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Insect Resistant Maize for Africa
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*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 Syngenta Foundation for Sustainable Agriculture.*

# IRMA Economics Study Reveals Farmer Selection Criteria for Maize in the Moist Mid-Altitude Western Kenya Zone

As part of a nationwide study, the IRMA Economics team conducted Participatory Rural appraisals (PRAs), in the moist midaltitude zone in Western Kenya. The team, comprising CIMMYT economist Hugo De Groote and KARI economists Martins Odendo, Omari Odongo, and Patrick Oucho undertook the research to identify the attributes farmers consider in choosing their varieties, the use of local and improved varieties in different areas and by different groups, and the constraints farmers face in maize production, in particular pests and diseases.

**Figure 1. Women farmers participate in maize variety evaluation in Kenya**

The surveys entailed focused group discussions with male and female farmers (figure 1) as well as key informants. In total, 143 villagers (of which 60 women) participated in eight group discussions in five villages, sampled from three districts. The districts were selected purposely to represent diverse ecological and socio-economic environments: Busia District (a *Striga* prone area), Butere-Mumias (a sugarcane and maize zone) and Homa Bay (a *Striga* and drought prone area). In each district, the villages were selected using multi-stage random sampling.

***The surveys entailed focused group discussions with male and female farmers as well as key informants. In total, 143 villagers participated in eight group discussions in five villages, sampled from three districts.***

The PRA results show that farmers have varied selection critera for maize varieties and use them in diverse combinations. The top three criteria are high yield, early maturity, and tolerance to *Striga*. Other important criteria are, in order of importance, the low cost of acquiring the seed, grain characteristics, low external input demand and resistance to field and storage pests. Criteria differ among sites and between groups, reflecting a different use of varieties between sites and between groups.

Farmers grow a wide range of varieties. In total, 20 different varieties were identified in the zone, eight of which are local. In each district, farmers grow 9 or 10 varieties, of which 3 to 4 are local. Local varieties are generally more popular than improved varieties. Only one of the local varieties, Shipindi, is grown in two districts. Three local varieties are grown by a majority of farmers in their district, but are not found in the other districts: Ka-Buganda in Busia, Msamaria in Butere-Mumias, and Nyamula in Homa Bay. Shipindi and Nyuamula are popular yellow varieties, although their price is generally lower than for white maize varieties.

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*(IRMA Economics Study cont'd...)*

The heterogeneity of the zone is also reflected in the range of improved varieties. They include late maturing hybrids such as H622 (grown by 28% of farmers present at the meetings) and H614D (21%), the OPV Katumani composite (second in popularity with 26%), and intermediate maturing hybrids such as Pioneer 3253, H513, and H511. There is also a clear gender distinction: within a locality, more women usually grow the local varieties, and more men grow hybrids.

The most important constraints to maize production, as reported by the farmers, are low soil fertility, cash or credit availability to purchase inputs, and poor extension services. The farmers explain that the cash constraint is a major problem, and its alleviation would lead to mitigation of many other constraints. The next group of constraints is the lack of farm implements (tools) and the related lack of labor. The stresses frequently cited are unreliable rainfall and insect pests. Quality of seed was also mentioned often, though not the cost of seed. Diseases did not usually rank high on the farmers' perceived constraints list. As a result of cash and other constraints, farmers recycle seed of cultivars for several years including the local varieties and the hybrids. They apply little or no fertilizer and no pesticide in maize fields.

*Striga*, a parasitic weed typical for the zone, was generally perceived as the most important pest, followed by stem borers and weevils, a major storage insect pest. In Busia, stem borers were ranked first, and in Butere-Mumias, weevils were considered more important than stem borers. Losses due to stem borers were estimated at 20-30%. Farmers would be interested in buying varieties resistant to stem borers, but the price should not be more than 25% higher than that of existing varieties.

Farmers are clearly interested in new varieties, and will adopt them as the recently introduced Pioneer 3253 shows. However, new varieties and crop management practices need to take into account not only farmers' preferences, but also their conditions. These conditions include erratic rainfall, low soil fertility, and low input use, exacerbated by lack of credit and available labor, all within a framework of unclear agricultural policies. The IRMA team determined that there are clearly opportunities for a wide range of varieties in the zone, and that breeders should focus on hardy varieties that are not dependent on high input use, with resistance to *Striga*, stem borers, and weevils.

The government can improve conditions for maize producers by setting and implementing clear policies. Policies are needed to encourage new companies to develop new varieties, while at the same time setting high quality standards and providing the regulatory agencies with the means for quality control. The government needs to provide the means for agricultural extension, and set policies to promote the provision of extension and credit by NGOs and the private sector. Seed producers and distributors need to operate efficiently so that the price of new varieties does not increase by more than 25%, the maximum increase farmers are willing to accept.

**— Martins Odendo, Hugo De Groote , Omari Odongo, and Patrick Oucho**

# Bt Gene Expression Determined for Second-Generation "Clean" Events

Generation of insect-resistant, transgenic maize plants through the production of insecticidal crystal proteins (Cry proteins) of *Bacillus thuringiensis* (*Bt*) is one of the approaches the IRMA Project is evaluating. First generation events that contained both the Bt (*cry*) gene and selectable marker (*bar*) genes were used for the leaf bioassays to determine the effectiveness of each gene against the targeted stem borers. Efforts are now focused on producing second-generation transgenic plants that contain so-called "clean" events without marker genes. Following extensive screening of over 3,000 initial transgenic plants, nine "clean" events have been identified; six of these contain the *cry1B* gene and three contain the *cry1Ab* gene.

In preparation for submitting an application to import these for further evaluation and use in Kenya in the Biosafety Greenhouses that are being constructed, information about the complexity of the transgene insertions and data on the expression of the introduced *cry* genes is necessary. Therefore, these nine transgenic lines were tested for Cry protein expression in root, leaf, and whorl tissue in CIMMYT's Applied Biotechnology Center. Detection and quantitative determination of the amount of Cry1B and Cry1Ab protein was monitored by enzyme-linked immunosorbant assays (ELISA). An untransformed tropical maize hybrid (CML216 x CML72) and commercial E176 transgenic maize were used as the negative and positive controls.

Each event had a range of expression in the different tissues. Of particular interest was Event 127 (*cry1B*) that had the highest levels of Cry protein in the leaves and whorl, with low levels in the roots. In addition, the protein amounts in the leaves and whorls were equivalent to those found in some of the commercial events.

These data provide important information for the development of tropical maize varieties adapted to Kenya with protection against insects, while at the same time minimizing the expression of the *cry* genes in seed and root tissues.

**—Alessandro Pellegrineschi**

# IRMA Project is Presented at the Second Inter-Institutional Seminar on Biotechnology In Kenya

On 14 March, 2002, Dr. Stephen Mugo, Coordinator KARI / CIMMYT IRMA Project made a presentation entitled "Harnessing Bt Technology for Stem Borer Control: The KARI / CIMMYT IRMA Project Approach," to the Second Inter-Institutional Seminar on Biotechnology in Kenya.

Sponsored by the African Biotechnology Stakeholder Forum, the seminar covered a wide range of subjects in biotechnology in addressing institutional capacity for biotechnology research, local institutional collaboration, and priority for biotechnology R&D projects in Kenya. The seminar attracted 50 participants from 25 institutions in Kenya. Intense discussions followed the seminar presentation.

Mugo in his IRMA presentation stressed the considerable damage wrought by stem borers in Kenya (maize grain losses averaging 15%, a loss valued at US$ 60-90 million annually, depending on the price of maize). He went through the various stem borer control strategies, including cultural, chemical, biological (micro-organisms and predators or parasites of the pest), and host plant resistance derived through conventional breeding or using biotechnological tools such as marker assisted selection (MAS) and genetic engineering.

CIMMYT, Mugo said, recognizes the potential of biotechnology to develop robust germplasm for developing countries. It also recognizes that relevant biosafety, environmental, and community concerns need to be addressed in the development and routine use of GMOs.

To apply that recognition to the situation on the ground, the IRMA Project was created, based on a collaboration between CIMMYT and KARI, with funding from the Syngenta Foundation for Sustainable Agriculture. The project's goal is to increase maize production and food security through the development and deployment of insect resistant maize to reduce losses due to the stem borer. The project focuses on identifying the best technologies or combination of technologies (developed conventionally or through biotechnology) to combat stem borers.

Mugo then articulated the main activities of IRMA project.

**Product development**: Bt-maize lines have been developed through genetic engineering using Bt source lines from private companies and synthesized with other partners. The molecular characterization of first-generation events has been carried out. Second-generation "clean" events, involving the removal of selectable markers to retain only the gene of interest, are in progress.

Experiments have been conducted to identify Bt Cry proteins that are effective against Kenyan stem borers. Leaf bioassays performed with Kenyan stem borers found *Bt* genes that were effective against *Chilo partellus*, *Chilo orichalcociliellus*, *Sesamia calamistis*, *Eldana sachharina* stem borers but *Busseola fusca* was controlled only 60%. Research is currently underway to identify and develop sources of insect resistance from several genotypes and lines from CIMMYT and KARI

**Product dissemination**: The dissemination of insect resistant Bt maize takes into account the need to study (1) potential effects of Bt maize on nontarget organisms, (2) development of appropriate insect resistance management strategies including the importance of refugia and (3) gene flow studies.

The characterization of target and non-target arthropods has been carried out in all five major maize growing environments in Kenya including the humid lowland coastal, the midaltitude dry, the midaltitude moist, the midaltitude transitional, and the highlands. Evaluation of a Bt-biopesticide compared to the conventional chemical insecticide for the management of stem borers and their effects non-target parasitoids, predators, and general insect diversity in a maize / bean cropping system has been conducted. Preliminary results indicates that the Bt-biopesticide preserves a diversity of all these threes groups of organisms. This further confirms that Bt maize technology is compatible with biocontrols in integrated pest management systems.

**Impact assessment:** Thus is an effort to shed light on how the Bt technology and insect resistant maize will fit into and influence the socio-economic factors in the farming community in Kenya. This has involved several approaches, including participatory rural appraisals (PRAs), crop loss assessments, a survey of stockists in agriculture, seed quality and regulations, know-how and extension, maize seed industry study, and assessment of crop losses due to stem borer damage.

**Technology transfer:** The IRMA Project has assisted in establishing a biosafety greenhouse, an open quarantine field, and facilities for molecular analyses. The project has also provided training for KARI and other scientists and journalists in a number of skills relevant to the project. In addition, it has provided support for irrigation facilities, an insectary, and an entomology laboratory.

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**Communication and documentation**: This component of the IRMA project aims to produce materials to inform diverse audiences about insect resistant maize technologies. Communication is effected through Stakeholders meetings, press releases, the IRMA Updates newsletter, workshops, public relations materials, and the IRMA website which is now linked to the KARI website. The project has supported ABSF in meeting their objectives of raising awareness.

**Human health and environmental concerns of Bt maize**: Concerns on the technology within the IRMA project are addressed within the activities where feasible as well as reliance on information generated elsewhere.

Mugo addressed some of the environmental concerns about Bt maize including the loss of genetic diversity through erosion of local varieties, creation of insect resistant super weeds through gene flow from Bt maize, the development of insect resistance to Bt, impact of Bt crop residues on soil biota, and impact of Bt on non-target organisms.

Future IRMA activities were also presented by Mugo. They include an application to import Bt maize seeds for second-generation events for tests in the biosafety greenhouse and open quarantine fields. Gene-flow studies to determine "contamination" distance, criteria used by farmers to select seed for recycling and estimation of the rate of introgression at the farm level. A baseline survey is planned to build a database for impact assessment, and regional workshops for extension staff was set for July. Other activities will focus on creating greater awareness about the technology among policymakers, the development of fact sheets, and training in report writing.

Mugo concluded his presentation with the hope that the IRMA project will serve as a positive example to other nations on how institutions and projects in the region can develop partnerships to safely deliver the technology for the betterment of the nations and their people.

Mugo's talk stimulated discussion on a number of issues including local capacity for monitoring biosafety concerns, the need to improve institutional collaboration, linkages to strengthen capacity and availability of equipment, and the need to address intellectual property rights issues relating to the IRMA project.

**—Stephen Mugo**

# Developing Maize Varieties to Meet the Needs of Farmers: Advances in Storage Pest Resistance

Based on surveys conducted as part of the IRMA project, farmers ranked insects attacking stored maize as one of the major pest problems facing small-scale farmers. Small-scale farmers are often forced to sell maize shortly after harvest to minimize losses during storage. Selling maize into a saturated market results in low pricing, in some cases well below world market prices. The farmer then has to purchase maize later in the season at elevated prices. Moreover, recurrent drought in Kenya has created a need for long-term grain storage as a food security strategy. However, this strategy is somewhat compromised by attack of the stored grain by storage pests, accounting for an estimated 10 to 15% grain weight loss.

Kenyan farmers cited two major storage pests in the surveys: the maize weevil (*Sitophilus zeamais*) (Figure 2) and the larger grain borer (LGB)(*Prostephanus truncates*). One way in which the IRMA project tries to minimize the impact of storage pests is to screen elite lines, open pollinated varieties (OPVs) and hybrids to identify those that are most resistant to storage pests. The objective is to reduce insect damage in the field and grain store to improve both the quantity and quality of stored grain for human consumption.

**Figure 2. Maize weevil (*Sitophilus zeamais*)**

To address this problem, the IRMA project established a screening facility at the Kiboko Research station under the leadership of Paddy Likhayo (Figure 3). Likhayo has extensive experience working on the LGB and has now set up his lab for large-scale screening of maize for weevil evaluations.

In the current study, 408 inbred lines, 70 open pollinated varieties (OPVs) and 115 maize hybrids were screened from the Kiboko 2001B planting. Harvested maize genotypes were disinfested by fumigatation in plastic drums using Phostoxin™ (phosphine gas) for 7 days.

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**Figure 3. Paddy Likhayo and David Bergvinson inspect kernel damage by maize weevil at Paddy's lab at Kiboko, Kenya**

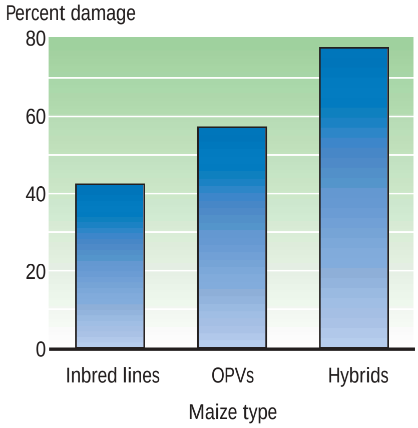



**Figure 4. Comparison of damage levels caused by the maize weevil (*Sitophilus zeamais* Motsch.) to maize germplasm from season 2001B plantings at Kiboko.**

*(Developing Maize Varieties cont'd...)*

The maize grains were cleaned using a 25-mesh screen and a hundred grams of each maize genotype placed into 500 ml jars with a perforated lid. Twenty 4-week old unsexed adult weevils were introduced into each jar. After three months of incubation under ambient conditions, the contents of each jar were sieved to separate grains, insects, and powder. The dust was weighed and expressed as a percentage of the original weight. Adult weevils were counted (live and dead) and the grains were sorted into damaged (grains with holes and / or tunnels) and undamaged grains. Grains in each fraction were counted and weighed and expressed as a percentage.

Significant differences in grain damage and adult emergence were observed between inbred lines, OPVs, and hybrids (Figure 4). This is likely related to seed size, with the larger hybrid seeds providing a more suitable substrate for weevil development. Maize inbred lines that sustained 5% or less grain damage were deemed to be highly resistant to the maize weevil and are listed in Table 1. Among these are some lines with conventional stem borer resistance developed at CIMMYT, which could complement Bt resistance to provide a broad-based resistance to both stem borers and storage pests. Although more susceptible than lines, the hybrid CML384-B / CML78 showed a moderate level of resistance (Table 2). A similar level of resistance was found in several OPVs such as MATUBA, NIP25, and ECA-EE-38.

It is hoped that the results from these laboratory post-harvest studies will help breeders to promote maize genotypes that are more resistant to storage pests that pose a major constraint on food security for small scale farmers. Screening for weevil resistance will continue and expand to LGB screening in the next round of characterization.

— **Paddy Likhayo, Stephen Mugo, and David Bergvinson**

**Table 1. Mean[1] percent grain damage (N/N) caused by *S. zeamais* to maize cultivars and number of adult insects emerged**

| Maize cultivars | Damage | No. adults |
|---|---|---|
| MBR C6 Bc F408-2-B | 0.6 | 23.7 |
| MBR Et/MBR Bc C1 F12-2-1-1-B-B-B | 1.3 | 20.7 |
| MBR C6 Bc F293-1-B | 1.3 | 22.3 |
| MBR C6 Bc F406-1-B | 1.3 | 20.3 |
| MBR/MDR C3 Bc/MBR C5 Bc F84-1-B | 2.0 | 26.7 |
| MBR Et/MBR Bc C1 F1-1-1-1-B-B-B | 3.0 | 27.3 |
| P390bco C3/254/247 F74-2-2-B | 4.1 | 30.7 |
| P390bco C3 F13-1-2-1-1-B-B-B-B | 5.0 | 34.0 |
| P390bco C3 F191-1-1-1-4-B-B-B-B | 6.0 | 47.7 |
| MBR C6 Bc F28-1-B | 7.0 | 42.7 |
| MBR C6 Bc F393-2-B | 8.0 | 47.0 |
| MBR/MDR C3 Bc/MBR C5 Bc F102-2-B | 9.0 | 51.0 |
| CML247-# | 20.3 | 136.0 |
| LSD (p=0.05) | 17.2 | 79.0 |
| CV | 38.6 | 34.3 |

**Table 2. Mean[1] percent grain damage (N/N) caused by *S. zeamais* to maize cultivars and adult insects emerged**

| Maize type | Damage | No. adults | Initial MC |
|---|---|---|---|
| NIP25 | 18.1 | 96.0 | 11.8 |
| FAW/NON TUXPE | 28.8 | 162.3 | 12.0 |
| MATUBA | 15.0 | 99.3 | 11.7 |
| DLC1 (LOCAL CHECK1) | 18.6 | 84.7 | 11.2 |
| POOL 16 SR ZAMBIA | 23.1 | 127.7 | 11.7 |
| CML384-B/CML78 | 27.8 | 154.5 | 12.4 |
| LSD (p=0.05) | 38.5 | 195.8 | |
| CV | 22.0 | 22.7 | |

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**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 (s.mugo@cgiar.org).**