

Review

Developing Bt maize for resource-poor farmers – Recent advances in the IRMA project

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Accepted 17 October, 2019

This paper presents an overview of the advances in the IRMA project, which develops insect resistant maize varieties for resource-poor farmers, using both conventional breeding and genetic engineering. The project started in 1999 and is active in product development, impact assessment, and communication, all within the Kenya regulatory framework. So far, four application for introduction of tissue or commencement of field research were made to and approved by the National Biosafety Committee (NBC), and Bt maize leaves or seeds genes imported for testing against different stem borer species in bioassays on cut leaves in a biosafety laboratory, in potted plants in a Biosafety Greenhouse, and as whole plants in confined field trials in the Open Quarantine Station (OQS) at KARI Kiboko. All these biosafety facilities were specially built by the project for these evaluations. So far, good control has been realized against four of the five major stem borer species: *Chilo partellus*, *Chilo orichalcociliellus*, *Eldana saccharina* and *Sesamia calamistis*. Economic impact assessment demonstrated that stem borers are major constraints and cause substantial losses. Resistant maize varieties are likely to be adopted and to provide major returns to the investment if resistance against the economically most important species, *Busseola fusca*, can be found. Otherwise, returns would still be positive but small. Environmental impact research indicate that build-up of resistance against the Bt genes has not developed after that sufficient natural refugia exist in most areas, but suitable strategies acceptable to farmers need to be developed for some. Surveys, stakeholders meetings and other communications indicate that farmers, consumers and other stakeholders are cautiously optimistic about technology. Frequent interaction with the stakeholders and regulatory agencies assures a participative decision-making process and compliance with the strictest scientific and regulatory standards.

Key words: Maize, Bt, stem borers, Kenya, genetic engineering.

INTRODUCTION

Since the 1960s, world food supplies have increased tremendously, thanks to increased agricultural productivity, brought on by the green revolution (Evenson and Gollin, 2003). As a result, the world is more able to feed its growing population, reducing overall malnutrition

(Rosegrant et al., 2001). Sub-Saharan Africa, however, did not have the same experience, and is now the only region where both the number and the proportion and the absolute number of malnourished children has been consistently increasing in recent years.

In East-Africa, maize is the major staple food, but land is limited and yield increases have leveled off. Agricultural productivity has not been able to cope with population growth, leading to annual imports and food insecurity. It is increasingly becoming clear that the

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expected maize green revolution in Africa did not take off (De Groote et al., 2005). The reasons are diverse and complex, one being the high costs of inputs compared to decreasing cereal prices. Breeding for more hardy and stress tolerant varieties that require fewer inputs can therefore make a contribution. Stem borers, with storage pests, are the major pest problem maize farmers face in Kenya (De Groote et al., 2004b). Genetically engineered (GE) maize, in particular Bt maize, can provide good protection against a range of stem borers.

From its introduction in 1996, GE crops now cover 81 million ha, of which 15 million ha are in Bt maize (De Groote et al., 2005; James, 2004). Although an increasing proportion is being grown in developing countries, in Sub-Saharan Africa only South-Africa is growing GE crops, including Bt maize. In East Africa, progress in the development and release of GE crops has been much slower.

In 1999, the International Maize and Wheat Improvement Centre (CIMMYT) and the Kenya Agricultural Research Institute (KARI), with the financial support of the Novartis Foundation for Sustainable Development (which later gave these responsibilities to the Syngenta Foundation for Sustainable Agriculture, SFSA), launched the Insect Resistant Maize for Africa (IRMA) project. The goal of IRMA is to increase maize production and improve food security through the development and deployment of insect resistant maize to reduce losses due to the stem borers. Lepidopteran stem borers are economically important pests of maize, a major staple in Kenya (Seshu Reddy and Sum, 1991). Both host plant resistance and genetically engineered maize (e.g. Bt maize) have been identified as possibilities to help resource poor farm families combat these destructive pests and meet their food requirements. The IRMA project focuses on identifying the best methods to properly combine these mechanisms and ensure that African farmers will be able to take advantage of modern approaches to this problem.

To guide IRMA project through the biosafety processes, some six principles were adopted. First, IRMA was conceived as a model of good practice, especially its biosafety aspects, from which other countries can learn. Second, IRMA uses both conventional breeding and biotechnology, so the farmers and consumers can choose the technology they prefer. Third, the project will provide the hands-on experience that is essential for Africa to understand and use biotechnology, including genetic engineering, and make informed decisions. Fourth, it will serve as a pilot project for public-private partnership and cooperation. Fifth, IRMA will employ state of the art technology and methodology, from both the natural and social sciences. Finally, IRMA was to be transparent and open through ongoing stakeholders consultations.

To develop and deploy Bt maize, important technical, regulatory, proprietary, and stewardship issues needed

to be addressed. Consequently, IRMA activities fall into four major categories: product development, product deployment, impact assessment, and communication and promotion, all carried out by interdisciplinary teams of scientists. Product development comprise of the chain from the identification of candidate gene(s), development of the appropriate gene construct, transformation of the appropriate maize germplasm. The putative transformants (events) are then tested for their efficacy in biosafety facilities including the laboratory (molecular characterization) and greenhouses (testing against the targeted pests). Once an event has been validated to be effective and to be inserted into the maize genome, classical backcrossing is used to move the gene from the Transformed plant (CIMMYT uses CML216xCML72) into elite maize lines. Throughout the repeated backcrossing, inheritance of the gene is monitored to ensure that Mendelian inheritance is observed to indicate that the event is stable. All of these activities are carried out following strict biosafety procedures at various authorized facilities.

Given the high sensitivity of the technology, the project has to study the environmental, social and regulatory systems. Potential effects on non-target organisms and insect resistance management strategies are important environmental concerns. Socio-economic impact assessment is emphasized to find out the need for Bt maize products, their fit in the farming communities, acceptability by farmers and consumers, and the level of demands by farmers and the maize industry. Finally, GM crop technology is highly regulated and major attention is paid to the regulatory system in Kenya as well as intellectual property rights (IPR) issues that arise from use in research and commercialization of Bt maize in Kenya and in Africa. As a new technology, Bt maize is subjected to debates that require careful, proactive, as well as defensible, and transparent communication regarding the pros and cons of GE maize.

In this paper, we present an overview of the IRMA project, including progress made and experiences gained, followed by a discussion of the issues still lay ahead, and the prospects of the different technologies.

THE REGULATORY ENVIRONMENT IN KENYA

The regulatory process for GE crops in Kenya

Kenya is signatory to the Convention to Biological Diversity (CBD) and the Cartagena Protocol on transboundary movement of GMOs. This requires a policy on biotechnology and biosafety, which usually consists of a Bill or Act in combination with implementing regulations. In efforts to implement the two protocols, Kenya has put in place a regulatory regime for biosafety; including rules and regulations as a system to handle applications for approvals to tests GMOs in Kenya. The regulations also

cover the monitoring and inspection system, and a public information system.

Kenya issued an Interim Regulation In 1998, enabling the establishment of the National Biosafety Committee (NBC) and providing guidelines for the establishment of Institutional Biosafety Committee (IBC) in those organizations active in research, import or utilization of GE products (NCST, 1998). The National Council for Science and Technology (NCST) is currently coordinating the development of a Biotechnology Policy and a Biosafety Bill, through the participation of a broad spectrum of stakeholders in different fora and awareness workshops (BIOEARN, 2003). The final drafts of the Policy and the Bill is with the Minister of Education, Science and Technology in readiness for presentation for cabinet approval. The NCST through UNEP-GEF funding is in the process of procuring GMO detection kits. The Kenya Plant Health Inspectorate Services (KEPHIS) is also procuring similar equipment through FAO grants, for use in the country's major entry points, including the seaport and airports.

Relevant regulatory institutions currently in place include the KEPHIS for phytosanitary issues, the National Environment Management Authority (NEMA) for environmental issues, the Public Health Department for food and feeds safety, the Kenyan Bureau of Standards, and the Department of Veterinary Services (DVS) for animal related issues.

Applications for GE crops

Applications for approval to import or use GE crops follow a three-tier process. The initial application is made to the institutional biosafety committee (IBC) of the institute where the application is being prepared, through a peer review process. The IBC appoints 2-3 reviewers to make recommendations, and the applicant may be asked to appear before IBC to defend the application. The IBC also employs the services of the Kenya Standing Technical Committee on Exports and Imports (KSCTCIE) to inspect any special facilities which may be required for the proposed introduction and research. Changes may be made before the application is forwarded to the NBC.

The NBC is comprised of about 20 representatives from as many institutions who review applications that have been already approved from recognized government research bodies such as KARI's Institutional Biosafety Committee (IBC). If approval is granted, the NBC requests the relevant regulatory institution (KEPHIS for GE crops and DVS for animals) to develop conditions under which the research will be conducted and to prepare compliance documents before granting a permit to import or commence the proposed research. The relevant institution then follows the research project to ensure compliance within set conditions.

Several applications for GE crops have now been approved, including transgenic sweet potatoes resistant to feathery mottle virus disease, Bt cotton for insect pest control, Bt maize for stem borer control, transgenic cassava to control the cassava mosaic disease, and a rinderpest vaccine to eradicate rinderpest disease in animals.

Applications for Bt maize

The IRMA project, over the last five years, has made four applications for importing and use of Bt maize (Table 1). The first application, in February 2000, involved the import of maize leaves to screen different Bt events for effectiveness against Kenyan stem borers. It was followed, in February 2002, by an application to import leaves with cross combinations of Bt events. In 2003, an application for the import of maize seed was approved for testing in a biosafety greenhouse. Finally, in 2005, the first use of Bt maize in the field, under open quarantine conditions, was approved.

The history of the project's applications shows that Bt maize seed is now available in Kenya. Most importantly, the required time for the processing of applications has shortened substantially. While the first applications took almost one year, the last two applications were approved in progressively shorter time ranging from nine to three months (Table 1). This was attributable to several factors. First, the KARI IBCs and NBC have established a structured and systematic ways to review applications. Secondly, the effects of the extensive trainings yielded fruit as the regulatory systems get to know and understand biotechnology.

Contributions to the regulatory process

The IRMA project has contributed to the development of the regulatory process in more than one way. The most direct has been training of regulators from KEPHIS and NBC through formal courses and visits to research sites in Kenya, Mexico and the USA. Second, the availability of a product through which applications has offered opportunities for interaction with NBC and IBC on Bt maize dossiers. IRMA scientists have also participated in the development of the biotechnology and biosafety policy and bill. The results as noted above have been shortening of time between application and decisions, increased science-based decision making, improved communication among stakeholders, and an increase in the number of applications.

However, there are still outstanding issues. First, the decision time is not as rapid as desired. Second, there have been cases of conditions becoming stricter, and non-science based decisions influencing the process (e.g. requirements of one year post-harvest monitoring

Table 1. Regulatory processes for Bt maize in Kenya.

Period	Activity	Time from application to approval (Months)
Feb. 2000	1 st application, "Application to introduce Bt maize leaves from first generation CIMMYT events to screening cry proteins using leaf bioassays for activity against Kenyan maize stem borers" submitted to NBC	
Jan. 2001	Approval for the first application "Application to introduce Bt maize leaves from first generation..." granted by the NBC. The Bt maize leaves from seven CIMMYT's first generation Bt maize events were imported and leaf bioassays performed and the effective genes against Kenyan stem borers identified.	12
March. 2002	2 nd application: "Application for an import permit to introduce Bt maize leaves from cross combinations of CIMMYT first generation Bt maize events to screen for cry proteins for activity against Kenyan maize stem borers using leaf bioassays" submitted to the NBC	
Dec. 2002	Approval of the 2 nd "Application for an import permit to introduce Bt maize leaves from cross combinations CIMMYT first generation Bt maize events to screen for cry proteins for activity against Kenyan maize stem borers using leaf bioassays" granted by NBC. Leaves from seven Bt maize straight events and 10 of their cross combinations were imported, bioassays done, and effective cross combinations identified.	9
April 2003	3 rd application "Application to introduce maize seeds containing nine second generation Bt maize events from genes <i>cry1Ab</i> and <i>cry1Ba</i> for evaluation, seed increase and crossing into other maize lines under biosafety greenhouse containment" made to the KARI IBC.	
Sept. 2003	Approval for the 3 rd application "Application to introduce maize seeds containing nine second generation Bt maize events from genes <i>cry1Ab</i> and <i>cry1Ba</i> for evaluation, seed increase and crossing into other maize lines under biosafety greenhouse containment" approved by NBC. The seeds were imported and evaluated in the biosafety level 2 greenhouse complex.	5
Dec. 2004	4th application "Application for Field Evaluation, Leaf Bioassays, Seed Increase and Backcrossing Maize Containing the <i>cry1Ab</i> or <i>cry1Ba</i> (Bt) Genes Under Confinement in the open quarantine site (OQS) at Kiboko" made to KARI IBC.	
Feb. 2005	Approval for 4th application "Application for Field Evaluation, Leaf Bioassays, Seed Increase and Backcrossing ..." granted by NBC.	3

for maize volunteers). Dispersal of maize seed far from mother plant is not possible without human or animals, and maize is a very poor competitor with weeds and therefore cannot survive through a season without human interception. Since maize seed does not have dormancy period, all seeds would germinate after the first irrigation and no more volunteers would be expected beyond two weeks of sustained irrigation. We hope that the requirement of one year monitoring will be reduced.

Intellectual property rights

Before engaging in a new technology, it is important to analyze the Intellectual Property Rights (IPRs) that are involved. IPRs are designed to protect one's investment into intellectual property and the products that are derived from these advances so as to provide economic returns to research to stimulate additional investment in research and product development. They usually increase the cost of using the technology, commonly referred to as 'technology fees' that not only cover

development costs but also the costs associated with defending IP claims. To ensure that the Bt technology developed will be accessible to farmers without being prohibitively expensive, the IRMA project commissioned a review of IPRs, including a Freedom to Operate (FTO) review. The study concluded that no patents had been filed in Kenya concerning the Bt technology, and therefore no patent restrictions are expected in Kenya (SwiftReviews, 2001). Moreover, when a company files for a patent in one country, it has a limited time (one year from the time of filing in the US) to file the patent in another country that is a member of the "Paris Convention". A tool to reduce the complexity of foreign filing issues is another treaty called the Patent Cooperation Treaty (PCT). Under this treaty, most members of the Paris Convention have agreed that the filing of a single English-language PCT application in the US Patent and Trademark Office, will be sufficient as a filing in other designated countries which must be filed within one year of the US filing date. Since all patented technologies under consideration by the IRMA project have been patented for more than one year, these

patents can no longer be filed in Kenya.

Even though there are no patent restrictions, other agreements might apply. Most importantly, when CIMMYT obtained Bt genes and constructs from different collaborators, in particular the University of Ottawa, it signed a material transfer agreement which stipulates for “research purposes only”. Problems might arise if IRMA uses these constructs to develop varieties, and wants to license Bt lines or varieties to commercial companies for seed production. A request was made to the University of Ottawa to provide an agreement for commercial use of the genes and constructs that have been used by CIMMYT. However, given the complexity of different IPR components belonging to different companies, it remains unclear if the University of Ottawa can provide an agreement for commercial use. CIMMYT has since approached all the major seed companies (Monsanto, Syngenta, and DuPont) with a letter of intent to ensure that the act of CIMMYT releasing these constructs to Kenyan farmers through the different national seed businesses will not result in litigation against the University of Ottawa, CIMMYT or the national seed producer.

As a result of these developments, IRMA will be able to provide the technology at low cost to local seed companies (De Groote et al., 2004a). Moreover, the gene is dominant and can be recycled by farmers. However, the use of Bt genes in farmer-saved seed is not allowed in many countries. Therefore, this issue needs further attention to ensure that seed recycling is allowed within the licensing agreement and that farmers are provided with the appropriate information to promote product stewardship such as maintaining a refugia and managing their maize so as to maintain the efficacy of Bt maize for the future generation.

PRODUCT DEVELOPMENT

Transformation and obtaining genes

With the advent of genetic engineering, genes that confer resistance to pest organisms have been inserted into various crop plants (Bennett, 1994; USDA, 1995). Among the biological pesticides, bacteria have been the most successful group of organisms identified as a source of biological insecticide for commercial crops. The best example comes from the soil bacterium, *Bacillus thuringiensis* (Bt) (Gill et al., 1992; Charles et al., 1996). Insecticidal crystal proteins, called δ -endotoxins, produced by Bt are highly toxic to specific pests; yet cause no harm to humans, to animals, or other non-target organisms such as beneficial insects (Croft, 1990). After being activated by midgut proteases, the Bt proteins bind to epithelial brush border membrane vesicles, creating pores that result in cell lyses (Gill et al., 1992). Incorporation of genes encoding δ -endotoxins

into maize has provided astonishing levels of resistance to insect pests. Bt transgenic plants containing insecticidal proteins have featured prominently in agricultural systems in both developed and developing countries (James, 2004).

CIMMYT acquired Bt genes from the private and public sectors, and has also synthesized other Bt-genes with partners. Various Bt cry genes (*cry1Ab*, *cry1Ac*, *cry1B*, *cry1E*, *cry1Ca*, and *cry2Aa*) have been used to develop constructs carrying the maize ubiquitin and rice actin promoters. *cry1Ab* was obtained from the University of Ottawa in Canada, *cry1Ba* and *cry1Ca* were synthesized by CIRAD in France, while *cry2Aa* was obtained elsewhere in Canada. These constructs have been used to transform embryos from a CIMMYT maize hybrid (CML216x CML72), thereby developing various Bt maize events. Backcrosses were made to CML216 to develop an inbred line carrier of the Bt genes, resulting in a number of useful Bt maize events, and the lines have shown high levels of resistance to pyralid stem borers such as the spotted stem borer *Chilos partelus* Swin. As the transformation program at CIMMYT matured and in light of public concern regarding the use of selectable markers, such as herbicide or antibiotic resistance to assist in the identification of events, CIMMYT has strived to develop “clean events” that do not carry the selectable Basta herbicide resistance (the bar gene) marker, thus addressing some of the concerns raised earlier about this technology (KARI and CIMMYT, 2001).

Identification of efficient genes through bioassays on imported leaves

Efforts were made to identify Bt- genes and their cry protein products that are effective against each of the target stem borer species. Insect bioassays of maize leaves containing the different cry genes were conducted. Given the early state of biosafety in Kenya and the lack of proper infrastructure in KARI to handle transgenic maize in the green house and the field at the time, Bt maize leaves that were grown in CIMMYT's biosafety greenhouses in Mexico were imported into Kenya and leaf bioassays performed in the KARI Biotechnology Center (Mugo et al., 2004). To carry out these bioassays, a specially biosafety level 2 laboratory was built and approved by the KSTCIE in 2001. Leaf bioassays were first carried out using first generation Bt maize events, i.e. Bt maize events that carried the bar gene as a selectable marker, to identify the events (and genes), to quantify their efficacy against the five major Kenyan stem borers: spotted stem borer (*C. partellus*), coastal stem borer (*Chilo orichalcociliellus* Strand), African stem borer (*Busseola fusca* Fuller), African sugarcane borer (*Eldana saccharina* Walker), and the pink stem borer (*Sesamia calamistis* Hampson). Cry1Ab protein was the most active against all species as

demonstrated by the least area consumed and the highest larval mortality (KARI and CIMMYT, 2002; Mugo et al., 2004). *C. partellus* was controlled by all cry events, except cry1E. Cry1E protein was not effective against any stem borer species. None of the events provided complete control of *B. fusca*. These results indicated the specificity of Bt toxins even among lepidopteran stem borers. These results also showed that the most aggressive and widely distributed borer, *C. partellus*, could be controlled with Bt events within the public domain but additional tests would be required to identify an effective gene for *B. fusca*, the pest that causes the greatest economic loss within Kenya.

To test if combinations of different Bt cry toxins would control *B. fusca*, a second set of Bt maize leaves was introduced, carrying both single gene events and cross combinations of these events (KARI and CIMMYT, 2003; Mugo et al., 2002). Bioassays were carried out to evaluate the efficacy of two-gene combinations in controlling the five major stem borer species, using maize tissue with seven straight Bt genes maize and 10 two-gene crosses. The results indicated that combining either the *cry1Ac* or *cry1Ba* gene to *cry1Ab* (from Novartis's Event 176) enhanced the level of control for *B. fusca* while remaining effective against *C. partellus* and the other species. This is likely due to either more total Bt protein being produced and/or complementarities of the two proteins. Though complete control of *B. fusca* was not achieved, the results indicated that two-gene combinations could be useful in development of Bt maize varieties that would offer complete control of the major stem borer species. This approach of complete control is a key assumption of the high-dose resistance management strategy in which all the stem borer larvae that do not contain resistance alleles will be completely killed by these Bt gene(s). However, the use of two genes in a single variety requires individual regulatory dossier to be prepared for each event as well as for the binary (combined) product, thus complicating the regulatory processes by requiring more information for the regulatory dossier, and increasing the cost to commercialize the final product.

Confirming efficacy in potted plants in the biosafety greenhouse

After the bioassays, the next logical step was to test the genes and constructs in live plants. Therefore, a special biosafety level 2 greenhouse complex (BGHC) was constructed. Important biosafety features of the BGHC were pollen screens, soil traps, restricted access with 24-h security, double door system into greenhouses, a sterilizer for soil and plant material, and a disposal system for plant materials. The BGHC was constructed and approved by KSCTCIE in 2004.

Evaluations in the BGHC have demonstrated the

efficacy of the Bt toxins from *cry1Ab* and *cry1Ba* when compared with the non-transformed CML 216 (Figure 1). All events showed very low leaf damage scores of one (completely resistant) when compared with CML216 which had a damage rating of over 4 (on a 1 to 9 scale, with 9 being highly susceptible) after one week of feeding by first instar larvae of *C. partellus* that were placed on plants at the 5-leaf stage of development.

These bioassays on cut leaves and on seedlings in the BGHC show that Bt maize technology is effective against Kenyan stem borers. However, tests remained to be performed on the stem borer species under field conditions in the major maize growing areas of Kenya.

Testing under field conditions in the open quarantine site

To allow trials under confined conditions, a special open quarantine site (OQS) was built on the KARI station in Kiboko. Particular features of the OQS are location at more than 400 m away from other maize growing plots, secure fencing to prevent entry by animals, locked gates and 24-h security system to restrict unauthorized access by humans, an irrigation system to allow crop evaluations through the year, and a team of trained staff to manage the facility. A confined field trial was initiated in May 2005 to test the efficacy of nine Bt maize events carrying *cry1Ab* and *cry1Ba* Bt genes against major stem borers in Kenya. Plants were infested with black head eggs of *C. partellus* at the four-leaf stage of growth by placing the egg mass in the plant funnel. Stem borer damage was assessed two weeks after infestation from each plant on a 1-9 scale (1 least damage to 9 most damage). Results showed that control was found for *C. partellus* (Figure 2). It is not possible to obtain meaningful data after infesting maize with more than one stem borer species. Therefore, leaf bioassays were used to test for efficacy against the other species: *B. fusca*, *E. saccharina*, and *S. calamistis*.

Leaves harvested from each of the representative plants in the OQS were transported by scientists and regulators observing biosafety for bioassays in the BGHC in Nairobi. Bioassays were performed with larvae from the three stem borer species following protocols previously described (Mugo et al., 2004). Large leaf area consumed and the low larval mortality rated observed for *B. fusca* point to very low resistance among the Bt maize events against this pest. However, *E. saccharina* and *S. calamistis* were well controlled by both *cry1Ab* and *cry1Ba* delta-endotoxins from the Bt maize events tested here (Figure 3). Therefore, tests using the leaf damage scores on plants after field infestations with *C. partellus* and from leaf bioassays with the three other pests in the biosafety greenhouse complex indicate that control was found for *C. partellus*, *E. saccharina* and *S. calamistis* but *B. fusca* was not controlled. Additional Bt genes or events will need to be sought and tested for effective

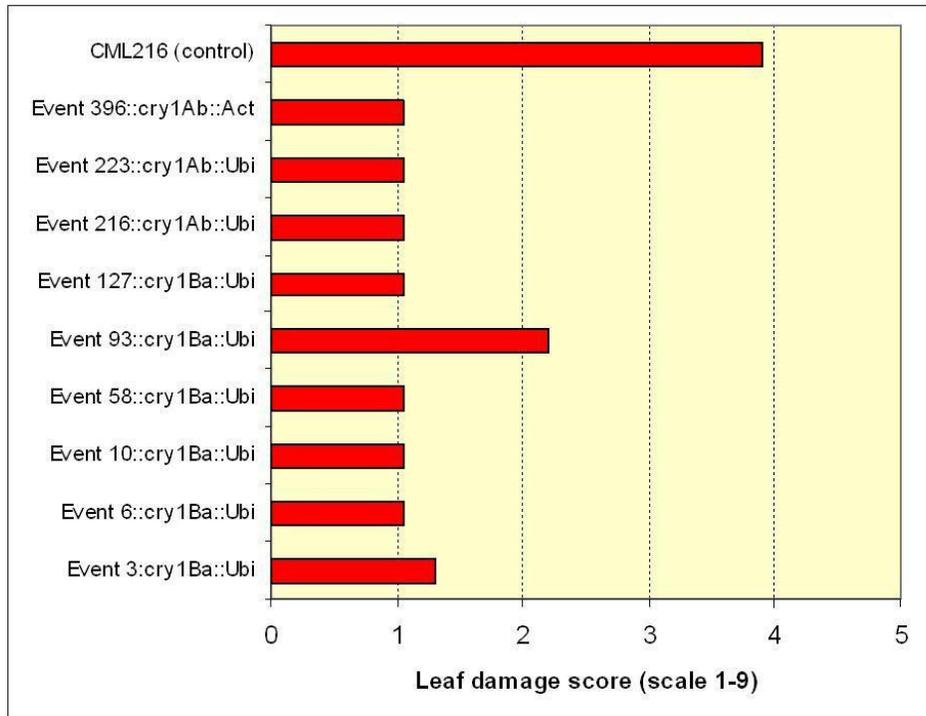


Figure 1. Leaf damage scores from spotted stem borer (*Chilo partellus*) for Bt maize events and a non-Bt control in potted plants at the biosafety greenhouse five days after infestation in 2004.

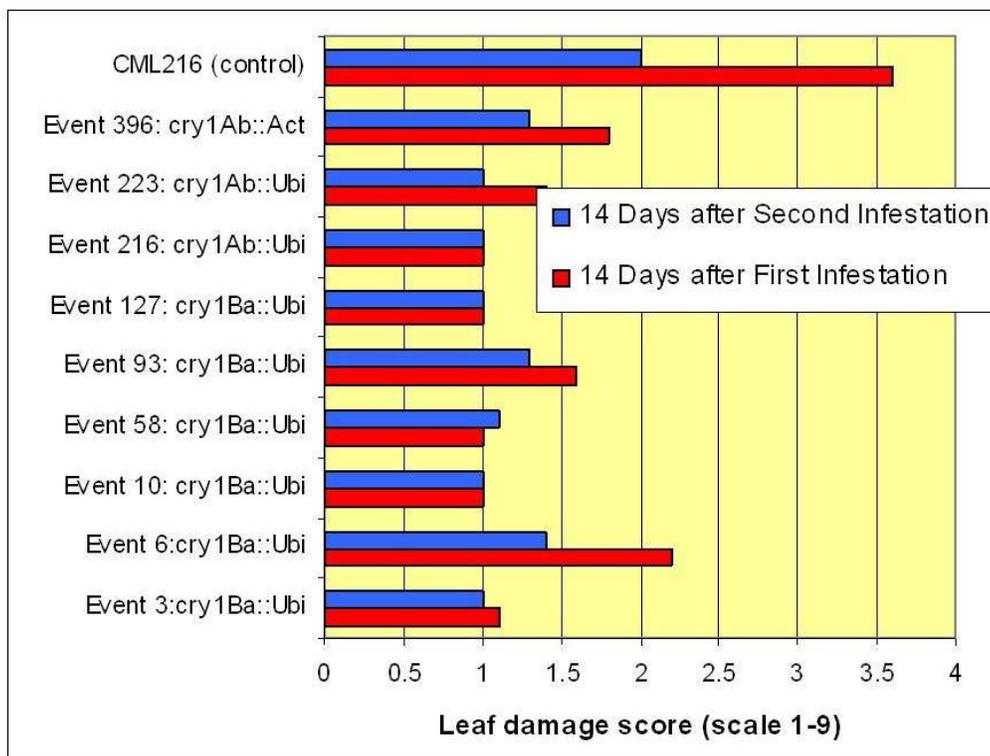


Figure 2. Leaf damage scores 14 days after the first and 14 days after the second infestation with *Chilo partellus* at the Bt Maize confined field trial at KARI CFT at Kiboko during 2005.

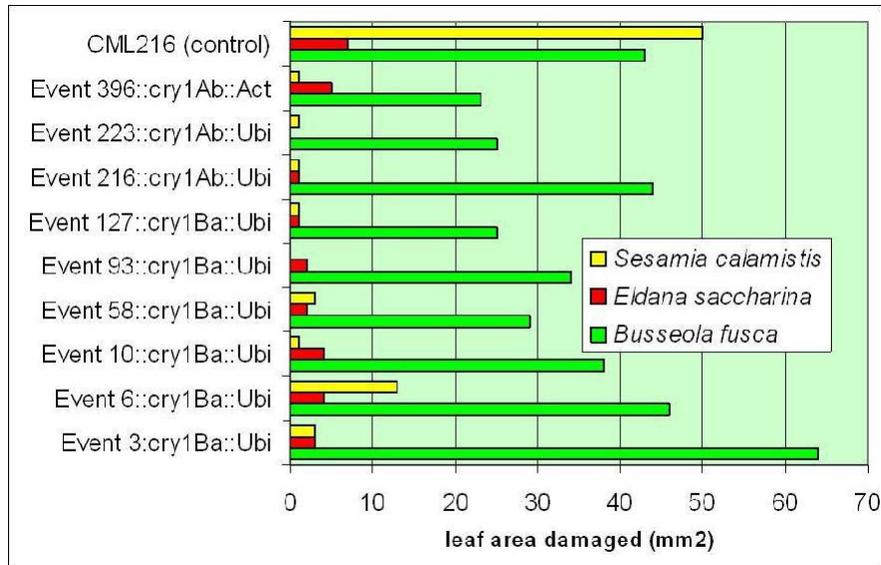


Figure 3. Leaf area consumed by three stem borer species after feeding on maize leaves for five days.

stem borer control in all maize growing ecologies in Kenya.

Variety development

A breeding program to develop Bt maize cultivars was initiated in the BGHC with the development of 20 backcross hybrids (BC0F1s) in early 2005 involving five Bt maize events, six maize inbred lines and two maize open pollinated varieties (OPVs). BC0F2s of these crosses are being developed at the OQS at KARI Kiboko. BC0F1 of three events and involving an additional nine maize inbred lines are being developed in the BGHC. Conversion will follow the backcrossing to the BC3 generation, and it is planned that lines coming from this work will cover the major maize growing areas in Kenya. A supportive study for a master's degree thesis research to investigate as to whether expression of Bt toxins changes with generations of breeding.

Inbred lines, F1, F2 and F3 generations (i.e. various stages of hybrid recycling) of Bt x Bt, Bt x non-Bt and non-Bt x non-Bt plants will provide useful information for breeding programs involving Bt maize and to develop seed recycling strategies that maintain the efficacy of the Bt technology in recycled seeds.

IMPACT ASSESSMENT

Ex ante impact assessment

To assess if insect resistant maize varieties address a problem perceived by small-scale farmers, IRMA organized participatory rural appraisals (PRAs) in 43

villages spread over the different agro-ecological zones. More than 900 farmers participated in group discussions (De Groote et al., 2004b) (Figure 4). Constraints in maize production as expressed by the farmers differ substantially between zones. Pests problems rank high in the low-potential zones (between first and third constraint), but are only of medium importance in the high potential zones (ranking fifth or sixth), after cash constraints, lack of technical know-how and extension, and expensive seed, often hard to obtain and of poor quality. The two major pest problems maize farmers encounter are stem borers and weevils (storage pests), which rank in the top three in all the agroecological zones.

After establishing that stem borers are a major constraint to maize production, an attempt was made to quantify the crop losses they cause. Based on estimates obtained from 1400 farmers in a 1992 nation-wide survey, maize yield losses due to stem borers could be estimated at 12.9% of the potential yield (De Groote, 2002). Crop losses were also measured in 150 farmers' fields during four seasons starting in 2000, using a simple experimental design in which half of each field was protected with a systemic insecticide, and the other half was left unprotected (De Groote et al., 2004c). The weighted average yield loss for all zones was calculated at 13.5% of the potential, ranging from 11% in the highlands to 21% in the dry areas. Total losses were estimated at 0.41 million tons, valued at US\$ 79 million (2001 US\$).

These results were combined with data on stem borer species prevalence in a GIS based model to calculate losses per zone per species (Figure 5) (De Groote et al., 2003). The results show that three quarters of the losses

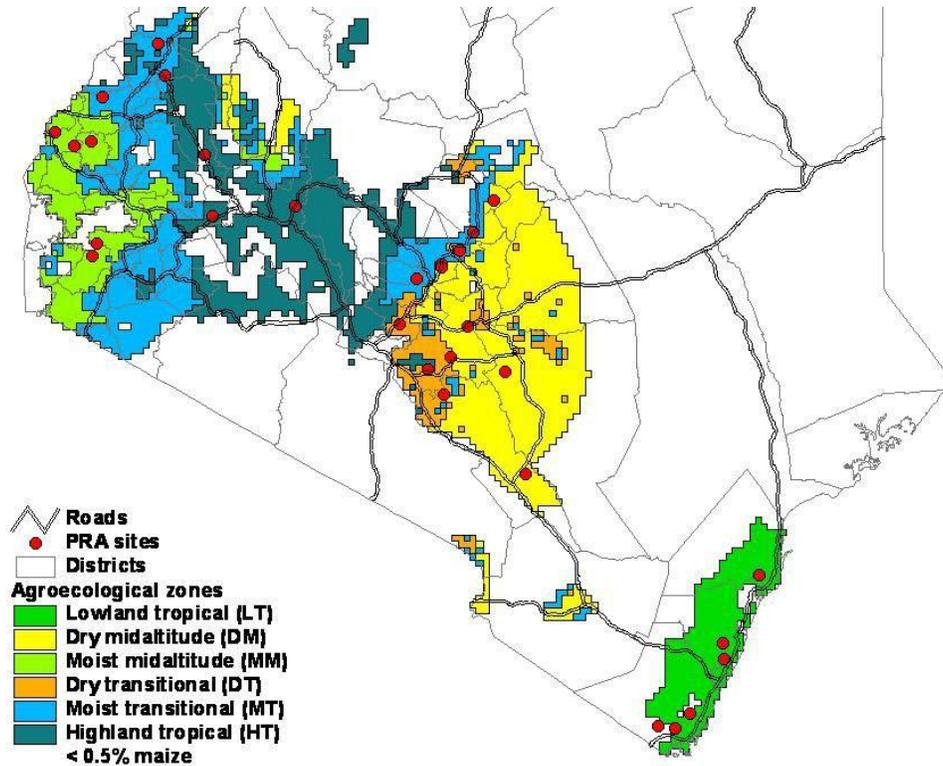


Figure 4. Maize agroecological zones and PRA sites.

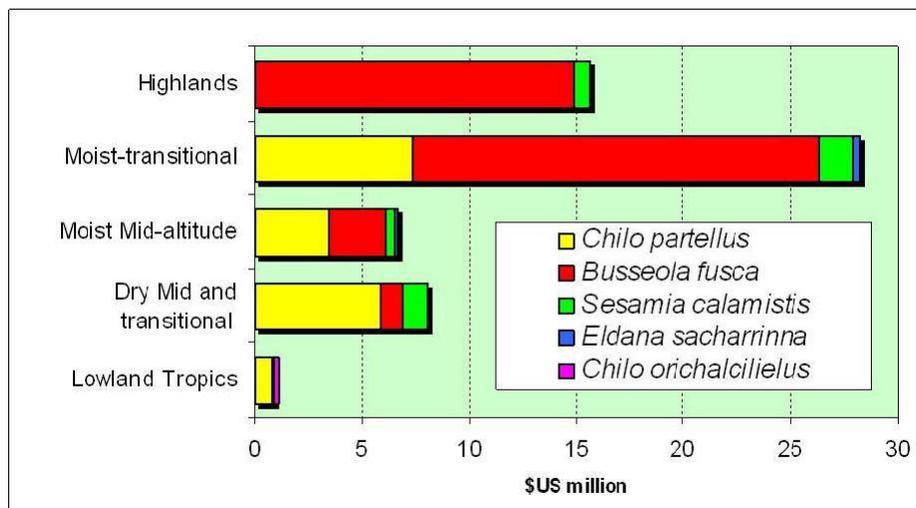


Figure 5. Value of crop losses from stem borers, by agroecological zone and species.

occur in the high-potential zones. Three species are responsible for 98.9% of the losses: *B. fusca* (63%, dominant in the highlands), *C. partellus* (29%, dominant in the lowlands), and *S. calamistis* (7%). These results were now used to evaluate two scenarios. First, assuming the new *Bt* maize varieties are efficient against all stem borers, and two-thirds of farmers who previously

adopted improved varieties will also adopt *Bt* maize varieties. Annual production is expected to increase by 250,000 ton (+9.4%), at a value of US\$ 48 million. Second, if no resistance against *B. fusca* is found, farmers in the high potential areas are unlikely to adopt the new varieties. In this scenario, production would only increase by 29,000 tons (+1.1%), valued at US\$ 5.4

million.

Total benefits of the projects can be calculated using the conventional economic surplus model, and compared to the project's costs, about US\$ 1 million per year over 10 years. In the first scenario, complete control of all stem borers, the yearly benefits reach \$49 million per year, of which two thirds go to the consumers. Discounted benefits over 25 years reach \$208 million, compared to discounted costs of \$6.76 million, or a very good benefit/cost ratio of 31. In this scenario, higher impact is expected from the high production moist-transitional zone. In the second scenario, with no resistance to *B. fusca*, the technology would only be effective in the low potential areas, and adoption rates would be fairly low. Yearly benefits only reach \$5 million, or \$24 million over 25 years, with a modest benefits/cost ratio of three, although these benefits would be concentrated in the low potential areas, and benefit the poorer farmers.

Impact on poverty

IRMA, as a public research project, is particularly concerned with its impact on poverty and livelihoods. The available evidence indicates that the technology is very likely to benefit the poor, either as deficit producers or consumers (De Groote and Mugo, 2005; De Groote and Mugo, 2005.). The major factors are that poor families spend more than half of their available income on maize, that stem borers are a real and serious problem in maize production, especially in the low-potential areas, and that effective Bt genes have been identified for stem borer control in those areas. However, to reach the larger group of poor households, genes which control *B. fusca* that attacks maize grown in the higher altitudes need to be found.

The characteristics of the technology, in particular the dominant expression of the Bt gene, make it especially attractive to both the poor farmers and the local seed industry. Farmers and seed companies are understandably eager to try and test the technology, and consumers and the general public are cautiously optimistic.

Insect resistance management

One concern of utilizing Bt maize technology is the likelihood of development of resistance to the Bt toxins by the target stem borer species. However, the rate of evolution of this resistance can be slowed or stopped through the use of appropriate resistance management strategies. To minimize the possibility of resistance development to Bt maize by Kenyan stem borers, the IRMA project is developing varieties of maize that carry multiple forms of resistance – both Bt-based and conventional resistance. In addition, resistance management strategies (IRM) are being developed, the primary strategy being providing refuge to stem borers by

ensuring that alternate host plants are in sufficient abundance to provide a toxin-free food source to ensure that “homozygous susceptible” moths (those whose progeny will be completely killed by the Bt variety) remain abundant within the maize cropping system. The “high-dose –refugia” management strategy requires that the Bt variety kills all susceptible moths and the few “heterozygous” (single copy of the resistance allele) moths that do emerge will mate with the susceptible moths that emerge in large numbers from nearby refugia crops. To be accepted by farmers, IRM strategies must conform to existing cropping systems, and the refugia crops must be economically viable and socially acceptable to farmers.

To select suitable crop species to be used as refugia, recommended forages, sorghum and maize varieties were evaluated for stem borer preference and survivorship in the field in four locations representing different agro-ecological zones in Kenya, over four seasons between 2000 and 2003. Results from field trials indicate higher borer damage rating and exit holes in all sorghum and maize varieties. Grass species with many exit holes included Sudan grass, Columbus grass, giant setaria and panicums. Laboratory bioassay for larval development rates and fecundity were conducted using four stem borer species: *C. partellus*, *B. fusca*, *S. calamistis* and *E. saccharina*. Differences between borer species were significant. In the case of *B. fusca*, the species for which resistance development is a major concern, the highest survivorship was observed on sorghums and maize and lowest in Napier grass. Egg production per female was highest in maize and lowest for Napier grass. The total borer life cycle was shorter on maize and longer on Napier grass (Table 2).

Vegetation surveys were conducted in major maize growing districts in Kenya to quantify the percent area covered by different natural refugia in order to estimate the availability of refugia in existing maize cropping systems. During this survey 850 farmers were interviewed with all interviews being geo-referenced and GIS maps on existing refugia generated for both cropping seasons. Kwale district at the coastal region of Kenya had maize equivalent refugia of 18%, a level comparable to the 20% recommended for commercial maize in the USA. However, the same region during the short rains has less than 10% refugia during the long rains. Some districts, such as Makeni district had less than 5% refugia in both cropping seasons. Such regions will require structured or augmented refugia to attain the 20% refugia. This is due largely to an almost exclusive planting of maize and very little area planted to alternate hosts, including sorghum.

To complement the researchers' efforts and increase the chances probability of responsible stewardship of the Bt, maize and refugia concept being accepted by the farmers, the KARI and CIMMYT scientists in the IRMA project have organized workshops to sensitize the

Table 2. Life cycle and reproductive potential for *Busseola fusca* and *Sesamia calamistis* reared on different classes of alternate hosts under laboratory conditions (Kitale, 2002).

Crop type	<i>Busseola fusca</i>			<i>Sesamia calamistis</i>		
	Life Cycle (days)	Percent Survival	Egg Production	Life Cycle (days)	Percent Survival	Egg Production
Napier Grass	64.5	2.8	5.0	60.9	3.3	93.0
Local Sorghum	60.3	37.8	184.8	56.5	13.3	67.0
Maize	53.2	18.5	246.6	51.7	27.5	629.3

farmers and extension discussed Bt maize, resistance management and the role of refugia within IRM input into the project. A group exercise was conducted to rank refugia species in experimental plots by farmers, extension agents and researchers based on their criteria. There were differences in the criteria and the ranking of the varieties for use as pastures and refugia. When all the criteria listed by the three groups were combined the most common criteria used by all was resistance to stem borers, alternative uses (food, pasture, refugia, hay) and the ability to attract and support stem borers. Farmers also mentioned availability of seed as important criteria which should not be ignored.

A study to screen for resistance development in *C. partellus* to Bt delta-endotoxins showed that no development of resistance to cry proteins over four generations of selection has occurred in *C. partellus* which indicates that field resistance can likely be managed for this species (Tende et al., 2005).

Non-target organisms

One of the concerns about utilization of Bt maize in reducing losses due to stem borers is the potential impacts on non-target arthropods. Therefore, the key non-target arthropods in major maize growing regions in the country need to be identified. However, arthropods found in a given maize habitat may be influenced by the prevailing environmental conditions, the maize cropping system, the varieties of maize and that of the association crops, and also by the crop husbandry practices used. IRMA, therefore, engaged in research to i) conduct on-farm surveys to understand the maize production systems, especially with respect to stem borer damage and management in each of the five major maize growing regions in Kenya; ii) identify and determine the relative abundance of the target and non-target arthropods of Bt maize in each of the five regions; iii) establish a reference collection of arthropods in the major maize cropping systems in Kenya; iv) determine the key non-target arthropods on which to focus the impact studies; and v) determine the impacts of Bt maize on major non-target arthropods in a biosafety green house and in a confined field.

On-farm surveys were conducted in each of five major maize growing regions including the lowland tropics (Kilifi), dry mid-altitudes (Machakos), moist mid-altitude (Embu) (Figure 4) and information collected on the major maize cropping systems, common varieties of maize and the association crops, and on the key crop management practices. On-farm studies were also conducted in all zones to identify the major target and non-target arthropods of Bt maize, through weekly monitoring with pitfall, water and sticky traps, and by destructive sampling of maize plants three times a season. The target arthropods (stem borers) in each of the regions in descending order of abundance were for the Kakamega: *B. fusca*, *C. partellus*, *S. calamistis* and *Cryptophlebia leucotreta*; in the Lowland Tropics at Kilifi they were: *C. partellus*, *C. orichalcociliellus*, *S. calamistis* and *Cr. Leucotreta*; at Kitale: *B. fusca* and *S. calamistis*; at Machakos: *C. partellus*, *S. calamistis*, *Cr. leucotreta* and *B. fusca*; and at Embu: *B. fusca*, *C. partellus*, *S. calamistis* and *Cr. leucotreta*. This information on the key stem borers in each region was essential for targeting of the Bt genes to be used in maize for each specific region.

Out of the wide range of arthropods recovered from farmers' maize fields in the different regions, five categories of non-target arthropods of interest were identified including non-target lepidopteran herbivores (*Helicoverpa armigera* and *Cryptophlebia leucotreta*), non-target non-lepidopteran herbivores (leafhoppers, crickets, aphids and *Prostephanus truncatus*), parasitoids (*Cotesia flavipes*, *C. sesamiae*, *Goniozus indica* and *Dentichasmias busseolae*), predators (ladybirds, earwigs, rove beetles and ants), and pollinators (honey bee *Aphis Melinifera*). The relative importance of the foregoing non-target arthropods varied among the different regions, with some of the arthropods being limited to certain regions. For example *G. indica* was limited to the Kenyan Coast, at Kilifi. An arthropod reference collection, comprising of voucher specimens of various specific identified arthropod groups, collected from farmers' maize fields in the five maize growing regions has been established (Songa et al., 2004). This collection will serve as technical reference during the monitoring phase in Bt maize fields. The next step is to conduct studies on non-target effects of Bt maize in the

green house and in confined fields. This information provides important baseline information on the current status of arthropods in farmers maize fields before the Bt maize is deployed in the fields. Preliminary controlled studies on the non-target effects of Bt maize will focus on the key arthropods in the respective regions. This information also serves as a bench mark against which comparisons will be made in determination of impacts of Bt maize on specific arthropod groups.

Since Bt maize was only allowed into Kenya for field trials in 2005, Bt's impact on non-target arthropods in maize was studied using Bt-sprays, thuricide (a Bt-biopesticide) in comparison with conventional insecticides (Dimethoate and Bulldock). The impacts of thuricide (a Bt-biopesticide) and conventional insecticides on the abundance of different non-target arthropods in a maize bean cropping system were determined. Results showed that both the bio-pesticide and the conventional insecticides were effective in reducing the stem borer damage in maize. However, the insecticides appeared to have more negative impacts on the non-target arthropod diversity (families) and abundance. The insecticides also had a greater negative impact on the stem borer parasitoid diversity and on some of the predator groups such as the ladybird beetles. Considering that thuricide and Bt maize have similar modes of action, the results of this study give an indication of the potential impact of Bt maize when compared to commonly used insecticides, on the target and non-target arthropods of Bt maize (Songa et al., 2002), this study will be repeated with Bt maize.

To study the potential effects of the Bt maize technology on target and non-target arthropods in maize-based cropping systems in Kenya, field collections of these organisms were established, using different trap types to determine the diversity and relative abundance of target and non-target organisms in the five major maize agroecologies. Bi-weekly collections from farmers' fields were made and all insects within each sample were classified to genus and a dry collection of specimens for each family of insects was established. In addition, a digital database for the specimens was developed, including a digital photograph and the coordinates of the location, to enable other researchers in other research stations to classify specimens in the future to at least the family level to facilitate monitoring document the impact of Bt maize on arthropod diversity and abundance. This information is now being used to (i) identify groups of arthropods that may be adversely affected by Bt maize, (ii) to quantify the impact of Bt maize on these non-target species in the greenhouse, prior to field testing, and (iii) serve as a baseline for impact assessment studies to ensure the technology is not adversely affecting arthropod diversity while at the same time providing stem borer control (Songa et al., 2002).

COMMUNICATION

Importance

From its initiation, IRMA project has recognized the need for effective communication to create public awareness. It is also important for education at various levels and creating public awareness. The major objectives of the communication work are to plan, monitor, and document processes and achievements for dissemination to the Kenyan public and developing countries. Public Awareness has been created through annual project stakeholders meetings since 2000, (Mugo et al 2002, 2002, and. Regular discussions with farmers, consumers and institutions during annual stakeholders meetings, group discussions and other forums, reveal that farmers are generally very enthusiastic about Bt maize, while scientists, consumers and the general audience are cautiously optimistic (Mugo et al., 2001; Mugo et al., 2004a). Interestingly, farmers requested that the project also consider transformation of their local varieties. These activities also show how small-scale farmers, consumers and the general public can be actively involved in the decision making process. Press events have been conducted and press releases made for major developments in the project. Scientists organized and participated in workshops to enhance the science/media interface. A quarterly newsletter was produced and distributed in hard copy and on the Internet. Kenyan print media monitored to follow trends in coverage and editorial positions on GM crops and the IRMA project. IRMA scientists regularly engage in broadcast and newspaper interviews. Networking has been maintained with organizations involved with GM products (ABSF, ISAAA, BTA). Videos have been produced on the project and its interaction with farmers in Kiboko. Seminars were conducted in five agro-ecological regions introducing approximately 120 extension officers to Bt technologies and related issues. Fact sheets on various issues have been developed and feedback from extension agents obtained. Posters have been developed for use in agriculture shows and other venues in production. A series of documents have been produced to capture the project's progress on a year-by-year basis.

Consumer surveys

Given the sensitivity of GE technology, it is important to gauge the awareness and attitudes of farmers, but also consumers. During the PRAs, in 2000, we observed little awareness among the farmers. At the end of 2003, however, we conducted a survey of 600 consumers in Nairobi, and found that almost half of the respondents (38%) were aware of GM crops, and more so in the high-income groups (Kimenju et al., 2005). Main sources of that information were the media, in particular

newspapers, followed by schools. Newspapers are more important to high-income and more educated consumers, but radio is most important to the low-income and less-educated groups. Consumers appreciate the technology's potential positive impacts, with more than 80% agreeing that it increases productivity. Most respondents (68%) would buy GM maize meal at the same price as their favorite brands, although many are concerned with environmental and health risks, and with ethical and equity issues. Consumers fear that GM crops technology can lead to a loss of traditional maize varieties (50% of respondents), and affect untargeted insects (51%). Some fear that consumption of GM foods can damage one's health (37%) or cause allergic reactions (40%). These results indicate that more effort is needed to further inform consumers about the technology.

Training

The capacity of Kenyans to research on genetic engineering has been enhanced through extensive training of staff from KARI, ministry of agriculture and KEPHIS on biotechnology, biosafety, management of biosafety facilities, and regulatory issues. Hands on training have been emphasized in Mexico and in Kenya. Training has been extended to scientists, and extension officers. Infrastructure has also been developed including a biosafety level 2 laboratory, a biosafety level 2 greenhouse complex, both at KARI-NARL Kabete, and an Open Quarantine Site OQS at KARI-Kiboko.

Distribution of the results

For a project using sensitive technology it is important to document and distribute the results rapidly. Therefore, IRMA collaborators are very active in national, regional and international conference to present our results.

PLANNING FOR THE FUTURE

Given the long and costly procedure to bring GE maize to the farmers' fields, careful planning is necessary, and scenarios need to be developed and adjusted based on the available information. As KARI and CIMMYT developed a business plan in 2003 to clearly define the various steps and partnerships required for the delivery of Bt maize, it became apparent that contingency plans also needed to be made to ensure that a viable product reached Kenyan farmers in a reasonable timeframe. The two major drivers that will impact on the delivery of Bt maize will be its efficacy against *B. fusca* and the ability of KARI, CIMMYT and its partners to have freedom to operate using the construct CIMMYT has used to

develop Bt varieties to date. It was felt that while the business plan that the IRMA project had developed for its second phase to deliver Bt maize varieties to the commercial seed sector and farmers represented our "Plan A". Alternate strategies should be discussed in the event that this technology does not provide adequate control of *B. fusca* or is not available for commercial release due to restrictions imposed by IPR.

Maize varieties with conventional resistance have been and continue to be developed and will not be discussed further apart from the possible option of pyramiding conventional resistance with Bt maize in order to deliver maize varieties resistant to *B. fusca* that combine single gene (Bt) and quantitative (conventionally breed maize varieties) resistance so that more than one mortality mechanism is employed is a resistant variety with the prospect of being less prone to developing insect resistant populations.

Our Plan "A" would be the release of a cry1Ab event, with event 216 being our lead event which contains Ubi-cry1Ab-nos with no selectable marker. Recent field tests showed that the event provides complete control of *C. partellus* but does not provide control of *B. fusca*. This has now taken us to our next option (Plan 'B') will be to stack event 216 with and event 127 (Ubi-cry1Ba-nos) that also controls *C. partellus* but on its own was less effective against *B. fusca* than cry1Ab. Crosses are now being made to stack these two events for further testing against *B. fusca*. Should this provide effective control of *B. fusca* the development of a regulatory dossier with two events stacked into one variety would require almost three times the work and expense of preparing a dossier compared to a single gene event.

Our Plan 'C' would be to work with the private sector towards the release of existing commercial events in Kenya, with KARI and CIMMYT providing the technical support for dossier development, monitoring and developing stewardship strategies such as IRM strategies for small-scale farmers. Of the commercial events that are currently used, Mon810 (35S-cry1Ab-nos) has already demonstrated good levels of field resistance to *B. fusca* in commercial maize varieties planted in South Africa; however, the efficacy of this event against *B. fusca* drops dramatically as the maize plant approaches physiological maturity. Dow AgroSciences has developed a commercial event called Herculex I (cry1Fa) that in addition to stem borers also controls pests such as cutworms and armyworms, pests that belong to the same insect family as *B. fusca*.

Given the difficulty the IRMA project has had in identifying an effective gene construct against *B. fusca*, it will be in the best interests of both the private and public sectors to ensure the few effective events that exist against *B. fusca* remain effective for future generations of maize farmers in Africa. One of the important roles for research centers, such as KARI and CIMMYT, will be to generate information and protocols that will enhance GM

product stewardship and develop educational material for both farmers and extension providers so they have the tools in hand to ensure that Bt maize varieties remain effective for both small-scale and commercial farmers.

CONCLUSIONS

Over its first six years, the IRMA project managed to develop facilities, submitted four applications and obtained permits to import and test Bt maize from cut leaves in a biosafety laboratory, as whole potted plants in a biosafety greenhouse, and as whole plants under field conditions in confined field trials in an open quarantine field site. The research identified efficient genes and events against four out of the five major stem borer species in Kenya, in particular *C. partellus*, *C. Orichalcociliellus*, *E. saccharina* and *S. calamistis*.

Economic impact assessment demonstrated that stem borers are a top constraints and cause major losses to small-scale farmers. Resistant maize varieties are likely to be widely adopted, and providing major returns to the research investment if resistance against the economically most important species, *B. fusca*, can be found. In the other case, returns would still be positive, but not large.

Environmental impact research indicate that build-up of resistance against the Bt genes is slow, that Bt maize is likely to be less harmful to non-target organisms than conventional insecticides, and that sufficient natural refugia exist in most areas. However, the areas where refugias are not sufficient need to be clearly mapped, and strategies developed that are acceptable to farmers.

Finally, IRMA's research and activities has demonstrated that farmers, consumers and other stakeholders are cautiously optimistic about technology. While concerns exist, and need to be addressed, the frequent interaction with the stakeholders and regulatory agencies assures a participative decision-making process and compliance with the strictest scientific and regulatory standards.

A communication strategy has served to raise awareness about biotechnology in general and Bt maize in particular, while Kenya scientists in KARI and other government bodies have received extensive training.

The project is, of course, not without its problems. The results, while encouraging, came slower than anticipated. Moreover, while control of most stem borers was achieved, it was not realized for the an economically important stem borer, *B. fusca*. Finally, the IPR issues were not as clear as initially expected. These delays increased the cost of the project and the time it will take to bring the products to the farmers. However, different scenarios were developed to address these concerns. Our research results indicate that the goal of the project, bringing insect resistant varieties to small-scale farmers, can be achieved within a reasonable time frame.

ACKNOWLEDGMENTS

The authors would like to thank the Syngenta Foundation for Sustainable Development and the Rockefeller Foundation for their financial support. We highly appreciate the work of our technicians in the laboratory, the biosafety greenhouse and the open quarantine station. This work would not have been possible without the help of our scientific collaborators of KARI, CIMMYT, the Nairobi University and Egerton University. We appreciate the effort of the regulatory agencies, in particular KEPHIS, the KARI IBC and the NBC, in studying and approving our applications. Finally, we highly appreciate the numerous farmers, consumers and other stakeholders who took the time to answer our questions, evaluated our trials and contributed their opinion to the decision making process in developing this new and exciting technology.

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