

BIOTECHNOLOGY FOR THE IMPROVEMENT OF MAIZE FOR RESOURCE POOR FARMERS: THE CIMMYT APPROACH

S. Mugo¹ and D. Hoisington²

¹CIMMYT, P.O. Box 25171, Nairobi, Kenya; ²CIMMYT-Mexico

INTRODUCTION

A major challenge facing developing countries is access to adequate food supply to cope with increasing demand driven by population growth. Sub-Saharan Africa's 1995 population of 600 million people is expected to double to 1.2 billion by 2020. Approximately 40% of the population, or around 225 million people, currently live on less than one dollar a day. Food production in sub-Saharan Africa grew at half (1.5% per year) the rate of population growth (3% per annum) from 1970-85. Since then, the situation has continued to deteriorate. During 1988-93, 33 African countries experienced a reduction in *per capita* food production. Food crop productivity growth, while preserving renewable resources, is essential for enhancing food security and for overall income and economic growth. This food must also have good nutritional qualities as demanded by increasingly enlightened communities. Increased awareness of environmental health means that food production must be undertaken with greater care to minimize negative impact on the environment.

Maize is a major staple in Africa as evidenced by the large land area under maize, high per capita consumption, the high calorific intake maize provides to the population, and increasing demand for maize. However, average maize yields are low. A host of biotic and abiotic stresses to which available germplasm are susceptible limit maize production. The major stresses in African countries include drought, low soil fertility, pests, diseases, and soil acidity/salinity. The key to renewed growth in the agricultural sector is rapid technological change in food production. Biotechnology can and is being used to address these concerns, although biotechnology should be viewed as only one part of a comprehensive sustainable poverty alleviation strategy, not a technological "quick-fix" for world hunger and poverty. Further, the need for more food has to be met through higher yields per unit of land,

water, energy and time. Thus, there is a growing need to mobilize science to raise the biological productivity ceiling without associated ecological harm (Swaminathan, 2000). The United Nations Development Program states that: "Biotechnology offers the only or the best tool of choice for marginal ecological zones - left behind by the green revolution - but home to more than half of the world's poorest people, dependent on agriculture and livestock (UNDP, 2001).

This paper explores the potential for biotechnology and the opportunities developing countries have to exploit the benefits of modern biotechnology.

Broadly defined, biotechnology is a wide array of technologies that includes techniques that use living organisms or substances from these organisms to make or modify a biological product or to improve plants, animals, or microorganisms for specific uses. Modern biotechnology applications in crop improvement can be divided into two major categories: molecular genetics and genetic engineering. Molecular genetics focuses on the use of molecular markers and genetic fingerprinting to identify the presence of specific genes already present in an organism and which govern traits of interest. Genetic engineering involves the insertion of native or foreign gene(s) into a host organism (microorganism, plant or animal) in order to increase the value or usefulness of the organism. Products of genetic engineering are called genetically modified organisms (GMOs).

Scientists can utilize genes derived from various sources, including related and unrelated species, those identified through genetic mapping experiments, and from efforts of functional genomics (Hoisington, 2000). Through the application of molecular genetics and genetic engineering, coupled with conventional crossing approaches, these genes can be efficiently incorporated into modern maize varieties (Fig. 1).

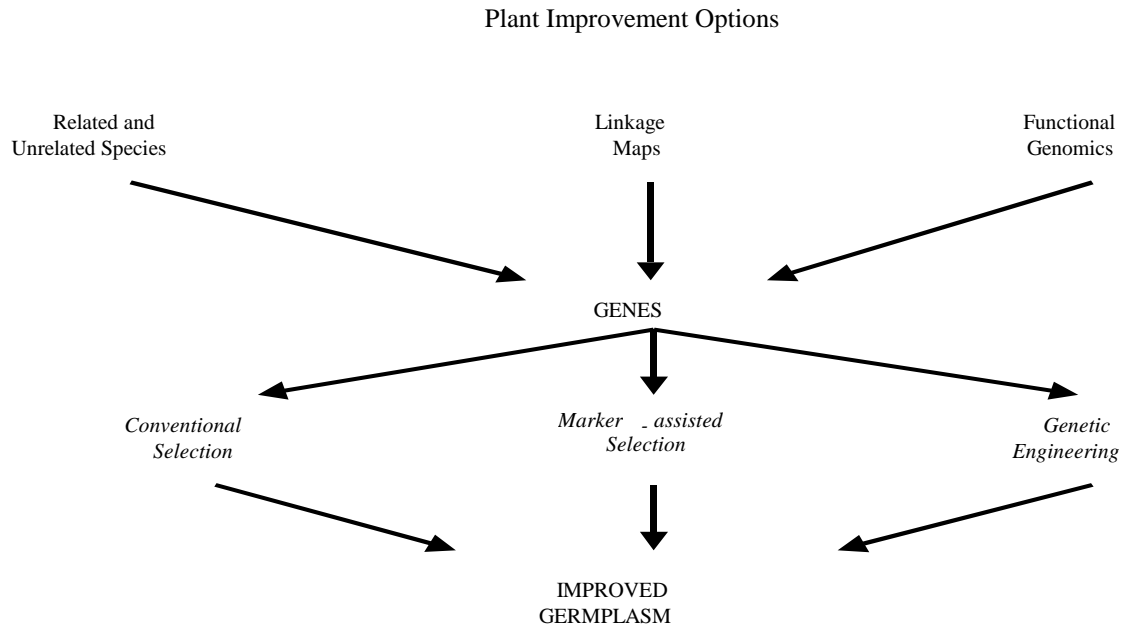


Figure 1. Plant improvement options

Tissue culture, a process whereby whole plants are propagated from minute amounts of plant tissue is an important step of genetic engineering. Functional genomics is aimed at understanding the function of all genes in an organism, and is a growing science that will become an important tool in biotechnology. Recent advances in genomics are bringing about a revolution in our understanding of the molecular mechanisms of disease, including the complex interplay of genetic and environmental factors.

The rest of this paper will present biotechnology research in maize improvement at CIMMYT and the approach taken to extend the technology and its products to benefit farmers in developing countries in Africa.

VIEWS OF BIOTECHNOLOGY BY THE CGIAR AND CIMMYT

CIMMYT is one of the 16 international agricultural research institutes of the Consultative Group on International Agricultural Research (CGIAR), and has the global mandate for improvement of maize and wheat systems. These 16 centers are part of Future Harvest®, a public awareness campaign that builds understanding about the importance of agricultural issues and international agricultural research. The CGIAR's mission is to contribute to food security and poverty eradication in developing countries through research, partnership, capacity building, and policy support. The CGIAR

promotes sustainable agricultural development based on the environmentally sound management of natural resources. Agriculture is recognized as playing a major role in development and over 70% of people in developing countries depend on the land for their livelihood (<http://www.cgiar.org>). Further, research is recognized as the means by which the world's knowledge of agriculture is increased and improved.

The CGIAR focuses on the following five major research thrusts: increasing productivity through genetic improvements in plants, protecting the environment through conserving natural resources, saving biodiversity through holding one of the world's largest *ex-situ* collections of plant genetic resources, improving policies for production, and strengthening national research.

The CGIAR centers view modern biotechnology as a tool that is to be used in conjunction with traditional or conventional agricultural methods to develop improved and sustainable agricultural systems. The critical roles for the CGIAR are as: 1) a protector of the interests of the poor and a facilitator and bridge-builder in biotechnology partnerships, and 2) a facilitator of public policy and innovative institutional arrangements (Persley, 2000). In fulfilling these roles, the CGIAR could contribute to research and development in biotechnology in the developing countries through direct biotechnology research within the centers, facilitating information sharing, identifying problems and priority setting, supporting national capacity building, ensuring

compliance with agreed biosafety standards, managing intellectual property, public/private partnerships, and communicating and addressing public concerns (Mugo *et al.*, 2001).

BIOTECHNOLOGY AT CIMMYT

CIMMYT has recognized the potential of biotechnology to develop robust germplasm for developing countries, but also recognized that the development and routine use of genetically modified organisms (GMOs) would require addressing relevant biosafety, environmental, and community concerns. Hence, the following policy statement was developed to regulate research activities aimed at producing and utilizing GMOs:

“CIMMYT is convinced that the application of biotechnology will have a direct beneficial impact on its crop improvement programs and on their contribution to the improved welfare of developing countries. The Center emphasizes the application of appropriate biotechnology techniques in its research, training, and collaborative activities. CIMMYT has formulated institutional guidelines in accordance with international standards. These are designed to prevent the release of hazardous organisms and/or their products and to protect employees involved in such research” (CIMMYT, 1994).

One of CIMMYT’s five research programs is devoted to biotechnology: the Applied Biotechnology Center (ABC). ABC has helped to apply biotechnology to increase the effectiveness of maize improvement and to conserve and use genetic resources. Through human resource development, technology transfer, information dissemination, and research partnerships, the ABC has helped agricultural scientists in developing countries apply biotechnology to address major challenges faced by maize farmers. Such challenges include drought, insect pests, infertile soils, and diseases.

CIMMYT also set up an institutional biosafety committee to advise the center management, and to handle biosafety-related issues for the center. Specifically, the committee advises center management on aspects of GMO research: establishment and implementation of laboratory safety; development of guidelines for CIMMYT’s greenhouse and field research with GM plants; evaluation of benefits and risks of conducting research with GMOs; national and international biosafety issues and activities; the operation of appropriate physical containment facilities; and new biotechnology projects requiring approval.

Although GMO research is well known at CIMMYT, the use of molecular genetics has a wider application in developing stress tolerant and

nutritionally enhanced maize germplasm for use in developing countries.

Molecular Genetics in Maize at CIMMYT

Molecular markers are DNA signposts that allow crop geneticists and breeders to locate on a plant chromosome the genes for a trait of interest. Molecular markers speed and facilitate the development of useful varieties intended to possess specific traits. Maize is an important target for biotechnology due to its importance as food and feed globally, due to the presence of hybrid technology that enables private sector investment, and due to significant and favorable scientific advantages. Such advantages include the availability of an enormous collection of known loci and genetic/cytogenetic stocks (Coe *et al.*, 1988), and the ease with which molecular studies can be accomplished - both genetic and biological (Hoisington, 1992).

CIMMYT adapts large-scale, low-cost, non-radioactive molecular marker techniques that meet its needs and those of its research partners in developing countries. Biotechnology research focuses on traits whose improvement or transfer is slow or expensive using conventional methods alone. The following traits are those targeted in current research: resistance to maize stem borers, rootworm, *Fusarium* ear rot, maize streak virus, witchweed (*Striga* spp.), acid soils, and tolerance to drought. Another biotechnology application is in the study of the genetics of apomixis and photoperiod.

One of the earliest applications of molecular markers was in fingerprinting crop varieties. This implies the unambiguous identification of individuals in a population or set of lines. Such measures of genetic identity are used for assessing duplication within genetic resource collections and for varietal protection (Melchinger *et al.*, 1990). Fingerprinting is most easily applied to homozygous (i.e., genetically uniform) lines. However, through the bulking of individuals or the use of polymerase chain reaction (PCR)-based techniques, fingerprinting data may be used to classify more genetically diverse materials: populations or groups of related lines, open pollinated varieties (OPVs), and germplasm bank accessions. Such studies can help elucidate the genetic structure of populations, the overall genetic diversity of a group of lines, and shed light on pedigrees and evolutionary relationships. Comparison of temperate maize germplasm to the great diversity in the tropics would provide the baseline information necessary to access the myriad of genes and useful alleles present. CIMMYT is undertaking molecular fingerprinting of germplasm accessions in the genebank in Mexico, and can offer fingerprinting as a

service to collaborators. We are in the process of characterizing 930 bulks of pools/populations with 8 microsatellite (SSR) primers. CIMMYT is exploring several applications of fingerprinting, among them the prediction of maize crosses that will result in outstanding hybrids.

Maize was one of the first major crop species for which a complete molecular map was developed (Helentjaris *et al.*, 1986). Since the first map publication, many other maps have been produced, and are now consolidated into a consensus map using a “bin” allocation to chromosome segments (Gardiner *et al.*, 1993). Given the high level of

polymorphism found even between highly related lines, this consensus map allows one to rapidly identify possible markers for use in further saturating a region of interest, or for developing alternative (e.g., PCR-based) marker systems. Efforts are now under way to add thousands of SSR markers to the map, and maps composed of dense amplified fragment length polymorphisms (AFLP) loci are available for a number of maize populations. Most of the molecular data is with the private sector, but the public sector has developed rather detailed QTL and single gene maps for traits that are of importance to developing countries (Table 1).

Table 1. Important traits (for East Africa) in maize located using molecular genetics

Trait	Genetics	Reference(s)
Fungal resistance		
Northern leaf blight	QTLs	Freymark <i>et al.</i> , 1993, 1994; Dingerdissen <i>et al.</i> , 1996
Fusarium ear rot	QTLs	Chungu <i>et al.</i> , 1996
Grey leaf spot		Maroof <i>et al.</i> , 1996
Viral resistance		
Maize streak virus	Major gene	Kyetere, 1995
Abiotic stresses		
Drought	QTLs	Lebreton <i>et al.</i> , 1995; Ribaut <i>et al.</i> , 1996, 1997; Agrama and Moussa 1996
Agronomic and morphological factors		
Yield	QTLs	Stuber <i>et al.</i> , 1992; Schön <i>et al.</i> , 1994; Veldboom & Lee, 1994, 1996a; Causse <i>et al.</i> , 1995; Ajmone-Marsan <i>et al.</i> , 1995, 1996; Austin & Lee, 1996a, 1996b; Bohn <i>et al.</i> , 1996; Ribaut <i>et al.</i> , 1996
Plant height	QTLs	Koester <i>et al.</i> , 1993; Ajmone-Marsan <i>et al.</i> , 1995; Berke & Rocheford 1995; Austin & Lee, 1996b; Bohn <i>et al.</i> , 1996; Veldboom & Lee, 1996b
Anthesis	QTLs	Koester <i>et al.</i> , 1993; Berke & Rocheford, 1995; Austin & Lee, 1996b; Veldboom & Lee, 1996b; Ribaut <i>et al.</i> , 1996
Silking	QTLs	Koester <i>et al.</i> , 1993; Berke & Rocheford, 1995; Austin & Lee, 1996b; Veldboom & Lee, 1996b; Rebai <i>et al.</i> , 1997; Ribaut <i>et al.</i> , 1996

CIMMYT embarked on the development of insect resistant germplasm using molecular methods including the use of quantitative trait loci (QTL) to select for improved stem borer resistance in elite lines. A consensus molecular marker map exists from which possible markers are identified using PCR-based ones, SSR, and even AFLP markers. CIMMYT, in collaboration with the University of Hohenheim, Germany, developed QTL maps for the southwestern corn borer (SWCB, *Diatraea gradiosella*) and the sugarcane borer (SCB, *Diatraea sacarralis*) (Groh *et al.*, 1998). Initial attempts to transfer a number of major QTLs to susceptible varieties using marker-assisted selection have been successful, especially when compared to conventional selection (CIMMYT, unpublished

results). Insect pests of major importance to Kenya are currently being investigated in joint projects with Kenya and Zimbabwe. Marker assisted selection (MAS) may help improve the efficiency of selection for resistant germplasm.

Maize streak virus (MSV), a major viral disease in Africa, appears to be controlled by a single major gene located on chromosome 1 (Kyetere, 1995). Although this has led to the easy development of a marker-assisted selection (MAS) strategy for MSV, it prompts a significant question about the durability of resistance to MSV, because all sources of resistance appear to be derived from alleles present at the same locus.

Drought and nitrogen stresses are important limitations to maize production in developing

countries. Several studies (Lebreton *et al.*, 1995; Ribaut *et al.*, 1996, 1997; Agrama and Moussa, 1996) indicate that tolerance to drought stress is correlated with a reduced anthesis-silking interval, and is controlled by five to six genes each contributing approximately an equal effect to drought tolerance. QTLs for other physiological traits of interest are being identified in five different crosses and more than 20 different environments. This information will be compiled in a consensus linkage map for maize. Particular emphasis will be given to genomic regions where consistent QTLs for a given trait are identified among crosses, as well as the regions where QTLs for different traits of interest are identified. If such regions can be identified, this information can be used to develop an efficient MAS strategy which will not require the construction of a linkage map for each new cross, or field evaluation to identify the target QTL.

The weakness of the quantitative genetic approach is that it provides very little information about the mechanisms and pathways involved in drought tolerance, or about the multitude of genes involved in the plant's response. The recent development of functional genomics should help to overcome this problem because these new approaches allow the simultaneous study of the expression of several thousands of genes. Considering the complexity of the genetic answer to the problem of a plant under water-limited conditions, a better understanding of the genes and the pathways involved in plant response will be crucial to accelerate, sustain, and complement conventional breeding programs. Therefore, research activities aimed at identifying and characterizing genes and pathways that are over- or under-expressed in water-limited conditions have been initiated at CIMMYT following the candidate gene approach and by conducting profiling experiments on contrasting materials derived from segregating populations. A mixed approach that combines phenotypic germplasm characterization, QTL identification and gene expression profiles will lead to efficient and effective strategies to develop maize with higher productivity under water-limited conditions.

Biotechnology-mediated techniques are being used to develop *Striga* resistant maize to complement other efforts to control *Striga*: 1) the use of imidazolinone-resistant synthetic maize lines which when treated with the herbicide are not infected by *Striga* plants (Kanampiu *et al.*, 1999), and 2) use of crosses of maize with teosinte and *Tripsacum* accessions.

Transposon tagging involves generating a population of maize where the expression of a number of random genes has been limited by the

insertion of transposon DNA into the genes. A small number of these transposon-tagged maize lines have been identified from a population of 8000 lines which show no or limited emergence of *Striga* in the field. Some of these lines have been subjected to laboratory testing at the University of Sheffield. In glasshouse trials, the lines tested had no emergence of *Striga*. The plants were, however, still infested by *Striga*, but the growth and development of the *Striga* plants were perturbed. Infection by *Striga* had very little impact on the growth of transposon-tagged hosts in contrast to susceptible maize controls.

Efforts are underway to stabilize those transposon tagged genotypes which demonstrate tolerance to *Striga* by immobilizing the transposable elements. We are beginning to convert a set of 5 to 10 inbred lines, including some of the best locally adapted inbred and CIMMYT maize lines, with transposon-tagged material. A genetic marker for the tolerant phenotype would accelerate the conversion of lines. Thus, work is currently under way to develop markers to use in marker-assisted selection. In order to fully characterize tolerant transposon-tagged lines, we aim to clone those genes which have null function due to the presence of a transposon insertion. This information will not only enhance our biological understanding of the host-*Striga* relationship, but will also provide the opportunity to generate more maize lines with tolerance and to introgress the trait into other cereals.

Markers are being used in the development of quality protein maize (QPM). QPM line conversion faces two main constraints. First, one cannot determine lines with high protein quality at the vegetative stage. This has to be done with kernels via assays for total nitrogen or using enzyme-linked immunosorbent assays (ELISA) for EF1A protein, which is highly correlated with the level of lysine present in the grain endosperm. Second, the mutant allele that confers elevated levels of lysine and tryptophan is recessive, so heterozygous plants cannot be identified phenotypically because they do not express altered amino acid levels. CIMMYT, therefore, uses SSR markers to test DNA extracted from leaf tissue of very young plants, allowing QPM plants to be identified early in the breeding cycle. SSR markers distinguish between homozygous recessive and heterozygous plants, reducing the length of period for the breeding process.

The ultimate utility of QTL mapping to a breeding program is in transferring specific QTLs via MAS. Several MAS strategies have been proposed, from simple backcross programs to more complex population improvement strategies. Unfortunately, there are no published results to date demonstrating success with MAS. Many of the published QTL

mapping papers conclude with a statement that MAS will be useful, but the results of a successful MAS effort have not yet been published. Encouraging results have been obtained at CIMMYT in MAS for insect resistance and drought tolerance.

One difficulty is that, for each new set of parental materials, QTLs (or at least the desired allele at each QTL) must be located before attempting MAS. Methods to circumvent the need to repeatedly map QTLs for each set of parental lines are being developed, and will allow MAS to be applied to quantitative traits. Another major limitation has been the available marker systems. For example, RFLPs are well suited for QTL mapping studies, but not for analyzing massive numbers of samples very quickly, a requirement in MAS. For this, PCR-based markers (sequenced-tagged sites, SSRs and AFLPs) hold promise (Ribaut and Hoisington, 1998). Otherwise, markers remain a viable option for the rapid backcrossing of single genes (for example, MSV resistance and transgenes) into a large array of genetic materials. Future use for more complex traits awaits further study.

A study showed that the relative cost-effectiveness of conventional breeding methods as compared to MAS for QPM line conversion differs depending on whether breeders can identify segregating materials by visual inspection (Dreher *et al.*, 2000). MAS is still attractive where phenotypic screening is expensive or difficult, including breeding projects involving multiple genes, recessive genes, late expression of the trait of interest, and seasonal considerations, or geographical considerations. Continuing refinement of molecular marker technologies will make MAS cheaper and more effective in coming years.

Genetic Engineering Activities in Maize at CIMMYT

Transgenic crops represent a promising technology that can make a vital contribution to global food, feed, and fiber security. During 1996-2000, global adoption rates for transgenics were unprecedented. This reflected grower satisfaction with products that offered significant benefits ranging from more convenient and flexible crop management, higher productivity, and a safer environment through decreased use of conventional pesticides, which collectively contribute to a more sustainable agriculture (James, 2001). From 1996-2000, a substantial share, up to 85% of the global area of transgenic crops, was grown in industrial countries. However, the proportion of transgenic crops grown in developing countries has increased consistently from 14% in 1997 to 24% in 2000 (Fig. 2).

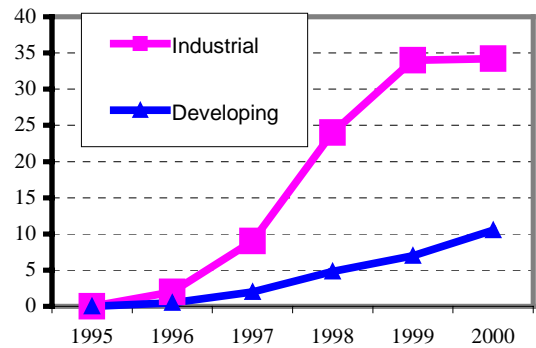


Figure 2. Global area of transgenic crops, 1996 – 2000: industrial and developing countries (million hectares). Source: James (2000)

CIMMYT's genetic engineering capabilities allows the center's scientists to draw on a range of useful genes for agriculture that will eventually become available, and to pass the products of such cutting-edge technologies to research partners in developing countries. Capacity was developed for genetic engineering of enhanced resistance to stem borers in maize using proteins that disrupt digestion in borer larvae with genes from the common gram-positive soil bacterium, *Bacillus thuringiensis* (*Bt*) (Mugo 2001a). Commercial transgenic maize containing one or more *Bt* genes was planted to 8.2 million ha globally in year 2000 (James, 2001). These "Bt genes" are synthetic versions of the gene from *Bt* that codes for a delta-endotoxin or insecticidal crystal protein. These proteins, used for years in biopesticides, are toxic to insect larvae, many of which are major crop pests. The first successful transformation of maize involved using biolistics or a gene gun (Kozziel *et al.*, 1993). It took several years for *Bt* maize to reach farmers' fields. Most of the delays were due to regulatory issues, primarily environmental and food safety. Recently, the successful transformation of maize mediated by *Agrobacterium tumefaciens* has created the possibility of enhancing the efficiency by which novel transgenics can be developed (Ishige *et al.*, 1996). It remains to be seen whether this method will replace biolistic transformation, particularly considering the intellectual property issues (i.e., the biolistic transformation patent expires in 2-3 years) and the advantage of chloroplast transformation via biolistics.

Insect resistance has been and will continue to be an important target for seed companies (Krattiger, 1997). The primary insect pest targeted is the European corn borer (ECB, *Ostrinia nubilalis*) and

other pests in America, Asia, and in many parts of Latin America. Even with the high specificity of the Bt proteins, control of other Lepidopteran species has been found for the Bt gene (*cryIAb*), which is contained in the U.S. Bt maize lines (Fietelson *et al.*, 1992; Bohorova *et al.*, 1997). These studies indicate that several of the Bt genes could be useful in providing multiple gene sources for insect control, and efforts are now underway in various laboratories to develop synthetic versions of these for use in developing appropriate transgenic varieties. As more commercial Bt maize lines become available, relatively simple greenhouse or field trials can be performed to quickly determine if the included gene will provide control of the targeted insect pest. In Kenya, prospective controls have been found for four major stem borer species: *Chilo partellus*, *Chilo orichaociliellus*, *Eldana saccharina*, and *Sesamia calamistis*.

There is a great deal of controversy regarding the use of herbicide-resistant crops in agriculture. Issues such as gene flow that creates "super weeds", and increased use of and dependence on agricultural chemicals, prompt arguments both for and against the deployment of herbicide-resistant crops. One area which has been largely overlooked is the problem of parasitic weeds in Africa. *Striga* (*Striga* spp.) inflicts considerable losses to maize production, and little or no resistance has been found within the maize genome. Some evidence is available (Kim *et al.*, 1994; Berner *et al.*, 1995) that levels of resistance or tolerance are found in some wild relatives, but the transfer of these traits into high-yielding maize varieties will take considerable time. Several non-selective herbicides can be used to control *Striga* infestation. When combined with the corresponding herbicide-resistant maize variety (non-transgenic), low-dose seed treatments can provide good levels of control (Gressel, 1992). This approach, while requiring additional research to develop, may provide a 'short-term' option to greatly increase the amount of food available for poor farmers in many African countries. The use of Roundup Ready® maize to control striga has been proposed, although CIMMYT is not currently involved in this activity.

Biotechnology also offers methods to enhance other qualities of maize. Currently, outside CIMMYT, work is under way to increase the levels of vitamin A, iron, and folic acid in the maize grain.

Apomixis – asexual reproduction through seed – results in plants that are exact clones of the mother plant. Having apomictic versions of high-yielding crop varieties and hybrids would mean that farmers could replant seed from their own harvest each year instead of having to purchase fresh seed. The potential implications of this for farmers in

developing countries, many of whom cannot afford commercial seed, are attractive. Scientists at CIMMYT are using conventional crossing methods and advanced molecular genetics techniques to study, isolate, and transfer the gene complex that controls apomixis from *Tripsacum*, a grassy relative of maize, to maize and other crops.

Apomixis research is organized in three sub-projects aiming at: (1) the recovery of apomictic maize via either interspecific hybrids or genetic engineering, (2) the elucidation of the molecular bases of apomixis, and (3) understanding the mechanisms governing deleterious dosage effects in the maize endosperm, a non-desirable consequence of the expression of apomixis.

CIMMYT's Genetic Engineering Strategy

In developing the tenets of its genetic engineering strategy for wheat and maize, CIMMYT has emphasized the needs of its partners at the national level and the usefulness and safety of its products at the farmer level. The points stated below guide the efforts of the Center's genetic engineering program:

- Plant varieties that are genetically engineered by CIMMYT are developed in concert with a national program partner to meet a delineated need.
- CIMMYT provides only transformed plants that carry "clean" events, meaning that only the gene of interest is inserted into the final product.
- No transformed plants that carry selectable markers, such as herbicide or antibiotic resistance, are provided to national programs.
- CIMMYT's focus on possible genes for transfer is on plant and bacterial genes.
- CIMMYT works only in countries that have biosafety legislation or regulations.

Special research partnerships are designed to share useful technology and develop products for farmers by efficiently focusing resources from many quarters on high priority problems. Examples are: 1) establishing applied biotechnology programs in Kenya and Zimbabwe; 2) using DNA markers and other biotechnology tools to generate locally-adapted drought tolerant and insect resistant maize; 3) developing improved maize that resists the parasitic flowering plant *Striga* for farmers in sub-Saharan Africa; 4) establish a collaborative research and training network among national maize and biotechnology research programs in Asia; and 5) developing and deploying insect resistant maize varieties in Africa. Details of this last project will be given as an example of national program approaches to acquiring and using biotechnology.

The Insect Resistant Maize for Africa (IRMA) Project

The Insect Resistant Maize for Africa (IRMA) Project aims at increasing maize production and food security through the development and deployment of insect resistant maize to reduce losses due to stem borers. The Project is a joint venture between CIMMYT and the Kenya Agricultural Research Institute (KARI), with financial support from the Novartis Foundation for Sustainable Development. Lepidopteran stem borers are economically important pests of maize, a major staple in Kenya, with annual losses estimated at \$90M (De Groote, 2000). Both host plant resistance and genetically engineered maize (e.g., Bt-maize) have been identified as possibilities to help resource poor farmers combat stem borer damage and meet their food requirements. The project focuses on identifying the best methods to properly combine these mechanisms and ensure that Kenyan farmers will be able to take advantage of modern approaches to this problem (Mugo *et al.*, 2001).

The overarching goals of the project are to develop insect resistant maize varieties for the major Kenyan maize growing environments, and to establish procedures to provide insect resistant maize for resource poor farmers in Kenya. During the implementation of the IRMA project, relevant technologies will be transferred to KARI and continuously evaluated.

The specific objectives of the project are as follows:

- **Product Development:** Develop insect resistant maize varieties for the major insect pests found in Kenyan maize production systems.
- **Product Dissemination:** Establish procedures for providing insect resistant maize to resource poor farmers in Kenya.
- **Impact Assessment:** Assess the impact of insect resistant maize varieties in Kenyan agricultural systems.
- **Technology Transfer:** Transfer technologies to KARI and Kenya to develop, evaluate, disseminate, and monitor insect resistant maize varieties.
- **Project Documentation and Communication:** Plan, monitor, and document processes and achievements for dissemination to the Kenyan public and developing countries.

Upon successful completion of the importation of Bt leaves, bioassays of these against five African maize stem borers were done. The results indicated that the cry1Ab protein is effective against all of the Kenya borers except for *Busseola fusca* (which was only moderately controlled). Other genes showed

various levels of control from total to none. From the data, it appears that a combination of cry1Ab and cry1B proteins may be the most effective in controlling the targeted stem borers. For verification, an application is being submitted to allow the importation of seeds from these events as well as crosses that would combine the various Bt genes. These would then be planted in a quarantine field site for verification of the lab results and further testing of gene combinations following approval by the Kenyan Biosafety Committee. Second generation ('clean') events are being produced for cry1Ab and cry1B genes. Currently, we have several events in which the selectable marker, bar gene, has not been inserted. These are the most desirable events, as they would involve only the gene of interest. Efforts are continuing to identify similar events for all cry genes known to be effective against the targeted insect species.

Development of insect resistant germplasm is going on by screening germplasm from diverse sources with host plant resistance developed through conventional breeding approaches. Identification of germplasm to be backcrossed to the Bt maize is going on through screening elite germplasm from KARI and CIMMYT.

Ecological work is focusing on establishing the diversity and relative abundance of target and non-target organisms that could potentially be affected by the introduction of Bt maize. Studies were also initiated on developing an insect resistance management (IRM) strategy that incorporates both vertical resistance mechanisms (through the "pyramiding" or "stacking" of resistance genes and the development of refugia) and horizontal resistance through more conventional crop development and agronomic measures.

Economic impact assessment includes studies of the seed sector. Farmers estimated the losses due to stem borers at 15%, with a value of US\$90 million. Group interviews with farmers in all agro-ecological zones revealed that stem borers are always placed among the three most important pest problems for maize. Farmers are very interested in testing resistant varieties.

In project documentation and communication, considerable effort has been given to creating dialogue and raising public awareness about Bt and insect resistant maize, and about biotechnology in general.

PROSPECTS FOR RESOURCE POOR FARMERS

A major issue for developing countries, which pertains to all transgenic activities, relates to the safe

and sustainable deployment of transgenic varieties. Controversy persists regarding the most appropriate strategies, both for safe and sustainable deployment. Strategies in response to these concerns have been proposed, and are in place in some countries. Will they work in developing countries? The answers are not simple or easy to obtain, but answers must be provided if transgenics are to be safely deployed and ultimately accepted in developing countries.

To tap this emerging technology, developing countries have to take certain measures:

- Develop a research agenda with governments committing resources, and setting priorities for the traits that need to be addressed, as well as set strategies to attain the objectives. This way, African countries will avoid adopting research agendas developed elsewhere or dictated by donors.
- Build capacity through strategic training of scientists to address the identified problems.
- Develop infrastructure, including laboratories and field facilities, to develop and test the products from biotechnology.
- Develop a policy framework, including biosafety legislation and regulations to govern the development, testing, and deployment of products in a safe way for humans and the environment.

African governments should take care not to let research products from biotechnology pass them by. Of course, governments must prepare themselves with the necessary legislation and regulations to ensure proper testing of genetically modified crops. However, they must ensure that farmers have adequate access to the new technologies that come from these scientific developments (Borlaug, 2000).

CONCLUDING REMARKS

Although the potential of biotechnology has often been exaggerated in the past, a high level of optimism is clearly justified for its use in the improvement of maize, even for developing countries. Undoubtedly, molecular genetics and functional genomics will revolutionize the way in which plant breeding is undertaken in the future. Basic research is leading to an improved understanding of the genetic mechanisms operating within a plant in response to the diverse stresses that it is exposed to, as well as the overall production of biomass and grain. New marker technologies such as micro-arrays are offering the opportunity to understand the presence and even expression of thousands of genes within a plant. This knowledge clearly offers promise for making germplasm improvement faster, cheaper, and more effective.

Emerging genetic engineering techniques are providing breeders with the never-before-seen capability to create novel plants by combining genetic materials from a wide array of sources. Although not without controversy, the options seem limitless and with the proper oversight and understanding, should provide extremely powerful options to develop durable and highly productive plant varieties for almost any production environment.

The challenge for African countries is to tap as much of this emerging technology as possible. This does not necessarily mean that countries must establish in-house capabilities. What is required is that nations recognize the importance of the new approaches, and ensure that appropriate legislation and regulations are enacted to allow the country to acquire, evaluate, and most importantly, deploy the new plant varieties produced via biotechnology. CIMMYT has developed the facilities, technology, and strategies that would enhance the development of suitable maize germplasm for production in developing African countries. The DGIS-funded insect resistance and drought tolerance projects with Kenya and Zimbabwe, the Rockefeller Foundation supported *Striga* projects, and the Insect Resistance Maize for Africa (IRMA) project supported by the Novartis Foundation for Sustainable Development are examples of national program approaches to acquire and use biotechnology.

REFERENCES

- Agrama, H.A.S. and M.E. Moussa. 1996. Mapping QTLs in breeding for drought tolerance in maize (*Zea mays* L.). *Euphytica* 91:89-97.
- Ajmone-Marsan, P., G. Monfredini, A. Brandolini, A.E. Melchinger, G. Garay and M. Motto. 1996. Identification of QTL for grain yield in an elite hybrid of maize - repeatability of map position and effects in independent samples derived from the same population. *Maydica* 41:49-57.
- Ajmone-Marsan, P., G. Monfredini, W.F. Ludwig, A.E. Melchinger, P. Franceschini, G. Pagnotto and M. Motto. 1995. In an elite cross of maize a major quantitative trait locus controls one-fourth of the genetic variation for grain yield. *Theor. Appl. Genet.* 90:415-424.
- Austin, D.F. and M. Lee. 1996a. Comparative mapping in F2:3 and F6:7 generations of quantitative trait loci for grain yield and yield components in maize. *Theoret. Appl. Genet.* 92:817-826.
- Austin, D.F. and M. Lee. 1996b. Genetic resolution and verification of quantitative trait loci for flowering and plant height with recombinant inbred lines of maize. *Genome* 39: 957-968.

- Berke, T.G. and T.R. Rocheford. 1995. Quantitative trait loci for flowering, plant and ear height, and kernel traits in maize. *Crop Sci.* 35: 1542-1549.
- Berner, D.K., J.G. Kling and B.B. Singh. 1995. *Striga* research and control - a perspective from Africa. *Plant Disease* 79: 652-660.
- Bohn, M., M. M. Khairallah, D. González-de-León, D.A. Hoisington, H.F. Utz, J.A. Deutsch, D.C. Jewell, J.A. Mihm and A.E. Melchinger. 1996. QTL mapping in tropical maize. I. Genomic regions affecting leaf-feeding resistance to sugarcane borer and other traits. *Crop Sci.* 36: 1352-1361.
- Bohorova, N., M. Cabrera, C. Abarca, R. Quintero, A.M. Maciel, R.M. Brito, D. Hoisington and A. Bravo. 1997. Susceptibility of four tropical Lepidopteran maize pests to *Bacillus thuringiensis* CryI-type insecticidal toxins. *J. Economic Entomology* 90: 412-415.
- Borlaug, N. 2000. Meeting Africa's food production challenges in the 21st Century. Address given to ICRAF campus staff on June 4, 2000. Nairobi, Kenya.
- Causse, M., J.P. Rocher, A.M. Henry, A. Charcosset, J.L. Prioul and D. de Vienne. 1995. Genetic dissection of the relationship between carbon metabolism and early growth in maize with emphasis on key-enzyme loci. *Molecular Breeding* 1(3): 259-272.
- Chungu, C., D.E. Mather, L.M. Reid and R.I. Hamilton. 1996. Inheritance of kernel resistance to *Fusarium graminearum* in maize. *Journal of Heredity* 87: 382-385.
- CIMMYT. 1994. Biosafety at CIMMYT. Internal Guidelines. Mexico, D.F. Mexico. April 1994.
- CIMMYT. 2000. CIMMYT in 1999-2000. Science and Substance. Mexico D.F.: CIMMYT. ISSN:0188-9214.
- Coe, E.H., Jr., M.G. Neuffer and D.A. Hoisington. 1988. The genetics of corn. In: *Corn and Corn Improvement*, Third Edition, G.F. Sprague and J.W. Dudley (eds.). American Society of Agronomy, Madison, WI, USA. pp. 81-258.
- De Groote, H. 2000. Maize Yield Losses from Stem Borers in Kenya. Paper presented at the International Workshop on Biological Control of Cereal Stem Borers. October 15-22, 2000 at ICIPE, Duderuville Nairobi, November 20, 2000.
- Dingerdissen, A.L., H.H. Geiger, M. Lee, A. Schechert and H.G. Welz. 1996. Interval mapping of genes for quantitative resistance of maize to *Setosphaeria turcica*, cause of northern leaf blight, in a tropical environment. *Molecular Breeding* 2: 143-156.
- Dreher, K., M. Morris, M. Khairallah, J.M. Ribaut, S. Pandey and G. Srinivasan. 2000. Is Marker-Assisted Selection Cost-Effective Compared to Conventional Plant Breeding Methods? The Case of Quality Protein Maize. Paper Presented at The Fourth Annual Conference of The International Consortium on Agricultural Biotechnology Research (ICABR), "The Economics of Agricultural Biotechnology," Ravello, Italy, 24-28 August, 2000.
- Fietelson, J.S., J. Payna and L. Kim. 1992. *Bacillus thuringiensis*: insects and beyond. *Biotechnology* 10:271-275.
- Freyemark, P.J., M. Lee, C.A. Martinson and W.L. Woodman. 1994. Molecular-marker-facilitated investigation of host plant response to *Exserohilum turcicum* in maize (*Zea mays* L.) - components of resistance. *Theor. Appl. Genet.* 88: 305-313.
- Freyemark, P.J., M. Lee, W.L. Woodman and C.A. Martinson. 1993. Quantitative and qualitative trait loci affecting host-plant response to *Exserohilum turcicum* in maize (*Zea mays* L.). *Theor. Appl. Genet.* 87: 537-544.
- Gardiner, J., S. Melia-Hancock, D.A. Hoisington, S. Chao and E.H. Coe. 1993. Development of a core RFLP map in maize using an immortalized-F2 population. *Genetics* 134: 917-930.
- Gressel, J. 1992. The needs for new herbicide-resistant crops. In: *Achievements and Developments in Combating Pesticide Resistance*, Dehholm, I., A.L. Devonshire and D.W. Hollomon, (eds.). Elsevier London. pp. 283-294.
- Groh, S., D. González-de-León, M.M. Khairallah, C. Jiang, D. Bergvinson, M. Bohn, D.A. Hoisington and A.E. Melchinger. 1998. QTL mapping in tropical maize: III. Genomics regions for resistance to *Diatraea* spp. and associated traits in two RIL populations. *Crop Sci.* 38: 1062-1072.
- Helentjaris, T., T. Weber and S. Wright. 1986. Use of monosomics to map cloned DNA fragments in maize. *PNAS USA* 83: 6035-6039.
- Hoisington, D. 1992. Maize as a model system. In: G.P. Chapman (ed.), *Grass evolution and domestication*. London: Cambridge University Press.
- Hoisington, A.D. 2000. Maize and biotechnology - The importance to Africa. Paper presented at the African Biotechnology Stakeholders Forum: Mass media and policymakers workshop on biotechnology. Feb. 29-March 3, 2000. Panafric Hotel, Nairobi, Kenya.
- Hoisington, D., C. Jiang, M. Khairallah, J.-M. Ribaut, M. Bohn, A. Melchinger, M. Willcox and D. González-de-León. 1996. QTL for insect resistance and drought tolerance in tropical maize: prospects for marker assisted selection. *Society for Experimental Biology Number L*. pp. 39-44.
- Ishige, Y., H. Saito, O. Ohta, Y. Hiei, T. Komari and T. Kumashiro. 1996. High efficiency transformation of maize (*Zea mays* L.) mediated by *Agrobacterium tumefaciens*. *Nature Biotechnology* 14:745-750.
- James, C. 2001. *Global status of Commercialized Transgenic Crops: 2000* (Preview). ISAAA Briefs No. 21. ISAAA: Ithaca, NY.
- Kanampiu, F.K., J. Ransom and J. Gressel. 1999. Advantages of seed-primed imazapyr for *Striga hermonthica* control on maize bearing target-site resistance. In: *Maize Production Technology for the Future: Challenges and Opportunities*. Proceedings of the Sixth Eastern and Southern Africa Regional Maize Conference, 21-25 September, 1998. Addis Ababa, Ethiopia: CIMMYT and EARO. pp. 172-179.
- Kim, S.K., A.Y. Akintunde and P. Walker. 1994. Response of maize, sorghum and millet host plants to infestation by *Striga hermonthica*. *Crop Sci.* 34: 900-907.
- Koester, R.P., P.H. Sisco and C.W. Stuber. 1993. Identification of quantitative trait loci controlling days

- to flowering and plant height in two near isogenic lines of maize. *Crop Sci.* 33: 1209-1216.
- Kozziel, M.G., N.B. Carozzi, T.C. Courrier, G.W. Warren and S.V. Evola. 1993b. The insecticidal proteins of *Bacillus thuringiensis*: past, present and future uses. *Biotechnology and Genetic Engineering Reviews* 11:171-228.
- Krattiger, A.F. 1997. Insect resistance in crops: a case study of *Bacillus thuringiensis* (Bt) and its transfer to developing countries. ISAAA Briefs No. 2. ISAAA: Ithaca, NY. 42 p.
- Kyeteere, D.T. 1995. Genetic basis of tolerance in maize to maize streak virus using molecular markers. Ph.D. Thesis, Ohio State University, Wooster, Ohio. 169 p.
- Lebreton, C., V. Lazicjancic, A. Steed, S. Pekic and S.A. Quarrie. 1995. Identification of QTL for drought responses in maize and their use in testing causal relationships between traits. *Journal of Experimental Botany* 46: 853-865.
- Maroof, M.A.S., Y.G. Yue, Z.X. Xiang, E.L. Stromberg and G.K. Rufener. 1996. Identification of quantitative trait loci controlling resistance to gray leaf spot disease in maize. *Theor. Appl. Genet.* 93: 539-546.
- Melchinger, A.E., M. Lee, K.R. Lamkey, A.R. Hallauer and W.L. Woodman. 1990. Genetic diversity for restriction fragment length polymorphisms and heterosis for two diallel sets of maize inbreds. *Theor. Appl. Genet.* 80: 488-496.
- Melchinger, A.E., M.M. Messmer, M. Lee, W.L. Woodman and K.R. Lamkey. 1991. Diversity and relationships among U.S. maize inbreds revealed by restriction length polymorphisms. *Crop Sci.* 31:669-678.
- Mugo S.N., D. Bergvinson and D. Hoisington. 2001a. Options in Developing Stemborer-Resistant Maize: CIMMYT Approaches and Experiences. Accepted 20 August 2001. *Insect Sci. Applic.* Vol. 21, No. 3
- Mugo S., D. Hoisington, D. Poland and H. De Groote. 2001b. The Role of International Agricultural Research Centers of the CGIAR in Research and Development in Biotechnology: Examples from CIMMYT. Paper presented at the workshop on "Safety in biotechnology of food and feeds in Kenya", National Council for Science and Technology (NCST) and Stockholm Environmental Institute, held at Panafric Hotel, Nairobi, Kenya, October 17-18, 2001.
- Mugo, S., D. Poland, G. Kimani and H. De Groote (eds.). 2001c. Creating Awareness on Biotechnology Based Technologies: Report on a Workshop. IRMA Project Document No. 5. Nairobi, Kenya: KARI and CIMMYT.
- Mugo, S. 2001. The Role of CIMMYT - a Future Harvest Center - in the Development and Deployment of Biotechnology Products. pp. 28-37. In: Wafula, J.S. and D.M. Kimoro (eds.). Experiences with and the Future of Kenya's Biosafety System. Biosafety workshop held at Grand Regency Hotel, Nairobi, Kenya, April 26, 2001. ABSF Document No. 1. ABSF: NCST.
- Persley, G.J. 2000. Agricultural Biotechnology and the Poor: Promethean Science. In: G.F. Persley and M.M. Lanti (eds.). *Agricultural Biotechnology and the Poor: Proceedings of an International Conference*, Washington, D.C., 21-22 October 1999. Washington: CGIAR.
- Rebai, A., P. Blanchard, D. Perret and P. Vincourt. 1997. Mapping quantitative trait loci controlling silking date in a diallel cross among four lines of maize. *Theor. Appl. Genet.* 95: 451-459.
- Ribaut, J.M., C. Jiang, D. González-de-León, G.O. Edmeades and D.A. Hoisington. 1997. Identification of quantitative trait loci under drought conditions in tropical maize. I. Yield components and marker-assisted selection strategies. *Theor. Appl. Genet.* 94: 887-896.
- Ribaut, J.M., D.A. Hoisington, J.A. Deutsch, C. Jiang and D. González-de-León. 1996. Identification of quantitative trait loci under drought conditions in tropical maize. II. Flowering parameters and the anthesis-silking interval. *Theor. Appl. Genet.* 92: 905-914.
- Ribaut, J.M. and D. Hoisington. 1998. Marker-assisted selection: new tools and strategies. *Trends in Pl. Sci.* 3:236-239.
- Schön, C.C., A.E. Melchinger, J. Boppenmaier, E. Brunklaus-Jung, R.G. Herrmann and J.F. Seitzer. 1994. RFLP mapping in maize: Quantitative trait loci affecting testcross performance of elite European flint lines. *Crop Sci.* 34: 378-389.
- Schön, C.C., M. Lee, A.E. Melchinger, W.D. Guthrie and W.L. Woodman. 1993. Mapping and characterization of quantitative trait loci affecting resistance against second-generation European corn borer in maize with the aid of RFLPs. *Heredity* 70: 648-659.
- Stuber, C.W., S.E. Lincoln, D.W. Wolff, T. Helentjaris and E.S. Lander. 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics* 132: 823-839.
- Swaminathan, M.S. 2000. Genetic Engineering and Food Security: Ecological and Livelihood Issues. In: G.F. Persley and M.M. Lanti (eds.). *Agricultural Biotechnology and the Poor: Proceedings of an International Conference*, Washington, D.C., 21-22 October 1999. Washington: CGIAR.
- UNDP. 2001. Human Development Report 2001. Making new technologies work for human development. Oxford University Press, New York, USA.
- Veldboom, L.R. and M. Lee. 1996a. Genetic mapping of quantitative trait loci in maize in stress and nonstress environments. 1. Grain yield and yield components. *Crop Sci.* 36: 1310-1319.
- Veldboom, L.R. and M. Lee. 1996b. Genetic mapping of quantitative trait loci in maize in stress and non stress environments. 2. Plant height and flowering. *Crop Sci.* 36: 1320-1327.
- Veldboom, L.R., M. Lee and W.L. Woodman. 1994. Molecular marker-facilitated studies in an elite maize population. 1. Linkage analysis and determination of QTL for morphological traits. *Theor. Appl. Genet.* 88: 7-16.