Insect Sci. Applic. Vol. 21, No. 4, pp. 409–415, 2001
Printed in Kenya. All rights reserved 0191-9040/01 $3.00 + 0.00

OPTIONS IN DEVELOPING STEMBORER-RESISTANT MAIZE: CIMMYT'S APPROACHES AND EXPERIENCES

S.N. MUGO[1], D. BERGVINSON[2] AND D. HOISINGTON[2]
[1]International Maize and Wheat Improvement Center (Centro Internacional de Mejoramiento de Maíz y Trigo, CIMMYT), P. O. Box 25171, Nairobi, Kenya;
[2]CIMMYT, Lisboa 06600, Mexico D.F., Mexico

(*Accepted* 20 *August* 2001)

Abstract—Various insect pests, of which stemborers are the most widely distributed and damaging, affect about 30 out of the 35 million hectares planted with maize in developing countries. Chemical control, biological control, cultural methods and host plant resistance constitute the four general approaches to stemborer control. The use of stemborer-resistant maize increases farming efficiency by both reducing yield losses from stemborer damage and reducing or eliminating the cost of insecticides and other inputs. In the past, CIMMYT followed conventional breeding methods to develop germplasm resistant to stemborers and molecular technology, quantitative trait loci (QTL) in marker-assisted selection (MAS) to select for improved stemborer resistance in elite lines. More recently, CIMMYT has developed the capacity to produce transgenic maize with resistance factor(s) derived from genes that encode delta-endotoxins derived from the soil bacterium *Bacillus thuringiensis (Bt)*. A sub-tropical source population with multiple borer resistance (MBR population) was developed by recombination and recurrent selection under infestation with four stemborer species. Marker-assisted selection is being used in two African countries to promote the transfer of resistance into elite and adapted germplasm. The Insect Resistant Maize for Africa (IRMA) project is pursuing the transfer of *Bt*-based resistance to adapted maize germplasm, initially in Kenya, but later to other interested African countries. CIMMYT's varietal release strategy is to pyramid *Bt* genes into maize populations with existing multigenic pest resistance, in order to enhance both the levels and durability of plant resistance to maize pests. This paper discusses the various approaches used at CIMMYT to develop stemborer-resistant maize germplasm.

Key Words: stemborers, maize, maize breeding, host plant resistance, marker assisted selection, transgenic maize, delta-endotoxins, *Bacillus thuringiensis*, multiple borer resistance maize populations

Résumé—Plusieurs espèces d'insectes infestent environ 30 sur 35 million d'hectares plantés de maïs dans les pays en voie de développement. Parmi ceux-ci, les foreurs sont les plus destructifs et les plus largement repartis. La lutte chimique, biologique, les méthodes culturales et l'utilisation des plantes résistantes forment les quatres approches courantes de lutte contre les foreurs. L'utilisation du maïs résistant aux foreurs augmentent l'efficacité culturale en réduisant les pertes en récoltes dues aux foreurs grâce à la réduction ou l'élimination des dépenses en insecticides ou autres entrants agricoles. Dans le passé CIMMYT a suivi la méthode conventionnelle des multiplications pour développer du matériel génétique résistant aux foreurs, la technologie moleculaire, l'aspect quantitatif du loci (QTL) des marqueurs assistés (MAS) aide à selectionner dans les lignes elites une meilleure resistance aux foreurs. Plus recemment, le CIMMYT a dévéloppé la capacité de produire

Corresponding author: SNM.
E-mail: smugo@cgiar.org

un maïs transgénique avec des facteurs de résistance dérivés des gènes qui codent pour une delta-endotoxin dérivée des bactéries du sol *Bacillus thuringiensis (Bt)*. Une souche sous tropicale avec une résistance multiple aux foreurs a été dévéloppée par recombinaison et selection recurrente sous une infestation de quatres espèces de foreurs. La sélection par marqueur assisté est actuellement utilisée dans 2 pays africains pour promouvoir le transfert de la résistance dans le matériel génétique élite adapté. Le projet sur la résistance du maïs aux insects (IRMA) suit le transfert de la résistance basée sur *Bt* au matériel génétique de maïs adapté, initiallement au Kenya, et après aux autres pays africains qui s'y intéresseraient. La stratégie de propogation des variétés de CIMMYT est d'insérer le gène de *Bt* dans les populations de maïs ayant des gènes multiples de résistances aux ravageurs dans l'optique d'améliorer les niveaux et la durée de la résistances des plantes aux ravageurs du maïs. Cet article discute des différentes approches utilisées par CIMMYT pour dévélopper un materiel génétiquement résistant aux foreurs de tiges de maïs.

Mots Clés: foreurs, maïs, selection des plantes maïs resistantes, selection par marqueur assisté, maïs transgénique, delta-endotoxins, *Bacillus thuringiensis*, population de maïs avec resistance multiple aux foreurs

INTRODUCTION

Approximately 30 out of the 35 million hectares planted with maize in developing countries are seriously affected by insect pests, which cause variable losses in grain yield and quality. Among the major groups of insects that infest maize, stemborers are the most widely distributed and damaging to maize worldwide. In Africa, there are several economically important stemborer species. These include the pink stemborer, *Sesamia calamistis* Hampson, found throughout Africa; the African stemborer, *Busseola fusca* Fuller and the African sugarcane borer *Eldana saccharina* Walker, found in nearly all of sub-Saharan Africa; and the spotted stemborer, *Chilo partellus* (Swinhoe), present in most parts of eastern Africa.

The four general approaches to crop pest control—chemical control, biological control, cultural control and host plant resistance—are all associated with specific advantages and disadvantages. For instance, chemical control, the most widely used method, exposes the farmer to health risks and can result in pesticide loading in the environment. Biological control often requires trained personnel to identify and deploy control agents, and the cooperation of the farming community. Cultural control measures are best when used in combination with other control measures, and rarely stand alone.

Host plant resistance is availed to farmers encapsulated in the seed, which ensures that after purchasing the seed, farmers need not invest in any more inputs in order to control stemborers. In this way, stemborer-resistant maize reduces yield losses from stemborers as well as reduces or eliminates the expense of insecticides and their associated health risks.

Plant resistance to stemborers is a genetic trait which manifests itself as antibiosis, in which the biology of the pest is adversely affected after feeding on the plant; non-preference (antixenosis), whereby the plant is not desirable as a host and the stemborer seeks alternative hosts; and tolerance, where the plant is able to withstand or recover from stemborer damage. Resistance may be controlled by different allelochemicals that kill or impair the growth of the pest. In addition, morphological factors, including increased leaf fibre and silica content as a defence against the European corn borer (ECB) (Bergvinson et al., 1997a; Rojanaridpiched et al., 1984), surface wax and high hemicellulose against the southwestern corn borer (SWCB) (Hedin et al., 1993), or a thickened cuticle against the sugarcane borer (Ng, 1988) have been identified as resistance mechanisms.

CIMMYT has pursued both resistance and tolerance mechanisms to stemborers (Kumar, 1997). Screening and breeding maize for oviposition non-preference is not done, mainly because moth oviposition behaviour can evolve to overcome the oviposition resistance of germplasm and because soil and environmental factors interact to make adult oviposition behaviour measurements difficult to predict reliably (Mihm, 1989).

APPROACHES IN DEVELOPMENT OF INSECT-RESISTANT MAIZE

Historically, CIMMYT followed conventional breeding methods to develop germplasm resistant to stemborers. However, more recently CIMMYT has initiated the development of insect-resistant germplasm using molecular and transformation technologies, including the use of quantitative trait loci (QTL) to select for improved stemborer resistance in elite lines. CIMMYT has also developed the capacity to produce transgenic maize with resistance factor(s) derived from genes that encode delta-endotoxins—proteins derived from the soil bacterium *Bacillus thuringiensis* (*Bt*). The protein binds to the brush border membrane vesicles of the peritrophic membrane resulting in pore formation and larval mortality of susceptible insects (Gill et al., 1992). CIMMYT's strategy is to pyramid *Bt* genes into maize populations with existing multigenic pest resistance, in order to enhance both the levels and durability of their pest resistance.

Conventional breeding

A sub-tropical source population with multiple borer resistance (MBR population) was developed by recombination and recurrent selection under infestation with southwestern corn borer (SWCB), sugarcane borer (SCB, *Diatraea saccharalis*), European corn borer (ECB, *Ostrinia nubilalis*) and fall armyworm (FAW, *Spodoptera frugiperda*) (Mihm, 1985). MBR was developed on the premise that new germplasm with resistance to the complex of insect problems in a given area is more useful than one with resistance to only a single species of insect pest. In addition, the resistance must be relatively durable, and the germplasm acceptable for yield and other agronomic characteristics in its intended area of use.

The breeding procedure used to develop MBR is detailed in Fig. 1, adapted from Smith et al. (1989). In summary, sources of resistance to SWCB were gathered from Mississippi State University, CIMMYT population 47 and the Islands of Antigua for resistance to SWCB, Cornell University and University of Missouri for resistance to ECB. The sources were screened for resistance to SWCB. In general, ECB sources were not resistant to SWCB but were included to introduce sources to other pests. Initial recombination was undertaken in the absence of any deliberate selection for insect resistance. This allowed maximum recombination of genes or blocks of genes without early-generation pressure, which might have fixed sub-optimal combinations of genes.

The next two recombinations were done with full-sib progenies with poorly adapted sources as males and with high numbers of families. The next generations were screened with SWCB and SCB with full-sib and half sib families. Screening for fall armyworm (FAW) was included later. Self pollinations were made during the fourth cycle to allow more precise selection for insect resistance than would be possible in full- or half-sib families. Selfing also allowed initiation of concentration of genes through a gradual S1 recurrent selection to avoid rapid fixation of sub-optimal gene combinations.

Crossing to testers was initiated to begin investigating heterotic patterns. Selected families were also recombined in a full-sib fashion, to provide a group of full vigour materials to be tested internationally for their reaction to a broad range of insect pest species. Data from 6 locations (Figs 2 and 3 for *C. partellus* and *B. fusca*) showed that although the initial mixture of materials included only sources of ECB, SCB, SWCB and FAW resistance, and although selection prior to the international testing only involved these species, high levels of resistance to *C. partellus* and *B. fusca* are present in MBR.

The extent of resistance to multiple species in the MBR families tested internationally is presented in Fig. 4. All but 22 of the 2000 families tested were intermediate or better in resistance to all the insect species for which they were evaluated. This suggested that there is generally a high level of multiple resistance in the MBR population.

Using information from international testing experimental varieties were made. Lines were developed by selfing. Heterotic patterns were established using the S1 testing. Gene pools were then developed from the resulting progenies. MBR populations continue to be the source germplasm for resistance to stemborer species in developing countries.

Success in developing MBR through recurrent selection is due to two factors. First, tropical maize resistant to lepidopteran pests appears to be controlled by polygenic genes and involves primarily additive variation (Hinderliter, 1983). Second, inbred lines from MBR have shown general combining ability as the most important source of variation among F1s for leaf feeding resistance and grain yield (Thome et al., 1992, 1994).

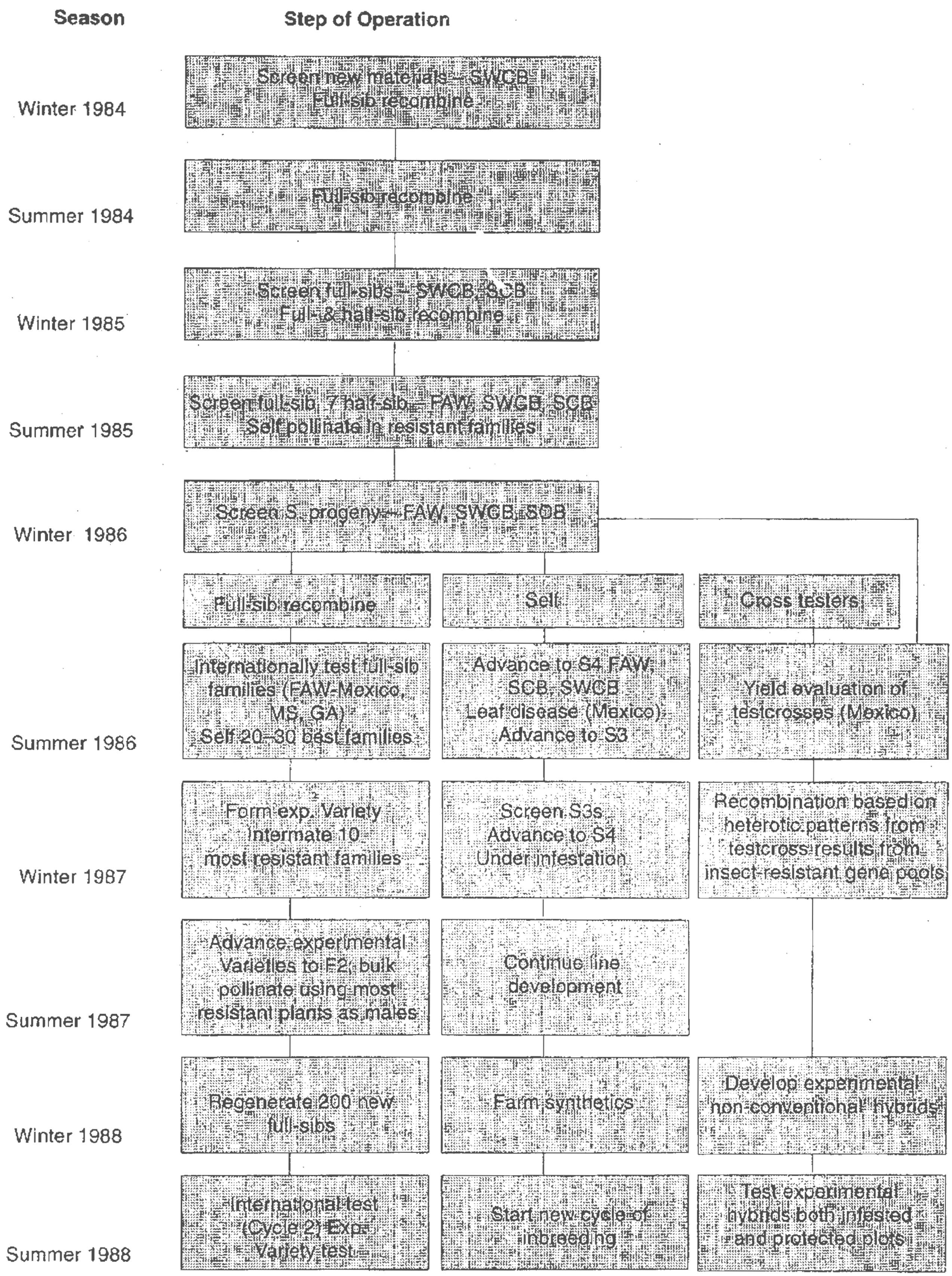

Fig. 1. Flowchart showing operations and breeding methodologies used in developing the Multiple Borer Resistance (MBR) populations, inbred line extraction and the formation of experimental varieties and 'non-conventional' hybrids (Adapted from Smith et al., 1989)

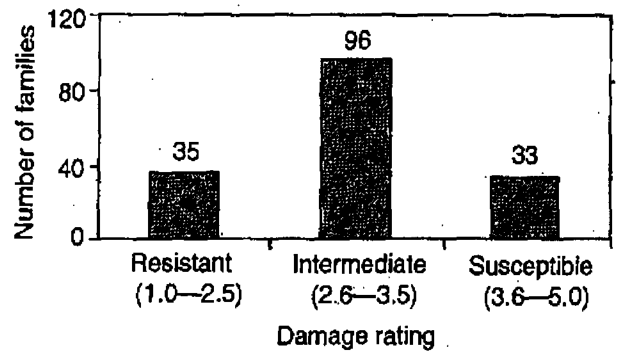

Fig. 2. Damage rating of 200 full-sib families of MBR for *Chilo partellus*

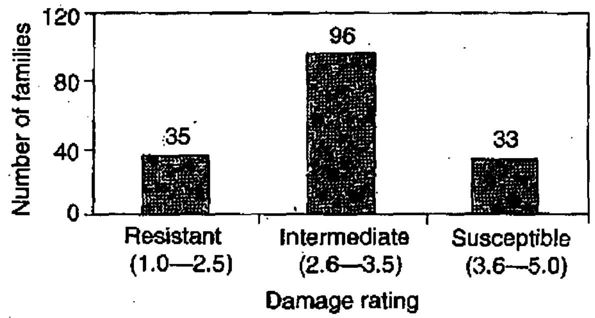

Fig. 3. Damage rating of 200 full-sib families of MBR for *Busseola fusca*

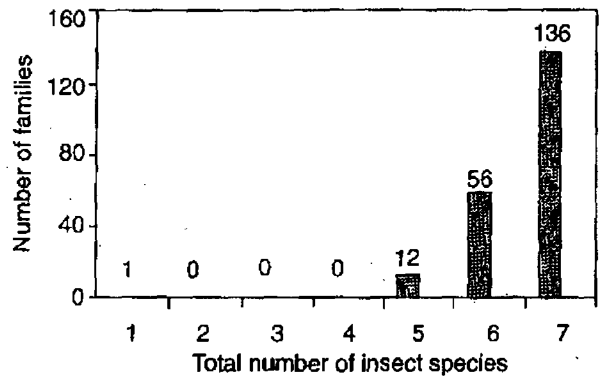

Fig. 4. Number of families vs. total number of insect species for which they carried resistance

Improvement of MBR populations through recurrent selection was further enhanced by the findings from studies of temporal and spatial changes in biochemical composition that maize resistance is toxin-related during early stages of tissue development and structurally related in mature tissue (Bergvinson et al., 1995). The immature tissue is ephemeral and employs qualitative defences such as DIMBOA (2,4-dihydroxy-7-methoxy-(2H)-1-,4-benzoxazin-3(4H)-one), an allelochemical affecting maize resistance to arthropod pests. As the plant matures, more quantitative resistance mechanisms such as phenolic fortification of cell wall carbohydrates are employed. Bergvinson et al. (1997b) concluded that biochemical composition would assist in identifying resistance mechanisms for maize improvement programmes in future.

Bergvinson et al. (1997b) evaluated MBR genotypes and found that the resistance mechanism appears to be nutritional in nature. Leaf tissue of MBR genotypes is tougher due to thicker epidermal cell walls, which restrict feeding by early instars. MBR genotypes also tend to have reduced nutritional value (lower nitrogen content) and elevated levels of fibre and cell wall phenolics, which contribute to elevated leaf toughness. Hence proteins, fibre and diferulic acid content in leaf tissue at the mid-whorl stage in plant development accounted for approximately 80% of the variation in field leaf damage scores for ECB (Bergvinson et al., 1997b).

Maize germplasm resistant to stemborers, adapted to African environments and improved through conventional breeding techniques continues to be available from CIMMYT's breeding programmes in Mexico, Zimbabwe, and Kenya. The most important ones are source populations (F1 through S4), multiple borer resistant MBR (Sub-tropical population 590), multiple insect resistant tropical MIRT (Population 390), second-generation borer (sub-tropical population 591), second-generation borer (tropical population 391), MBR elite and several subtropical insect-resistant synthetics. However, breeding for resistance to SWCB and SCB is laborious and time-consuming because it requires recurrent selection with at least four to five cycles of infestation in order to recover and verify a desirable level of resistance.

Biotechnology-mediated development of maize germplasm resistant to stemborers

Marker-assisted selection for resistance to stemborers

Quantitative trait loci (QTL) involved in resistance to SWCB and SCB have been identified in two mapping populations (Groh et al., 1998). Most of the QTLs showed additive and dominance effects and most of the QTLs common to both insects were identified from MBR populations. Marker-assisted selection technology for resistance to stemborers is now available and is being used in a collaborative project involving among Kenya, Zimbabwe and CIMMYT. MAS may help improve the efficiency of selection for resistant germplasm, but it still requires a significant investment of resources and given the complex genetic nature of resistance, may be of limited usefulness at this time.

Transgenic maize resistant to stemborers

Transgenic plants expressing *Bacillus thuringiensis* δ-endotoxins are now being used commercially in several crop species. These toxins have demonstrated good control of temperate (*Ostrinia nubilalis*) and tropical (*Diatraea grandiosella* and *D. saccharalis*) stemborers in maize. Incorporation of genes encoding δ-endotoxins into maize has provided extremely high levels of resistance.

Transgenic plants containing insecticidal proteins are already featuring prominently in agricultural systems in both developed and developing countries. Entomologists, breeders, molecular biologists, and population ecologists need to determine how best to deliver this technology to provide good pest control and reduce environmental hazards (including gene flow and retarding the development of resistance in pest populations). To achieve these objectives, we need to better understand the pest biology, behaviour, and response to insecticidal proteins; the temporal and spatial expression of toxins in transgenic plants; the dynamics of different refugia strategies in resistance management; the impact of toxin-producing plants on biological control; and how to deliver this package to resource poor farmers.

The Insect Resistant Maize for Africa (IRMA) Project

The Insect Resistant Maize for Africa (IRMA) project—a joint venture between the International Maize and Wheat Improvement Center (CIMMYT) and the Kenya Agricultural Research Institute (KARI), with financial support from the Novartis Foundation for Sustainable Development —was launched by CIMMYT and the Kenya Agricultural Research Institute (KARI) in 1999, to develop and deploy maize, specifically for Kenyan and East African conditions. A major component of its resistance management strategy to control major tropical pests is the generation of maize germplasm that possesses resistance based on co-expression of different synthetic versions of the *cry1B* and *cry1Ac* genes, which confer resistance to SWCB and SCB. In addition, the synthetic *cry1E* gene active against FAW and the translational fusion *cry1B-1Ab*, which is active against SWCB and SCB, have been introduced into a tropical maize background. Molecular analyses confirmed the integration, copy number, and transmission of the introduced *cry* gene. In T4, the transformed plants with *cry1Ac* and *cry1B* are resistant to the three insects and show the expected Mendelian segregation, as did the T1 transgenics with the *cry1E* and *cry1B-1Ab* genes. The new tropical maize carrying *cry1Ac* and *cry1B* provides breeders with another resource for their germplasm.

By using tropical germplasm that expresses the *Cry* genes, we can test the effectiveness of modified proteins against the major African pests. Leaves from transgenic maize lines will be used for bioassays with the Kenyan stemborers *Chilo partellus*, *Busseola fusca*, *Sesamia calamistis* and *Eldana saccharina*, in order to identify the effective *Bt* transgenes against each species. Future studies will determine the most effective combinations of *Bt* genes.

High priority is given to the identification and development of gene constructs and transgenic events that do not contain herbicide or antibiotic selectable markers. In addition, each *Bt* gene is isolated from its associated vector prior to transformation. These events are referred to as 'clean events' since the plants carry only the *Bt* gene and associated regulatory sequences. Only transgenic plants carrying the purified *Bt* gene(s) will be used for further progeny tests and for supplying breeding materials to Kenya in the future.

The IRMA project will be implemented initially in Kenya and the experiences and information gathered will be supplied to other African countries. A major focus of the project involves impact assessment and socioeconomic analyses. These studies, conducted jointly by KARI and CIMMYT, involve a number of aspects such as assessing the demand for insect resistant maize varieties through studies of the different maize-based farming systems, a survey of farmers' perceptions and preferences, and of consumers' preferences. Farmer participatory trials at all stages are planned to ensure that the technology is appropriate and acceptable. The cost-benefit ratio of the new technology at different levels (the seed company, the maize producer, the consumer, and society as a whole) will be assessed.

The project also aims to transfer appropriate technologies to KARI and to develop, evaluate, disseminate and monitor insect-resistant maize varieties.

References

Bergvinson D. J., Hamilton R. I. and Arnason J. T. (1995) Leaf profile of maize resistance factors to European corn borer, *Ostrinia nubilalis*. *J. Chem. Ecol.* 21, 343–354.

Bergvinson D. J., Arnason J. T. and Hamilton R.I. (1997a) Phytochemical changes during recurrent selection for resistance to the European corn borer. *Crop Science* 37, 1567–1572.

Bergvinson D. J., Arnason J. T., Mihm J. A., and Jewel D. C. (1997b) Phytochemical basis for multiple borer resistance in maize, pp. 82–90. In *Insect Resistant Maize: Recent Advances and Utilization*. Proceedings of an international symposium, 27 November–3 December 1994, Mexico, D.F. (Edited by J. A. Mihm). CIMMYT.

Gill S. S., Cowles E. A. and Pietrantonio F. V. (1992) The mode of action of *Bacillus thuringiensis* endotoxins. *Annu. Rev. Entomol.* 37, 615–636.

Groh S. D., Gonzalez-de-Leon M.M., Khairallah C., Jiang D., Bergvinson M., Bohn D.A., Hoisington D. and Melchinger A.E. (1998) QTL mapping in tropical maize: III. Genomic regions for resistance to *Diatraea* ssp. and associated traits in two RIL populations. *Crop Science* 38, 1062–1072.

Hedin P. A., Davis F. M. and Williams W. P. (1993) 2,4-dihydroxy-7-methoxy-(2H)-1-,4-benzoxazin-3(4H)-one (N-O-ME-DIMBOA), a possible toxic factor in corn to the southwestern corn borer. *J. Chem. Ecol.* 19, 531–542.

Hinderliter D. G. (1983) Host plant resistance in two tropical maize, *Zea mays* L., populations to the southwestern corn borer, *Diatraea grandiosella* Dyar, and the sugarcane borer, *D. saccharalis*. PhD thesis, University of Wisconsin, Madison, Wisconsin, USA.

Kumar H. and Mihm J. A. (1997) Mechanisms of resistance in maize to southwestern corn borer, pp. 55–56. In *Insect Resistant Maize: Recent Advances and Utilization*. Proceedings of an international symposium, 27 November–3 December 1994, Mexico, D.F. (Edited by J. A. Mihm). CIMMYT.

Mihm J. A. (1985) Breeding for host plant resistance to maize stemborers. *Insect Sci. Applic.* 6, 369–377.

Mihm J. A. (1989) Evaluating maize for resistance to tropical stemborers, armyworms, and earworms, pp. 109–121. In *Toward Insect Resistant Maize for the Third World*. Proceedings of the international symposium on methodologies for developing host plant resistance to maize insects. CIMMYT.

Ng S. S. (1988) Southwestern corn borer, *Diatraea grandiosella* Dyar and the fall army worm, *Spodoptera frugiperda* Smith: Biology and host plant resistance studies. PhD Dissertation, Mississippi State University, Mississippi State, MS.

Rojanaridpiched C., Gracen V. E., Everett H. L., Coors J. C., Pugh B. F. and Bouthyette P. (1984) Multiple factor resistance in maize to European corn borer. *Maydica* 29, 305–315.

Thome C. R., Smith M. E. and Mihm J. A. (1992) Leaf feeding resistance to multiple insect species in a maize diallel. *Crop Science* 32, 1460–1463.

Smith M. E., Mihm J. A. and Jewell D. C. (1989) Breeding for multiple resistance to temperate, sub-tropical, and tropical maize insect pests at CIMMYT, pp. 222–234. In *Toward Insect Resistant Maize for the Third World*. Proceedings of the international symposium on methodologies for developing host plant resistance to maize insects. CIMMYT.