



CIMMYT

International Maize & Wheat Improvement Center

**Global Trends in Agricultural Biotechnology and CIMMYT's
Biotechnology Programme**

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1. Introduction

Despite new developments agricultural developments, yields in Africa have not been able to keep up with population growth. Currently 35% of the global population live in Africa and are food insecure (IFPRI 1997). Yields in food crops have not increased over the last decade, and one of the major problems facing Sub-Saharan Africa is the poor performance of its agriculture, leading to poverty and malnutrition (Ndiritu 2000). Agricultural scientists are facing a major challenge: how to improve food security, increase productivity, conserve biodiversity, reduce pest management costs, deal with increasing urban migration and reduce poverty. Increasingly, they are looking to the latest developments in science to address this problem, and biotechnology had been welcomed as the solution to the food security problem.

Specific issues related to biotechnology are how to develop institutional capacity for risk assessment and management, to access information on developments in biotechnology elsewhere that may have application in Africa, and to develop the necessary human resources and infrastructure (Persley 2000). There is an urgent need for a focused debate on the role of modern agricultural biotechnology in developing countries, a debate that should and is being led by people from developing countries themselves (Pinstrup-Anderson and Cohen 2000).

Lately, biotechnology has come under scrutiny by certain groups, especially environmental activists, who object mostly to the new Genetically Modified Organisms. These organisms, although widely used in North America and a several other countries, are not accepted in Europe. Major reasons cited are fear for human toxicity, effect on the environment, and the development of resistance.

For the International Maize and Wheat Improvement Centre (CIMMYT), the prudent use of biotechnology, is only one of the tools we use to pursue our mission, to help alleviate poverty by increasing the productivity, profitability, and sustainability of maize and wheat farming systems. We understand that there are advantages and disadvantages to different approaches, but think that the debate should center on a careful assessment of the risks, and compare them to the benefits. However, we think one cannot stay on the sidelines and make useful contributions.

To contribute to the debate, in this paper we present the global trends of biotechnology development in agriculture in the world, with emphasis on Africa. We present CIMMYT's work in the field, again with emphasis on Africa, and try to try some conclusions as to where we think the development is going.

2. Biotechnology

2.1. General trends in 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. It 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 allow us to identify the presence of specific genes already present in an organism that 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).

Tissue culture is a way of propagating plants starting from minuscule amounts of plant tissue. Because of the origin and sterile environment in which these plants grow, plantlets are stronger and reach maturity earlier than other plants. They are also free of pests and non-viral diseases, giving them a distinct yield advantage. The use of tissue culture is now widely accepted and practiced, and bananas from tissue culture are particularly popular in Kenya. The private sector has adopted the technology, and small companies are setting up shop to produce this material in Kenya and other African countries.

Genetic markers are sequences of DNA that are linked to certain traits of an organism. Over the years, different techniques have been developed to detect the markers, making it possible to screen plants at a very early stage. This technique has become a very important tool for plant breeding.

The third, and most controversial, biotechnology tool is genetic manipulation of organisms. Typically, a gene with useful traits is transferred from one organism to an unrelated organism, often from a different species. The result is a Genetically Modified Organism or GMO. Some of the most popular GMOs use genes from a bacterium, *Bt*, that generate toxins to insects. These genes, popularly called *Bt* genes, have been incorporated into maize and cotton varieties. These varieties were first introduced in the USA and spread very quickly, and now take about half the acreage in that country. Worldwide, xx ha have been planted.

GMOs have their share of controversy. They are perceived as dangerous manipulations of nature resulting in unnatural organisms (“Frankenstein foods”) that scientists do not sufficiently understand, its toxicity to humans was feared, as well as its effect on the environment, especially toxicity to non-target organisms, leakage into related species, and development of resistance. A large body of literature has been produced since the release of those varieties. Fears for toxicity to humans have been laid to rest, and significant impact on non-target organisms has not been demonstrated. Leakage into wild species, however, has been demonstrated, and the development of resistance is a real possibility.

The latest development in biotechnology is functional genomics. Its purpose is the complete picture of the roles and functions of plant genes of interest and how they interact with one another and the environment to produce an individual plant type. This work is just starting. At an international workshop at CIMMYT in Mexico, four principal areas of work were identified: alleviating abiotic stresses, alleviating biotic stresses, adding value to cereals, and improving yield potential, specifically by modifying photosynthesis.

2.2. GMOs: the debate

The use of GMOs holds much potential in enhancing food production through technologies that lead to reduced input use, reduced risk to biotic and abiotic stresses, increased yields, and enhanced quality of agricultural products (Mann 1999, National Academy of Science, 2001). Genetically modified (GM) crops have seen rapid adoption in countries such as the United States, Canada, Argentina and China. Area under GM crops increased from 2 million ha in 1996 to 40 million ha in 1999 with crops grown in over seven countries (James 1999). In Europe, however, a strong coalition of farmers' and environmental lobbys has stopped the GMOs from being deployed. The question arises, which path should Africa follow? We would like to contribute to the debate with scientific arguments, avoiding the emotions that unfortunately cloud the debate.

It is argued that developing countries stand to gain from products of genetic engineering. Dr. Norman Borlaug (2000) said recently, "The new tools of genetic engineering—if scientists are permitted to use them—will permit accelerated development of food crop varieties with greater tolerance to drought, heat, cold, and soil mineral toxicities; greater resistance to menacing insects and diseases; and higher nutritional quality levels. African governments should take care not to let these research products pass them by. Of course, governments must prepare themselves with the necessary legislation and regulations to ensure proper testing of genetically modified crops. But they also must ensure that farmers have adequate access to the new technologies that come from these scientific developments".

Moreover, the United Nations Development Program states in its latest annual report 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).

The legislation and regulations are necessary due to concerns about products developed using genetic engineering techniques. Concerns and issues that need to be addressed wherever GMOs are being developed or introduced include: 1) risks to human health; 2) ecological and environmental risks; 3) build-up of resistance to Bt-toxins in target insect pests; and 4) ethical concerns.

But why, in the face of these risks, adopt biotechnology? For three reasons: potential benefits, costs of inertia versus costs of change, and the means of managing those risks (UNDP, 2001, pp. 65-66).

There is an urgent need for a focused debate on the role of modern agricultural biotechnology in developing countries, a debate that should and is being led by people from developing countries themselves (Pinstrup-Anderson and Cohen 2000).

3. CIMMYT's work in biotechnology

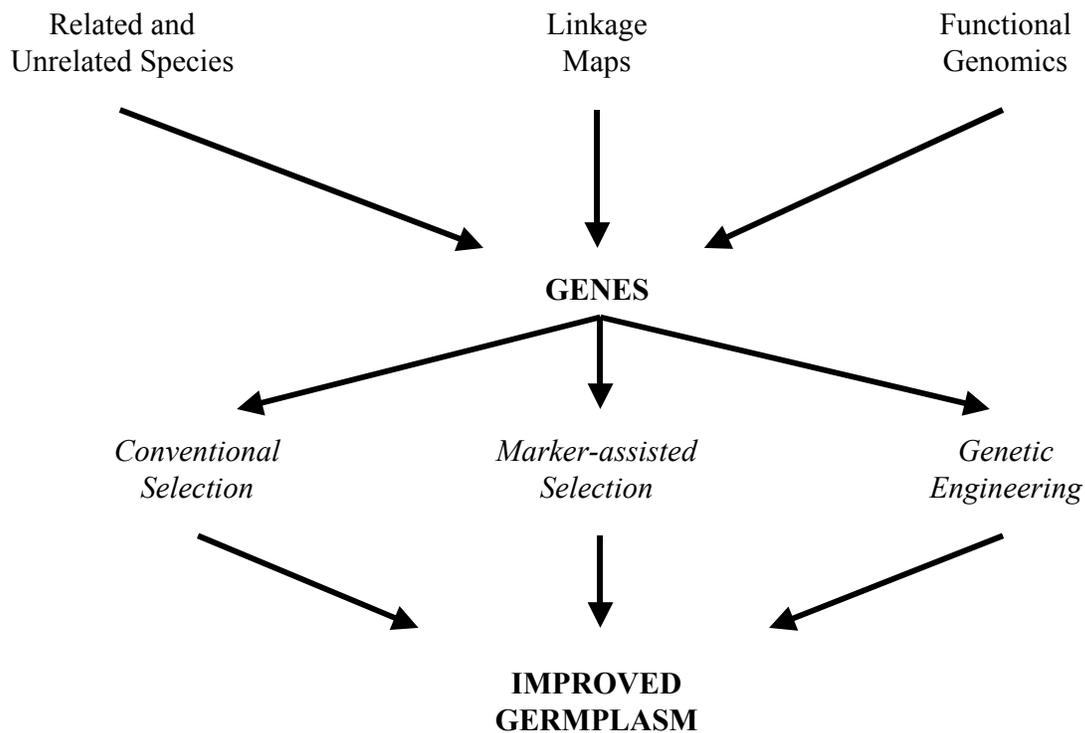
3.1. CIMMYT – mission and organization

CIMMYT's mission is to help alleviate poverty by increasing the productivity, profitability, and sustainability of maize and wheat farming systems. Scientists concentrate on three fields. New efficient maize and wheat varieties are being developed, in particular with built-in genetic resistance to important diseases, insects, and other stresses. A second activity is the conservation and utilization of maize and wheat genetic resources from throughout the world. Finally, we work on the development of more sustainable maize and wheat production systems. In this work we take a holistic approach, where teams of scientists of all disciplines work the whole range from high power biotechnology labs all the way down to testing new methods with farmers in their fields and under their conditions.

The impact of our work is especially observed in the success of our varieties. Over 55 million hectares are planted to CIMMYT-related varieties in developing countries (80% of total production), and more than 21 million hectares in developing countries are planted CIMMYT-related varieties (50% of the area in improved varieties).

The multidisciplinary is reflected in CIMMYT's structure. Five programs work closely together: the wheat, maize, economics, natural resources and biotechnology programs. The biotechnology program, also called the Applied Biotechnology Center, takes the lead in the upstream research, but collaborates with the other programs to bring its products to the end-users. The work concentrates on plant improvement (Figure 1). Apart from research, the ABC also provides services and training.

Plant Improvement Options



The most important current biotechnology activities focus on striga, insect resistance, quality/nutrition, All CIMMYT's work in biotech is a combination of ABC, with the respective commodity program (breeders, agronomists, and other scientists) and the economics program.

3.4. 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.

4. Current biotechnology activities at CIMMYT

4.1. *Striga* control

Striga spp. (*Striga hermonthica*, *Striga asiatica*) is an obligate parasitic flowering weed which, upon stimulation, germinates and attaches to the roots of important gramineae species including maize, sorghum, millet, rice, sugarcane and Napiergrass. Upon attachment to the roots of its host, *Striga* competes with the host plant for water and nutrients, and exerts a potent phytotoxic effect on the host, causing severe stunting and a characteristic "bewitched" (hence, the common name 'witchweed') and chlorotic whorl. An estimated 20 to 40 million hectares of farmland in sub-Saharan Africa are infested with *Striga* where it affects the welfare and livelihood of over 100 million people.

Agronomic practices, including hand weeding, trap and catch cropping, improved and managed fallows, inter-cropping, and use of organic and inorganic fertilizers have all been shown to help alleviate *Striga* problems by reducing *Striga* seedbanks and emergence, and by enhancing soil fertility. However, they have not been widely adopted because their effects are long term and require investment in resources, time and space before realizing benefits. Farmers also have problems to conceptualize how the effects of *Striga* on a current crop affect future crops, making adoption rates very low.

Genetic variability for *Striga* tolerance does exist in maize, but the level and stability of the tolerance have not been acceptable or fully exploited. Current conventional selection and breeding efforts to improve maize tolerance to *striga* in eastern Africa have produced varieties which are capable of producing 200% greater yields under both natural and artificial levels of *Striga* infestation. However, the absolute yield gains are still not sufficient to offset the added cost of seed.

Herbicides such as ethylene and dicamba can effectively control *Striga* but have not been adopted in Africa due to logistical difficulties (Ransom *et. al.* 1997) or cost effectiveness. Furthermore, general "over-the-top" herbicide applications may have little immediate effect since *Striga* inflicts most of its damage before the parasite emerges from the soil. In the longer term, appropriately targeted herbicide applications may reduce the *Striga* seedbank in soil and alleviate the problem.

In summary, agronomic practices, host plant resistance and herbicide use through conventional delivery methods, while often effective, have seen poor adoption for several reasons: (i) their effects are only seen in the medium to long-term requiring several seasons of use before benefits are realized, (ii) they require an understanding of the biology of *Striga* parasitism which farmers usually lack, (iii) they require rotating land out of maize production at a time when increasing population pressure requires increasing intensity of land use for food production, (iv) while host plant resistance exists, the gains are not sufficient to inspire widespread adoption at the benefit-cost ratios expected, and (v) conventional 'over-the-top' herbicide applications are ineffective or prohibitive in cost.

Among these multiple approaches, CIMMYT in collaboration with IITA, the Weismann Institute of Science, the University of Sheffield and KARI has in recent years undertaken several innovative biotechnological approaches to addressing the seemingly intractable problem of *Striga* parasitism of maize in Africa. These include the identification of alternative sources of resistance to *Striga* among its wild relatives, *tripsacum* and teocinte, the evaluation of mutator-induced resistance in maize, and the use of low-dose herbicide seed treatments on herbicide-resistant maize varieties.

Both teocinte and *Tripsacum* accessions have been identified that show good levels of tolerance to *Striga* (Gurney et al., 2001). In the field, some plants have shown seemingly complete immunity to *Striga*, i.e. no *Striga* emergence, season-long. Under more stringent conditions in the laboratory, these same materials were clearly better than the maize checks but moderate *Striga* germination and attachment was still observed. Further work is underway to determine whether the reduction in germination and attachment also reflects in reduced phytotoxicity at the plant level.

The identification of transposon-induced tolerances in maize appears to be a very promising longer term approach for *Striga* control. Mutator-containing families with *Striga*-free phenotypes have been identified and verified through stringent genetic and lab-based assays. CIMMYT is presently investigating further the potential value of those resistant alleles, both at the phenotypic and molecular levels, with the ultimate goal of introducing the most promising ones into adapted materials (Kanampiu et al, 1999).

One of the most promising short-term approaches to *Striga* control that CIMMYT has undertaken involves the use of low-dose herbicide seed treatments. This approach utilizes maize varieties developed from a natural mutant of maize containing resistance to ALS-inhibiting herbicides such as imidazolinones. These varieties do not fall under transgenic GMOs. Imazapyr (imidazolinone, tradename) is highly effective against *Striga*. When applied as a seed dressing on imidazolinone resistant (IR) maize, imazapyr is imbibed by the germinating seed and absorbed into the growing maize seedling (Kanampiu et al, 2001; Kanampiu et al., 2000). *Striga* seeds, stimulated to germinate by maize roots, attach and are killed by systemic imazapyr in the maize seedling before any damage is inflicted on the host plant. Additionally, imazapyr from the seed-coat that is not absorbed by the maize seedling diffuses into the surrounding soil and kills ungerminated *Striga* seeds. Very small quantities of imazapyr (as little as 30 grams a.i./ha, costing less than US\$4/ha) applied using this method have been found to be highly effective in providing season-long control of *Striga* and more than doubling maize yield under the conditions found in western Kenya. The cost of the coating is about \$0.16 per kg of maize seed, currently selling at \$2/kg, or a very modest increase of the price by 8%.

Finally, while each of these innovative approaches have shown considerable promise in controlling *Striga* parasitism, it is important to recognize that *Striga* infestation is only a symptom of a much greater malaise in African agriculture. *Striga* infestation has been caused and exacerbated by years of monocropping with cereals and declining soil fertility. As a consequence, areas have developed ultra-high levels of long-lived *Striga* seeds in the soil with only some breaking dormancy each season when stimulated by crop exudates. Biotechnological solutions to *Striga* will neither be successful nor sustainable without addressing these root causes of the problem.

4.2. Insect Resistance

4.2.1. The problem

Insect pests, of which stem borers are the most widely distributed and most damaging, seriously affect about 30 out of 35 million hectares of maize in developing countries. There are several economically important stem borer species in Africa. These are the pink stem borer (*Sesamia calamistis* Hampson), found throughout the continent, the African stem borer (*Busseola fusca* Fuller) and the African sugarcane borer (*Eldana sacharina* Walker) both found in nearly all sub-Saharan Africa, the spotted stem borer (*Chilo partellus* Swinhoe) found in most of Eastern Africa.

There are four general approaches to insect control, each with its own advantages and disadvantages: (1) chemical control by application of insecticides; (2) biological control through identification and introduction of natural enemies; (3) cultural control, which includes a broad range of field and crop management techniques; and (4) host plant resistance, by which the plant itself is resistant to the stem borers. For reasons of cost and labor, farmers often resign themselves to using no control measures at all.

4.2.2. Control methods

The chemical control method is the most widely used, but this method exposes the farmer to health risks and can result in pesticide loading of the environment. Sprays of bacillus thuringiensis (Bt) have been used as topical applications against lepidopteran insects in high value crops such as vegetables but no commercial use in maize has been observed. These are organic substances with little negative effects on the environment.

Biological control often requires trained personnel for identification and deployment of control agents and commitment of the farming community to enhance the establishment of biological control. Biological control often employs integrated pest management techniques and parasitoidal organisms such as other insects that prey on the target pest. In Kenya, work is being done with a small wasp called *Cotesia flavipes*, which attacks the *Chilo partellus*. Great care must also be taken to make sure that the predators do not have adverse effects on non-target plants and animals in the environment.

Crop management strategies such as stover management are best when used in combination with other control measures and rarely stand-alone. A crop management approach that shows potential is based on planting maize together with other plants, usually forage grasses that either attract and distract the borers away from the maize plants or repel them away from the plot where the maize is being grown. This approach is called push-pull in that the farmer grows plants around the border of the field that pull the borers away from the maize, and other plants are grown among the maize that push away or repel the pests.

Host plant resistance is the option favored by CIMMYT. Resistance is available to farmers encapsulated in the seed, a fact that ensures the technology is cheap, safe, and that farmers need not purchase more than the seed. Plant resistance to stem borers is a genetic

trait that enables a plant to resist or tolerate insect feeding. In development of maize varieties resistant to stem borers, resistance may be controlled by different allelochemicals that kill or impair the growth of stem borers. Morphological factors, including increased leaf fiber and silica content as a defense against the European corn borer (ECB) (Bergvinson et al 1997a; Rojanaridpiched et al, 1984), surface wax and high hemicellulose against Southwestern corn borer (SWCB) (Hedin et al, 1993), or a thickened cuticle against sugarcane borer (Ng, 1988) have been identified as resistance mechanisms. CIMMYT has pursued both resistance and tolerance mechanisms to stem borers (Kumar 1997).

4.2.3. Approaches in development of insect resistant maize.

CIMMYT followed conventional breeding methods to develop germplasm resistant to stem borers. Recently, however, CIMMYT has initiated development of insect resistant germplasm using both molecular and transformation technologies, 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 University of Hohenheim, Germany, developed QTL maps for the southwestern corn borer (SWCB, *Diatraea grandiosella*) and the sugarcane borer (SCB, *Diatraea saccharalis*) (Groh et al., 1998). Initial attempts transfer a number of major QTL to susceptible varieties using marker-assisted selection has 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. 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.

CIMMYT has also developed the capacity to produce transgenic maize. The resistance factor(s) in this maize are synthetic versions of the gene from the gram-positive soil bacterium *Bacillus thuringiensis* (*Bt*) that codes for a delta-endotoxin or insecticidal crystal protein. 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). Most Bt toxins are active against lepidopteran pests as well as some Coleopterans, but are not toxic to mammals.

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*) stem borers in maize. Transgenic plants containing insecticidal proteins will feature 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, behavior, 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.

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.

4.2.5. The Insect Resistant Maize (IRMA) project

A major project - the Insect Resistant Maize for Africa (IRMA) project - has been launched by CIMMYT and the Kenya Agricultural Research Institute (KARI) to enhance insect resistance in tropical 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, were transformed into tropical maize germplasm. 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 enhancing their germplasm.

By using tropical germplasm that expresses the *Cry* genes, we can test the effectiveness of modified proteins against the major African pests. In accordance with Kenyan national biosafety regulations, an application to introduce maize leaves carrying each of the *Bt* genes into the country has been prepared and is now under review by the Kenyan National Biosafety Committee. Leaves from transgenic maize lines will be used for bioassays with the Kenyan stem borers *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. We are referring to these events 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.

4.3. Enhanced Nutrition by improving maize quality

Traditional maize has a poor protein quality, it is deficient in lysine and tryptophan. In 1963, scientists at Purdue University found a mutant maize variety with much higher levels of those amino acids: Opaque 2. Unfortunately, this variety had a low yield potential. Many years of research work at CIMMYT led to the improvement of these varieties, now called Quality Protein Maize or QPM, making its yields competitive with conventional varieties of improved maize (Lauderdale, 2000). The CIMMYT scientists involved received the prestigious Food Prize for their effort. The improved protein of QPM can enhance the nutritional status of people who consume a great deal of maize as their staple food. There is also high potential of the varieties to be used in animal feed. Presently KARI and CIMMYT are developing QPM varieties suited for Kenyan conditions.

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

4.4. Apomixis

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 harvests each year instead of having to purchase fresh seed. The potential implication of this for farmers in developing countries many of whom cannot afford commercial seed is nothing short of revolutionary. Scientists from the French National Research Institute for Development Cooperation (ORSTOM) working at CIMMYT, along with ABC and Mexican researchers are using a sophisticated suite of conventional crossing methods and advanced molecular 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 four 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) the understanding of the mechanisms governing deleterious dosage effects in the maize endosperm, a non-desirable consequence of the expression of apomixis.

The work in interspecific hybrids is now entirely focusing on the analysis of maize-*Tripsacum* addition forms (i.e., combining the maize chromosomes and a few *Tripsacum* ones) derived from apomictic mother-plants. Molecular and cytogenetical characterization and progeny-testing revealed that some of them might have retained at least part of the apomictic process. Characterization of diplospory, the apomixis type in *Tripsacum*, at the molecular and cellular levels has produced new knowledge regarding both the mechanisms and their regulation. Such work will allow us to identify candidate genes which involvement in apomixis will be finally determined using the genomic tools already developed at CIMMYT (mutagenesis population). Cloning and identification of

one of the best candidate has been completed but this work still needs further analyses for determining the full sequence and for expression studies.

Within the artificial apomixis sub-project, search for genes and promoters which manipulation could result in formation of somatic embryos in the nucellus has been undertaken. Critical information was gained from our private sector partners and will be used in forthcoming experiments.

Deleterious dosage effects in the maize endosperm, a non-desirable consequence of the expression of apomixis, have been shown to result from alterations in the cell cycle regulation. Candidate genes were identified and will be more in-deep analyzed. This will be done through expression studies and the use of a mutagenized population that was especially designed and developed at CIMMYT.

4.5. Abiotic Stress Tolerance

Different options exist to combat abiotic stresses such as drought. Farmers can provide additional water for their crops through irrigation, or they can make better use of available rainfall through agronomic practices such as terracing or tied ridges. These methods are usually expensive or labor intensive, so farmers and scientists have searched for drought tolerance in maize varieties. Next to classical breeding, biotechnology can contribute substantially to this goal. CIMMYT is putting a lot of effort in this endeavor.

The major effort has been on the genetic dissection of drought tolerance through quantitative trait loci (QTL) identification of yield components secondary morphological traits of interest (e.g., anthesis-silking interval) and more recently physiological parameters. By December 2001, QTLs for drought-related traits will have been 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, nor the field evaluation to identify the target QTL.

The weakness of this quantitative genetic approach is that it provides very little information about the mechanisms and pathways involved in drought tolerance nor about the multitude of genes involved into 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 of a plant under water-limited conditions, a better understanding of the genes and the pathways involved into plant response will be crucial to accelerate, sustain, and complement conventional breeding programs. Therefore, research activities aimed to identify and characterize 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 cereals with higher productivity under water-limited conditions.

CIMMYT also works on other abiotic stresses. Low-nitrogen QTLs have been mapped in maize, and aluminum tolerance QTLs is currently being mapped in maize.

4.6. Durable disease resistance

Rust diseases, specifically leaf rust and yellow rust are globally important foliar fungal diseases in wheat. Enhanced resistance to these fungal pathogens plays a major role in the adaptation of improved wheat cultivars with high yield potential into different agro-climatic regions. Fusarium head blight is another fungal disease of global significance. In contrast to the rich genetic resources available for breeding better varieties, molecular genetic tools and a knowledge base for understanding factors conditioning disease resistance and tools for manipulation of such resistances using biotechnology approaches are lacking in wheat compared to other cereals of global importance. Characterization of genes conditioning durable resistance to rust diseases and fusarium head blight remained the focus of activities during the current year.

Although 10-12 slow rusting genes are known to be present in the CIMMYT spring wheat germplasm, only two such genes, namely *Lr34* and *Lr46* have been designated. We have been able to further characterize *Lr46* and have been able to pin point genomic location using bulked segregant analysis and partial linkage mapping using existing linkage maps. It has also been demonstrated that as in the case of *Lr34/Yr18/Bdv1* complex, this is also a complex of genes that have effects on at least leaf rust and yellow rust commonly. Further, markers have also been identified for *Lr34/Yr18* complex. We have been able to characterize several loci conditioning durable resistance in improved CIMMYT wheats such as Pavon 76, Parula and Tonichi. New gene designations have been obtained for two such genes and designate as *Yr29* in chromosome 1BL and *Yr30* in chromosome 3BS. Several quantitative loci conditioning resistance to fusarium head blight have also been identified.

We are using candidate gene approaches, using resistance like sequences isolated from rice and maize to find possible homologies with genes conditioning resistance in wheat. We believe that better characterization of gene complexes in wheat that would confer durable resistance to rust diseases would help in better understanding of the functional aspects of disease resistance not only to the rusts but likely for other pathogens as well since these genes are known to confer resistance to multiple diseases. We are exploring the possibility of utilizing markers identified for *Lr46/Yr29* and *Lr34/Yr18* in applications in the wheat breeding activities.

4.7. Training and Services

CIMMYT has extensive training activities. Three specific courses are organized regularly: molecular markers, genetic engineering, and applied biotechnology. CIMMYT also received regularly visiting scientists and students.

CIMMYT is also very active in different networks that promote the responsible use of biotechnology such as the Asian Maize Biotechnology Network (AMBIONET), the African Biotechnology Information Network, African Biotechnology Stakeholders Forum (ABSF), among others.

CIMMYT also provides services through its Molecular Genetics Service Lab. Different activities take place, such as DNA sequencing, QPM (*o2*) selection, *Ph1* pairing gene in wheat, cereal cyst nematode (CCN) marker in wheat, BYDV resistant *T. intermedium* segment introgression in wheat.

5. Conclusions

CIMMYT makes use of modern biotechnology to accomplish its mission of reducing poverty in the world. It continuously identifies fields where biotechnology can be used, listening directly to the needs of small scale farmers through its field workers and collaborators in the national programs.

CIMMYT makes use of the best science available, through its own high level scientists and through extensive networks with other research institutes, private as well as public. The applications are developed in teams of molecular geneticists, breeders, agronomists and social scientists, and are tested all the way until the farmers' fields, by farmers under their typical circumstances. Technologies are also carefully evaluated to fit local regulatory networks and distribution networks.

CIMMYT is well aware of the controversy around biotechnology, especially GMOs, and tries to make a contribution by offering solid science, proper risk assessment, respect for the regulatory framework in which the technologies will be deployed, as well as respect for the opinion of the public at large. We only work in those countries that have proper biosafety regulations in place, and we respect those rigorously. Where needed, we help regulatory agencies develop policies, and organize discussions and stakeholders meetings. All our projects are also accompanied by risk assessment and impact assessment studies. Finally, we like to contribute to the debate by engaging actively in discussion with the general population, as well as through presentations to the scientific community such as the present paper.

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