

**MAIZE ♦ PROGRAM ♦ SPECIAL ♦ REPORT**

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**The CIMMYT Maize  
Germplasm Bank: Genetic  
Resource Preservation, Regeneration,  
Maintenance, and Use**

**S. Taba, Technical Editor**



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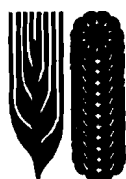
**CIMMYT**

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# MAIZE·PROGRAM·SPECIAL·REPORT

## **The CIMMYT Maize Germplasm Bank: Genetic Resource Preservation, Regeneration, Maintenance, and Use**

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**CIMMYT**

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CIMMYT is an internationally funded, nonprofit scientific research and training organization. Headquartered in Mexico, the Center is engaged in a worldwide research program for maize, wheat, and triticale, with emphasis on improving the productivity of agricultural resources in developing countries. It is one of 17 nonprofit international agricultural research and training centers supported by the Consultative Group on International Agricultural Research (CGIAR), which is sponsored by the Food and Agriculture Organization (FAO) of the United Nations, the International Bank for Reconstruction and Development (World Bank), and the United Nations Development Programme (UNDP). The CGIAR consists of some 40 donor countries, international and regional organizations, and private foundations.

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Responsibility for this publication rests solely with CIMMYT.

**Abstract:** Originally developed as background information for participants in a 1992 external review of the CIMMYT Maize Germplasm Bank and related activities, this publication recounts CIMMYT's role in the early work of marshalling maize genetic resources, tells how the materials collected have been utilized, summarizes current activities of the Maize Germplasm Bank, and describes directions in which CIMMYT activities on maize genetic resources are evolving to encourage more effective and widespread use of bank accessions.

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## **Foreword**

### **Global Implications of Germplasm Conservation and Utilization from a Plant Breeder's Perspective**

Saving endangered plant genetic resources is one of the most compelling issues today. Present and future attempts to address questions of sustainability and biodiversity in agriculture are certain to involve breeders and improved crop cultivars. Plant breeders are the primary initial users of germplasm bank accessions, since they can identify useful traits and incorporate them into adapted and useful cultivars. But breeders are generally less inclined to incorporate germplasm bank materials in their research than to use germplasm that has already been improved and adapted for a given production area. The reason for this is simple: along with any useful traits that germplasm bank sources provide, there are usually many undesirable, deleterious genes that keep a breeding program from reaching its objectives very quickly.

So, one might ask, what good are plant genetic resources? Why spend precious dollars and time to collect and maintain them?

The answer is that, despite the promise of splicing in genes from alien sources and the drawbacks of using germplasm bank materials, those materials are and will be the main source of crop genetic diversity and specific traits of interest to plant breeders for some time to come. The challenge is actually one of finding practicable ways to facilitate the use of germplasm bank accessions in conventional breeding programs. This special report briefly describes directions in which CIMMYT activities on maize genetic resources are evolving to meet that challenge (Chapters 5 and 6, Appendix 1). It also recounts our role in the early work of marshalling maize genetic resources (Chapter 1), tells how we have utilized the materials collected (Chapters 3 and 4, Appendices 2 and 3), and summarizes current activities of the Maize Germplasm Bank (Chapter 2).

Certainly there are numerous success stories involving the use of bank accessions. CIMMYT maize breeders have made significant improvements in cultivars by using materials from our germplasm bank. One example dates back to the 1960s, when a search was begun for host plant resistance to several corn borer species. After thousands of accessions were evaluated, it was evident that the Caribbean landrace, Antigua, contained genes that were favorable for resistance to one or more species of borers. Further work with the Antigua germplasm has resulted in the transfer of resistance to other agronomically acceptable germplasm. Currently marker assisted selection techniques are being used to enhance the transfer of these favorable genes to a wide range of maize germplasm. It now appears very likely that this use of bank sources will have a global impact, since it will reduce the need for harmful pesticides and in many environments considerably increase the production of food.

Other examples of successful use of bank materials in the CIMMYT maize program include the following:

- Outstanding improved populations have been generated by intercrossing selected accessions of the Tuxpeño maize race. The resulting varieties are some of the most widely sown tropical germplasm in the world.
- Extremely early-maturing tropical maize based on the early landrace "Oaxaca 256" is suited to a broad range of tropical environments and allows farmers to harvest early in the season, when food might otherwise be in critically short supply.
- Significant improvements in drought tolerance in CIMMYT maize have been accomplished through research involving populations formed partly from germplasm bank sources. The more than 300 bank accessions screened for this work were identified using the bank database. Specifically, entries were sought from areas where annual rainfall was less than 600 mm or which were noted as especially dry.
- Germplasm Bank accessions have also been used extensively at CIMMYT in breeding for tolerance to low soil fertility conditions. Recent results indicate that landrace materials show potentially useful genetic variation for high nitrogen uptake under nitrogen stress conditions as well as for several other characteristics associated with maize grown under low soil fertility conditions.

As evident from these examples, maize breeders have often found productive uses for germplasm bank holdings. But their value to breeders would increase greatly if improvements were made in the following areas:

- Information gathered during the collection and regeneration of accessions should be as complete and readily available as possible. This means that, during collection and/or regeneration, proper care must be exercised to record all characteristics likely to be of interest to subsequent users.
- Supplemental collection efforts are needed to fill gaps in bank holdings for given crops. In the case of maize, for example, we know of uncollected Caribbean landraces that could add useful diversity to the crop's gene pool, both for temperate and tropical areas.
- Information systems must be updated as improved technologies become available. Data banks should be maintained in such a way that accessions with very specific traits or combinations of traits can be identified quickly and accurately. In so far as is practical, this data should be made available for users on diskettes or by other data transmission methods to search as they desire.
- We should undertake as much "prebreeding" or germplasm enhancement as possible, especially for accessions that appear useful on a wide scale. This would include such efforts as converting accessions to acceptable plant heights and daylength responses. A prebreeding exercise that has proven quite useful to CIMMYT maize breeders is the formation of "core subsets" of large groups of accessions, such as the Tuxpeño race complex, based on agronomic acceptability. Materials from subsets can be made available either as individual entries or populations in which selected accessions are recombined.

- We should also include and describe as much improved germplasm as possible in banks. As mentioned, breeders have found improved cultivars very useful as parents in their programs. Obsolete improved varieties are often lost unless a conscious effort is made to preserve them. At CIMMYT we have decided to place in the germplasm bank seed of every fourth cycle of selection of maize breeding populations. Of course, care must be taken to keep such accessions at a manageable number. In this regard, it might be worthwhile to re-evaluate older collections to eliminate duplications or very similar entries.
- Finally, additional funding must be sought for these and other activities relating to the conservation and utilization of maize genetic resources.

As we move into the 21st century, the importance of genebanks to plant breeders will increase significantly. Improved cultivars will continue to replace landraces, and wild relatives will disappear as their niches are threatened by urbanization and changes in agricultural land-use patterns. Though the plants may disappear, however, the genetic diversity they embody need not vanish from existence. Careful preservation of improved cultivars will in fact retain the genes in a more useful form.

One way to promote genetic diversity in crops is to ensure the unhampered distribution of germplasm bank materials. The plant breeding community is truly global. The pedigree of the famous wheat variety, *Veery*, for example, contains 46 landraces from 18 countries. Those who develop improved cultivars should in some cases have property rights to market their product. But the germplasm should always be freely available for breeding research and should eventually be stored in genebanks for the benefit of future generations. As mentioned in Chapter 2, this principle is a key feature of CIMMYT policy on maize genetic resources and follows guidelines of the International Undertaking on Plant Genetic Resources (FAO Resolution 8/83).

In summary, germplasm conservation is extremely important to plant breeders and, in consequence, to the clients they serve. The value of germplasm bank accessions will grow with time. We must do all that we can to see that valuable genetic resources and information about them are preserved and made freely available to interested parties. For additional information about the maize materials and research mentioned in this special report, please contact the authors or the Director's office of the Maize Program.

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Associate Director

**The CIMMYT Maize Program**

## List of Acronyms

<b>CA</b>	Cluster analysis	<b>LGB</b>	Larger grain borer ( <i>Prostephanus truncatus</i> )
<b>CD-ROM</b>	Compact disc, read only memory	<b>MWv</b>	Maize weevil ( <i>Sitophilus zeamais</i> )
<b>CEW</b>	Corn earworm ( <i>Helicoverpa zea</i> )	<b>NAS</b>	The US National Academy of Sciences
<b>CIRAD</b>	The Center for International Cooperation in Agricultural Research for Development (CIRAD), France	<b>NCB</b>	Neotropical corn borer ( <i>Diatraea lineolata</i> )
<b>CRW</b>	Corn rootworms ( <i>Diabrotica</i> spp.)	<b>NRC</b>	The US National Research Council
<b>CTA</b>	The Technical Centre for Agricultural and Rural Cooperation (The Netherlands)	<b>NSSL</b>	The USDA National Seed Storage Laboratory
<b>DTP</b>	Drought tolerant population	<b>OPV</b>	Open pollinated variety
<b>Eto</b>	Maize race developed at <i>Estación Experimental Tulio Ospina</i> , Medellín, Colombia.	<b>ORSTOM</b>	The French National Institute for Development Cooperation
<b>FAO</b>	The Food and Agriculture Organization of the United Nations	<b>OSS</b>	The Office of Special Studies (a research unit established in Mexico in 1943 by the Rockefeller Foundation and the Mexican Ministry of Agriculture)
<b>FAW</b>	Fall armyworm ( <i>Spodoptera frugiperda</i> )	<b>PBS</b>	Población blanco semidentado
<b>GIS</b>	Geographic information systems	<b>PCA</b>	Principal components analysis
<b>GR/CIDS</b>	The Genetic Resources Communication, Information, and Documentation Systems project (University of Colorado, USA)	<b>PCIM</b>	Programa Cooperativo de Investigación de Maíz, Peru
<b>IBPGR</b>	The International Board for Plant Genetic Resources (now IPGRI, the International Plant Genetic Resources Institute)	<b>PET</b>	Preliminary evaluation trial
<b>ICTA</b>	Instituto de Ciencia y Tecnología, Guatemala	<b>PTC</b>	Población tardío Caribe
<b>INIA</b>	The Mexican National Institute of Agricultural Research (now INIFAP, the National Institute of Forestry, Agriculture, and Livestock Research)	<b>RAPDs</b>	Randomly amplified polymorphic DNA probes
<b>INIAP</b>	Instituto Nacional de Investigaciones de Agropecuaria, Ecuador	<b>RFLP</b>	Restriction fragment length polymorphism
<b>INRA</b>	The French National Institute of Agricultural Research	<b>SCB</b>	Sugarcane borer ( <i>Diatraea saccharalis</i> )
<b>LAMP</b>	The Latin American Maize Evaluation Project	<b>SM</b>	Spider mites ( <i>Oligonychus/Tetranychus</i> spp.)
		<b>SNLP</b>	San Luis Potosí (state in Mexico)
		<b>SWCB</b>	Southwestern corn borer ( <i>Diatraea grandiosella</i> )
		<b>UNAM</b>	The National Autonomous University of Mexico
		<b>UNDP</b>	United Nations Development Programme
		<b>USAID</b>	The US Agency of International Development
		<b>USDA</b>	The US Department of Agriculture
		<b>VAX</b>	Virtual address extended



## History of CIMMYT's Maize Germplasm Bank

*M. Listman, S. Taba\**

As an institution dedicated primarily to plant breeding, CIMMYT since its inception has been inherently both a user and generator of crop genetic resources. It was not until the mid-1980s, however, that the Center explicitly recognized major curatorial responsibility for an important part of the world's maize and wheat germplasm. This has occurred not as part of some master developmental plan, but rather in response to evolving perceptions (both within CIMMYT and without) about the role of genetic resources in breeding and as part of humanity's heritage. Succinctly stated, the story of CIMMYT's Maize Germplasm Bank may be said to comprise a progression through four major stages: 1) the collection, acquisition, and storage of holdings, 2) their organization for utilization, 3) systematization, evaluation, information dissemination, and the acceptance of global responsibility for New World maize landraces, and 4) movement toward functioning as a pro-active preserver and promoter of genetic resources.

### **Beginnings**

The current holdings of CIMMYT's Maize Germplasm Bank can be traced to samples of landraces collected and regenerated over 1943-1959 by the Office of Special Studies (OSS), a research unit operated jointly by the Rockefeller Foundation and the Mexican Ministry of Agriculture (Wellhausen, 1988). Collections were begun mainly to amass raw material for breeding improved maize in Mexico. That utilitarian aim was soon broadened by the Rockefeller-Mexico team to include preserving, as an endowment to humanity, farmer-crafted landraces against the day they would be replaced by improved maize.

Members of the group zealously combed the Mexican outlands, maize's center of genetic origin, to obtain some 2,000 representative samples of the crops' tremendous diversity. These they later classified into 25 races and some yet to be defined (Wellhausen et al. 1951, 1952), adding greatly to knowledge about the origin and evolution of maize in Mexico. In addition, their study inspired a subsequent project by the US National Academy of Sciences-National Research Council (NAS-NRC) to collect and characterize maize throughout the Americas (NAS-NRC 1954-1955). The more than 11,000 samples obtained from that effort were stored in the regional banks of Brazil, Colombia, Mexico, and Peru, and the US Regional Plant Introduction Station, and are listed in the Maize Preservation Committee reports. Racial classifications of the collections were set forth in a series of 11 bulletins cast in the mold established by Wellhausen et al. (1951).

The Office of Special Studies, closed in 1959, resulted in two important research initiatives: The National Institute of Agricultural Research (INIA), formed by Mexico to further the country's agricultural development; and the Inter-American Maize and Wheat Programs, launched by the Rockefeller Foundation to extend accomplishments of the Office of Special Studies beyond the borders of Mexico. As part of the new arrangement, maize collections gathered previously by the Office of Special Studies entered the patrimony of INIA.

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One of the first projects of Inter-American Maize Program was to assist INIA in regenerating its collections. In performing its charge, the Rockefeller program retained an extra set of the renewed seed of unique collections, compositing similar samples into groups, and stored it in a refrigerated facility at Chapingo. This material was used for activities of the Inter-American Program and, augmented by 618 entries from that group's collection work in the Caribbean, Mexico, and Central America, subsequently formed the inaugural holdings of the CIMMYT Maize Germplasm Bank.

Several other major sets of maize collection were added to the Inter-American Program collections after CIMMYT was formed in 1966: 1) backup samples of maize gathered from the Andean region and Central America under the NAS-NRC initiative and held temporarily at the US National Seed Storage Laboratory, Fort Collins, Colorado; 2) NAS-NRC collections originally stored in the Brazilian bank and eventually sent to CIMMYT; 3) CIMMYT collections from missions in the Andean region in the late 1960s; and 4) part of collections gathered from Brazil and Uruguay with support of the International Board for Plant Genetic Resources (IBPGR).

As mentioned, staff of the Inter-American Maize Program germplasm bank, concerned about the large volume of collections to be maintained, established several racial and geographical "groups" by compositing similar collections. In addition, the breeding program made composite populations—comprising both inter-racial and intra-racial crosses—from which elite fractions could be selected. For example, the population Tuxpeño Crema 1, created in 1965, originally comprised 8 collections, including materials from Michoacan with possible Celaya introgressions. In an early mixing generation, the population La Posta (formed in 1963 and including 14 Tuxpeño lines and 1 ETO line) was incorporated into Tuxpeño Crema 1. In the last four decades, Tuxpeño Crema 1 and La Posta have been used at CIMMYT to produce diverse but related germplasm populations (Srinivasan et al. 1992).

### **Consolidation and organization**

With the completion at El Batán in 1971 of a new office and laboratory complex, including suitable facilities for medium-term seed storage, all samples in the temporary bank at Chapingo were transferred to CIMMYT headquarters. Dr. Mario Gutiérrez, head of the CIMMYT Maize Germplasm Bank from 1966 to 1976, was responsible for organizing the materials and associated records and for overseeing their transfer to El Batán.

Despite lacking proper storage facilities during its initial years of operation, up through 1973 the CIMMYT Maize Germplasm Bank had sent some 470 shipments involving nearly 15,000 items to researchers in 80 countries, as well as growing for regeneration and/or propagation more than 8,000 accessions and recording basic information about them. That same year some 2,000 Bank collections were sent to locations in Africa, Asia, and Latin America for replicated field tests of yield, disease and insect resistance, and other agronomic traits.

The active collection of the Bank was initiated in 1972. Shortly after the new storage facility was completed at El Batán, the Bank continued the task of putting its house in order by documenting all

accessions as part of the Genetic Resources Communication, Information, and Documentation Systems (GR/CIDS) project of the University of Colorado, USA. The first passport<sup>1</sup> catalog was developed in the course of that work.

Attempts were also made during the 1970s to multiply and regenerate backup samples of the NAS-NRC materials received from NSSL. Initially, it was found that many of the collections, excepting those from Brazil and some of lowland precedence, were unadapted to conditions in Mexico and could not be grown at CIMMYT stations there. Later Dr. Gutiérrez sent seed of these collections to the banks of Colombia and Peru that had originally accepted responsibility for its maintenance from NAS-NRC, in the hopes that these institutions would have better luck at growing it out and thus replenishing their collections. These banks, though, also had difficulties regenerating the collections which, while they had originated on the same continent, were often adapted to conditions not found in the banks' experiment fields. CIMMYT received seed only from PCIM, Peru, as a result of the above effort. Moreover, once the outside funding associated with the NAS-NRC initiative ceased, conserving genetic resources often took a back seat to other agricultural research priorities in national program budgets. Thus, some of the original NAS-NRC collection may have been lost, and what remains comprises very small samples whose viability, after more than four decades of storage, has fallen to alarmingly low levels.

The next round of systematic collecting was sponsored by the IBPGR a few years after that institution's establishment in 1974 (Reid and Konopka, 1988). On the recommendation of an IBPGR-CIMMYT crop advisory committee on maize genetic resources, collections were made in Latin America and parts of Africa, Asia, and Europe, concentrating on areas where landraces were being displaced by modern agriculture. Some 15,000 samples were obtained, some of which now form part of CIMMYT collections, as mentioned.

After Dr. Gutiérrez left CIMMYT in 1976, a Maize Program scientist was assigned part-time responsibility for Bank oversight. This circumstance persisted until the mid-1980s and reflected the secondary role of genetic resource conservation at the Center during that time, when breeding for increased production was seen as CIMMYT's overriding priority (CIMMYT, 1992).

One of the main focuses of Bank activities then was to provide resources for breeding, not only to researchers at CIMMYT but worldwide. From 1979 to 1984, 163 cooperators in 40 countries requested nearly 4,000 Bank accessions, amounting to more than a million seeds (CIMMYT, 1985). Apart from responding to seed requests, staff regenerated nearly 1,000 accessions over this period to ensure the continuing viability of the collections. The best materials grown for regeneration were selected for further evaluation and crossing with appropriate gene pools in the breeding program.

Given the frequent difficulty of regenerating accessions of non-Mexican origin, added to the expense of conducting regeneration plantings, CIMMYT has received assistance in the upkeep of our stocks from outside sources on several occasions. In one case, during 1981-86 Dr. Wilfredo Salhuana of

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<sup>1</sup> Passport data comprise the records of origin for all collections, groups, and composites in the Bank.

Pioneer Hi-Bred International assisted CIMMYT in regenerating more than 1,600 accessions of tropical germplasm (Salhuana, 1987). Backup samples of regenerated seed were sent to NSSL for storage as CIMMYT accessions. CIMMYT collections from Ecuador in the late 1960s were also grown and regenerated at the Santa Catalina Station of INIAP (that country's national program) during 1986-88. In 1987, ICTA of Guatemala increased some original collections.

### **Ensuring conservation, facilitating utilization**

At the outset of the 1980s, the subject of genetic resource conservation—previously submerged in academic obscurity—was thrust into the public and political spotlight. Genetic vulnerability and related issues surfaced increasingly in the mass media as well as in scientific journals. As a leader in the development of maize and wheat germplasm for the developing world, CIMMYT came in for criticism regarding its management of the genetic resources under its care. But far from concluding that CIMMYT was unworthy of its trust, Center directors saw the commentary rather as an indirect mandate for a larger role in genetic resources.

Thus in 1984 the Maize Program began modifications in one of its cold storage rooms that made it possible to keep the temperature at  $-15^{\circ}\text{C}$ , at least doubling the lifetime of seed placed there. Each Bank accession was subsequently divided, one portion earmarked for long-term storage as a “base collection” in the new facility and the remainder to be kept in the intermediate storage, “active” chamber. This two-tiered partitioning of holdings represented a significant refinement in the Center's notion of how genetic resources should be managed: on the one hand, conservation of invaluable seed collections was assured, with the risk of genetic drift minimized; on the other, given that germplasm when simply “stored” is like a dead file in a dusty archive, utilization was afforded due importance as a separate-but-related function.

To further the latter, in 1986 the Center undertook a thorough overhaul of the maize germplasm collection. A new curator, Suketoshi Taba, was appointed full-time head of the Bank. Garrison Wilkes, a leading expert on genetic resource issues, was brought in to assess the condition of the Bank and to help organize into accessible computer data an overwhelming backlog of handwritten information about its accessions. CIMMYT staff began developing a database system for compiling and accessing information about germplasm stored in the Bank. This system, supported by the Center's mainframe VAX, contains files for all the major categories of Bank information—passport, regeneration, evaluation, and storage. It was developed in two principal phases: 1) software for managing passport data and regeneration and storage information; and 2) an evaluation database.

It was likewise proposed that CIMMYT should be responsible for certain parts of the world base collection of maize germplasm, a line of thinking that the Program Committee of the Center's Board of Trustees strongly endorsed in 1986, stating that “CIMMYT should continue to collect, conserve, document, and evaluate these races and their wild relatives...in cooperation with, and with the support of, IBPGR...thus playing CIMMYT's part in the network of conserving maize genetic resources” (CIMMYT, 1992). Later that year, discussions with IBPGR led to CIMMYT's acceptance of responsibility for maintaining a base collection of landraces of maize native to the Western Hemisphere.

*The wild relatives of maize* — Scientists and others have written about maize's nearest relative, teosinte, since the Spanish Conquest—the plant is mentioned in at least two Aztec codices as an ingredient in a medicinal preparation, and there are many accounts of expeditions to locate teosinte and take samples. Beginning in 1962, though, Wilkes systematically collected seed of more than 70 naturally occurring populations in Mexico and Central America, examined a subset of that collection for various agronomic and morphological characters, and described half-a-dozen major races of teosinte and their distribution (Wilkes, 1967). Some of this seed, along with other collections obtained later in Mexico and Guatemala, entered CIMMYT's Bank and has been used to fill requests from researchers around the world. CIMMYT also received seed deposits of teosinte from Mexican institutions (e.g., *Zea diploperennis* from the University of Guadalajara) (Taba, 1990).

CIMMYT's present system of distribution by race was established in recent years, along with the practice of checking the status of teosinte populations in Mexico and Central America through periodic monitoring visits. In this regard, valuable contributions came from Dr. Wilkes, USDA, and our national program colleagues. In particular, scientists from the Mexican National Institute of Forestry, Agriculture, and Livestock Research (INIFAP) and Dr. Angel Kato of the Graduate College of Chapingo have made outstanding progress in collecting, documenting, and characterizing Mexican teosinte populations in recent years, and IBPGR has provided valuable assistance in disseminating the information compiled.

The more distant wild relative of maize, Eastern gamagrass or *Tripsacum*, also figures among genetic assets long maintained and distributed by the Bank. During the early 1970s, Dr. Gutiérrez, in collaboration with L.F. Randolph, Division of Biological Science, Cornell University, established a *Tripsacum* garden on the CIMMYT research station at Tlaltizapán using clones from Mexico and introductions from Florida, about 70 of which are maintained in current Bank holdings. CIMMYT's most notable effort with *Tripsacum*, though, began in 1989 and involves visiting scientists from the French National Research Institute for Development Cooperation (ORSTOM). They have assembled some 1,500 accessions and are employing cytological, biochemical, and molecular biology techniques to characterize the genetically diverse collection, in hopes of using it as a wellspring of traits for maize breeding (see Chapter 4, "Wild Relatives of Maize.")

### **User-oriented bank management**

Current thinking about the role of the CIMMYT Maize Germplasm Bank depicts a service-oriented operation with values that closely parallel those of modern libraries. The overriding principle is getting one's product out to users—in our case breeders and other researchers, whose work benefits developing country farmers and consumers. Strategies for achieving this include 1) reaching out to users with targeted, special services; 2) personal contact with users to identify their needs; 3) networking with alternate suppliers to optimize resources; 4) structuring access to be as "automatic" (i.e., without intermediaries) as possible; and, above all, 5) active promotion of the products available. Several recent activities of the Maize Germplasm Bank illustrate our efforts to apply those strategies.

A major networking initiative occurred in 1988, when CIMMYT and INIFAP jointly hosted the Global Maize Germplasm Bank Workshop. The event attracted more than 50 specialists from nearly 30 nations and resulted, among other important outcomes, in a critical assessment of the status of major collections of maize landraces held in Latin American banks.

Following recommendations that emerged from this event, Bank staff have taken several steps with the broad goal of establishing a global network for the conservation and distribution of maize genetic resources. In 1991, representatives from 13 maize germplasm banks throughout Latin America convened at CIMMYT to discuss operational procedures of a project to regenerate endangered landrace collections under their responsibility. Genetic erosion in these holdings had reached serious proportions (the conventional preservation activity in many countries was to regenerate collections every 4-5 years). The Latin American Maize Evaluation Project (LAMP), begun in 1986, and IBPGR provided the participating national banks with some assistance for regeneration. CIMMYT supported this effort but was not a direct participant and none of the regenerated accessions were deposited in the CIMMYT Bank. To ensure the rescue and long-term preservation of the landraces described above, many of which no longer exist outside germplasm banks, in 1992 USAID and the USDA National Seed Storage Laboratory (NSSL) granted funds for CIMMYT to begin coordinating the regeneration by Latin American banks of 7,000 endangered accessions in their collections.

In addition to maintaining CIMMYT's landrace collections and assisting other banks in preserving theirs, we have recently begun making periodic visits to check the status of major Mexican landraces in situ—a strategy similar to that applied in the case of teosinte. Our staff travelled to Jalisco state, for example, in 1991-92 to monitor and take samples of the landraces Tabloncillo, Tabloncillo Perla, Tampiqueño, and Jala (Listman and Pineda, 1992).

*Documentation and characterization* — As a special service to users—previously constrained to sorting through massive, unwieldy catalogs when framing requests to our Bank—in 1988 we made passport information on some 10,500 Bank holdings, along with user-friendly inquiry software, available on a CD-ROM that essentially put the Bank in users' pockets. Manufactured and distributed with funding from the Technical Centre for Agricultural and Rural Cooperation (CTA) of The Netherlands and in collaboration with CGNet Services, the compact disc allows any researcher with access to a CD-ROM reader to quickly "zero in" on germplasm of interest, thus helping us to respond more effectively to requests for seed. During 1989-92, we updated maize descriptors jointly with IBPGR and national program collaborators and, with funding from IBPGR, developed a global maize database which was distributed by USDA in 1992 on a CD-ROM to national programs and the maize research community at large. Among other things, this product essentially updated the information distributed through the first CD-ROM.

Another recent line of work intended to enhance the utility of Bank collections is the formation of core subsets of race complexes. According to Frankel (1988), the sheer size of germplasm collections, while comforting, paradoxically poses a barrier to using them: "...these very large numbers...inhibit rather

than facilitate the close study needed for effective utilization of a collection. The problem is how to reduce numbers while holding the loss of genetic information within tolerable limits." A recent evaluation of the Tuxpeño landrace complex, for example, has allowed us to establish representative subsets of that rather extensive collection (see Appendix 1, "Forming core subsets from the Tuxpeño race complex"). By searching within a subset, breeders can more easily locate useful genetic diversity present in the larger body of material.

Finally, given the enormous interest of late in genetic resource conservation and utilization, the work of the Maize Germplasm Bank figures largely in CIMMYT publications and public awareness activities. For example, CIMMYT's 1988 annual report featured the theme of the conservation and management of maize and wheat genetic resources (CIMMYT, 1989). Disseminating information about work in the area of maize genetic resources helps to promote the products and services of the Maize Germplasm Bank to a range of potential users.

### **Conclusion**

Looking over the nearly three decades of existence of CIMMYT's Maize Germplasm Bank, it is apparent that the Bank has not only fulfilled the aim that Wellhausen and his team envisioned when they began their collection efforts—that of conserving genetic diversity destined to disappear before the onslaught of a growing human populace—but is now going a step further and, through concerted work to get useful germplasm from its collections into breeders' hands, actually returning some of that genetic diversity to farmers.

Serious challenges remain. A major one is to address the tremendous backlog of important collections, such as those which resulted from the NAS-NRC initiative, that require regeneration. Another significant task will be new collecting missions to fill large gaps in our germplasm holdings, thus achieving a truly representative global collection for maize. Finally, we need to promote the duplication of our accessions at other banks worldwide to guarantee their long-term safety and use.

If one strategy holds promise for tackling these difficulties, it is international cooperation. With this in mind, the CIMMYT Maize Germplasm Bank will continue to promote and strengthen the regional and global frameworks already in place for the conservation and management of maize genetic resources.

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## Current Activities of CIMMYT's Maize Germplasm Bank

S. Taba\*

### Our mandate

CIMMYT's Maize Germplasm Bank maintains base and active collections of landraces, the former for long-term storage and the latter (kept in medium-term storage) for seed distribution. Each will eventually incorporate the world's largest representation of landraces, with emphasis on those originating in the Western Hemisphere.

CIMMYT's conservation mandate also includes teosinte and *Tripsacum*. An active collection of teosinte is maintained by collecting seed during in situ monitoring tours of the remaining populations in Mexico and Guatemala and by regenerating previously collected samples. Collection is done in cooperation with national programs. An active collection of *Tripsacum*, representing the variation in this genus, is maintained at one of the Center's experiment stations, and seed is available from these plantings. In the future, in situ monitoring can be done along with that for teosinte.

In addition to conserving landraces, the Maize Germplasm Bank occasionally augments its collections with seed of elite lines and populations, developed by breeding programs at CIMMYT or other institutions, under proper agreements.

### Current holdings

A common practice in reporting CIMMYT holdings in the past was to include old collections where only 100-200 seeds were available. The number now reported—10,956, with some accessions under regeneration—includes only accessions with sufficient, viable seed. We also have some 2,200 collections comprising small amounts of seed. As has been mentioned, our stocks come from four major maize collection initiatives: 1) the Rockefeller and NAS-NRC efforts in the 1940s and 50s; 2) Inter-American Maize Program collections in the early 1960s; 3) CIMMYT collections in the late 1960s; and 4) the IBPGR-coordinated work of the 1970-80s (Reid and Konopka, 1988). In addition, collections gathered in Africa, Asia, and elsewhere were added to the store of seed. CIMMYT now preserves the collections of Rockefeller-OSS, NAS-NRC (partial), and the Inter-American Maize Program (PIM), as well as seed from Brazil and Uruguay collected with the support of IBPGR and a much smaller fraction from other parts of the world (Table 1).

### Long-term preservation

Base collection seed is kept in sealed containers at subzero temperatures (-15°C or lower) and low humidity, allowing it to remain viable for 50-100 years. The purpose of such long-term storage is to avoid "genetic drift"—possible changes in the genetic composition of an accession when it is grown out to replenish supplies or replace less viable seed. To further guarantee the safety of our base collection, a small sample of every accession will be deposited at the US Department of Agriculture's National

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Seed Storage Laboratory (NSSL) in Boulder, Colorado, and some seed will be duplicated in the holdings of developing country banks through the USAID-NSSL cooperative regeneration project now under way.

### **Distribution from the active collection**

Seed in the active collection, kept at just above freezing (0-2°C), constitutes the working bank from which all seed requests are filled. Our normal practice is to send from 25 to as many as 100 or 200 seeds per accession to any bona fide researcher free upon request, depending on availability (occasionally users are asked to wait while a collection is regenerated).

CIMMYT abides by national policies limiting or directing seed distribution to organizations within the country, assuming that a written statement of these policies is received from the appropriate authorities.

*Seed health* — Upon their arrival at CIMMYT in Mexico, incoming collections are inspected and treated before planting with a combination of insecticide and fungicide to safeguard against seedborne insects and diseases. To qualify for storage, seed must come from plots that are free from seedborne diseases of maize and/or undergo inspection by CIMMYT's Seed Health Unit. At harvest ears are treated with an insecticide solution in the field and air dried. After shelling and drying, seed is examined and unhealthy grains are discarded. We distribute only seed grown at one of our experiment stations or elsewhere under collaborative arrangements, with the exception of seed of teosinte populations from wild stands in Mexico and Guatemala (before storage that seed is treated with insecticide and fungicides). In the case of *Tripsacum*, we also supply small amounts of material from our clonal garden at Tlaltizapán. All CIMMYT experimental plots or wild-relative gardens are monitored from planting to harvest by program pathologists and entomologists. For all shipments, CIMMYT's Seed Health Unit conducts various tests following international quarantine guidelines. Every possible precaution is taken to ensure that the seed is alive, healthy, and free of diseases and insects, and all shipments are accompanied by appropriate phytosanitary documentation from CIMMYT and the government of Mexico.

### **Bank staff**

Germplasm Bank operations are the responsibility of one senior staff person, supported by a research assistant and two field helpers, one at Tlaltizapán and the other at El Batán. Two temporary helpers have also worked with us for the last five years, the last four under funding from the global database project. They will be kept on, either through core funding or as part of the USAID-NSSL regeneration project.

### **Seed shipment and internal use**

During 1988-92, from 500,000 to 1,000,000 seeds were used each year (Tables 3-7). Internal use was primarily for Bank evaluations. Evaluation and enhancement are suggested to promote the utilization of the germplasm preserved.

## **Preservation and regeneration**

The current Bank storage facility was built in 1972. Funds from IBPGR were used to modify half the storage space for the base collection in the mid-1980s, allowing seed there to be kept at  $-15^{\circ}\text{C}$ .

The storage vault now used for the active collection measures nearly  $140\text{ m}^3$ , with a storage capacity of 10,920 accessions in one-gallon tin cans. All available space there is currently being used. The base collection vault measures  $145\text{ m}^3$ , and can hold 17,820 accessions in half-gallon cans of 1-1.5 kg, or 5,000 seeds. Extra space is available there, since both active and base vaults presently hold the same number of samples—i.e., all Bank accessions. Excess seed from regeneration plantings (saved for exchange) is now stored in the Wheat Program Bank, but will soon have to be moved elsewhere. A seed drying unit has been in operation since 1991.

Field space for Bank use at CIMMYT experiment stations is normally allocated as follows: 3,500-4,000 rows at Tlaltizapán (state of Morelos, 940 masl,  $18^{\circ}\text{N}$ ), 1,000-1,200 rows at Poza Rica (state of Veracruz, 60 masl,  $20^{\circ}\text{N}$ ), and about 2,000-2,500 rows at El Batán (headquarters, 2,240 masl,  $19^{\circ}\text{N}$ ).

Since 1988 we have regenerated 893 entries, 548 of which were collections and 345 accessions (Table 2). Of the collections regenerated, most are new introductions collected recently in Brazil and Uruguay with IBPGR funding and old collections taken in Venezuela under the NAS-NRC initiative. We regenerate a collection when one or both of the following conditions exists: 1) low amounts of seed or 2) poor germination in evaluation trials. Fewer regenerations were performed in 1990-91 because of a lack of field space and an increased emphasis on race evaluations, but in 1992 we greatly augmented the number of regeneration plantings.

*Regeneration methodology and policies* — We employ chain crosses in 150-250 plants sown in 16 rows of 5 m and repeat regeneration plantings from which fewer than 100 ears are harvested (Table 2; approved vs not approved). Some failed entries are replanted the following season in either 8 rows or a full 16 rows and the harvest combined with that of the previous cycle to reach the 100-ear minimum. The harvested ears are treated immediately with insecticide at the field station, dried to 13-15% moisture, and shelled. Fifty seeds of each ear are used to make a balanced composite. Two balanced composites are made for each collection—one for long-term storage at CIMMYT and the other for backup at NSSL. The remaining seed is used for another balanced bulk by volume from each ear for the active collection. As of 1991, the three seed bulks for each regeneration are then placed in a drying room at low relative humidity for three-to-four weeks before packing. Germination and seed moisture (4-6% optimum, depending on texture) are determined before storage in the base collection.

Excess seed bulk from regeneration has recently been stored in CIMMYT's Wheat Germplasm Bank. In 1992 we cleared out as much of this as possible, leaving less than 3 kg per collection and only those regenerated after 1980. A list of the remaining seed is being compiled. One alternative for handling excess seed from regeneration is to offer it to other banks or programs. In 1986, for example, some of this seed was "repatriated" to the Latin American countries whence the particular collections originated.

As for the regeneration backlogs that exist, it has also been suggested that banks in the countries of origin for specific collections assist in renewing seed of those holdings. Most comprise highland material acquired from NSSL in the late 1960s as part of the original NAS-NRC collections and those made by CIMMYT in the Andes at that time; having been in storage for so long, their viability is uncertain. During the last few years, we have returned all original collections from Guatemala to the national program of that country, where in subsequent plantings only about 20-30% of the seed germinated. Portions of seed from those regeneration plantings have been sent back to CIMMYT. We also returned to Costa Rica entries originating there that we were unable to regenerate. To date there has been no word on actions taken with those collections, but we will include them in cooperative work under the CIMMYT-USAID-NSSL regeneration project for Latin American landraces.

Some Bank accessions in the past have been established without prior regeneration. Our current procedure for new introductions is: 1) observation plantings, 2) regeneration plantings, and 3) registration as an accession.

Teosinte collections in the Bank are regenerated when the supply of seed reaches a certain minimum level. We have begun regenerating some accessions in pots at Tlaltizapán during the off-season for maize, and were able to regenerate the Guatemala population *Zea luxurians* by artificially shortening the daylength exposure. We will continue to experiment with regeneration methods for other populations. Requests for teosinte can also be met by providing samples of seed collected in situ.

*Duplicate storage* — Up through the mid-1980s Pioneer Hi-Bred International assisted in regenerating CIMMYT accessions and shipping samples to NSSL for registration as CIMMYT deposits. After that, shipments were held until completion of the new NSSL facility. In 1992, we shipped 599 newly regenerated accessions to NSSL. We are preparing duplicate samples of materials regenerated in the last 10 years. To date 6,388 duplicate samples of CIMMYT accessions are held at NSSL.

In 1986 we offered excess seed in our holdings to banks in Latin America, distributing it to the countries of origin. Thus far we have sent backup samples of our stocks largely to the NSSL International Base Collection Storage Bank. We will continue to send duplicate sets there, as well as announcing the availability of excess seed to interested banks.

Approximately 2,200 accessions need regeneration to ensure viability and/or sufficient seed (Table 8). Some NAS-NRC collections in this category could be included in the USDA-NSSL regeneration project. In addition, we need to regenerate collections recovered by Dr. Gutiérrez in the late 1960s and early 1970s. Finally, we have other samples of non-accessioned seed that have never been regenerated, some requiring growing conditions very similar to those of their native habitats.

## **Evaluation**

After compiling passport data on Bank accessions in 1986-87, we began intensive efforts to evaluate our holdings for general characteristics such as ear morphology and plant architecture. Some 40 trials were conducted in race groupings established using the newly compiled passport information. We

summarized results for the Tuxpeño race complex and used them to develop core subsets of that complex. A subsequent comparison, through the global passport database project, of Tuxpeño race accessions in the CIMMYT and INIFAP banks showed that the Mexican institution has an additional 570 accessions which were not included in our evaluation trials. The Tuxpeño complex of the western and northern state of Mexico contains introgressions from the Mesa-Central-maize germplasm complex. In the southern parts of Mexico, the Zapalote germplasm complex influenced Tuxpeño (Kato 1988, 1984). The INIFAP collections contain additions of Tuxpeño races from Chiapas, Tabasco, and Quintana Roo.

Our evaluations also covered Mexican dent complexes such as Celaya and Vandeño, as well as the highland races Conico, Conico Norteño, and Chalqueño. In cooperation with Dr. Fernando Castillo of the Graduate College of the University of Chapingo, and a student of his, Conico core subsets were selected using data from 1991-1992 evaluation trials. Data from evaluation trials for other races will be summarized and core subsets established through similar types of collaboration. Additional trials in other environments may have to be conducted before enough data are available, especially since only part of the race complexes are preserved at CIMMYT. Complementary evaluation trials with the accessions preserved at the Mexican bank would be desirable.

The narrow-ear complex of western Mexico—which includes Tabloncillo, Reventador, Dulcillo, and Harinoso de Ocho—was evaluated at Tlaltizapán in 1989. The top performing accessions were used to test combining ability with ETO and Tuxpeño experimental varieties (see appendices 2 and 3, “Evaluation of Tabloncillo Ancho and Tabloncillo Tampiqueño Full-sibs in Crosses with Poza Rica 8749” and “Combining Ability of Mexican Narrow-ear Races with Tuxpeño and ETO Testers”). The lowland tropical and subtropical maize subprograms of CIMMYT are conducting research using the Bank collections evaluated (see Chapter 3 “Utilization of Bank Materials”). Bank staff have also performed informal evaluations of certain collections under regeneration, with an eye to enhancing them and, in one instance, looking for Mexican yellow flint and dent source materials.

### **Bank information management**

We have an in-house database system developed in 1986-87 (Rosales et al. 1990) for day-to-day Bank activities such as seed shipments, regeneration, evaluation, and maintaining passport and seed storage information. This is constantly updated. In addition, in 1989-91 we developed a PC-operated global database system, in collaboration with CGNet and with IBPGR funds, that has been distributed throughout the international maize conservation network.

### **Collaboration with other institutions**

The Bank's strong emphasis on collaboration as a way to use research resources and disseminate results efficiently is illustrated by several recent or ongoing efforts related to the conservation and utilization of maize genetic resources. It should be mentioned that these endeavors build on previous collaborative work by the CIMMYT Bank and its predecessor of the Inter-American Maize Program in collecting, evaluating, and facilitating the use of maize genetic resources. These early networking linkages have gradually been strengthened with funding from agencies such as USAID, Pioneer Hi-Bred International, and IBPGR.

In 1989 the IBPGR seed physiology unit granted the Boyce Thompson Institute of Cornell University funding for research on maize seed longevity in cooperation with the National Autonomous University of Mexico (UNAM) and the CIMMYT Maize Germplasm Bank. Our main contribution to the project is seed of landraces, in return for which we receive associated data.

The global passport database development project funded by IBPGR was carried out in close collaboration with Latin American maize banks and has resulted in the documentation of many accessions for which information was previously difficult to obtain. Regarding the Latin American Maize Evaluation Project (LAMP), CIMMYT was not a direct participant, but we have made our collections available to research programs in the USA, Guatemala, and Uruguay for LAMP trials, have cooperated with LAMP-USA in the evaluation of Caribbean collections (20%) and topcross trials, and have planted other LAMP trials on our experiment stations at El Batán and Tlaltizapán. Finally, we have participated in several LAMP-related events, including a seminar on genetic resources and meetings in Chile and the USA.

*The Latin American regeneration project* — This project is the centerpiece of our efforts to establish an effective international network for the conservation and utilization of maize genetic resources. Through it, CIMMYT is coordinating the regeneration by Latin American banks of more than 7,000 maize landrace samples in danger of being lost. The work is funded by USAID and USDA-NSSL (the latter as a continuation of previous regeneration work in Colombia, Mexico, and Peru conducted by North Carolina State University with grants from USDA). The banks will keep the collections they regenerate. To strengthen international conservation, backup samples will be stored at CIMMYT and NSSL in Fort Collins, Colorado, USA. CIMMYT will also maintain active holdings of the landraces, together with computerized information about them, for distribution to interested researchers worldwide. In addition, participants will eventually possess a complete electronic database on materials they hold, a fundamental precondition for a hemisphere-wide maize germplasm network.

Given the amount of germplasm in Latin American banks and the status of those holdings, it would be logical to expect that there will be an extension of this project. It has been estimated, for example, that some 5,000-6,000 Latin American and Caribbean accessions under long-term storage at NSSL now require regeneration (Eberhart 1991), suggesting that many of the NSSL duplicates should be replenished through the USAID-NSSL regeneration project.

### **Monitoring teosinte**

As mentioned, our major strategy vis a vis teosinte, apart from maintaining collections in our Bank, is periodically to check the status of populations in situ. In 1991, for example, staff monitored and took samples of teosinte in Guatemala, in collaboration with Dr. Wilkes and the Guatemalan National Genetic Resources Program, finding that several populations were smaller than when last observed. On a similar trip in Mexico in 1992, we found that all populations were stable and discovered new populations at previously unexplored sites. Future trips are planned to seek out unknown populations, especially in the area of Oaxaca and the Balsas River basin (Wilkes, personal communication). The Bank has recently begun to regenerate teosinte seed in its collections to ensure

the continuing viability of base stocks and to supply seed for active use. Finally, we would like to thank the genetic resource programs of INIFAP, Mexico, and Guatemala, and express our sincere appreciation to colleagues from those programs for their extraordinary interest and cooperation in this work.

### **Training**

Our staff engage in a range of activities designed to strengthen the capacity of national programs to preserve and utilize the maize genetic resources at their disposal. Personnel of El Salvador's maize bank, for instance, recently spent a month with us to learn about our management system and field operations. We also work with researchers who attend in-service training courses at CIMMYT headquarters, providing information on Bank contents and procedures for regeneration, characterization, and evaluation. Our staff participated in a course on genetic resources at the University of California, Davis, in 1990, and in a joint training course at that institution in 1992 in which we gave hands-on instruction in bank management. We intend to offer similar short courses from time to time. Finally, we consider the attention afforded the many visitors to the Bank throughout the year as a type of training activity.

### **Collecting**

Little collection has been done in recent years, except for samples of teosinte obtained during monitoring visits in Guatemala and Mexico. The samples from Guatemala have not yet been deposited in the Bank due to that country's policy on seed exchange of wild plants. We gathered seed of Tampiqueño and Tabloncillo Amarillo and Azul during recent visits to Jalisco state to monitor the status of maize landraces, and are growing them out and checking their appearance against the descriptions in the passport database. Several unique ones, especially of the Jala and Tampiqueño races, have been registered as accessions. Dr. Kato of the Graduate College has also provided us with samples of Jala and Jala mixtures from near the point of origin of that landrace. We plan a monitoring visit to Chiapas to check the status of Ollotillo and collect samples in the near future.

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**Table 1. Current CIMMYT Maize Bank accessions by country of origin.**

Country	No. of accessions	Country	No. of accessions	Country	No. of accessions
AFG	19	ETH	29	PAN	149
AGO	6	GLP	21	PER	263
ARG	90	GRP	21	PHL	3
ATG	10	GTM	468	PRI	32
AUS	1	GUF	9	PRY	99
AUT	7	GUY	15	SCX	13
BHS	1	HND	18	SLA	7
BOL	98	HTI	38	SLV	79
BRA	2390	IND	2	SUR	11
BRB	13	ISR	4	SVT	16
CHL	63	JAM	9	THA	2
CHN	25	KEN	2	TOB	19
CIM	114	LBN	7	TRI	40
COG	1	MEX	3532	UGA	4
COL	120	MLI	58	URY	972
CRI	375	MTQ	2	USA	32
CUB	171	MWI	5	VEN	332
DEU	7	NGA	1	VGB	53
DOM	99	NIC	74	YEM	2
ECU	578	NPL	212	ZWE	4
EGY	1	PAK	2		
				<b>Total</b>	<b>10,956</b>

\* Country codes of three letters are used; CIM = CIMMYT.



**Table 2. Regeneration of Maize Bank collections/accessions at CIMMYT in 1988-1992.**

Year	No. of entries planted	No. of entries approved <sup>1</sup>	No. of entries for repeat
1988	317 (301 Coll. <sup>2</sup> + 16 Acc. <sup>3</sup> )	280 (264 Coll. + 16 Acc.)	37 (37 Coll. + 0 Acc.)
1989	462 (422 Coll. + 40 Acc.)	238 (199 Coll. + 39 Acc.)	224 (224 Coll. + 1 Acc.)
1990	172 (69 Coll. + 103 Acc.)	107 (34 Coll. + 73 Acc.)	65 (65 Coll. + 30 Acc.)
1991	117 (31 Coll. + 86 Acc.)	107 (26 Coll. + 81 Acc.)	10 (10 Coll. + 5 Acc.)
1992	342 (128 Coll. + 214 Acc.)	161 (125 Coll. + 138 Acc.)	181 (181 Coll. + 76 Acc.)
<b>Totals</b>	<b>1410</b>	<b>893</b>	<b>597</b>

<sup>1</sup> Approved = At least 100 usable ears harvested.

<sup>2</sup> Coll. refers to the number of collections (undergoing 1st regeneration).

<sup>3</sup> Acc. refers to the number of accessions with accession number.

**Table 3. Seed distribution of accessions from the CIMMYT Maize Germplasm Bank, 1988.**

Country	Number of shipments	Number of packets	Number of maize seeds	Number of teosinte seeds	Number of <i>Tripsacum</i> seeds
Argentina	2	13	1000	750	
Brasil	1	5	1000		
CIMMYT	48	4341	542453 + (5.9)*		250
Canada	2	14	1600 + (39)*		
Costa Rica	1	316	161792		
Ethiopia	2	17	3200		
Spain	1	15	3000		
France	1	7	-	350	
Germany	1	14	450	250	
Hungary	2	18	3600		
India	3	105	18000		375
England	1	14	-	240	180
Mexico	6	382	156900		
Portugal	1	1	-	25	
Philippines	1	5	-	150	100
Thailand	1	1	+(0.25)*		
USA	20	499	135650 + (33.1)*	360	
Zimbabwe	3	650	77888 + (0.5)*		
<b>Totals</b>	<b>97</b>	<b>6417</b>	<b>1,106,533 + (78.75)*</b>	<b>2125</b>	<b>905</b>

\* Seed weight in kg.

**Table 4. Seed distribution of accessions from the CIMMYT Maize Germplasm Bank, 1989.**

Country	Number of shipments	Number of packets	Number of maize seeds	Number of teosinte seeds	Number of <i>Tripsacum</i> seeds
Argentina	4	37	1600	490	
Brasil	3	40	4300	400	500
Bolivia	2	12	1100 + (1)*		
CIMMYT	47	5093	509542 + (2.3)*	1400	
Costa Rica	1	51	255		
Cuba	1	6	1500		
China	1	14	2100		
Ecuador	1	5	500		
Spain	1	9	150	150	150
Honduras	2	119	11900		
India	1	74	14800		
Indonesia	1	10	1500		
England	1	2	400		
Japan	1	22	2400	300	
Korea	1	28	1400		
Mexico	13	239	30148 + (0.5)*	50	150
Nepal	1	18	1800		
Nigeria	4	514	75600	200	150
Peru	1	7	7000		
Philippines	1	6		150	
Thailand	5	60	11700 + (1.0)*		
USA	14	1317	52784 + (64.3)*	350	280
Uruguay	1	3	+ (4.5)*		
Yemen	1	4	1000		
Zimbabwe	3	19	2100		
<b>Totals</b>	<b>112</b>	<b>7709</b>	<b>735579 + (73.6)*</b>	<b>3490</b>	<b>1230</b>

\* Maize seed weight in kg.

**Table 5. Seed distribution of accessions from the CIMMYT Maize Germplasm Bank, 1990.**

Country	Number of shipments	Number of packets	Number of maize seeds	Number of teosinte seeds	Number of <i>Tripsacum</i> seeds
Argentina	3	16	3850		
Bulgaria	1	7	350		
CIMMYT	47	3450	506894		250
Colombia	1	1	+(0.5)*		
Canada	1	4	2000		
Cote de Ivoire	1	582	74496		
Spain	2	5	1200	20	
France	1	1	200		
Guatemala	1	338	18748		
Germany	2	18	1550	20	
Hungary	1	2		100	
India	1	20	2000		
Iran	1	9		120	60
Mexico	6	203	21016	160	
Peru	1	4	4800		
Rep. Dominicana	1	10	2000		
Thailand	1	1	+(0.5)*		
USA	12	650	33500 + (138.9)*		
Zimbabwe	3	219	41728		
<b>Totals</b>	<b>87</b>	<b>5540</b>	<b>714332 + (139.9)*</b>	<b>420</b>	<b>310</b>

\* Maize seed weight in kg.

**Table 6. Seed distribution of accessions from the CIMMYT Maize Germplasm Bank, 1991.**

Country	Number of shipments	Number of packets	Number of maize seeds	Number of teosinte seeds	Number of <i>Tripsacum</i> seeds
Argentina	2	6			
Brasil	4	24	5600	75	60
Bolivia	1	2		40	
CIMMYT	27	2231	389756	35	
Canada	2	20	2000 + (10)*		
Colombia	1	10	2000		
Costa Rica	1	50	7650		
China	1	2		10	10
Spain	1	3	750		
England	1	1	100		
Ghana	1	23	1150		
Honduras	1	17	1700		
Italy	2	4	150	15	
India	1	5	500		
Mexico	6	320	44466		
Peru	1	2	50		50
Thailand	3	47	4000	30	20
USA	10	89	10040+(2.5)*	445	
<b>Totals</b>	<b>66</b>	<b>2856</b>	<b>469.912+(12.5)*</b>	<b>650</b>	<b>140</b>

\* Maize seed weight in kg.

**Table 7. Seed distribution of accessions from the CIMMYT Maize Germplasm Bank, 1992.**

Country	Number of shipments	Number of packets	Number of maize seeds	Number of teosinte seeds	Number of <i>Tripsacum</i> seeds
Argentina	3	60	10400	360	
Brasil	1	7	100	150	
Costa Rica	1	2	600		
CIMMYT	25	1970	404480		
China	2	4	300	50	
Czechoslovakia	1	3		300	
Spain	1	2		50	
Ecuador	2	4	900 + (0.5) *		
France	1	6	600		
England	1	1		50	
Ghana	1	2	100		
Kenya	1	2	300		
Iran	1	97	14550		
Mexico	11	252	48944	40	
Nigeria	1	105	+(0.5) *		105
Sweden	1	5		125	
Thailand	1	1	+(1.0) *		
USA	10	693	26400 + (837.5) **	175	
<b>Totals</b>	<b>65</b>	<b>3216</b>	<b>507674 + (839.5) *</b>	<b>1300</b>	<b>105</b>

\* Seed weight in kg.

\*\* Duplicate sample shipment to NSSL (599 accessions).

**Table 8. Accessions for regeneration, CIMMYT Maize Germplasm Bank.**

Country	Count	Country	Count
Angola	4	Malawi	3
Argentina	36	Mali	16
Austria	2	Mexico	275
Bolivia	34	Nepal	76
Brazil	614	Nicaragua	9
British V. Islands	35	Pakistan	1
Colombia	81	Panama	30
Costa Rica	153	Paraguay	62
Chile	26	Peru	47
China	11	Philippines	2
Cuba	41	Puerto Rico	2
Dominican Republic	2	Saint Croix	3
Ecuador	89	Saint Lucia	3
Egypt	1	Saint Vincent	1
El Salvador	4	Suriname	8
Ethiopia	12	Tobago	1
Germany	3	Trinidad	1
Guatemala	214	United States	7
Guyana	11	Unknown	3
Honduras	10	Uruguay	156
Israel	4	Venezuela	93
		<b>Total</b>	<b>2186</b>

## Utilization of Germplasm Bank Materials: The CIMMYT Maize Program

### **The development of general purpose and hybrid-oriented source germplasm for the tropical lowlands (S.K. Vasal)\***

Bank materials have been used quite effectively in developing useful maize germplasm at CIMMYT. One good example is Tuxpeño Crema-1, which was formed by intercrossing outstanding accessions of the Tuxpeño race. This material is being used widely in different parts of the world, especially developing countries. We now have different versions of Tuxpeño Crema-1 that combine attributes such as drought tolerance, short plant stature, streak resistance, brachytic-2, tolerance to inbreeding, and improved crossbred performance in hybrid combinations. Bank materials have also been used in developing new populations, pools, and germplasm complexes of particular races. The following describes the systematic use of bank accessions to develop general purpose and hybrid oriented source germplasm for the tropical lowlands.

*Special considerations* — The possibility of using Bank materials to generate useful source germplasm has been recognised for a long time. Unfortunately, the poor agronomic character of most of Bank materials makes their frequent use impractical in active breeding programs. Bank accessions often have excessive plant and ear height, a tendency to root and stalk lodging, too much foliage, barrenness, greater pollen shedding and silking interval, and a lack of inbreeding tolerance. One must resort to prebreeding before this germplasm can be used effectively or introgressed into other germplasm. Its use also requires special breeding skills and a definite germplasm management system to keep the total number of source populations to a more or less determined level.

*The evolution of Program breeding philosophies* — Beginning in 1973-74, the CIMMYT Maize Program devised a two-tiered germplasm management strategy involving back-up gene pools and front-line, "advanced" maize populations. The germplasm at both levels was intentionally kept open-ended to permit the introgression of new and superior germplasm from time to time. The germplasm also followed a more or less predetermined "flow" through the system: materials from the maize germplasm bank and the national programs were generally added to gene pools, which featured a broad genetic base. The decision to introgress materials depended on ecological adaptation, maturity, grain color, and grain texture characteristics. In most advanced elite populations, only superior families from each corresponding pool were identified and later introgressed following systematic evaluation.

The major emphasis of the Maize Program up until the mid-1980s was on population development and improvement efforts to derive open-pollinated products (OPVs). No emphasis was placed on hybrid-oriented products, other than evaluating the combining ability of existing maize germplasm with respect to Tuxpeño and ETO testers. Thus in both germplasm development and improvement activities, intrapopulation improvement schemes were used. Beginning in the middle of the decade,

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\* Breeder, lowland tropical maize.

though, a modest effort in hybrid development was initiated in response to a perceived need for hybrid-oriented products, and this focus has grown steadily since then. As a result, the use of Bank materials must be based on well recognised heterotic groups and follow a stepwise progression involving evaluation, use in developing new source germplasm, introgression into already existing germplasm, and finally hybrid development.

*Evaluation of germplasm accessions* — A systematic evaluation of Germplasm Bank accessions in multisite tests was necessary to identify superior collections for use in breeding over the past 25 years. At least a few thousand Bank accessions have been evaluated (Table 1). In the past four years we have used several germplasm accessions and also S1 lines identified through such testing.

*Introgression of bank accessions into existing gene pools* — Once superior accessions are identified, they are used mainly to broaden the genetic base of existing gene pools. In four tropical, late maturity pools, several materials from the Bank have been introgressed over the past 20 years. Table 2 lists the materials involved in such pools. The introgression process may take various forms.

*Introgression of selected accessions* — Since most gene pools are handled and improved using a modified half-sib system, materials to be introgressed are planted the first year as female rows and the entries are detasselled and forced to pollinate with male rows representing bulks of families or selected ears from the previous cycle. In the following season, the crosses of the bank materials x pools are either advanced separately by pollinating only the desirable plants or by planting the F1 crosses again as female rows. The performance and combining ability of bank accessions are judged visually in relation to male rows alternating with the female rows. Ears are selected from the desirable plants to enter the main body of that particular pool the next season. A number of bank accessions have been introgressed into pools 23, 24, 25 and 26 (Table 1).

*Formation of new source populations* — Bank accessions have also been used in forming new source populations. In recent years the tropical late germplasm development unit headed by Dr. W. Villena has developed two new, broad-based materials involving superior Bank accessions. These populations have been tentatively named Población Blanco Semidentado (PBS) and Población Tardío Caribe (PTC). PBS drew on criollos Argentinus landraces from the area of Villa Valencia in Veracruz, especially accessions of tall plant type but good yield potential, as evidenced by ear size. To improve the agronomic type of this germplasm, it was subsequently crossed to CIMMYT lowland tropical, short stature maize and to some ear-rot-resistant, white flint materials.

The PTC resulted from the crosses among Caribbean selections in turn crossed to short plant type testers (NPH). The population is of mixed grain color and, after a few more cycles of recombination, will likely result in a unique set of materials characterized by good standability, husk cover, and grain yield potential.

*Mini-pools* — A few Bank materials have been crossed to testers, evaluated for their combining ability, and used to form “mini” pools.

*Development of hybrid-oriented source germplasm and progenitors* — With the initiation of our hybrid development effort, the combining ability of germplasm at all levels has started receiving greater attention. Heterotic patterns of tropical pools and populations have been identified. In addition several inbred-based populations and new heterotic groups have been formed. In the future heterotic patterns of Bank accessions will be evaluated before introgressing them into appropriate materials.

Based on the performance of Bank materials in multilocation evaluation nurseries, some were suggested for use in breeding lowland tropical maize. We are inbreeding selections of these and evaluating them, particularly the accessions Veracruz 23 and Sinaloa 21. In addition, S1 seed from the Bank's own inbreeding research, initiated in 1989, was shared with lowland tropical maize breeders. In 1989, 1,134 S1 lines of various accessions from Brazil, the Dominican Republic, Guatemala, Puerto Rico, and Venezuela, as well as the Mexican states of Chihuahua, Coahuila, Colima, Jalisco, Michoacán, Nayarit, Nuevo León, Oaxaca, Puebla, Queretaro, San Luis Potosí, Sinaloa, Sonora, Tamaulipas, and Veracruz were grown and selections advanced to S2. Further inbreeding and utilization of these lines in various ways will be pursued in the future.

*Development of special trait germplasm* — Bank materials having special traits will be used to improve lowland tropical maize. We have received 66 Olotillo accessions that possess a thin cob, deep kernels, and excellent shelling percentage. The best ones were subjected to inbreeding and the S1s are currently being evaluated.

**Table 1. Bank accessions evaluated, 1972-1981.**

Year	No. of accessions	(Test sites)
1972	663	(PR, TL, BA)
1974	1891	(PR, Thailand)
1975	2048	(PR)
1976-77	456	(PR, TL, OB)
1980-81	300	

**Table 2. Materials introgressed into four CIMMYT tropical, late maturity pools.**

**Pool 23. Tropical Late White Flint**

(Mix.1 Col. Gpo. 1) Eto x Sint. 10 líneas; Sint. 10 líneas; Compuesto IACP blanco (Advanced generation crosses among materials such as J<sub>1</sub>; Colleege white x Tuxpeño; UPCA Var.1, 2, 3; Bogor Comp. 2; E.H. 4207; Jawahar syn. 44; Kisan; Cuprico x Flint Compuesto, Kisan Syn. 70, Ganga 5, Sona Syn. 72, Vijay, Composite D, Metro, Guatemala P.B. 5, Harapan); Nicarillo x Sint. 10 líneas; Tuxpeño P.B. x Sint. 10 Líneas; Compuesto blanco Central Americano (Salco; Sint. Nil. 2; Nicarillo; Tocumen 70; H3, 5, 101; Poey T23, 27, 66 and 72; ICA-H104, 154, 207, 302; Comp. precoz SM/H111; Pioneer X304A; X306A; XB101, XB101A, X-354; TR-1; Desarrural H-B101, 105; A-Doble 6; B-Doble 2; Cuyuta H2; and H507); Tuxpeño-Caribe 2; Mezcla tropical blanca; Compuesto Caribe; materials resistant to downy mildew; materials from Colombia; Trinidad 34; White flint segregates - selections from Colombia, Across 7442 x Suwan 1, DMR, Phill. DMR Comp. 2 x Suwan 1, Maíz dulce, RPM x C17-C<sub>2</sub>, Tung, Yue-12 (Temp.Y.D.), Rampur Yellow, Uruguay 263A, Uruguay 260A, Uruguay 704A, EVA-(MDR-I) 76, EVA-(MDR\_-II)76, Fam. 400 Phyll. Resist., Milho CV-CMS-04, Milho CV-CMS-11, Pioneer hybrids, Milho CV-CMS-13; SQH 154 (San Miguel), SQH 156 (San Miguel); AB-2087, X304C-TFI x 304CF15, PR7843- SR.

**Table 2. (cont'd).**

**Pool 24. Tropical Late White Dent**

Tuxpeño P.B.C<sub>11</sub> x La Posta C<sub>2</sub>; Tuxpeño-Caribe 2; Mezcla tropical blanca; Eto Mix. 1-Col.Gpo.1; Blancos cristalinos; Pfister hybrids; Compuesto IACP (Blanco); Compuesto resistente a Pudrición; Compuesto resistente a tallo; Compuesto grano duro: Nicarillo; Compuesto blanco Central Americano; V520C; A6; A21; N12; PD(MS)6 - Selecccion blanca; IDRN, materials from Zaire; materials from Colombia; materials resistant to downy mildew; materials resistant to tar spot; Guatemala 88, 104; Pool 24 - Selections from Colombia; FR-804W x FR805W; Sarhad W x Dholi 772; Tuxp. 1 (Reducir hoja)C4; White Dent x DMR; Haxami x Suwan 1W; Tuxp. 1 (Reducir espiga), Suwan 1; NN14B x Pool 24; C190A; A445N; A503N; FSHMR x FSHMR (DMR)1; FSHMR-2; Caisan x C17 (Sel. blanca); RPM x C17 C<sub>2</sub> (Sel. Maz. colgante); Khumaltar Yellow; Amarillo dentado; EVA (MDRI)76; Fam. 400 Resist. Phyll.; Milho CV-CMS-04 (Am. Dent.); Milho CV-CMS-11 (Pool 21); Milho CV-CMS-12 (Pool 22); Pioneer hybrids; SHQ 154 (San Miguel); EVA (MDR-11)76; Milho CV-CMS-14 (Pool 25); Milho CV-CMS-30 (Comp. amplio); AB 2087; Pioneer hybrids; SHQ 154 (San Miguel); PR 7843-SR; Milho CV-CMS-13 (Comp. cerrado); AB2087 X304C TF1 x 04C F15; Sint. braquítico Lucio Blanco; PR 7822-SR; Across 7729-SR.

**Pool 25. Tropical Late Yellow Flint**

Mix.1-Col.Gpo.1; Eto; Sint. 10 lineas; Compuesto Ecuador, Mexico, Argentina, and India; Mezcla amarilla x varios amarillos (Ant. x Ver.181, Comp. Amarillo Central Americano, IACP, V520C, Pob. Cristalina, Crist. Dentado, and Nicarillo); Republica Dominicana; Serie Cris.; Tuxpeño, Nicarillo; Tuxpeño Caribe-2; Amarillo Cristalino-1 (Yellow flint segregates recovered from advanced generation crosses among materials such as Cuba 11J, Eto amarillo, PD(MS)6, Granos amarillos & Tuxpeño amarillo - sel. crist. C3); Cogollero; Materials resistant to downy mildew; materials resistant to tar spot; Costa Rica 71; Cuba 2, 3, 13, 65, 16; Panama 64, Surinam 800; Pool 25 - Selections from Colombia; (Suwan 2 x Sarhad) Suwan 1; (D741 x Ind. Pool 3) Suwan 12; (CM115 x B79) Suwan 1; (BS11 x Suwan 1) L4 Suwan 1; Sarhad x Suwan 2# (OB7446 x Suwan 1)Suwan 2#; Suwan 1; Compuesto Amplo; Kwangsi No.16 Kwangsi Ping-Ko (WF); Rampur composite; Kakani Yellow; Hetanda composite; Khumaltar Yellow; Rampur Yellow; EVA-(MDR-I)76; EVA-(MDR-II)76; Lin. Ill. x Ganesh 2; Comp. Hawaii x Khumal Yell., Kakani local; Fam. 400 Resist. a Phyll., Milho CV-CMS-04(Am. Dent.); Milho CV-CMS-11 (Pool 21); Milho CV-CMS-14 (Pool 25); AB2087; Pioneer hybrids; SHQ 154 (San Miguel); Milho CV-CMS-12 (Pool 22); Pioneer hybrids; SHQ 156 (San Miguel); X304 CTF1 x 304 CF15; Sin. Braq. Lucio Blanco; Across 7728-SR; Tocumen (1)7835-SR; PR7843-SR.

**Pool 26. Tropical Late Yellow Dent**

Amarillo Bajío; Sint. 10 lineas; Mezcla amarilla x varios amarillos (same as in Pool 25); Compuesto Central Americano; compuesto IACP (Amarillo); Tuxpeño; Nicarillo; Tuxpeño Caribe-2 (yellow segregates); Dentado amarillo P.B. (same as in Pool 22); Amarillo Dentado 2 (involves materials such as Cuba 11J, Eto amarillo, Gr. amarillo, Tuxpeño amarillo sel. dentado, V520C, A6, A21, Nicarillo); Comp. materials resistente achap. (Advanced generation crosses of mezcla amarilla and Ant. x Ver. 181, Cuba x Republica Dominicana); Cogollero; IDRN; Materials resistant to downy mildew; materials resistant to tar spot; Puerto Rico 2; Trinidad 20; Cuba 25, 47, 56, 95, 107, 121; Dom. Rep. 150, 206; Brazil 820; Cuba 33, 167; Puerto Rico 9; Haiti 30; St. Vincent 2; Pool 26 - Selections from Colombia. Sarhad x Suwan 1; BS16 x Suwan 1; Indonesia x CB x Suwan 1; (Eto x CBC Flint) Suwan 1, (FR 13 x FR 13A)FR; B73-Suwan 1; (CM 115 x 079) Suwan 1; B57 x FRMo17) Suwan 1; Am. Bajío x Suwan 1; Yellow Dent x DMR; Suwan 1; K64 Ht N; A697 N; FSHMRxFSHMR (DMR)1; CM 109; Hetanda Composite; Rampur Yellow; Caisan x C17 (Sel. Blanca); RPM x C17 (Late-recombination); RPM x C17 (Sel. Maz. colgante); Amarillo Dentado; Pool 25; Uruguay 569A; Uruguay 713A; Fam. 400 Phyll. Resist. Milho CV-CMS-04 (Am. Dent.); Milho CV-CMS-11 (Pool 21); Milho CV-CMS-12 (Pool 22); Milho CV-CMS-14 (Pool 25); AB-2087; Pioneer hybrids; SHQ 155 (San Miguel); SHQ 156 (San Miguel); EVA-(MDR I)76; X304C TF1 x 304 CF15; Sint. braquítico Lucio Blanco; Rampur Comp., Comp. Hawaii x Khum. Yellow; Milho CV-CMS 30 (Comp. Amplo); 6-33144722 R22; GA-8151 R23; Across 7729-SR; Tocumen (1)7835-SR.



### **Early-maturing maize for the tropics (H. Córdova)\***

Early maturing maize can provide significant advantages to farmers in developing countries, including greater flexibility in time of planting, the possibility of avoiding biotic and abiotic stresses that occur at certain times in the cycle, the ability to fit more easily into intensive and intercropping systems, and a chance to market the harvest earlier and thus obtain a higher price (Beck, 1991). However elite early maturing germplasm is relatively scarce in lowland tropical environments, where the vast majority of developing country maize is produced.

To provide our cooperators in national programs with new germplasm sources for use in developing early maturing maize for the tropics, we are systematically screening accessions from CIMMYT's Maize Germplasm Bank. During the 1988 winter and summer seasons, we evaluated more than 1,000 of these materials. Most of the early-maturing bank accessions tested so far have shown low yield potential per se. As a result, we are placing greater emphasis on crossing accessions that show reasonable yield potential to elite tropical early sources. In the 1989A season, 50 of the best early accessions were crossed to Poza Rica 8530 and in 1989B the topcrosses were evaluated in replicated yield trails. Only four crosses performed as well or better than the check entries. We are now improving these four materials through recurrent selection with mild selection intensity. Using this methodology, we hope to introduce new and useful genes into more desirable agronomic backgrounds.

Oaxaca 256 was identified as the earliest collection and used as a source of extra earliness. Crosses among high yielding late stable genotypes (Pools and Populations) and sources of earliness from the germplasm bank allowed us to identify new gametic recombinations for forming new early genotypes that yield well and possess superior agronomic traits. After four cycles of recurrent selection for earliness and yield, we have made encouraging progress on both counts with two extra early and early populations, 101 and 102 (Table 2).

Synthetic varieties formed from the superior families have been tested in several locations in 1991 and 1992. A new early variety from population 102 outyielded all varieties tested in the preliminary evaluation trial PET-1 in 1992.

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\* Breeder, lowland tropical maize.

**Table 1. CIMMYT maize gene pools: Constitution and % germplasm bank accessions.**

<b>Germplasm</b>	<b>Bank accessions (%)</b>	<b>Selection emphasis</b>
Pool 15 (TEWF)	32	Earliness, yield, stalk quality
Pool 16 (TEWD)	37	Earliness, yield, ear rot resistance
Pool 17 (TEYF)	37	Earliness, yield, stalk quality
Pool 18 (TEYD)	23	Earliness, yield, stalk quality
Pool 19 (TIWF)	10	Ear rot resistance, yield
Pool 20 (TIWI)	50	Ear rot resistance, yield
Pool 21 (TIYF)	32	Stalk quality, yield
Pool 22 (TIYF)	22	Stalk quality, yield
Pool 23 (TLWF)	21	Ear rot resistance, yield, <i>E. maydis</i>
Pool 24 (TLWD)	24	Stalk rot, yield
Pool 25 (TLYF)	19	Ear rot, yield
Pool 26 (TLYD)	31	Stalk rot, yield
TLWFD	23	Stalk quality, yield

**Table 2. New early and extra early maize populations: Number of materials and % germplasm bank accessions.**

<b>Germplasm</b>	<b>No. of materials</b>	<b>Bank accessions (%)</b>	<b>Selection emphasis</b>
Population 101 (Super precoz blanco)	4	25	Extra earliness, yield
Population 102 (Precoz blanco)	48	15	Earliness, yield, ear rot resistance

## **Evaluation of Tuxpeño accessions from subtropical areas**

*(M. Bjarnason and K. Pixley)\**

Germplasm of the Tuxpeño race is widely used in tropical and subtropical maize programs because of its productivity and good combining ability. CIMMYT maize populations have included Tuxpeño germplasm from mainly the lowland tropical region along the Gulf of Mexico. Agronomically-promising Tuxpeño accessions from subtropical areas in Mexico have been identified in Germplasm Bank evaluation trials in recent years, and we are looking at the best ones more closely in our program. The objective of this work is to identify new sources of Tuxpeño more specifically adapted to subtropical areas. These regions are dryer and have production constraints that differ from those common in the tropical areas from which most Tuxpeños previously utilized at CIMMYT originated.

*Materials and methods* — Fifteen accessions from the Mexican states of Hidalgo, Michoacán, San Luis Potosí, Tamaulipas, and Veracruz were evaluated and increased at Tlaltizapán during 1991A. The three most promising white and yellow accessions were grown in 1992A, and plant-to-plant sibbing was made among selected plants in each accession. Full-sib ears of the most promising accession of each grain color were shelled individually. Among the whites, a Celaya Tuxpeño from San Luis Potosí (Acc. 438) was selected, and a Tuxpeño also from the state of San Luis Potosí (Acc. 1909) was selected among the yellows. In 1992B, 153 full-sib families from the white accession and 165 from the yellow were evaluated for yield and other agronomic characters together with checks in simple alpha (0,1) lattices at Tlaltizapán. The same families were evaluated for resistance to rust and *E. turcicum* at El Batán. Data are presented here for trials involving full-sib families and for a trial of subtropical, late maturing populations that included these and other promising bank accessions.

*Results and discussion* — The means of the full-sib families evaluated and of 25 families selected for yield, ear height, lodging, and husk cover are presented in Table 1. The performance of the best check entry, 91SLWF, a new white flint population, is also included. The mean yield of all the full sibs evaluated was lower than that of the check entry for both trials, but the mean of the selected fraction exceeded the check for yield. Ear height was above acceptable levels in both populations, which is typical of many Bank accessions. The yellow population had very attractive ears with deep yellow dent kernels. The *E. turcicum* inoculations in El Batán were not successful, but severe natural rust (*P. sorghi*) infection showed that both populations were highly susceptible, and very limited variability was observed for resistance to this disease.

In the trial of late maturing populations (Table 2), which included both established and new populations, one of the white Bank accessions, a Tuxpeño Olotillo from Tamaulipas (Acc. 411), showed very promising yield potential. The better yellow accession, SNLP Tux. Y, yielded more than the yellow advanced population 45, but was 11 days later in flowering. All Bank accessions had high ear placement and poor rust resistance.

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\* Breeders, subtropical maize.

We are evaluating 166 full-sib progenies from accession 411 (Tamaulipas Tux. Olotillo), and the 25 progenies selected from each of the accessions listed in Table 1, for resistance to *E. turcicum* at Poza Rica in the winter cycle 1993A. Also in 1993A, the best 15-20 of the 25 full sibs will be recombined at Tlaltizapán after selecting for decreased ear height and other traits within each family.

We plan to continue working with the accessions Tamaulipas Tux. Olotillo (white) and SNLP Tux. (yellow). For improvement of these materials per se, there is enough variability for plant and ear height, and the heritability of those traits is high, so we would expect rapid progress for those traits

**Table 1. Population mean and mean of 25 selected full sib (FS) families from two Bank accessions.**

	Yield (Mg/ha)	Days to silk	Ear height (cm)	Lodging (%)	Bad husk cover (%)
<b>SNLP Celaya Tuxp. White (SNLP GP6)</b>					
Mean 156 FS	7.54	61.4	203	15	14
Mean 25 sel. FS	9.05	60.6	196	7	11
Best check 91SLWF	8.76	61.0	172	16	16
SE of an entry mean	0.91	1.1	9	8	7
<b>SNLP Tux. Yellow (SNLP 104)</b>					
Mean 165 FS	9.20	64.7	191	4	26
Mean 25 sel. FS	10.21	63.8	181	3	20
Best check 91 SLWF	9.77	64.0	133	0	5
SE of an entry mean	0.70	1.3	9	4	9

**Table 2. Results of trial of subtropical late populations, 1992 (36 entries).**

Entry	Rank	Grain yield (Mg/ha)	Days to silk	Ear height (cm)	Rust (El Batán) (1-5)
SPMAT/P500	1	8.15	60	104	4.0
SPMAT/PL32{Y}	2	8.12	58	105	3.5
TMPS TUX. Olot. W (TAMA 37)	3	8.12	60	125	5.0
91SLWFCO	10	7.65	71	109	3.5
Pop. 42C8	11	7.61	62	113	1.0
H430 (DC Hybrid)	14	7.38	70	106	4.0
Pop. 47	15	7.32	63	106	2.5
Pop. 44	17	7.22	69	102	1.5
SNLP TUX. Y (SNLP 104)	20	7.08	71	146	4.0
SNLP Cel. TUX. W. (SNLP GP6)	25	6.30	69	135	4.0
Pop. 45 C8	28	6.11	60	96	2.5
SNLP TUX. Olot. Y (SNLP 114)	29	5.90	71	142	3.5
LSD .05		1.44	4	15	
mean		6.86	65	106	
CV, %		12.1	3.9	7.8	

through some type of recurrent selection. However, because of limited variation for rust resistance, other strategies, e.g. a conversion program, will be more efficient for improvement of this trait. We do not know yet about *E. turcicum* resistance in these materials, but this is another trait of importance in many subtropical areas. For hybrid development, a complementary heterotic group could be established by crossing these accessions to elite lines known by pedigree to be of different genetic background and selecting the best lines for recombination. Experience at CIMMYT with tropical Tuxpeños has shown that they combine well with many other materials, so we are cautiously optimistic that these subtropical Tuxpeños will be useful in heterotic combinations with germplasm already in use in hybrid-oriented programs for the subtropics. Line development beyond the S1 or S2 stage will not be attempted in these accessions until their agronomic performance has been enhanced.

We envision a chronologically two-tiered implementation of this germplasm: 1) an intermediate-term approach in which agronomic deficiencies will be addressed by crossing to elite material; and 2) a longer-term approach in which we will maintain as pure as possible the genetic identity of the materials. We expect that these pure Tuxpeño types of different origin than those presently used will broaden the germplasm base and provide new options in germplasm development for subtropical areas.

An alternative approach for use of Germplasm Bank accessions in subtropical germplasm development would be to implement an early testing scheme for combining ability with a less select group of candidate accessions. This would identify accessions most likely to contribute new and useful genes for hybrid-oriented work, but would also likely result in accessions with poor per se characteristics. Some form of back-cross conversion program would be needed to improve accessions while limiting the theoretical genetic contribution of non-recurrent parents to 25% or so. An important consideration, if this approach were utilized, is the greater investment of resources during the early stages (evaluation of accessions). Perhaps this type of evaluation could or should be a primary task of the Germplasm Bank.

### **Breeding for cold tolerance in highland germplasm development (J.E. Lothrop)\***

Natural and farmer selection in cool highland areas has led to the development of maize types that are uniquely adapted to cooler regions, as documented recently in experiments carried out in growth chambers (Ellis et al. 1992) where it was shown that optimum growing temperatures for cultivars based on Cónico and Cacahuacintle germplasm were 6-12°C cooler than those for lowland tropical Tuxpeño germplasm, subtropical germplasm, and temperate germplasm. On the other hand, the two highland cultivars were the only cultivars killed by a constant temperature of 37°C. Besides a lack of heat tolerance, Germplasm Bank accessions of highland materials often are deficient in several important agronomic traits, and there may be problems with grain texture, low tolerance to inbreeding depression, and photoperiod sensitivity.

In research on highland maize, we recognize that there is a gradient of niches to which our germplasm is targeted, from extremely cold environments with growing season mean temperatures as low as 12.5°C to relatively warmer environments which have means approaching 20°C. Night temperatures are extremely important in determining cold tolerance. To simplify matters somewhat, CIMMYT has defined three highland mega-environments based on temperature: 1) the tropical highland transition zone, with mean growing season temperatures of 17-20°C and minimum night temperatures usually above 15°C; 2) the temperate highlands, with means from 15-20°C and widely fluctuating night temperatures and photoperiods; and 3) the tropical highlands, with means from 12.5-17°C and night temperatures below 10°C and occasional frosts. To reduce genotype-by-environment interactions for breeding, the latter mega-environment may be divided into the very cold (12.5-15°C) and cold (15-17°C) zones. It is important to realize that temperatures and rainfall determine the adaptation of maize pathogens and insects as well, and maize for each zone must carry the appropriate resistances as well as temperature adaptation.

The most important Bank accession for the relatively warm tropical highland transition zone has been Ecuador 573. It was introduced in Kitale, Kenya, in 1959. When crossed to the local variety, Kenya Flat White, the hybrid manifested excellent heterosis. Hybrids based on this heterotic pattern have had a great impact in Kenya (Gerhart 1975) and in other countries of East Africa—including Tanzania, Ethiopia, Zaire, Burundi, and Rwanda—in areas at altitudes of 1600-2000 masl.

No single Germplasm Bank accession has had a large impact in the temperate highlands. CIMMYT has developed populations for this zone using crosses between temperate germplasm (mainly Corn Belt Dents and European Flints) and improved tropical highland populations which are about 60% improved Cónico types, 20% temperate, and 20% subtropical.

Bank accessions have been very useful in breeding morocho, floury, and semi-dent maizes for the tropical highlands. In this zone the need for cold tolerance is so important that no exotic germplasm can be used directly. Rather, it must be used in small doses of up to 40% in warmer zones (15-17°C) and less than 10% in cooler zones (12.5-15°C) to correct shortcomings of the indigenous highland germplasm.

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\* Breeder, highland maize.

Floury tropical highland germplasm accounts for some 4% of the 6 million hectares of highland areas worldwide. Cacahuacintle is the most important race in Mexico and has contributed greatly to early floury populations for the Andean highlands. Cuzco Gigante has been the most important late floury landrace. In developing gene pools for the Andean highlands (both floury and morocho) about 50% of the initial germplasm was from CIMMYT's 1970's highland pools (Taba 1992). These pools were predominantly Cónico (early, hard grain), Chalqueño (late, hard grain), and Cacahuacintle (early and intermediate, soft floury grain).

Morocho tropical highland germplasm represents about 3% of the highland area worldwide. Early morocho landraces from the Peru-Bolivia border area and late Montana types from Columbia and Ecuador were key components of the Andean morocho gene pools. About 50% of the original germplasm of these pools was from 1970s CIMMYT highland pools with predominantly Cónico and Chalqueño germplasm.

Hard grain types account for more than 90% of the world's highland areas. The major landraces used for the tropical highlands have been Cónico (early) and Chalqueño (late). These landraces were collected in Mexico's central plateau beginning in the early 1940s by the Rockefeller Foundation funded Office of Special Studies (OSS) of the Mexican Ministry of Agriculture (Wellhausen 1952). After evaluation in yield trials, the best accessions were improved by mass selection and attempts were made to derive inbred lines. Since Cónico and Chalqueño exhibit severe inbreeding depression, all commercial hybrids except one (involving one S3 line) from 1950-1992 have been made using S1 lines (Arellano 1984; Espinosa 1991). Mexico's first hybrid, H-1, was a three-way cross of S1 lines from Chalqueño that was released in 1950 for irrigated areas. Altogether, the OSS released six hybrids and four open-pollinated varieties (OPVs) based on either Cónico or Chalqueño germplasm during 1944-1956. In 1957 Gilberto Palacios de la Rosa, head of the OSS highland program, discovered the "latency" genotype in an S1 line derived from the Cónico-Chalqueño Bank accession Michoacán 21. Early generation lines from Michoacán 21 formed part or all of the pedigrees of six commercial hybrids and one synthetic OPV in the period 1965-1992 (Espinosa 1991). Starting in 1971, some 640 landraces from medium-to-low yield potential areas of the central plateau outside of the state of Mexico were collected and evaluated. The best 16 of these Cónico types were selected and improved by stratified mass selection, and six OPVs were released in 1980 (Arellano, 1984; Mendoza and Carballo 1980). One of these OPVs was based on the Cónico landrace Tlaxcala 151. Early generation lines from this source combine well with Michoacán 21 early generation lines (and CIMMYT highly inbred lines), and it forms the base of one heterotic group used for breeding early and intermediate hybrids (Valdivia 1992).

Recently, Mexican highland maize breeders have become concerned about the narrowness of the highland germplasm base (Cónico and Chalqueño). CIMMYT developed broad-based gene pools using these races in the 1970s. Since neither Cónico nor Chalqueño possesses sufficient variation to allow breeding progress in improving root strength, eliminating tillering, and tolerating high levels of inbreeding, the CIMMYT program since 1984 has been judiciously introgressing exotic germplasm (primarily temperate and subtropical) to correct these faults while maintaining cold tolerance and adaptability. Populations containing a mix of indigenous and exotic germplasm and improved by

recurrent selection have been the source of many excellent OPVs, and in 1993 CIMMYT made available inbred lines (S5-S8) derived from these populations for areas with mean growing season temperatures of 15-17°C (valleys of Mexico, Puebla, areas of Yunnan province, P. R. China, etc.). These lines are approximately 60% Cónico, although RFLP studies will be necessary to estimate the percentage accurately. Lines that are approximately 90% Cónico will soon be ready for cooler areas (12.5-15°C) such as the valley of Toluca, as well as lines that contain approximately 60% Chalqueño for longer season areas.

For the future, improved hard grain maize varieties for highland tropical areas will continue to be based on indigenous maizes. Over the short term, hybrids will be formed using as one parent an early generation line derived from a Bank accession and the other a highly inbred line containing exotic germplasm. For the longer term, it is expected that improved lines with exotic germplasm and approaching homozygosity will be used to form more modern hybrids and synthetic OPVs. Still, these improved lines will contain approximately 60-90% indigenous germplasm.

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## **Improving drought tolerance in tropical maize (G.O. Edmeades)\***

CIMMYT's strategy in breeding for drought tolerance is twofold. First, we focus on the rapid improvement of drought tolerance in elite germplasm, and seek to increase the low frequency of drought-adaptive alleles that normally exists within elite maize populations. This approach can result in a 30% increase in grain yield under conditions of drought at flowering and during grain filling that would otherwise reduce potential yields from 6 t/ha to about 2 t/ha (Bolaños and Edmeades, 1993). Much of this gain has come about by changes in partitioning of biomass to the developing ear at flowering, resulting in an increase in grain numbers per ear and a decrease in barrenness. No attention has yet been paid to problems encountered during the establishment of elite germplasm. Selection in elite populations has not changed total biomass production and so has not changed water use efficiency. As well, there seems little genetic variability in elite populations for delayed senescence or osmotic adjustment under drought. Finally, there were indications that gains were declining in advanced selection cycles for improved drought tolerance in elite populations (Bolaños and Edmeades 1993).

These observations led to a second approach in research on drought tolerance: the identification of donors for characteristics that would increase production under drought at no great cost to performance under well-watered conditions. We sought to establish two populations as repositories for a wide array of genes related to performance under drought. The first of these, Drought Tolerant Population - 1 (DTP1), was established in 1986. The second, DTP2, is 50% DTP1, and has extensive introgression from superior source materials. These populations are, as expected, very heterogeneous for grain type, grain color and maturity. Agronomic performance was also initially poor, so our first emphasis was to improve standability and disease resistance. In order to reduce bias from continuous selection at Tlaltizapán in the dry winter cycle, we have sent  $S_1$  progenies of DTP1 to interested collaborators in national programs for screening under the drought conditions they normally encounter (Edmeades et al. 1991).

*Identifying sources for drought tolerant populations* — We have screened about 200 potential sources of drought tolerance (elite, landrace, hybrid and OPV) under drought and well-watered conditions at Tlaltizapán. The elite sources were from countries other than Mexico (especially southern Africa, Thailand, USA, as well as CIMMYT's own program), and most were included on the basis of their reputed performance under drought elsewhere.

A total of 300 potential sources of drought tolerance were identified from CIMMYT's Germplasm Bank. These came from sites described in the passport data as below 1000 m elevation and which had either an annual rainfall of less than 600 mm or were from sites which were noted as dry. These were usually prescreened in the summer for agronomic performance (stalk strength, susceptibility to disease, low yield), and a total of 160 were advanced for testing under drought in either late or early flowering trials. The criteria used in selection under drought were limited to what we could manage at Tlaltizapán. Each entry was grown in a replicated 3-row plot under two levels of drought stress during the winter, as well as normal irrigation to observe yield potential. In the stressed water regime

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\* Physiologist/agronomist.

irrigation was withdrawn 3 weeks before anthesis and the crop was severely stressed at flowering and during grain filling. A selection index was used to identify superior entries. This index placed weight on delayed foliar senescence, high osmotic potential (when measured), high grain yield and high ears per plant under stress, a short anthesis-silking interval, and lodging resistance under stress. The index maintained yield and flowering date under well-watered conditions. Compared with selection in elite germplasm, less emphasis was placed on performance under unstressed conditions, and more on delayed senescence and high osmotic activity.

There are many problems encountered in such trials. The site used (winter, Tlaltizapán) limited the traits we could observe. We were unable, for example, to examine variability in ability to emerge from depth in a dry soil. In a number of trials the variability in flowering date among entries made it hard to distinguish escapes from those with genuine drought tolerance. We used the summer prescreening trials to stratify entries by flowering date, and then grew all early (or late) flowering entries in the same trials (see Table above). The passport data was not a precise guide to maturity, and many of the accessions are very photoperiod sensitive. Thus it was often difficult to get a fair comparison of ability to withstand stress at flowering, even with a prescreening trial the previous summer.

*Utilization* — Of the 13 components used to form DTP1, one was a direct Bank accessions (Michoacan 21), and another (Latente x Latente) was directly traceable to a Bank accession (also Michoacan 21). When DTP2 was formed, two of the 25 components used in this introgression into DTP1 were drawn directly from the Germplasm Bank (Sinaloa 31, and Tamaulipas 25). Thus 15% of the germplasm used in DTP1 and 11% used in DTP2 could be directly traced to Germplasm Bank accessions. The population DTP1 is now in its second cycle of testing internationally and has been used by several NARS (e.g., Zambia, Malawi) as a further source of drought tolerance. The CIMMYT sub-station at Harare has also extracted some promising lines from DTP1. At present S<sub>1</sub> lines from DTP1 are being

**Details of six trials involving Bank materials conducted between 1987 and 1993.**

Cycle	Trial number	# Germplasm Bank entries
86A	1283	1
87A	1612	46
88A	1609 (late)	35
89A	1609 (early)	32
90A	1613 (late)	34
91A	1612 (late)	12
93A	1619	-
<b>Total</b>		<b>160</b>

screened for their capacity to germinate and establish under a gradient of moisture stress at Tlaltizapán. We anticipate that DTP2 will be superior to DTP1, especially in the midaltitude environments. At present the drought tolerance of these populations is almost to the standard of our best elite selections, and their unstressed yield potential in subtropical environments is about 10-11.5 t/ha. We anticipate that the adaptation of DTP1 will be oriented towards the lowland tropics, while that of DTP2 will be modified to suit the mid-elevation tropics.

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## **Use of Germplasm Bank accessions in breeding for tolerance to low fertility conditions (H.R. Lafitte)\***

The availability of nitrogen limits maize yields throughout the developing world. At the same time, most breeding nurseries are grown under non-limiting levels of nutrients in order to minimize the effects of soil heterogeneity during the selection process. It might be argued that improved materials have lost certain adaptive traits for performance on low fertility soils which were present in the landrace materials from which they were derived. In our search for sources of germplasm which perform well under conditions of low N fertilization, we turned to the Germplasm Bank to examine the performance of accessions which had not undergone much selection under high fertility conditions. Our objectives were twofold: 1) to contrast the strategies of unimproved and improved maize under conditions of low soil N, and 2) to identify accessions to form populations adapted specifically to low N environments.

These low N populations represent an attempt to see what would have happened had breeders, like small scale farmers, worked under low N conditions. We also hope that the agronomic performance of the populations can be improved rapidly in order to provide a source of useful germplasm for those national programs that are addressing the problem of low soil fertility through improved varieties. These populations represent a long-term approach to the problem of low soil fertility. We are simultaneously working to improve the performance of an elite population under low N conditions.

*Evaluation of Bank accessions under different levels of soil nitrogen* — A total of 209 Bank entries have been evaluated under high and low N. The low N treatment received no fertilizer N while the high N plots received the normal station application of 200 kg N as ammonium sulfate. The evaluations were made over a period of 3 years, and a variety of criteria were used to select the accessions. In one year, entries were selected on the basis of race: entries represented races of maize which are common to low fertility soil groups in Mexico and Central America, and the accessions were collected at elevations below 1000 masl. In another year, materials of Caribbean origin were evaluated. And in the final experiment, the entries were selected on the basis a low fertility rating in the collector's notes. It should be noted that these accessions, while classified as landraces for the purpose of this study, could all have been improved to some extent prior to their collection. In one trial, improved materials were included as well. In these trials characters measured included: plant and ear height, ear leaf area, flowering dates, ear leaf chlorophyll at flowering, grain yield, biomass yield, and Kjeldahl N concentrations in the stover, cob, and grain.

*Contrasting behavior of improved and landrace genotypes* — Grain yields of improved entries tended to be greater than the yields of Bank accessions under both N levels. Landrace entries tended to have a lower harvest index, but the harvest index of Bank materials was less affected by N stress than was that of improved materials, and the nitrogen harvest index of Bank materials actually increased with N stress while it decreased in improved entries.

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\* Physiologist/agronomist.

When fertilizer N was applied, the improved materials accumulated considerably more N in the grain than did the Bank entries. At low N, however, N partitioning within the plant was similar for the two germplasm groups. The greater dry matter yields in the improved materials under low N was associated with a greater dilution of the grain N, that is, from a lower N concentration in the grain.

With N stress, the leaf area of Bank entries was reduced more than in improved materials, but leaf chlorophyll was maintained at higher concentration. This finding suggests that the advantage of the improved materials lay in the production of leaf area of a lower N content, rather than in an improved ability to absorb N from the environment.

In summary, it appears that landrace materials show potentially useful genetic variation for:

- High N uptake under N stress
- Maintenance of leaf chlorophyll concentration -N
- Maintenance or increase in harvest index (HI) and nitrogen harvest index (NHI) with N stress
- Maintenance of grain N concentration with N stress

Negative correlations among these traits were not observed.

*Formation of populations with specific adaptation to low N environments* — Landrace entries were selected independently in each of the three evaluations, and were combined to form an early population (32 accessions) and a late population (22 accessions). The populations have undergone five cycles of half-sib mixing under low N with mild selection to improve agronomic characters in the last two cycles. Recurrent selection under low N began in 92B with strong emphasis on reducing ear height and lodging and improving yield.

Preliminary observations of the low N populations reveal that they are very tall and suffer from extreme lodging under high N, such that they show little positive response to N application. In their target low N environment, however, their height is not excessive and their yields are fairly good. We feel that the populations show sufficient promise to continue their improvement under recurrent selection for several more cycles, at which point their performance will be evaluated alongside that of elite germplasm under low N conditions.

### **Using sources of multiple insect and disease resistance (J.A. Mihm)\***

Pest management and plant protection research in the Maize Program have concentrated on identifying and improving materials with host plant resistance to the major insect pests and pathogens of the crop in the developing world. An essential component for breeding and improving host plant resistance is germplasm with genetic variation for resistance to a species of interest. Insect resistant source germplasm from CIMMYT's Bank has been utilized after evaluation by our headquarters breeding program. In the case of materials from outside banks, screening and evaluation has been conducted by non-CIMMYT scientists at other locations, and our researchers have collaborated in different aspects of these efforts.

Essential components to improve maize for insect resistance include:

- A healthy colony of the pest(s)
- The capability to mass rear pest(s)
- Germplasm with variation for resistance
- The capacity to artificially infest large numbers of plants efficiently and uniformly
- Methods for assessing damage (i.e., rating scales)
- An effective selection/breeding scheme
- A multidisciplinary team comprising an entomologist, a breeder, and a pathologist

Target pest complexes of importance in the developing world include:

<b>Pest species</b>	<b>Common name</b>	<b>Abbreviation</b>
<i>Diatraea grandiosella</i>	Southwestern corn borer	SWCB
<i>D. saccharalis</i>	Sugarcane borer	SCB
<i>D. lineolata</i>	Neotropical corn borer	NCB
<i>Spodoptera frugiperda</i>	Fall armyworm	FAW
<i>Helicoverpa zea</i>	Corn earworm	CEW
<i>Diabrotica sp.</i>	Corn rootworms	CRW
<i>Sitophilus zeamais</i>	Maize weevil	MWv
<i>Prostephanus truncatus</i>	Larger grain borer	LGB
<i>Oligonychus/Tetranychus sp.</i>	Spider mites	SM

Germplasm collections have been screened and utilized for these pests at various locations, as shown below:

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\* Entomologist.