KARI
CIMMYT

IRMA Updates

Insect Resistant Maize for Africa

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Contents

The IRMA project was launched in 1999 with the primary goal of increasing maize production and food security for African farmers through the development and deployment of improved maize varieties that provide high resistance to insects, particularly stem borers. To achieve this goal, KARI and CIMMYT scientists will identify conventional and novel sources of stem borer resistance and incorporate them into maize varieties that are well suited to Kenyan growing conditions and to farmer and consumer preferences. Major funding for the project is provided by the Novartis Foundation for Sustainable Development.

Good Progress Reported at IRMA Project Annual Meeting

Good progress on IRMA project goals was reported at the Annual Meeting held 4-8 December 2000 in Nyeri, Central Kenya. Approximately 30 participants from KARI, CIMMYT, the Ministry of Agriculture, and the Novartis Foundation for Sustainable Development attended the meeting to report results in their respective areas and to further develop workplans for the coming year.

Several things stood out about the meeting, observes David Hoisington, IRMA Project Director and Director of CIMMYT's Applied Biotechnology Center (ABC). "The high level of interactions and discussions that took place, both in the small groups and also with the entire project team, represents a promising start to the team effort and serves as a good indication that everyone is really committed to the project and sees the benefit of working together."

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It has also become evident, Hoisington says, "the IRMA project has a broad range of activities that complement one another well and that address the major issues and concerns raised by the technology. This broad and conscientious approach should serve us well as the actual products move closer to the field in Kenya."

Five specific areas were addressed at the meeting: (1) **Product development** of insect resistant maize varieties for Kenya; (2) **Product dissemination** through the establishment of procedures to provide insect resistant maize to resource poor farmers; (3) **Impact assessment** of these maize varieties on Kenyan agricultural systems; (4) **Technology transfer** of key technologies to KARI and Kenya to develop, disseminate, and monitor insect resistant maize varieties; and (5) **Documentation and communication** of project goals and achievements and dissemination of information to diverse stakeholders.

IRMA project scientists from KARI, CIMMYT, and CIMMYT-Kenya, joined by representatives of the Novartis Foundation for Sustainable Development, met in Nyeri Kenya for the project's Annual Meeting.

Although 2000 was the project's first full year underway, there were a number of highlights to report. Considerable baseline data had been gathered from more than 900 farmers in Kenya's five maize growing ecological zones. Among other things, data from these surveys firmly established that Kenyan farmers perceive stem borers as a major insect pest and that they were keenly interested in new insect resistant varieties—even if the cost was moderately higher—provided that they met their other selection criteria.

Surveys carried out in the field provided important information about the organism ecology in major maize producing areas (see Dr. Songa's report in this issue). Prior to these studies, little information was available about these organism ecologies, specifically, the populations of non-target and beneficial

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(Good Progress cont'd...)
organisms The finding from this research will form the basis for assesing impacts of Bt maize on non-target organisms.

Work on developing resistance in the lab proceeded well with the successful transformation of a number of *Bt* genes into elite CIMMYT maize lines at CIMMYT-ABC in Mexico. CIMMYT scientists are now pursuing the production of second generation transgenic plants that carry only *Bt* gene(s), without any herbicide or antibiotic markers, so-called "clean" events. Once developed, these maize plants will be crossed with selected Kenyan varieties to meet the needs of farmers in various growing regions of Kenya. Meanwhile, KARI and CIMMYT-Kenya maize breeders have run numerous on-station and on-farm trials to identify maize varieties that not only offer some natural resistance to borers, but that also show tolerance to drought, low nitrogen, and a host of common diseases, while providing high yields.

A Stakeholders Meeting in March 2000, the initiation of an IRMA Project Document series, and considerable press coverage of issues surrounding biotechnology and genetically modified crops represented positive movement in keeping the public well informed.

Finally, permission to import Bt maize leaf tissue for bioassays against Kenyan stem borers cleared most, though not all, of the regulatory hurdles by the end of the year. While the process was somewhat slow, it was pursued with the requisite care and attention warranted by a new technology. It also served as a beneficial learning experience for both those submitting the application and the boards that considered it.

Not resting on their laurels, sub-groups at the Annual Meeting put together ambitious draft workplans and budgets for 2001. These were reviewed by the IRMA Steering Committee at the end of the workshop week. Among the initiatives coming out of this session were a proposal for a follow-up Stakeholders Meeting and the need to identify and coordinate activities related to other GMOs that might be proposed for Kenya.

—**David Poland,** CIMMYT Biotechnology Writer/Editor

Biosafety Level II Laboratory Developed at the KARI Biotechnology Center

Several large steps were recently taken toward the establishment of a biosafety laboratory for KARI. Properly designed and equipped laboratory and greenhouse facilities are important for the appropriate handling of transgenic maize in Kenya.

Dr. Diego González de León was contracted by IRMA to develop a plan for converting the existing Kenya Agricultural Research Institute (KARI) Biotechnology Center building at the National Agricultural Research Laboratories (NARL) into a functioning biotechnology laboratory with attached biosafety greenhouses. Dr. González de León was formerly a molecular geneticist at CIMMYT's Applied Biotechnology Center (ABC) and was involved in the original design and construction of the ABC. During a visit to the NARL facility in May 2000, he met with KARI scientists to determine needs for the facility, took detailed measurements of all existing infrastructure and inventoried all major equipment that would be available for use in the new laboratories. From this data, he developed a detailed set of architectural drawings of the existing building and the proposed modifications to develop a facility that would meet the immediate and future needs of KARI in animal and plant biotechnology. In addition, a suite of biosafety greenhouses was designed, modular in nature to allow for expansion.

A laboratory for insect bioassays has since been constructed in accordance with the plans developed by the consultant. A construction consultant was employed to make the necessary modifications to meet the requirements of the national biosafety regulations and guidelines for a biosafety level II laboratory. The bioassay laboratory was inspected and approved by the Kenya Standing Technical Committee on Imports and Exports (KSTCIE) for use as a biosafety (hazard) level II facility. Since completion, mock bioassays have been conducted to prepare for the evaluation of Bt maize leaves, pending the issuance of an import permit by KEPHIS.

—**Dr. Stephen Mugo,** IRMA Project Coordinator

Introduction of Bt Maize Leaves

One of the IRMA project's highest priorities at this time is the identification of the most effective *Bt* genes against each of the targeted insect pests.

Several methods may be used to determine the activity of *Bt* genes, including insect bioassays using isolated Bt proteins or immunological assays of labeled Bt proteins against isolated insect mid-guts (to determine whether receptors are present in the mid-gut). The protein bioassays are easy but often do not indicate the most effective proteins. The immunological tests are highly accurate, but are technically challenging and require special expertise and infrastructure. Ultimately, the best assay is obtained by subjecting Bt maize plant material to an "infestation" of the target pests and observing the results.

CIMMYT has produced a number of first generation *Bt* maize plants, each containing an individual *Bt* gene. These provide excellent material for testing against African insect pests. As of 2000, given the early state of biosafety in Kenya and the lack of proper

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Identification of Candidate Germplasm Recipients of Bt *cry* Genes

With the growing of a nursery at Kiboko during the 2000B season, the IRMA project initiated the search for candidate germplasm to receive select *Bt* genes.

The nursery comprises 216 genotypes acquired from KARI centers, the Africa Maize Stress (AMS) project, and from the maize physiology and maize entomology units at CIMMYT-Mexico. The germplasm came from (1) elite stem borer resistant OPVs and inbred lines from CIMMYT, (2) elite drought tolerant inbred lines from AMS project, (3) elite low nitrogen tolerant inbred lines from the AMS project, (4) commercial cultivars, and (5) elite OPVs, hybrids, and inbred lines from the Kenya National Maize Breeding Program.

The germplasm included genotypes with the following traits: drought escaping/tolerant, low-N tolerant, Striga resistance, stem borer resistance, *Turcicum* blight toleranance, GLS resistance, and high yielding capabilities. The genotypes were observed, evaluated, and increased and/or advanced by CIMMYT and KARI scientists. Evaluation was for maturity, response to natural stem borer infestation, plant aspect, ear aspect, grain yield, post-harvest weevil resistance, plant and ear heights. The nursery was harvested in September 2000.

Sixty-eight inbred lines and 70 full vigor materials were selected from the nursery for further evaluation as putative genotypes to receive *Bt* genes. The lines are at different stages of inbreeding ranging from S_3 to S_8. These selected genotypes are being screened for tolerance to abiotic and biotic stresses as well as their combining ability as inbred genotypes for November 2000 plantings. The inbred lines were planted at Kiboko for observation, evaluation, and advance to the next generation of inbreeding.

A separate planting at Kiboko of early and late germplasm will be screened for drought resistance. The 68 lines were also planted at Embu for screening for resistance to *Chilo* and *Busseola* stem borers, and at Muguga for screening for resistance to foliar diseases. These lines will also be crossed to two testers, CML78 and CML 444. This is necessary to test their worth in hybrid combinations and their heterotic grouping.

The 70 full vigor genotypes that were selected are being evaluated at Kiboko for tolerance to low N and to drought stress. The early and late maturing genotypes are being evaluated in different trials for drought screening to enable proper management of watering. These full vigor materials are also being evaluated at Embu for screening for resistance to *Chilo* and *Busseola* stem borers, and at Muguga for screening for resistance to foliar diseases.

The nurseries and evaluations are being done in collaboration with the KARI breeders who contributed the germplasm. The breeders visited Kiboko during harvesting and made the selections. Those present were Dr. Jane Ininda (NARC Muguga), Mr. Wesley Chivatsi (RRC Mtwapa), Mr. Dickson Ligeyo (NARC Kitale), Mr. Zachary Muthamia (NDFRC Katumani), and Dr. Stephen Mugo (CIMMYT-Kenya).

—**Dr. Stephen Mugo,** IRMA Project Coordinator

(Introduction of Bt cont'd...)

infrastructure in KARI to handle transgenic maize (in the lab and the field), the IRMA project determined that the simplest procedure would be to import Bt maize leaves (grown in CIMMYT's biosafety greenhouses in Mexico) into Kenya and perform leaf bioassays in the KARI-NARL Biotechnology Laboratories.

The application to Kenyan authorities to import Bt maize leaves has progressed as follows. The application that was lodged with the KARI Institutional Biosafety Committee (KIBC) was considered and queries made. The applicants (Dr. Benjamin Odhiambo, Dr. David Hoisington, and Dr. Stephen Mugo) responded to the queries. The applicants also appeared before the committee to defend the application. The application was approved by the KIBC and forwarded to the National Biosafety Committee (NBC). The application went through a similar procedure of consideration and queries. Meanwhile the biosafety level II laboratory was being constructed. After KSTCIE inspected the laboratory, the NBC approved the application and advised KEPHIS to issue the permit to introduce leaves of Bt maize into Kenya for use in insect bioassays. This has been a long and tedious process but it ensures that the current rules and regulations guiding introduction of genetically modified organisms are followed. The process also ensures that a wide cross-section of stakeholders is involved in the process of introducing Bt maize into Kenya.

The objective of the bioassays is to screen and identify the effective *Bt* genes (*cry*) in maize (*Zea mays* L.) leaves for resistance to Kenya stem borers (*Chilo partellus, Busseola fusca, Eldana saccharina, and Sesamia calamistis*). This information will then allow us to better target the development of Kenyan maize varieties with the appropriate combinations of genes for resistance to these stem borer species and to identify the appropriate *cry* genes for backcrossing to Kenya maize cultivars.

—**Dr. Stephen Mugo,** IRMA Project Coordinator

Impacts of Bt Maize on Non-Target Organisms

Stem borers are one of the major causes of low on-farm maize yields among small-scale subsistence farmers in Kenya. Bt maize offers farmers an effective, affordable and practical option for controlling stem borers. However, to deploy Bt maize to Kenyan farmers, it is desirable that its effects on non-target organisms in major maize cropping systems be determined.

Impacts on non-target organisms could have implications for the preservation of biodiversity and on natural control of pests through possible effects on natural enemies (parasitoids and predators). Information on the magnitude of these effects (if present) on the major non-target organisms is useful to the biosafety regulatory bodies in order to make informed decisions on deployment of Bt maize in Kenya.

The organisms found in a given maize habitat are influenced by the prevailing environmental conditions and by crops intercropped with maize. Therefore, the first step in determining the effects of Bt maize on non-targets in Kenya is to identify the range and relative abundance of the target and non-target organisms in the country's five maize growing regions. This information will be used to determine which organisms to focus on in studies on non-target effects of Bt maize.

Potential arthropods to be selected include natural enemies, pollinators, and decomposers of organic material. Following up on the cited need for information on non-target effects of Bt-maize, on-farm studies were initiated by KARI through the IRMA project in two maize growing regions in coastal and western Kenya in the latter half of 2000.

The same study is being extended to the nation's three other maize growing regions. At each participating farm, various traps were set up to collect arthropods from the maize plot area, on and in the maize plant, and from the soil surface. The on-farm plots were managed by the farmers, while extension staff and the KARI/IRMA team was responsible for the weekly collection of arthropods (from traps) and their identification.

Results of this study showed that in western Kenya the stem borers that attacked maize were *Busseola fusca, Chilo partellus*, and *Sesamia calamistis* in descending order of abundance. In the coastal site, the stem borers were *C. partellus*, *Chilo orichalcociliellus*, *S. calamistis*, and *Cryptophlebia leucotreta*, in descending order. Stem borer parasitoids recovered in the coastal area were *Cotesia flavipes*, *Cotesia sesamiae*, *Goniozus indicus*, *Chelonus curvimaculatus*, and *Dentichasmias busseolae*; in the western region they were *Cotesia sesamiae* and *Denstichasmias busseolae*.

Other potential parasitoid groups recovered from the traps at both sites were Tachinid flies, hump-backed flies, Muscid flies, flesh flies, and frit flies. Potential predator groups were ants, ear wigs, spiders, carabid beetles, lady bird beetles, and rove beetles. The only pollinator was the honey bee and the decomposers were termites and earthworms. Crickets were abundant and were thus considered to contribute to biodiversity. The non-target effects on arthropods will be tested using a bi-season list of potentially important arthropods derived from this preliminary research. The studies on non-target effects will be conducted in three stages: (1) in a biosafety laboratory where the direct and indirect effects will be tested; (2) in open quarantine, where populations of the important arthropods will be monitored in Bt maize and non-Bt maize fields; and (3) in farmers' fields, where arthropods will be monitored in Bt maize grown in Kenya's major maize production regions. This study will provide information that will contribute to the safe deployment of Bt maize in Kenya. It will also increase awareness and thus chances of adoption of Bt maize by the farmers since they are directly involved with the survey, arthropod characterization, and field monitoring.

KARI entomologist Josephine Songa and KARI breeder Dr. Odongo collect insects from a trap placed in a farmer's field to determine the types of insects and other organisms present, and their prevalence.

—**Dr. Josephine Songa,** KARI Entomologist

Do you have a question or comment about the IRMA project or the quarterly newsletter articles? Or perhaps you have an article you would like to contribute. If so, please contact the IRMA Quarterly Newsletter editor at d.poland@cgiar.org or IRMA Coordinator Stephen Mugo (contact information on back page).

Gene Constructs Developed to Fight Kenyan Stem Borers

The CIMMYT Applied Biotechnology Center (ABC) has generated maize germplasm that may likely possess resistance to many of the borer pests found in Kenya.

Different synthetic versions of the *Bt* genes that confer resistance to southwestern corn borer (SWCB) and sugarcane borer (SCB) (specifically, the *cry1B* and *cry1Ac* genes) were used to transform tropical maize germplasm.

Progress was made on other fronts as well. The synthetic *cry1E* gene was found to express a protein in transgenic maize and possibly to be active against fall armyworm (FAW). In addition, the translational fusion *cry1B-1Ab* gene produced a single fusion protein and showed resistance to the SWCB and SCB. In the T4 (BC4) generation, the transformed plants with *cry1Ac* and *cry1B* showed resistance to the three insects, with expected Mendelian segregation, as did the T1 transgenics with the *cry1E* and *cry1B-1Ab* genes.

The IRMA project, in accordance with CIMMYT-ABC policy, has given high priority to identifying and developing gene constructs that do not contain herbicide or antibiotic selectable markers. Insect resistant tropical maize varieties produced by IRMA will carry only the modified *Bt* genes. In conventional screening for transgenic Bt plants, the *Bt* gene is linked to one or more "marker" genes, such as the *bar* gene (which confers resistance to the herbicide Basta™) or antibiotic genes (that confer resistance to antibiotics such as ampicillin). By applying either the herbicide or the specified antibiotic to the experimental maize plants, researchers can then determine which plants actually carry the marker gene and hopefully the associated *Bt* gene. Those with marker/*Bt* genes will survive the treatment, and those without them, will die. The difficult next step is removing the marker gene.

One hundred and seventy-seven transgenic maize plants carrying the purified *cry1B* gene have been obtained and grown in the CIMMYT biosafety greenhouse. Two infestations of neonate larvae on the small plantlets were used to select plants that are resistant to SWCB. A simple test, in which leaves of insect resistant plants are painted with herbicide, selected 51 transgenic plants that either do not have or do not express the *bar* gene. Molecular tests using PCR or Southern blots will allow us to completely eliminate plants that contain *bar* genes. Only transgenic plants carrying the purified *cry1B* gene will be used for further progeny tests and for supplying breeding materials to Kenya in the future.

During the latter half of 2000, the maize transformation group at CIMMYT transformed tropical maize with the "clean" *cry1Ab* gene, which is at the regeneration stage; and "clean" *cry1E* gene, which is at the callus selection stage. Currently, the group is working on transforming maize with the "clean" translational fusion gene *cry1B-1Ab*.

—Dr. Alessandro Pellegrineschi,
CIMMYT Cell Biologist

Editors Note: Dr. Pellegrineschi has replaced Dr. Natasha Bohorova who was previously conducting the transformation work for the IRMA project. Dr. Bohorova is now working with a biotechnology company in the United States.

Cell biologist Alessandro Pellegrineschi examines transformed maize callus tissue. After successfully transferring various *Bt* genes into maize, CIMMYT's ABC lab is now working on producing strictly "clean events" that do not carry either herbicide or antibiotic markers.

IRMA Project Document Series

The following project documents were prepared and published:

- KARI/CIMMYT IRMA project. Strategic plan. IRMA Project Document No. 1. Nairobi, Kenya
- *Development and Deployment of Insect Resistant Maize: Proceedings of a Workshop.* IRMA Project Document No. 2. Siambi, M., S.N. Mugo, and J.A.W. Ochieng (eds.). 2000. Nairobi, Kenya: KARI and CIMMYT.
- Stakeholders Meeting: Insect Resistant Maize for Africa (IRMA) Project. IRMA Project Document No. 3. Mugo, S.N., D. Poland, H. De Groote, and D. Hoisington (eds.). 2000. Nairobi, Kenya: KARI and CIMMYT.

IRMA On-Farm Yield Assessment Trials Attract Extension Staff and Farmers

Recent on-farm and on-station trials focused on stem borer damage drew substantial interest from extension staff and local farmers alike. Trials in Kakemega and Kitale were the first of a series to assess regional and national maize yield losses resulting from stem borers, specifically average and maximum yield losses due to *Chilo partellus* and *Busseola fusca.*

This information will encourage development of resistant germplasm well suited to Kenya's diverse maize growing regions, and also serve as basis for assessing the impacts of such new technology and germplasm. Such data is currently available for a few areas, but only for leaf damage. Other data is from farmer perceptions of damage from field surveys. However, very little data is available on estimates of yield loss from paired experiments. These trials are aimed at generating data on losses from artificial and from natural infestations against protected control treatments.

Yield loss assessment trials were grown on-station and on-farm. On-farm, farmer grown and managed yield loss assessments plots were grown at 30 locations in the country by IRMA project socioeconomists during 2000B season. On-farm variety evaluation trials were grown at four sites located on four farms in different sub-ecologies. Selected farms were accessible, with level and uniform soil types, and had a good chance of being properly managed. Although farmers managed the trials, our collaborators were responsible for insect control, data collection, and harvesting. The cultivars grown included commercial releases, popular local varieties, and experimental hybrids in each region. Only natural infestation and a protected control were used on-farm so as not to introduce borers into farmers' fields through artificial infestation. Protection was accomplished with Bulldock, a popular insecticide.

The trials at two sites in Kakamega and two sites in Kitale were very good. The extension and research staff were interested in the treatments and management and decided to hold farmer field days to demonstrate improved varieties showing response to diseases, plant aspect, and yield potential. The Kakamega field day was held on July 25, 2000 at Mrs. Otinga's farm in Lurambi Kakamega. A turnout of more than 100 farmers and primary and secondary students as well as local authorities attended. In Kitale Dr. George Ombakho organized a field day at Mrs. Florence Nabwera farm in Kimilili Bungoma district on September 12, 2000, which was equally well attended.

—**Dr. Stephen Mugo,** IRMA Project Coordinator

Dr. Odongo, maize breeder KARI/RRC Kakamega and principal collaborator in the KARI/CIMMYT IRMA Project explains insect resistant maize variety trials to researchers and Ministry of Agriculture extension staff in a farmer's field in Kakamega.

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