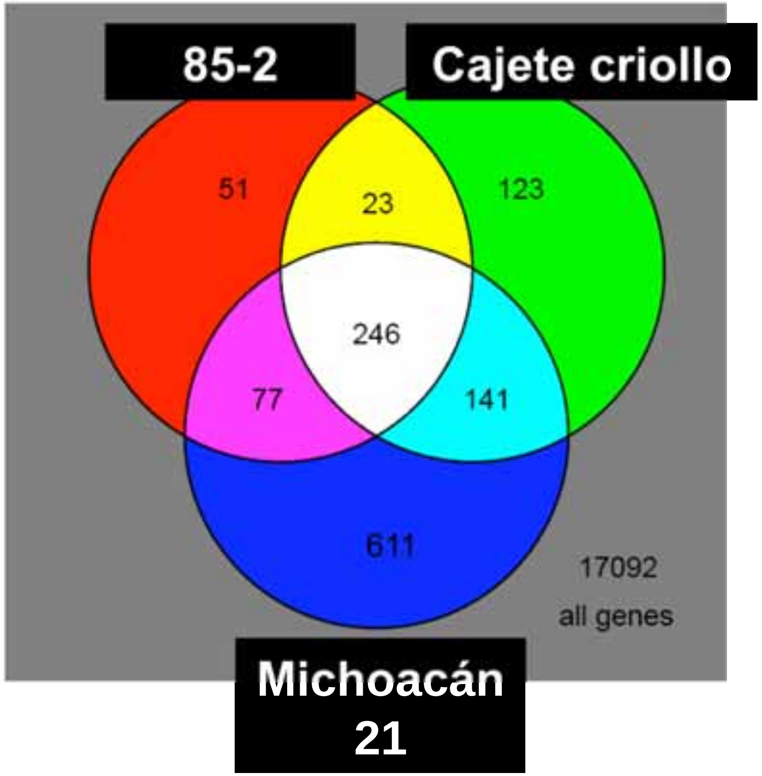
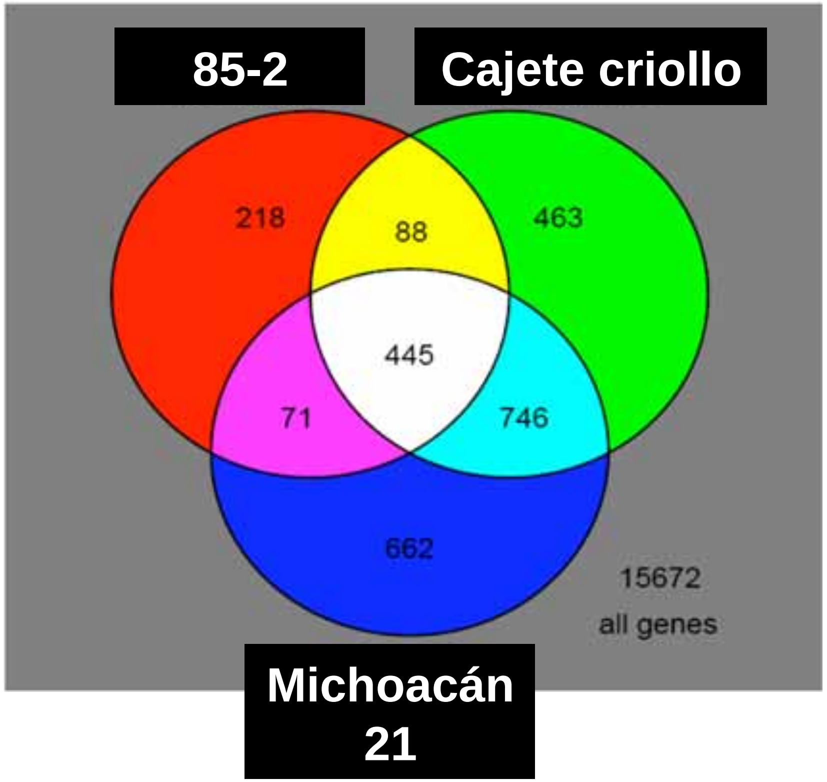
Induced Genes
during drough treatment



Repressed Genes
upon recovery irrigation



Hayano et al, 2009

Do transgenes have a different impact on genome expression, structure or stability than naturally occurring processes?

Mutations induced by physical and chemical factors.

Aberrations in the recombination process

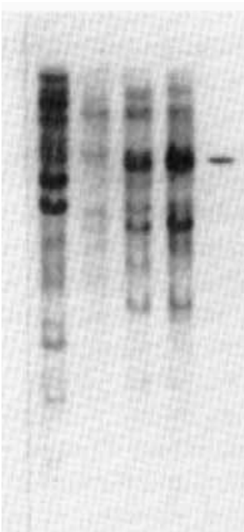
Insertion of genes from organelle genome into the nuclear genome

Movement and amplification of transposons

Transposable elements

Many classes of transposons and retrotransposons exists in a single organism.

Ac in different maize lines



Copy number varies from 0 to over 1 million

The number and chromosomal position of a given transposon is variable

Transposons comprise over 50% of the maize genome.

Effects of transposons on genome function.

Mutations by insertion into and excision from genes
(80% of spontaneous mutations detected in Drosophila and maize are cause by transposons).

Activate and suppress transcription of neighboring genes.

Alter transcript processing by introducing donor and acceptor splice sites.

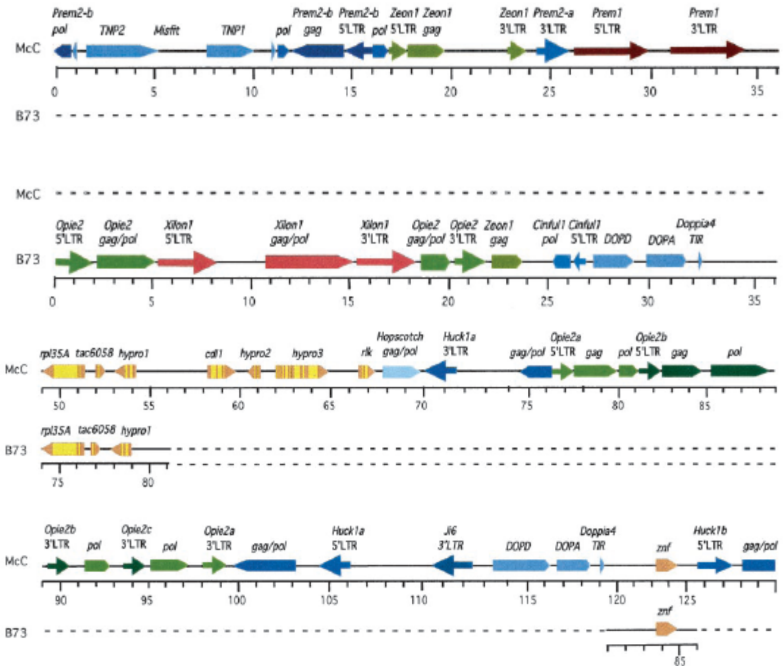
Can move exons and promoters into existing sequences to create new genes.

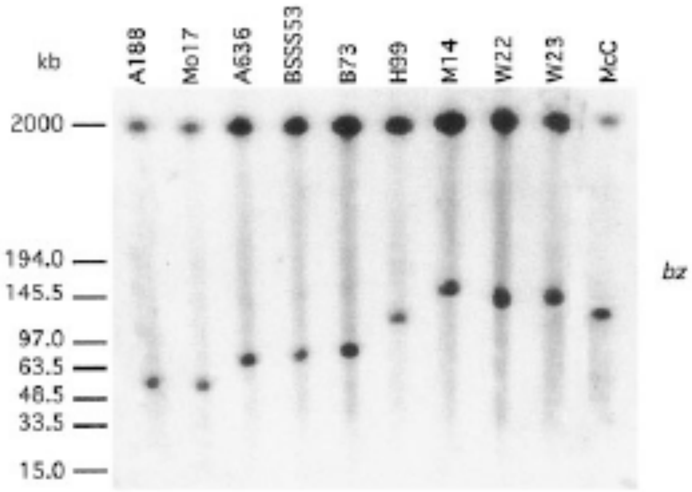
Transposition and amplification events enlarges genomes.

Intraspecific violation of genetic colinearity and its implications in maize

Huihua Fu* and Hugo K. Dooner*†‡

*The Waksman Institute, Rutgers University, Piscataway, NJ 08855; and †Department of Plant Science, Rutgers University, New Brunswick, NJ 08901





We have found that the *bz* genomic regions of two North American maize lines differ extensively in the organization and content not only of the intergenic retrotransposon clusters in the region, but also of the genes themselves. A central issue raised by these findings, with potential practical implications, is the meaning of allelism in a region such as the one where *bz* resides.

nature genetics

Gene duplication and exon shuffling by helitron-like transposons generate intraspecies diversity in maize

Michele Morgante[1], Stephan Brunner[2], Giorgio Pea[2,3], Kevin Fengler[2], Andrea Zuccolo[1,3] & Antoni Rafalski

Table 1 Estimates of frequency of shared and nonshared genes and gene fragments between inbred lines B73 and Mo17

	Total	Shared	Unique to B73	Unique to Mo17
Absolute number	20,656	16,408	2,353	1,895
Proportion (%)	100.0	79.4	11.4	9.2

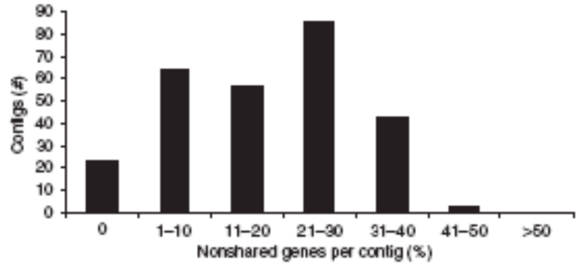


Figure 1 Genome analysis of nonshared genic regions in maize. Distribution of the proportion of nonshared genes and gene fragments between inbred lines B73 and Mo17 in 274 large contigs containing at least 20 shared overgo probes.

The maize genome is in flux, and its genetic diversity is profoundly affected by the presence of polymorphic insertions due to DNA transposons that have modulated the gene content of a species to an unanticipated extent.

Our conservative estimate of 10,000 nonshared gene fragments in the maize genome, a large fraction of which are likely to have been mobilized by helitrons, reflects only those that are polymorphic between the two inbred lines we studied. Extrapolating the total number of insertions and of gene fragments involved to the existing maize population is impossible.

The involvement of the genic insertions in the modulation of the expression of the ancestral intact genes through transcriptional silencing mechanisms could be important for the heterosis phenomenon that does not have yet a satisfactory molecular explanation.

Research article

Open Access

Maize haplotype with a *helitron*-amplified cytidine deaminase gene copy

Jian-Hong Xu and Joachim Messing*

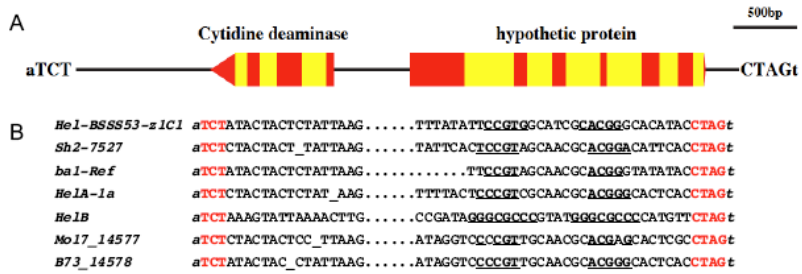


Figure 1
***Hel-BSSS53-z1C1* on maize chromosme 4S**. A) A physical map of *Hel-BSSS53-z1C1* is presented. Putative genes are shown as pentagons pointing in the direction of transcription. Exons are shown in red and introns in yellow. Conserved nucleotides at the 5'-TCT and 3'-CTAG termini and 5'-a, 3'-t target sites are highlighted at the ends. B) Termini of the maize *Helitrons Hel-BSSS53-z1C1* from BSSS53 4S [10], *HelA-1a* and *HelB* from McC 9S [22], *Mo17_14577* from Mo17 9L, *B73_14578* from B73 6S [11], the *Helitron* insertions in mutants *sh2-7527* [24] and *bal-Ref* [25, 49]. *Helitron* sequences are in uppercase letters and the invariant host nucleotides, where the *Helitrons* inserted, are in *italic* lowercase letters. Conserved nucleotides at the 5' and 3' termini are in red bold uppercase letters and inverted repeats at the 3' termini are underlined.

Plant genomes

Massive changes of the maize genome are caused by *Helitrons*

SK Lal and LC Hannah

Heredity (2005) **95**, 421–422. doi:10.1038/sj.hdy.6800764; published online 12 October 2005

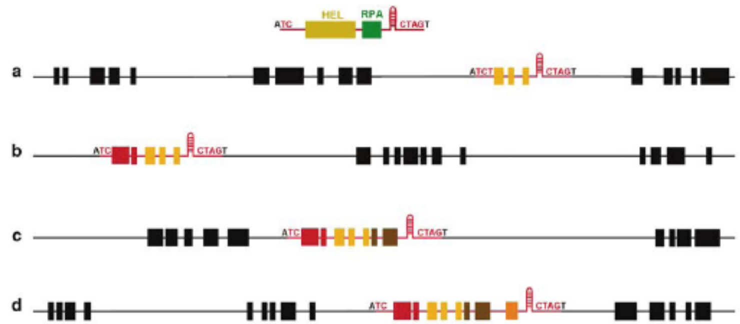


Figure 1 Hypothetical journey of a *Helitron* through the maize genome. The upper panel displays the structure of autonomous maize *Helitron* inserted between nucleotide A and T. The invariant terminal nucleotides and the coding regions of DNA helicase (HEL) and single-stranded DNA binding protein (RPA) are indicated. The loop near the 3′ end of the *Helitron* represents the palindrome sequence. The lower four panels represent a hypothetical journey of a non-autonomous *Helitron*, lacking the HEL and RPA genes, as it inserts sequentially into four different regions of the genome and grows in size by capturing gene sequences. The exons of the wild-type maize genes are represented by black boxes. The red lines represent the *Helitron* insertion and the colored boxes represent the exons of the captured genes. Exons of different genes are given different colors.

PNAS

Microarray analyses reveal that plant mutagenesis may induce more transcriptomic changes than transgene insertion

Rita Batista*†‡, Nelson Saibo†, Tiago Lourenço†, and Maria Margarida Oliveira†§

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We found that the improvement of a plant variety through the acquisition of a new desired trait, using either mutagenesis or transgenesis, may cause stress and thus lead to an altered expression of untargeted genes.

In all of the cases studied, the observed alteration was more extensive in mutagenized than in transgenic maize plants.

We propose that the safety assessment of improved plant varieties should be carried out on a case by-case basis and not simply restricted to foods obtained through genetic engineering.

Conclusions and questions

-With the available information it can be considered that GM crops are safe and do not endanger biodiversity, however we still need more information.

-Do we have strategies to contain gene flow in case of an unlikely disaster?

- With increased demand and higher prices of food, particularly cereals, can Mexico and other developing countries afford NOT using GM technology?

Conclusions and questions

-We need to preserve maize genetic diversity.

Shall we do it only by *ex situ* conservation?

For *in situ* conservation, who is going to be responsible, the farmers, the government?

-Sustainable agriculture often overlooks, social, economical aspects of the problem. To have sustainable agriculture it must be economically sustainable for the farmer. How shall we do it, subsidies, specialty markets?

If we want farmers to benefit from this technology in centres of origin and diversity, we have to accept the responsibility of altering plant evolution in an unprecedented way.

This is Esperanza (Hope) and her brothers. Her family practices organic farming, not because they want but because they have no choice. They have no money to buy fertilisers, herbicides or pesticides.

She cannot attend school because she has to help her family farming. Her job is to help her father getting ride of weeds.