African Journal of Biotechnology Vol. 10(23), pp. 4682-4693, 1 June, 2011
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 ©2011 Academic Journals

Review

Experiences with the biosafety regulatory system in Kenya during the introduction, testing and development of Bt maize

Stephen Mugo[1]*, Simon Gichuki[2], Murenga Mwimali[2], CatherineTaracha[2], and Harrison Macharia[3]

[1]International Maize and Wheat Improvement Center (CIMMYT), P.O. Box 1041 - 00621, Nairobi, Kenya.
[2]Kenya Agricultural Research Institute (KARI) Biotechnology Centre, P.O. Box 57811 - 00200, Kenya.
[3]National Biosafety Authority, P.O. Box 30623 Nairobi, Kenya.

Accepted 10 January, 2011

The introduction of genetically modified (GM) crops in Kenya is governed by a biotechnology policy, a biosafety law, and a biotechnology awareness strategy to enable research and development of GM crops. In addition, Kenya has the National Biosafety Authority (NBA) that regulates plant biosafety through technical institutions including the Kenya Plant Health Inspectorate Services (KEPHIS). *Bacillus thuringiensis* (Bt) maize for stem borer pest control was imported and tested under this regulatory system. The Insect Resistant Maize for Africa (IRMA) project aimed at developing and deploying transgenic Bt maize for stem borer control in Kenya. The IRMA project developed and implemented an internal biosafety strategy. To comply with and implement the biosafety regulations, IRMA set-up biosafety facilities to develop, maintain and test the Bt maize. These included a biosafety level 2 laboratory, a biosafety level 2 greenhouse containment and a confined field trial site, which also provided valuable models to national and international interests. IRMA also developed protocols for the use and disposal of transgenic plant materials, and also generated data for guidance on post-harvest monitoring at Bt-maize trial sites. IRMA also trained Kenyan administrators, scientists, technicians and regulators on conducting and handling GM trials. Training included informal courses, seminars, scientists' visits to established institutes, and support to research for degree related training. The project, therefore, provided major inputs in the development and advancement of biotechnology framework in Kenya. This paper reports on the achievements realized, challenges encountered by IRMA, and lessons learnt in research and development of GM crops in Kenya.

Key words: Regulatory, biosafety facilities, *Bacillus thuringiensis* (Bt) transgenic maize, Kenya, genetically modified (GM) crops, biosafety waste disposal.

REGULATING BIOTECHNOLOGY – THE KENYAN STRATEGY

Modern biotechnology provides important tools for crop, and livestock improvement and novel uses of plants, animals and micro-organisms. This includes plant transformation technology, which has become an adaptable platform for cultivar improvement as well as for studying gene functions in plants. Bio-safety regulatory frameworks are intended to serve as mechanisms for ensuring safe use of biotechnology products without risk to human health, environment, or unintended constraints to technology transfer. Kenya is a signatory of the Convention on Biological Diversity (CBD) which was implemented in

*Corresponding author. E-mail: smugo@cgiar.org. Tel.: +254 (020) 722 4610, or +254 733 720 297, or +254 723 621 909.

1993. The key obligations of parties to the CBD included the establishment of a competent authority and a focal point.

The focal point includes a policy on biotechnology and biosafety, a biosafety law, a system for handling requests or notifications, a system for monitoring and enforcement, and a mechanism for public awareness, education and participation. The Kenyan Biosafety Framework includes the Policy on Biotechnology adopted by the cabinet in 2006, the Biosafety Law 2009, a system for monitoring and enforcement developed by National Council for Science and Technology (NCST) in 1998 (NCST, 1998). The National Biotechnology Awareness Strategy (Bio AWARE) developed in 2008 by the Ministry of Agriculture is a mechanisms for public awareness, education and participation (Republic of Kenya, 2008). The enactment of the Biosafety Law in 2009 (Government of Kenya, 2009) enabled the establishment of the National Biosafety Authority (NBA) in 2010. However, by February 2011, the eight implementing regulations stipulated in the Biosafety Act required to get the Act in use had not yet been gazetted.

The many steps in the regulatory process for the *Bacillus thuringiensis* (Bt) maize GM trials in Kenya has been reported (Falck Zepeda and Zambrano, 2009; Mugo et al., 2005). The research pro-posals are developed by a multidisciplinary team of scientists and forwarded to the Kenya Agricultural Research Institute's (KARI) Institutional Biosafety Committee (IBC). Generally, the IBC reviews the application and requests Kenya Plant Health Inspectorate Service (KEPHIS) to inspect the target experimental facility through the Kenya Standing Technical Committee on Imports and Exports (KSTCIE). The IBC may review the application with or without appearance by the applicants. The IBC approves or disapproves the application and forwards it to the NBA. The NBA may also review with or without the applicant, and may request KEPHIS to inspect the facilities. Once the NBC approves the applicant, KEPHIS indicates the conditions included in a compliance document to KARI. Upon satisfaction with the compliance document, KEPHIS issues a letter of authorization and an import permit, if necessary. The applicant then signs a letter of commitment to KEPHIS. Research commences under the supervision of KEPHIS and using standard operating procedures (SOPs) issued by KEPHIS. After research is concluded, a report is written to the NBA.

The IRMA project presented four applications all of which were approved (Mugo et al., 2005), Mugo et al., 2010a, b). These were: 1) "Application to introduce Bt maize leaves from first generation International Maize and Wheat Improvement Center (CIMMYT) events to screening Bt δ-endotoxins using leaf bioassays for activity against Kenyan maize stem borers; 2) 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 Bt δ-endotoxins for activity against Kenyan maize stem borers using leaf bioassays; 3) application to introduce maize seeds containing nine second generation Bt maize events from genes c*ry1Ab* and *cry1Ba* for evaluation, seed increase and crossing into other maize lines under biosafety greenhouse containment, and 4) application to introduce maize Hybrid DKC8073YG and DKC8053YG (with Bt event MON 810 containing *Cry1Ab* gene) to carry out greenhouse containment trials to evaluate the efficacy of the Bt δ-endotoxins against major maize stem borers in Kenya.

COMPLIANCE ON BIOSAFETY REQUIREMENTS IN THE IRMA PROJECT

The main regulatory milestones relate to a number of applications and necessary approvals made by the NBC. These included approval for growing Bt maize in the biosafety level 2 greenhouse complex at the National Agricultural Research Laboratories (NARL), Kabete; at the open quarantine sites (OQSs) at Kiboko, at KARI research stations and on-farm trials, and inclusion in the national performance trial (NPT) system of variety release. Finally, commercial seed production and distribution of Bt-maize to Kenyan farmers was to be approved. To achieve this, a multidisciplinary regulatory team was established, biosafety physical facilities and working protocols were developed. A comprehensive training for Kenyan scientists and regulators was done in compliance with the regulatory requirements.

ESTABLISHMENT OF APPROPRIATE BIOSAFETY FACILITIES FOR RESEARCH AND DEVELOPMENT OF BT MAIZE IN KENYA

The application of biotechnology and its wide range of tools pose new challenges to governments that require biosafety frameworks to be established for the safe and responsible use of the genetically modified organisms and their products. Biotechnology is a relatively new science and has raised more biosafety issues than other sciences. Biotechnology has also raised passionate debates due to concerns in health, biodiversity and the environment. This has forced countries to propose biosafety frameworks that require specific facilities for development, implementation and stewardship.

The Government of Kenya through the NCST developed and published the Guidelines for Biosafety in Biotechnology (NCST, 1998). The guidelines require that all institutions dealing with biosafety develop IBC. The KARI-IBC developed the KARI Guidelines and Regula-

tions for Biosafety in Biotechnology Research in 1994 (KARI, 1994). These biosafety guidelines outline the general biosafety policies and procedures of KARI to ensure the safety of research with genetically engineered organisms, their products, the personnel, agriculture, environment and biodiversity. As IRMA aimed at developing and deploying insect resistant maize developed using Bt technology, it was advised to develop facilities for physical containment. This refers to the use of specially constructed buildings, equipment and procedures to prevent the unintended transmission or escape of organisms covered by good practice codes or guidelines. Specific laboratory practices, containment equipment, and special laboratory design were made to achieve level 2 of physical containment. The facilities developed were a biosafety level 2 laboratory (BL2 Lab), a biosafety level 2 greenhouse complex (BGHC), and an open quarantine site (OQS). These were all developed following the NCST guidelines used in Kenya as well as other guidelines available internationally (Traynor et al., 2001).

Currently, there is neither a source on practical guidance on the management of biosafety greenhouses and laboratories containing transgenic plants nor the requirements for building or renovating plant growth facilities to make them appropriate for containment of transgenic plants, their products, and associated organisms. The biosafety level 2 laboratory (BL2) Lab and the biosafety level 2 greenhouse (BGH L2) standards were established by the National Institute of Health (NIH) in the United States and are widely adopted as a standard. For plant biosafety, levels 1 through 4 specify the physical and biological containment conditions and practices required for greenhouse experiments utilizing transgenic plants. Level “1” is the lowest and level “4” is the highest containment level.

Biosafety level 2 refers to facilities suitable for work with agents in Risk Group 2. The facility has to be located a distance away from/ and restricted to the public and biohazard signs have to be posted at the entrances. Work surfaces and floors must be made of impervious and easily cleanable material, while biological and all other wastes have to be heat sterilized within the building and incinerated within close vicinity of the facility. The Level 2 facilities are either for containment or confinement, denoting that the safe methods for managing biohazardous agents are both in-door or outdoor, environments. Hence the BL2 Lab and BGH L2 are containment facilities, while the OQS is a confinement facility. For both containment and confinement facilities, the two pillars of risk mitigation for confined field trials are preventing gene flow through measures to ensure genetic confinement and prevent entry to the food and feed chain.

The three facilities (BL2 Lab. BGH L2, and OQS) have been described here, detailing their dimensions, functions, construction costs, process of construction, intended use, maintenance and the measures of mitigation for genetic and material confinement, as well as key uses.

BIOSAFETY FACILITIES DEVELOPED IN KENYA

Biosafety level 2 laboratory for insect bioassays

The biosafety level 2 laboratory is located in the Biotechnology Centre at the KARI and NARL along Waiyaki Way, Kabete, Nairobi. The BL2 lab was renovated from an existing office block at an approximate costs of US$ 50,000. A contractor constructed the BL2 laboratory under the supervision of the KSTCIE. It is a physical containment facility indicating special buildings, equipment and procedures to prevent the escape of organisms. The BL2 Lab was developed to enable biocontainment of insects, including insect-proof window screens, an insect-o-cutor, floor sealing, autoclaving, and disposal facilities. The laboratory has double door system with an alarm to alert if both doors are left open at the same time. The double door space is used for changing into laboratory coats used in the lab. The windows have sealed panes and small mesh wire covers the vents. An insect killer with hot and colored filaments is provided to attract and kill flying insects. The doors have rubber seals all round the edges to prevent the entry or exit of crawling insects. Any chemical work is performed in the laminar flow hood. An autoclave is provided for sterilization of plant tissue, insects, and other wastes as described by Mugo et al. (2004a). After completion of the bioassays, all larvae are collected in water containing a detergent in preparation for autoclaving and disposal. The petri dishes and other reusable equipment are soaked in a disinfectant (sodium hypochlorite) for one hour, and then washed with a detergent. All plant tissue; insects and disposable items are placed in biohazard bags and autoclaved at 15Psi pressure, 121°C for 20 min.

The autoclaved biohazard bags were then incinerated, using kerosene, then buried in an open pit outside the BGHC. The sterilization and disposal is carried out under the supervision of a KEPHIS plant inspector. The laboratory was constructed to test transgenic maize during the development of insect resistant maize in Kenya. It was first utilized for leaf bioassays to test stem borer larvae on first generation Bt maize public events. An application to introduce leaves from maize transformed with Bt genes was made to the National Biosafety Committee (NBC) through the KARI Institutional Biosafety Committee (IBC) in February 2000. A permit to allow importation of the leaves was granted by KEPHIS in January 2001. Bioassays were carried out to identify the effective Bt proteins against five Kenyan stem borers: *Chilo partellus*, *Chilo orichalcocliiliellus*, *Busseola fusca*, *Eldana saccharina*, and *Sesamia calamistis* (Mugo et al.,

2004b). The bioassays provided the necessary information for the development of Kenyan maize varieties with the appropriate combinations of genes for resistance to these stem borer species. A second set of tests were carried out to screen Bt δ-endotoxins from single and combined Bt events in maize leaves. An application was made to the National Biosafety Committee (NBC) for a plant importation permit to introduce Bt maize leaves from first generation events and combinations of these events to test the efficacy of a two-gene combination in controlling Kenyan stem borers. Permission was granted in October 2002.

The Bt leaf samples arrived in Kenya in December 2002, for use in the bioassays with the five major stem borers. The maize leaf tissues introduced were from five Bt transgenic events, their cross combinations and the non-transformed CML216 maize inbred line (Mugo et al., 2004a). Data derived from these experiments promoted better targeting of Kenyan maize varieties slated for further development with the appropriate combinations of genes for resistance to the various stem borer species.

Since then, many studies have been conducted including the screening for the development of resistance in maize stem borers (*B. fusca* (fuller)) and (*C. partellus* (Swinhoe)) to Bt maize toxins, and studies on the tritrophic interactions among parasitoids, lepidopteran stem borers and Bt maize in Kenya (Obonyo et al., 2008a). Also, bioassays were conducted to screen nine public Bt maize events from CFT at OQS in Kiboko against Kenyan stem borer species, bioassays in screening maize Hybrid DKC8073YG and DKC8053YG to evaluate the efficacy of Bt *Cry1Ab* gene Event MON810 Bt δ-endotoxins against maize stem borers in Kenya (Mugo et al., 2005).

Biosafety level 2 greenhouse complex (BGHC L2)

The BGHC is also located at the Biotechnology Centre at Kabete, Nairobi. The construction was completed in 2003 and the facility was inaugurated on 23 June 2004 by the president of Kenya. The total cost is estimated at KES 8.4 million (US$ 175,000). KEPHIS advised on the desired biosafety features of the BGHC through the KSTCIE. Since the biosafety greenhouse was the first to be constructed in Africa outside South Africa, there were consultations among engineers, KARI and CIMMYT scientists, KEPHIS plant inspectors, and the KSTCIE to ensure successful completion of the facility. The KSTCIE inspected the facility three times before certification and final approval in 2003.

The physical requirements for BL2 are:1) A location away from public areas; 2) biohazard signs posted at entrance; 3) work surfaces made of impervious and easily cleaned material; 4) protective clothing provided; 5) sterilizer installed for devitalization and 6) secure double door system which are self-closing. The operational requirements for BL2 are: 1) Limited access to the facility; 2) facility to be managed by biosafety officer; 3) a biosafety manual; 4) personnel trained on standard operating and emergency procedures and 4) installations subject to annual emergency checks. The greenhouse has special features to prevent the transfer of pollen, seed, or other plant material from transgenic plants to the outside environment. The BL2 greenhouse has special features, approved by the KEPHIS and consistent with international standards, to ensure that transgenic materials do not enter the external environment before approval is obtained for testing in a controlled OQS.

The greenhouse complex was constructed to test transgenic maize during the development of insect resistant maize in Kenya. Specifically, the BGHC was intended to be used for: 1) Verification of the effects of Bt cry genes on target and non-target insects, 2) maintenance and seed increase of transgenic maize lines, 3) making crosses of transgenic and locally adapted maize lines, 3) storage of transgenic seeds, and 4) training in greenhouse operations and management. The greenhouse covers approximately 394m^2 head house, a large greenhouse covering 149m^2 and four small greenhouses each covering 74 m^2. The head house was made to carry three other such sets. Two small units have been added by the African Biofortified Sorghum and the BioSafeTrain projects. The main features are a covered walkway, six security doors, a head house, three greenhouses, two emergency exits, an electric soil sterilizer, an incinerator, a visitor's pavilion, soil storage bins, and a disposal trench. The basic structural features include steel frame, stone wall for the head house, concrete terrazzo floor for easy cleaning, 6 mm thick glass panes, pollen screens (5 microns), window vents and sun filter nets. Containment is maintained by restricted access to all, except trained and authorized personnel. To prevent pollen escape, windows are fitted with 50 microns fine mesh screens that will not allow pollen (usually 80 microns) to escape. Controlled pollination through bagging of tassels of transgenic maize is used as a additional defense for pollen escape. Transgenic plants are also confined in plant rooms with double-entry doors to greenhouse rooms to prevent inadvertent movement of pollen. The green house complex has a seed store approved for storage of transgenic seeds consisting of a secure air conditioned room. The seeds are held in seed envelope as primary container, which are well labeled showing the type and quantity, placed in lockable metal cabinets. Seeds are accounted for and every harvest is supervised by KEPHIS plant inspectors. Similarly, issuance of seeds and disposal is properly recorded. Bt cotton seeds, Bollgard I and Bollgard II, have also been stored at this seed store.

The greenhouse has a separate sewer from the national grid. Water draining from the floor enters the sewer system through traps that hold solid materials, while the liquid wastes have an exit above the bottom of the trap. These solids are then disposed through the sterilization system. Additional risk management strategies include: 1) Reserve pollen screens, reserve glass panels, polythene and masking tape for covering glass panes in case of accidental breakages; 2) flooding the floors with water as pollen is very sensitive to moisture; 3) regular insecticidal pest management. For the control of crawling pests, there is a moat with water surrounding the greenhouse; 4) available biosafety greenhouse operation manual (Murenga et al., 2008a). The BGHC has been in use since its inauguration, with the major use being mostly for the testing of Bt maize events. Nine second generation public Bt maize events in 2004 through PIP number PH/408/2004 were received to allow the importation of transgenic maize seed from CIMMYT-Mexico into Kenya for testing in the BGHC (Mugo et al., 2005). The Bt maize seed arrived in June 2004, and was received and inspected by KEPHIS. The transgenic seeds of nine events were sown in pots at the BGHC to evaluate the efficacy and effects of these Bt genes against the target stem borer species; they were also increased for future experiments and for conversion of locally adapted germplasm to Bt maize. These initial experiments in the green house involved growing plants from Bt maize seed to confirm results obtained from cut leaf bioassays carried out in the biosafety laboratory. In a subsequent trial, the Bt Cry1Ab gene Event MON810 Bt δ-endotoxin was tested for efficacy in controlling Kenyan maize stem borers in greenhouse containment trials through PIP KEPHIS/6099/2007(Mugo et al. 2008). In addition, recently, there was an importation of transgenic drought tolerant maize seed by the Water Efficient Maize for Africa (WEMA) project through Plant Import Permit number KEPHIS/9799/2010. Several other studies have been conducted: 1) Screening for the development of resistance in maize stem borers *B. fusca* and *C. partellus* to Bt maize toxins; 2) studies on the tri-trophic interactions among parasitoids, lepidopteran stem borers and Bt maize in Kenya (Obonyo et al., 2008b); 3) expression of *Bacillus thuringiensis* (Bt) δ-endotoxins in successive generations of crosses involving tropical Bt maize inbred lines breeding continued.

CONFINED FIELD TRIAL (CFT) SITE

The Confined Field Trial (CFT) or earlier known as the Open Quarantine Site (OQS) was designed for development, testing, seed increase and dissemination of transgenic maize varieties, and risk assessment studies under open quarantine conditions prior to deployment and large scale commercial release. The CFT is located in KARI Kiboko Center at Kiboko market 150 km from Nairobi. The quarantine field is located at 1 km from Kiboko town. Kiboko field station located at 020 15' S and 370 75' E at an elevation of 975 m asl with sandy clay soils and 530 mm annual rainfall, spread over two very short rainy seasons.

The one-hectare area borders on bush land. The Kiboko field station is easily accessible, has facilities for growing food crops, has adequate land for isolation by distance, and has trained scientists. There is a good water supply for irrigation from a permanent spring. The field site was fenced using 2 m high chain-linked fences topped with 3 strands of barbed wire. The CFT was equipped with lockable gates, water trough with a disinfectant, irrigation covering the whole 1 hectare, troughs for dispersal and burning of plant waste, and soaks pits for dispersal of exhausted disinfectant water. The Kiboko CFT site was selected as the test site considering accessibility and availability of facilities for irrigation for growing crops, trained personnel and sufficient land for controlling gene flow with border rows, and possibilities of isolation by distance from other maize fields. A letter of request to use the test site was sent to the Director KARI and approved in 2001. Construction started in 2001 and was completed in 2002 at a cost of US$125,000. KEPHIS through the KSTCIE guided the construction on behalf of the NBC.

The CFT was developed within the national biosafety framework, and meets the biosafety level II international standards. The inspection by KSTCIE in 2003 gave the facility a full OQS status, with the statement that "use of the facility for work on GMOs will require approval by the NBC". KSTCIE inspected the OQS further in 2004 and gave it the full mandate to test Bt maize there. The CFT main features and functions are to achieve genetic and material confinement. Genetic confinement is meant to prevent seeds, pollen, as well as vegetative materials from getting into the surrounding breeding and growing system. Hence, the CFT has secure fencing to restrict access by people; 24 h security enhanced by locked gates, an isolation distance of more than 400 m from other maize plots. The site is also managed by staff trained on biosafety, while proper destruction and disposal of plant and other wastes is practiced. Transgenic maize seeds harvested and intended for storage are counted, packaged in line with biosafety requirements and escorted by KEPHIS plant inspectors to the seed store in the BL2 Greenhouse in Nairobi. Proper records are kept at the site. Further tools, equipment, and containers are cleaned before leaving the site, effective material disposal methods are practiced, and trial sites monitored (Murenga et al., 2008a). Waste materials are dried and burned in the trenches within the site using a sandwich of charcoal, waste materials and diesel.

The ashes are buried in the trench. Fifteen mock trials have been conducted at the CFT since its approval in January 2003. The objectives of the mock trials were to calibrate various activities, train staff and to establish that isolation distance is to avoid unplanned crossing of Bt maize to other cultivars. Four Bt maize confined field trial (CFTs) were carried out at KARI Kiboko open quarantine field site. A CFT is a small-scale experimental field trial of genetically engineered *Bt* maize for insect resistance being carried out to collect the data necessary to evaluate performance and collect data for further risk assessment. These were: 1) Test of efficacy of nine Bt maize events against Kenya stem borer in field conditions during 2005A and 2006A seasons (Mugo et al. 2010 this volume), and 2) backcrossing Bt maize events to convert eight maize types to Bt during 2005A and 2006A seasons. Other research conducted at the site was: 1) The evaluation of effects of Bt cry genes on target and non-target insects (Songa et al., 2010); and 2) training in OQS operations and management for Kenya and the ECA region. Considerable resources have been used to develop biosafety facilities for immediate use by the IRMA project and for use by other current and future projects. These facilities have enabled research to identify and develop useful technology. These facilities have also provided means for extensive training for personnel in research, extension, farmers, seed industry and regulators from Kenya and other African countries. These biosafety facilities have provided models from which similar facilities in Kenya and other countries have been constructed. The facilities represent the tangible evidences of research in a novel technology and provides an axis for development of policy in Kenya and the rest African countries.

FACILITIES AND PROTOCOLS FOR DISPOSAL OF PLANT AND OTHER MATERIAL IN BIOSAFETY FACILITIES IN KENYA

Biotechnology applications and its wide range of tools pose new challenges to governments to develop biosafety framework for the safe and responsible use of the genetically modified organisms and their products. Despite the concerns, biotechnology specifically genetic engineering, continues to be applied in improvement of crop quality and agronomic performance. Important traits namely insect resistance, herbicide resistance, drought and salt tolerance, improved colors in fiber and flower crops, resistance to water logging, high nutritional value such as high vitamin A rice, and longer shelf lives have been incorporated into many plant species through transformation. The BL2 GHC and the CFT research generate waste from transgenic plant material, and their disposal at the end of the experiments should be done safely and responsibly. Plant and insect tissues from BL2 Lab are disposed by first autoclaving, burning in an incinerator followed by burying the ashes in an open pit outside the BL2GHC. The bulky waste such as soil, and whole maize plant materials from the BL2 GHC is sterilized in a soil sterilizer, burned, and ashes buried. In the CFT, more voluminous plant wastes are generated over time. Disposal is carried out by drying, burning the organic with charcoal and liquid fuels and burying the ashes in trenches within the CFT.

POSTHARVEST MONITORING FROM BT MAIZE TESTING FACILITIES IN KENYA

After the termination of experiments at the CFT, it is a biosafety requirement that disposal and post harvest monitoring for maize volunteers be carried out for one year. After harvesting, all plant materials are dried in the field for a week and later incinerated in trenches provided in the CFT and ashes buried. The trial area is irrigated to germinate any transgenic Bt volunteer maize seeds. Germinating seedlings are uprooted and destroyed; monitoring for volunteers is carried out every 2 weeks for 6 weeks and later once per month over a period of 12 months after termination of the trials. Post harvest monitoring of volunteers was carried out at the four trial sites in the OQS by scouting for maize seedlings by plot areas, borders, and the isolation. No volunteer maize seedling was found in the trials 1 area in the 2006A season as the crop was harvested prior to seed maturity (Figure 1). The one year monitoring period for trial 1 2006 A ended in April 2007. These observations are consistent with the biology of maize. Maize seed dispersal from the mother plant is not possible without the help of humans, birds or other animals. Maize is a very poor competitor with weeds and cannot survive through a season without human intervention. Maize kernels have no dormancy period and germinate readily when conditions are optimal after physiological maturity. Once the data was obtained, it was suggested to the National Biosafety Committee (NBC) to reduce the post harvest monitoring period in future trials at the confined field trial sites.

TRAINING OF KENYA'S RESEARCH SCIENTISTS, REGULATORS, AND EXTENSION STAFF IN BIOTECHNOLOGY AND BIOSAFETY RESEARCH AND DEVELOPMENT

Biotechnology and biosafety are relatively new sciences that demand knowledge and skills in its development, implementation and stewardship. The levels of scientific skills including biotechnology in Africa still remain below, compared to other continents. Since the Insect Resistant

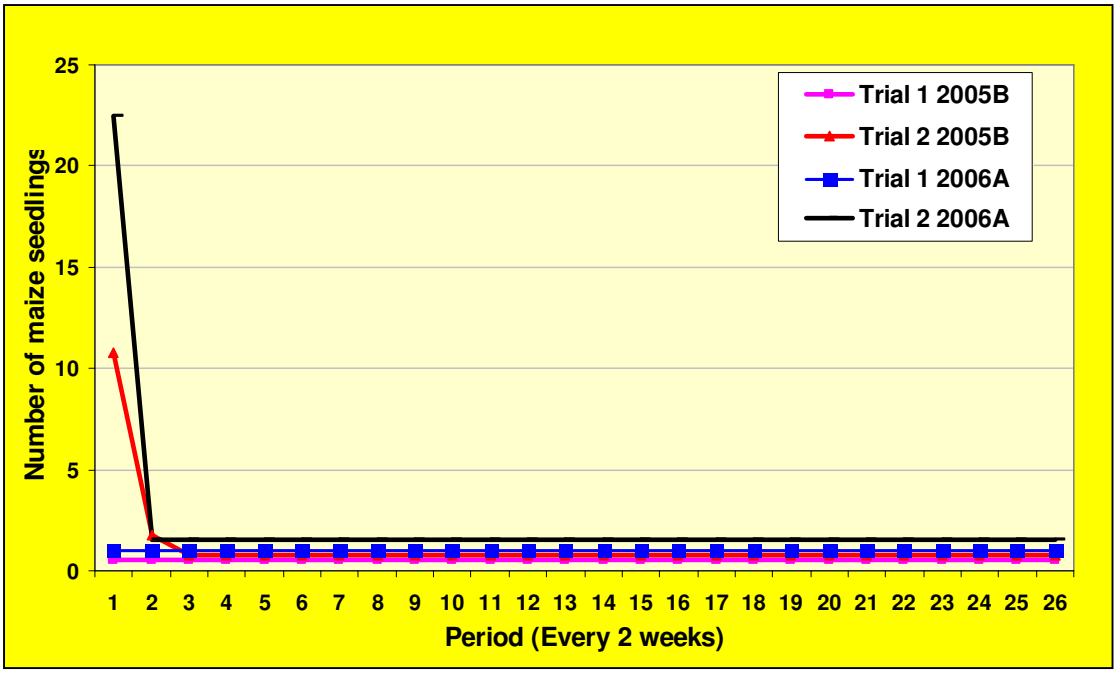

Figure 1. No. of volunteer maize seedling during post harvesting monitoring period of Bt maize CFT at Kiboko 2005B and 2006A seasons (Aug 2005-Oct 2007).

Maize for Africa (IRMA) project aimed at developing and deploying insect resistant maize developed using Bt technology, training of Kenyans involved in the development, use, and regulation of transgenic technology was necessary. The immediate need was met through informal approaches that involved (i) short term visits to locations within and outside Kenya where the technology was being developed, tested or used, (ii) short term courses of 1-2 days, and (iii) short term visiting scientist model of maximum of six months. Informal learning was found to be relevant in all facets of biotechnology and its applications. Formal MSc and PhD degree related trainings were also included. This resulted in a large numbers of scientists from Kenya Agricultural Research Institute (KARI) and other institutions, plant inspectors from Kenya Plant Health Inspectorate Service (KEPHIS), extension staff from the Ministry of Agriculture, journalists from various media houses, farmers and other stakeholders exposed to the technology development regulation and use.

Training scientists on biotechnology and biosafety

Training of scientists on biotechnology and biosafety was met through short term visiting scientist model of maximum of six months (Table 1). The courses had the following objectives: 1) To familiarize the scientists with the protocols used for rearing of stem borers and field infestation of the same at CIMMYT, conduct insect bioassays in the laboratory, learn about biosafety standards maintained at the CIMMYT Applied Biotechnology Center (ABC), learn about the development of Bt maize via biolistic transformation, molecular analysis of putative transgenic plants, and to learn methodology, activities, and biosafety protocols practiced in the ABC biosafety greenhouses. Additional objectives were: 1) Learn about the management of biosafety facilities at CIMMYT-Mexico and at Biotechnology Center in Kenya; 2) learn about post harvest pest (maize weevil) resistance breeding activities at CIMMYT Harare. Still others were: 1) Biosafety Assessment and Regulation of Agricultural Biotechnology, 2) management of biosafety screen houses and open quarantine sites; 3) planting Bt maize confined field trial; 4) on-site compliance training for trial managers and inspectors of confined field trials; 5) study tour on growing and management of Bt maize in South Africa; 6) learn procedures followed and methodologies used to manage insect resistance and conduct environmental risk assessments; 7) FAO/KEPHIS-sponsored training workshop on Biosafety for Regulatory Officers to enhance the participant's understanding of the concepts and issues associated with modern agricultural biotechnology; 8) understand the Kenya's policy and regulations on GMOs; 9) evaluate Kenya's preparedness to monitor GMO-related activities; 10) facilitate the acquisition and

Table 1. Training courses in IRMA Project (2000-2008).

S/N	Title	Location	Objectives	Duration / dates	Participants names	Participants' institution
1	Protocols of insect bioassays	Applied Biotechnology Center, CIMMYT Mexico	1) To familiarize with the protocols used for rearing stem borers the timing of field infestations; 2) learn about insect bioassays in the lab, 3) learn about biosafety standards maintained at the ABC, development of Bt maize, and 4) to learn about methodology, activities, and biosafety protocols practiced in the ABC biosafety greenhouses.	June-July 2001	Catherine Taracha, and Josephine Songa	KARI
2	On-the-job training in the areas of biosafety, greenhouse operations, and in transformation and molecular analysis	Applied Biotechnology Center, CIMMYT Mexico	To familiarize with biosafety, greenhouse operations, and in transformation and molecular analysis.	6 months (2002-2003)	Catherine Taracha and Murenga Mwimali	KARI
3	Management of biosafety facilities at CIMMYT-Mexico.	Applied Biotechnology Center, CIMMYT Mexico	Training on management of biosafety facilities at CIMMYT-Mexico.	2 weeks (4th – 20th October 2002)	Kennedy Onchuru, Benson Kuria, Catherine Taracha	KARI, KEPHIS
4	Training on biosafety and management of the open quarantine site (OQS) field facilities	Nairobi and Embu	Training on biosafety and management of the open quarantine site (OQS) field facilities	Sept 23-24, 2002	10 KARI, 2 persons - CIMMYT, and 1 person - KEPHIS	KARI, KEPHIS, CIMMYT
5	Observe the maize weevil resistance breeding project activities	CIMMYT-Zimbwabwe	To familiarize with the maize weevil resistance breeding project activities	28th-31 August 2002	Paddy Likhayo	KARI, CIMMYT Zimbabwe
6	Study the design and functions of the biosafety greenhouse facilities	Applied Biotechnology Center, CIMMYT Mexico	To study the design and functions of the biosafety greenhouse facilities	2002	Christopher Ngichabe David Hoisington	KARI, CIMMYT
7	Training on Biosafety Greenhouse (BGH) Operations	KARI, NARL	To familiarize with biosafety Greenhouse (BGH) Operations	8–12 March 2004	25 – KARI, and 5 - KEPHIS	KARI, KEPHIS
8	"Biosafety Assessment and Regulation of Agricultural Biotechnology, Ghent University	Ghent University, Belgium	To understand regulatory issues and to boost the knowledge and skills among scientists on IRMA II project planned regulatory issues as well as during implementation of the project.	2 weeks, 9-20 August 2004	Allensandro Pellegrineschi, Stephen Mugo, Margaret Mulaa, Rose Onamu, and Simon Gichuki	CIMMYT, KARI, and KEPHIS
9	Management of biosafety screen houses and open quarantine sites for cassava	Kakamega	To familiarize with management of biosafety screen houses and open quarantine sites for cassava	Nov-03	Joel Mbithi, David Karuri, Murenga Mwimali, Simon Gichuki, The late Benjamin Odhiambo	KARI, CIMMYT

Table 1. Contd.

S/N	Title	Location	Objectives	Duration / dates	Participants names	Participants' institution
10	Operations and management of Bt maize confined field trial	Kiboko	To familiarize with operations and management of CFT at Kiboko	Monday 18 April 2005	13 – KARI, 5 – CIMMYT, 2 – KEPHIS, 1 – UoN, 1 – ICRISAT, 1 – NCST, 2 – MOA Makueni, 1- OP Kiboko Location, 1 – Kiboko, 8 – Kiboko farm	As indicated
11	On-site compliance training for trial managers and inspectors of confined field trials		To familiarize with for trial managers and inspectors of confined field trials	26-27 April 2005	S. Gichuki, S. Mugo, Joel Mbithi, Evans Mwasame	KARI, CIMMYT, KEPHIS, PBS
12	Biosafety for Regulatory Officers	KEPHIS Headquarters, Karen	To improve the understanding of Kenya's policy and regulations on GMOs To evaluate Kenya's preparedness to monitor GMO-related activities To facilitate the acquisition and use of correct technical information for making informed decisions on matters of biotechnology & biosafety and To establish institutional linkages for continued collaboration on issues relating to the application of modern biotechnology and the associated concerns on biosafety	29 November to 1 December 2005	Murenga Mwimali Wanjala Bramwell	KARI, KEPHIS
13	To learn procedures followed and methodologies used to manage insect resistance and conduct environmental risk assessments	University of Nairobi	To learn procedures followed and methodologies used to manage insect resistance and conduct environmental risk assessments	28 May-1 June 2005	Dennis Ndolo	University of Nairobi, KARI
14	CGIAR Student Research Induction, and took a research methods course	World Agroforestry Centre (ICRAF campus), Nairobi	To equip the students with essential skills for their research: conceptualization, data collection, analysis, and presentation	1 week, April 2006	Haron Karaya, Murenga Mwimali, Regina Tende, Dennis Ndolo	KARI, CIMMYT, ICRAF, Royal Kingdom of the Netherlands
15	'Writing to Be Read'	World Agroforestry Centre (ICRAF campus), Nairobi	To improve writing and presentation skills for a range of materials, from research articles, to project proposals, to journalistic fare for a lay readership	3 days, April 2006	Murenga Mwimali, Regina Tende, Dennis Ndolo, Jane Wamaitha, Jayne Binnot	KARI, ICRAF, Royal Kingdom of the Netherlands
16	Biosafety at the KARI Biosafety laboratory and greenhouse Complex	KARI - NARL, Nairobi	To familiarize with biosafety laboratory and biosafety greenhouse (BGH) operations	15-16 Feb. 2007	15 – KARI, 1 – KEPHIS, 2 – CIMMYT, 4 - JKUAT	KARI, CIMMYT, KEPHIS, and JKUAT

use of correct technical information for making informed decisions on matters of biotechnology and biosafety; and 11) establish institutional linkages for continued collaboration on issues relating to the application of modern biotechnology and the associated concerns on biosafety, Other purposes for training scientists were to help them: 1) Formulate dependable guidelines for use in decision making on matters of biotechnology and biosafety; 2) equip students with essential skills for their research conceptualization, data collection, analysis, and presentation; 3) improve writing and presentation skills for a range of materials, from research articles, to project proposals, to journalistic fare for a lay readership, and equip participants with information and skills that would enable them effectively address issues of biosafety with regard to agricultural biotechnology and effectively conduct biosafety risk assessment studies.

Visiting scientists training at CIMMYT Kenya

Visiting scientist arrangement is a proven way of imparting hands-on research capability to collaborators. The approach is to have a scientist attached to a program for a period that spans a crop season, such that the trainee participants in experimental planning and designs, planting, field management data, taking, harvesting, data analysis, selections and developing way forward in the activity. Scientists in IRMA attended to many students on such attachment to CIMMYT AMS and DTMA projects, and these from Kenya, Uganda, Tanzania, DRC, Ethiopia, and Sudan. IRMA has also had one visiting scientist (August – November 2008) on plant breeding and DTMA projects, and these from Kenya, Uganda, Tanzania, DRC, Ethiopia, and Sudan.

Informal training for administrators in science and development

Four high-ranking Kenyan officials from KARI, Ministry of Agriculture, and KEPHIS visited CIMMYT and Mexico government in 2000 to learn how research and development on biotechnology is done. Six management level Kenyan scientists from KARI, KEPHIS, the Ministry of Agriculture, and CIMMYT visited the government and private sector institutions dealing with development and regulation of biotechnology and biosafety facilities in the USA and Mexico in 2002. The objective was to familiarize themselves with biotechnology development, deployment and regulation and to view physical facilities as well as to discuss possible areas of collaboration. Six other management level Kenyan officials from the Ministry of Agriculture, NCST, KEPHIS, KARI and NBC took a Biotechnology Study Tour to Syngenta in the USA, in July 2008. The objective was to enable Senior Kenya Government officials in charge of seed development including biosafety regulation and certification to experience how these processes are done in the USA and to share progress in biosafety issues and general food situation in Kenya with their hosts at Syngenta.

Support to research for BSc, MSc and PhD degree related training.

Support was given to students in formal degree courses for BSc projects, BSc student attachments and MSc thesis and PhD dissertation where the proposed research fitted within the objectives of the IRMA project. Two BSc. students were attached to IRMA Project at CIMMYT Kenya for one month in 2006. Numerous numbers of students from middle level diploma and university colleges have been attached to the BGHC complex, for exposure to general principles of biotechnology applications and biosafety since construction. Twelve students have had their masters' degree thesis research done with IRMA support. Four were in plant breeding, one in agronomy, one in entomology, two in biotechnology, and four in socio-economics. One PhD student completed his studies in entomology. Eight theses were published from this work (Obonyo Dennis, 2009, Kimenju, 2007, Tende, 2008, Ndiso, 2008, Murenga Mwimali, 2008, Haron Karaya, 2006, Makonde, 2008, Nambiro 2004, Owuor, 2003). Several peer reviewed papers were published from this training (De Groote and Kimenju, 2008; De Groote et al., 2008; Kimenju and Groote, 2008; Kimenju and De Groote, 2008; Kimenju et al., 2005; Murenga et al., 2008b; Nambiro et al., 2004).

Training of farmers, extension staff and media and general public in seminars and visits to biosafety facilities

Seminars were held to enlighten farmers about the OQS at Kiboko during its development as well as its operations. A series of five one-day seminar/workshops were held in July 2002 for lead extension staff of the Kenyan Ministry of Agriculture in the major maize growing regions of Kenya (Mombasa, Machakos, Embu, Kitale, Kakamega). Approximately 120 extension personnel attended. The seminars were aimed at informing extension staff about Bt technology the IRMA project and to identify areas the project needs to address that may not have previously been considered, identify good communicators among extension staff, and effective messages, for possible later use in media communication via video and/or radio productions. The extension staff helped refine a series of fact sheets on Bt maize through extension Feedback. Media and scientists training workshop were co-organized by IRMA and African biotechnology

stake holders forum (ABSF) in 2001 and in 2006. Extensive education and its application on biotechnology was shared with the media participants. The biosafety facilities mainly, the BGHC and the OQS, were focal points for informal training through visits by major groups. A total of 575 entrants were registered represent-ting 55 national and international institutions at the OQS during the May – October 2005. Likewise, the BGHC recorded more than 150 visitors from 62 institutions and nationalities during the Jan – March 2006 period.

OUTCOMES AND IMPACTS OF THE TRAINING IN IRMA PROJECT

The trainings resulted in large numbers of scientists from KARI and other institutions, plant inspectors from KEPHIS, extension staff from the Ministry of Agriculture Journalist from various media houses, and farmers and other stakeholders exposed to the technology development regulation and use. Further, research was done largely using skills from these trainings including evaluation of Bt maize technology in the lab, greenhouse and field facilities, development of insect resistance management strategy, assessment of environmental impacts mainly non-targets organisms, breeding conventional resistance maize, and assessment of impacts on maize farming systems. Due to this training and other trainings instituted by other biotechnology stakeholders' institutions, there was a marked improvement in science based regulatory as well as decreased time interval to make decision from applications for genetically modified organisms (GMOs) testing in Kenya. A rather informal training approach was adopted due to the prevailing low level of knowledge about the biotechnology and biosafety sciences as well as almost non-existent of institutions for training in theses sciences when IRMA Project were initiated. Nevertheless, IRMA was able to fill in an important void by offering degree relating students with practical research areas. Training was also diversified so as to meet the multi-disciplinary project needs in the IRMA Project. The project has built a critical mass for its own research and development as well as for partners in regulatory and extension roles. These trainings resulted in a large numbers of scientists from KARI and other institutions, plant inspectors from KEPHIS, extension staff from the Ministry of Agriculture, Journalists from various media houses, and farmers and other stakeholders exposed to the technology development regulation and use. In Kenya, informal learning was found to be relevant in all facets of biotechnology and its applications, because it often had an immediate effect on work performance and it covered a topic directly related to the immediate needs. Often, informal learning complemented formal learning. Training in project specific knowledge and skills are necessary. New projects need not follow similar trend as there are now trained personnel in Kenya.

Experiences in applications for introduction and testing Bt maize

The IRMA regulatory achievements include: 1) Approvals for biosafety facilities (Bioassay Laboratory, Biosafety greenhouse and open quarantine site), introduction of transgenic Bt maize leaves and seeds for the first time in Kenya, and 3) approvals for transgenic Bt maize confined field trials, evaluations and backcrossing.

Moving the biotechnology agenda in Kenya - IRMA project's contributions to the regulatory system

The IRMA project contributed to moving the biotechnology agenda in Kenya in many ways. A direct contribution was through training of regulators from KEPHIS and NBC through formal courses and visits to research facilities in Kenya, Mexico and the USA. IRMA also provided Bt maize as a product through which the ground work was laid down for the first time in Kenya for a Biosafety regulatory system. Key KARI and CIMMYT scientists' were instrumental in the development of the biotechnology and biosafety policy, bills, and biosafety law. The IRMA project's regulatory compliance and implementation aided in a noticeable reduction of time between application approvals and regulatory decisions. There was an increased science based decisions made making at various levels of the regulatory system. Also, there were improved communication among the various stakeholders, an increase in the number of biosafety applications, a decline in regulatory costs, preparedness for contingencies, and amelioration in stakeholders trust.

ABBREVIATIONS

GM, Genetically modified; **CBD,** convention on biological diversity; **NBA,** National Biosafety Authority; **Bt,** *Bacillus thuringiensis*; **KARI,** Kenya Agricultural Research Institute; **IBC,** Institutional Biosafety Committee; **KSTCIE,** Kenya Standing Technical Committee on Imports and Exports; **SOPs,** standard operating procedures; **NPT,** national performance trial; **NCST,** National Council for Science and Technology; **BGHC,** biosafety level 2 greenhouse complex; **OQS,** open quarantine site; **NARL,** national agricultural research laboratories; **WEMA,** water efficient maize for Africa; **CFT,** confined field trial; **IRMA,** Insect Resistant Maize for Africa; GMOs, genetically modified organisms, **NIH**, National Institute of Health; **KEPHIS**, Kenya Plant Health Inspectorate Service.

REFERENCES

De Groote H, Kimenju SC (2008). Comparing consumer preferences for white, yellow and fortified maize: Application of a semi-double-bound logistic model on urban consumers in Kenya. Food Policy 33: 362-370.

De Groote H, Wangare L, Kanampiu F, Odendo M, Diallo A, Karaya H, Friesen D (2008). The potential of a herbicide resistant maize technology for Striga control in Africa. Agric. Syst. 97: 83-94.

Falck Zepeda J, Zambrano P (2009). Estimates and Implications of the costs of compliance with biosafety regulations for African agriculture.

Government of Kenya (2009). Biosafety Law, Government of Kenya, Nairobi, Kenya.

KARI (1994). Guidelines and Regulations for Biosafety in Biotechnology Research.

Kimenju SC, Groote HD (2008). Consumer willingness to pay for genetically modified food in Kenya. Agric. Econ. 38: 35-46.

Kimenju, SC, De Groote H, Karugia J, Mbogoh S, Poland D (2005). Consumer awareness and attitudes toward GM foods in Kenya. Afr. J. Biotechnol. 4: 1066-1075.

Makonde HM, Lenga FK, Masiga D, Mugo S, Boga HI (2008). Effects of *Bacillus thuringiensis* Cry1a(C) δ-endotoxins on nitrogen fixing bacteria in clay soil in Kenya. Pp IN: Mugo S, Gethi J, Ouma JO, Ajanga S, Beyene J, Murenga GM, Mulaa M, Likhayo P, Gichuki S, Kega V, De Groote H, Chavangi A (Eds). 2008. Book of abstract of the "Consolidating Experiences from IRMA I and II: Achievements, Lessons and Prospects", IRMA Project End-of-Phase II Conference, 28-30 Oct 2008, Nairobi, Kenya: IRMA Document No. 35: KARI and CIMMYT.

Mugo S, De Groote H, Bergvinson D, Mulaa M, Songa J, Gichuki S (2005). Developing Bt maize for resource-poor farmers - Recent advances in the IRMA project. Afr. J. Biotechnol. 4:1490-1504.

Mugo S, Taracha C, Bergvinson D, Odhiambo B, Songa J, Hoisington D, McLean S, I, Gethi M (2004a). Screening cry proteins produced by Bt maize leaves for activity against Kenyan maize stem borers. Pp 102-105. In Friesen DK, AFE, Integrated Approaches to Higher Maize Productivity in the New Millennium. Proceedings of the 7th Eastern and Southern Africa Regional Maize Conference, Nairobi, Kenya, 11 - 15 February 2002, Mexico, D. F.: CIMMYT.

Mugo S, Groote HD, Songa J, Mulaa M, Odhiambo B, Taracha C, Bergvinson D, Hoisington D, Gethi M (2004b). Advances in Developing Insect Resistant Maize Varieties for Kenya within the Insect Resistant Maize For Africa (IRMA) Project, p. 31-37, *In* Palmer FE, ed. Integrated Approaches to Higher Maize Productivity in the New Millenium. Proceedings of the 7th Eastern and Southern Africa Regional Maize Conference, Nairobi, Kenya, 11 - 15 February 2002. CIMMYT, Mexico, D. F.

Mugo SN, Murenga MG, Karaya H, Tende R, Taracha C, Gichuki S, Ininda J, M'mbijjewe K, Chavangi A. (2010b). Control of *Busseola fusca* and *Chilo partellus* stem borers by *Bacillus thuringiensis* δ-endotoxins from Cry1Ab gene Event MON810 in greenhouse containment trials. Afr. J. Biotechnol. (2011).

Mugo SN, Murenga MG, Taracha CO, Songa JM, Gichuki ST, Tende R, Karaya H, Bergvinson DJ, Pellegrineschi A, Hoisington DA (2010b). Testing public Bt maize events for control of stem borers in the first confined field trials in Kenya. Afr. J. Biotechnol. (2011).

Murenga GM (2008). Expression of *Bacillus thuringiensis* (Bt) δ-endotoxins in successive generations of crosses involving tropical Bt maize inbred lines, University of Nairobi, Kenya, Nairobi.

Murenga GM, Mugo S, Gichuki ST, Taracha C (2008a). Manual for Biosafety Level II Greenhouse for Research on Transgenic Plants at KARI Biotechnology Centre. Insect Resistant Maize for Africa (IRMA) Project. IRMA Project Document No. 14. Nairobi, Kenya: KARI and CIMMYT.

Murenga M, Mugo S, Githiri SM, Danson J, Olubayo F (2008b). Control of Chilo partellus stem borers by Bt maize δ-endotoxins in generations of tropical Maize. Pp IN: Mugo S, Gethi J, Ouma JO, Ajanga S, Beyene J, Murenga GM, Mulaa M, Likhayo P, Gichuki S, Kega V, De Groote H, Chavangi A (Eds). (2008). Book of abstract of the "Consolidating Experiences from IRMA I and II: Achievements, Lessons and Prospects", IRMA Project End-of-Phase II Conference, 28-30 Oct 2008, Nairobi, Kenya: IRMA Document No. 35: KARI and CIMMYT.

Nambiro E, De Groote H, Oluoch K'Osura W (2004). The Hybrid Maize Seed Industry in the Transnzoia District of Western Kenya, p. 474-479, *In* Friesen DK, Palmer AFE, eds. Integrated Approaches to Higher Maize Productivity in the New Millenium. Proceedings of the 7th Eastern and Southern Africa Regional Maize Conference, Nairobi, Kenya, 11 - 15 February 2002. CIMMYT, Mexico, D. F.

NCST (1998). Regulations and guidelines for biosafety in biotechnology for Kenya National Council for Science and Technology (NCST), Nairobi, Kenya.

Obonyo D, Lovei G, Songa J, Oyieke F, Mugo S, Nyamasyo G (2008a). Developmental and mortality responses of *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) following partial feeding on Bt-transgenic maize. J. Appl. Biosci. 11: 554-563.

Obonyo DN, Songa JM, Lovei GL, Oyieke FA, Nyamasyo G, Mugo S (2008b). Tritrophic interactions between parasitoids, lepidopteran stem borers and Bt maize in Kenya. Poster presented at the National Conference on the Environment 18-21 May 2008, Kenyatta University, Nairobi, Kenya.

Republic of Kenya (2008). National Biotechnology Awareness Strategy 2008 - 2013. 2008. GoK, Nairobi, Kenya.

Traynor PL, Adair D, Irwin R (2001). A practical guide to containment. Greenhouse research with transgenic plants and microbes, *In* Traynor PL, ed. Information System for Biotechnology. Virginia Tech.