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Between myth and reality: genetically modified maize, an example of a sizeable scientific controversy

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Abstract

Maize is a major crop plant with essential agronomical interests and a model plant for genetic studies. With the development of plant genetic engineering technology, many transgenic strains of this monocotyledonous plant have been produced over the past decade. In particular, field-cultivated insect-resistant Bt-maize hybrids are at the centre of an intense debate between scientists and organizations recalcitrant to genetically modified organisms (GMOs). This debate, which addresses both safety and ethical aspects, has raised questions about the impact of genetically modified (GM) crops on the biodiversity of traditional landraces and on the environment. Here, we review some of the key points of maize genetic history as well as the methods used to stably transform this cereal. We describe the genetically engineered Bt-maizes available for field cultivation and we investigate the controversial reports on their impacts on non-target insects such as the monarch butterfly and on the flow of transgenes into Mexican maize landraces.

Keywords: Genetically modified (GM); Maize; Plant transformation; Scientific controversy

1. Introduction

1.1. The plant revolution

The so-called *Green Revolution* was one of the great success stories of the second half of the 20th century. The introduction of higher-yielding varieties of rice, wheat and maize in the 1960s led to increases in food production in developing countries to keep pace with population growth, with both of these more than doubling over a short period of time. Nevertheless, the proportion of people in developing countries who are undernourished, which was 50% 40 years ago, remains unchanged today. These undernourished people now number approximately two billion, or 20% of the present world population. Despite the high incidence of under nutrition, world food prices have declined in real in terms by over 70% during the last 40 years. Those who have benefited most from this change are the most impoverished who spend the highest proportion of their income on food [1]. Though total world food production is high enough to feed the current world population, food distribution is, for reasons which seem inevitable, unequal. Consequently, if we are to feed the world's hungry people, food production must again be increased. The benefits of the original Green Revolution have already been integrated into the system and so a new solution must be sought elsewhere.

The need for a second Green Revolution is clear. This new Green Revolution has been termed "Doubly Green", because it must be both more productive and more environmentally friendly both in terms of the conservation of natural resources and the avoidance of pollution than was the first Green Revolution [1]. A new Green Revolution will necessitate application of recent advances in plant breeding, including new tissue culture techniques, marker-aided selection, and genetic modification. In the industrialized countries, the bigger life-science companies dominate the application of biotechnology to agriculture [2]. In 1999, 40% of all cotton, 35% of soya and 25% of maize grown in the United States were genetically modified (Norman E. Borlaug [3] won the Nobel Peace Price in 1970). In 2001, world production of GM crops occurred principally in the United States, Brazil,

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Argentina, Canada and China. By contrast, almost no GM crops were grown in Europe. This disparity can be explained largely by the greater level of controversy associated with GM crops in Europe [4], which has led to a slow uptake of GM technology in Europe. For example, the European Parliament is only now completing its first reading of two EC proposals on GMOs. These will establish a sound community system to trace and label GMOs and to regulate the marketing and labelling of food and feed products derived from GMOs (Brussels info No. 02/27–3 July 2002).

In this paper, we have chosen to focus our attention on GM maize for three reasons:

1. Maize was the first crop for which a GMO controversy was partially resolved in Europe, with the granting of permission for field culture in 1997.
2. Maize is among the three major world crops (with rice and wheat) with a production of 550–600 millions of tons in 2001.
3. Maize is a model plant that is intimately associated with our growing understanding of plant genetics.

1.2. Plant transformation

Before proceeding further, a few definitions should be established for the purposes of this review. The term "transformation" will be used to refer to the introduction of DNA into plant cells, while "transformed" will be used to describe plant cells after this process has taken place. The term "transgenic" will be used interchangeably with "transformed", but will be used also to refer to plant cells in which the DNA of interest (the transgene) is integrated into the host genome. The two main techniques for transformation that are commonly used are, on the one hand, biolistics and on the other, T-DNA insertion mediated by *Agrobacterium tumefaciens*. Today, there is legitimate concern that the presence of antibiotic marker genes in modified crop plants may be transferred to pathogenic organisms, which might in turn become resistant to antibiotics. To answer these concerns, several research groups have already developed techniques to eliminate such marker genes (see Charlie Scutt's paper in this issue and [5]).

1.3. Scientific and societal controversies

One of the major difficulties for policy makers who must take decisions on GM technology arises not from the inherent uncertainties, but from a fundamental lack of understanding of new phenomena at or beyond the frontiers of our present knowledge. In addition, public attitudes to risk can be hugely affected by the emotive nature of particular terms and expressions, such as the term GM itself. It is surprising that many people accept risk where they have the illusion of control (for example, driving cars) whereas they reject risks of a vastly lower magnitude over which they exert no control. In some cases, scientific publications arouse such emotional reactions [6]. Several such cases will be analysed in this paper, including that of transgenic Bt-maize and the monarch butterfly, and the case of alleged promiscuity between transgenic and wild maize plants close to their site of natural origin. The ecological risks of GM crops are indeed of greatest concern where transgenes can spread to weed species or to wild natural plant populations through hybridization. Finally, biotechnology can contribute to future food security if it benefits sustainable small-farm agriculture in developing countries (see discussion in [7]).

2. Maize: a plant species with a long genetic history

2.1. The origin of maize

The origin of maize (*Zea mays* L. ssp. *mays*) has been the subject of intense debate. Maize is believed to have been first domesticated some 7000 to 10,000 years ago in south-central or south-western Mexico and to have appeared in Europe only after colonization of the new world by Christopher Colombus. However, the botanical origin and evolution of maize has been the subject of much debate. During the last century, several hypotheses were proposed, only three of which persist [8]:

- The "Common origin" hypothesis, proposed by Randolph and Weatherwax, states that maize, and two annual Mexican plants (teosinte and *tripsacum*) all diverged from a common ancestor.
- The "Maize as an Ancestor of Teosinte" hypothesis, proposed by Mangeldorfs, contends that the annual teosintes of Mexico may have descended from maize or from a maize × perennial teosinte cross.
- The "descent from teosinte" hypothesis of Beadlle proposes that maize was derived directly from teosinte by human selection and that a small number of major genes selected by the prehistoric peoples of Mexico may have transformed teosinte into maize within the past 10,000 years.

In recent years, this third hypothesis, that maize is a domesticated form of teosinte, has been largely supported in spite of the profound morphological differences which exist between maize and the teosintes. They differ profoundly in both ear morphology and plant growth form. Teosinte plants have long axillary branches that are tipped by male inflorescences (tassels), whereas maize plants have fewer, short axillary branches tipped by female ears. A second difference concerns the architecture of the female inflorescences. In spite of their morphological differences, maize and teosinte are genetically close and they can be crossed easily. In fact, it was shown that pollen of diploid teosintes is able to fertilize dent maize after hand crossing, and the resulting F1 hybrids are highly fertile. Nevertheless, such hybrids are rarely found in the maize fields of Mexico and Guatemala where teosinte grows as a weed. Kermicle and Allen [9] have shown that pollen of dent maize was able to fertilize some, but not all, teosinte taxa. A combination of reasons may account for this

variable fertilization behaviour. For example, one system of incompatibility has been described and is governed by a single gene, the *Ga1* gene [9].

To elucidate the molecular basis of the morphological evolution of maize, Doebley et al. [10] used molecular marker loci mapping to determine the number, location and relative level of effects of genes controlling the morphological differences between maize and teosinte. They mapped five major loci and suggest that a relatively small number of loci with large effects were involved in the early evolution of the key traits that distinguish maize and teosinte. For two of the chromosomal regions, it has been determined that a single locus is responsible for major effects on the traits studied. One locus found on chromosome 4 controls differences in structures associated with the kernels and has been named t*eosinte glume architecture1* (*tga1*) [11]. A second locus, on chromosome 1, is responsible for differences in plant architecture, and corresponds to the gene *teosinte branched1* (*tb1*) [12]. This gene has been cloned by transposon tagging [13]; it encodes a protein with homology to the *cycloidea* gene of snapdragon and belongs to the TCP gene family. Members of this family encode putative basic helix-loop-helix DNA-binding proteins. The genes *tga1* and *tb1* are considered as regulatory genes and are probably involved in the morphological evolution of maize [14].

2.2. Genome origin, organization and evolution

Maize belongs to the grass tribe Andropogoneae, which has a haploid or basal chromosome number of 5. However, maize and some other members of the Andropogoneae, including sorghum, have a haploid chromosome number of 10.

Polyploidy is one mechanism which is supposed to be involved in the evolution of the maize genome. Maize has long been thought to be an ancient tetraploid whose genome has reverted over time to functional diploidy and thus lacks two sets of duplicated chromosomes. Gaut and Doebley [15] compared the divergence times for 14 pairs of duplicate maize loci, revealing two statistically different groups. Their results suggest a segmental allotetraploid origin for maize.

A second mechanism hypothesized to be involved in the evolution of the maize genome invokes the involvement of transposable elements (TEs). These mobile DNA elements were discovered in maize by Barbara McClintock during the 1940s. TEs are found in prokaryotes as well as in animals, plants, and yeast. In maize, it is estimated that 50% of the genome consists of repetitive elements [16]. Transposons are divided into two classes based on their mechanism of transposition [17]. Class 1 elements, or retrotransposons, transpose using an RNA intermediate. They often have long terminal repeats (LTRs) at their extremities. Class 2 elements transpose by means of a DNA intermediate. They possess terminal inverted repeats (TIRs) and can be divided into two groups: autonomous and non-autonomous elements. The best known of the class 2 elements are the Activator (Ac), Suppressor/mutator (Spm) and Mutator (Mu) transposons. MITE families (Miniature Inverted-repeat Transposable Elements) of transposons have also recently been described [18,19]. TEs of MITE families are small, reminiscent of non-autonomous DNA elements and have high copy numbers.

The position of TEs close to genes suggests that they might be involved as cis regulatory elements and that their insertion or deletion could drive the evolution of gene expression. They are thought to be involved in evolutionary change because their movement can be triggered by environmental or genomic stress (UV light, microbial or viral infection and inter-species hybridization).

2.3. Maize mutants: a powerful genetic tool

Maize has always been considered as an excellent genetic model. It possesses a large range of remarkable mutant phenotypes. It is also a good model for cytogenic investigations, studies which correlate gene transmission with observable features of the physical chromosomes. The long story of maize as a genetic model provided many characterized mutants of this species [20]. Furthermore, the discovery of transposons in maize and the understanding of their mechanisms of transposition have led, more recently, to the use of transposons to generate very large collections of novel maize mutants. The collection, screening and characterization of these maize mutants now represent a major activity of the research community. The mutator transposon is, today, one of the most useful tools for the generation of novel mutants of maize and the elucidation of the functions of maize genes.

3. Transformation of maize

Plant genetic engineering is a powerful tool used to answer fundamental biological questions: it provides a way to dissect plant processes and metabolic pathways, and has been used to determine gene function and to study tissue-specific gene expression. Transformation additionally allows the engineering of plants to confer potentially useful agronomic traits such as protection against insect pests or important diseases.

3.1. The tools and their limitations

The main techniques used for plant transformation are *Agrobacterium*-mediated transformation and methods that use direct DNA delivery for transformation [21].

Protoplast transformation was the first technique, using direct DNA delivery, to report the successful integration of foreign genes into a plant cell. Protoplasts can be generated from any plant tissue and have an ability, termed totipotency, to regenerate into mature plants. The delivery of naked foreign DNA can be achieved by treatment with polyethylene

glycol or by electroporation. A more common method of direct DNA delivery transformation is that of particle bombardment or biolistic transformation [22]. In this method, tiny particles of gold or tungsten are coated with DNA containing the gene of interest. Particle bombardment of the target tissues involves the acceleration of the coated particles by an explosive or electrical discharge, or by pressurized helium. Mechanical force, generated by high particle velocities, drives the foreign DNA through all the biological barriers, allowing for genomic integration into virtually any tissue. However, this method is limited in the size of DNA constructs that can be used. In addition, many studies have shown that in the vast majority of cases, biolistic transformation results in the integration of multiple, often rearranged, copies of the transgene. Moreover, the integration of multiple copies has been linked with gene silencing. In this poorly understood phenomenon, the expression of an introduced gene is detected and shut-off by the plant cellular machinery. Multiple copies of a transgene can also lead to an overexpression that can prove to be toxic to the plant.

The second method used to transform plants exploits the natural ability of the plant pathogen *A. tumefaciens* to transfer DNA. The transfer DNA (T-DNA) portion of a large Tumour-inducing endogenous plasmid (pTi) with defined ends becomes integrated into the nuclear genome of susceptible plants that are in contact with the bacterium [23]. This method is widely favoured due to its ease of use and low cost. Since monocotyledonous plants are not natural hosts for *A. tumefaciens*, genetic transformation in these species was achieved later than in dicots.

Agrobacterium-mediated transformation usually involves several steps, from the incubation of cells or tissues with the bacterium to the regeneration of fertile plants from the transformed cells via a callus stage. The foreign DNA sequence, consisting of gene(s) of interest and a marker gene, is inserted between the borders of the T-DNA cloned in a small plasmid (usually a binary vector able to replicate both in *Escherichia coli* and *Agrobacterium*). This construct is then introduced into an *A. tumefaciens* strain whose pTi contains an active virulence region for plant transformation but lacks a T-DNA region. Following co-cultivation of plant tissues with the bacterium, the T-DNA is transferred into a plant cell and the foreign gene or genes are incorporated into the nuclear genome. Improvements in culture and regeneration techniques, application of specific pretreaments according to the tissue of the species, as well as the development of supervirulent forms of plasmid vectors, have led to a drastic expansion in the number of transformable plant species.

In general, *Agrobacterium*-mediated transformation results in a greater proportion of stable, low-copy number of transgenes in plant genomes in comparison to direct DNA transfer. Another reason for the popularity of this method is that the T-DNA seems to integrate in transcriptionally active regions of the plant genome, increasing the likelihood that the transgene will be expressed.

3.2. Historical survey of maize transformation

In the mid-1980s, the recalcitrance of maize and most other monocot species to *Agrobacterium*-mediated transformation led to the development of direct DNA delivery method [24]. Nevertheless, the first challenge was to regenerate fertile plants from tissue cultures before envisaging to genetically engineered maize. This was achieved using protoplast-derived calli from the widely used inbred lines B73 and A188 as well as from an elite line [25,26]. Subsequently, several attempts to transform maize by protoplast electroporation yielded transgenic plants that were not fertile [27]. By bombarding embryogenic maize suspension cells, the first fertile transgenic maize carrying the selectable marker gene *bar* under the control of the CaMV 35S promoter was obtained [28]. This new ability to transform and regenerate monocot plants marked a significant advance in plant transformation. Additional work including improvements of the particle bombardment system and the use of an osmotic treatment of target tissues [29] led to the gradual adaptation of the biolistic transformation process for the production of transgenic maize from more highly differentiated tissues. For a long period, particle bombardment has been the method of choice to transform cereals.

Work in which *Agrobacterium* was used to deliver an infectious cDNA of maize streak virus convincingly demonstrated that maize is susceptible to T-DNA transfer from the bacterium [30]. However, it was only after the processes involved in T-DNA transfer were better understood that transgenic maize was produced. The first report of efficient transformation of maize by *A. tumefaciens* was published in 1996 [31]. Immature zygotic embryos co-cultivated with the bacteria carrying extra copies of virulence genes (super binary vector) produced transgenic maize inbred A188. This protocol allowed the transformation of other lines which included this inbred as one parent [32]. A more recent stage was reached with the use of L-cystein which allowed the transformation of immature embryos of HiII inbred [33]. This antioxidant moderates *Agrobacterium*-maize induced apoptosis, and allows the use of standard binary vectors to give an efficacy of 5.5%.

3.3. Bt-Maize

Over the past few years, maize transformation has been used with the aim of crop improvement, especially to increase insect and pathogen resistance. The European corn borer, an insect in the order Lepidoptera, is the most damaging insect pest of maize, and losses resulting from its damage and control are high. Selection and breeding of maize has steadily produced insect-resistant genotypes but the transgenic approach of plant genetic engineering provided a powerful means to complement and supplement the traditional methods. Therefore, insect-resistant transgenic plants which express *Bacillus thuringiensis* toxins were among the first

products of plant biotechnology to be approved for commercial use.

B. thuringiensis is a bacterium that produces fairly specific insectidial proteins known as crystal (Cry) proteins or δ-endotoxins [34]. These are part of a large family of homologous proteins active in only one or a few insect species. They were first introduced into tobacco and tomato by *Agrobacterium*-mediated transformation [35]. Using unmodified *cry* genes, the protein expression level was too low to confer sufficient protection against pests under field conditions. Typical of genes from a *B. thuringiensis* species, Bt *cry* genes have a high A/T content as compared to plant genes. Unmodified cry transcripts fail to accumulate to high levels in transgenic plants, probably as a result of mRNA splicing caused by the presence of cryptic introns [36]. However, effective resistance against the European corn borer was achieved in maize using a partial *cry1Ab* gene with modified codon usage and with the removal of polyadenylation signals [37]. The tissue-specific expression of Cry proteins in maize is dependent on the gene promoter used and the genotypic background. Since 1996, several companies have developed different Bt-maize lines and commercialized their use by licensing these to seed companies. All but one of the new lines use a CaMV 35S gene promoter that results in season-long expression of Cry1Ab (or Cry1Ac or Cry9C) in all plant tissues [34]. Tc1507, a new line (termed an "event") expressing the Cry1F coding sequence driven by a ubiquitin promoter, recently obtained registration from the American Environmental Protection Agency. The Bt transformation event 176 uses a combination of two maize-derived, tissue-specific promoters: a phosphoenolpyruvate carboxylase promoter that results in gene expression only in green plant tissues, and a calcium-dependent protein kinase gene promoter that targets the expression of Cry1Ab in pollen. These two constructs are inserted at the same locus. Results of field experiments consistently demonstrated that the currently available Bt events give an excellent control of European corn borer larvae and lower incidence and severity of insect-mediated maize diseases such as rot diseases.

4. "Long live monarch": story of a scientific polemic

When cornfields are threatened by the highly damaging insect pest, the European maize borer, farmers most commonly employ two techniques for pest management. The first of these makes use of synthetic chemical insecticides, while the second, which is considered as more friendly for the environment, uses microbial insecticide sprays. Microbial sprays are based on the use of toxins with insectidial effect, the Cry proteins naturally produced by *B. thuringiensi*s. For several years, GM maizes expressing the Bt Cry protein have been available. Although different bacterial strains have insecticidal effects against different groups of insects [38], the endotoxin used for maize transformation was considered as specific for the lepidopters and was expected to have a low impact on the environment.

4.1. Bt-maize causes a polemic

In order to evaluate the impact of Bt-maize on non-target insects, a preliminary toxicological study was conducted in the laboratory. Monarch butterfly larvae, used as non-target insects, were fed milkweed plants dusted with Bt-pollen [39]. The monarch butterfly, one of the most popular butterflies in the United States, leaves Mexico during spring to travel north where it lays its eggs. These hatch into caterpillars that eat exclusively milkweed, a plant commonly found in cornfields [40]. In areas where maize is intensively cultivated, milkweed grows at a higher density in cornfields than on non-agricultural land [41]. As a result, it is realistic to propose that milkweed may become dusted with maize pollen and in the case of a Bt-cornfield, that monarch larvae could ingest Bt-pollen with milkweed leaves. Losey et al. [39] found a 44% higher mortality in larvae fed with Bt-pollen compared to those fed with untransformed pollen, and a significant effect of maize pollen on monarch larvae feeding behaviour. These authors concluded that their results had "potentially profound implications for the conservation of monarch butterflies" and that it was imperative to "gather the data necessary to evaluate the risk associated with new agrotechnology and to compare these risks with those posed by pesticides and other pest-control tactics".

Despite the fact that these results have received considerable criticism from the scientific community, they were extensively used by the media who have grossly exaggerated the threat posed to the monarch butterfly by transgenic maize.

4.2. Impact of Bt-maize on the monarch butterfly

To accurately evaluate the impact of Bt-pollen on the monarch butterfly, it is necessary to place the conclusions reached by Losey et al. [39], in their proper context. The toxicity of Bt-maize pollen on non-target insects will depend on the Cry protein expressed and on the promoter used, as this will determine the level of expression of the endotoxin in pollen. Commercial Bt-hybrids variously express *cry1Ab*, *cry9C*, *cry1Ac* and *cry1F* genes [42]. Assays carried out with the different purified toxins revealed that Cry9C and Cry1F proteins are relatively non-toxic and that monarch larvae were sensitive to Cry1Ab and Cry1Ac proteins. These tests also indicate that monarch larvae are more susceptible to Cry protein during their first stage of development [42]. Pollen from the different hybrids were assayed and the only pollen which was found to consistently affect monarch larvae was from event 176 hybrid. Event 176 was engineered with a pollen specific promoter and expresses more than 78 times more Bt toxin in its pollen than do other hybrids [43] constructed with the cauliflower mosaic virus 35S promoter or the ubiquitin promoter. These results illustrate the heteroge-

neity of commercial Bt-maizes and their potential toxicity on non-target organisms. Results obtained from one maize variety are not applicable to all other varieties; Losey et al., however, are guilty of such a generalization. Another severe criticism of the work of Losey et al. concerns the amount of pollen used in their feeding experiments. In these experiments, pollen density was set to visually match densities on milkweed leaves from cornfields. Results obtained from experiments that used only one pollen density, which had not been accurately determined, must be considered as very preliminary. In addition, insufficient data were given by Losey et al. to accurately repeat their work. Average pollen density inside a cornfield has been estimated at 170.6 grains per cm^2, with a maximum of 1400 grains per cm^2[44]. Pollen density was found to fall to 14.2 and 8.1 grains per cm^2 at, respectively, 2 and 4–5 m from the edge of a cornfield [44]. Laboratory experiments using a pollen density >1000 pollen grains per cm^2 were designed to reproduce the results obtained by Losey et al. This study resulted in a 97% survival of larvae [42] instead of the 56% found in the original study. This result suggests that the pollen density used by Losey et al. was far in excess compared to pollen density in cornfields or that pollen may have been contaminated with non-pollen tissues. The only laboratory experiments that found maize pollen to be toxic to monarch butterflies at environmentally realistic pollen densities were performed with event 176 hybrids which use a pollen specific promoter.

In addition to the methodological problems described above, some other experimental details in the work of Losey et al. are also subject to question. These include the use of a control for feeding experiments which originates from an unrelated maize hybrid and the utilization of a no-choice test for feeding experiments.

Though the first stage of impact assessments may be performed in a laboratory setting and include toxicogical experiments to describe a "worst-case scenario", real impact on the environment cannot be extrapolated from such results without field experiments. Field work carried out with Bt-maize to assess the impact of Bt-pollen on non-target insects has revealed several important factors which should be taken into account.

One factor which is important to consider is the spatial and temporal overlap of monarch larvae exposure to pollen. Oberhauser et al.[41] found that in northern maize belt sites, about 40–62% of larvae overlapped with pollen shed, whereas in areas further south, about 15–20% of the larvae overlapped with pollen shed. Factors such as feeding behaviour of monarch larvae are also important determinants for risk assessment. Monarch butterflies laid the majority of their eggs on upper leaves, and 55% of first instars were found on upper leaves rather than on middle and lower leaves [44]. Due to higher rainfall, pollen density on upper leaves is at half the density found on middle and lower leaves [44]. As a consequence, the exposure of monarch larvae to Bt-pollen does not correspond to the average pollen density but is lowered by their feeding habits.

The lowest-observable-effect-concentration range for Bt11 and Mon810 hybrids (the predominant commercial hybrids) has been estimated to be encountered by larvae in only 0.7–0.1% of natural in-field situations [45]. The impact of Bt-pollen on monarch populations is restricted to event 176. As this hybrid will be phased out in 2003, and furthermore, represents less than 2% of the total maize planted in the US, the impact of Bt-maize on monarch butterflies should remain very low.

The evaluation of the risk encountered when using new pest management systems such as GM plants must be considered by comparison with the current practices. European maize borers are commonly controlled by organic farmers by spraying *B. thuringiensis* preparations used as a biopesticide, regardless of their deleterious effect on non-target organisms [38]. The insecticide, λ-cyhalothrin, has a dramatic effect on monarch larvae which died within hours [43] and due to insecticide drift, the survival of larvae outside the cornfield was also reduced. Therefore, compared to the currently used methods for pest management, Bt-maize represents an improvement for non-target organism survival.

The studies, which have integrated laboratory and in-field experiments, stressed the need for environmental and behavioural investigations before concluding on impacts for the environment. Despite the lack of such studies, the work of Losey et al. [39], which was based on very preliminary results, was entitled "Transgenic pollen harms monarch larvae". This paper was followed by extensive media coverage which further misinterpreted the laboratory results, leading non-scientists to a massive opposition to Bt-maize and biotechnologies.

5. Introgression of transgenic maize genes into Mexican maize landraces

In November 2001, *Nature* published evidence that transgenic maize genes had become introgressed by cross-pollination (permanently incorporated) into landraces of maize in Mexico [46]. The authors claimed that the transgenes were moving in the genome and thus, were unstable, and that such introgression may threaten the biodiversity of Mexican landraces. Since then, this work has been repudiated because of methodological errors, but the debate on maize gene flow and its consequences is still open.

Gene flow from maize can occur by pollen transfer and seed dispersal. Unlike its relative, the annual teosinte, maize kernels are held tightly on the cobs and do not come apart at maturity: maize seeds do not disperse by themselves. Pollen movement is the only effective means of gene escape from maize plants. All forms of *Zea mays* ssp. *mays* freely cross-pollinate forming fertile hybrids. Gene flow and introgression can also occur between teosinte and maize in both directions but at a low level [47]. Thus, when grown in close proximity, cultivated maize can cross with teosinte as well as with wild maize relatives. Gene dispersal between crop

plants and their wild relatives has been occurring since the beginning of plant domestication and is influenced by the plant and the environment [48]. If it does not pose any risk in itself, gene flow raises the question of the effect of the new gene–plant combination. The main concern is the fear for genetic diversity. Mexico is the global centre of maize diversity and the place of origin of strains grown commercially around the world. For 6000 years of traditional breeding, landraces have constantly changed due to human intervention and diversity has been promoted for the development of additional new varieties. To protect this diversity, the Mexican Government declared a moratorium in 1998 on planting transgenic maize anywhere in the nation. However, there is no scientific evidence that out-crossing from engineered crops could endanger maize biodiversity. Conversely, despite the potential for maize-teosinte crossing that has existed for millennia, teosinte populations have maintained their distinctness due to a gene cluster that limits crossing with maize [49]. The second worry is the possibility of introgression leading to an increase in the fitness of wild relatives. If transgenes confer an evolutionary advantage to the species, then their prevalence in wild population would be expected to increase progressively. With GM crops, the most likely traits to confer such advantages would be herbicide tolerance and insect resistance. Introgression of a herbicide resistance gene could lead to a potential weed problem and to a decrease of the herbicide efficiency, but this has meaning only in an agricultural setting, and in the absence of the herbicide pressure, the selective advantage would disappear rapidly. The effects of insect resistances, such as those conferred by Bt genes, are central to the debate as changes in these resistances, caused by plant–insect co-evolution, are difficult to predict.

Gene flow is constant and because maize varieties cross readily, almost everyone agrees that GM maize may be growing in Mexico. What science must now resolve is whether or not the flow of transgenes into maize landraces will have significant negative impacts on either maize genetic diversity or on the broader environment.

6. Conclusions

The past century has seen some great advances in biotechnology, and some well-intended applications that aim to improve living conditions for humanity. Due to the possibilities offered by GM technology in this new century, societies will need to make some important choices about the type of world that they wish to move towards. For the further development of GM technology, the reader is directed to a recent paper entitled “How safe is safe enough in plant genetic engineering?” [50]. In the present paper, we have described two examples of fallacious scientific arguments that attempt to demonstrate harmful effects for GM maize and to thereby heighten controversy (see also [51]). In a series of EMBO reports and in many other papers on GMOs, several scientists and sociologists of science try to elucidate the reality of the GMO debate. For some of these experts, the preponderance of myths concerning GMOs is a central feature of the controversy associated with them. These experts suggest that the problem of public acceptability of GMOs cannot be resolved without first deconstructing the associated myths [52].

From the purely scientific point of view, GM technology represents a means to learn more about gene function. The economic need for GMOs and their use as a pure science research tool are entirely distinct. Nonetheless, scientific questions relating to the widespread use of GMOs must be raised [50]. These include questions of the long-term behaviour of GMOs in natural habitats, the future of biodiversity in parallel with GM agriculture [53,54] and the long-term health effects of GM foods of human beings [51]. The GMOs of the future will be able to provide agricultural improvement in yield, plant breeding, and quality factors. In addition, GMOs can be used in “molecular farming”, providing a new and efficient means of production for medical and pharmaceutical products such as vaccines.

At the frontiers of scientific discovery and its potential applications, a level of future uncertainty is inevitable. The public’s perception of risk, however, can vary hugely, and can be adversely affected by the inadvertent use of unnecessarily emotive language. It must be kept in mind, also, that the level of social acceptability gained by a new technology such as GM is an entirely separate issue from public satisfaction with the social and scientific processes associated with its adoption [52].

To conclude this review paper, we would like to mention a recent paper published by Norman E. Borlaug [3], Nobel Prize Laureate for Peace, 1970: “... no food products, whether produced with recombinant DNA techniques or more traditional methods, are totally without risk. The risk posed by foods are a function of the biological characteristics of those foods and the specific genes that have been used, not of the processes employed in their development. The affluent nations can afford to adopt elitist positions and pay more for food produced by the so-called natural methods; the 1 billion chronically poor and hungry people of this world cannot. ... Most certainly, agricultural scientists and leaders have a moral obligation to warn the political, educational, and religious leaders about the magnitude and seriousness of the arable land, food, and population problems that lie ahead, even with breakthroughs in biotechnology”. The gains in food production provided by the Green Revolution have reached their ceiling while the world population continues to rise. Advances in plant biotechnology must be deployed for their benefit by a strong public-sector agricultural research effort [55].

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