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Research and field monitoring on transgenic crops by the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT)

David Hoisington · Rodomiro Ortiz

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Abstract The International Maize and Wheat Improvement Center (CIMMYT) aims to genetically enhance both crops and generate public sector-provided products for the resource poor, e.g., drought tolerant wheat and insect resistant maize, and through international–national partnerships facilitate the acquisition of improved germplasm for non-mandate crops in the cropping systems where maize and wheat thrives; e.g., GM-papaya through a national food security undertaking in Bangladesh. The Center also engages in public awareness campaigns in projects such as Insect Resistance Maize for Africa (IRMA), which includes food, feed and environmental safety, monitoring of resistance and establishment of refugia, non-target effects and gene flow. Monitoring of genetic resources is a wide concern among the centers of the Consultative Group on International Agricultural Research (CGIAR), with an emphasis on the quality of gene banks. Decisions, policies and procedures about monitoring should be science-based, and this requires education, an area where CIMMYT and other CGIAR centers can play an important role. There will be a need to continue to evaluate the need for, and type of monitoring, as new (and unique) products are developed and released in the emergent economies of the world.

Keywords Biosafety · GMO · Geneflow · Maize · Papaya · Wheat

D. Hoisington
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India

R. Ortiz (✉)
CIMMYT, Apdo. Postal 6-641, 06600 Mexico, DF, Mexico
e-mail: r.ortiz@cgiar.org

Introduction

Many of the world's poorest people are small-scale farmers, whose livelihood is at risk because of low productivity and insecure harvests. At the same time, poor urban and rural consumers suffer from malnutrition, the so-called hidden hunger, which impairs productivity. The International Maize and Wheat Improvement Center (CIMMYT) together with its partners works to solve these problems of poverty and food insecurity with a range of multidisciplinary research and capacity-building activities focused on food, agricultural, and natural resource in maize- and wheat-cropping systems.

In the last two decades, biotechnology has produced a number of valuable tools and techniques that can be used to help improve and conserve all crop species. Thus, CIMMYT believes that biotechnology has an important role to play in improving the productivity, stability, quality, and use of maize and wheat cultivars in developing countries while preserving the environment. CIMMYT, along with its sister centers of the Consultative Group on International Agricultural Research (CGIAR), is committed to making these new opportunities offered by biological sciences available as international public goods and thereby complementing private-sector research so that technologies can reach resource-poor farmers and malnourished poor consumers.

While plant breeding that utilizes non-transgenic approaches will remain the backbone of CIMMYT's crop improvement strategies, genetically engineered maize and wheat cultivars (popularly called genetically modified crops, GM-crops) will not be excluded as products capable of contributing to CIMMYT's principal goals. Indeed, in tackling certain intractable problems, using genetically engineered crops and transgenes (or genes introduced into a species through genetic engineering) may be the best available approach for meeting the challenges of food security and environmental protection. CIMMYT believes that it is important that any variety, genetically engineered or not, that is released to farmers is safe and effective. Thus, efforts will be focused on evaluating the environmental and food/feed safety aspects on all new cultivars. Equally important is to ensure the sustainability of the technology for farmers. Thus, efforts will also focus on issues such as resistance management strategies, intellectual property rights and seed saving technologies that allow farmers long-term benefits, inexpensive access to the varieties and the ability to save seed from generation to generation.

Recognizing that both the scientific community and the general public express a range of conflicting opinions on the use of genetic engineering, CIMMYT favors public dialogue based on transparency and science. CIMMYT will take a holistic approach in this debate by examining, to the best of our ability, biosafety, food safety, trade, intellectual property rights, and ethical and cultural aspects, all of which shape the science and policy actions related to the development and use of GM-crops (http://www.cimmyt.org/english/wps/transg/gmo_stmt.htm). In this regard, CIMMYT keeps in its Internet home page (http://www.cimmyt.org) a link under the icon "Transgenic Research and Statements", which provides updates both on policy guidelines and research (http://www.cimmyt.org/english/wps/transg/index_res.htm). Below we share examples of ongoing GM-crop research-for-development by CIMMYT and partners.

Assessment of transcriptional factor genes to enhance drought tolerance in wheat

A number of strategies are being followed to enhance the tolerance of maize and wheat to water-stress conditions, including the development of genetically-engineered cultivars containing various gene constructs to enhance the performance of these cultivars under water stress. While there are a number of issues that must be addressed if such transgenic cultivars are to be effectively deployed to farmers (e.g., intellectual property, biosafety, food, feed and environmental safety), if genetic systems based on transgenes can be found effective, they will provide an attractive and complementary option for improving a plant's performance under stress conditions. Particularly attractive is the single, dominant nature of the transgene that makes the transfer and maintenance of this system in any cultivars much easier than those based on polygenes.

Molecular mechanisms of water stress response have been investigated primarily in the model plant species *Arabidopsis thaliana* (Bennett 2003 and references therein). Analyses of the expression of dehydration-inducible genes have shown that at least four independent signaling pathways function in the induction of stress-inducible genes in response to dehydration (Gilmour et al. 1998): two are ABA-dependent and two are ABA-independent. Several stress-induced genes, such as *rd29A* in *A. thaliana*, are induced through the ABA-independent pathway (Liu et al. 1998). The Dehydration-Responsive Element Binding gene 1 (*DREB1*) and *DREB2* are transcription factors that bind to the promoter of genes such as *rd29A*, thereby inducing expression in response to drought, salt, and cold (Dubouzet et al. 2003; Kasuga et al. 1999).

The Japan International Research Center for Agricultural Sciences (JIRCAS) shared with certain CGIAR centers gene constructs containing the *AtDREB1A* gene under the control of various promoters. These were introduced into several crops with the expectation that *AtDREB1A* would recognize the DREs of endogenous genes and enhance stress responsiveness. For example, different transgenic groundnut lines were produced by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and the transgenic lines show delayed wilting under simulated drought by 20–25 days compared with non-transgenic controls (Mathur et al. 2004).

Likewise, transgenic wheat produced at CIMMYT and tested in small field plots in CIMMYT's biosafety greenhouse had a 10-day delay in drought-induced wilting (Pellegrineschi et al. 2004). On-going trials in CIMMYT's biosafety greenhouse will enable researchers to see whether the DREB-wheat responds well under more "natural" conditions. These trials are the first time that transgenic wheat has been planted under field-like conditions in Mexico, and rigorous biosafety procedures are being followed. CIMMYT also plans to test the *DREB* gene in a variety of drought-tolerant wheat developed through conventional breeding, to see if the resulting plants can use water even more efficiently (Iwanaga 2004). If the results from these trials are positive, DREB-wheat will provide a powerful option for improving the yield of wheat under water-stress conditions, and will demonstrate the genes potential usefulness in other crops such as rice, maize and barley.

Insect Resistant Maize for Africa

Maize is a major food crop in Africa, especially in the eastern and southern regions of the continent. Threats to this food source endanger food security, and stem borers pose just such a threat in much of Africa (De Groote 2002). To tackle this problem, the Insect Resistant Maize for Africa (IRMA) project was launched in 1999 by the Kenya Agricultural Research Institute (KARI) and CIMMYT, with funding from the Syngenta Foundation for Sustainable Agriculture (Mugo et al. 2005). The project is aimed at producing maize that is adapted to various Kenyan agroecological zones and is also resistant to key insect pests, primarily stem borers. Both conventional and biotechnology-based sources of resistance are being examined for their effectiveness against the borers. The project emphasizes public involvement and awareness through events such as its annual Stakeholders Meeting. Furthermore, major project objectives include environmental and socioeconomic impact studies, resistance management strategies, and project documentation. Based on the experiences and results generated in Kenya, appropriate technologies and varieties will be extended to other African nations.

At this stage, the project produced stable, low-copy events of *cry1Ab* and *cry1Ba*, which were backcrossed into CML216. A biosafety greenhouse (BGH) was established in KARI and seeds of the *cry1Ab* and *cry1Ba* events imported and growing in the BGH following approval by the Kenyan Government. Quarantine field site was established and is being used for mock trials and training of local staff and farmers. Testing of Bt-maize at the site is anticipated pending regulatory approvals. Events of *cry1Ca* and *cry2Aa* are now being produced.

Numerous experiments were conducted or are in-progress to determine effective insect resistance management strategies for Kenyan farmers. Environmental, food and feed safety aspects are also being investigated. Collecting baseline data is essential for effective monitoring and guiding of the project. Monitoring research includes efficacy of both products (determined in biosafety greenhouse, open quarantine site, and national performance trials), build-up of resistance to both products (for Bt maize, now being studied in the biosafety greenhouse), adoption of refugia strategy; efficacy of refugia strategy; potential environmental impacts, impacts on non-target and beneficial insects and other organisms, adoption of products, consumer and grower acceptance as well as media coverage. Baseline studies and activities, which serve as the basis for current and future monitoring, include:

- Baseline participatory research assessments (PRAs) with 1,800 farmers of five maize growing agro-ecologies to determine extent of losses due to stem borers and current insect management practices. Also the PRAs are undertaken to determine salience of the problem among the farmers and regions as well as the demand for solutions.
- Assays conducted with maize farmers' in the five maize agro-ecologies to identify the insects typically found and their relative abundance. Dry and digital collections were established for future reference. This undertaking can be regarded among the most extensive assay of its kind to date in Kenya.
- A large and diverse group of 880 farmers from the five maize growing agro-ecologies had their farms surveyed to determine the availability and quantity of plants that could serve as natural refugia in an insect resistance management scheme. Farmers were also queried about their potential acceptance of additional refugia plants based on their economic and practical implications.
- A survey was conducted in Nairobi at large and small supermarkets as well as posho mills, of urban

consumers to determine their knowledge, attitudes and acceptance of GM-crops at large and Bt-maize in particular.
- Since the project's inception, the print media has been monitored through a clipping service (news items, editorials, and letters to the editor) to discern trends in media coverage that could affect attitudes of policymakers, parliamentarians, and the general public.

Perhaps CIMMYT and IRMA's most important contribution to future monitoring has been in the area of capacity building, particularly in the areas of biosafety greenhouse management, insect field assays, and refugia plant surveys. Future monitoring efforts will clearly have to be conducted by national staff, although CIMMYT and other CGIAR centers might play a role in occasionally "monitoring the monitors" and providing training to update personnel in the latest procedures.

Recently, De Groote et al. (2005) assessed the risks and benefits of the IRMA project in Kenya. The authors indicated that most objections to Bt-maize cannot be substantiated. They recognized that is indispensable to work with Bt-maize and introduce it in an experimental setting so that farmers, consumers, and policy makers can make informed decisions. Their survey results indicate that Bt-maize responds to an important constraint, so farmers are very interested, and consumers are likely to benefit too. Furthermore, farmers do not express strong objections. In their ex-ante assessment, the poorer farmers in the low-potential areas seem to benefit relatively more, since they have relatively higher losses, and poor consumers will benefit relatively more since they spend proportionately more of their income on maize. According to the authors of this report, it seems that Bt-maize will be commercialized by local companies, since there are no restrictive intellectual property rights involved, and thus extra costs will be low. In this regard, because the *Bt* genes are dominant, farmers will not become dependent on the seed industry since they can recycle their seed. Their recycling methods, moreover, are likely to select for the *Bt* gene and, over time, incorporate the gene into local cultivars. As pointed out by De Groote et al. (2005), it will be difficult to inhibit this flow of trangenes into local landraces and cultivars and will be difficult to remove the transgenes once introduced. Hence, the IRMA project staff took samples of all local landraces and cultivars in the different agro-ecologies to deposit in the National Genebank. Their report also suggests that natural refugia might be insufficient in certain areas, but this could be countered by pyramiding several *Bt* genes in appropriate cultivars or mixing seed with sufficient amounts of non-Bt maize. Research of the effects of Bt-maize on non-target organisms has not yet been initiated, but identification of these organisms was started and comparative studies will start immediately with field trials.

Gene flow, genetic diversity and conservation of landraces in centers of domestication

A different aspect of baseline data and monitoring accompanies issues related to genetic diversity and conservation of landraces in centers of domestication, e.g., maize in Mexico (Serratos et al. 1995). This issue has come into sharp relief in public debates over the presence of GM-maize in Mexico, and transgenes being discovered in landraces therein.

Transgenic crops were originally created to meet the demands of intensive farming systems, not traditional farming systems. A key difference is that under intensive farming systems, new seed is usually purchased (or one could say replenished) on an annual basis, while under traditional farming systems, seed is recycled, exchanged, and selected by farmers. For this reason, monitoring under intensive systems is more controlled and easier than under farmer systems. Key to monitoring and modeling impacts and gene flow under the latter system is understanding it.

In the USA and Western Europe, research on gene flow in maize has focused mainly on measuring the distance over which wind-borne pollen can travel and still remain viable. In the case of maize in Mexico, however, gene flow is not just a biological phenomenon; it is a human one as well (Bellon and Berthaud 2004). Gene flow may result from inadvertent mixing of pollen, which frequently happens when many small adjacent fields are planted to diverse maize cultivars. But it may also occur when farmers deliberately mix seed from different sources with the express purpose of hybridizing them. Mexico is within the center of domestication and diversity of maize, and many landraces are still grown by small-scale farmers. Through their preferences and management practices, these farmers foster gene flow between distinct, sometimes genetically distant, maize populations. Maize diversity in farmers' fields therefore is not static; rather, it is dynamic and changes constantly as a result of biological and social processes. By fostering gene flow, these processes give rise to and sustain genetic diversity.

CIMMYT has been working for some time on characterizing the ways in which small-scale farmers in Mexico manage their maize germplasm and on describing how farmers' management practices affect gene flow, the genetic structure of maize landraces, their diversity and evolution (Aguirre Gómez et al. 2000; Bellon and Risopoulos 2001).

Mutations

An initial experiment conducted to measure the lethal and deleterious mutations present in these landraces detected high rates of deleterious mutations. On average, in the 17 elite landraces studied by the project, 53% of the plants showed a defect. The remaining landraces are being studied in an ongoing experiment, but preliminary results show a similar rate of accumulated mutations.

"Acriollamiento" or management of modern cultivars in traditional agriculture

In another project, management of modern cultivars within traditional systems has been studied on the coast of the state of Oaxaca (Bellon et al. 2003) and in Chiapas (Bellon and Brush 1994). In these areas, traditional farmers have access to improved modern cultivars derived from the tropical maize race Tuxpeño. This research shows that farmers apply the same management to the modern cultivars as that given to the local landraces, and that in many instances, they favor mixing the two types. This process is called "acriollamiento" or local adaptation.

Case study in Cuzalapa, Jalisco (Mexico)

Louette et al. (1997) conducted research in Cuzalapa, and their report indicated that seed exchanges between farmers and partial replacement were quite high. Of 484 fields in this research, planted with 25 local landraces, it was observed that farmers used their own seeds in only 53% of the fields. In the other fields, seeds were obtained either from the same village (36%) or neighboring villages (11%).

Learning from other continents: case study in Burkina Faso

In Burkina Faso, West Africa, maize cultivation may be classified into two very compartmentalized types. Early, yellow material is planted by women in their backyards; late, white maize is planted by men in larger plots, away from the village. Sanou et al. (1997) have shown that gene flow (genes from an improved modern cultivar distributed recently in this region) takes places between the two distinct types; genes from a modern cultivar, consistent with the second type of cultivation, were found in the landraces of the first type. We can conclude that this physical and cultural isolation is not effective in avoiding the exchange of genes between maize cultivars.

Sharing knowledge from partners: pollination between maize and teosinte

Gene flow occurs between maize and teosinte (*Zea* spp.) but at a low frequency. Recently Baltazar et al. (2005) investigated hybridization, flowering synchrony, pollen size and longevity, silk elongation rates, silk and trichome lengths and tassel diameter and morphology in gene flow research between a hybrid maize, landraces of maize and teosinte (*Z. mays* spp. *mexicana*, races Chalco and Central Plateau). Their research shows that crossing occurs mostly in the direction of teosinte to maize, and it supports the hypothesis that gene flow and subsequent introgression of maize genes into teosinte populations results likely from crosses where teosinte first pollinates maize. The resultant hybrids then backcross with teosinte to introgress the maize genes into the teosinte genome. Such an approach slows introgression and accounts for the co-existence of teosinte as a separate entity in the vicinity of large maize fields.

Sampling protocols for monitoring trasgenes

Maize and its wild species progenitor teosinte are wind-pollinated and capable of outcrossing. Hence, there a potential introgression of transgenes from commercial transgenic-derived hybrids into landraces and wild maize relatives may occur if they are grown nearby (Christou 2002). Furthermore, local farmers' behavior may have a significant influence on causing transgenes to diffuse, to be expressed differently, and to accumulate within landraces (Bellon and Berthaud 2006). Hence, the need for using appropriate protocols for planting and monitoring gene flow—particularly through pollen movement, especially when co-existence occurs (Brooks et al. 2004).

Pollen dispersal and viability, planting size, field shape, physical barriers, and wind speed or direction are among the factors to be taken into account for monitoring protocols (Brooks et al. 2004 and reference therein). Moreover, population genetic structure, representative sampling, and appropriate sampling and sub-sampling units for further statistical analysis are needed to detect transgene flow (Cleveland et al. 2005). For example, a sampling method that maximizes the probability of finding rare alleles in the reference population (Crossa et al. 1993) will be the proper to assess the frequency of trasgenes in a defined area. According to effective population size (*Ne*) theory, a balanced sample includes equal number of seeds from the largest possible number of maternal plants within each sampling level. Cleveland et al. (2005) suggested that for each reference population, its sampling should maximize the number of units (fields, location, ears within a field) sampled at each level, make a consistent use of balanced samples (number of ears, field area, or number of seeds per ear), and use *Ne* as the basis of any estimations of frequencies of rare alleles in the population. According to above approach, the conflicting reports about detecting transgenes in farmers' fields in Mexico (Quist and Chapela 2001; Ortiz-García et al. 2005a) result from inappropriate sampling methods and inconclusive statistical analysis due to the use of census population size (*n*) rather than *Ne*. Ortiz-García et al. (2005b) provide further details on using *Ne*, combined probability tests for data analysis across locations, and most appropriate null hypothesis for statistical testing. Nonetheless, monitoring of transgenes should also consider an understanding of local seed systems and farming practices that affect both population structure and dynamics, together with sound scientific methods, particularly when aiming as the basis for policy decisions.

Socio-economic inputs into transgene monitoring for decision-making processes

As pointed out by Bellon and Berthaud (2006) a human values-perception model will allow judgments on the potential impact of transgenes on biodiversity and the environment. They emphasize that farmers' or consumers' perceptions that transgenes are ''contaminants'' and that landraces containing transgenes are ''contaminated'' could cause these landraces to be rejected and trigger a direct loss of diversity. In this regard, Mugo et al. (2005) indicated that IRMA project surveys, stakeholders meetings and other communications indicate that farmers, consumers and other stakeholders are cautiously optimistic about GM-crop technology in Kenya. Similarly, participatory rural appraisals and surveys served to gauge the awareness and attitudes of farmers and consumers. For example, a survey of 604 consumers was conducted in Nairobi, Kenya at three points of sale (supermarkets, kiosks, and mills) to determine consumer awareness and attitudes towards GM-foods (Kimenju et al. 2005). The questionnaire sought information from maize consumers about their awareness and knowledge of biotechnology and GM-crops, their attitudes towards GM-food, and their willingness to pay for it. In excess of a third of the respondents were aware of GM crops, mostly from newspapers, television and radio. Likewise, from its initiation, IRMA project has recognized the need for effective communication to create public awareness and for education at various levels, which allows establishing on the ground stakeholder involvement and a participatory strategy for optimal use and monitoring of the technology.

IRMA also organized participatory rural appraisals in 43 villages spread over Kenya's agro-ecological zones that grow maize. More than 900 farmers participated in group discussions (Mugo et al. 2005 and references therein). Such participatory rural appraisals provided information to main constrains in maize production and although farmers could differ in their assessments, insect pests were high in low-potential areas whereas in high potential areas were of medium importance. After establishing that stem borers were a major constraint to maize production, their crop losses were estimated (12.9% of the potential yield) using a previous nation-wide survey and also measured in 150 farmers' fields during four seasons (13.5% of potential yield loss). Moreover, to complement the researchers' efforts and increase the chances probability of responsible stewardship of the GM-maize and refugia concept being accepted by the farmers, the IRMA project researchers held workshops to sensitize farmers and extension, in which views about GM-maize, resistance management and the role of refugia within insect resistance management were exchanged. Mugo et al. (2005) indicated that group exercises were undertaken to rank refugia species in experimental plots by farmers, extension agents and researchers based on their criteria. The ranking of the cultivars for use as pastures and refugia was not the same. Nonetheless, when the data for all groups were combined the most common criteria was resistance to stem borers, alternative uses (food, pasture, refugia, hay) and the ability to attract and support stem borers. The farmers also highlighted the availability of seed as important criteria which should not be ignored. In short, the frequent interactions of IRMA researchers with the stakeholders and regulatory agencies assure a participative decision-making process and compliance with the strictest scientific and regulatory standards.

Issues for a biosafety policy and monitoring in traditional agriculture

Due to permanent gene flow between different landraces, the probability is high that in these traditional agricultural systems, genes from introduced cultivars will find their way into the local landraces. We foresee at least two implications in terms of biosafety.

1. One could be tempted to establish strict rules and genetic barriers to restrict gene flow from the introduced cultivars, in order to keep the landraces free of their genes. However, before establishing such rules and policy, one should carefully study the impact of such measures on the flow of other genes, and on the viability of the current landraces. In effect, if we consider our hypothesis that gene flow is one element of the farmers'genetic system, modifying it will have consequences on the adaptability and acceptability of the currently cultivated landraces. In this traditional system, limiting the existing geneflow for biosafety or other reasons without changing other components of the farmers' management would lead to a loss of viability of the local landraces and their abandonment by farmers.
2. What if a gene diffusing from a variety that complies with all the biosafety requirements it is later found to be harmful long after the initiation of the diffusion process? Or that a gene from a transgenic plant created to produce pharmaceutical compounds inadvertently escapes? How can we return to the pre-diffusion situation? Or, how can this system be made reversible? Could this be accomplished by avoiding any new gene flow, or through more gene flow from landraces and cultivars that are free of the offending gene? Are other options available? Overall biosafety will increase when rules and strategies are defined to establish when reversibility is needed and how it should be implemented in traditional agricultural systems.

Although much has been learned, many important questions remain unanswered. What are the relative contributions of biological processes (e.g., pollen drift) versus social processes (e.g., seed mixing) in causing gene flow? Are the practices that foster gene flow similar across types of farmers and farming systems? What factors influence these practices and determine their impact? To what extent do farmers deliberately manipulate gene flow? Does gene flow enhance or reduce genetic diversity? Which characteristics enhance diversity, and which characteristics reduce diversity? Has gene flow from improved varieties affected the diversity of landraces? What is the impact of gene flow on the livelihoods of farmers that plant landraces? How can answers to these questions be used to answer related questions about the impact of transgenic maize in these systems?

Monitoring of gene flow in and of itself will not be sufficient to project diffusion of transgenes and potential impacts. Traditional farmers' management of diversity and traditional agriculture are not static, therefore, the traditional systems themselves need to be monitored as they evolve or outside forces bring changes to them (i.e., increased arrival of transgenic seed from migratory workers). If we want to have a framework of effective biosafety rules in these traditional systems, we must consider and monitor all of the relevant variables and components of these systems.

Adventitious presence of transgenes in ex situ collections

CIMMYT adds new maize and wheat genetic resources each year to those that are already conserved under long-term ex situ conditions, and the Center will continue to abide by the letter and spirit of its 1994 agreements with FAO concerning the management of collections of maize and wheat germplasm held "in trust." CIMMYT associates itself formally with the International Treaty on Plant Genetic Resources for Food and Agriculture and, as in Article 15.1(c) of that Treaty, recognizes "*the authority of the Governing Body to provide policy guidance relating to ex situ collections held by them and subject to the provision of this Treaty*," including guidance on the subjects covered by CIMMYT Guiding Principles for developing and deploying genetically engineered maize and wheat cultivars. Hence, the Center will continue to develop and implement measures that are feasible given current technology and funding to protect the genetic integrity of incoming (and already held) accessions and to maintain them according to international standards (e.g., see "Plant Genetic Resources Operational Manual" by Taba et al. 2004). The data arising from screening undertaken during the implementation of these measures will be made available as produced and without restriction. Recently, the Genetic Resources Policy Committee of the CGIAR issued a draft "Guiding principles for the development of Future Harvest Centers' policies to address the possibility of unintentional presence of transgene in *ex situ* collections, to which will adhere when formally issued by the CGIAR system.

Protocols for monitoring transgenes in experimental fields and genebanks

As pointed in above sections, sampling methods are very important for developing right transgene flow methods. Similarly, appropriate experimental design and lab protocols are needed to ensure sound evidence when monitoring transgenes or their impact in target and non-target organisms. Danson et al. (2006) provide details of a polymerase chain reaction (PCR) method and a FTA paper technology to detect genetically engineered *Bacillus thuringiensis* (Bt) maize in open quarantine fields in Kenya. Likewise, details for assessing the effects of successive seasons of GM-(herbicide-tolerant)-maize cropping on weeds and invertebrates are available elsewhere (Heard et al. 2006). Such protocols are useful tools in the monitoring of GM-crops into the farming systems and the environment.

CIMMYT took a leading role in the CGIAR system for developing protocols that ensure that transgenes are not inadvertently introduced into its genebank accessions or breeding materials. A two-tiered screening of maize landraces in CIMMYT genebank and candidate landraces indicate no presence of transgenes (http://www.cimmyt.org/english/wps/transg/tiered_17Oct02.htm). The sampling methodology involved grouping seeds from 105 accessions and candidate landraces into 24 bulks. Any of the bulks included a group of landraces or cultivars that share criteria based on an established classification strategy. For the latest screenings, the landraces were bulked by village or as several villages that possessed comparable topography. Between 1 and 12 samples, with considerable similarities but also displaying unique agronomic characteristics, were bulked. A finding of a transgene in a bulked sample would mean that the individual samples that constitute the bulk would then be screened to determine the source of the "positive" result.

For the first tier screen, the 24 bulks were germinated and DNA extracted according to the standard protocols (CIMMYT 2005). The DNA was amplified using a primer corresponding to the CaMV 35S promoter and another primer corresponding to the bar gene, two fragments of DNA found in most commercial transgenic maize and not known to exist naturally in the maize genome (sequence available upon request). One leaf was taken from each of the 100 plants to represent each bulk and broken down into 10 batches of 10 leaves for DNA extraction, processing, and analysis. In all, 2,400 leaf samples were used to test the 24 bulks. DNA isolated from a known transformed plant containing the CaMV 35S promoter was run as a positive control. To further ensure that the reactions were working correctly, all DNA samples were amplified using a primer corresponding to a fragment of DNA known to exist naturally in the maize genome. All positive controls amplified correctly, and no bulk of gene bank maize amplified the CaMV 35S promoter sequence or the bar gene sequence, indicating that, in the samples tested, neither of these was present. The second tier screen consisted of growing the plants from the 24 bulks in a greenhouse and spraying them with the herbicides Basta and RoundUp. To survive the treatment, a plant would have to express transgenically-based resistance to the herbicide. A total of 144 plants from each bulk were sprayed with Basta and a like number with RoundUp. No plants survived, indicating that the bulks did not include landraces carrying and expressing either of the herbicide resistance genes. As shown by the results posted on CIMMYT's public web site no transgenes were found to date.

In summary, CIMMYT's ability for monitoring the potential impacts of new cultivars builds on over 40 years of ensuring seed health of international nursery sets that reach partners around the world every year. The Center considers that monitoring is a national issue that needs critical attention in the short-term, and that it should be for all products not just those developed by a specific process. In this regard, decisions, policies and procedures should be led by facts of science, which requires education—an area where CIMMYT and its sister centers of the CGIAR can play a critical role. We hope that as more GM-crops, especially those ensuing from public efforts, are released, regulations and monitoring will be more rationale and based more on the traits released. Nevertheless, there will be a need to continue assessing monitoring issues as countries in the developing world deploy new (and likely unique) GM-crops able to tolerate better local stresses and that possess enhanced nutritional quality.

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