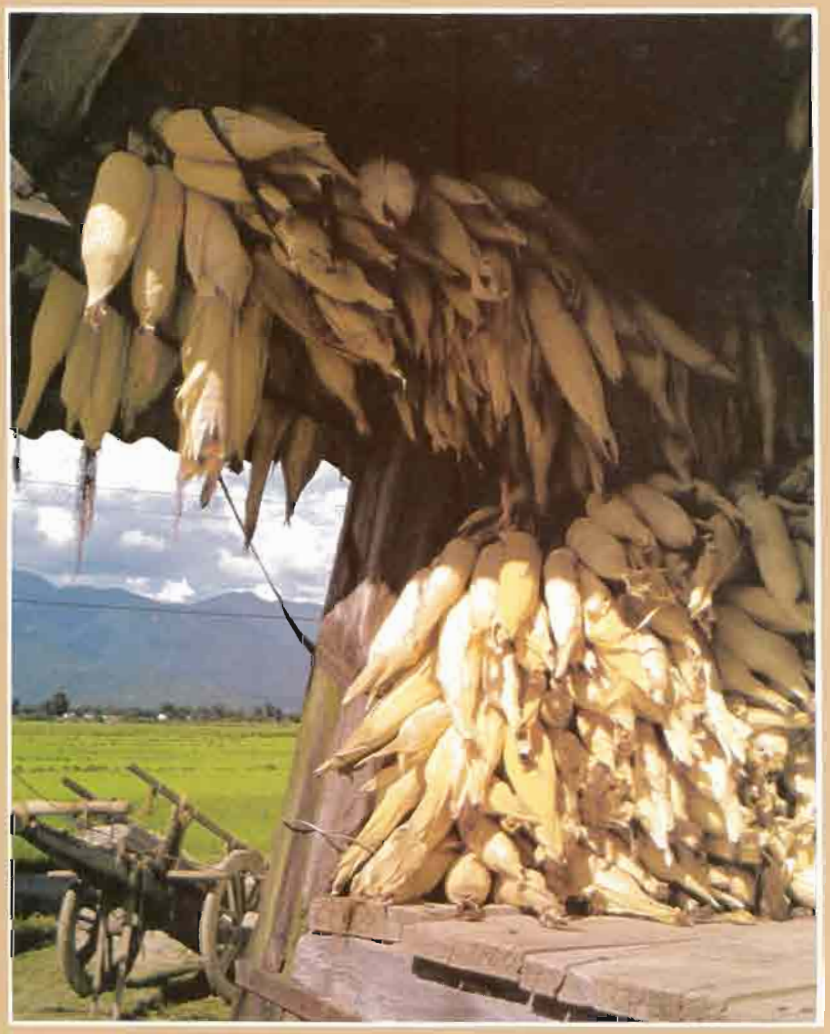
Recent Advances in the Conservation and Utilization of

GENETIC RESOURCES

Proceedings of the Global Maize Germplasm Workshop



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CIMMYT, Mexico, 6-12 March 1988

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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 13 nonprofit internaitonal 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 a combination of 40 donor countries, international and regional organizations, and private foundations.

CIMMYT receives core support through the CGIAR from a number of sources, including the international aid agencies of Australia, Austria, Brazil, Canada, China, Denmark, the Federal Republic of Germany, Finland, France, India, Ireland, Italy, Japan, Mexico, the Netherlands, Norway, the Philippines, Spain, Switzerland, the United Kingdom, and the USA, and from the European Economic Commission, Ford Foundation, Inter-American Development Bank, OPEC Fund for International Development, UNDP, and World Bank. CIMMYT also receives non-CGIAR extra-core support from Belgium, the International Development Research Centre, the Rockefeller Foundation, and many of the core donors listed above.

Responsibility for this publication rests solely with CIMMYT.

Correct citation: CIMMYT. 1988. Recent Advances in the Conservation and Utilization of Genetic Resources: Proceedings of the Global Maize Germplasm Workshop. México, D.F.

Editing: Nathan Russell and G. Michael Listman
Design and layout: Miguel Mellado E., José Manuel Fouilloux B., and Efrén Díaz Chias
Cover photo: Thomas Luba
Typing: María Teresa Pedroza, María Concepción Castro, and Ana Gloria Núñez

ISBN 968-6127-44-5

Contents

Preface

Even for the nonspecialist, maize genetic resources is an inherently interesting subject. It has an engaging history, shaped by people of unusual intelligence and foresight; it is currently being transformed by exciting new developments in several disciplines; and it offers future challenges that will place heavy demands on the technical and organizational capacities of national and international institutions. With the overall aim of more clearly identifying these challenges and helping prepare to meet them, the Global Maize Germplasm Bank Workshop was held at CIMMYT headquarters on 6-12 March 1988 under the joint sponsorship of Mexico's National Institute of Forestry, Agriculture, and Livestock Research (INIFAP) and CIMMYT. With funding from the United Nations Development Programme (UNDP), these Proceedings were published to provide a record of the presentations and in doing so to reinforce the workshop's essential message: namely that the conservation and utilization of maize genetic resources are global concerns calling for international cooperation in the sharing of ideas, responsibilities, technology, and germplasm.

It was at least partly this awareness that gave rise to early achievements in the collection and classification of maize landraces, which are the subject of papers by E. Hernández Xolocotzi and E.J. Wellhausen. The vast amount of germplasm they and others collected was first deposited in several national collections; Mexico, for example, received all of the original collections made in Mexico, Central America, and the Caribbean, to which it has subsequently made significant additions (see Country Reports). An important subsequent step was the establishment of a modern germplasm storage facility at CIMMYT in the early 1970s. This bank was first organized and managed by Mario Gutiérrez G., and it contains samples of the materials deposited in the Mexican bank and numerous additional accessions (see Wellhausen, these Proceedings, for a discussion of the contents and history of the Mexican and CIMMYT collections).

The international character of this work has been even more firmly established since the creation of IBPGR, which has organized numerous collection missions jointly with various national institutes. Reviewing these early and more recent efforts puts us in a better position for planning and setting the priorities of future work. A key point emerging from this review is that, largely as result of previous work, the maize genetic resources agenda has been considerably altered. From the 1940s to 1960s, the order of priorities was clearly: collection, preservation, evaluation, and utilization. By the 1970s the order had been reversed, with utilization taking first place.

This is not to say that no more challenges remain in the tasks that once headed the list. Some national maize collections are still not adequately preserved, and a vigorous debate about approaches to conservation has arisen, in part, from the discovery only a decade ago of a new diploid perennial species of teosinte (a wild relative of maize) and the rediscovery of another species that had been considered extinct. Current experiments with in situ conservation and monitoring of the maize wild relatives promise to be highly instructive.

Nonetheless, it is now widely recognized (and is expressed very forcefully in these Proceedings by M.M. Goodman) that investments in the collection and preservation of maize genetic resources will be increasingly difficult to justify unless more rapid progress can be made in their evaluation and utilization for the development of new cultivars with high and stable yields. The types of initiatives required to achieve this end are spelled out by various contributors to these Proceedings.

One precondition for increased utilization is a change in the orientation and activities of germplasm banks. They must be enabled to take advantage of new techniques for generating information about bank accessions (such as cytology studies and applications of biochemical gene markers) and to adopt more efficient systems for organizing and disseminating information. An example of the latter is CIMMYT's new, computerized bank management system and a CD-ROM version of the system's passport data file, which contains basic descriptions of all bank accessions.

A second requirement is much intensified cooperation between national and international institutes in germplasm evaluation and information exchange. This is far too large a task for any single country or institution. The Latin American Maize Project (LAMP), discussed in several of these papers, is an important move in the right direction. But workshop participants expressed hopes for even broader, global collaboration and considered a proposal put forward by IBPGR for an international network of active collections. They also took several other steps aimed at establishing a basis for future cooperation. One was simply to become better acquainted, through the country reports summarized here, with the holdings and operations of the majority of the world's national germplasm banks. Another was to draw up general conclusions and recommendations that should provide a set of common goals for future joint action.

We hope the information presented here will prove useful to workshop participants and other researchers interested in maize genetic resources, as they take up the many individual initiatives that will be required to fulfill the collective goals outlined in these papers. We are grateful to the workshop participants for sharing their work and insights and to the Technical Centre for Agricultural and Rural Cooperation (CTA) of The Netherlands, IBPGR, Pioneer Hi-Bred International, and UNDP for their financial support of the workshop.

October 1989

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Experiences in the Collection of Maize Germplasm

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Abstract

This paper describes key decisions that were taken in developing a methodology for collecting maize landraces in Mexico. Following a brief review of events that prompted this activity, the paper relates how a method was found for identifying and collecting populations that would adequately represent the genetic diversity of maize. The major steps were formulation of a scheme for classifying maize races and establishment of practical guidelines for germplasm collection. Finally, the paper describes experiences in collecting landraces and presents some general principles that were derived from those experiences.

Three events led to the collection and conservation of maize germplasm. The first was developments in genetics, especially in the theory and techniques of plant improvement. For biological reasons those developments involved maize, and for economic reasons they were centered on the USA. The second event was worldwide acceptance of the application of genetics to plant improvement, following spectacular increases in US maize production achieved during World War II through the use of double-cross hybrids. The third was the involvement of the Rockefeller Foundation in agricultural programs in Mexico and other Latin American countries, starting in the 1940s.

During that period the most logical strategy for increasing agricultural production was to start with adaptation trials of improved materials from the USA. That approach gave good results with wheat but not with maize. As a consequence, local material had to be collected for development of improved seed adapted to local conditions. The decision to do so was reinforced by Vavilov's findings on the world's centers of diversity of domesticated plant species and research results indicating that landraces were being lost in Iowa, USA, as a result of general acceptance of maize hybrids. It was soon realized that collecting maize germplasm would require an ethnobotanical approach and that a taxonomic scheme for identifying open-pollinated populations would be essential to the task.

The Search for a Method of Identifying and Collecting Populations

The task before us was to collect viable seed that would include the genetic diversity of maize. In the absence of genetic studies that would identify the variants, we had to reach an understanding of the morphology of the phenotypes as well as the causes of the variants. The latter were of special interest to P.C. Mangelsdorf, since the investigation of them provided him with an opportunity to test his hypothesis about the development of maize from its ancestral form. My own experiences emphasized the human role in the development of the variants of maize. I detected the following relationships between traditional farmers and maize variants:

- Farmers select variants with different responses to ecological conditions.
- They also select for variants according to the forms of maize consumed.
- Human activities disperse materials and isolate populations.

By 1940 a sizeable amount of knowledge had been accumulated on Mendelian characters and maize morphology, as a result of which the initial scheme for classifying maize, often based on simple genetic characters, was discarded. At that time, the work of Edgar Anderson and his collaborators at the Missouri Botanical Garden was becoming important in maize classification. Starting with his doctoral research on the nature of variation in the genus *Tradescantia*, Anderson explored the phenomena of introgressive hybridization and its morphological expression. This led to the development of graphic analysis of the segregating population resulting from hybridization of two populations.

Anderson's subsequent studies on grass morphology and on the maize populations of central and western Mexico finally led him to formulate the concept of maize race with Hugh C. Cutler. The basis of that concept is the recurrence of parental characteristics due to chromosomal linkage of Mendelian characters. The race can be identified by the morphological characteristics of the more important plant structures. Several studies indicated that characters of the reproductive organs--the ear and tassel--are more constant than those of the vegetative structures. Anderson preferred the tassel because it has undergone less condensation and fasciation.

As a result of his studies on the effect of *Tripsacum* introgression on maize through teosinte and of archeological evidence, Mangelsdorf proposed a series of characteristics indicating the degree of *Tripsacum* influence in maize. And on that basis, he established a natural classification of maize races. The following morphological characters were selected for the purposes of collecting:

Ear:

- Form (pyramidal, cylindrical, big butt, oblong, spherical, and ramosa or ramified)
- Length and diameter at base, tip, and middle
- Number and regularity of rows of grain

Grain:

- Texture (pop, flint, dent, floury, sugary, and waxy)
- Length, width, thickness, and shape
- Color

Key Considerations In Collecting Maize Landraces

The following questions had to be answered to provide guidelines for collecting:

- What is the purpose of collecting?
- Where should one collect?
- What quantity of seed should be collected, and how should it be selected?
- When should the collecting be done?
- What should be the frequency of collecting over space and time?
- What data should be recorded?

We assumed that the purpose of collecting was to obtain maximum genetic diversity and that for this purpose morphological characters of the ear and grain would serve as indicators of the nature of the population found. The following criteria were used in determining where to collect:

- Ecological variation, on the assumption that the crop would have been selected for specific adaptation

- Technical variation (irrigated or rainfed, commercial or traditional, and so forth)

- Ethnic variation, or differences among specific uses of maize and metaphysical concepts surrounding its use

Since the collections from the 1940s were to be used for preliminary race identification, immediate planting, and storage at low temperature, it was decided that each sample should contain enough seed for 25 years and that it should be chosen from material selected for seed by the farmer or selected by the farmer upon request. That amount proved to be excessive in areas characterized by difficult ecological and economic conditions and by subsistence maize production. As a result, the sample size was set at 1 kg of seed from 25-50 ears. It has been suggested that collecting should be done in the field just prior to maize harvest, a procedure that involves a large investment of time and money. I decided instead to make collections from granaries and even in regional markets where ears are offered for sale. In Guatemala and Peru, interpreters were needed to collect from non-Spanish speaking indigenous farmers.

Within a geographic region, the frequency of collection was determined by the degree of variation found in the 50 km between sampling sites, when other factors were constant. As for frequency of collection over time, I have suggested that 25 years is an appropriate interval for studying changes in the materials used, genetic erosion, and evolution under domestication. On this point, Ortega Pazka's (1973) study in Chiapas is helpful. He found that in an isolated region the same races persist for as long as 25 years, that exotic material may be adopted for cultivation on new production areas, that improved varieties are available, that new varieties are formed through crosses and segregation of local and introduced varieties, and that the yield of local varieties generally increases as a result of pollination by improved materials.

By the time Columbus arrived in America, maize was an essential food crop in most of the indigenous cultures from the northeastern USA and southern Canada to northwestern Argentina and from sea level to 4,000 m above sea level (masl). After colonization and where indigenous human populations persisted, maize continued to be very important for human consumption, partly because of religious concepts associated with it. One of the results of colonization was that indigenous human populations were driven into areas of harsh ecological conditions. As a consequence, selection under domestication has tended toward ecological adaptation and increased fitness for the forms in which maize is consumed by humans.

Some of the races that show adaptation to special ecological conditions are:

- Gaspe--short growing period
- Guatemalan Big Butt--long growing period
- Tuxpeño, Celaya, Chalqueño, Cuban Yellow Flint, Cuzco Gigante, and Interlocked--conditions that are favorable for high production
- Chococeño, Enano, and Piricinco--macrothermal, high rainfall conditions
- Cónico Norteño--semiarid conditions
- Palomero Toluqueño, Cónico, Cacahuacintle, and Sabanero--high elevation, microthermal, humid conditions
- Nal-tel--juvenile calcimorphic soils

Other races and varieties that are suited to specific agricultural systems are: Tuxpeño (Rocamex V-520), Gordo, and Cónico for swidden agriculture and Chalqueño, Chinampa, and Cajete for early, deep planting.

Selection for the forms in which maize is used is well illustrated by the races listed in Table 1 and others that we collected in Chihuahua, Mexico. For the races Cónico Norteño, Cristalino de Chihuahua, Azul, Dulce, and Gordo, we recorded the following uses: *tortilla*, *tesguíno* (germinated, ground, fermented), *pinde* (roasted and ground), *marinillas* (ground and made into cakes), *esquite* (cooked and dried for later use in soups), corn on the cob, and protector of the *milpa* (maize field). To that list may be added Guatemalan Big Butt, whose thick resistant stalks are used for fences and the soft husk leaves for cigarettes.

A final consideration in collecting maize is that the crop is a result of human activity. The keen curiosity and constant observation of farmers has led them to intervene in the development of maize races by isolating them geographically and genetically, by diminishing their isolation, and by deliberately crossing materials. The formation of new variants has thus been a continuous and dynamic process.

Experiences in Collecting Maize Landraces

In maize production areas with indigenous populations, each farmer and farming community selects and maintains various landraces, based largely on the three considerations I have described above (response to ecological conditions, the forms in which people consume maize, and human activities). To collect the genetic variation of maize in a given community, one has to be persistent and to use a great deal of tact in dealing with farmers. I have reported elsewhere on some of my experiences in collecting and will add a few more here.

After graduating from the College of Agricultural at Cornell University, USA, I worked for three years with the government land-grant agricultural communities, mostly those of Mayan descent in Tabasco, advising, or rather learning, about their swidden system of producing maize, coconut, cassava, rice, sesame, and beans. Afterwards, I worked with the US Foreign Economic Administration in Mexico, collaborating first in the castor bean program, then in work on native oil-producing plants, and finally in the farm machinery program in Mexico. At the end of World War II, I joined the Government of Mexico-Rockefeller Foundation agricultural program, for which my main task was to continue maize collections initiated by E.J. Wellhausen in the central plateau of Mexico. I was given funds for travelling on public transport, purchasing materials and cloth bags for collection, and paying the costs of shipment, but no further instructions.

I suspected that Chiapas, at the southern extreme of Mexico within the Mayan region, would be a suitable place to start because of its ecological and ethnic diversity and so set out for the region with A.J. Sharp, who was interested in the mountain flora. I collected along the southern Sierra of Chiapas in a coffee producing area. After collecting Tuxpeño and Tepecintle, we descended to Mapastepec to continue our trip by railroad. But there we found that a railroad strike was on and had no way of knowing when the train would reach us.

The samples had been taken care of, we had eaten, and a local fair was in full swing. I decided to join in. Suddenly, several things happened at once: first, I slipped on a tequila rind and received a gash on the scalp with a beer bottle; about that time the train arrived, so we shipped the material and got on the train. The conductor looked at the blood on my head and said, "You can't travel

unless you are accompanied by a doctor." "He's a doctor," I replied, pointing to Dr. Sharp. "You are a doctor?" inquired the conductor. "Sure," answered Sharp, and we went on our way.

At the next stop on our trip, Tonala, a doctor shaved my scalp, cleaned the wound, and inserted nine metal stitches. Two months later, I went to George Harrar for my check. While I was signing the receipt, he looked at my head but made no comment.

In the upper part of Cerro Mal at 2,500 masl, I found Big Butt and Salpor, but the people would not sell me any, because if they run short of maize, they have to make a trip down to 2,000 masl and back. Finally, I practically stole two ears of each type, since I could not return empty-handed. On the way back, my guide and I stopped at a wayside stand to eat. The waitress kept staring at me while serving us. Doubting that my looks were the only reason for her interest, I finally asked her what was the matter. "Well, the brothers of the girl you robbed are looking for you to kill you," she said. We did not even finish our meal, but left in a hurry with our precious samples.

While collecting near Las Margaritas, Chiapas, center of variation of the large eared Comiteco, I found farmers walking along the main street of a neighboring town carrying several large, spectacular, golden yellow ears in their arms. With no further encouragement, people would gather and buy seed. Later, I saw people at Las Margaritas selecting ears from their harvest according to the size and appearance of the ears, the large ones getting the better price.

In the mountains of northwestern Mexico in the State of Nayarit, I could not persuade the indigenous Huichol population to sell me samples of their ceremonial maize varieties of the race Bofo. "This maize is like a member of our family," they said, "We do not want it to go and suffer and not be well attended." Nevertheless, we were finally given, not sold, some specimens after explaining that they would be used for experimental purposes.

The man who gave us the seed said, "Delay weeding." That seemed like a strange recommendation until we observed in our plantings that this race has an average of seven tillers. If the tillers develop under rainfed conditions in poor soils, the yield drops rapidly due to intraplant competition. And the competition of weeds (agrestics) prevents tillering.

Traditional maize plantings in Mexico include several red-eared materials, which symbolize protectors of the *milpa*. Red is chosen because it is the color of the east, abode of the favorable god; it is also the color of fertility, the color of menstruation. Farmers who sell seed produce a variety having red grain and add a certain amount of red grain to the total quantity of seed sold to a given farmer.

In Loja, Ecuador, I found a great variety of maize seed types displayed in the market. After purchasing a series, I realized that persons were being employed to sort out by hand uniform lots of grain from the main harvest to present a more attractive material and obtain a higher price. That practice seems to be related to the use of maize as *mote*, which consists of cooked grains. A similar practice is observed in Mexico, where beans are grown frequently in mixtures to assure a crop under rainfed conditions.

While collecting in Nariño, Colombia, I was asked by the research group of the Institute of Agricultural And Livestock Research to obtain additional material of a high yielding yellow flint previously collected there. Although I saw numerous maize fields, I was unable to collect seed. Most of the production was for corn on the cob (*choclo*), and since the farmers produced just

enough seed for the next planting, they were in no position to sell seed. It took time to arrange for purchase of plants near maturity to obtain seed at the proper season.

I had a similarly frustrating experience when I was asked to obtain material with the recessive opaque-2 or floury-2 genes. The best I could do was to identify materials with the lowest protein content among the numerous floury varieties from Colombia, Ecuador, and Peru.

When preparation of *Races of Maize in Mexico* was undertaken, P.C. Mangelsdorf took charge of the project. A large map of Mexico was drawn on the floor of the patio of the experiment station at El Horno, Chapingo, Mexico. When ears of the approximately 1,500 collections available were placed on the map, the various complexes stood out clearly, and with field data obtained from a preliminary planting, description of the races was begun. On one occasion more material was requested to confirm the existence of a race. Cytological and tassel data were obtained to confirm the initial identification. Finally, various tests were made to complement Mangelsdorf's categories of primitive, exotic, ancient, and modern races. The resulting publication (Wellhausen et al. 1950) served as a model for similar studies in Latin America. Other publications were prepared along somewhat different lines by Anderson (1946), Brown and Anderson (1947, 1948, 1960), Carter and Anderson (1945), and Brieger et al. (1958).

During a rest period in Florencia, Colombia, a hot humid site, in a wonderful hotel modeled on New York City's Waldorf-Astoria, I wrote a summary of my ethnobotanical experiences in Mexico, Cuba, Guatemala, Colombia, Ecuador, and Peru. The gist of those experiences is expressed in the following five principles:

1. There are always antecedents. The sites where we collect have often been visited by other collectors, although they may not have recorded all of their experiences. For instance, Victor Manuel Patiño never mentioned that he avoided collecting in Tolima, Colombia, because it was a frequent scene of guerrilla warfare. In Peru, Alexander Grobman found abundant pottery shards for study. In Mexico the existing codices and pottery shards show exact representations of maize races. Early historic reports include numerous references to maize in the USA, Mexico, West Indies, Peru, and Brazil, and we have rich archeological reports from the southwestern USA, Mexico, Guatemala, and Peru.

2. The ecological environment determines the phytogeography and is thus a good indicator of plant distribution and diversity.

3. Human activity has been and still is the most important factor in the development and maintenance of cultivars. It accounts for the races and varieties prevalent in the USA and that persist in Mexico, Guatemala, Colombia, Ecuador, Peru, and Bolivia, and it explains the variation of maize in Europe, Africa, and India.

4. Each plant population has distinct morphological characteristics and ecological adaptation.

5. It takes time to gather material and knowledge that has accumulated over thousands of years of selection.

6. Ethnobotanical exploration of maize germplasm is a dynamic endeavor. As scientific research proceeds, new questions arise. For instance, how does domestication occur under traditional agriculture? How does genetic erosion take place? How can we conserve genetic

diversity and at the same time allow evolution to continue? How have exotic cultivars been incorporated by traditional farmers? Often, to find answers to those questions, we have to make and study new collections.

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Table 1. Identification of maize races from samples obtained from farmers' fields in Babicora, Chihuahua, Mexico, 1975

Landrace (primary race) and its crosses with secondary races	Frequency	Frequency as percentage of total number of samples
Cristalino Chihuahua	28	35.44
x Apachito	14	17.72
x Gordo	6	7.59
x Híbrido Gringo	2	2.53
x Cónico Norteño	1	1.26
Subtotal	51	64.54
Apachito	6	7.59
x Cristalino Chihuahua	11	13.92
x Tabloncillo	6	7.59
x Gordo	3	3.79
Subtotal	26	32.89
Cónico Norteño	0	0
x Cristalino Chihuahua	2	2.53
Total	79	

IBPGR's Role in Collecting Maize Germplasm

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Abstract

Since 1975 IBPGR has supported systematic collection of maize landraces in Argentina, Bolivia, Brazil, Chile, South Korea, Morocco, Paraguay, Peru, Portugal, Spain, Thailand, and Uruguay. Maize has also been collected as part of multicrop collecting missions in many other parts of the world. Besides being stored in national gene banks, duplicate samples have been sent either to CIMMYT or the National Seed Storage Laborary (NSSL), USA. Since 1980 IBPGR has supported a regional cooperative project for characterizing and forming interracial composites of collections made in the Southern Cone of South America. Catalogs have been published that include evaluation data for Argentina (1983), Bolivia (1983), Brazil (1984), Chile (1984), Paraguay (1983), Peru (1984), and Uruguay (1983). In the future IBPGR will place more emphasis in its maize activities on collecting in areas throughout the world that are thought to represent gaps in the diversity of maize and its wild relatives.

IBPGR was established in 1974 by the Consultative Group on International Agricultural Research (CGIAR) to ensure the conservation of genetic variability in economic species, so that it can be used by plant breeders and other research workers interested in the evolution of cultivated plants and in agriculture generally. Soon after IBPGR was created, lists of global priorities were drawn up for germplasm collection. Since collecting was urgently required in numerous countries, the priority given to specific crops reflected not only their importance as staple foods for large numbers of people but also the need for immediate action where the threat of genetic erosion was particularly great. The priority lists served as working documents for guiding the progress of field work and were reviewed periodically as germplasm was acquired and as new information came to light.

Setting Priorities

From its inception IBPGR has placed a high priority on the genetic resources of maize (IBPGR 1976). In accordance with the Board's procedure of establishing crop committees to advise on the most urgent problems associated with a particular crop, a maize advisory committee was convened that comprised representatives of CIMMYT and IBPGR and leading scientists in the field of maize genetic resources. The group recommended that IBPGR 1) develop a coordinated inventory of all available material, 2) ensure the preservation of collections already made, and 3) identify areas that require further collection.

The recommendations were acted upon immediately, so that by the time the maize advisory committee met for the second time in 1977 all three of them were receiving attention. The inventory was prepared by surveying all gene banks known to be holding maize germplasm. Bank managers were asked to report on the number of accessions in their collections, the type of collections they held, their evaluation data and storage conditions, and many other items. In investigations of that type, it usually takes a considerable time to accumulate and check all the responses. But by 1980 IBPGR was able to publish a directory of maize germplasm collections (Ayad et al. 1980).

It also took some time to determine the status of the preservation of existing collections. In the course of that work, it soon became apparent that some collections were stored under poor conditions. In Brazil, for example, collections had been made in the early 1950s under the auspices of the Rockefeller Foundation and approximately 2,500 samples had been obtained from this country and some others in South America. No storage facilities were available, and the collection was reduced in 1959 by bulking to 90 accessions (see Wellhausen, these Proceedings), which were afterwards maintained at the University of Sao Paulo. In 1973 the Brazilian Agricultural Research Enterprise (EMBRAPA) provided funds for storing the material and two years later itself assumed responsibility for storage, assigning the material to the National Center for Maize and Sorghum. In another country, a large portion of the collection had been eaten by rats. Collections made in Spain had been lost completely, although they were documented and information about them published. In spite of some such difficulties, however, the situation was considered to be good overall, and it was felt that with continued cooperation between national programs and holders of the principal base collections, the future for storage of maize germplasm would be bright.

A number of areas were assigned high priority for further collection. It was decided that all of South America would require a great deal of attention, with the Southern Cone receiving highest priority. Three areas were identified as being in the greatest danger of genetic erosion as a result of introduction of new cultivars: 1) the western slopes of the Andes in Peru (1,500-2,000 m above sea level), 2) the Santa Cruz plains of Bolivia, and 3) Argentina. It was clear that in Europe the Iberian Peninsula would have to be recollected and that collections should be made for the first time in Albania and in areas adjacent to it in surrounding countries. In Asia the Himalayan region of Nepal, Bhutan, Sikkim, and Assam, which had been inadequately sampled, were given high priority because of the presence in the area of apparently unique types of germplasm. It was suggested that Indonesia and the Philippines probably contain uniquely adapted material as well and that Korea and China urgently require collection.

Exploration and Collection in South America

In 1978 a workshop was held in Sete Lagoas, Brazil, under the aegis of IBPGR to draw up a strategy and plans for collecting, conserving, and evaluating maize germplasm in the Southern Cone. Participants assessed the diversity of maize germplasm in the region and identified areas of maximum diversity. Following that meeting, collection progressed rapidly, so that by 1984, when the symposium Genetic Resources of Maize in Latin America was held in Buenos Aires, it was generally recognized that the missions conducted to date had been very successful.

With a few prominent exceptions, the collecting expeditions had covered the areas where maize germplasm was known or inferred to be at maximum risk. In Argentina the organization and execution of the missions were undertaken by the National Institute of Agricultural Technology (INTA) at Pergamino and the Faculty of Agronomy of the University of Buenos Aires. A total of 10 expeditions were made in most areas where maize is grown, including the southernmost areas of the country. Only the province of San Juan has not yet been collected.

In Bolivia national collecting activities were organized and coordinated by staff of the Phytoecogenetic Research Center at Pairumani and of the Bolivian Institute of Agronomic and Animal Husbandry Technology (IBTA). A total of five expeditions took place in almost all areas where maize is produced. A large number of races were recorded in the eastern plains region, justifying its inclusion in the original priority list.

Brazil has proved to be a difficult country in which to collect simply because of its size and problems in gaining access to remote regions. From the start collections have been conducted by EMBRAPA, which has completed a total of eight missions, with a ninth (to northeastern Brazil) now in progress. All of the missions reported a great deal of genetic erosion, with many of the indigenous races being contaminated by foreign germplasm.

Chile's National Insitute of Agricultural Research (INIA) at the La Palatina experiment station assumed responsibility for conducting four collecting missions. Samples were taken from all maize growing regions, and the country is now considered to be adequately collected.

Responsibility for collecting maize in Paraguay is shared by breeders of the National Agronomic Institute (IAN) and the Regional Center of Agricultural Research (CRTA). All areas of the country have been visited, and collection is now underway in the southwest. All of the indigenous colonies, which are rapidly disappearing, have been visited and collected, even those in remote areas that are normally very difficult to reach.

In Uruguay the collecting missions were organized by the maize program at Estanzuela. The entire country has been systematically collected, and 10 races have been obtained, a significant number considering the geographic homogeneity of the country.

Eastern Peru was designated as an area requiring urgent collection, since the region was rapidly being integrated into the modern agricultural economy. Staff of La Molina National Agricultural University, with support from the Ministry of Agriculture, completed 17 collection expeditions.

A more detailed study of the collection and evaluation of maize germplasm in the Southern Cone has been done by Sevilla (1984). All other collecting conducted in other parts of the Americas with IBPGR support has been organized as opportunities have arisen. That is, multicrop missions have acquired maize samples that were growing alongside other, targeted species. Table 1 clearly shows the emphasis given to the Southern Cone region.

Exploration and Collection Outside the Americas

IBPGR was able to support extensive collection of maize in Spain and Portugal during the late 1970s, and a successful mission to Korea was completed in 1982. During the second phase of the mission in Korea, it was found that the area planted to native maize lines was being steadily reduced and that area planted to foreign hybrids was increasing (Bong-ho Choe 1986). Bhutan was collected during a multicrop mission, but few samples were obtained. In Yugoslavia collection has taken place in the southern region adjacent to Albania, where it was found that an area where farmers are growing modern hybrids has not expanded (Radovic and Zivkovic 1983). Much of the maize germplasm collected in Africa, with the exception of Morocco, was acquired in the course of multicrop missions. Collection of material throughout the Old World with IBPGR support is indicated in Table 2.

Documentation

IBPGR's collecting activities are complemented by projects for characterizing, evaluating, and thoroughly documenting samples. In 1980 the Board began to support a cooperative project that is characterizing and making interracial composites of the germplasm collected from Peru and the

Southern Cone and is assisting in the organization of data. Catalogs for Argentina, Brazil, Bolivia, Chile, Paraguay, Peru, and Uruguay have been published that provide both passport and characterization data on the collections. More than 6,000 accessions were characterized on the basis of a large number of morphological, agronomic, and ecogeographic descriptors, and this information was used for racial classification in the seven countries of the region (Sevilla 1984). In Uruguay racial classification was based on numerical taxonomic studies. Support has also been provided to centers in Korea, Morocco, and Portugal for multiplying and characterizing collections, with particular emphasis on material collected through IBPGR-sponsored missions (Table 3). Besides being stored in national gene banks, seed from the Southern Cone has been deposited in duplicate storage at CIMMYT and the National Seed Storage laboratory (NSSL), USA.

To provide guidelines for standardizing documentation of maize germplasm, a descriptor list was prepared and published in 1980. In light of experience with characterizing and evaluating collections, however, a modification in the list was proposed, and the revised version is currently being circulated to crop experts for comments.

Current and Future Collection Missions

There are still some gaps in the geographical representation of maize diversity in South America. Currently, missions are underway in northeastern Brazil, southwestern Paraguay, and the Orinoco Delta region of Venezuela. The Amazon region as a whole has been rather poorly sampled. But within this vast region some small areas have been well collected, and some collecting has followed the new roads that were built in recent years. Exploration of remote areas is expected to lead to the discovery of new indigenous races that are still preserved by isolated tribes and may have a significant impact on our knowledge about the domestication and evolution of the crop. Because of the huge extent of the unexplored region, random sampling, probably through multicrop expeditions, is suggested. Until current collections are characterized and evaluated, maize collecting in the rest of the world will probably continue only as opportunities arise. Exploratory collection remains to be done in China, and the Himalayan region still needs attention. In Africa only Angola is considered likely to have landraces that are different from those collected in other parts of southern Africa.

The Wild Relatives

In all the meetings held to discuss maize germplasm, it has been suggested that it is not necessary to organize missions specifically to collect the wild and weedy *Zea* and *Tripsacum* species. The expectation was that those materials could be collected at the same time as cultivated material. That has not happened, however, and only a small number of such samples are known to be held in collections. A very limited number of samples have been collected in their areas of distribution (Mexico and Guatemala) mainly for botanical and biosystematic studies (Kato 1975; Smith and Lester 1980; Doebley 1984). But our knowledge about the diversity of the wild relatives of maize is still very poor. Intensive collecting missions are urgently needed to sample the diversity effectively, followed by characterization, evaluation, and classification.

First priority should probably be given to teosinte (*Zea mexicana* or *Zea mays* spp. *mexicana*), which is fully compatible with *Zea mays*, belonging to its primary gene pool, and has significantly contributed to the evolution of the crop species. The target area for collecting wild maize relatives is Mexico and Guatemala, where these species show maximum diversity.

It is generally recognized that collection of wild species is a much more difficult exercise than sampling farmers' fields for cultivated landraces. There is an urgent need for accurate and permanent documentation of plant populations, with detailed geographic and ecological passport data and voucher specimens deposited in scientific institutions (Iltis et al. 1986).

Suggested Research Activities

Emergency activities aimed at collecting and effectively preserving the endangered germplasm of most cultivated species are nearing completion. So, recently, IBPGR has shifted its emphasis to studies of the genetic diversity of germplasm, using models designed to identify gaps in the collection and areas for detailed collection. Other suggested research activities are described below.

Ecogeographic/areographic surveys--A prerequisite for these surveys is complete documentation of the germplasm in a centralized database for maize, with adequate characterization, evaluation, genetic, and ecogeographical data. It is suggested that case studies by Nabhan (1986) and Suarez and Vanderborght (1986) for *Phaseolus* be used as models for maize. Those studies describe methods that allow the investigator to detect gaps in ecogeographic representation and to identify areas of high diversity. The models can also be used in planning future missions for collecting the wild relatives of maize in Mexico and Central America.

Numerical taxonomy studies--Unlike earlier studies based on a few morphological characters, the compilation of large data sets that cover morphological, physiological, and agronomic attributes would permit high resolution classification. That degree of classification, done worldwide, would provide a basis for any future activities with genetic resources, from planning the collecting missions to effective breeding with the materials obtained.

Biochemical research--A number of methods have been used recently to assess the degree of affinity among races of maize or its wild relatives and to detect evolutionary and migration patterns in the germplasm. Isozyme variations and principle components analysis of the isozyme patterns were used effectively for racial classification in Bolivia by Goodman and Stuber (1983) and in Mexico by Doebley, Goodman, and Stuber (1985). Electrophoretic mapping of zein polypeptides was used to discriminate between maize inbred lines in the USA by Wilson (1985). Such methods should be used to assess the affinities among all maize races. Small scale assessment of local races within a country, though useful, is of limited scope since it includes only part of the diversity available.

Cytological and genetic research--Useful information on the evolution, distribution patterns, and affinities among maize races can be expected from extensive cytological studies on chromosome knob patterns. Work by McClintock (1959, 1960, 1978) and Kato (1975) indicates that such an approach shows considerable promise.

Conclusion

IBPGR has in large part accomplished its first and most urgent task, which was to collect and preserve endangered genetic resources of cultivated plants, and has turned its attention to their wild relatives. Further maize collection missions in the centers of diversity of the main racial

complexes can be justified only if surveys detect gaps in the ecogeographical representation of areas where there is a high probability of discovering new races or if they are designed to collect germplasm possessing genes required in active breeding programs. Much of IBPGR's future involvement in maize genetic resources will depend upon the results of this workshop and the recommendations of its participants.

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Table 1. Collection of maize in Latin America since 1975, with support from IBPGR

Country	No. of samples
Argentina	2,003
Bolivia	1,568
Brazil	1,024 *
Chile	547
Colombia	47
Costa Rica	16
Ecuador	28
Guatemala	24
Honduras	1
Mexico	78
Nicaragua	25
Panama	21
Peru	1,064
Paraguay	236 *
Uruguay	859
Total	7,541

* Does not include results of missions now underway.

Table 2. Collection of maize around the world (except in the Americas) since 1975, with assistance from IBPGR

Country	No. of samples
Afghanistan	143
Algeria	37
Bhutan	47
Burkina Faso	190
Cameroon	25
Côte d'Ivoire	23
Egypt	84
Ghana	29
Greece	86
Guinea	140
India	3
Iran	3
Korea	2,895
Libya	13
Malawi	83
Mali	71
Mauritius	11
Morocco	95
Nepal	610
Pakistan	406
Portugal	786
Spain	499
Sudan	47
Syria	22
Thailand	177
Togo	90
Tunisia	5
Yemen	53
Zambia	369
Zimbabwe	36
Total	7,078

Table 3. Maize projects (excluding collection) supported by IBPGR since 1977

Institution	Activity	Years
Argentina		
National Institute of Agricultural Technology (INTA), Pergamino	Multiplication and evaluation of Argentine germplasm	1985-87
	Meeting on evaluation, management, and conservation in the Southern Cone	1980
University of Buenos Aires	Multiplication of seed samples	1983
Colombia		
Instituto Colombiano Agropecuaria, Tibaitata	Equipment and funds to maintain and augment seed collections	1978-79
Korea		
Chungnam National Univeristy, Dagjeon	Evaluation of Korean germplasm	1983
Mexico		
CIMMYT	Upgrading storage facilities for long term conservation	1983-84
Morocco		
Insitute Hassan II, Rabat	Evaluation of Moroccan germplasm	1986
Peru		
Universidad Nacional Agraria, Lima	Characterization and documentation of germplasm	1982-83
	Germplasm evaluation	1980-82
	Storage facilities	1977-78
Portugal		
National Institute of Agricultural Research (INIA), Braga	Characterization, evaluation, and documentation of Mediterranean germplasm	1987-90
INIA, Oeiras	Evaluation of Portuguese germplasm	1983
Thailand		
Thailand Institute for Scientific and Technological Research	Facilities for long term seed storage	1981

The Indigenous Maize Germplasm Complexes of Mexico

Twenty-five Years of Experience and Accomplishments in Their Identification, Evaluation, and Utilization

Edwin J. Wellhausen, Former Director General of CIMMYT

Abstract

This paper outlines the process by which the races of maize in Mexico were collected and classified during the 1940s and explains how that work led to further efforts to collect the maize races throughout Latin America and elsewhere in the world. Then follows a description of what was learned through that work about the origin and evolution of maize and the implications of the findings for modern breeding programs. The paper next relates how the Mexican collections were employed in varietal improvement and in a program for raising Mexican maize production. The paper concludes with a challenge for current breeding programs to exploit more effectively heterotic patterns observed in the Mexican races during early evaluations of these materials. Two appendices describe the early history of the Mexican and CIMMYT maize germplasm banks.

There are many reasons why I am very pleased to be here. Foremost among them is that this workshop deals with a subject very close to my heart. It gives me an opportunity to get reacquainted with many of my colleagues. And most of all, I am pleased to see so many people here today interested in and concerned with the further preservation and utilization of the vast maize germplasm complexes brought together in the 1940s, first in Mexico and then in Latin America as a whole.

As Mario Gutiérrez well knows, if we had tried to organize a workshop of this kind in the early 1960s, when he took charge of the CIMMYT maize bank, we would have had difficulty in bringing together more than 10 people. Let me congratulate the organizers of this conference, Suketoshi Taba and Francisco Cárdenas, for their success. I am glad the maize germplasm banks again have been placed on the front burner.

As most of you know, my own role in the collection, characterization, and preservation of the vast germplasm complexes at hand began in Mexico in 1943 with the initiation of the Government of Mexico-Rockefeller Foundation (RF) cooperative agricultural research and training program. This program operated as the Office of Special Studies of the Ministry of Agriculture and was first aimed at increasing the production of the basic food crops in Mexico. My initial mandate in this program was to increase maize production with a select team of young Mexican agricultural scientists. Mexico then had a maize deficit of about 20% with a population of 25 million.

In September 1943 we started our program with a plan for the systematic collection of indigenous varieties in Mexico's principal maize growing areas. Our objectives, initially, were primarily utilitarian: namely, to determine major production constraints and to identify the outstanding cultivars and germplasm complexes with which breeding and production programs could be immediately launched.

Fortunately, P.C. Mangelsdorf, our program advisor, convinced us that, as maize breeders, we were obligated to collect and preserve for future use all the existing native indigenous germplasm that the introduction of our own improved varieties would someday most certainly replace and in some cases extinguish. This then became our principal objective in collecting.

Although the collection, characterization, and classification of the indigenous strains of maize in Mexico was basically a sideline effort, it was one of the most exciting, fundamental, and far-reaching things we did. Little did we realize at the time that these activities would explode into an international campaign for the collection and preservation of the varieties of maize in all of Latin America, the Caribbean islands, and southern Europe.

Today, a combination of the seeds collected and preserved in the Mexican and other seed banks of Latin America during the 1940s and 1950s represents a diversity of types unequaled in any other cultivated species. This diversity is a product of thousands of years of evolution under domestication in a myriad of different ecological situations and is definitely one of the great natural resources of the Western Hemisphere. To lose any part of this diversity obviously would be a major disaster.

Furthermore, the characterization of the early collections in Mexico, their classification into races, and a study of the origin and relationships produced a volume of information that clearly indicated how this vast diversity came about. The evolutionary factors involved were revealed. Distinct heterosis patterns became apparent. The most productive races were defined. All of this in the early years was of tremendous help in the development of a fruitful maize breeding program for Mexico and later, with the advent of CIMMYT, for the world as a whole.

As the racial complex of Central and South America and the Caribbean area unfolded and with the striking chromosome knob studies by Barbara McClintock, Angel Kato, and Almiro Blumenschein at hand, plus an examination of the ancient archeological plant remains discovered in the caves of Tehuacán by P.C. Mangelsdorf, it became very clear that maize was domesticated in South Central Mexico beginning about 7,000 years ago.

The chromosome knob studies amazingly revealed the paths of migration and origin and relationships of all the races of maize (about 300 or more) in the Western Hemisphere. This knowledge is being poorly exploited, yet it is of fundamental importance in the worldwide improvement of maize. The stage is now beautifully set for a very intelligent and more complete exploitation of the germplasm reserves being stored in the maize banks. The big question is "Why are the available materials and information so poorly utilized today?"

In this paper I shall briefly outline for you what we did, what we found and learned, and how we made use of the germplasm collected and knowledge acquired in the early years of our breeding program in Mexico and later throughout the tropics in general. There were many young outstanding students and potential maize breeders and some eminent biologists, botanists, and geneticists involved in these endeavors in one way or another. The pronoun "we" used herein includes them all. Finally, I would like to outline for you what I belive is in the maize banks and how it might be more fully exploited. Also, I will at least try to partially answer the above question.

What Did We Do?

Insofar as possible, we collected samples directly in farmers' fields at harvesttime (15 to 20 ears per samples), usually first in the main maize producing regions situated in a strip extending across Central Mexico for more than 1,200 km between 18° and 22° latitude. Later we collected in more remote areas.

Students in the National School of Agriculture at Chapingo collected most of the samples in the commercial maize production regions. They travelled by bus from village to village. From each village they combed the surrounding fields, using locally available transportation and collected samples every few kilometers. Professor Efraím Hernández Xolocotzi, a renowned botanist at Chapingo, collected in the more remote areas. Hernández was especially good at ferreting out the rare types grown and preserved for ceremonial and other reasons.

When we started collecting, improved technologies were still unknown. Most of the maize was grown by small farmers, on all kinds of unfertile soils, in areas with different rainfall regimes, and at altitudes varying from sea level to 3,000 m above sea level (masl). In most areas maize was subject to some kind of stress, such as drought, floods, heat, cold, insect pests, and a range of soil problems. Generally, drought was feared most.

Then, as now, maize provided the basic food for the people of Mexico. It was a subsistence crop. Most rural families were more concerned with having enough to eat in poor agricultural years than maximum yields in good years. The varieties grown were selected or formed on the basis of their ability to resist the often extreme stresses of a particular environment rather than their yield capacity.

Ears of each sample were photographed, two or more rows of grain were shelled from each ear, and the resulting seed sample was placed in refrigerated storage in jars at about 8% moisture. What remained was stored intact in rat proof containers properly identified for future studies. Each sample was indexed in our book of records, together with pertinent data on the date and place at which it was collected and other special observations made by the collector. All samples were grown and compared in controlled experiments at one, two, or three of four different testing sites. One year we had 80 ha in replicated tests. Test sites covered a wide range of conditions from very favorable to often extremely unfavorable.

By 1947 we had observed and compared about 2,000 collections. We were overwhelmed and a bit bewildered by the wide range of genetic variability that these collections exhibited. It soon became apparent that if this diversity was to be intelligently used in our breeding programs, some kind of classification would be needed. From our studies of these materials we discerned certain basic types. One day we drew a large map of Mexico on the ground in a field near our laboratories at Chapingo delineating the states. We placed representative ears of each sample collected, approximately at a point on the map where it had been collected. We studied and restudied this display, together with data obtained in the field tests. Distinct racial complexes were clearly discernible.

This stimulated us to some precise action. We invited Dr. Paul Mangelsdorf to come to Mexico. Together, we recognized 25 races, determined which collections were most typical of each race, and proceeded to describe them. Along with their descriptions, we postulated their origin and relationships. Results are summarized in the well-known bulletin *Races of Maize in Mexico: Their Origin, Characteristics, and Distribution*, which was published in Spanish in 1951 and English in 1952. Most of our postulations were later confirmed by the events that followed.

This publication sparked the formation of a committee by the Agricultural Board of the National Academy of Sciences (NAS)-National Research Council (NRC), Washington, D.C., to promote the collection and preservation of indigenous strains of maize throughout Latin America. Funds

were supplied from what was then the Office of Inter-American Affairs (US government) and the project got underway in the early 1950s patterned after the work in Mexico.

From our base in Mexico, we promoted the collection and classification of the indigenous varieties of Central America and Panama. Lewis Roberts, Ulysses Grant, Ricardo Ramírez, David Timothy, and various coworkers collected and described the races in Colombia, Venezuela, Ecuador, Bolivia, and Chile; Alexander Grobman and Wilfredo Salhuana collected and described races in Peru; F.G. Brieger and Ernesto Paterniani in Brazil, Paraguay, and Uruguay (later supplemented by Paterniani and Major Goodman); Carlos Rossi in Argentina; William Brown in the West Indies; and William Hathaway in Cuba. Results were published in numbered publications by the NAS-NRC. In this effort over 10,000 collections were made and examined, and more than 200 races were described.

Seed samples of the collections made in Mexico, Central America, and the Caribbean region were stored in the Mexican bank at Chapingo (El Horno). This bank was established in 1944 by the Office of Special Studies and operated by it until 1959. In 1959 the Mexican Ministry of Agriculture, with the encouragement of the Rockefeller Foundation, formed the National Agricultural Research Institute. The cooperative program was terminated and all its assets, including the Mexican seed bank, were transferred to the new institute. The Ministry of Agriculture, through its research institute, has continued to maintain this bank, but a new bank sprouted with the establishment of CIMMYT in the early 1960s. The contents and early history of the two banks are briefly described in Appendices A and B.

With the collections in South America, other seed banks originated. Seeds of collections made in Colombia, Venezuela, Ecuador, Bolivia, and Chile were stored in the Colombian bank first established at Medellín by the Government of Colombia-Rockefeller Foundation cooperative program initially headquartered there. Seeds of the Peruvian collections were stored and maintained by Peruvian maize breeders at the agricultural university near Lima. Seeds of collections made in Brazil, Paraguay, and Uruguay were stored and maintained by the Institute of Genetics at Piracicaba. Seeds of Argentina collections were maintained by maize breeders at Pergamino. Seeds samples of the Caribbean collections made by Brown in the West Indies and by Hathaway in Cuba were sent to Mexico for storage in the Mexican bank. All of these banks, perhaps with the exception of the Mexican and Colombian ones, were poorly equipped for maintaining the viability of seeds in storage and had to rely on frequent renewal in the field. As indicated in Appendix B, the Brazilian collections were eventually sent to CIMMYT for maintenance.

Small samples of seed of all collections sponsored by the NAS-NRC were sent to the US National Seed Storage Laboratory (NSSL) at Fort Collins, Colorado, for safekeeping. Samples of each of the Mexican collections were also sent. What happened to those collections is indicated in Appendix B.

I would be remiss if I did not also mention the collections and classifications made by Brandolini and his colleagues in Italy, by Covor in Romania, by the Agricultural Research Institute in Spain, by the Agricultural Research Institute in Hungary at Morton Vassar, and in Nepal by Japanese botanists. I do not know the status of these collections today, but the classifications and description of these samples have contributed much to our knowledge of the origin and migration of maize in those areas of the world.

What Did We Find and Learn?

We found the high yielding varieties with which our production program could be immediately launched and much more. We found that nowhere in the world was the total spectrum of variation in maize as great as it was in Mexico, and nowhere was maize so deeply entwined with the social and economic life of the people. We were especially intrigued by the tremendous variability in productivity, ranging all the way from the low yielding primitive forms described as Nal-Tel and Chapalote to the high yielding races of Tuxpeño, Chalqueño, and Celaya, which we found growing on the more fertile river bottoms and old lake beds. Varieties of these races without further improvement were capable of yielding 6-7 t/ha in their areas of adaptation, with good care and chemical fertilizers.

Furthermore, our own characterization of the variation at hand, coupled with the studies by Mangelsdorf of prehistoric plant remains discovered in the caves of Tehuacán and the chromosome knob studies of McClintock, Kato, and Blumenschein, confirmed that maize was domesticated in Mexico. All evidence points to South Central Mexico as the center of origin. A little to the west of this area the close relatives of maize, teosinte and *Tripsacum*, still abound. Where maize was domesticated is no longer debatable.

Even more important than where maize originated is to know how it evolved from the tiny cobs found in the Tehuacán caves (dated by radiocarbon laboratories at about 5,000 B.C.) to the modern, highly productive cultivars collected in the 1940s about 7,000 years later. Unraveling this story and the spread of maize from its center of origin to all parts of the world has been very exciting.

The prehistoric evolutionary sequence, from the initial semblances of maize to the precursors of the Nal-Tel-Chapalote complex and other primitive races identified in our collections in Mexico, was clearly revealed by Mangelsdorf in his examination of the archeological plant remains discovered in the Tehuacán Valley by MacNeish in the early 1960s. With this information as a point of departure and with all that obtained from an intensive study of collections now stored in the maize banks, the evolution of maize can be briefly related as indicated below. It has not changed much from concepts expressed in our 1952 publication *Races of Maize in Mexico*. It is certain that early forms of the races Nal-Tel and Chapalote or their close derivatives became widely distributed at least several thousand years ago. They were taken by early man into many different environments and ecological niches, and many distinct, more or less homogeneous varieties developed in the isolation of separated regions. The principal factors involved in this early evolution were the relatively frequent gene mutations and a partial release from the pressure of natural selection through human intervention.

In this early evolution, progress in productivity came about very slowly. In sharp contrast, however, during the last 500 years there has been an explosive increase in productivity resulting from the interhybridization of distinct varieties brought together by chance through the peregrinations of man. Then as today, much of the hybrid vigor that often results from the intercrossing of distinct varieties persisted into advanced generations, and many new higher yielding cultivars came into being.

Repeated cycles of this random hybridization led to the chance development of such highly productive races as Tuxpeño, Chalqueño, and Celaya even without much direct or conscious selection by man. The pedigrees of the high yielding varieties we found were generally very

complex, encompassing many lesser yielding races in repeated cycles of intercrossing. We often thought, if such varieties had arisen through random interhybridization, what could modern plant breeders do with more than 200 well-described races at their disposal and with their knowledge of genetics and modern breeding technologies. This question is yet to be answered. There is no reason to believe that the high yielding germplasm complexes that nature has created through chance combinations are the best that can be attained. It seems to me that this is something that ought be challenged by CIMMYT and its international cooperative maize improvement programs.

We owe much to McClintock, Kato, and Blumenshchein (see Appendix A). They have provided us with a large amount of positive information on the history, centers of origin, paths of migration, and genetic relationships of all the races of maize in the Western Hemisphere. If we take into consideration the findings of Japanese botanists in the Himalayan region, those of Brandolini and others in southern Europe, and our own observations in Southeast Asia and Africa, I believe it can be truthfully said that we now know the origin and relationship of all the germplasm complexes in the world. This revelation is of tremendous value and of fundamental importance in the more complete and efficient exploitation of the germplasm reserves at hand in the further intelligent improvement of maize throughout the world.

Utilization of Collections in Varietal and Production Improvement

In our collecting campaign, it became very clear that there were three major constraints limiting maize yields in Mexico: 1) low soil fertility, 2) limited yield capacity of prevailing varieties, and 3) limited rainfall and its often erratic distribution. The first two could be modified with chemical fertilizers and the use of fertilizer responsive varieties; the third generally was not modifiable, although its effects might be alleviated.

Accordingly, and concurrently with our collection and classification activities, we launched our production campaign almost immediately, with fertilizer and the elite varieties that had evolved on the very limited more fertile soils of river bottoms and old lake beds. As a first step in our production campaign, we multiplied and distributed these varieties directly to farmers for widespread use with fertilizer and good husbandry. Later, as our program broadened, we modified them through recurrent selection or the formation of synthetics and hybrids using tested S_1 lines (with one generation of inbreeding) to meet the requirements of different areas. These lines were derived from a wide range of different germplasm complexes. In all of our breeding work, emphasis was placed on resistance to the stress of erratic rainfall patterns. We found that getting a high yield was easy, but getting it with all the other characteristics needed for stability of yield under the limited and erratic rainfall conditions prevailing in most of Mexico's maize belt was a major problem.

It was exciting to find that Mexico's germplasm complexes are rich in gene based mechanisms for drought resistance and adaptation to changing environments. There are many indications of this, but I have time to mention only two.

Seedling drought resistance--Most of Mexico's maize is planted with the first rain after a 6- to 8-month dry season. The first rain will generally wet the soil to a depth of 8-10 cm, enough to germinate the seed and sustain the seedling to a height of about 15 cm. Often, the rains do not continue as they should, and there may be a long interval between the first and second rains. In that case the plants wither and may die, and the farmer needs to replant. In the evaluation of collections, we found that some varieties will endure the state of withering much longer than

others, in extreme cases up to 3 or 4 weeks. We found that this ability is due to an easily transferable gene based mechanism. Varieties carrying this trait surprisingly are also resistant to frost (for example, -2°C). That the same genetic mechanism should function for two very different traits may seem strange at first, but the two are related. A variety delayed in its early growth may get caught by frost at the end of its cycle, especially at high altitudes above 2,200 masl, where frost generally comes early in October and occasionally in September. Once during my 41 years in Mexico, the maize in the Central Mesa was frost killed in August. Natural selection obviously would favor the development of such traits.

Drought resistance in the middle of the growth cycle--Drought, of course, may occur at any stage of the plant's growth. One of the most striking gene controlled, drought resistance mechanisms is the one that exists in the hybrid Celita developed for the Bajío area. This hybrid (H220) is a three-way cross made with two S_1 lines derived from a Celaya-Conico Norteño germplasm complex and a third derived from a collection of the race Bolita, which evolved in Oaxaca in a valley with very limited and erratic rainfall.

It is an amazing hybrid. Under conditions of severe drought during initial periods of growth, it will yield more grain than any collection or improved variety in the Bajío (Mexico's central breadbasket). Just as striking is its capacity to yield up to 5 t/ha in good rainfall years. This is the kind of elasticity needed in rainfall areas in which rains are often poorly distributed. Under severe drought plants are short and the ears are short--maybe not more than 5 cm long--but the grain on the cob is its normal size and shows no signs of stress. This variety has become widely distributed in the central valleys between 1,200 and 1,800 masl. It is probably one of the best varieties we developed. How its drought resistance mechanism functions is still to be determined. Prolificacy is part of it.

I cannot resist mentioning another mechanism that I think contributes to a plant's flexibility in yield. Varieties with ear-bearing tillers generally are more flexible. Under adverse conditions tillers do not develop, but under favorable conditions they do and give high yield. This is a mechanism through which the plant may adjust its productivity to changing moisture conditions. Most all varieties in the erratic rainfall areas of Mexico tiller. This character is worthy of further study.

From our studies of the origin and evolution of the enormous diversity of maize, we learned that maize is self-improving. We exploited this knowledge to the utmost. Varieties released to farmers looked good and were highly productive in years with good rainfall distribution and adequate fertilization. We knew that what we introduced would upgrade local *criollo* varieties through the random interhybridization that was sure to occur.

We never got more than 10% of the main maize production areas planted with improved seed of our own making. But this was enough to catalyze the indirect development of a whole series of new fertilizer responsive varieties by the farmers themselves through random interhybridization with local types. Some of these were embarrassingly better suited to their conditions than those we introduced.

As the use of improved seed and fertilizer spread throughout Mexico's highly commercialized maize belt, idle land disappeared. The low yielding, poorly rooted Conico types at one time so highly prevalent in the high plateau were absorbed. Likewise, the Conico Norteño types that prevailed in the Bajío and the Tabloncillo varieties once so prevalent in Jalisco were swamped out. By the early 1950s a green revolution was in full swing. Annual production soared, steadily

rising from 3.5 million t in 1943 to 8.8 million by 1958, an increase of 2.5 times. During this same period, Mexico's population increased from about 25 million in 1940 to about 45 million in 1958. For the first time in many years, Mexico had reached self-sufficiency. By 1960 there even was a small surplus.

Many people were involved in this success. However, major credit is due to the farmers. They were the ones that talked about their successes, not us. They were the extension agents, not us. Although we might have helped, they were the ones that pressured government officials to increase the supply of fertilizer at reasonable prices, not us. It was they who continuously clamored for a favorable relationship between cost of inputs and the price they received for their product, not us.

The Job Ahead

In the identification and exploitation of the many existing heterosis patterns, we have only scratched the surface. In the early 1960s when our inter-American Maize Project got underway, the late Mario Castro evaluated the performance of all possible crosses among the 25 described Mexican races. His efforts were most revealing.

It was a bit of a surprise to find that the hybrid between the low yielding race Chapalote and Tuxpeño, the most productive of all races, yielding equal to the high parent. This tells us a lot about the genetic power of Chapalote. Even more striking, which of the 25 races do you suppose came up with the best combinability? It was not Tuxpeño but Harinoso de Ocho, an ancient race found in northwestern Mexico. Both of these races have been the progenitors of many more modern races. Both of them played a role in the development of the race Bolita, a major source of germplasm in the drought resistant hybrid Celita described earlier.

There were other surprises, such as the cross between representative collections of the races Celaya and Comiteco. Celaya is the highest yielding race found in the Bajío. Comiteco is a high yielding race found near Comitan, Chiapas. The two areas are far apart. The cross yielded 50% more than Celaya, the high yielding parent in tests made in the Bajío. A similar result was obtained in a cross between the race Pepitilla, which evolved in the state of Guerrero, and the race Celaya. These heterosis patterns are still to be exploited. Unfortunately, due to the green revolution rush, only part of Castro's results ever got published.

There are other examples of strong heterosis patterns involving germplasm complexes that developed outside of Mexico. One spectacular example is provided by the cross between Kitale Flat White of Kenya (Africa) and a variety from Ecuador (Ecuador 573). This cross was made and tested in Kenya by Michael Harrison. It yielded about 40% more than Kitale Flat White, the best of the varieties grown in Kenya.

The most widely useful heterosis pattern for the further improvement of maize in the lowland tropics (worldwide) is the one that exists between Tuxpeño and the Cuban and coastal tropical flints of the Caribbean. Although it constitutes the basic germplasm of the international maize improvement network spearheaded by CIMMYT, it has been poorly exploited, except perhaps in the case of Brazil, Central America, and Venezuela. In my opinion, it might be more effectively exploited through the establishment and execution of well-planned reciprocal recurrent selection programs in which the flint and dent population are upgraded along with the combining ability between them. All the heterosis patterns described above support the theory that the greater the

difference in relationship between varieties the greater the amount of heterosis expressed in crosses between them.

So far, we have exploited mainly the elite materials in bringing about a worldwide green revolution, mostly on the better soils in regions with good rainfall distribution. Only about 20% of the world's agricultural land falls into this category. Although at the moment there is enough food to feed 5 billion people, this is no time for complacency. In a few more years there will be 6-8 billion people to nourish. We urgently need a second green revolution.

Although still more can be produced in the better agricultural environments, this second green revolution will have to come in the *unfavorable* agricultural regions, which in the tropics include about 80% of the total agricultural land. In this second green revolution, maize will come into its full glory. There is no other grain crop that is as efficient in its photosynthetic activity as maize.

In this second green revolution, we will need a new set of varieties, perhaps a different variety for each of a hundred different ecological situations. We need much tougher varieties with greater tolerance of stress, especially periodic droughts, problem soils, and insect attacks. We will need varieties that are more efficient in the use of both water and chemical fertilizers. We will need to pay more attention to fitting the variety to the soil rather than the soil to the variety.

The building blocks or genes for this new set of varieties are in the seed banks. They are apt to exist in higher frequencies in the races that up to now have been ignored by the breeders, especially the more ancient types (such as Chapalote, Harinoso de Ocho, Zapalote Chico, Bolita, and Salvadoreño) collected on the west coast of Mexico and in Central America.

There are three reasons why the materials in the banks have not been effectively used:

1. They look bad.
2. Breeders can still make progress with materials they are now using.
3. Very few people know what is in the bank and how to use it.

In the early years of the maize seed bank, the breeders of Mexico were also the collectors, evaluators, and users of the germplasm being stored. They developed a feeling for the potential value of these materials and an enthusiasm for unraveling the mysteries of its origin. Their successes were based on the intelligent use of the wide range of diversity at hand.

These breeders are no longer doing the breeding work. For some reason, in the green revolution rush of the late 1960s, the enthusiasm, the feelings, and knowledge gained were not passed on to the younger generation that replaced them. Critics say that the banks today serve only as seed depositories, that they are morgues. Unfortunately, it is true. This urgently needs to be changed.

We senior maize scientists have left you younger maize breeders a legacy of materials and information that no generation of breeders has had before. It should make maize breeding in the tropics tremendously more exciting and fruitful than ever before. The glory, excitement, and payoff from this endeavor are all yet to come. Let me leave this thought with you. I think all of us working with maize in the tropics *can take much pride* and *satisfaction not for what we have accomplished but for what we can achieve* with the germplasm reserves waiting to be exploited. The best is yet to come.

Appendix A: Early History of the Mexican Seed Bank, 1944-1959

The Mexican seed bank was established in 1944 by the Office of Special Studies (Government of Mexico-Rockefeller Foundation cooperative agricultural research program) and operated by it until 1959, when the Ministry of Agriculture created its National Institute of Agricultural Research. At that time the Office of Special Studies was terminated, and all its assets, including the maize bank, were transferred to this new institute (see Appendix B).

Seed of collections made in 1943-1945 were first stored at room temperature in a temporary adobe building constructed at Chapingo (El Horno), pending the completion of more permanent refrigerated storage facilities in 1946. Temperature in this building varied from a low of about 15°C in winter to a high of about 22°C in summer. Seed stored under these conditions in capped jars at 8% moisture (air-dried) maintained its viability for about five years.

All jars were transferred to the refrigerated facility at El Horno when it became available in 1946. Temperatures were maintained at 5° to 8°C; humidity was not controlled. As before, seed was stored in quart jars at 8% moisture. This facility served the Office during its 16 years of existence. During this period the seed samples were regenerated at least twice.

Plantings for seed renewal were made in either of two places with irrigation. Collections from areas with elevations above 2,000 masl were renewed at Chapingo (2,200 masl). Collections made in areas below 2,000 masl were revitalized in the state of Morelos at about 1,200 masl. Plantings at both locations were made in 60-plant plots at Chapingo during April and May and in Morelos during December and January. All varieties planted in Morelos on these dates during the dry season grew well.

All plots were hand-pollinated, sib matings. As many plants as possible were pollinated in this way. At harvest equal amounts of seed were taken from each ear and mixed, and a representative sample of the mixture was placed in a quart jar at 8% moisture, properly identified, and returned to the bank. The remaining seed was stored at room temperature and used for breeding and evolution purposes.

This bank provided the basic materials for the early stages of the green revolution in maize, first in Mexico, and then in other countries of Latin America, Asia, and Africa. It provided seed of many of the individual collections examined in the chromosome knob studies by Barbara McClintock and coworkers, studies which confirmed the origin of maize in Mexico and its migration from there to all parts of the Western Hemisphere.

The bank is being continued by the Ministry of Agriculture. It contains samples of all the original collections in Mexico, Central America, and those made in the Caribbean region by William Brown. Many new collections made in Mexico since 1959 have been added.

Appendix B: Early History of the CIMMYT Seed Bank, 1960-1972

In 1959 the National Institute of Agricultural Research (INIA) was formed within the Ministry of Agriculture. The Office of Special Studies was terminated, and all its staff and assets were transferred to INIA. It was agreed that the new institute would be responsible for the further

development of agricultural research in Mexico and that the Rockefeller Foundation would continue to assist Mexico in the organization and establishment of INIA and at the same time give her a hand in extending previous accomplishments in the improvement of maize and wheat more broadly to other interested countries.

Thus, in 1960 the Inter-American Maize and Wheat Programs were born. These were the forerunners of the International Maize and Wheat Improvement Center (CIMMYT), established first in 1963 as a joint venture between the Government of Mexico and Rockefeller Foundation and then in 1966 as a truly international research center operated by an international board of directors.

One of the first projects of the Inter-American Maize Program was to renovate the seeds in the Office of Special Studies bank, which had become INIA's responsibility under the new arrangements. In the process we took 100 seeds of each collection in the bank, including those from Central America and the Caribbean. The high altitude collections were planted at Chapingo and the low altitude ones in the state of Morelos in 60-plant plots. As in the case of all previous seed renewals, we sib pollinated as many plants as possible within each plot, and at harvest equal quantities of seed were taken from each hand-pollinated ear and mixed. This then became the renewed sample.

The renewed samples were utilized in two ways: 1) one quart of seed of each sample was returned for storage in what had become the INIA bank or Mexican bank in the new arrangement, and 2) the remaining seed of each sample was used as described below.

For years I had been concerned about the need to eliminate the many duplicates that existed in the maize bank. These duplicates were replicate (or nearly so) samples of the same variety collected every few kilometers in a given valley or area. Insofar as possible in this renewal project, equal quantities of seed of these duplicates were composited. Each composite was indexed as a group (for example, Jal. Group 1, Gto. Group 10, Antigua Group 2, and so on). Up to 850 groups were formed. There should be a record book among those transferred to CIMMYT indicating the collections included in each group. Certain groups included as many as 15-20 or more collections. In each group the most representative collection was identified, and although included in the composite it was also maintained separately. In those groups having a large number of collections, usually three representative samples were singled out for separate maintenance. Since the INIA bank could not handle additional materials, arrangements were made with the National School of Agriculture at Chapingo for the construction of a temporary refrigerated seed storage facility in the basement of their soil science building.

This facility served the Inter-American program and CIMMYT in its early years until the present modern seed storage center at El Batán became available in 1971. A minimum of one gallon of seed of each composite and a quart of each individual collection to be maintained were stored in this temporary bank. These materials constituted the initial contents of the CIMMYT bank.

Another early project of the Inter-American program was the re-collection of a larger supply of seed of the prevailing cultivars of the Caribbean region. The Caribbean region was recognized as being rich in types that could be of immediate importance in launching the international maize improvement program. Pablo Daza, who collected most of the indigenous maize samples in Colombia, was chosen to do this job in all the islands except Cuba. José Jiménez was sent to re-collect in Cuba. Efraím Hernández X. made a number of collections a year or so before. All seed

collected was sent directly to Mexico, and when it arrived a representative sample of each collection was placed in the CIMMYT bank. These second collections were marked with the letter D, such as Antigua 2D or Saint Vincent 3D, to identify those collected by Daza and with the letter J, such as Cuba 1J, to identify those collected by Jiménez. Those collected by Hernández X. were marked EHX. This was done to distinguish these seed samples from the ones sent to the bank by William Brown.

In the early 1960s another event occurred that swelled the volume of accessions in the CIMMYT bank. During the collecting campaign sponsored by the NAS-NRC, a small sample of seed of each collection was sent to the US National Seed Storage Laboratory at Fort Collins, Colorado, for safekeeping. we also sent to this bank a sample of each of the Mexican collections. Sometime in the early 1960s, a letter was received from the director of the Fort Collins bank informing me that due to shortage of space the international collection could no longer be stored. I suggested that seed of all collections, except those from Mexico, be sent to me in Mexico City. What seemed to be tons of it came. This was about the time that Mario Gutiérrez G. joined the international program and took charge of the bank. The records will show how many of these individual collections are now being maintained in the CIMMYT bank.

Still another event happened, I believe, before Mario Gutiérrez took over. Ernesto Paterniani wrote me about his troubles in maintaining the Brazilian maize collections at Piracicaba. Could he ship what he had to Mexico? I went to Piracicaba. Together we composited collections that obviously were of the same variety, much as was done in Mexico. We earmarked those which should be maintained separately. What we came up with was shipped to Mexico. Again, the records should show how much of this shipment is being maintained in the CIMMYT bank.

In the early 1960s, I became deeply involved in the organization and development of CIMMYT. The Center's new office and laboratory complex at El Batán was completed in the early 1970s. The new installations included modern facilities for long term seed storage, with ample space and the latest in shelving and equipment for temperature and humidity control. All seed samples in the temporary bank were transferred to this new, hopefully, permanent location. I would like to highly commend Mario Gutiérrez for his efforts in this transfer and the reorganization of the records. As a matter of fact, the world as a whole is greatly indebted to Gutiérrez for his magnificent efforts in putting the bank in order from the mid-1960s to the mid-1970s, when he resigned his position in CIMMYT to take on a maize breeding job in Brazil. It would be well if he and others that followed him could bring this brief history of the CIMMYT bank up to date.

Seed Increase and Germplasm Evaluation

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Abstract

This paper explains why the genetic resources of maize, though well collected, preserved, and classified, have not been widely used in breeding programs. An important step toward increasing the utility of these resources in breeding programs is evaluation through cooperative efforts like the Latin American Maize Project (LAMP). The paper describes the various stages in LAMP's evaluation plan and offers practical advice on increasing maize seed.

An extensive collection of maize has been organized in various Latin American countries under the auspices of the National Academy of Science and National Research Council of the USA and, more recently, IBPGR. The collection is being stored in various germplasm banks in several countries (Table 1).

Systematic morphological and cytological studies have been carried out with some collections to determine which groups or races occur in each country. Anderson and Cutler (1942) proposed the use of race information as another level of classification and defined the term as a group of individuals with several characteristics in common. Morphological studies have led to the classification of more than 280 landraces (Table 2): Wellhausen et al. (1952); Hatheway (1957); Roberts et al. (1957); Wellhausen et al. (1957); Brown (1960); Ramírez et al. (1960); Grobman et al. (1961); Timothy et al. (1963); Grant et al. (1963); Brandolini (1969); Hernández and Alanis (1970); Brown and Goodman (1977); Paterniani and Goodman (1977); Richieri et al. (1979); and Torregroza (1980, 1981).

Efforts to collect, maintain, conserve, and classify the genetic resources available have not been matched by efforts to use them. Brown (1975) states that maize breeders in the USA are using only 2% of the available germplasm, and thus a minimal proportion of the genome has been employed.

Reasons for Limited Use of Germplasm

The main reasons that maize germplasm has not been used widely are set forth below. Some of the problems have been partially resolved through cooperation among several institutions and persons interested in genetic resources, but much remains to be done. It is important to point out that the different operations involved in genetic resources cannot be undertaken by a single institution, much less by an individual, but can only be accomplished through joint, coordinated efforts.

Insufficient quality and quantity of seed--If the amount or viability of seed in a germplasm bank is insufficient, then the material must be increased. Since it is virtually impossible for a single country or institution to have every environment required for increasing accessions from all over the world, it is necessary to establish a system for increasing seed in which various institutions and countries participate. This approach ensures adequate quality and amounts of seed for carrying out studies in genetic resources. Such joint actions are now being undertaken, and for a significant number of accessions, adequate amounts of seed are now available. I am referring to the regeneration project initiated three years ago with germplasm banks in Colombia,

Mexico, and Peru under the leadership of M. Goodman, with financing from the Agricultural Research Service of the US Department of Agriculture (USDA). The project is only a beginning, and many other germplasm banks not currently participating in the project also need to increase seed. Moreover, it would be advisable to have backups for all accessions in another germplasm bank as insurance against accidents.

Lack of documentation--Documentation is important not only in germplasm banks but in many other activities as well. The primary purpose of documentation in banks is to create a record of the material available and its origin. Reference documentation is known as passport data, which include information on the collection and the donor. Without such data it would be impossible to ascertain which environment is suitable for increasing a given type of seed and to carry out subsequent evaluation. When passport data are not available, accessions must be sown at various sites to determine which is the most suitable. To avoid confusion about introduced accessions, it is important to note the codes used by the country in which the accession originated. Although many countries have their own methods of recording accessions, it is worth keeping the original codes, so that information about the materials can be cross-indexed. Such an approach will help prevent data from being lost, something that occurs all too often.

The efforts that several countries have made to organize and publish information on genetic resources deserve special mention. Catalogs that include passport information and other data gathered over the years (for Argentina, Brazil, Bolivia, Chile, Paraguay, Peru, Uruguay, and the USA, among others) have been published and distributed. Passport information on most of the collections in the CIMMYT and Mexican banks has been recorded on computers and will presumably be distributed in electronic form or in catalogs.

Agronomic and adaptability problems--Most collections from farmers have not been selected and are poor in agronomic traits. The most common criticisms aimed at such collections are that they are prone to lodging, show excessive plant height, produce barren ears, exhibit slow grain drying, and so forth. Some accessions, on the other hand, are highly promising but are adapted only to specific growing conditions, so that it is difficult to use them in other environments (the variety Cuzco is an example). Altitude and latitude also pose adaptation problems, since it is not easy to transfer material from high to low altitudes or from regions having short days to those having long.

Lack of evaluation data--One method of increasing the use of genetic resources is to provide information on traits that could be useful in research programs. For that purpose it is necessary to carry out evaluations of genetic material, bearing in mind the traits that researchers seek.

Lack of methodology for incorporating germplasm into improvement programs--Many characteristics of interest are governed by unknown genes that interact in unknown ways, making it difficult to develop a specific methodology for incorporating germplasm into material used in improvement programs. Several methods may be used, depending on the complexity of the heredity of the trait to be included and on the crop.

Classification

The concept of race is not easily understood. Races are distinguished from one another by variation in sets of plant characteristics, though the level of differentiation between races is not necessarily the same for all. It seems that a race can pass through many generations without

loosing its identity. There is a lack of information about the origin of races, and the accuracy of what we do know is reduced by the practice of basing classification on quantitative data that vary according to environment. Goodman and Paterniani (1969) found that ear and grain characteristics are the least affected by environment, followed by tassel traits and vegetative traits, the latter of which show the most interaction. Ear and grain characteristics therefore seem to be the most appropriate for classification. The concept of race is helpful in examining the relationships between materials and can be of use to breeders in selecting germplasm.

Increasing Seed

Accessions should be stored indefinitely under conditions that ensure the lowest possible risk of genetic drift. Seeds should be kept in cold chambers at 5° C and 30 to 40% relative humidity. The temperature should be lowered to -20°C for long term storage. If the quantity or viability of seed is inadequate, the material must be increased, an operation that can involve the following problems:

1. Seed increase imposes a large demand on labor and facilities, which may be difficult for a single institution to meet.

2. Accessions may be lost as a result of poor adaptation, disease, insects, or natural disasters.

3. Mixtures may be introduced through error or contamination.

4. Selection may occur naturally through adaptation or artificially during pollination as pollinators try to choose the best plants.

5. It is important to take into account the seed sample used for sowing and that used for storage. As shown in Figure 1, sampling is used in various genetic resources activities and can be very helpful, as long as the population is as representative as possible.

6. Using a low number of plants in each regeneration leads to a high degree of gene loss and to changes in the original variability of the material through genetic drift. The effective size of a population is an important factor in germplasm maintenance. The degree to which a population maintains its genetic properties depends on the number of seeds or individuals in a population, N, and primarily on the number of individuals that were intercrossed in previous generations, which reflects the effective number Ne.

7. Changes in genetic composition may be brought about by mutation. Although the occurrence of mutations is fairly low, such changes can take place in some geographical zones.

Listed below are some suggestions for increasing seed:

1. Use an appropriate sampling method to select seed for sowing and for cold storage. In seed increase one must bear in mind the number of seeds for sowing, the number of plants to be pollinated, and the number of plants harvested to obtain a balanced mix of seeds and a suitable amount for storage. In general, 300-400 plants should be planted and some 200 pollinated. At harvest take the same quantity of seeds from each pollinated ear to form a balanced sample.

2. Choose an environment for increasing seed that corresponds to the site at which the collection was obtained. This practice lessens the possibility of natural or artificial selection and increases the amount of seed produced.

3. Use an appropriate pollination technique that avoids contamination and selection.

4. Increase a sizeable amount of seed and if possible increase only once. If seed is increased several times, avoid using only a small number of plants in each regeneration; make sure an appropriate, constant number of plants is used in each succeeding regeneration.

5. At sowing, group accessions according to race, maturity, or some other characteristic for more efficient pollination, harvesting, and preliminary evaluation. The evaluation might be aimed at grouping materials by race, determining similarities among materials, or studying traits that might be worth improving. One can take fuller advantage of the seed increase cycle by keeping records on the characteristics that may be of use in selecting materials for improvement.

6. After similarities among materials have been established, they have been grouped according to race, or the most suitable accessions have been identified, racial composites and/or elite materials can be formed.

Evaluation

Evaluations provide information on the various characteristics of bank accessions and help scientists select material for use in research programs. It is important to remember that since the material has never been improved, it will not be easy to obtain from it a product that is comparable to those already on the market, such as improved varieties, synthetics, and hybrids. Any effort to use genetic resources must be undertaken with the understanding that obtaining a final product will inevitably be a long term process.

Though highly important, the task of evaluating accessions is not simple and cannot be accomplished single-handed by one country, international center, or private party. The complexity and scope of this work help explain why to date it has not been carried out systematically. Proper evaluation must be achieved through joint, coordinated action by all groups concerned with plant genetic resources. In view of the need for and importance of such efforts, Pioneer Hi-Bred International decided to finance the evaluation of maize germplasm. The Agricultural Research Service of the USDA is implementing the Latin American Maize Project (LAMP) with these funds under the direction of Quentin Jones. The project has reached its second stage, and over 14,000 accessions are being evaluated in cooperation with 11 countries: Bolivia, Brazil, Chile, Colombia, Guatemala, Mexico, Paraguay, Peru, Uruguay, the USA, and Venezuela. Those countries and the personnel involved in this project are to be congratulated for their dedicated efforts, which will no doubt lead to increased use of local and foreign genetic resources in maize improvement programs and eventually to increases in the productivity of the crop.

Described below is the evaluation plan being followed by LAMP.

Stage 1--To avoid confounding environmental factors and maturity differences among accessions, it was necessary to group the materials, first according to elevation--high (greater than 2,500 m above sea level, masl), intermediate (2,000-2,500 masl), low (less than 2,000

masl), and jungle--and, second, according to maturity and plant height. Those groupings make it possible to choose accessions for an experiment that show similar adaptation and crop development and thus to control experimental error more effectively. Various experiments can then be carried out in a single region. To ensure that evaluation sites were representative of those regions, care was taken to plant accessions in environments similar to those where they were collected.

Experiments were carried out in each region at a single site in one-row plots with two replications. It is necessary to have data on the environment of the evaluation sites, so one or two checks were planted for comparison or to make adjustments according to soil variability or environmental conditions. Data on the accessions evaluated are collected on sheets designed to facilitate entry of data in the computer. The characteristics included are:

Number of seedlings
Days to male and female flowering*
Plant and ear height (cm)
Number of plants
Number of broken and lodged plants
Number of tillers
Number of ears
Ear appearance
Yield
Moisture content of the grain
Grain type
Grain color
Race and shelling percentage

*If facilities exist for obtaining maximum and minimum temperatures, it is preferable to express this characteristic in terms of growing degree units utilized by the plant.

Those are the basic characteristics that should be recorded, but others could be included if necessary. For example, if plants show disease symptoms, it would be a good opportunity to record their level of resistance.

Once the data have been entered in the computer, any conversions should be made, and the information should be organized as follows:

Country
Region
Evaluation site
Stage
Plot
Entry number
Repetition
Pedigree/ accession
Race
% of no. of seedlings
No. of days to male flowering
No. of days to female flowering
Plant height
Ear height
No. of plants
% of broken plants
% of lodged plants
Degree of tillering
No. of ears per plant
Ear appearance
Yield (kg/ha)
Moisture content of grain
Grain type
Grain color

On the basis of selection criteria chosen by the evaluator, 20% of the accessions are selected.

Stage 2--The selected accessions, grouped on the basis of maturity, are sown at two sites, with two replications in each region. The same checks used in the first stage are sown every 20 rows for the same purposes as those described above. The characteristics recorded are the same as those listed previously, but the evaluator should include any other important traits. Between 5 and 10% of the accessions are selected according to the selection criteria established by the evaluator.

During this stage it is possible to carry out selection for special traits in separate experiments; these could include evaluations of reaction to diseases, insects, aluminum toxicity, or other stresses that researchers consider important for their countries. For that purpose separate experiments should be carried out at sites where the stress under study is known to exist; if that is not possible, the stress will have to be created artificially, a more expensive approach. It is advisable that stress reactions be studied by interdisciplinary groups of specialists.

Twenty percent of the accessions are sown at locations where the stress under study occurs naturally. Each of the accessions, grouped according to maturity, are sown in a row, with two replications. Susceptible, intermediate, and tolerant checks are sown every 20 rows. In the case of an evaluation for disease reaction, that approach achieves good distribution of the pathogen (by means of the susceptible check) and enables one to determine disease incidence. Where possible, data on flowering are expressed in growing degree units required for flowering. Stress resistance or tolerance is rated on a scale of 1 to 9 (1 = bad and 9 = very good). Resistant accessions and those showing intermediate resistance (rated greater than 5) are selected.

Stage 3--Germplasm exchanges will be initiated during this stage, so it is extremely important to have sufficient seed of selected accessions for use in this and the following stage. Selected material will be sent to the countries that have suitable environments for evaluation. The material does not have to be well adapted. What is more important is that it perform satisfactorily without being closely adapted to the evaluation site. Adaptation will be achieved later when the material is crossed with local germplasm.

Selected local and foreign accessions will be grouped according to maturity and sown in the appropriate region at two sites with two replications. The checks used in the previous stages should be planted every 20 rows, and the same characteristics should be monitored. In addition, crossing of accessions should be initiated in an isolated field; plants used as females should be detasseled, and a tester that is adapted to the region should be chosen to serve as the male.

The main reasons for crossing accessions with a tester are as follows:

1. The yield of accessions per se indicates the quantity of favorable genes they contain, but cross evaluation indicates the contribution of new favorable genes that are not contained in the tester.

2. Nonadditive gene action can be important.

3. Crossing gives foreign accessions the adaptation that is necessary for comparison.

4. Crossing may help identify new sources of heterosis.

Selecting a good tester is thus of basic importance. A good tester is one that provides a reasonably correct range of performance in the accessions, permits accurate discrimination among the genotypes under study, and shows minimum interaction with environment. In addition, one should bear in mind certain practical considerations in choosing a tester; it should combine well with the material being used in improvement programs, so that the material can subsequently be incorporated into breeding efforts without much difficulty.

A tester can be a variety, composite, synthetic, double cross, triple cross, single cross, or inbred line. The first three show fairly high levels of genetic variability, which make it harder to discriminate among progeny of the accessions and to ascertain the real genetic contribution of the accessions. The ideal tester would be genetically uniform, as is an inbred, but this would imply an overly specific cross that would be too subject to variation caused by the environment. It would therefore be more appropriate to choose a material that is intermediate in variability, such as a double-cross hybrid involving two single crosses that are of different origin and combine well. Good results from a cross between an accession and a double-cross hybrid would indicate that the accession crosses well with either single cross, showing a different heterotic source, or that the accession crosses more effectively with one of the single crosses. One can determine which of those possibilities is correct by crossing the accessions selected with the two single crosses.

During stage 3 separate experiments on resistance to disease, insects, aluminum toxicity, and so forth should be continued. Resistant accessions and those showing intermediate resistance should be sown in one-row plots, with two replications, at two sites. Data should be collected on flowering, degree of resistance, and any other traits of interest. Tolerant accessions can be identified by analyzing combined data from the second and third stages.

Stage 4--During this stage two yield trials will be planted in each region, in which materials will be grouped according to maturity. One trial will include the accessions, testers, and local and foreign checks. Local and foreign hybrids that will serve as a basis for assessing the performance of entries in the different trials will also be included in this experiment. Another trial will be planted that contains crosses between the accessions, the testers from each country, and local and foreign hybrids. Trials in each region will be planted at three sites, with two replications and two rows per plot. The characteristics to be recorded are the same as those in previous stages. Combined analysis across sites over time will be carried out by region and by country and across countries growing the same trial. The material to be used in improvement programs will be selected after the results have been analyzed. Evaluation of special traits can continue during this stage. Tolerant local and foreign accessions and crosses between them should be included in a trial having two replications. Susceptible, intermediate, and resistant checks should be planted every 20 rows, and resistant accessions should be selected.

I would not like to give the impression that the approach I have outlined here is the only way to increase the use of germplasm bank accessions. The LAMP project is only a start toward that end, and various problems have to be resolved before it can produce results. For example, the commercial cultivars currently available are superior to the unimproved germplasm as a result of having undergone numerous cycles of selection. So, even if promising germplasm is detected, breeders may still be unwilling to use it. It is therefore necessary to undertake recurrent selection in accessions per se, in crosses, or in backcrosses to provide breeders with a better product for use in their programs.

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Table 1. Maize accessions in germplasm banks

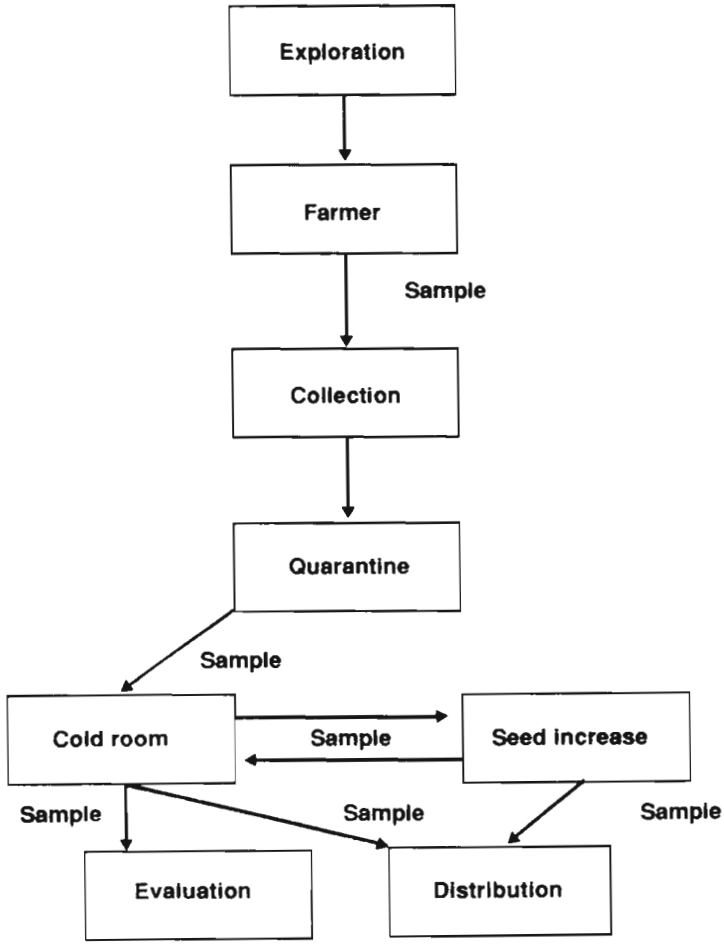
Number of accessions	Storage period	Institution	Site
15,084	Medium, long	VIR[a]	Leningrad, USSR
15,000	Medium	IMR	Belgrade, Yugoslavia
11,100	Short, medium	CIMMYT	El Batán, Mexico
10,000	Medium[b]	INIFAP	Mexico
7,619	Long	NSSL	Fort Collins, USA
7,145	Medium	UNA	Lima, Peru
5,000	Short	ICA	Medellín, Colombia
3,200	Short	RICTP	Fundulea, Rumania
3,000	Medium, long	INTA	Pergamino, Argentina
3,000	Medium	ISU	Ames, Iowa
2,800	Long	PGRO	Ottawa, Canada
2,654	Medium, long	NIAS[a]	Tsukuba, Japan
2,220	Medium	CIFEP	Cochabamba, Bolivia
1,678	Medium, long	IPB	Los Baños, Philippines
1,571	Short	IARI	New Delhi, India
1,500	Medium	NARS	Kitale, Kenya
1,368	Medium	CRIFC	Sukamandi, Indonesia
1,306	Medium	MRI	Trnava, Czechoslovakia
1,040	Medium, long	INIA	Madrid, Spain
1,000	Short	CNU	Daejeon, South Korea
1,000	Medium[b]	MI	Braga, Portugal

Source: Plucknett et al. 1987.
[a]Designated as base collection by IBPGR.
[b]Long term facility under construction.

Table 2. Distribution of catalogued maize races in the Western Hemisphere and Europe

Country	Number of maize races	Country	Number of maize races
Argentina	13	Mexico	37
Bolivia	32	Paraguay	7
Brazil	20	Peru	49
Chile	19	Uruguay	9
Central America	13	Venezuela	19
Colombia	23	Antilles	7
Cuba	7	USA	9
Ecuador	23	Europe	11
Total			298

Figure 1. Genetic resources activities.



User Oriented Bank Management

Suketoshi Taba, Maize Germplasm Bank, CIMMYT

Abstract

The concept of user oriented bank management is being developed and implemented in the CIMMYT maize germplasm bank. Central to this concept is a computer system that contains data pertaining to each phase of the bank's work (including the compilation of passport data and regeneration, characterization, preliminary evaluation, and storage of bank accessions) and from which the bank manager and users can retrieve data fairly easily. Greater exchange of information and active collection of germplasm by bank managers and users should promote a sharing of responsibility for preservation of maize genetic resources by all national and international banks as well as breeding programs. That approach opens up new opportunities for bank managers to take part in maize improvement programs on a continuous basis, mainly by generating information that helps breeders make decisions about breeding materials.

CIMMYT's maize germplasm bank now preserves a large assembly of germplasm that includes 10,475 accessions in its active collection. Many of these materials have been regenerated through cooperative arrangements with other research institutions (in Peru and Ecuador, for example) and with Pioneer Hi-bred International, Inc. Collections that have not yet been regenerated will undergo this process in due course. Because of the size and importance of these resources, it is particularly important that CIMMYT now develop a more user oriented bank management system.

The first task of the bank, of course, is to secure seed samples of its collections through regeneration and storage. But its next responsibility is to compile passport information and additional data derived through regeneration and agronomic evaluations. Ready availability and effective dissemination of such information will to a large degree determine the extent to which the bank's holdings are used by researchers. Collegial relationships between the bank manager and users also promote greater use of the bank. The essential thing is to ensure a steady flow of information among all parties interested in conservation and use of maize germplasm. For that reason the work of the bank must extend beyond preservation of accessions to the generation of information about them.

The computer files currently being developed at CIMMYT include passport information as well as data on regeneration and storage of the seed available and on its agronomic performance as judged from preliminary evaluations. That information will give users a more precise means of choosing and requesting materials from the bank. Most often, the database will suggest a group of collections that a user can evaluate with a certain objective in mind. The key to successful use of the database is for its manager and the requester to infer correctly which accessions are probably most relevant for the task at hand. With the user oriented database that we have developed, the process of choosing bank materials consists of sequential phases, as shown in Figure 1.

This paper describes the current status of the various information files that make up the database (for further information on data management, see Konopka, J., and J. Hanson, eds. 1985. *Documentation of Genetic Resources: Information Handling for Gene Bank Management.* Rome: IBPGR and Nordic Gene Bank) and describes the role we envision for germplasm banks,

preservation of the CIMMYT bank's base and active collections, and international collaboration in the regeneration of accessions.

Passport Data

During the 1970s an effort was made at CIMMYT to compile the bank's passport information. Fresh seed was stored in the newly constructed concrete chamber, with the temperature set at 0°C. An orderly system for storing the accessions was developed by Mario Gutiérrez G. The active collection currently maintained in our storage facility was initiated in 1972. Efforts to document the accessions were coordinated by the Genetic Resources Communication, Information, and Documentation Systems (GR/CIDS) Project of the University of Colorado, USA. The first passport catalog was developed in the course of that work. More recently we have been urged to publish a new catalog of passport data to meet the needs of all bank users outside CIMMYT. (See the passport data sheet in Appendix 1.) We have responded by developing the current catalog, which includes all of the information taken by the collectors of the accessions that we currently hold. That is supplemented by information on topography and maize culture at the collection site.

One of the most important descriptors in the catalog is racial classification, which we have established from various sets of bank field books. In some cases we have treated commonly recognized names as race names. Our racial classifications are based on the most reliable information that we could glean from bank records, but they are subject to modification depending on the results of future studies.

As indicated in Tables 1-3 and Figure 2, the passport database is nearly complete. As more information becomes available in the future, it will be incorporated into the database. We have fairly complete passport data on the accessions from Mexico and Central America but somewhat less information on those from South America and other parts of the world. All of the information compiled so far will be available soon on CD-ROM.

Some of the main features of the passport file are that 1) it includes all of the passport information on CIMMYT accessions that is available in the banks at CIMMYT and Mexico's National Institute of Forestry, Agriculture, and Livestock Research (INIFAP), 2) variation in grain color and type is coded to indicate variability within an accession, 3) the file allows users to conduct searches in the database using single or multiple criteria, (4) it can include the associated accession numbers of other banks, and 5) it has an open-ended file structure, allowing future addition of information.

Characterization

Characterization is usually performed at the time of regeneration or seed increase. In the past data taken by the CIMMYT bank manager were not always standardized, so the data compiled so far are not consistent. To avoid that problem in the future, we have made a regeneration data sheet that will be printed by the bank's computerized management system. A new introduction will first be observed to determine the feasibility of its becoming an accession. If that seems possible, it will be increased and regenerated. At the same time, a racial characterization of the accession will be entered on the regeneration data sheet.

As shown in Appendix 2, a regeneration data sheet includes qualitative characters, some passport information, and characters for which one can expect genotype x environment

interaction. Some passport information indicated on the data sheet will allow a bank manager to identify an accession in the field. Data on qualitative characters (such as grain color and texture and plant color), on characteristics of the tassel, and on plant and ear morphology will enable the bank manager to make sure that the right accession is being regenerated and that it has not become contaminated. The data sheet also includes information to be taken before seed storage. The complete set of data will be recorded in the regeneration data file in the computer.

New regeneration data collected for an accession will replace the previous regeneration data in the same file. Information from the computer data file is in accordance with the seed available from the bank. Past regeneration records for each accession are being filed in the bank and will be used as a bank management tool. If the bank wants to evaluate accessions kept over the long term, the individual records of an accession that are filed over time must be available to future bank managers. Regeneration data on an accession should be used for that purpose. Criteria for discarding accessions (duplicates, for example) would be developed based on the records.

Data currently missing from the regeneration file can be supplied before the next regeneration. If some accessions are to be planted for evaluation, the characterization data of the bank descriptors can be taken when the evaluation is done and recorded in the file. In conjunction with the bank's preliminary evaluation, field weight per regeneration plot and overall agronomic ratings can be taken at regeneration. The information available in the data file is somewhat limited at the moment. For example, we have data on stalk lodging for only 14.93% of the current bank accessions, root lodging 27.5%, and plant height 23.96 %, compared to 72.73% for ear height. From the regeneration file, data can be retrieved using single and multiple search criteria to meet users' requests.

Evaluation

A computerized information management system makes it possible for germplasm bank managers to devote more effort to active evaluation of germplasm bank accessions, in addition to the more routine activities of preservation and curatorship. In CIMMYT's maize germplasm bank, agronomic ratings on accessions are taken at the time of regeneration. Regenerated accessions with high ratings are placed in preliminary evaluation trials, in which accessions are grouped by maturity and other criteria. Constant checks, including both collections and improved varieties, are included in the trials. The primary objective of the evaluations is to generate agronomic data that provide the bank manager and users with a stronger basis on which to choose accessions that meet bank users' needs. The preliminary evaluation following each regeneration will be repeated over time. The data collected will be stored in an evaluation data file, which will be integrated with other bank files and made available to the bank manager and users. Further evaluations can be done as needed to provide more genetic and agronomic information about the accessions. A few bank evaluation trials were conducted at CIMMYT in 1974, 1984, and 1986 (CIMMYT 1975, 1985; Salhuana, these proceedings).

Choice of Breeding Materials and National Germplasm Banks

In conjunction with evaluation, breeders' success depends, to a large extent, on the source materials they use. That is why the exchange and evaluation of breeding materials among breeders is so common. The genetic diversity needed by breeders can often be found in their own breeding stocks, which to some extent are improved populations derived from original collections. Breeders' first choice is for source materials that have been enhanced through past

breeding efforts. However, when sufficient diversity is not present, then it becomes crucial to obtain information on potential source materials in a germplasm bank.

Breeders' inventories, which include information on the performance of foundation stocks for breeding, are similar to the inventory of a germplasm bank. In managing the inventory and preserving seed for a breeding program, breeders are performing essentially the same functions as those of a germplasm bank. The difference is that the germplasm bank manager performs those functions over a longer period of time with collections and obsolete, elite populations. Nevertheless, both contribute toward the same end. By assembling potentially useful germplasm, breeders and germplasm bank managers ensure that the national crop improvement program has a sound basis on which to choose breeding materials. The work of germplasm preservation and evaluation is thus an important part of crop development research.

In order to assemble potentially useful germplasm in its active collection, a national germplasm bank must have free access to the materials in other germplasm banks. Ready availability of the germplasm promotes the preservation of active collections in all banks and a sharing of the responsibility for preserving the genetic diversity of maize by various national and regional banks and breeding programs. During 1986-87 the CIMMYT maize germplasm bank began to encourage this activity by providing national collections to Guatemala, Paraguay, and Uruguay for evaluation as part of the Latin American Maize Project (LAMP). In 1986 we also offered CIMMYT accessions from each of the Latin American countries to their national breeding program or germplasm bank.

Seed Storage and Preservation

From 1972 to 1984, the CIMMYT maize germplasm bank had two cold storage rooms that were maintained at 0° to 3°C. In 1985 one of the cold rooms was designated for storage of a base collection containing seed of the same materials included in CIMMYT's active collection. After rearrangement of storage cans and racks, storage of the base collection at -15°C was started on January 20, 1988. One-gallon tin cans (holding 3.5 kg of seed) are used to store the active collection and half-gallon cans (containing 1.5-2 kg of seed) to maintain the base collection. Seed moisture content in storage is kept in the range of 10-12%. Care is taken to store accessions of floury grain types at a higher moisture content in the active collection. Accessions for which we have large seed samples are stored in the base collection for future studies. Seed viability, quantity, and shipment of individual accessions will be monitored using the data management system.

Users' seed requests are filled from the active collection. The base collection serves as a back-up for replenishing the active collection as needed. The base collection also includes regeneration seed packets (six for each accession) in which a balanced number of kernels from each regenerated ear is bulked. The balanced bulk is placed in the laminated aluminum foil bags after the seed has been dried to a moisture content of less than 8%, depending on the seed type. Newly regenerated and increased seed are stored in both the active and base collections. At each regeneration 100 ears are considered sufficient to represent the genetic diversity contained in the accession.

As I suggested earlier, preservation of active collections must be undertaken by numerous groups, including both maize germplasm banks and concerned breeding programs worldwide, in order to safeguard the existing maize genetic resources. We strongly encourage exchange of the

accessions among maize germplasm banks and preservation by each bank of those materials judged to be best.

International Cooperation in the Regeneration of Collections

The regeneration of collections held by maize germplasm banks requires large amounts of labor, land, and other resources but is an extremely worthwhile investment. During the late 1960s, Brazilian collections were saved in large part by regeneration of these materials at CIMMYT. We have not been so successful, however, in regenerating other collections, specifically those made during the 1950s in the Andean region and in the mid- to high altitude regions of Guatemala and Costa Rica. Neverthless, we continue to regenerate as many materials as our resources permit, as indicated in Table 4, which gives the numbers of accessions and collections to be regenerated in the near future. Regeneration of some of those early collections will require international cooperative arrangements with national programs and banks. We have been in contact with some of those groups and hope to recover collections that are still viable. We are quite optimistic about this work since in the past CIMMYT has received invaluable assistance in regenerating collections, mainly from programs in Ecuador and Peru and from Pioneer Hi-bred International, Inc.

Generally, the amount of seed produced through regeneration surpasses a bank's capacity to store and use it in the foreseeable future. Rather than discard extra seed, cooperating banks could exchange it and thus reduce their costs and improve the management of regeneration. Standards for regeneration will be discussed during the workshop with that possibility in mind.

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_____. 1985. *CIMMYT Research Highlights.* Mexico, D.F.

Table 1. Summary of bank accessions' racial classification and of collection site data

Origin of accessions	Number of accessions with: Primary race	Primary and secondary races	No race classification	Data on location and altitude
Mexico	1,579	1,469	456	3,261
Central America and Caribbean	801	609	450	1,682
South America and others	2,784	297	2,071	3,031
Total	5,164	2,335	2,976	7,974
As % of total accessions	49.30	22.29	28.41	76.12

Table 2. Summary of bank accessions' grain texture

Origin of accessions	Number of accessions with: Dent	Flint	Floury	Sugary
Mexico	2,994	235	172	63
Central America and Caribbean	1,139	653	33	0
South America and others	2,443	1,920	748	11
Total	6,576	2,808	953	74
As % of total accessions	62.78	26.81	9.10	0.70

Table 3. Summary of bank accessions' primary grain color

Origin of accessions	Number of accessions with: Yellow	White	Orange yellow	Blue	Other
Mexico	654	2,379	7	219	205
Central America and Caribbean	869	738	68	38	147
South America and others	3,163	1,394	302	37	255
Total	4,686	4,511	377	294	607
As % of total accessions	44.47	43.07	3.60	2.80	5.79

Table 4. Accessions and collections due for regeneration at CIMMYT in 1987

Country of origin	Number of accessions	Number of collections
Argentina	1	4
Brazil	-	217
Bolivia	2	86
Cuba	2	-
Colombia	15	82
Costa Rica	53	1
Chile	2	18
China	7	-
Guatemala	87	338
Honduras	-	1
Mali	1	-
Mexico	16	12
Morocco	-	93
Nepal	-	76
Paraguay	1	2
Panama	2	-
Peru	2	1,064
Uruguay	2	122
Venezuela	6	325

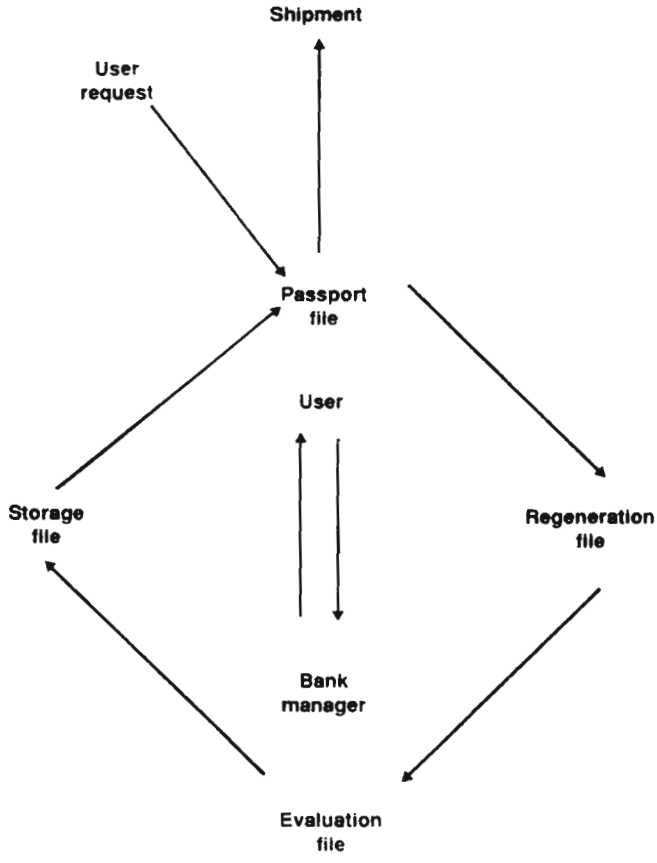

Figure 1. How seed requests are met by the CIMMYT maize germplasm bank.

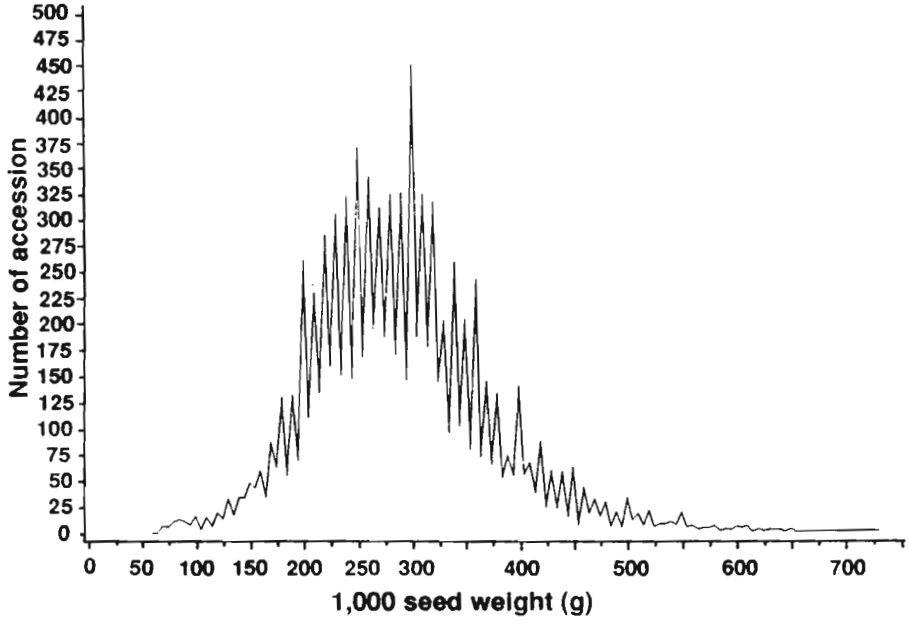

Figure 2. 1,000 seed weight of bank accessions.

Appendix 1: Passport Data Sheet

Maize Germplasm Bank System

Passport Form Id. Number _____

Accession Number _____ Status _____

Name _____

Donor Inst. _____ Donor's Id _____ Country _____

Races

I _____
II _____

Associated Numbers

PI	NRC	CIMMYT	PERU	BRAZIL	COL

Pedigree _____

Pop Name _____ _____

Type ___ ___ ___

Color ___ ___ ___

Popul. Type _____
Acquisition _____
Intro. Date _____

Location

Prov _____
Site _____

Coordinates

Latitude _____ __
Longitude _____ __

Altitudes

C _____ P _____
M _____

Observations _____

Collection

Local Name _____ Inst. _____
Coll Number _____ _____

Collector _____ Coll Date _____ Frost _____ _____
Coll Gr. _____ Coll Ears _____ Grow __ __ __ __

Matur ___ Vigor ___ Topo ___ Temp ___ Rain ___
Hail ___ Wind ___ Fert ___ Moist ___ Cult ___
Pred ___ Sources _____

Codes for grain types:

1 floury
2 dent
3 flint
4 popcorn
5 sugary
6 tunicated
7 morocho
8 soft crown
9 opaque

Codes for grain colors:

a) yellow
b) white
c) red
d) purple
e) variegated
f) sun red
g) dark blue
h) brown
i) yellow orange
j) mottled
k) blue
l) white cap (yellow endosperm)

Codes for climate, culture, and growing conditions:

Maturity: 1 early, 2 late, 3 very late
Plant vigor: 1 robust, 2 intermediate, 3 weak
Growing period: month-month
Topography: 1 slope, 2 plain, 3 valley, 4 fertile plain
Temperature: 1 cold, 2 temperate, 3 hot
Rain: 1 abundant, 2 regular, 3 scarce
Hail: 1 frequent, 2 regular, 3 none
Wind: 1 strong, 2 moderate, 3 calm
Frost: month-month
Soil fertility: 1 fertile, 2 regular, 3 poor
Soil moisture: 1 abundant, 2 regular, 3 poor
Culture: 1 irrigated, 2 supplemental, 3 nonirrigated
Predominance: 1 large, 2 medium, 3 small, 4 rare

Appendix 2: Regeneration Data Sheet

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" CIMMYT Maize Germplasm Bank                                                    Page |_|_|_|_|_|
  Regeneration Data "

                                                          |                                    R R R R R R P  R = Race and
Identification number ........ |_|_|_|_|_|_|              | Primary race classification .. |_|_|_|_|_|_|_| P=Purity (5-9).
Coll. Num./Pop. Num. ......... |_|_|_|_|_|_| |_|_|_|_|_|_| | Secondary race classification  |_|_|_|_|_|_|_|
Field book number ............ |_|_|_|_|                  | Adaptation zone .............. |_|
Collaborator ................. |_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_| |  (1) Tropical lowland,
Location ..................... |_|_|_|_|_|_|_|_|_|_|       |  (2) T_intermediate,
Year ......................... |_|_|_|_|                  |  (3) T_highland,
                                L L # # L   # # # #   # # # |  (4) Subtropical,
Plot number .................. |_|_|_|_|_|-|_|_|_|_|-|_|_|_| |  (5) Temperate,
Number of regenerations ...... |_|_|                      |  (6) Specific site:__________
Number of increases .......... |_|_|                      | Agronomic scale .............. |_|  Rating 1 - 5:
Density ...................... |_|_|_|_|_|                |                                      (1) Good,        (2) Poor.
N P K ........................ |_|_|_| |_|_|_| |_|_|_|    | 1000 kernel weight (gr) ...... |_|_|_|_|
Planting date/% Field germin.. |_|_| |_|_| |_|_|_|  DD MM %%% | Storage adress ............. |B|_|_|_|_|_|_|_|_|
Number of plants/plot ........ |_|_|_|                    | Storage adress ............... |C|_|_|_|_|_|_|_|_|
Seedling vigour .............. |_| Rating 1 - 5: (1) Good, (5) Poor| Available seed (gr) in bank .. |A|_|_|_|_|
Tillering/plant .............. |_|.|_|                    | Available seed (gr) in bank .. |B|_|_|_|_|
Leafiness .................... |_| Rating 1 - 5: (1) Leafy, | Available seed (gr) in bank .. |C|_|_|_|_|
Days to anthesis (50%) ....... |_|_|_|       (5) Less leafy. |                                 D D M M Y Y Y Y   % % %
G. D. U. anthesis ............ |_|_|_|_|                  | Storage date/% Initial germin. |_|_|_|_|_|_|_|_|-|_|_|_|
Days to silking (50%) ........ |_|_|_|                    | Germination (%)/Date ......... |_|_|_|_|_|_|_|_|_|_|_| %%% MMYYYY
G. D. U. silking ............. |_|_|_|_|                  | Kernel moisture (%)/Date ..... |_|_| |_|_|_|_|_|_|_| %% MMYYYY
Number of plants pollinated .. |_|_|_|                    | Origin for multiplication .... |_| |_|_|_|_|  Pack_(1-6) YYYY
Pollination type ............. |_| 1-Chain, 2-Bulk, 3-Self, 4-Pair | Field book status ............ |_|
Effective population.......... |_|_|_|                    |          (1) Approved,        (2) Repeat,      (3) Rejected
Plant height ................. |_|_|_|                    | Previous multiplication site . |_|_|_|_|_|_|_|_|_|_|_|
Ear height ................... |_|_|_|                    | Previous multiplication plot . |_|_|_|_|_|-|_|_|_|_|-|_|_|_|
Number of leaves above ear ... |_|_|                      | Previous multiplication
Leaf senescense .............. |_|_| No. of days after pollination | planting date ................ |_|_| |_|_|  DD MM
Plants stalk lodged .......... |_|_|_|  %%%               | Previous multiplication year . |_|_|_|_|
Plants root lodged ........... |_|_|_|  %%%               +-----------------------------------------------------------
Root development (volume) .... |_|              Rating 1 - 5:          (1) Large,              (5) Small.
Tassel type .................. |_|              (1) Primary,           (2) Primary_secondary,  (3) Primary_secondary_tertiary.
Sheath pubescense ............ |_|              (1) Exist,             (2) Segregating,        (3) Non exist.
Plant Color (stalk) .......... |_| |_| |_|      [1] Green,             [2] Dilute sun red,     [3] Sun red,
                                                [4] Purple,            [5] Dark purple.
Leaf desease ................. |_|              (A) H. maydis,         (B) H. turcicum,        (C) P. sorghi,
                                                (D) P. polysora,       (E) P. maydis,          (F) Other.
Husk cover ................... |_|              Rating 1 - 5:          (1) Good,               (5) Bad.
Harvest date ................. |_|_| |_|_|  DD MM
Field weight ................. |_|_|.|_|   Kg.
Number of ears represented ... |_|_|_|
Ear rot ...................... |_|              Rating 1 - 5:          (1) Clean,              (5) Rotten.
Easiness of kernel shelling .. |_|              Rating 1 - 5:          (1) Easy,               (5) Difficult.
Glume size ................... |_|              Rating 1 - 5:          (1) Large,              (5) Small.
Kernel type .................. |_| |_| |_|      [1] Floury,            [2] Dent,               [3] Flint,
                                                [4] Pop,               [5] Sweet,              [6] Tunicate,
                                                [7] Morocho,           [8] Soft crown,         [9] Opaque.
Kernel color ................. |_| |_| |_|      (A) Yellow,            (B) White,    (C) Red,      (D) Purple
                                                (E) Variegated,        (F) Sun red,  (G) Dark blue, (H) Brown
                                                (I) Orange yellow,     (J) Mottled,  (K) Blue,      (L) White cap (yellow endosperm)
Kernel row number ............ |_|              (1) Less than or equal to 10,                  (2) Between 10 and 16,
                                                (3) Between 16 and 22,                         (4) Greater than 22.
Kernel row type .............. |_|              (1) Regular,      (2) Irregular,      (3) Straight,      (4) Spiral.

Observations ................. |_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|_|

          () Seedling vigour,          () Root system,               () Stalk strength/quality,     () LEAF/angle/number/size,
          () EAR/size/length/shape,    () GRAIN/texture/type/size,   () FOLIAR/disease/insect,      () EAR/disease/insect,
          () Plant morphology,         () Prolificacy,               () Dought tolerance,           () Cold tolerance,
          () Yield,                    () Combining ability,         () Adaptation,                 () Tassel size/shape,
          () Glume texture,            () Ear quality,               () Average kernel row number.
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Long-Term Storage of Plant Germplasm

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Abstract

The National Seed Storage Laboratory (NSSL) is responsible for long term preservation of valuable plant germplasm as the base collection of the National Plant Germplasm System (NPGS). NSSL seed stocks include plant introductions, recently released and obsolete cultivars, open-pollinated cultivars, parental lines, and genetic stocks. In addition, NSSL supports its conservation activities with long range research on biochemical, physiological, and genetic changes in seed during storage and the effects of seed moisture content, storage environment, and containers on seed longevity. Seed from NPGS is available to researchers worldwide.

The National Seed Storage Laboratory (NSSL), a joint facility of the US Department of Agriculture and the Agriculture Research Service located on the campus of Colorado State University at Fort Collins, has been in operation since 1958. NSSL is responsible for maintaining plant germplasm as a base collection of seeds for the USA and for the global network of genetic resource centers coordinated by IBPGR. NSSL is part of the National Plant Germplasm System (NPGS), which coordinates the efforts of federal, state, and private sector research units to collect, maintain, and preserve plant germplasm for agricultural and industrial use.

NPGS working collections are divided among four Regional Plant Introduction Stations (RPISs) at Pullman, Washington; Geneva, New York; Experiment, Georgia; and Ames, Iowa. The maize working collection is at Ames. Samples of each accession are kept in both an RPIS working collection and the NSSL base collection. Seed of any NPGS accession is available to researchers worldwide from the working collections. International germplasm exchange is coordinated through the Plant Introduction Office, USDA/ARS/NER, Germplasm Services Laboratory, Room 322, Building 001, BARC-West, Beltsville, MD 20705. Scientists also have direct computer access to information on NPGS accessions through the Germplasm Resources Information Network (GRIN), and printed information on maize can be obtained from the Director, North Central Regional Plant Introduction Station, Ames, IA 5001l.

The principal mission of NSSL is long term preservation of valuable plant germplasm. NSSL aims to store good quality seed and to maintain viability above 85% for as long as possible. Thus, only clean seed with a high germination rate is suitable, although lower viability material may be held tentatively until the donor or NPGS can provide acceptable replacement seed. Seed accepted for storage is handled under four general categories: 1) the base collection, 2) quarantined germplasm samples, 3) genetic stocks and special collections, and 4) plant variety protection voucher samples. Present categories of seed stocks include plant introductions, recently released and obsolete cultivars, open-pollinated cultivars, parental lines, genetic stocks, and virus indicator stocks.

NSSL also supports its mission with long range research on the biochemical, physiological, and genetic changes in seed during storage and the effects of seed moisture content, storage environment, and containers on seed longevity. For each crop there are special laboratory procedures to monitor seed viability during storage. NSSL is also responsible for basic research on the cryopreservation of clonal germplasm--including pollen, buds, apical meristems, and plant

and cell tissue cultures--in order to develop the technology necessary for establishing a clonal germplasm base collection at NSSL.

The Crop Science Society of America requires that samples of all cultivars, germplasm, and genetic stocks registered in *Crop Science* be sent to NSSL. In order to have enough seed for both the working and base collections, NSSL requests 5,000 seeds in the case of parental inbreds and self-pollinated accessions, and 7,500 seeds of cross-pollinated accessions. Donors are asked to contribute 3,000-4,000 seeds for cross-pollinated species and collections involving a mixture of genotypes, and 1,500-3,000 seeds for pure-line accessions. Moisture content should be as low as feasible within the 6-12% range. Germination of 95-100% (85% minimum), together with a high level of purity, is required for the pure seed component. Accessions not meeting these standards are considered on an individual basis. Donors are asked to provide passport and supplemental information. NSSL measures and records percent purity, average weight per 100 seeds, total seed number, seed moisture content and germination percent of pure seed. Seed is dried to 6% ($\pm$ 1%), and each accession is sealed in a moisture proof container and placed in storage. One of the small seed storage vaults has been converted to a low temperature seed dryer equipped with silica-gel dehumidifiers. Maize seed dried there has gone from 7.5% to 6% moisture in three weeks, and wheat seed from 7.9% to 5.8% in two weeks, at 5°C. All verified accession data are added to GRIN.

Seed is stored in vaults cooled to -18°C or in cryotanks with liquid nitrogen at -196°C. Seed viability and moisture content are monitored every 5 or 10 years, depending on species longevity. When viability drops below standards, the accessions are regrown at an RPIS. Accessions may also be increased at an RPIS when donated samples do not consist of the required number of seeds upon intial acquistion.

Back-up material from international centers and other institutions is imported and stored but not distributed. Wheat from CIMMYT and rice from the International Rice Research Institute (IRRI) are stored under quarantine restrictions. Accessions intended for the base collection from countries with quarantine import restrictions are held in quarantine until seed increases can be made under quarantine grow-out protocols.

Certain genetic stocks--including genetic marker lines, aneuploid stocks, etc.--are also preserved because of their genetic characteristics or scientific value.

Under the Plant Variety Protection Act, a voucher sample of each cultivar submitted for protection must be sent to NSSL for storage. These samples are not available for distribution. There are 113 parental inbred lines of maize at NSSL, and that number is expected to increase over time.

With 232,000 accessions as well as back-up germplasm from CIMMYT and IRRI already in storage, NSSL seed vaults are expected to be filled within two years. Congress has appropriated funds to plan for the needed expansion, and the NSSL administrative budget request to Congress for the 1989 fiscal year includes a provision for construction. A National Academy of Science Board on Agriculture committee, chaired by T.T. Chang, was appointed to help develop specifications. Seed vaults with approximately four times the current capacity have been requested to provide conventional storage at -20° to -36°C, as well as cryogenic storage. These facilities will provide protection against natural disasters--such as tornados and floods--and vandalism. Expanded research facilities are also needed to develop techniques for the early detection of declining seed viability and the preservation of clonal germplasm plant tissue.

To ensure efficient use of present and future facilities, electrophoresis and HPLC technologies show promise for eliminating duplicate samples and evaluating genetic diversity among accessions of certain species.

Of the 17,000 maize accessions at NSSL, 11,000 are collections from Central and South America, 5,100 are open-pollinated or synthetic varieties, 300 are inbred lines from 13 universities and 17 seed companies, and 600 are genetic stocks. The average germination rate of a sample from 1,500 maize accessions placed in storage in 1965 was 93%. This had declined to 88% when tested in 1983. At that time 74% of the samples still exceeded 85% germination, and these had declined only 1% in viability from the original 95% germination rate. Seed vaults were maintained at 5°C and less than 40% relative humidity until 1978, when seed was transferred to moisture proof containers and vault temperatures lowered to -18°C.

Back-up maize accessions of approximately 1,500 kernels each from CIMMYT are entered in the base collection at NSSL (but not the RPISs). There are 5,800 accessions from 30 countries in this category. Major Goodman of North Carolina State University and Wilfredo Salhuana of Pioneer HiBred International are increasing non-NPGS race collections and supplying seed for both the working and base collections. In 1987, 1,074 maize accessions were added to NSSL inventories.

A Local Germplasm Bank Information Management System

Pedro M. Rosales, Data Processing Services, and Suketoshi Taba, Maize Germplasm Bank, CIMMYT

The purpose of this paper is to discuss the advantages of using a computerized information management system to assist in the operations of a germplasm bank. Storage of both seed and relevant information is a vital task of germplasm banks, and it is equally important that bank managers provide seed samples and the corresponding documentation to users. The information system developed for CIMMYT's maize germplasm bank is a local system for handling passport information, records on seed regeneration, agronomic data obtained from evaluation and characterization, and records on storage and utilization of the accessions.

The Information System Environment

The maize germplasm bank involves relationships among the various elements shown in Figure 1. The circle in the middle of the figure represents the information system, and the boxes represent related elements, which are as follows:

- Seed donors--Institutions or individuals that send materials to the bank
- Field book system--Computer system that provides field books needed by bank management
- Bank management--Persons responsible for the performance of the germplasm bank
- Bank users--Insitutions or individuals that are interested in receiving materials from the bank

The information system helps maintain the relationships among these elements and is designed to meet the requirements of management and users efficiently.

Data Management

The functions of the germplasm bank information management system are as follows:

- Passport data--Allows new material to be accessioned. Bank management decides about the final disposition of the new material, that is, whether to accept or reject it.
- Storage--Assigns a storage location in the active and base collections to new material that has been accepted by bank management.
- Regeneration--Handles information generated through characterization and preliminary evaluation of accessions and monitors seed viability in the storage chambers to identify possible candidates for regeneration.
- Evaluation--Allows bank management to retrieve information obtained through further evaluation of accessions, so that they can be compared according to traits of interest.

- Seed shipment--Provides information on the shipment of accessions to users requesting bank material.

- Field books--Produces field books needed for collecting data on bank material. Data collected in the field can be added to that already stored in the information system.

These data management activities are designed to fit the current requirements of bank management. Some features of the system, however, such as the number of storage chambers and the conditions that warrant seed regeneration, can be altered to meet future needs. The system is thus flexible and can readily be adjusted according to future developments in the germplasm bank.

Passport data--The steps by which new material is incorporated into the bank's holdings are shown in Figure 2. Bank management first adds information on new material to the passport data stored in the information system. If information about the incoming material is missing, the seed donor is notified. Upon creating its passport, the system assigns a unique number to the new material, which is employed in any further management operations. This number is independent of the accession number, which is not assigned until the material is finally accepted as a bank accession. Bank management next decide about the final disposition of the material, that is, whether it should undergo preliminary observation and regeneration or not be accessioned.

In the observation phase, the material undergoes a preliminary check to determine whether the bank will accept or reject it. If the material is to be maintained, it is scheduled for regeneration, the main purposes of which are to obtain the amount of seed and characterization data required in order for the material to be accessioned. As discussed below, field books are provided for data collection during observation and regeneration.

At the end of each maize growing cycle, the system updates previous information on the material according to data collected in the field. Depending on field results, the information system indicates that the new introductions are ready for the next phase or will remain at the current one. The system will also check materials for which observation or regeneration has been completed and indicate that they are ready to be accessioned.

Seed storage--New material completing the passport phase has to be stored and preserved. For that purpose each material accessioned is placed in the base and active collections. The storage process is closely related to the regeneration and seed shipment processes, as indicated in Figure 3. The information system identifies each material that has completed the passport phase and, using its identification number, assigns the material to storage locations, records the amount of seed stored in each, and indicates that the material is an accession available for shipment to users. The seed viability of the accession is monitored to anticipate the need for regeneration and seed increase. The information system also allows bank management to specify the amount of seed transferred from the base to the active collection, a procedure that is useful for updating inventories of seed in the two collections, and to determine the number of new accessions that have entered the bank over time. All of these capabilities contribute to more efficient use of scarce storage space.

Regeneration--To ensure that bank holdings remain in good condition, it is important to periodically check certain characteristics of the seed in storage. The information system will perform these checks and produce an audit report showing the accessions for which seed

amounts or viability are low enough to warrant seed increase or regeneration. The system will not schedule the material automatically for regeneration but will let bank management take the final decision as to which materials should enter the regeneration phase. When an accession is regenerated, data are collected and entered in a field book for storage in the information system (these steps are covered below). After the maize growing cycle, the information system updates the amounts of seed in storage, and accessions that have been increased or regenerated are once again designated as being available for shipment to users.

Seed distribution--Helping users decide which accessions to use and shipping these materials to users, along with the corresponding documentation, are key activities of bank management. The Maize Germplasm Bank Inquiry System, which is available on CD-ROM (compact disk, read-only memory), was developed to help users choose accessions. With this system they can actively search for potentially useful accessions on the basis of multiple criteria derived from the passport and regeneration data.

The information system helps bank management make various decisions about seed shipment. The system displays incoming requests, along with any stored data on seed availability, thus preventing shipment of any accession for which the quantity of seed is low. If a request can be met, passport and regeneration data are produced and included in the seed shipment. The system also produces a packing list that specifies the accession being sent, its location in the storage chambers, and the amount of seed of each accession being sent. One can also generate reports on the amounts of seed provided to particular institutions and countries.

Further evaluation--The main objective of this task is to provide bank management and users with information on the agronomic performance of accessions. A computer system is being developed that will facilitate management of information pertaining to field trials of bank materials. Future releases of the information system will be able to extract information from this trial system. Bank management will then have a better means of comparing materials, and the data will also be added to the documentation that is sent with seed shipments to users.

Field books--The information system provides field books for recording data on materials (during observation, regeneration, or evaluation in the field) as well as a means of entering the data in the system. The information system first inquires what kind of field book is needed. The current options are:

- Observation of introduction field book
- Regeneration of introduction field book
- Regeneration of accession field book
- Evaluation of accession field book

The information system allows bank management to enter the accessions to be included in the field book. It then checks the materials to determine whether they can be included in the field book that has been specified. For example, an introduction that is ready for observation cannot be included in a regeneration of accessions field book. The information system also allows for modifications in the list of accessions.

The field book contains certain items of passport data that help bank management identify accessions, and it includes various columns where data collected in the field can be entered. The field books requested can be reprinted later. Thus, the bank manager can prepare a set of field books, modify the list of entries as necessary, and then reprint the field book for the upcoming crop cycle.

Upon entry of data in the information system, it inquires what type of field book is to be entered. In adding the observation, regeneration, or evaluation data to information already stored, the system will check whether the accessions on which data are being entered are actually scheduled for that type of operation. The system also allows for correction of typing errors that occur during data entry.

For further information on documentation in germplasm bank management, see: Konopka, J., and J. Hanson, eds. 1985. *Documentation of Genetic Resources: Information Handling for Gene Bank Management.* Rome: IBPGR and Nordic Gene Bank.

Acknowledgments

The authors would like to thank Felipe Pineda, assistant in the maize germplasm bank, for his thorough testing of the maize germplasm information system during its development.

Figure 1. The Maize Germplasm Bank.

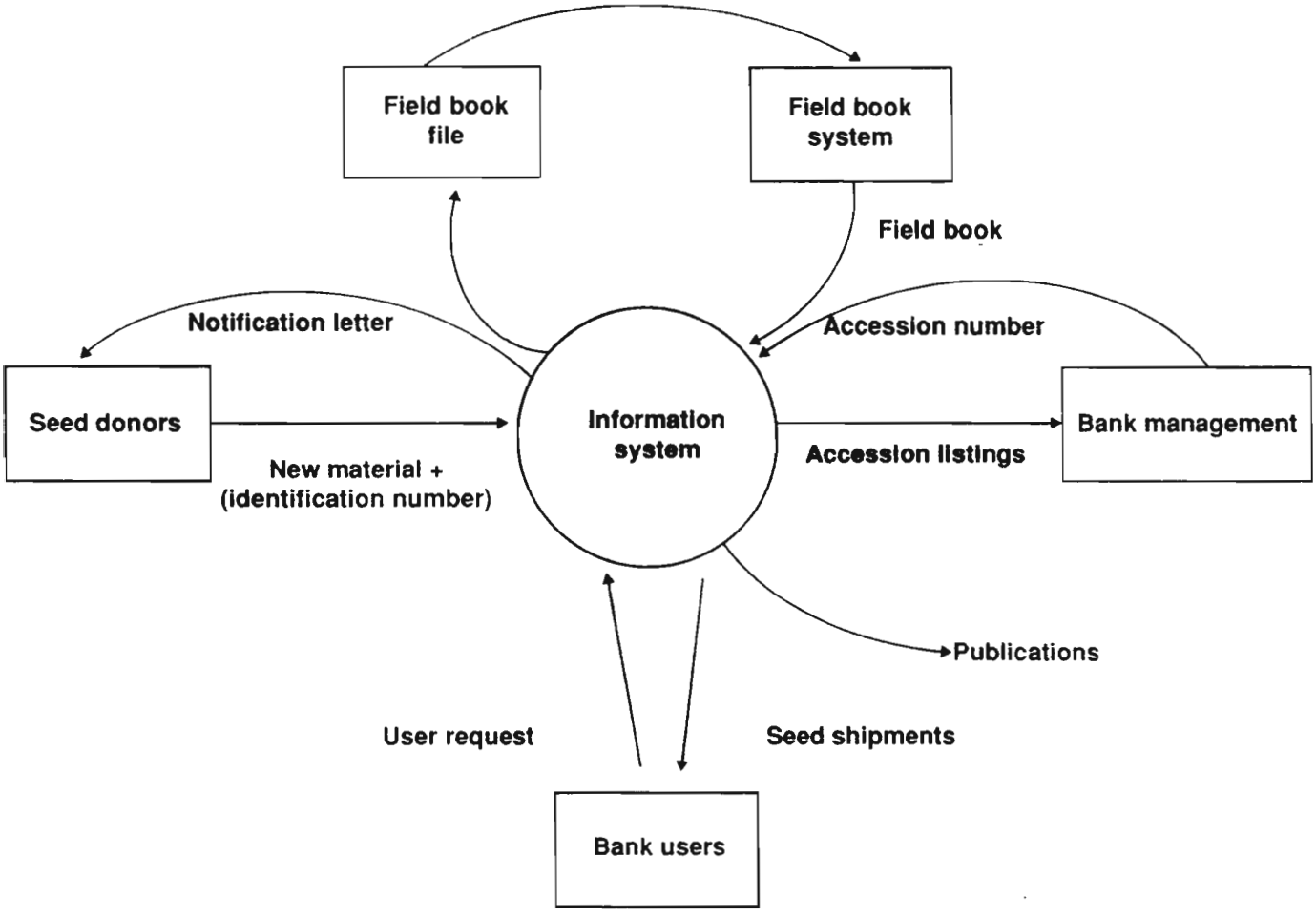


Figure 2. Introduction of new material in the maize germplasm bank (passport phase).

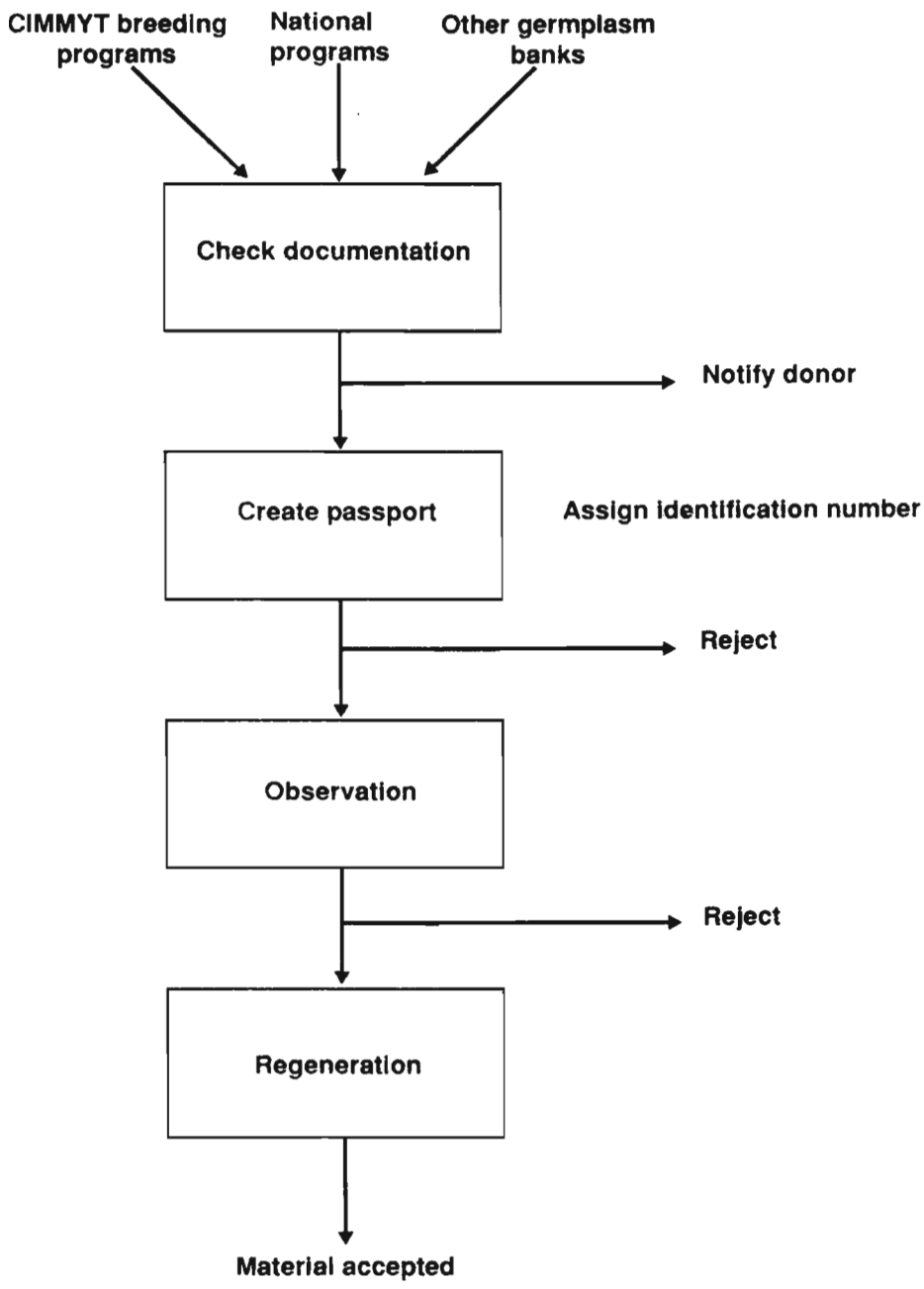
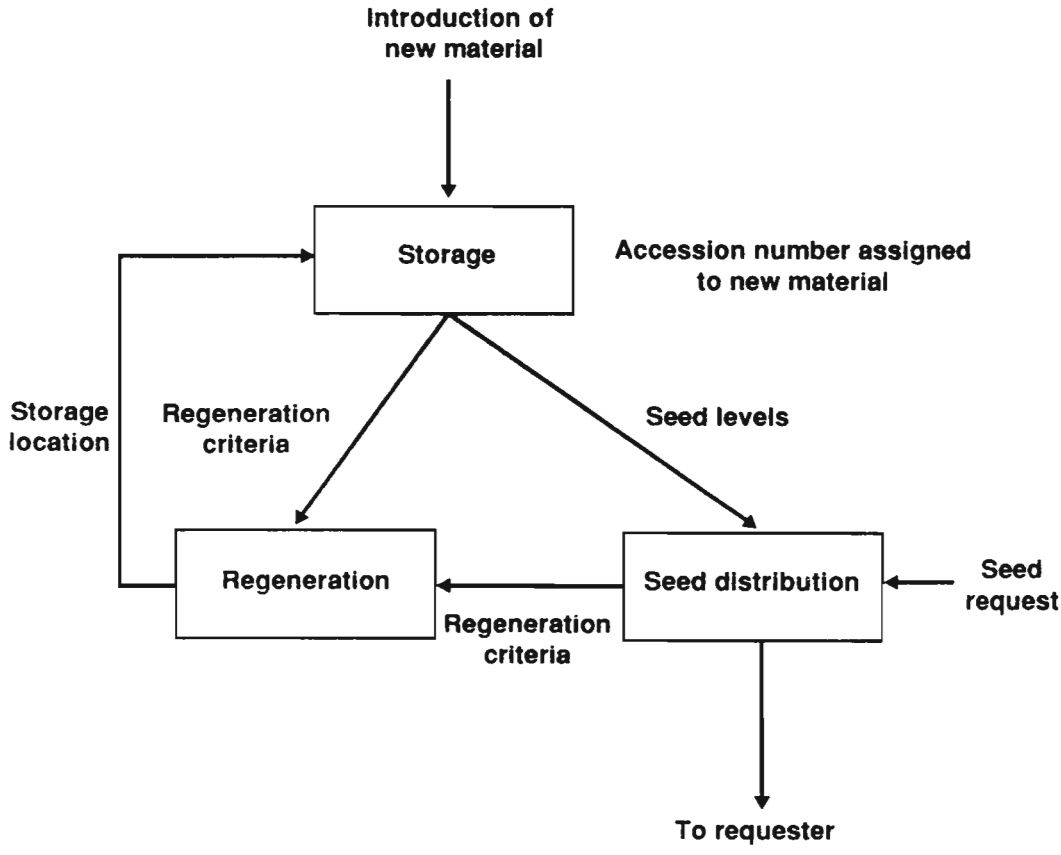


Figure 3. Interrelation of bank activities.



In Situ Conservation of the Genus *Zea* in the Sierra de Manantlán Biosphere Reserve

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Abstract

The possibility of creating a perennial maize and improving the resistance of maize to viral and spiroplasmal diseases, using genes derived from Zea diploperennis *Iltis, Doebley and Guzman, helped gain support for the idea of conserving this species in its natural habitat. The Sierra de Manantlán Biosphere Reserve was created by presidential decree in 1987 and incorporated into the international network of Biosphere Reserves of UNESCO-MAB in 1988 to conserve the endemic perennial* Z. diploperennis *in situ. It is also hoped that the Reserve will conserve and protect populations of the wild annual relative of maize,* Zea mays *subsp.* parviglumis *Iltis and Doebley, as well as germplasm of the traditional maize races Tabloncillo and Reventador.*

In situ and Ex Situ Conservation

The conservation of germplasm can be managed according to two models: in situ, in its place of origin, or ex situ, outside its place of origin, as in zoos, botanical gardens, and germplasm banks. In situ conservation, clearly the more complex of the two, attempts to protect species under the natural conditions in which they are normally found, be they pristine or anthropogenic habitats. In contrast with ex situ conservation, which saves germplasm under artificial conditions, in situ conservation seeks to maintain the genetic diversity of the species under the conditions in which it evolved so as to allow the process of adaptation to continue.

What follows is a discussion of plans for the conservation of genetic resources of the genus *Zea* in the Sierra de Manantlán of western Mexico, where two species of wild *Zea* and two traditional races of cultivated maize are found. The background and arguments provide justification for conserving both the "wild" and cultivated species in situ.

Background

Unfortunately, in situ germplasm conservation has been described by various international organizations (UNESCO-MAB 1974) in reference to the conservation of ecosystems and communities, with the result that its objectives have been confused with those of various types of reserves, such as biosphere reserves and wild protected areas. It has thus been mistakenly assumed that in situ germplasm conservation implies the maintenance and management of the existing genetic diversity of all organisms in entire ecosystems and/or diverse tropical plant communities. We do not have even a general idea of the biological diversity of the majority of these communities, let alone the genetic diversity of the component organisms. Although in situ conservation of a few species with recognized value has been tied to biosphere reserves (Oldfield and Alcorn 1987; Ingram and Williams 1984), the *World Conservation Strategy* (IUCN et al. 1980) endorses this practice only for wild species, stating that the conservation of cultivated plant and animal germplasm in reserves is not possible.

The concept of germplasm conservation (that is, of the genetic diversity that exists in a species, be it wild or cultivated) was developed during the 1940s in part by plant breeders and agronomists. These scientists recognized the need to safeguard genetic material of cultigens and

their wild relatives against displacement by improved varieties and newly introduced cultigens; they hoped to prevent the complete loss of this genetic material and make it more readily available for use by breeders (Frankel 1988). In situ conservation was later considered as a means of making up for the potential faults of ex situ conservation, such as loss of seed viability, selection under conditions different from those in which the genetic material occurs naturally, among others (Dinoor 1976; Iltis 1974; Wilkes and Wilkes 1972; Prescott-Allen and Prescott-Allen 1981).

Generally, investigators of germplasm conservation--for the most part agronomists, geneticists, and plant breeders--have not considered in situ conservation of germplasm feasible or justifiable. In their view the displacement of traditional cultigens and cultivars is a necessary result of development programs that promote improved varieties and/or different crops as a means of increasing agricultural production and making it more "stable." Implicit in this approach is the belief that leaving the traditional cultigens/varieties in place instead of replacing them with improved seed denies traditional agriculturalists an opportunity to better their lives (Ingram and Williams 1984; Williams 1988; Frankel 1983).

The rate of genetic erosion is increasing with the introduction of improved seed of local cultigens and with increased planting of cultigens other than the traditional crop (sorghum in place of maize, for example). In view of these trends, is the ex situ method of germplasm conservation meeting the requirements of saving, conserving, and evaluating the germplasm collections now in storage? Some of the country reports presented at this workshop (Colombia, Argentina, and Peru) and the observations of Lyman (1984) and Mitgang and Raeburn (1989) suggest that it is not. We can only hope that development programs have not totally displaced the traditional cultigens/varieties, so that we might repeat the actions necessary to conserve them effectively either in or ex situ.

Germplasm banks have sought to conserve the complete range of genetic variation (or the majority of it), based on collections that contain a representative sample of the phenotype. It is assumed that the original collections contained the majority of variability existing in the population under natural conditions. Though we do not actually know, it is very likely that, in the course of seed increase by banks, the diversity in the original or present-day populations has not been maintained. In theory replanting the populations can maintain their diversity (Crossa, these proceedings), but this procedure is conducted outside the system in which a species or cultigen evolved and does not provide the sociocultural or natural conditions that created and have maintained existing diversity (Nabhan 1985).

The functions, achievements, and faults of germplasm banks are well known and are described in these Proceedings. I am not arguing here against them but merely wish to call attention to a bias in the conservation ethic of scientists who have played an important role in the conservation and development sphere of international political economy (Stakman et al. 1967; Wilkes 1989). While criticizing ex situ conservation of germplasm, I am not promoting in situ conservation as a panacea. It is more important that we compare the objectives, methods, and needs of the two approaches to clarify the differences between them and to establish their complementarity.

Wild Relatives

Conservation of the wild relatives of crops has been an important topic at least since the early 1970s, when Harlan and De Wet (1971) called attention to the usefulness of the various gene

pools. It was hoped that one could transfer from the parent to the cultigen characters that might increase production, confer resistance to disease or drought, or improve the quality or taste of the cultigen (Wilkes 1977). Conserving germplasm of wild species is a common goal of various international, national, and local organizations, but, as with the cultigens, there is still controversy as to whether the germplasm should be conserved in situ or ex situ (Iltis 1974; Wilkes and Wilkes 1972; Ugent 1970; Frankel 1974; Frankel and Bennett 1970; Frankel and Hawkes 1975; Ingram and Williams 1984; Williams 1988; Oldfield and Alcorn 1987; Altieri et al. 1987). Obviously, each cultigen and each of its relatives is a special case, and the manner of conservation depends on the conditions under which each is found.

Conservation of wild relatives is somewhat like that of crops, although the former is complicated by logistical problems and frequently by a lack of understanding of the taxonomic relationships between cultigen and relative. Fortunately, the taxonomy of the genus *Zea* is now quite well understood (Wilkes 1967; Doebley and Iltis 1980; Iltis and Doebley 1980; Doebley, in press); its ecogeografic aspects have been amply described; and germplasm has been collected very recently (Sánchez G. and Ordaz S. 1987). For the most part, the majority of the taxa are well represented in various germplasm banks (CIMMYT, INIFAP, the US Department of Agriculture, and the herbarium of the University of Wisconsin). Ex situ conservation of *Zea* germplasm over the short term is assured. As for the wild taxa, ex situ conservation presents logistical problems over the long term, arising from seed increase of the accessions. Spontaneous seed dispersal occurs in the experimental plots, and the populations must be isolated to avoid outcrossing with neighboring populations. CIMMYT's (1986) plan for in situ monitoring of various populations of two of the annual species is a first step toward a more sustainable model for conserving germplasm of these wild relatives.

It appears to some (Lyman 1984) that the wild relatives are less threatened with displacement by improved varieties and new cultigens than are traditional cultivars. Nonetheless, we cannot be sure that this is so in the case of *Zea* (Benz et al., in press). If all the wild relatives of crops were inhabitants of "pristine" ecosystems, they might be saved, perhaps, by setting the ecosystems aside and protecting them (as with the nuclear zone of biosphere reserves), so that the species and ecosystems could continue undergoing natural processes of change. This view of conservation is naive, however, considering that man is the most prevalent force in nature today and that, as a result, it is difficult to find ecosystems that are really pristine. This is not to say that setting areas aside should not be a goal of conservation projects but only that such areas are nearly nonexistent and that establishing them would very likely require active management. Leaving them to the forces of nature may result in further destruction or longer recuperation times.

Another problem with this approach to protecting wild relatives is that not all, nor even the majority, of them occupy pristine environments. The wild, or spontaneous, relatives of maize, like those of many other important cultigens, such as wheat, rice, potato, and sorghum, have evolved jointly with the cultigen and, as a result, do not occupy niches outside habitats disturbed and/or maintained by humans. How can we hope to conserve a wild relative in situ, if its survival depends on human disturbance in the form of traditional agriculture? In this case in situ conservation of the wild relatives requires conservation of the whole agricultural system, including the spontaneous relatives as well as the traditional crops.

Conservation of crops' wild relatives does not necessarily imply denial of opportunities for advancement to the people directly or indirectly responsible for creating and managing the

system under which the wild relative survives. But it does place certain limits on the introduction of external inputs that might endanger the wild relative. An example might be to promote the use of an improved maize variety in the habitat of *Z. diploperennis*. With its requirements of a weed-free field, fertilizers, and herbicides, the introduction of such a variety necessarily implies displacement of the traditional cultigen (genetic erosion in the strict sense) and of *Z. diploperennis*.

Zea diploperennis and the Sierra de Manantlán Biosphere Reserve

With the publication of the discovery of *Z. diploperennis* (Iltis et al. 1979), the creation of some form of reserve to protect it seemed inevitable (Vietmeyer 1979; Iltis 1983; Guzman et al. 1985). The discovery resolved the debate about the existence of a diploid perennial species ancestral to *Z. perennis* (Hitchc.) Mangelsdorf and Reeves (Reeves and Mangelsdorf 1942), and it helped improve our understanding of the phylogenetic relationship between *Zea* and *Tripsacum* (Doebley 1983; Doebley et al. 1987), giving us a better idea how, where, and from what the genus *Zea* evolved (Guzman 1982).

The discovery of *Z. diploperennis* also gave support to the belief, at times slightly exaggerated, that a perennial maize might be developed (Iltis et al. 1979; Fisher 1982; Vietmeyer 1979). Studies to date have indicated that a perennial maize with adequate production probably is not possible, due to the conflict in resource allocation between production of seeds and perennial roots (van der Walt 1982; Price et al. 1987). The real utility of this species probably lies more in its immunity or resistance to numerous viral, mycoplasmal, and spiroplasmal diseases of maize (Nault 1980; Nault et al. 1982). If we estimate that about 2% of total maize production is lost to viral diseases (Ullstrup 1977), the disease resistance alone would make *Z. diploperennis* worth US $34 million annually in the USA and US $2.7 million in Mexico. It is not known whether *Z. diploperennis* might also contribute to increasing production of elite lines or improving the nutritional quality of maize for humans or animals. But what we do know of its biology leads us to think that it does have great value, perhaps even more than is now realized. The potential of *Z. diploperennis*, together with the importance of maintaining the associated ecosystems, made it obvious that the species should be conserved in situ (Guzman M. 1985; Guzman M. et al. 1985; Santana C. et al. 1987).

Apart from *Z. diploperennis*, two populations of annual teosinte (*Z. mays* L. subsp. *parviglumis* Iltis and Doebley) are also found within the Biosphere Reserve (Sánchez and Ordaz 1987). One of these looked very healthy in 1982, associated with cultivation of Tabloncillo, one of the races traditionally planted in the area. In the last few years, people have nearly stopped planting this variety, having received credit and a technological package of improved seed and fertilizer. Whether this has affected the local population of *Z. mays* L. subsp. *parviglumis* is currently being investigated. This annual species normally occupies places within the farmer's field (in this case, the *coamile*), often spontaneously hybridizing with maize, or is found along field margins, stone fences, or paths. The second population, found outside the village El Sauz (Sánchez y Ordaz 1987), is still not very well known; it does not seem to be as vigorous or as dense as other populations of this species. According to Wilkes (see CIMMYT 1986), other populations are steadily contracting. For that reason and because it is the closest relative of maize (Doebley et at. 1984), the Reserve also seeks to conserve this species in its natural habitat.

The population of *Z. diploperennis* and the two populations of *Z. mays* subsp. *parviglumis* are found on the slopes and in the valleys of the Sierra de Manantlán of Jalisco, Mexico (Iltis 1983;

Guzmán 1982; Sánchez and Ordaz 1987; Benz et al. in press). Reaching an altitude of 2,900 masl, this massif dates to the Cenozoic era. Its relief has provided such a wide range of sufficiently protected environments that it probably has acted as a refugium for a multitude of plant and animal species during the Neocene (Toledo 1982). Within the Sierra de Manantlán, 17 species of plants alone have been collected that are endemic to western Mexico (Jardel, personal communication). The Sierra de Manantlán and adjacent Cerro Grande form the headwaters of numerous drainages that feed the Armeria, Marabasco, and Purificación Rivers. These are very important in the region, because they provide water for the numerous irrigation systems in the surrounding valleys. Because of their richness in plant species, including *Z. diploperennis*, their economic importance as a source of water, and their immensely valuable and highly exploitable forests, the Sierra de Manantlan and Cerro Grande (Figure 1) were set aside as a Biosphere Reserve by presidential decree in March of 1987, and the Reserve was incorporated into the international system of Biosphere Reserves of UNESCO-MAB in January of 1988 (Santana C. et al. 1987; Jardel, personal communication).

The Reserve consists of *ejido* and communal land (72%) and private property (26%). During the last 40 years, the forests of the Sierra have been intensively exploited for the local and international timber markets. Prior to and during this century, the Sierra has also provided ample pasture for cattle. Millenia of human activity in the New World, the Sierra de Manantlán, and its surroundings fostered the development of agriculture based on the cultivation of maize, beans, and to a lesser extent squash (Kelly 1945, 1949, 1980). According to Warman et al. (1982), the agricultural technology used in the area is still very traditional. It remains so because the area is not readily accessible and simply because the inhabitants do not want to change, having more confidence in their traditional ways. In view of this resistance, it makes no sense to try introducing nontraditional practices, such as fertilizer and insecticide application and the use of improved varieties of maize or other crops, without first examining more closely why and how the system has so far resisted the onslaught of modern technology. We have much to learn from the traditional system before discarding it in favor of a modern one that seems enormously productive but requires extremely high external inputs, calling into question its long term stability, efficiency, and adaptability (Pimental et al. 1973).

In traditional agricultural systems, we find two of seven of the most primitive races of maize in Mexico, perhaps in the world (Benz 1986): Tabloncillo and Reventador, following the nomenclature of Wellhausen et al. (1951). Based on the distribution of these races, it is very likely that maize has been present in the area since shortly after its origin, if in fact it did not originate here. Furthermore, during a systematic search of Jalisco in 1982-1984 to obtain collections of Reventador, this race was found at only one of the various sites mentioned by Anderson and Wellhausen et al. (1951), where it had been encountered in the 1940s. For this reason it is very likely that Reventador is in danger of extinction under conditions of traditional cultivation (Benz 1986).

As mentioned above, the occurrence of these two races within the Reserve is associated with a more traditional way of life and a general lack of outside influence. This situation somehow persists in spite of the growing pressure of new technologies and the forces of an international political economy driven by the government (and in some cases by the people of these very same communities). These forces stem from policies promoting increased agricultural productivity and national self-sufficiency in food production. As long as the political situation creates a sense of economic urgency, the pressure to displace traditional varieties will increase genetic erosion.

Under the traditional system practiced in the Reserve, the farmer achieves a guaranteed minimum production from a diverse agroecosystem composed of a principal traditional cultigen, squash, chile, beans, weeds, such as the husk tomato and other edible greens (*quelites*), as well as other products. All this takes place under a single system of conscious management attuned to the myriad biotic and abiotic interactions and interrelationships existing within the cultivated field (Hernández X. 1985). In situ conservation of primitive races of maize (and its spontaneous relatives) implies conserving the entire agricultural ecosystem (Oldfield and Alcorn 1987; Altieri et al. 1987; Altieri and Merrick 1987). To introduce something new into this system, even though it might appear more productive, causes marked ecological changes that endanger the equilibrium of the traditional system and assure its complete destruction in one step.

The primitive races also constitute a source of genes coadapted over the centuries to produce under unfavorable and favorable environmental conditions. It is not known whether the genes contained in these races can be useful outside the system in which they evolved, because the holdings of germplasm banks have not yet been fully evaluated. To leave the races within the agroecosystem where they originated will allow them to continue evolving under the social and environmental conditions to which they are adapted. In this way we hope to conserve the coadapted gene complexes that are in large part responsible for the survival of the races.

Farmers' perceptions (that is, their empirical knowledge) of the traditional system and its components is extremely important, because they are responsible for continued cultivation and thus to a large extent for the survival of the traditional races. Although opinions vary among farmers, they all seem to recognize the importance of maintaining their own and well-known traditional varieties. They certainly want more production, but they also appreciate the security of sowing something familiar. Traditional agriculturalists are careful about taking risks; they will continue to sow traditional varieties until they find something more productive, and the new material must produce as much or more within the farmer's social and agroecological system (Bartlett 1980).

Many questions still need to be answered before we can confidently assume the task of conserving the traditional varieties in situ. Which races have greater genetic variability, the traditional or improved ones? Of the traditional races, which populations contain greater genetic diversity, those conserved in situ or those conserved ex situ. And the most difficult question of all, which genes are useful now, and which will be useful in the future? We know that of all the representative populations of Mexican maize races in CIMMYT's germplasm bank, Reventador and Tabloncillo are the most isoenzymatically homogeneous (Doebley et al. 1985). This does not mean that they are less valuable than more heterogeneous material. To evaluate the relative utility of homo- or heterogeneity, we would require equivalent studies of improved varieties that are being distributed by government institutions and are displacing the traditional varieties and of populations of the traditional varieties that have been maintained in their natural habitat. Population genetics studies would help us define minimum population sizes of the traditional varieties, determine the probability of losing rare alleles with the introduction of improved varieties, and compare populations conserved in situ with those still existing ex situ.

The right of traditional farmers to improve their lives obviously has to be considered if we seek to conserve the traditional varieties whereever we find them. If the improved varieties are more productive, do not greatly alter the existing agroecosystem, and do not present too great a risk to the agriculturalist (i.e., do not require annual purchase of seed), do we have the right to ask farmers to maintain the status quo, simply because we seek to conserve traditional varieties in

situ? One alternative might be to have only a few families continue sowing the traditional varieties, while the rest sow nontraditional seed, as is done in Yugoslavia. Subsidies would most likely be required to make such a scheme work. Unfortunately, the in situ conservation program would then be less reliable, since survival of the varieties would depend more on the subsidy than anything else. If we follow this path, we will have to be sure that traditional varieties are maintained as before and not mixed with introduced seed. A second alternative, which might satisfy our needs and the farmers', would be to designate community centers in which traditional varieties would be maintained in situ by farmers trained in the goals, methods, and theory of germplasm conservation (Oldfield and Alcorn 1987). In these centers we could save, study, and use genetic resources and at the same time create opportunities for improving part of the populations of traditional varieties within their natural habitat. In establishing such a program, one would have to consider the genetics of minimum viable population size, population structure, as well as the social and economic forces that make in situ conservation possible.

We stand a good chance of conserving in situ *Z. diploperennis, Z. mays* subsp. *parviglumis* and the traditional races found within the Sierra de Manantlán Biosphere Reserve. But the conservation of the first two depends primarily on the survival of the traditional agricultural system, which in turn implies conserving the traditional varieties in situ. For example, the largest population of *Z. diploperennis* is located in the vally of San Miguel, where almost all of the 320 ha are under cultivation. Apparently, *Z. diploperennis* not only withstands but actually prospers under grazing and cultivation (Benz et al., in press). However, where stands of the species are left to the processes of secondary succession, as is the case with the population at Las Joyas Scientific Station, the stands appear to diminish over time (J. Cruz, pers. comm.). These findings suggest that, for the purposes of in situ conservation, *Z. diploperennis* and its agroecosystem are inseparable.

How feasible is in situ conservation of crop germplasm, specifically that of maize? Various authors (Iltis 1974; Olfield and Alcorn 1987; Altieri and Merrick 1987; Nabhan 1985) have noted the value and importance of conserving and developing traditional agricultural systems as a means of conserving the genetic resources they contain. However, none of these authors has demonstrated the feasibility of conserving a traditional system containing numerous sources of germplasm, jointly with its biological diversity, stability, and adaptation. Research currently being conducted in the Reserve seeks to evaluate the agricultural production capacity and economic and social factors that either promote continued use of traditional germplasm or force farmers to stop planting this material. We hope that the results of these investigations will aid in evaluating the feasibility of conserving traditional varieties of maize and other cultigens in situ in the Reserve.

Epilogue

The question whether in situ conservation of crop germplasm is incompatible with development programs (Williams 1988; Frankel 1983) can be resolved only if we find a way of producing more without destroying the habitats of potentially useful germplasm, whether in natural or anthropogenic ecosystems. The complexity of the problem is demonstrated by the case of Plan Puebla (CIMMYT 1974), which perhaps also provides us with a potential solution.

It is well known that a sense of urgency about conserving traditional maize germplasm arose from efforts to increase maize production (Stakeman et al. 1967). In a production program focusing on the valley of Puebla during the 1960s, hybrid and synthetic maize varieties were promoted, along

with the use of fertilizers and increased plant densities (Winkelmann 1977). The latter two measures did increase production, but neither the hybrids nor the synthetics proved to be more productive than the local traditional varieties. Although fertilizer application and the increased plant density created nonlocal dependencies and brought about marked changes in the traditional agricultural system, the traditional germplasm persisted and was conserved in situ. The introduction of such technologies may not be compatible with in situ conservation of *Z. diploperennis* or *Z. mays* subsp. *parviglumis*, because of increased competition in denser stands. Nonetheless, this case does illustrate that conserving traditional germplasm in situ does not necessarily preclude increased production and accompanying benefits for the human population.

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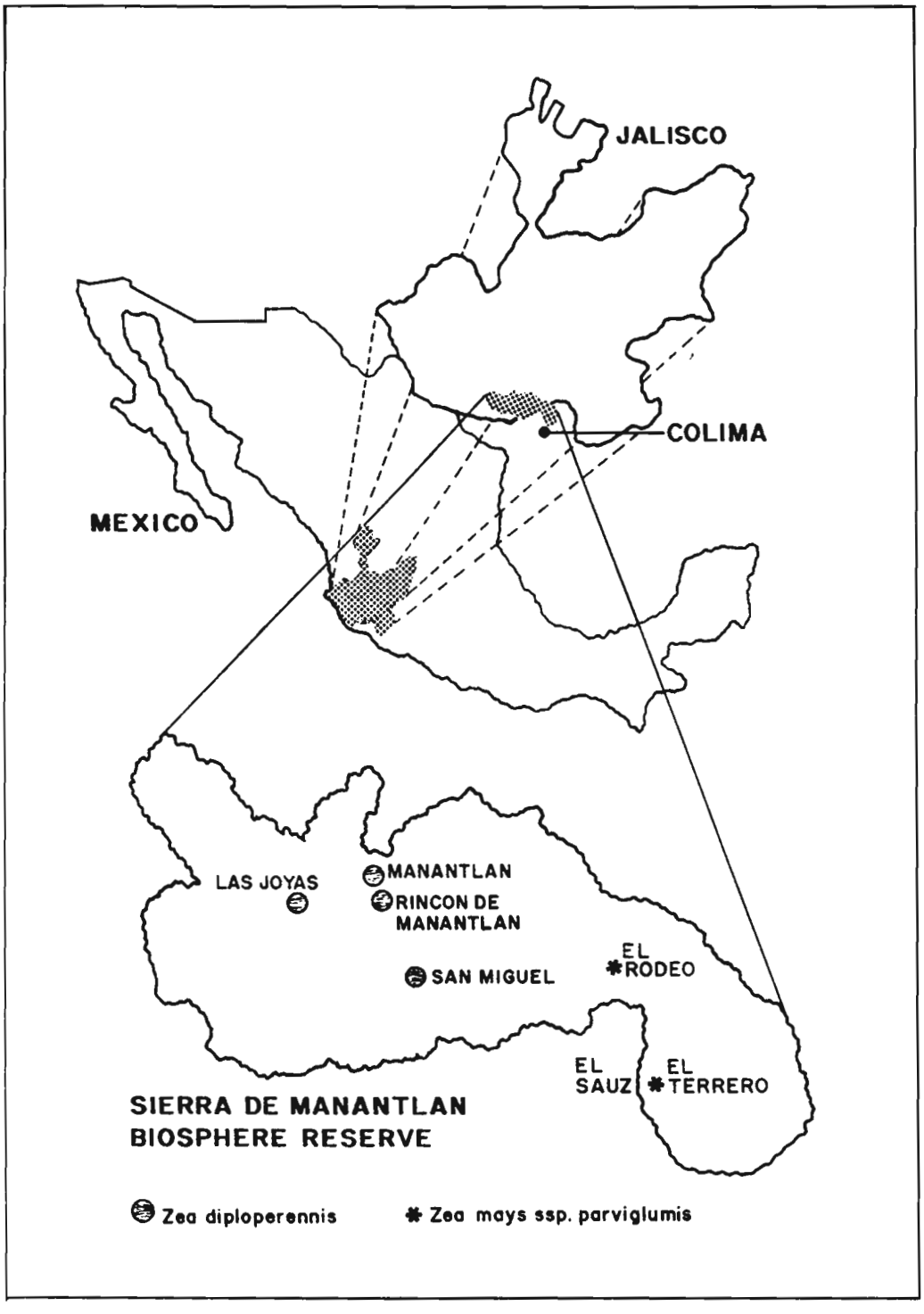

Figure 1. The Sierra de Manantlán Biosphere Reserve in Jalisco, Me

Teosinte and the Other Wild Relatives of Maize

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Abstract

This paper provides a review of the wild relatives of maize and a personal account of field work covering 25 years of study with the Meso-American grass, teosinte--the closest relative of maize. It comments on the threat of extinction of the wild relatives and on measures being taken to preserve them.

Maize is a New World domesticate that has spread worldwide, while teosinte, its closest relative, has remained in the highland and western escarpment of Mexico and Guatemala. Teosinte is an amazing plant, being one of the few widely recognized wild relatives of a domesticate. Maize is also an amazing plant, because so many of its landraces in Mexico and Guatemala contain teosinte germplasm. Yet, the two are never confused, since they have remained morphologically distinct. The evidence of tripsacoid germplasm in maize from the more distantly related genus *Tripsacum* is less clear. There are approximately 15 taxa in this genus, and all are New World perennial grasses, whose center of distribution, like that of teosinte, appears to be the western escarpment of Mexico and Guatemala.

Maize the Food Plant and Teosinte the Wild Relative

Both teosinte and maize are unique among the grasses, because they bear the male and female flowers in separate places. The male flowers are borne terminally in the tassel and the female flowers in the ear or spike, which occupies a lateral position on the plant. The individual kernel or fruit of teosinte is enclosed in a hard rachis segment enclosed in the front by the enlarged lower glume. The individual rachis segments disarticulate at a brittle zone of abscission between the segments. This ability to sow its seed separates teosinte as a wild plant from maize, the cultivated crop. The individual kernel of maize is unique among grasses, in that the seed is not covered by a floral bract or glume but is borne fully exposed in a massive stiff axis, the cob. The spikes of teosinte and the cob of maize are both enclosed and protected by modified leaf sheaths, which make up the familiar husk.

Tripsacum, the More Distant Relative

The approximately 15 species in the genus *Tripsacum* are more distant relatives of maize and do not share the same chromosome base number. The male and female flowers are borne separately but in the same inflorescence. The seed are scattered as single rachis segments. Several of the members of the genus have been crossed with maize successfully, and the resulting F_1 hybrids have been highly male sterile, although backcrossing in both directions has been accomplished. There have been extensive and comprehensive attempts to hybridize members of the genus with various teosinte taxa, but all have failed (Tantravahi 1968). The genus is fertile across many of its recognized taxa, and some taxa possess diploid ($2n = 36$), triploid, and tetraploid ($2n = 72$) members. The genus is fully perennial, and all taxa possess either well developed fleshy rhizomes or wirey root systems. There are three geographic clusters: 1) the South American taxa; 2) the pan-US, Mexico, Central America and northern South America group; and 3) the western escarpment of Mexico and Guatemala group. The last cluster includes the ancestral diploids and is probably the center from which the genus has radiated.

The Origins of Maize

The transformation of wild plants to domesticated ones has not occurred in the same way among all plants. No uniform evolutionary pattern has been followed in the origin of our cultivated crops. Similarly, there is no uniform evolutionary pattern for wild/cultivated plant hybridization or for the crop/weed complex. Some are ancient domesticates; others have only become domesticated in historic times; some have long term introgressive hybridization patterns; and others have recently formed weed/crop complexes.

I emphasize this point because maize, which has diverse endosperm types and lends itself to different cooking styles and uses and has adapted to both highland (its native zone as a wild plant) and lowland habitats (where teosinte germplasm has been important), has a distinctive origin each time a landrace is formed. Heterosis between the unique gene systems found in distinct landraces accounts in part for the yield potential of both maize and teosinte. Clearly, the evolution of maize has resulted more from a sequence of genetic changes over time, some of which are dependent on teosinte, than from the fixation of a particular trait. In maize the change from a wild to a domesticated plant has been more a process interwoven with teosinte than an event or "big bang." The genus *Tripsacum* appears not to have been a player in the origin and evolution of maize as a domesticate.

Geographic Center of Maize and Its Wild Relatives

Mexico and Peru are the centers of racial diversity for maize recognized by N.I. Vavilov (1926). Mexico is the primary center of genetic diversity and the Andean Zone a secondary center where the maize crop has undergone rapid evolution. Of the 50 races found in Mexico, 7 have counterparts in Guatemala, 6 in Columbia, 5 in Peru, and 2 in Brazil. Clearly, Mexico has been a center for landrace diffusion, although 27 or nearly half of these landraces remain endemic to Mexico. Much the same pattern of endemism exists in Peru, where 30 of the country's 48 races occur only within its borders. Based on over 2,800 collections of maize made by the Vavilov Institute in the New World, Mexico was recognized as the center of greatest diversity (Kuleshov 1933). Like the Vavilov centers for other crops, Mexico was characterized by mountainous regions that bordered the tropics and had long been populated by agricultural people who were isolated by steep terrain, arid regions, or other natural barriers. Wellhausen et al. (1952) accounted in the *Races of Maize in Mexico* for the racial diversity of maize in much the same way, citing a) the preservation of primitive races, b) the influx of exotic races from countries south of Mexico, c) hybridization with teosinte, and d) the geography of Mexico with its varied habitats and isolating factors conducive to rapid evolution.

Essentially the same conditions prevail in Peru, but since teosinte was native to Mexico and not found in Peru (although *Tripsacum* is found), Vavilov considered Mexico to be maize's site of origin (primary center). He also considered teosinte to be the progenitor of maize, an idea shared by Beadle (1972), Doebly (1983), Galinat (1977, 1983, 1985), Harlan and de Wet (1972), Kato (1984), and Iltis (1972, 1983), and Vavilov attached considerable significance to the fact that teosinte was fully fertile with maize and that the hybrids occurring naturally between the two could be found in Mexico (Vavilov 1931).

Using evidence very different from that of Vavilov, Harshberger had concluded years before that maize originated in Mexico and, more specifically, that it had once occurred as a wild plant in

central Mexico at elevations of more than 1,500 m above sea level (masl) in a semiarid region with summer rains of approximately 35 cm (Harshberger 1893). His conclusions were remarkable because, unknown to him, he had exactly described those areas of Mexico where the closest relative of maize, annual teosinte, occurs and had pinpointed the sites where archaeological evidence of wild and early maize were to be found.

The naturally occurring teosinte populations are limited to the western escarpment of Mexico and Central America in a seasonally dry, subtropical zone that is 800-1,800 masl and receives summer rains and to the Central Plateau of Mexico (1,650-2,000 masl), also with summer rains. The teosinte population in the Valley of Mexico at Chalco (2,250 masl) is an anomaly. The vegetation is deciduous thorn scub to oak woodland. The growing season of June through October (August through January in Huehuetenango) begins with the summer rains and by August or September (November/December in Huehuetenango) has reached the midflowering stage. The habitat preferences of *Tripsacum* spp. are very similar to those of teosinte but less restrictive. All teosinte collection sites are sympathetic with taxa of the genus *Tripsacum*

A highly variable wild plant, teosinte has both annual and perennial taxa. The seed producing annuals, all of them diploid (2n = 20), are found in eight geographically isolated population clusters, six in Mexico and two in Guatemala. Some of the populations are small, the smallest occupying less than 1 km^2, while the largest covers thousands of square kilometers. There are two perennial teosintes, both of which produce rhizomes, occurring only in restricted habitats in the state of Jalisco, Mexico. One, *Zea diploperennis*, is diploid (2n = 20) and the other, *Z. perennis*, is tetraploid (2n = 40).

Throughout the rest of this report, the name "teosinte" refers to the annual form, except where otherwise specified. A conscious effort has been made here to avoid the use of taxonomic terms (races or species and subspecies) for the various taxa of annual teosinte. Instead, reference is made to teosinte populations, which together include all named taxa. I feel this is appropriate, since in plant breeding with genetic resources the population, not the named taxa, is used.

My Early Days With Teosinte

In 1960 when I started as a graduate student of P.C. Mangelsdorf's, the botanical literature on teosinte was fragmentary, and the geographic distribution and field biology was known only to E. Hernández Xolocotzi. In fact, when I started my field work in Mexico in 1962, I was not sure teosinte could be located except at the well-known site at Chalco. I also wondered why a field/taxonomic study of teosinte had not already been undertaken by someone else. At the time I was unaware that C.L. Gilly at Iowa State University had begun studies a little more than a decade and a half earlier. In 1962 there was only the distribution map in the *Races of Maize in Mexico.* I have subsequently learned that the map is based on a report prepared by Hernández X. at the Mexican National School of Agriculture, Chapingo, and by C.L. Gilly. The original copy submitted to E.J. Wellhausen of the Rockerfeller Foundation in Mexico (now CIMMYT) formed the basis of the Spanish version of the *Races of Maize in Mexico* published in 1951 but was lost. The copy retained by Hernández X. was loaned to a student at Chapingo preparing a seminar on teosinte and was also misplaced (Hernandex X. and Mario Castro G., oral communication, 1962). So, in 1962 the knowledge of teosinte populations was either with Hernández X., who freely shared it with me, or in the older literature. Today the most complete distribution maps for teosinte are those published by T. Angel Kato Y. (1976) at Chapingo and Jesús Sanchez and

Lorenzo Ordaz (1987) of Mexico's Institute of Forestry, Agriculture, and Livestock Research (INIFAP).

The Habitats of Teosinte

The distribution of teosinte along the escarpment of Mexico and Guatemala parallels that of the ancient Mexican and Mayan civilizations, whose staple food was maize. Teosinte hybridized readily with the crop, producing a robust and fertile F_1 hybrid. Even today the habitats of teosinte are found on some of the country's best agricultural land. In the states of Jalisco. Guanajuato, and Michoacan, the plant grows mostly along stone fences bordering maize fields, not as a weed invading the fields, but as a rare survivor making its last stand in this narrow strip of untilled soil. In a few places, such as Chalco, teosinte has successfully invaded maize fields as a "mimic" of the cultivated crop. The two, teosinte and maize, are so similar in appearance that farmers cannot readily distinguish them and thus do not weed teosinte from their fields during the early part of the crop cycle. The largest population, and the one least likely to disappear in the near future, is that which occupies hundreds of square kilometers in the mountains around the Rio Balsas, primarily in the state of Guerrero.

Teosinte is not uniformly distributed on the western escarpment of Mexico and Guatemala, but the races exist as semi-isolated populations, each more or less in geographic isolation (Table 1). Most of the geographical populations are spatially isolated by the broken topography of high mountains and deep valleys. The environments of these geographic populations are not identical, and annual teosinte has developed several physiologically different races, each of which has acquired a limited morphological, ecological, chromosomal, and genetic distinctness. The bulk of my thesis became the documentation of these differences. The thesis went directly to the printer and became the 1967 monograph, *Teosinte: The Closest Relative of Maize*. Others have given Latin names to the races and arranged them hierarchically (Table 2), but the groups as such have proved to be remarkably durable (Iltis and Doebley 1984; Doebly et al. 1984).

Certainly the recently discovered perennial diploid (*Z. diploperennis*) and the long recognized tetraploid perennial (*Z. perennis*) deserve binominal status. For the annual teosintes, it is still not obvious in my mind that clarity is served by such distinctions. The strongest case can be made for the teosinte race Guatemala, which significantly differs from other annual populations in that its morphology is the most *Tripsacum*-like and possibly the most primitive of all (Wilkes 1972). The fruitcase is elongated (trapazoidal and not triangular), and the male flowers in the tassel are distinct from all other annual teosinte races. The chromosomes are characterized by terminal knobs, a condition not found in maize. The plant has a tendency to branch on the central stalk from the bottom up (a *Tripsacum* trait), whereas all other annual teosintes branch from the top down. In addition, this is the only race of teosinte that exhibits a tendency for perennialism. Also, when crossed with maize the race Guatemala retains in the F_1 more teosinte traits, such as single spikelets, than the other annual races. Clearly, Jutiapa is the most distinct of all the annual teosintes, but this does not necessarily mean it is ancestral. Jutiapa teosinte is also the least maizoid of the annual teosintes (Wilkes 1972), but it is genetically compatible with the other races and does not in my opinion justify separate species status (Bird 1978).

The Threat of Extinction

At several sites on Mexico's central plateau, teosinte has died out in recent times because of more intensive land use and pasturage. In general, the distribution of the plant is contracting, and some vulnerable populations are nearing extinction, though none is threatened with immediate elimination. The chief reasons for the contraction of teosinte populations are 1) intensified land use (which is related to the development of roads), 2) genetic "swamping" of small, isolated stands that outcross to maize and thus lose their ability to disperse seed, and 3) cultivation of a cash crop such as short stature sorghum instead of maize, making the presence of teosinte as a weed more obvious. By the best estimates, the current distribution of teosinte is about half of what it was in 1900. The disappearance of the remaining populations will accelerate as more roads are built and land use is intensified. Just 20 years ago, a third of the teosinte populations were far from any road, and another third were accessible by dirt roads that could be travelled only with difficulty. Now all are accessible by roads that can easily be negotiated by automobile.

Although it is difficult to define danger of extinction with much precision, some helpful criteria have been established by the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources (IUCN) in Switzerland. Six categories proposed by the IUCN--extinct, endangered, vulnerable, rare, indeterminate, and stable--can readily be applied to teosinte. Some populations (Honduras C.A., for example) are already extinct, and at least one (Guatemala) is endangered and cannot be expected to persist much longer as a naturally occurring population. Most of the teosintes, though (the exception is Balsas), can be considered vulnerable; that is, they are declining to such an extent that if nothing is done they will become endangered. The populations thought to be rare (Nobogame, Durango, and Oaxaca) are scarce enough that they could be eliminated easily, but they are currently under no immediate threat and are more or less stable. Indeterminate populations (Central Plateau and Huehuetenango) are those whose status is uncertain.

Diversity in the Genus *Tripsacum*

The taxonomy of the genus is not well understood, although it is widely recognized that it has evolved by polyploidy and separately from maize and teosinte in recent times. Two diploids are found on the western escarpment of Central Mexico, from which the genus has most probably radiated out. One (*T. zopilotense*) has wirey perennial roots and occurs in a zeric habitat, while the other (*T. maizar*) possesses fleshy rhizomes in a much more mesic habitat (Table 3). The two are morphologically distinct and allopatric in their distribution and within the distribution range of the Balsas race of teosinte. All the other polyploid taxa of Central Mexico are not so easily distinguishable on either a morphological or geographical basis.

In all, there are seven diploids (2n = 36) in the genus. Besides *T. zopilotense* and *T. maizar*, there are *T. floridanum* of southern Florida, *T. laxum* of west southern Mexico and Guatemala, *T. bravum* in Mexico and *T. cundinamarce* in Colombia (both narrow endemics), and *T. australe* of northern South America. In addition, one species or species complex, *T. dactyloides*, has both diploid (2n = 36) and tetraploid (2n = 72) forms. *T. australe* of South America was once included under *T. dactyloides*, and quite possibly the taxonomy of this taxa, ranging from the eastern USA through Mexico and Central America to northern South America, will be further subdivided as our understanding of the complex increases.

The tetraploid species (2n = 72) of Mexico and Guatemala form an almost continuous range of variation, and field studies indicate that they hybridize readily and that sometimes these populations are clonally propagated. Morphologically, it is often extremely difficult to separate *T. lanceolatum* from *T. pilosum* in Central Mexico and sometimes *T. pilosum* from the diploid *T. maizar*. *T. bravum* is geographically narrowly defined in a zone where teosinte is found in the Rio Bravo Valley of the state of Mexico, bordering the Balsas region of the State of Guerrero. *T. andersonii* has been split out of *T. lanceoletum* in Central America, and in the field the two are not so easily identified. *T. latifolium* is also Central American and limited to the Caribbean watershed of Guatemala and Honduras. *T. latifolium* is in many respects very similiar to *T. lanceolatum* in the field, and the two are most easily distinguished by their allopatric distribution. All of the above taxa have a decided preference for limestone soils and a seasonally dry habitat at elevations of 1,200-1,850 masl. Indeed, when clonal collections were made in 1964 for the Botanical Museum of Harvard University (Tantravahi 1968), these were the habitat markers used to discover new population sites. *Tripsacum* is a very frequent roadside and road cut colonizer in Mexico and Central America, given these two factors: limestone and midelevation. In South America there are both small to midsized plants of *T. peruvianum* and the luxuriant, sugarcane sized *T. cundinamarce*. The relationships and diversity of *Tripsacum* in South America are not well understood. At present it is difficult to know with certainty the evolutionary relationships in the polyploid speciation in the genus. It is hypothesized that, from the presumed center of diversity and present center of diversity on the western escarpment of Mexico (at elevations between 800 and 1,600 masl), two divergent diploids moved northward into what is now the USA and that these diploids gave rise to *T. dactyploides* (2n = 72). Similarly, these same taxa moved east and south, giving rise to additional taxa not distinguished from *T. dactyloides* in Central America and South America. The tetraploid complex of the Central Plateau of Mexico (*T. lanceofatum-T. pilosum*) is probably of hybrid origin, as are the *T. latifolium* of Central America. The origin of *T. laxum* and the South American taxa is not known with enough certainty to even form a hypothesis about evolutionary relationships.

Exchanges between maize and *Tripsacum* chromosomes have been established, and the evidence is quite good for *Tripsacum* x *Tripsacum* exchanges. It was with *T. dactyloides* (Kansas 2n = 72) that Mangelsdorf and Reeves first successfully hybridized maize and *Tripsacum*. Since then, *T. floridanum*, *T. dactyloides* (2n = 36), and *T.* ????????? have been hybridized with maize. Studies of the hybrids have indicated that certain segments of *Tripsacum* chromosomes can be substituted for corresponding segments in maize chromosomes and the plants remain both viable and fertile. Galinat has mapped more than 25 homologous loci on the chromosomes of these two genera. The accumulated information on maize-*Tripsacum* hybrids and their derivatives indicates that the respective genetic architecture of maize (2n = 20) and *Tripsacum* (2n = 36, 72), while quite different, is more similiar than their karyotypes would suggest.

The naturally occuring populations of *Tripsacum* in Mexico and Central America appear to be holding their own and are not presently threatened by possible extinction. Clearly, grazing has cut back on the abundance of plants covering the hillsides, but *Tripsacum* is an aggressive colonizer and can often be found on road cuts and steep hill slopes that are not grazed. The only exceptions might be the two Mexican diploids, *T. zopilotense* and *T. maizar*, which have very confined distributions in their type localities. A perennial garden of clones from the USA, Mexico, and Central and South America has been established at CIMMYT's Tlaltizapán station. This living collection represents much of the variation in the genus, and thanks to the work of F. Randolph in the 1970s, it is available for interested researchers, both now and for the future. The CIMMYT maize bank is unique among germplasm banks in the international agricultural research centers,

because it is the only major one that monitors the wild relatives in situ and maintains a perennial off-site garden of selected clones. Thirty years ago we knew relatively little about the populations of teosinte in Mexico and Guatemala. Hopefully, the genus *Tripsacum* will soon be well enough known to assure us that the CIMMYT garden is in fact a representative sample.

Curbing the Threat to Extinction

Although neither teosinte nor *Tripsacum* is likely to provide any means for short term or intermediate agronomic improvement of maize, they probably contain genetic variation not found in any maize breeding program. Thus, when they are gone, there will be no close, living relative of maize to serve as a source of such variation. So as better to protect this vital part of the genetic foundation of the world's maize crop, the CIMMYT Maize Program has begun in situ monitoring of the teosintes and promoting distribution of *Tripsacum* spp.

Maintenance in the germplasm bank--The two perennial teosinte taxa are being maintained in two forms: 1) as clones in a perennial garden along with *Tripsacum* spp. and 2) as seed in the bank's cold storage facilities. The *Tripsacum* garden is kept at Tlaltizapán as part of the Maize Program's work on wide crosses. This garden is being enlarged in an attempt to include all the known taxa, and additional collections are being made by maize staff. The bank will monitor the quality and documentation of the *Tripsacum* holdings and conduct some research in conjunction with work on the teosintes. Seed of the perennial and annual (seed propagated) teosintes can be regenerated by the gene bank only with some difficulty, since their pollination cannot be controlled as easily as that of maize and since they disperse their seed at maturity. For those reasons the bank continues to hold teosinte collections dating back to the 1960s that have not been regenerated and accessioned. In 1985 it was decided that wild collected seed of teosinte should be accessioned directly and that only endangered populations should be regenerated. In addition, large accessions of 5 kg (or more than 100,000 seed) of teosinte are being put into long term storage for future research using laboratory techniques.

In general, requests for seed of the teosintes will be filled largely from the wild seed that is being held in the active collection, which contains 1.5 kg of every accession. Teosinte seed will be regenerated to meet requests only for populations that have become very small and are nearing extinction. For all other populations, fresh seed of teosinte will be collected during in situ monitoring of populations, which was begun during 1985 in cooperation with staff of INIFAP's maize seed bank and with the national maize program of Guatemala.

In situ monitoring--The aim of this conservation activity is to keep a watch on the populations in their natural habitats. This work is not to be confused with in situ preservation, which would be a much more time-consuming and complicated endeavor. The approach chosen by CIMMYT maize staff and their colleagues in the national programs is simply to make yearly checks on the status of each population. Since most of the monitoring sites are no more than a day's drive from Center headquarters, this clearly is the most flexible and efficient approach and yet provides a sufficient guarantee that the material will be closely watched. If the status of a recognized population appears to be changing and it has been placed in immediate danger of extinction, there will be time for Maize Program staff to respond in cooperation with the appropriate national organizations. In situ monitoring offers other advantages as well. As mentioned previously, the monitoring can be done in conjunction with seed collection. Moreover, since the distribution of *Tripsacum* parallels that of teosinte, scientists can check sites of the former while making the rounds of teosinte populations.

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Table 1. Teosinte populations in Mexico and Guatemala * ● - rare, occurring at a single location; ■ - indeterminate; ▲ stable; ○ endangered.

Population and its status*	Common name	Location	Extent	Habitat
Nobogame ●	*maicillo*	Tarahumare Valley in the Sierra Madre of the state of Chihuahua about 16 km northeast of Guadalupe y Clavo.	No more than 50 km^2 on the valley floor.	Along margins of maize fields and in willow thickets bordering streams.
Durango ●		Valley of Guadiana 10 km outside Durango in the state of Durango	No more than 50 km^2.	Limited to wasteland along irrigation canals.
Central Plateau ■	*maiz de coyote*	Isolated populations throughout the entire central plateau in the states of Jalisco, Michoacán, and Guanajuato. Largest continuous population is in region north of Lake Cuitzeo.	Once a continuous population covering thousands of square kilometers but now occurs in scattered, isolated pockets rarely larger than 10 km^2	Occurs in cultivated fields and along field margins or stone walls and in areas protected from grazing.
Chalco ▲	*accece* or *acece* (unwanted or disagreeable)	Valley of Mexico from Amecameca to Xochimilco, Chalco, and Los Reyes. Isolated populations around Texcoco.	Main population centered in a 300-km^2 area around Chalco.	Found almost exclusively in maize fields as a "mimic" of the maize but also as a weed along roadsides.
Balsas ▲	*maiz de huiscatote* (roadrunner) *maiz de pajaro* (bird) *atzitzintle*	Hills surrounding Río de las Balsas Basin. Population distributed discontinuously, with one located south of Chilpancingo in the state of Guerrero and the other on the northern rim of the basin.	Population south of Chilpancingo covers hundreds of square kilometers, while the other extends over thousands of square kilometers in the states of Guerrero, Michoacán, and Estado de México.	Sometimes observed in maize fields but generally in dense stands on hillsides, especially along gullies or other areas where there is rain runoff.
Oaxaca ●	*Cocoxle* (ground dove or roadrunner)	San Francisco de Honduras, 5 km from San Pedro Juchatengo in the Sierra Madre del Sur of Oaxaca.	No larger than 50 km^2, although there may be outlying, isolated pockets.	Grows on hillsides and in maize fields surrounding the town.
Huehuetenango ■	*milpa de rayo* (where lightning strikes the fields) *salic*	Hills and valleys of the Departamento de Huehuetenango around the Guatemalan town of San Antonio Huista, near the Mexican frontier.	Probably not larger than 300 km^2.	Found along trails, in fields, and on hillsides of deserted *milpas*, or maize fields.
Guatemala	*milpa silvestre* (wild corn) *teocintle*	Distributed discontinuously in southeastern Guatemala across the hills and valleys of Jutiapa, Jalapa, and Chiquimula.	Was once distributed continuously, covering 500 or more km^2, but is now fragmented, the largest population covering no more than 1 km^2	Occurs at small, isolated sites along the margins of fields or in other areas protected from grazing.

Table 2. Two classification systems for *Zea*

Wilkes (1967)	Iltis and Doebly (1984)
Sect. *Euchlaena*	Sect. *Luxuriantes*
	Z. diploperennis Iltis Doebly & Guzman
Z. perennis (Hitchc.) Reeves & Mangelsdorf	*Z. perennis* Hitch Reeves & Mangelsdorf
Z. mexicana (Schrader) Kuntze race Guatemala	
	Sect. *Zea*
	Z. mays subsp. *parviglumis* Iltis & Doebly
race Huehuetenango	var. *huehuetenangensis* Iltis & Doebly
race Balsas	var. *parviglumis* Iltis & Doebly
	subsp. *mexicana* (Schrader) Iltis
race Chalco	race Chalco
race Central Plateau	race Central Plateau
race Nobogame	race Nobogame
Sect. *Zea*	
Z. mays L.	*Z. mays* subsp. *mays*

Table 3. Taxa of the genus *Tripsacum*

	Growth habit	Habitat
T. dacylopides L. Most wide ranging, from eastern USA to Mexico and Central America to northern South America. Species complex with diploid, triploid, and tetraploid members.	Robust clones up to 3 m high, long-lived, die out in center and form rings several meters in diameter.	Waterways and moist areas to open fence rows.
T. floridanum Porter Vasey Everglade region of south Florida and Cuba. Diploid (2n = 36).	Narrow leaved perennial, seldom tall.	Pine hummacks.
T. lanceolatum Rupr. ex Fourn. Western escarpment of Mexico and border of USA at Gila Bend, Texas. Mexican form is more robust than arid zone members of border area, which have been made taxa in their own right.	Robust perennial, forms dense clones.	Stream banks or north slopes of hillsides (Arizona mesic), sympathetic with teosinte on Central Plateau (mesic) in Guanajuato/ Jalisco.

Continued on next page

Table 3. Continued

	Growth habit	Habitat
T. zopilotense **Hernandez and Randolph** Western escarpment of central Mexico, often on steep slopes of shifting talus. Diploid (2n = 36).	Narrow leaved perennial with wirey roots in very zeric zone, seldom over 1 m high in seasonal growth.	Zeric dry hillsides.
T. maizar **Hernández and Randolph** Western escarpment of central Mexico. Diploid (2n = 36).	Tall (4-5 m), wide-leaved colonizer of open sites in moist forest of Pacific slope, thick rhizomes.	In Guerrero is sympathetic with Balsas taxa, teosinte (also true in Oaxaca).
T. pilosum **Scibner and Merrill** Mexico's western escarpment, generally in mesic sites, where *T. maizar* is found.	Very robust clone, generally less tall than *T. maizar*, open fields.	Mesic oak woodland, marginally sympathetic with teosinte in Jalisco/Zacatecas.
T. bravum **Gray** Narrow endemic from pine/oak interface in Valle de Bravo, Edo. de México. Diploid (2n = 36).	1- to 2-m clone with moderate leaf width.	Mesic woodland, not far from Valle de Bravo.
T. latifolium Found in moist zones of Guatemala (Caribbean slope). Tetraploid (2n = 76).	Wide-leaved, robust, tall clone with luxuriant growth.	Moist, protected places.
T. andersonii **Gray** Probably a segregate of *T. latifolium*. Widely grown forage grass, male sterile. Found in Central America.	Mostly found under cultivation, very luxuriant.	Sympathetic with teosinte in Huehuetenango, Guatemala.
T. laxum **Nash** Not to be confused with *pilosum* or *maizar*, because its glabrous leaf sheaths more typical of interior of Mexico and Guatemala. Diploid (2n = 36).	Large (3-4 m), moderately robust, wide-leaved plant. Inflorescence less branded than *maizar* or *pilosum*.	Moist areas.
T. australe **Cutler and Anderson** Separated out of *T. dactyloides* for eastern South America. May be a species complex. Diploid (2n = 36).	Large (4 m), robust clones.	Moist woodlands.
T. peruvianum **de Wet and Timothy** Eastern slope of Andes. Gametophytic apomict (2n = 72, 90, 108).	Stream-side plant in dense forest.	
T. cundinamarce **de Wet and Timothy** Colombia. Diploid (2n = 36).	Very robust, heavy-caned plant found along river banks at moderate elevation.	

The Physiology and Genetics of Seed Ageing

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Abstract

Two maize breeding lines were compared for in-storage survival capacity using mean generation analysis (MGA). The results indicate that a set of nuclear dominant genes determines performance during storage, and the segregation ratios of polyamine levels support this evidence. There was a correlation between high polyamine content and superior storage performance. Segregation ratios of 3:1 indicate dominance for high levels of spermidine and putrescine in the F_2 population. Electrophoretic profiles of megabase fragments from embryo DNA were obtained using pulsed field gel electrophoresis (PFGE). Two genetic lines of Zea mays *L. with markedly different life spans during storage showed electrophoretic DNA polymorphism before and after ageing. A pronounced change in the short-lived line upon loss of viability could indicate DNA breakage associated with this loss. Since PFGE profiles after ageing show discrète regions of DNA, it is suggested that the changes in DNA mobility observed after ageing may be site-specific and are not caused by random breakage. Our experiments suggest the possible involvement of polyamine metabolism and DNA fragmentation in the physiological and genetic characteristics relevant to maize storage.*

With the introduction of high yielding maize varieties in developing countries, postharvest losses have tended to increase, because the new varieties are not as well adapted to postharvest conditions as traditional materials. In Mexico maize (*Zea mays* L.) losses during storage range from 10 to 25% of total production, which in 1984 was approximately 12 million t (FAO 1977; CONACYT 1985). The country's annual certified seed production is estimated at around 150,000 t (Sobrino-Ansa 1985).

It is generally recognized that to reduce postharvest damage requires research aimed at exploiting genetic variability among seed populations (NAS 1978). Among maize seed lines, there are marked differences in survival during storage (Moreno et al. 1978), though little is known about the mechanisms accounting for these differences. Studies on seed quality loss during storage have not traditionally taken advantage of genetic polymorphism within species. By comparing the physiology of lines showing marked differences in susceptibility to seed ageing, performance related changes among cultivars have been detected. This paper reports on advances using this approach to study aspects of seed performance during storage.

Materials and Methods

Two maize lines, NYC0113 X NYPA33 (C2) and NY4503 (C6), were used in these experiments, the former showing good and the latter poor storage characteristics. Both lines, at neutral inbreeding equilibrium, were obtained from the New York Seed Improvement Cooperative at Ithaca. The seeds were surface sterilized twice with 3.5% sodium hypochlorite for 3 min and rinsed with sterilized deionized water. Moisture content was equilibrated for 2 wk at 5°C with a saturated NaCl solution to maintain 75% relative humidity. The seeds were then transferred to an incubator at 30°C and 75% relative humidity. To measure leacheate conductivity (LC), 15 seeds were steeped in 5 ml of sterile distilled water per gram of seed. Readings were made after 20 h of constant agitation at 25°C. These same seeds were used to measure oxygen consumption rate (OCR) at 25°C with a Clark type O_2 electrode. The linear part of the oxygen consumption curve

was used to calculate the OCR. Germination tests were conducted at 25°C in sterile petri dishes with two seed germination blotters (blue Anchor paper 8.5 cm in diameter). Enough water was added to reflect light from the blotter surface, and this was checked every 24 h. Germination was recorded at 5-h intervals. Each experiment was performed at least three times using 50 seeds per replication. Seeds with a 5-mm protrusion of the radicle were considered germinated.

Analytical Measure of the Vigor of Germination

A new quantitative assay for seed vigor was used to evaluate storage performance. It was developed from the product of two parameters: the maximal rate of germination ($\triangle$max %G/t), and the ratio of maximal achievable germination (%G_∞) and the time to reach it (t_∞). Two linear functions, a Gompertz progression and the probit transform of the kinetics of germination, allow analytical estimation of these values. As a measure of the vigor of germination (VG), we have proposed the relationship: VG = ($\triangle$max %G/t)(%G_∞/t_∞) (Lozano and Leopold, in preparation).

VG was significantly different for the two lines throughout the entire experiment (Figure 1). During equilibration viability did not decline, and there were no differences in viability among seed lines. Even when the germination rate (%G) remained constant during equilibration, VG values showed a marked difference between lines of good and bad storage characteristics. Loss of vigor can be precisely described as a function of germination rate, leakage, and respiration during storage.

The analytical VG has proven sensitive to vigor differences between seed lots over a wide range of percent germination values. The VG can be considered a linear function (R2 = 0.99) of storage time, OCR, and LC. Thus, it should be an excellent analytical measure of seed lot quality. The proposed VG has the advantage of relying on values based on the response distribution, location in time, and dispersion related parameters, which are important properties in a vigor test (Scott et al. 1984). We now have a computer program that can convert experimental data into VG values for germination or emergence tests. This program, prepared by Errol Jones, is available upon request.

Changes in Physiological Characteristics During Storage

Evaluation of the performance during storage of maize seed lines with different storage characteristics could give us some clues about the physiological basis of seed deterioration. We are also interested in accurately measuring and predicting seed quality and its decline during storage.

Neither OCR nor LC differed between seed lines during equilibration or storage at 30°C. Coleoptile growth decreased during storage but did not significantly differ for the two cultivars. The results of these experiments show that the kinetics of the several physiological indicators of seed quality that change during storage are even more important than simultaneous measurement of these parameters. This approach may allow us to detect parameters related to storage performance by comparing seed lines with markedly different storage characteristics.

Lipoxygenase Activity During Seed Storage

Lipid peroxidation has been considered a central component of seed ageing (Harrington 1970; Villiers 1973; Wilson and MacDonald, in press). Products of lipid peroxidation have pronounced effects on other cellular systems (Priestley 1986). DNA, for example, is denatured by these

effects on other cellular systems (Priestley 1986). DNA, for example, is denatured by these products (Reiss and Tappel 1973; Fujimoto et al. 1984). Lipoxygenase (LOX) activity has been detected at very low moisture contents (Brockman and Acker 1977) well within sorption region I (Leopold 1986); therefore, free water might not be an absolute requirement for this activity (Potthast 1978). Differences in LOX activity could explain differential germplasm survival.

The LOX activity of dissected maize embryos was measured for the two populations (C2 and C6) of contrasting storage performance at 30°C and 75% relative humidity. Embryos were taken from seeds under optimal storage conditions and from seeds subjected to accelerated ageing until their %G fell below 5%. LOX activity was gauged by the initial linear increase in OCR upon adding linoleic acid (LA). Oxygen uptake rate was measured with a Clark type O_2 electrode (Yellow Springs Instruments) fitted to a 2-ml reaction vessel (Gilson Instruments) at 25°C. Individual, homogenized, dissected embryos from dry maize seeds were added to the vessel, using 100 microliters (ul) of homogenate (x mg embryo/ 10x ul of phosphate buffer pH 6). After equilibration was reached, 100 ul of LA solution (3.6 mM) were added to the O_2 reaction vessel to initiate O_2 uptake. LA was combined with Tween 20 (1:1 v/v) under nitrogen in degasified solutions and kept frozen at -10°C in 1-ml aliquots. Three readings of the pooled, freshly dissected embryos were taken and averaged.

Two LOX isozymes were detected in each population (Figure 2). Optimal LOX activity was detected at pH 6 and 7.5 in the good performer (C2) and at pH 5.5. and 7.5 in the poor performing (C6) population. LOX activity measured in individual embryos is highly variable for both isozymes. The coefficients of variation were 10% for the C2 and 45% for the C6 population. LOX activity in C2 seeds remained constant for the first 20 days of storage. After 50 days there was a 10-fold decrease in LOX activity, even though the germination rate remained constant. A concomitant decline in VG was also observed (Figure 3). In contrast, the decrease in LOX activity in the poor performer was detected only after the germination rate began to drop. The decrease in vigor precedes that in LOX activity (Figure 4). We suggest that in these maize lines no correlation exists between LOX activity and seed performance during storage.

Evaluation of the Genetic Stability of Seed Populations by Mean Generation Survival Analysis

Assessing genetic stability characteristics is important in selection and population studies. Mean generation survival analysis (MGSA) can be used to estimate the genetic components for seed survival under constant storage conditions. The seeds must be maintained under aseptic conditions to allow a true estimate of genomic stability among different populations. Since a negative cumulative normal distribution accurately describes seed survival, the slope of the probit linear equation is equal to the inverse of the standard deviation of the speed of seed deaths (Roberts and Ellis 1982). Those authors showed that this slope is a function of controlled storage conditions and the genetic makeup of the stored population.

The survival kinetics were determined for each of the two parental populations (C2 and C6), the F_2 generation, and the two backcrosses of the F_1 to each parental population. The two parental populations, C2 as female and C6 as male, were crossed by controlled pollinations, and an F_2 was obtained by random sampling of seeds from 50 selfed F_1 plants. The F_1 was backcrossed to each parental population to obtain the F_1 x C2 and the F_1 x C6. Seeds were stored aseptically at 30°C and 75% relative humidity. At least 10 determinations involving three samples of 36 seeds each were germinated for each variety to assess survival.

The MGSA of these populations shows that the ageing characteristics of seeds in storage are regulated by nuclear dominant factors (Figure 5) and that superior storage quality is inherited as a dominant trait. No maternal inheritance patterns were detected.

Polyamine Titers as a Storage Index for Maize

The polyamine (PA) contents of embryos from MGSA populations were measured to identify seed survival mechanisms (Lozano et al. 1988). Many physiological functions of PAs may result from the pronounced cationic nature of these molecules, which are strongly protonated at physiological pHs (Herbst and Tanguay 1971). They can thus interact electrostatically with nucleic acids, notably polyanionic molecules, as well as with ionized functional groups in proteins or DNA (Slocum et al. 1984). Because of PAs' ability to stabilize DNA structure and supramolecular conformation (Flink and Pettijohn 1975; Behe and Felsenfeld 1981) and to strongly influence DNA metabolism (Kaur-Sawhney et al. 1980; Shoemaker et al. 1983; Sen and Ghosh 1984), we suggest that PAs may enhance the ability of seed populations to survive during storage.

Embryos from individual kernels were weighed and placed in 200 ul of cold 5% perchloric acid and homogenized. After centrifugation (1,250 x g), 100 ul of supernatant were used for HPLC polyamine analysis. PAs were benzoylated using a modified version of Redmond and Tseng's method (1979). HPLC analysis of the benzoylated PAs and standards was performed using a programmable Varian model 5500 liquid chromatograph. The benzoylated PAs were cromatographed through a 4.6- x 250-mm, 5 micromolar, C18 reverse phase column (ODS ultrasphere, Beckman) and eluted with a water:methanol gradient. A Hewlett-Packard 3390A integrator was used to quantitate polyamines.

The segregation ratios for PA levels were measured in dissected embryos by assessing individual embryo titer value in relation to the confidence interval of the sample population ($P>0.95$). A correlation was found between high PA contents and superior survival characteristics within the MGSA populations analyzed. High PA titers and high seed survival showed the same inheritance pattern (Figure 6). The segregation ratio was 3:1 dominant for high levels of spermidine (SPD) and putrescine (PUT), but SPM content was not related to the survival inheritance pattern. Given the survival kinetics of the MGSA populations, it is suggested that both PA titers, SPD and PUT, and seed survival characteristics are determined by nuclear factors. No maternal inheritance effects were detected for PA titers or mean generation analysis survival. On the strength of these results, we would emphasize the potential of SPD and PUT titers as an index of storage performance, as well as the fact that the character for their presence breeds true, with high heritability.

Evaluation of DNA Integrity During Seed Storage

The instability in the nucleic acids of seeds during storage has been evaluated in terms of chromosomal breakage (Roos 1982) or the electrophoretic mobility of DNA extracts (Osborne 1982; Cheah and Osborne 1978). The fraction of damaged chromosomes and the change in the electrophoretic mobility of spoolable DNA has been studied during seed deterioration. The relationship between moisture content and chromosomal aberrations during seed ageing has not been fully studied. The electrophoretic pattern of spoolable DNA does change as seed viability falls below 30%. To detect early changes in DNA stability, the resolution of electrophoretic techniques must be increased. One method is pulsed field gel electrophoresis (PFGE), which can

separate DNA molecules of up to 9 million base pairs (Mbp) (Schwartz and Cantor 1984; Carle and Olson 1984, 1985, and 1987). An important improvement has been the use of DNA samples of nearly intact chromosomal size (Smith and Cantor 1987; Carle and Olson 1987) instead of spoolable DNA preparations.

In order to determine the megabase DNA electrophoretic profile of aged maize embryos, whole seeds of the C2 and C6 genotypes were stored under aseptic conditions at 30°C and 75% relative humidity. After a month in storage, the C2 genotype showed 92% germination and the C6, 0%. A nuclear fraction was prepared from dry embryos by differential centrifugation (Spiker et al. 1983), and the number of nuclei obtained were assessed by DAPI fluorescence (Brunk et al. 1979). The nuclei fractions were embedded in 0.4% low-gelling-temperature agarose (gel inserts) and digested with protease and RNAse in situ. During processing the osmoticum was kept high. Calcium chelators were used and the pH maintained above 8 in order to eliminate DNAse activity and preserve DNA integrity.

The DNA electrophoretic profile of these samples was obtained using PFGE at a constant voltage (6 V/cm), 0.7% agarose. The frequency of field inversion was linearly decreased from 60 to 24 s in the forward direction and from 20 to 8 s in the opposite direction. The total running time was 12 h. After ethidium bromide staining, the gels were photographed, and the actual picture was density scanned using a digital scanner. This serves as a preliminary analysis of the PFGE profiles.

The electrophoretic profile of megabase DNA from viable embryos was obtained for the MGSA parental populations described above (Lozano and Leopold 1988). Lower DNA stability was observed in short-lived maize embryos. The PFGE profiles for the two genetic lines show electrophoretic DNA polymorphism before and after ageing. Since the electrophoretic patterns obtained before and after storage show discrete regions of DNA mobility, we suggest that DNA fragmentation is not caused by random breakage processes. The short-lived line displays an increase in the number of these well-defined bands upon loss of viability (Figure 7).

Acknowledgments

This research was funded by grants from the IBPGR and PSPA-UNAM.

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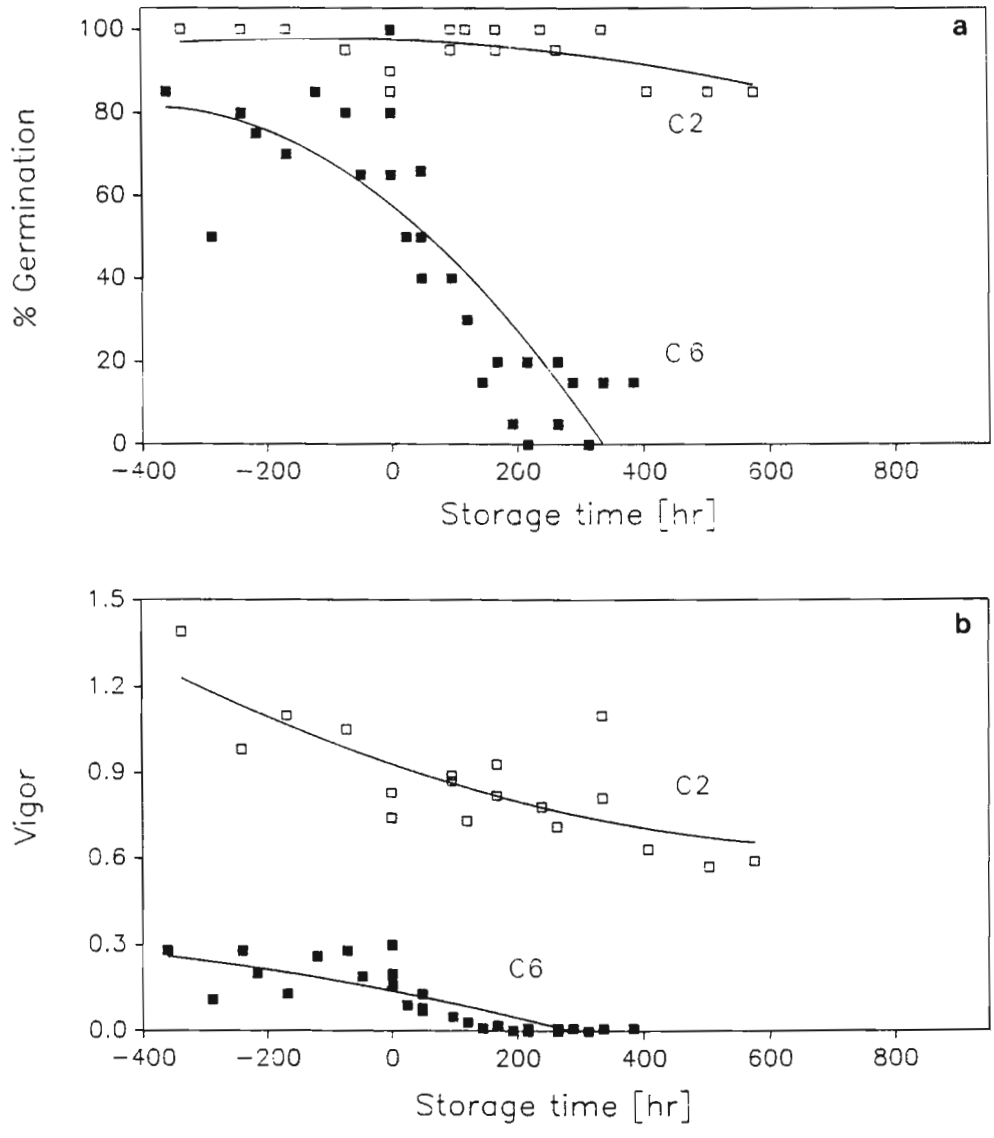


Figure 1. The performance of whole maize seeds aseptically stored at 30°C and 75% relative humidity, showing percent germination and vigor of germination for two lines with good (C2) and poor (C6) storage characteristics. Each point represents the average of three independent replications. Negative time values correspond to a moisture content equilibration period at 5°C. Positive values indicate storage at 30°C. Percent germination was determined at t_{∞}, the time at which no further increments in %G are expected.

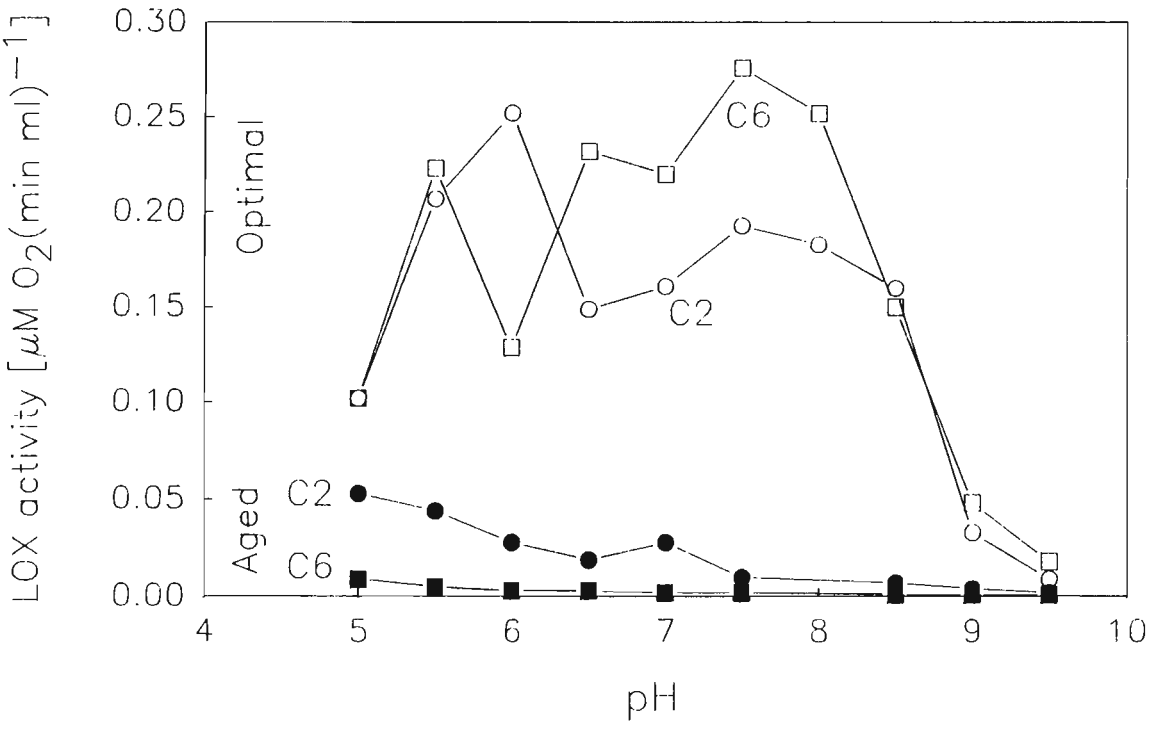


Figure 2. Lipoxygenase activity as a function of pH for dissected embryos of two maize lines with good (C2) and poor (C6) storage characteristics from seeds under optimal storage conditions and seeds subjected to accelerated ageing. Each point represents the average of three readings of the pooled, dissected embryos.

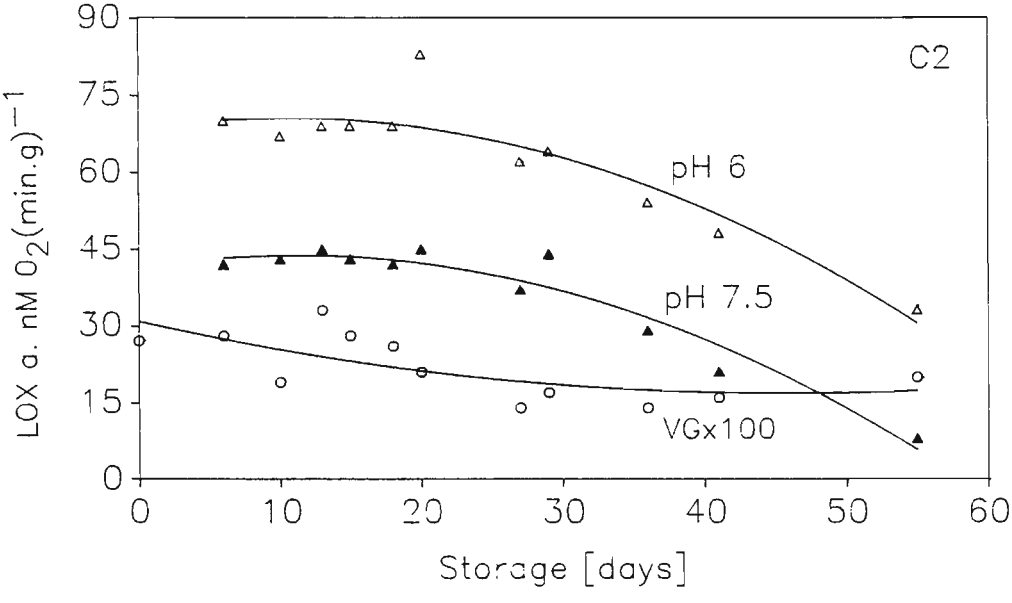


Figure 3. Lipoxygenase activity (LOXa) at pH 6.0 and 7.5 of individual C2 (good storer) embryos as a function of storage time, with simultaneous measurements of vigor. Each point represents the average LOXa of at least 10 dissected embryos.

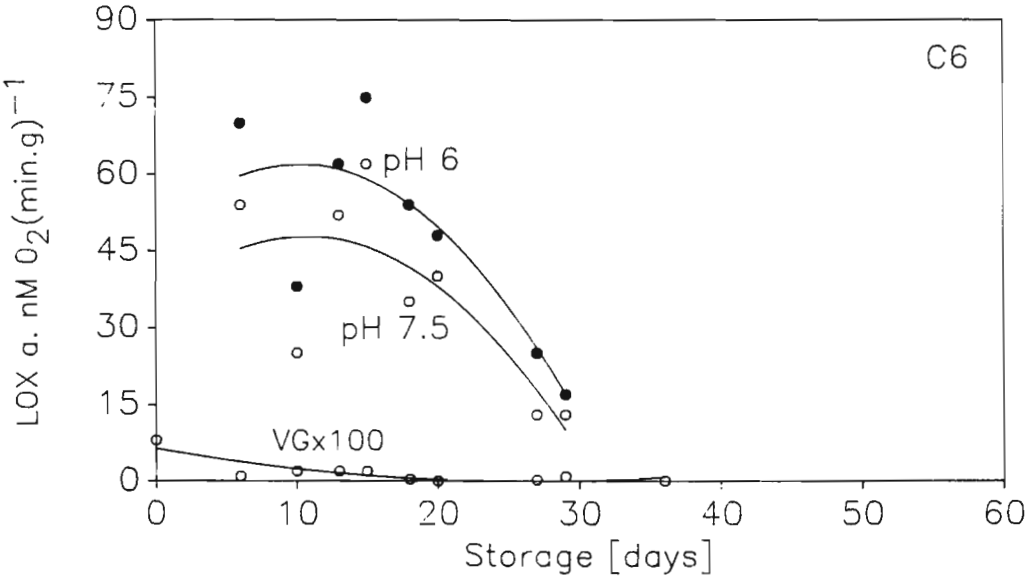


Figure 4. Lipoxygenase activity (LOXa) at pH 6.0 and 7.5 of individual C6 (poor storer) embryos as a function of storage time, with simultaneous measurements of vigor. Each point represents the average LOXa of at least 10 dissected embryos.

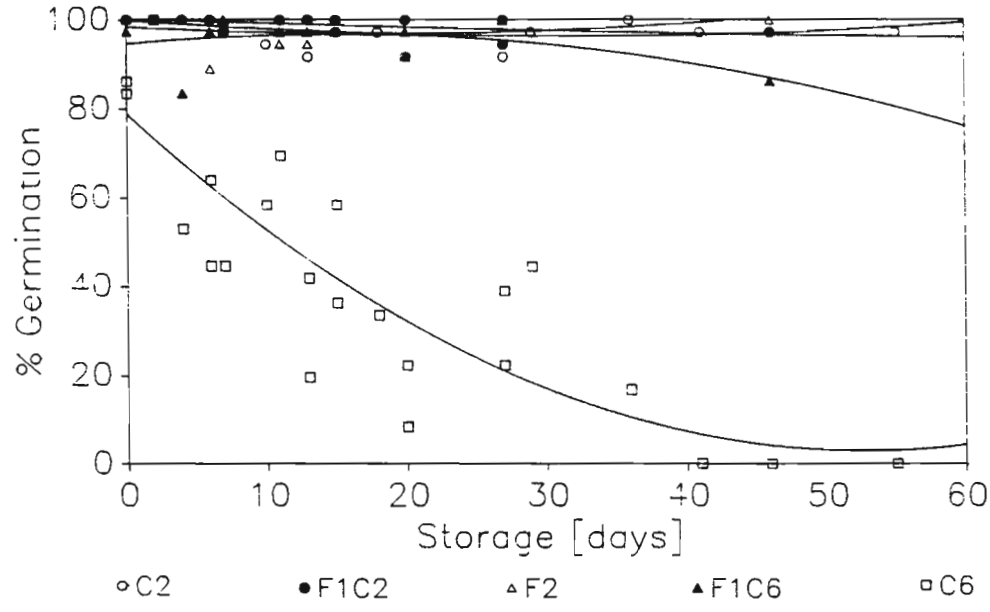


Figure 5. Survival curves of maize lines during storage for the two parental populations C2 and C6, the F_2 generation, and the two backcrosses of the F_1 to each parent. Each point represents the germination of three samples of 36 seeds each.

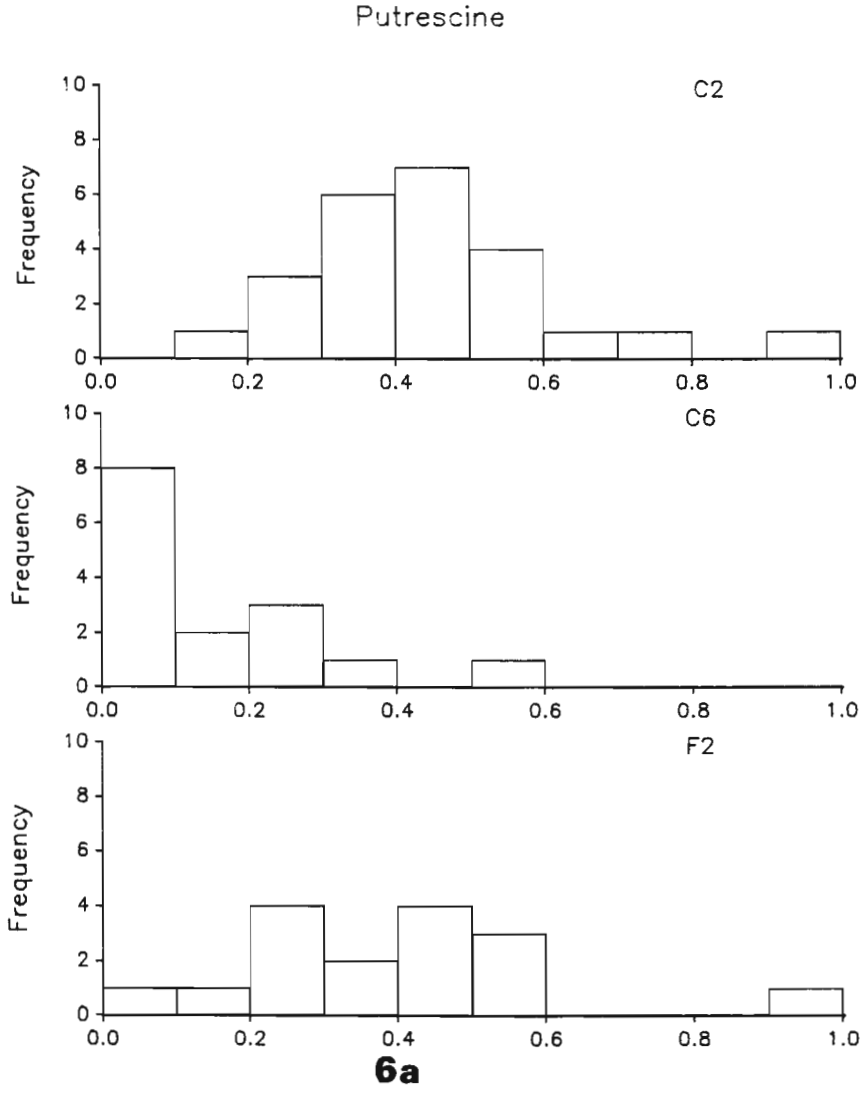
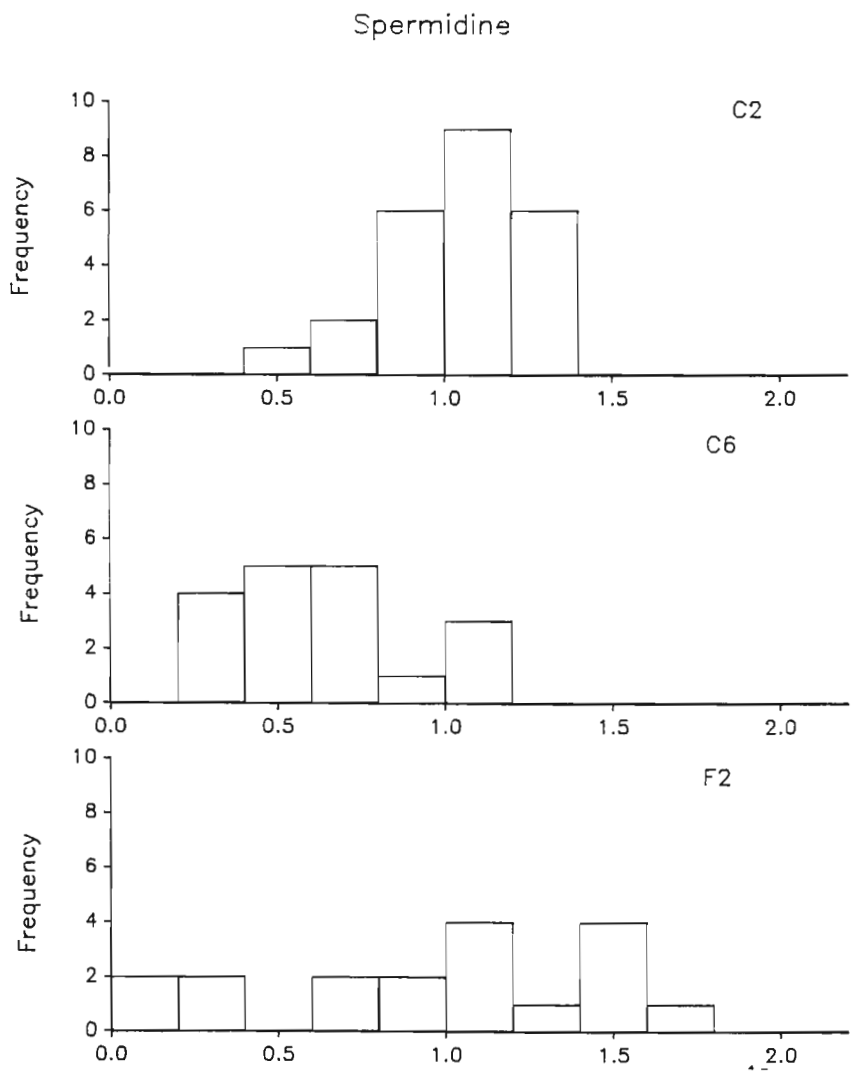
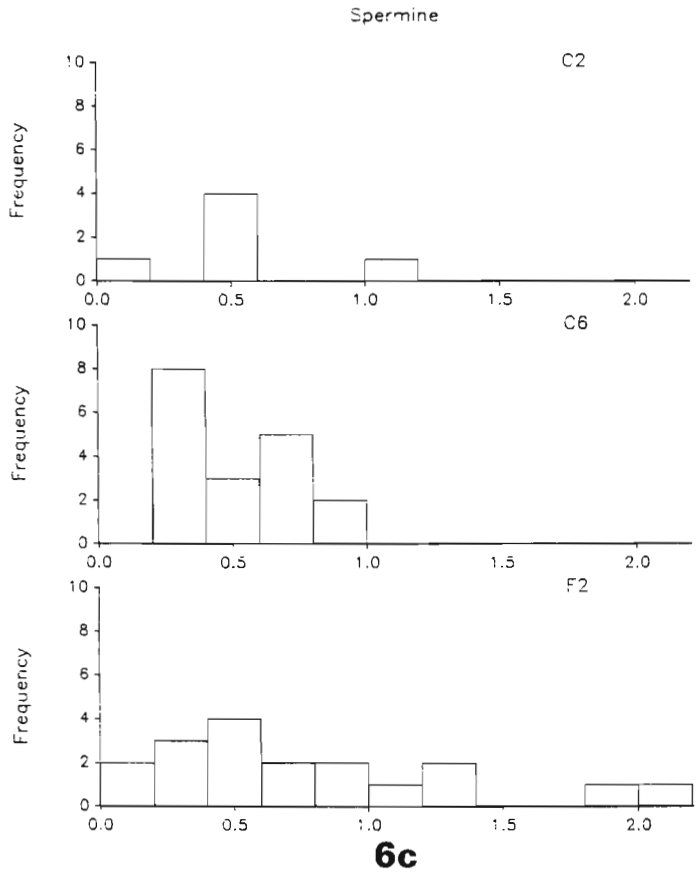


Figure 6. Density distribution of polyamine titers for mean generation survival analysis (MGSA) populations. Frequency distributions were constructed from individual embryo polyamine titers.


Spermidine
C2
C6
F2
Frequency
Frequency
Frequency
10
8
6
4
2
0
0.0
0.5
1.0
1.5
2.0

6b



6c

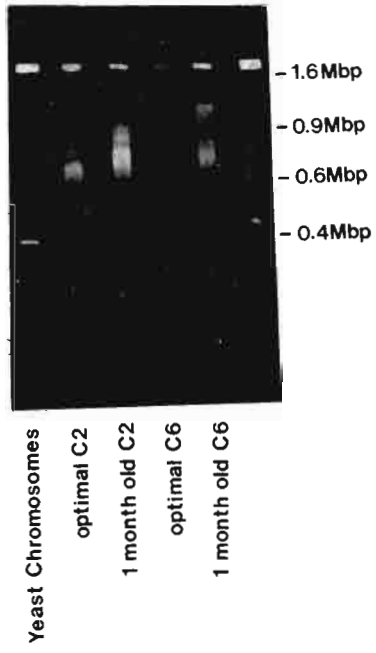


Figure 7. Pulsed field gel electrophoresis of intact nuclei preparations from the C2 and C6 lines.

Comparison of Mexican Maize Races Stored Under Adverse Humidity and Temperature

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Abstract

*A total of 105 collections of 39 Mexican maize (*Zea mays *L.) races, with eight replications of 100 seeds each, were stored for 90 days at 85% relative humidity and 27°C. At the end of the storage period, wide variation was observed in the germination of the different races and collections. On the basis of germination data, the collections were classified as "resistant," "intermediate," and "susceptible" according to their ability to withstand adverse storage conditions.*

Mexico possesses a wide range of maize genotypes, which have adapted over the centuries to highly varied ecological niches. It is important to know the degree of variation in these materials for ability to maintain viability under storage.

Materials and Methods

A total of 105 collections of maize races were increased by the germplasm bank of the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP) at Celaya, Guanajuato. All of the collections were increased during the same growing cycle and therefore under the same growing, harvest, and postharvest conditions. At the outset of the study, the seed of the 105 collections had germination rates of 90-98% and moisture content of 9-11%. The moisture percentage was achieved by drying two samples of 5-10 g from each replication in a forced-draft oven at 103°C for 72 h (USDA 1976). None of the collections showed evidence of storage fungi, indicating that they were properly handled from the time they were harvested until they were placed in storage.

The germination percentage was tested by placing 100 seeds from each of the eight replications between humid paper towels and incubating them at 25°C for seven days. Normal germinating seeds were counted after four and seven days. Eight replications of 100 seeds each from each collection (a total of 800 seeds) were randomly distributed in chambers with relative humidity at 75%, which was maintained with a saturated solution of potassium chloride (Wink and Sears 1950), and temperature at 26°C. Single samples were taken after 90 days of storage to determine germination percentages.

Bayesian statistical analysis (De Groot 1970) was used to divide the 105 maize collections into three categories: 1) resistant (with germination higher than 75%), 2) intermediate (40-75% germination), and 3) susceptible (germination less than 40%).

Results and Discussion

According to our classification criteria, 31 collections of 17 races were designated resistant (Table 1), 54 collections of 28 races as intermediate (Table 2), and 20 collections of 10 races as susceptible (Table 3). The data obtained in this study show that great differences exist between races in the ability of their seed to maintain viability. The majority of the collections classified as

resistant are of tropical adaptation, and dent is the predominant grain type among resistant materials. On the other hand, the collections classified as susceptible are from intermediate or high elevations and possess floury or sweet grains. These findings are indicative of the selection brought about by the tropical environment in varieties stored under conditions of high humidity and temperature, which favor deterioration in the ability of maize seed to germinate.

In addition, variability was observed in collections from a single race, even though all seeds were sown, cultivated, and harvested in the same cycle and the same plot. This suggests that there are genetic differences between such collections, which are manifested in their varying longevity in storage. This variability should be taken into account in the maintenance and multiplication activites of germplasm banks.

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Table 1. Maize race collections showing resistance to adverse storage conditions

Race	Collection	Pr ($p \geq 0.75$)	Germination percentage[a]
Tepecintle	Oaxaca 177	1.000	91.0
Dzit Bacal	Chiapas 447	1.000	87.8
Tuxpeño Norteño	Chihuahua 50	1.000	87.6
Tamaulipeco	Nuevo Leon 37	1.000	84.8
Vandeño	Chiapas 114	1.000	84.8
Vandeño	Chiapas 30	1.000	84.8
Nal-Tel de Alt.	Oaxaca 301	1.000	83.5
Zapalote Grande	Chiapas 236	1.000	82.8
Celaya	Guanajuato 265	1.000	80.8
Dzit Bacal	Veracruz 96	1.000	80.6
Tuxpeño Norteño	Coahuila 21	1.000	79.6
Tuxpeño Norteño	Chihuahua 13	0.998	78.9
Celaya	Guanajuato 101	0.990	78.0
Zapalote Grande	Chiapas 521	0.987	77.9
Tamaulipeco	Tamaulipas 3	0.972	77.4
Conico Norteño	Querétaro 9	0.966	77.3
Zapalote Grande	Chiapas 664	0.942	76.9
Tamaulipeco	Tamaulipas 29	0.942	76.9
Tuxpeño Norteño	Chihuahua 121	0.920	76.6
Tuxpeño	Oaxaca 9	0.797	75.8
Tuxpeño	Tamaulipas 125	0.748	75.5
Tamaulipeco	Coahuila 25	0.722	75.4
Reventador	Nayarit 39	0.722	75.3
Chapalote	Sinaloa 2	0.693	75.2
Zapalote Chico	Oaxaca 52	0.635	75.0
Chalqueño	Hidalgo 7	0.604	74.9
Tehua	Chiapas 204	0.604	74.9
Comiteco	Chiapas 340	0.518	74.5
Palomero Toluqueño	Chihuahua 150	0.467	74.4
Celaya	Guanajuato 88	0.467	74.4
Cónico Norteño	Guanajuato 16	0.467	74.4

[a]Mean for eight replications of 100 seeds each.
Mean of standard deviation for the resistant group = 4.65.

Table 2. Maize race collections showing intermediate performance under adverse storage conditions

Race	Collection	Pr (0.4<p<0.75)	Germination percentage[a]
Elotero Sinaloa	Sinaloa 17	0.623	74.0
Cónico Norteño	Guanajuato 22	0.710	73.6
Palomero Toluqueño	Chihuahua 135	0.850	72.9
Cónico Norteño	Guanajuato 165	0.844	72.6
Onaveño	Sonora 105	0.912	72.4
Coscomatepec	Veracruz 404	0.953	71.9
Celaya	Guanajuato 69	0.980	71.2
Tepecintle	Chiapas 76	0.989	70.9
Tabloncillo Perla	Nayarit 16	0.989	70.9
Mushito	Michoacán 328	1.000	69.4
Chalqueño	Guanajuato 167	1.000	69.4
Motozinteco	Chiapas 650	1.000	69.0
Cónico Norteño	Zacatecas 246	1.000	68.2
Cónico Norteño	Zacatecas 218	1.000	68.1
Jala	Nayarit 54	1.000	68.1
Tabloncillo Perla	Sinaloa 12	1.000	67.9
Cristalino de Chihuahua	Chihuahua 154	1.000	65.6
Bolita	Oaxaca 44	1.000	65.4
Chalqueño	Durango 241	1.000	65.4
Chalqueño	Zacatecas 251	1.000	64.4
Chalqueño	Mexico 35	1.000	63.9
Cónico Norteño	Guanajuato 73	1.000	63.6
Cónico Norteño	Guanajuato 50	1.000	63.1
Chapalote	Sinaloa 6	1.000	62.4
Reventador	Sinaloa 60	1.000	62.1
Cónico Norteño	Guanajuato 34	1.000	61.9
Bofo	Durango 94	1.000	61.6
Nal-Tel de Alt.	Oaxaca 310	1.000	61.2
Arrocillo	Veracruz 359	1.000	60.9
Blandito	Sinaloa 61	1.000	60.9
Arrocillo	Veracruz 308	1.000	60.8
Celaya	Guanajuato 36	1.000	59.9
Tabloncillo Perla	Nayarit 41	1.000	59.0
Oloton	Chiapas 238	1.000	58.6
Chalqueño	Puebla 87	1.000	57.5
Bolita	Oaxaca 205	1.000	57.2
Cristalino de Chihuahua	Chihuahua 254	1.000	57.2
Elotes Conicos	Querétaro 618	1.000	56.1
Chalqueño	México 37	1.000	56.1
Mushito	Michoacán 371	1.000	54.8
Tabloncillo	Jalisco 100	1.000	54.7
Zamorano	Michoacán 66	1.000	54.2
Dulcillo	Sinaloa 34	1.000	53.9

Continued on next page

Table 2. Continued

Race	Collection	Pr (0.4<p<0.75)	Germination percentage[a]
Conejo	Guerrero 17	1.000	53.8
Chalqueño	Zacatecas 4	1.000	53.6
Bofo	Durango 95	1.000	51.5
Cónico Norteño	Guanajuato 68	1.000	50.4
Cónico Norteño	Guanajuato 42	1.000	50.2
Bolita	Oaxaca 40	1.000	48.9
Dulce	Jalisco 300	1.000	47.1
Cristalino de Chihuahua	Chihuahua 128	1.000	46.9
Jala	Nayarit 6	1.000	46.9
Elotes Occidentales	Querétaro 94	1.000	42.9
Tabloncillo	Jalisco 269	0.842	42.2

[a]Mean for eight replications of 100 seeds each.
Mean of standard deviation for the races rated intermediate = 5.33.

Table 3. Maize race collections susceptible to adverse storage conditions

Race	Collection	Pr (p≤0.40)	Germination percentage[a]
Elotes Cónicos	Puebla 510	0.644	39.8
Bolita	Oaxaca 28	0.927	38.0
Zamorano	Michoacán 5	0.936	37.9
Bolita	Oaxaca 180	1.000	36.4
Tabloncillo	Jalisco 43	1.000	35.0
Bofo	Nayarit 191	1.000	34.0
Tabloncillo	Jalisco 63	1.000	33.2
Bolita	Oaxaca 221	1.000	33.1
Jala	Nayarit 131	1.000	32.5
Dulce	Jalisco 304	1.000	30.9
Elotes Occidentales	Zacatecas 210	1.000	26.6
Dulce	Zacatecas 182	1.000	25.1
Dulce	Jalisco 78	1.000	23.5
Zamorano	Guanajuato 191	1.000	23.1
Ancho	Guerrero 307	1.000	21.5
Bofo	Jalisco 289	1.000	21.1
Ancho	Guerrero 326	1.000	15.0
Elotes Occidentales	Zacatecas 180	1.000	14.9
Pepitilla	Morelos 102	1.000	10.2
Pepitilla	Morelos 99	1.000	7.1

[a]Mean for eight replications of 100 seeds each.
Mean of standard deviation for susceptible races = 4.96.

Active Collections Network: A Proposal for Maize Germplasm Conservation and Use

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Abstract

The current global network, focusing on base seed collections, is described, followed by an explanation of the functions of active collections, a summary of past maize collection activities, and discussions of the current status of maize genetic resources and of the Latin American Maize Project. A proposal is presented for establishing a global network of active collections, whose central feature would be a database providing all cooperators with ready access to information about the location and composition of collections worldwide.

Genetic resource centers (GRCs) are primarily responsible for conserving the genetic diversity of crop plants and making genetic resources available to those who can evaluate and use them. Since the 1960s and early 1970s, these centers have handled either base collections of germplasm, active collections, or both. Base collections are designed for long term conservation of genetic resources, whereas the day-to-day requests of plant breeders and other scientists for germplasm samples are normally met with material from active collections. Besides the multiplication and distribution of samples, GRCs with active collections are also responsible for regenerating seed stocks in base collections, when required. Although a worldwide, collaborative group of centers has already been charged with maintaining long term base collections of maize germplasm, this paper makes concrete recommendations for establishing a global network of GRCs, served by a central database, to handle active maize germplasm collections.

The Current Global Network

IBPGR was established by the Consultative Group on International Agricultural Research (CGIAR) in 1974 to support, coordinate, and promote an international network of GRCs. The newly formed center immediately implemented programs to further the collection, conservation, evaluation, documentation, and use of crop genetic resources. In the planning and organization of this global network, IBPGR first put major emphasis on building up representative collections of germplasm--especially landrace populations and primitive cultivars--with a view to preserving endangered genetic diversity. Later, emphasis was shifted to the wider gene pool, and IBPGR adopted a priority system--based in part on advice from crop committees cosponsored by IBPGR and CIMMYT--for allocating support to field collection of crops in different regions (IBPGR 1976 and 1981).

In the mid-1970s, few centers had suitable long term storage facilities, and IBPGR promoted the establishment of GRCs that could provide adequate conditions for the collected materials. At the same time, attention was given to long term preservation methods, human resource development through training, and documentation of sample data. In this way a network concept emerged, and in structuring the network IBPGR's strategy was to involve a broad spectrum of the world's scientific community. In the main, national genetic resource programs and global agricultural research centers such as CIMMYT became the basic operational units of the network. IBPGR acted as a catalyst in promoting new programs in both developed and developing nations. By the end of the first decade of IBPGR's operation, the global network had become a reality, involving about 100 collaborating countries and a number of gene banks set up by national programs.

Base Seed Collections

To provide a framework for the global network, IBPGR designated national, regional and international centers as holders of either world or regional base collections for a particular crop or group of species. Backup duplicates of all accessions in a particular base collection are also held in another center--preferably in a different country or even on another continent. Agreements are currently in effect between IBPGR and 39 centers in 31 countries, regarding the international responsibility for base collections of seed crops, including major cereals, legumes, vegetables, forages and a few industrial crops. At the recommendation of the IBPGR and IBPGR/CIMMYT Crop Advisory Committee on Maize Genetic Resources, the following centers have agreed to hold base collections of maize germplasm (IBPGR 1985).

*Institute**	*Scope of collection*
NSSL, Fort Collins (USA)	New World
NIAR, Tsukuba (Japan)	Asian
TISTR, Bangkok (Thailand)	Asian
VIR, Leningrad (USSR)	European
PGB, Braga (Portugal)	Southern Europe
CIMMYT (Mexico)	Backup storage (New World)

*See appendix.

Storage conditions for base collections should promote longevity and thus reduce the need for regeneration. The preferred standards for long term seed conservation are sealed containers at 5 + 2% moisture content (wet weight basis) at a temperature of -18°C or less.

Active Collections

Apart from breeders' working collections, which fall outside the scope of genetic conservation, users' most frequent contact is with active collections. Centers having such collections distribute germplasm to plant breeders and other scientists upon request. IBPGR's policy is to promote free and full availability of germplasm and information. Material from active collections is also used for regenerating samples (a task that should be done in environments as similar as possible to those from which the original material was obtained) to replenish base collection stocks and keep enough material on hand to meet user requests. Finally, centers with active collections characterize and evaluate samples in collaboration with plant breeders and other scientists.

Active collections can be stored under the same conditions as base collections if sufficient resources are available, but less stringent temperature standards are normally applied, since it is assumed that active materials are held only on a medium term basis. Accordingly, they are often stored at 0°-10°C, preferably in sealed containers and at a moisture content similar to that used for base collections.

A major prerequisite for the effective use of active collections is proper documentation of collection data, achieved through database systems permitting the storage and retrieval of information on available germplasm. Passport, management, characterization, and evaluation data should all be entered into the database whenever seed samples are registered at gene banks. Unfortunately, few curators have vigorously sought passport data; hence there are gaps in many database records.

Plant breeding and/or crop improvement centers often have active collections, since these centers can take advantage of in-house multidisciplinary specialists to help evaluate samples. Most base collection centers in the current network are attached to or collaborate with crop improvement programs and maintain active collections as well. Many of the CGIAR centers (including CIMMYT) exemplify this integration of base and active collections with breeding.

The Case of Maize

Maize is a good example of indigenous germplasm from the centers of diversity being well collected, described, and conserved. From 1943 on, the Rockefeller Foundation cooperated with Latin American countries to gather maize germplasm from major centers of diversity, forming the basis for maize collections in Mexico, Colombia, and Brazil. Subsequently, through the efforts of the National Academy of Sciences/National Research Council Committee on Maize, collecting was extended to other countries of South America, most of Central America, and the major islands of the Caribbean. Efforts were also made to augment the North American maize collection and duplicate the Latin American maize collections of the NSSL (Brown 1975). By 1955 a total of about 12,000 samples had been assembled. From these accessions approximately 250 races were named in a series of monographs (Goodman 1983). While parts of Africa, Asia, and Europe are considered secondary centers of diversity, by far the greatest variation in maize is represented by Latin American races.

Following this work, there were several years of limited activity in maize germplasm preservation, and a lack of financial and technical support resulted in the loss of some materials. After the establishment of IBPGR, additional collections were made in Latin America and parts of Asia, Europe, and Africa. About 15,000 samples in all have been collected from different countries of Latin America, Africa, and Asia (Reid and Konopka, these Proceedings).

Current Status of the Collections

Major collections of Latin American maize germplasm are currently held at the following institutes.

Institute	*Number of accessions*
CIMMYT (Mexico)	12,500
INIFAP (Mexico)	9,000
ICA (Colombia)	6,000
NSSL (USA)	5,000
NCPRIS (USA)	4,225
PCIM (Peru)	3,444
INTA (Argentina)	3,000
CIF (Bolivia)	2,200
EMBRAPA (Brazil)	2,150
INIA (Chile)	855
ICTA (Guatemala)	800
UR (Uruguay)	600
IAN (Paraguay)	400

In addition, significant collections of maize germplasm are kept at:

Institute	*Number of accessions*
VIR (USSR)	15,000
NIAR (Japan)	2,650
PGRC (Canada)	1,700
IARI (India)	1,750
INIA (Spain)	1,200
PGB (Portugal)	1,100
ORD (South Korea)	3,000

Other GRCs in Africa, Asia, and Europe possess various amounts of maize germplasm, including old cultivars from Latin America and some local collections. Many of these centers have inadequate storage facilities, and detailed documentation of the samples is not available. There could be extensive duplication in these collections. Of the 20,000 to 25,000 maize germplasm accessions in the Latin American collections, only about one-fourth are deposited in the base collection at NSSL, USA. Similarly, the collections from several national programs in Europe and Asia have not been properly duplicated in other designated base collections.

A detailed examination of the maize collections around the world reveals that maintenance has never been accorded high priority, and consequently some collections have been lost (Goodman 1983). In many cases there is an urgent need to regenerate and multiply samples. Adequate storage facilities should be a priority, and duplicate sets of materials should be kept in an IBPGR designated base collection at a center other than the one where the original set is held.

Many national programs, backed by the technical and financial support of IBPGR and some bilateral donors, have recently built facilities of either the cold storage or deep freezer type. Both are equally suitable when used with the appropriate, IBPGR recommended technology for seed drying, seed packaging, and long and medium term sample storage. The first priority is reducing seed moisture content. It was once generally believed that maize seeds are damaged when their moisture content is brought below 10%, but Ellis and Roberts (personal communication) have shown in several studies that most species can be dried to a moisture content of 5-7%, and they are currently working to verify an even lower limit for maize seed. Once dried, material should be stored in hermetically sealed containers.

The CIMMYT Maize Germplasm Bank

The largest maize collection is maintained by CIMMYT (about 12,500 accessions) and consists of the original Rockefeller collections and selected collections from other centers. The CIMMYT collections appear to suffer major geographical gaps, poor representation of some germplasm (Goodman 1983), and a backlog of accessions in need of seed increase. Policy at CIMMYT has recently changed, however, and the center has computerized all the available passport information on its collection and is in the process of publishing a catalog of accessions from Central America and the Caribbean. CIMMYT has also agreed to provide backup base storage for New World materials.

Latin American Maize Project

In 1986, GRCs from Bolivia, Brazil, Chile, Colombia, Guatemala, Mexico, Paraguay, Peru, Uruguay, the USA, and Venezuela joined the US Department of Agriculture (USDA) in a five-year cooperative program--called the Latin American Maize Project (LAMP)--to characterize and evaluate Latin American maize germplasm collections. The USDA Agricultural Research Service receives US$1.5 million (US$300,000 per annum for five years) from Pioneer Hi-bred International, Inc., to administer LAMP, which is carried out primarily by Latin American scientists. The Pioneer grant will greatly expedite and enhance the assessment of the valuable diversity in about 15,600 accessions of maize germplasm from the centers of greatest genetic diversity. This project lays out a specific plan for rescuing (through multiplication), evaluating, and conserving Latin American maize germplasm. The principal researchers involved in the project held their first meeting at INIA, Santiago, Chile, in 1987 to assess the progress made during the first year and to finalize plans for the future. Those attending pointed out the urgent need for better seed storage facilities to guarantee the security and availability of germplasm.

Proposal for a Network of Maize Genetic Resources Centers

The efforts of CIMMYT, LAMP, and another IBPGR program in South America have laid the basis for a collaborative network of centers. Priority must be given not only to the security of materials in collections but to the availability of materials and information for users. In such an endeavor, a crucial element is the cooperation of all GRCs holding collections of local, regional, and international importance. Moreover, IBPGR believes that a central database is the keystone of any international genetic resource network, enabling that network to carry out more effective conservation and render better service to users.

In planning a network of active centers for a given crop, the following points are important:

- Basic documentation on the collections of a particular crop spread among various national, regional, and international centers
- Assurance of secure long term storage for samples
- Full availability of materials and information to bona fide users

Obviously, documentation plays a pivotal role. The experience of IBPGR attests to the importance of a central database, located at an institute having adequate computing facilities, for each major crop. The database would provide ready access to information on *who* holds *what* and how the variation is partitioned, allowing curators to allocate their work in a mutually supportive manner.

Crop databases compile comprehensive information on the dispersed collections in the network and provide a way of assessing the status of collections. They help to identify gaps in collections, redundant duplicates between and within collections (as opposed to backup duplicates), and distinct samples in the individual (national and international) collections. Distinct materials include local landraces or primitive cultivars and their attendant wild relatives, old and obsolete varieties or modern cultivars developed by respective national or international institutions, and any other important germplasm--including breeding lines, genetic stocks, etc.

In a network of centers holding active collections, all genetic resource programs (national and international) with local, regional, or global sets of materials should participate. The network should be *crop-specific*, not national gene bank specific, and partners for each crop in the network will differ. For some crops (especially those with many thousands of accessions in different centers, such as wheat, rice, barley, and maize), it may be necessary to formulate regional groupings to improve coordination and accessibility and to avoid the occasional quarantine problems. Such groupings, however, can only stem from the collective goodwill of scientists and curators.

In such a scheme, each national genetic resources program or international agricultural research center would be responsible for storing and maintaining its materials, meeting national and international user requests, and depositing subsamples of its distinct samples in another collection with long term storage facilities (preferably an IBPGR designated base collection) as backups. Participating centers would be expected to maintain a routine flow of information and updates--including passport, characterization, and evaluation data on their holdings--to the central database. Goals for all GRCs in the network would include:

- An adequate medium term storage facility with a suitable staffing structure for the conservation of germplasm
- A policy guaranteeing free availability of germplasm and information to users
- Reasonably adequate physical facilities--including land, screen houses, etc.--to grow out samples for regeneration, characterization, and evaluation
- Computing facilities suitable for data acquisition, analysis, and exchange
- A close association with plant quarantine authorities
- Cooperative links with plant breeders and other scientists to facilitate evaluation
- Availability of continuous, adequate funding for proper maintenance, characterization, evaluation, and distribution of materials and information

Those attending the present workshop could play an important role in deciding such issues as the size and configuration of the network (i.e., a single network to cover all relevant maize germplasm centers or regional networks to cover New World, African, Asian, and European collections); who decides on participation and funding; and which center(s) will serve as headquarters for the central database and coordination of activities (CIMMYT, for example, could undertake such a responsibility).

Once a central database is in place, participating centers could agree on a strategy for identifying and eliminating redundant duplicates between and among collections, as well as detecting and filling in gaps (through carefully planned field missions) in existing collections. The centers could also be made responsible for the identification, conservation, and exchange of distinct materials, including backup duplication of materials for storage in an IBPGR designated center.

A functional network, however, must be built on current strengths and not on wishes for the future. It is obvious that some national GRCs, especially in developing countries, may need

financial and/or technical support to acquire the expertise and infrastructure essential to a commitment as full partner in the proposed network.

In planning and organizing such a cooperative venture, it is important that IBPGR and CIMMYT act jointly to coordinate and link efforts. Help in setting up the central database could come from IBPGR, and LAMP could serve as a model cooperative venture. Perhaps the proposed network could even be built around LAMP, expanding its coverage to include other Latin American and Caribbean countries. In that case similar efforts could be initiated to cover centers in Europe, Africa, and Asia.

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Appendix: Acronyms for Institutions With Major Collections of Maize Germplasm

Acronym	*Institution and location*
CIF	Centro de Investigaciones Fito-ecogenéticas, Bolivia
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo, Mexico
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuaria, Brazil
IAN	Instituto Agronómico Nacional, Paraguay
IARIA	Indian Agricultural Research Institute, India
ICA	Instituto Colombiano Agropecuario, Colombia
ICTA	Instituto de Ciencia y Tecnología Agrícolas, Guatemala
INIA	Instituto Nacional de Investigaciones Agrarias, Chile and Spain
INIFAP	Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias, Mexico
INTA	Instituto Nacional de Tecnología Agropecuaria, Argentina
NCPRIS	North Central Regional Plant Introduction Station, USA
NIAR	National Institute of Agricultural Research, Japan
NSSL	National Seed Storage Laboratory, USA
ORD	Office of Rural Development, South Korea
PCIM	Programa Cooperativo de Investigaciones en Maíz, Peru
PGB	Portuguese Gene Bank, Portugal
PGRC	Plant Genetic Resources, Canada
TISTR	Thailand Institute of Scientific and Technological Research, Thailand
UR	Universidad de la República, Uruguay
VIR	N.I. Vavilov All-Union Institute of Plant Industry, USSR

Cytological Classification of Maize Race Populations and Its Potential Use

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Abstract

*The ample information available on the chromosome knob constitution of maize (*Zea mays *L.) races is interpreted as follows: 1) maize was domesticated from ancestral populations of the extant Mexican annual teosinte,* Z. mexicana *(Schrader) Kuntze, in several parts of Middle America; 2) each center of domestication gave rise to primordial germplasm with a particular combination of chromosome knobs (knob complex); 3) the majority of knob types were present in more than one of those complexes, but each complex had one or more specific knobs; 4) each complex was dispersed over particular territories, and under varied environmental conditions, diverse varieties and races arose; and 5) when two or more complexes coincided in a given region, intercrossing generated further diversification.*

Based on this interpretation, a means of classifying maize race populations is proposed that combines the traditional, morphological racial classification and information about specific knobs of the complexes. The possibility of using this morphological-cytological classification in investigations of heterosis patterns, combining ability, adaptability, etc., is also discussed, with the aim of determining its value for maize breeding.

Almost three decades ago, research was initiated on the chromosome morphology of the maize (*Zea mays* L.) races cultivated in the Americas. The scope of this work was later broadened to include chromosome analysis of teosinte, *Z. mexicana* (Schrader) Kuntze, populations in Mexico and Guatemala. The point of departure for these investigations was the work of McClintock (1959, 1960), who determined, by analyzing the chromosome knob constitution of representative plants of selected races from Mexico, Guatemala, and South America, that through a similar but more extensive study one could trace the dispersion of maize races and the relationships among them. The results of this work have been published by Longley and Kato (1965), Kato (1976, 1984), McClintock (1978), and McClintock et al. (1981).

Those studies led to new propositions about the origin of maize and its races and the relationships among them and with teosinte. The available information was studied using numerical analysis by Smith and Goodman (1981) and Smith et al. (1982). But none of the findings have been applied toward more practical ends, especially in maize breeding. The purpose of this presentation is to review the results and interpretation of the above-mentioned cytological studies and on that basis to present a new way of classifying populations (collections) within different races and some possible practical applications of this classification scheme.

Origin of Maize and of Knob Complexes

In studies of the knob constitution of American maize and various teosintes, the following patterns were observed: 1) both maize and Mexican annual teosinte tend to have intercallary knobs, and those found in maize have their exact homologue in teosinte, though in teosinte some knobs have been observed in positions not found in maize; 2) Guatemalan teosinte and the perennial teosintes of Jalisco, Mexico, have only terminal knobs, some of which (those in the $4S_2$, 7S, and

9S positions) have also been found in maize and those at $3S_2$, $4S_2$, $5S_2$, 7S, 8S, and 9S in the Mexican annual teosinte.

In maize and Mexican annual teosinte, there are two special chromosomes: abnormal-10, which differs from the normal by having an extra segment with a subterminal knob at the end of its long arm, and the B chromosome. Both the extra segment of abnormal-10 and the B chromosome are additional or supernumerary elements of the normal genome and are of unknown origin. Since neither has been found in Guatemalan teosinte or in the perennials, they have been taken as evidence of the involvement of Mexican annual teosinte in the phylogeny of maize.

It is well known that there is complete homology between the chromosomes of maize and teosinte (except that from southern Guatemala) and that the gene sequence is similar, if not the same, in the genomes of both species. On the other hand, given the evidence that the knobs are very conservative structures from the evolutionary point of view, I have proposed elsewhere (1976, 1984) that maize could have derived from the original populations of the present Mexican annual teosinte through a domestication process and that neither the Guatemalan teosintes nor the perennials were involved in such a process.

When knobs of different types and positions were mapped according to the geographic origin of the maize collections in which they were observed, distinct patterns of distribution were found: 1) some knobs showed more general distribution by region and race, while others showed somewhat more restricted distribution to varying degrees; and 2) the knobs also showed relatively greater concentration in certain races and territories.

Analysis of chromosome knob data in a study of more than 5,000 maize plants of 1,343 collections from all over the Americas identified the geographical regions where knobs of restricted distribution were concentrated. These were considered to be the places in which the domestication of primordial maize germplasm occurred. It is believed that from these distribution or domestication centers domesticated materials were dispersed along particular routes corresponding to human migratory movements during and after domestication. The maize races native to the regions where the knobs appeared more frequently have been considered representatives of the primordial germplasm. This does not necessarily mean that they are "pure" descendants of the maize originally domesticated but that possibly they are closely related to it.

Based on analysis and interpretation of the chromosome knob data, five domestication centers were identified in central Mexico and Guatemala. The germplasm originating in each center had a knob combination different from the others, with some knobs being observed in all cases, some present only in certain combinations, and still others being specific to the original germplasm. These combinations of chromosome knobs in the original germplasm were named "knob complexes," of which there are five: Zapalote, Mesa Central, Pepitilla, Tuxpeño, and Guatemala Highlands.

Knobs that are specific to the Zapalote complex, whose domestication center lies somewhere within the states of Oaxaca and Chiapas, are those found on the short arms of chromosomes 4, 5, and 7 and the medium and large knobs at the position $6L_2$. This germplasm was distributed along the Pacific coast of Mexico in two directions: 1) up to the northwest and 2) to the south toward the lowlands of Guatemala and from there to other Central American countries. In the northwest the specific knobs of this complex appeared to be concentrated in representatives of

various races, including Reventador, Chapalote, Tabloncillo, Harinoso de Ocho, and Vandeño. The influence of this germplasm was also observed in maize populations from southern Veracruz.

The Mesa Central complex, with its distribution center in the highlands of central Mexico, was dispersed mainly toward the northern plains of the states of San Luis Potosi, Zacatecas, Durango, Nuevo León, Coahuila, and Chihuahua and toward the west to the states of Guanajuato, Michoacán, and Jalisco. From there it was taken, together with germplasm of the Zapalote complex, to northwestern Mexico and the Southwest of the USA. Among the races that developed from this complex are Chalqueño, Palomero Toluqueño, Cónico, and Cónico Norteño. The knobs specific to this complex are the large knob on the long arm of chromosome 1; the small, medium, and large ones at the most proximal position of the long arm of chromosome 6 ($6L_1$); and the small knob most distal on the long arm of chromosome 10 ($10L_2$).

The Pepitilla complex, characterized by a large knob at the most distal position on the long arm of chromosome 6 ($6L_3$), has its center of origin in the midhighland regions of Morelos, Guerrero, and Edo. de México. From there it was dispersed mainly to western Mexico in Michoacán and Jalisco, where it joined the maizes of the Zapalote and Mesa Central complexes and together with them was distributed to the north along the Pacific coast. Extant representatives of this complex are the races Pepitilla and Maíz Ancho.

The Tuxpeño complex, possibly domesticated in the Oaxaca-Chiapas region of southern Mexico (independently from the Zapalote complex, which also originated there), is now distributed mainly on the eastern coast of the country and is typically represented by the race Tuxpeño. This germplasm is also found in southern Mexico in the states of Guerrero, Oaxaca, and Chiapas (represented there by the race Vandeño), in the central region referred to as the *Bajío*, and in part of western Mexico, where it is represented by the race Celaya. From northern Veracruz this germplasm has also influenced maizes of the Central Mesa, especially those of Hidalgo, Querétaro, and Guanajuato, possibly giving rise to the race Celaya. The specific knob characterizing this complex is the large one at the distal position of the long arm of chromosome 9 ($9L_2$).

The Guatemala Highlands complex is characterized by the predominance of a combination of knobless and small knobbed chromosomes. The small knob at the more proximal of the two positions that can have knobs on the long arm of chromosome 10 ($10L_1$) is specific to this complex. The representative races of this primordial germplasm are San Marceño, Serrano, Quicheño, Negro, Imbricado, and Salpor. This complex was dispersed from its center of domestication in the highlands of Guatemala to the Oaxaca-Chiapas region of Mexico, where it met the Zapalote complex, as well as to the rest of Central America.

Formation of Races and Their Varieties

The foregoing demonstrates how the great variability of extant varieties and races of maize might have originated. Three fundamental processes that could have led to this variability can be summarized as follows:

1. If maize was domesticated from Mexican teosinte at different locations, the populations of teosinte must have been well distributed in the Mexican territory and shown a wide diversity of races and varieties. That teosinte is basically allogamous would lead one to think that maize has possessed great morphological, cytological, and genetic variability since its origin.

2. Within the limits of each center, domestication could have occurred simultaneously at several different sites or at the same places but at different times. If this were the case, it is very probable that the primordial germplasm of each center has been highly variable since its origin. Furthermore, as the primordial germplasm was taken to other territories, it met new ecological conditions, under which human populations selected different materials according to their needs and preferences. In this way the first racial variants of the primordial germplasm could have been formed.

3. As people became established as farmers and improved their food supply, especially by domesticating plants such as maize, the human population increased and began to migrate in search of new lands for producing food and other necessities. Through migration inhabitants of different territories came into contact with one other, initiating a chain of maize seed exchanges along specific migration routes. When two or more migration routes, each carrying maizes of differing origin, coincided in a given territory, the maizes were crossed, creating opportunities for additional selection of new varieties and races.

Different migrants, over space and time, possibly carried distinct racial and varietal variants. In this way a range of varieties with similar general characteristics could have developed somewhat independently. These varieties can now be grouped in racial categories, with some populations being typical and others atypical or intermediate.

Association Between Chromosome Knobs and Phenotypic Characters

Some studies have shown an association between chromosome knobs and agronomic, morphological, and physiological plant characteristics. In general, this association has been thought to result from linkage between the knobs and certain gene combinations. Other studies, however, have considered only the correlations between several characteristics and the number of knobs present in the plants and not that with specific knobs, making it more difficult to offer a concrete explanation of these associations from the viewpoint of genetics (Blumenschein 1964; Brown 1949; Cutler and Cutler 1948; Ibrahim 1960; Lorenzoni 1965; Mangelsdorf and Cameron 1942; Moll et al. 1972; Murdy 1963; Tavcar 1957; and Vachhani 1950).

There are even cases in which the results are contradictory, if it is assumed that specific knobs or groups of them must be linked to the same genes and therefore condition the same characteristics. Thus, in Guatemalan maizes Mangelsdorf and Cameron (1942) found that a high number of knobs are correlated with straight rows of kernels on the ears and a low number of knobs with hairy leaf sheaths, whereas Brown (1949), studying US maize varieties, reported just the opposite, namely that a high number of knobs was associated with irregular rows and a low number with glabrous sheaths. Cutler and Cutler (1948) also found a correlation between prominent rachis flaps and high number of knobs in some maizes and no association at all in others.

Given the multicentric origin of maize and its races and varieties and that many knobs occur in several of the knob complexes, the same knob or combination of them may or may not show an association with certain agronomic, morphological, or physiological characteristics in different maizes. Whether this is so depends on the original complex or complexes from which the knobs derive. The same knob type may be linked to different allelic combinations of the same genes originating in diverse primordial germplasm. Thus, if the studies cited above were repeated with

different materials, one could expect apparently contradictory results, like those of Brown (1949), Cutler and Cutler (1948), and Mangelsdorf and Cameron (1942). Even if a single knob type at the same chromosome position in a given population is considered, the expression of knob linked genes may differ, because knobs in different plants may be of diverse origin. Therefore, no significant phenotypic correlations may appear, or their values may be very low.

In an effort to find a relationship between the chromosome knob number of inbred lines and their combining ability for grain yield in crosses with a tester, Wellhausen and Prywer (1954) found that lines from lowland varieties with a high number of knobs tended to show better combining ability than those with a low number of knobs. The reverse was true for lines from highland varieties, although with both lowland and highland materials some exceptions to these patterns were found. One problem in considering only the number of knobs instead of specific combinations of knobs in the different lines is that two lines with the same number of knobs may have knobs of different size and at different positions in different chromosomes. Consequently, these knobs might be linked to different gene complexes, causing the derived lines to show diverse behavior. In any case these studies show that it should be possible to learn more about relationships between the phenotypic characteristics of plants and their chromosome knob complement, once the morphological characteristics of these knobs and their probable origin are better understood. One approach is to consider the knob contribution of the diverse complexes in light of the geographic origin of the populations in question (Kato 1984). This approach is explained in more detail in the following sections.

Potential Use of Chromosomal Information

Assuming that racial and varietal formation occurred through the interaction of primordial germplasm and that one or more specific knobs identify this germplasm, then determining the presence of specific knobs in collections of a given race should indicate what primordial germplasm could have participated in its formation. The presence of a given knob need not be associated with one or more specific characteristics. Furthermore, the frequencies of those knobs in given populations could serve as estimators of the relative proportions of different primordial germplasm involved in their formation. This information could help determine whether populations (collections) formed from only one primordial material express narrower general variability than those possessing the specific knobs of two or more primordial materials. If the populations do differ in genetic variability, the differences may be reflected in variable patterns of heterosis, both general and specific combining ability, and/or adaptability.

In the USA the Corn Belt maizes are the most productive. Anderson and Brown (1952) have shown that these varieties are the product of a cross between northeastern flints and southern dents, based on historical, cytological, and genetic evidence. The cytological evidence shows that the northeastern flints have almost no chromosome knobs, whereas the southern dents have 5-12 of these structures. The chromosome knob numbers of Corn Belt varieties are intermediate between those of their ancestors. Upon separating inbred lines from Corn Belt varieties according to chromosome knob number and the combining ability of lines tested in single crosses, Anderson and Brown (1952) found a high correlation between yield and chromosome knob number. This is an example of the practical use of knowledge about the origin of Corn Belt varieties.

The US southern dents did not derive from a single, uniform source but rather show evidence of complex origin through crossing of various distinct materials (Anderson and Brown 1952). This

has been confirmed by the chromosome morphology studies of McClintock et al. (1981), who show that the southern US maizes arose from at least three original sources or complexes: Mesa Central, Pepitilla, and Tuxpeño. In the classification of inbred lines by Anderson and Brown (1952), the results of single-cross yield tests could have given more information, if the knobs in the complexes from which the lines were derived had been studied in detail.

Cytological information could be used not only to elucidate the origin and evolution of maize and its races but also to classify populations phylogenetically, either individually or as racial groups or in other groupings. This new classification should be closely associated with previous racial classification using the morphological system developed by Wellhausen et al. (1952) for the Mexican maizes. Once morphological-cytological classification has been accomplished, studies could be conducted to determine whether such grouping is also associated with aspects of population performance, such as heterosis patterns or combining ability, morphological or physiological variability, adaptability, etc. If such associations are found, the chromosome analysis would provide a more specific criterion for the selection of materials and may become a valuable instrument for future genetic studies and for maize breeding.

Nonetheless, caution should be exercised in using the information provided by McClintock et al. (1981), especially the knob data on individual populations. The reason is that the knob constitution--particularly knob frequency at different chromosome positions--of five or six plants (often there are fewer plants of a given collection) may not represent that of the population, resulting in considerable sampling error. It is important to obtain chromosome information from a greater number of plants of each collection.

Analysis of the Race Tuxpeño

To illustrate the potential use of chromosome information, a brief analysis is presented of Tuxpeño data provided by McClintock et al. (1981). This Mexican race was chosen because more Tuxpeño collections have been studied than of other races and because the collections come from a wide range of locations. The 57 collections studied have been separated into four groups (Table 1), each corresponding to a geographical region: 1) north central Mexico (the states of Chihuahua, Coahuila, Durango, and San Luis Potosí); 2) the eastern coast of the Gulf of Mexico (Tamaulipas, Veracruz, and Yucatán); 3) western Mexico (Colima, Jalisco, and Michoacán); 4) and southern Mexico (Oaxaca and Chiapas) and Guatemala.

This analysis was based on the presence or absence of knobs that are specific to the distinct knob complexes (Kato 1984). The small knobs at 2S, 3L, and 7L, listed in Table 1 under the Mesa Central complex, also occur in the Guatemala Highlands complex. Nevertheless, they can be considered specific to each complex within its territory of influence. The abnormal chromosome 10 is also included, since it derives only from the Zapalote and Mesa Central complexes and did not figure in the origin of either of the other complexes, Tuxpeño included. In the analysis of Tuxpeño, the presence or absence of this chromosome may provide important information.

The first thing that can be observed in Table 1 is that not all collections studied show the large knob on the long arm of chromosome 9, even though it is specific to the Tuxpeño complex. The reason is probably sampling error. Increasing the number of plants analyzed by collection should confirm whether or not this type of knob is present in the population.

Another interesting point is that none of the collections show the knobs that are specific to the Pepitilla and Guatemala Highlands complexes. For reasons still unknown, Tuxpeño germplasm may never have been affected by those complexes, even though populations of this race are grown in parts of western Mexico that are influenced by the Pepitilla complex and in southern Mexico and Guatemala within the area influenced by the Guatemala Highlands complex.

The Tuxpeños from the Gulf of Mexico coast clearly form two groups. First, those from the southern region of the state of Veracruz (Ver. 44, 101, 123, and 128) show the influence of the Zapalote complex, judging from the presence of some of the knobs specific to it (large at 5S and small and large at 7S). In this respect the first group is similar to Tuxpeño collections from southern Mexico and Guatemala. The second group comprises collections from the northern half of Veracruz and from Tamaulipas, which do not exhibit the influence of the Zapalote complex but do show effects of the Mesa Central complex, as evidenced by the small knob at $10L_2$. In addition, three collections showed the small knob at 3L.

The collections of the north central and western regions of Mexico are similar, both showing a relatively high degree of influence by the Mesa Central complex, as evidenced by knobs observed at the positions 2S, 3L, $6L_1$, 7L, and $10L_2$. Apparently, these groups of collections have been influenced more by germplasm from the Mesa Central than by northern Gulf coast collections, since they show a greater variety of knobs of the Mesa Central complex. In the northern Gulf coast collections, the effect of Mesa Central maizes is reflected almost exclusively in the presence of the knob at $10L_2$. The differing influence of Mesa Central maizes may indicate that distinct variants of this complex came into contact with Tuxpeños in the different regions

As indicated in Table 1, the abnormal chromosome 10 was found only in collections from the northern Gulf coast and north central and western Mexico but not in those from southern Veracruz, Oaxaca, and Chiapas or those from Guatemala. As mentioned previously, the first groups of collections show evidence of introgression by Mesa Central germplasm, while the second groups have been affected only by the Zapalote complex. Thus, it appears that the chromosome was introduced into the Tuxpeños by maizes from the Central Mesa and not by those of the Zapalote complex, though it is known that the latter have possessed this chromosome since their origin.

In all four groups, some collections show none of the knobs considered here and lack the abnormal chromosome 10. This could be the result of sampling error. But if a larger sample of plants were analyzed and the results were the same, then it could be concluded that these collections were derived exclusively from Tuxpeño germplasm with no introgression of germplasm of other complexes.

These conclusions could be valid for regional groupings, but with respect to individual populations, they might vary to some degree, depending on the results of studies with more representative samples of plants. For instance, would Tuxpeño populations from a given region that do not show introgression from other knob complexes demonstrate a different general pattern of heterosis, combining ability, or adaptation from populations that do show such introgression? Or would those characteristics exhibit a specific tendency owing to the influence of each knob complex of different origin, and if such relationships exist, would variation in the frequency of the knobs specific to each alien complex indicate different degrees of genetic introgression?

We will not have answers to these questions until studies designed specifically to examine them are conducted by interdisciplinary teams of cytologists, geneticists, and breeders. The results of these studies would provide scientists in other disciplines (molecular geneticists, for example) with new opportunities to elucidate the phenomena discussed here and their relationship with the genetic structure of maize populations. Cytological information derived from these studies could also contribute to interpretation of the field performance of different maize collections. Such uses of cytological data would underscore the importance of conserving the variability of maize races currently maintained as collections in germplasm banks.

Acknowledgments

The author would like to thank Dr. Alfredo Carballo Z. for reviewing the manuscript and suggesting important modifications that much improved the final product.

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Table 1. Specific knobs of the different knob complexes present in collections of the race Tuxpeño native to various regions of Mexico and Guatemala

Collection	No. of plants	Chromosome knob complex: Zapalote $4S_2$ s	$4S_2$ m	$4S_2$ l	$5S_1$ s	$5S_1$ m	$5S_1$ l	$6L_2$ m	$6L_2$ l	Mesa Central: 7S s	7S l	$1L_1$ l	$2S_1$ s	$3L_1$ s	$6L_1$ s	$6L_1$ m	$6L_1$ l	$7L_1$ s	$10L_2$ s	Pepitilla $6L_3$ l	Tuxpeño $9L_2$ l	Guat. High. $10L_1$ s	Abn. 10
North-Central Mexico																							
Chihuahua:																							
35	5																						
Coahuila:																							
3	3														+				+				+
4	6																						
5	5														+				+				
7	5													+							+		
12	6													+				+			+		
14	4																						
19	4																						
40	6												+	+					+		+		+
64	3																				+		
81	6																						
Durango:																							
28	6														+								
San Luis Potosi:																							
78	6													+									
Western Mexico																							
Colima:																							
17	6																						
23	6																	+	+				
Jalisco:																							
12	6												+	+		+							+
150	5														+								

Continued on next page

Table 1. Continued

Collection	No. of plants	Chromosome knob complex: Zapalote 4S$_2$ s	4S$_2$ m	4S$_2$ l	5S$_1$ s	5S$_1$ m	5S$_1$ l	6L$_2$ m	6L$_2$ l	Mesa Central 7S s	7S l	1L$_1$ l	2S$_1$ s	3L$_1$ s	6L$_1$ s	6L$_1$ m	6L$_1$ l	7L$_1$ s	10L$_2$ s	Pepitilla 6L$_3$ l	Tuxpeño 9L$_2$ l	Guat. High. 10L$_1$s	Abn. 10
Michoacán:																							
162	6																	+	+				
166	6													+									
176	5																	+					
200	6																				+		
Gulf of Mexico coast																							
Tamaulipas:																							
1	4													+					+		+		
2	6																						
4	6																		+		+		
8	6																		+		+		+
28	5																						
32	6																				+		
39	6																		+				
41	4																		+				
44	5																						+
45	5																		+				
Veracruz:*																							
218	6													+									
215	5													+					+		+		
191	5																		+		+		
179	6																						
165	5																		+				
78	5																						
39	11																						
149	6																						
225	5																		+		+		
133	6																						
132	6																						
101	6					+		+															
44	6							+				+											
123	6							+									+						
128	6																						
Yucatán:																							
70	6																						

Continued on next page

Table 1. Continued

Collection	No. of plants	Chromosome knob complex: Zapalote $4S_2$ s	$4S_2$ m	$4S_2$ l	$5S_1$ s	$5S_1$ m	$5S_1$ l	$6L_2$ m	$6L_2$ l	Mesa Central 7S s	7S l	$1L_1$ l	$2S_1$ s	$3L_1$ s	$6L_1$ s	$6L_1$ m	$6L_1$ l	$7L_1$ s	$10L_2$ s	Pepitilla $6L_1$ l	Tuxpeño $9L_2$ l	Guat. High. $10L_1$ s	Abn. 10
Southern Mexico and Guatemala																							
Oaxaca																							
6	6																						
7	5													+				+					
11	6																				+		
20	5									+													
35	4									+													
Chiapas																							
7	6	+	+							+													
11	5																						
Guatemala																							
60	2																						
456	6							+										+					
809	2																						

Source: McClintock et al. (1981)

Note: 1L, 4S, $10L_2$, etc., indicate knob positions on the short (S) and long (L) arms of different chromosomes. The subscript numbers indicate the position of the knob in relation to the centromere when two or more knob positions are present on a chromosome arm (1 being the closest).

* Collections from Veracruz are listed in the order of their geographic origin from north to south.

Use of Biochemical Gene Markers for Measuring Maize Genetic Diversity

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Abstract

A variety of biochemical gene marker technologies offer an environmentally independent means of characterizing and quantifying maize genetic diversity in detail. The relatively inexpensive allozyme gene markers are widely employed but limited by the small number of mapped loci. More costly restriction fragment length polymorphism (RFLP) technology is developing rapidly and offers great potential for assessing genetic diversity in sufficient detail for plant breeders. Large numbers of polymorphic RFLP loci have been mapped, and a saturated maize genome map should soon be available. Two-dimensional protein electrophoresis and zein storage protein analysis have potential for further characterizing diversity, but their use is still experimental.

Biochemical gene markers can be applied to all germplasm bank operations. Data generated with this technology can help in prioritizing collections to be maintained and in identifying duplicate entries or those needing recollection. In addition, maintenance of genetic diversity during seed increase could be monitored using gene markers. Applications to germplasm evaluation include selecting entries for screening programs, identifying new heterotic pools, choosing testers, and accelerating elite/exotic backcrossing programs. Use of biochemical gene marker data in conjunction with current collection and plant breeding practices could improve effectiveness in measuring and utilizing maize genetic diversity.

Genetic diversity is the raw material with which breeders work to continually improve the agronomic performance of maize. Without diversity, the art and science of plant breeding would not exist. To effectively collect, preserve, and evaluate this precious resource, germplasm bank personnel require accurate means of characterizing and measuring genetic diversity, tasks in which biochemical gene markers could be of considerable help.

Biochemical gene markers are measurable biochemical characteristics that reveal the genetic information encoded at a single gene. Allelic variations in gene structure are reflected by changes in the marker trait. Calculation of linkage relationships with known loci using recombination data allows the positioning of additional biochemical markers on existing maize genome maps. To be useful for quantifying genetic diversity, mapped markers should ideally represent the entire maize genome. Map unit distances of 10 or less between each biochemically marked gene would ensure tight enough linkage between a marker and its surrounding genes for all segments of the genome to be represented (Tanksley 1983). According to current estimates, a minimum of 175-200 equally spaced gene markers would be required to provide total saturation of the maize genome. With such a gene map, indivduals and populations could be described in terms of allele frequencies at each biochemically marked locus. This description of genetic diversity would be genotypic and independent of confounding environmental effects, which have severely limited the use of morphological and physiological gene markers.

Allozyme Gene Markers

Allozymes are the type of biochemical gene marker most commonly used to study genetic variation in maize (Goodman et al. 1983; Kahler 1985; Wendel et al. 1985). These are enzymes coded at the same genetic locus, each showing similar enzymatic activity but differing in their chemical structures. Often, the difference is only a single amino acid in the primary protein structure. The term isozyme is often used synonymously with allozyme, but the two are not identical. The former includes any group of enzymes that have similar enzymatic activity but different chemical structures. Allozymes are isozymes that must meet the additional criterion of being coded at the same genetic locus, an example being the five allozyme groupings of malate dehydrogenase isozymes found in maize (Goodman et al. 1980).

The allozyme composition of an individual at a given locus is determined in the laboratory by electrophoretic separation of the various allelic forms (Stuber et al. 1988). Maize protein samples extracted from coleoptile tissue are allowed to migrate in an electrical field on a starch gel support medium. Allozyme migration rates are determined by their specific size and total electrical charge, which are unique for each allelic variant. Use of specific histochemical enzyme stains makes it possible to view allozymes in the gel. Allelic variants are then scored by measuring migration distances relative to known genotypes.

Allozyme analysis is a relatively inexpensive, straightforward laboratory technique but requires trained personnel. Major limitations to the use of allozymes in measuring genetic diversity are the limited number of marker loci available and a low level of polymorphism. Currently, only 35 commonly used allozyme loci have been characterized (Table 1 and Figure 1), and there is little potential for mapping new loci. Although the information provided by allozyme analysis is very useful and has been employed in numerous taxonomic, evolutionary, and genetic studies (Doebley et al. 1984; Doebley et al. 1987; Smith et al. 1985), it does not represent the entire maize genome in enough detail for plant breeders.

Allozyme studies of numerous maize races and teosinte collections have shown that most populations have a high proportion of common alleles found at high frequencies. Therefore, measurement of relationships between collections on the basis of allozyme data can be strongly influenced by a few rare alleles. Allozyme analysis provides an imperfect measure of absolute genetic diversity. Nonetheless, the data do provide the most convenient, objective, and economic means currently available for examining genetic diversity.

Restriction Fragment Length Polymorphism Gene Markers

Restriction fragment length polymorphisms (RFLPs) hold considerable promise for overcoming the limitations that prevent allozyme markers from adequately representing the entire maize genome. RFLP testing utilizes a group of enzymes, known as restriction endonucleases, that can cleave DNA at specific base pair sequences. There are hundreds of characterized restriction endonucleases, each recognizing a different base sequence region for cleavage. Using any one of these restriction enzymes, isolated DNA from a given individual can be cut into a specific, reproducible group of fragments. Individuals of different genetic makeup will have altered DNA structures, and therefore different restriction fragment patterns, when cut with the same restriction endonuclease. Analysis of changes in the composition of any one fragment allows one to examine genetic differences in that small region of the maize genome. Testing of enough fragments (RFLP loci) spread throughout the maize genome could provide the detailed gene

marker map needed to assess genetic diversity in sufficient detail for the purposes of plant breeding (Tanksley 1983).

Specific restriction fragments are monitored with a single or low copy number DNA fragment (referred to as a probe) that is contained within the restriction fragment and has been previously isolated and radioactively labeled. Since the labeled probe contains a large region of base pair similarity with the specific restriction fragment, it will selectively bind only that fragment. Use of such a probe permits an individual restriction fragment to be singled out of a mixture and studied independently. Because of genetic differences, the size of a given fragment will vary from one individual to another. Their size can be altered through the addition or deletion of genes within the fragment or, more commonly, the addition or deletion of restriction enzyme cleavage recognition sites. Genetic variation will result in a polymorphic group of restriction fragments differentiable by size. Thus, the restriction fragment represents the equivalent of a genetic locus, and the size variants are its alleles.

Restriction fragments varying in size are separated from one another by electrophoresis. Extracted DNA is cleaved with a restriction endonuclease, and the resulting fragments are placed in an electric field, using an agarose gel as the support media. Because of the sieving effect of the agarose gel, larger fragments migrate at slower rates in the electric field and are therefore separated across the gel. After electrophoresis a southern transfer is performed, which moves fragments from the agarose gel onto a nitrocellulose or nylon support membrane without disturbing their relative separation positions. Membranes are exposed to a radioactive probe, which binds to fragments having similar DNA base sequences, thereby making them radioactive. DNA fragments can then be viewed by exposing the membranes to X-ray film. Fragment size, and thus the allelic identity, are determined by migration distance relative to known standards. To date, over 350 RFLP loci have been mapped in maize, and many of these are highly polymorphic. A current listing of them is maintained in the *Maize Genetics Cooperation News Letter* (distributed annually by the Department of Agronomy and US Department of Agriculture at the University of Missouri, Columbia).

The disadvantages of RFLP gene markers are primarily monetary. With current technology the cost of analysis per locus is approximately ten times that of isozyme analysis. In addition, more ellaborate testing facilities are required. Costs may decrease as the technology improves. One way of reducing expenses would be to identify a small number of specific probes closely linked to traits of agronomic importance. Collections could be screened at these loci for novel genetic variants to choose a limited number of entries for more detailed laboratory and field testing.

Additional Types of Biochemical Gene Markers

Two additional techniques that provide gene markers in maize are two-dimensional protein electrophoresis and zein storage protein analysis. Although their use is currently limited, these methods have potential for supplementing isozyme and RFLP data.

Zein is a heterogeneous group of alcohol soluble proteins that make up over 50% of the total maize endosperm proteins. Zein is most commonly extracted with 55% isopropanol in the presence of a reducing agent and then separated by reverse phase, high performance liquid chromatography (Paulis and Bietz 1986; Smith 1986; Smith and Smith 1986) or by various forms of electrophoresis, primarily isoelectric focusing (Soave et al. 1978; Hagen and Rubenstein 1980; Righetti et al. 1977; Wilson, 1985; Izquierdo, 1984). Qualitative differences in zein polypeptide

composition have been used on a limited basis to explore genetic relationships between individuals (Smith and Smith 1987; Wilson and Larkins 1984; Nucca et al. 1978). The multigene family that codes zein polypeptides is located on only 2 of the 10 maize chromosomes (Soave and Salamini 1983), thus limiting their use for complete genome mapping.

However, since most zein genes are located on three chromosomal arms currently unmarked by allozymes (Figure 1), data obtained with the two techniques are complementary. Used in conjunction with allozyme data, zein HPLC analysis provided more detailed genetic grouping of hybrids commonly grown in the USA than could allozyme data alone (Smith 1988; Smith and Orman 1988). Zein analysis does provide a rapid, economical means of surveying limited genome segments, but futher research is needed to standardize data analysis and to map specific polypeptides.

Two-dimensional electrophoresis combines two separation techniques (O'Farrell 1975). Proteins are first separated according to their isoelectric points by means of isoelectric focusing. SDS electrophoresis in a second dimension, perpendicular to that of the first, further separates proteins by molecular weight. Two-dimensional electrophoresis can resolve a single tissue protein extract into 500 to 1,000 unique individual proteins based on structural differences. An extensive amount of research is needed to optimize the techniques, standardize computer data acquisition, and perform basic genetic analyses of the information generated. If two-dimensional electrophoresis data can be sorted, this one test could provide a large amount of genetic information at reasonable cost.

Calculations of Genetic Diversity Based on Biochemical Gene Marker Data

Marker data have two general uses: 1) to assess diversity within a collection and 2) to compare the genotypic identities of two or more collections. Calculations of diversity within a collection should take into account the number of alleles per locus, number of polymorphic loci, allele frequencies, and proportion of plants that are heterozygous at one or more loci. Genetic diversity within a population increases as the number of alleles per locus and number of loci that are polymorphic increase.

Comparisons of collections are based on similarities and differences in allele frequencies. These differences can be conveniently revealed by multivariate statistical techniques, such as principal component analysis, principal coordinate analysis (Figure 2), or cluster analysis (Figure 3). These analyses simplify complex data sets by reducing the number of original variables. Thus, associations between collections can be expressed in one, two, or three dimensions. It is important to note that valid interpretation of data following multivariate analysis requires a reasonable understanding of the mathematical and statistical bases of multivariate techniques.

Use of Biochemical Gene Markers in Germplasm Collection

Choice of collections to be maintained--With limited resources it is possible to maintain only a limited number of collections during a season. In most cases only those collections should be maintained that together encompass the broadest range of genetic diversity and most extend the range of conserved diversity. Collection data and field observations could be supplemented with biochemical marker data to select the most genetically diverse group of collections for conservation.

Identifying possible duplicates--Collections displaying similar biochemical gene marker profiles could be duplicate entries. However, the decision to remove a collection must be based on both lab and field data. Biochemical gene marker data should serve only for intitial screening to identify possible duplicates; they do not provide absolute proof of duplication.

Identifying entries to be recollected--Individual collections that show morphological and/or physiological diversity in the field but that display little diversity for biochemical gene markers may need recollection. In such instances the original sampling might have failed to capture the genetic diversity of the population.

Use of Biochemical Gene Markers in Germplasm Preservation

Biochemical gene markers offer an efficient means of detecting samples in which genetic diversity has been reduced or adversely affected during seed increase through genetic drift or selection. If diversity has been lost, the increase could be performed again, correcting the sample size, pollination procedures, and/or harvesting techniques to better maintain the original level of genetic diversity. Such monitoring would be especially useful for collections in which most of the diversity takes the form of rare alleles at very low (less than 0.05) frequencies. Poor pollen control or mislabeled rows can also alter diversity, causing large shifts in allele frequencies, including the introduction of new alleles. Such gross changes could be readily detected by laboratory analysis.

Use of Biochemical Gene Markers in Germplasm Evaluation

Choosing an appropriate range of diversity in a screening program--Often, the most appropriate collections to be included in a program of screening for agronomically useful traits are those encompassing the broadest range of genetic diversity available. Multivariate analysis of biochemical gene marker data could provide an additional set of criteria for identifying collections that together would represent the broadest possible range of diversity.

In some cases the search for agrononmically useful genes is best accomplished by concentrating upon a relatively narrow range of related germplasm. Again, biochemical gene marker data could help identify collections with similar genotypes at the marker loci. These collections might be those having related genetic backgrounds. Other data, including original collection information, should also be considered.

Identification of new heterotic pools--In hybrid maize breeding and production, it is extremely important to identify germplasm pools and to organize lines and populations into groups showing similar heterotic response. Detailed description and comparison methods provided by multivariate analysis of biochemical gene marker data offer additional means of categorizing collections. Used in conjunction with collection and heterosis data, gene marker data could be used to identify collections that represent new heterotic pools of germplasm.

Choice of testers in the development of hybrids--To obtain a true representation of a collection's potential as a source of inbreds that are useful in hybrid seed production, it is necessary to measure the combining ability of lines from that collection by crossing them to genetically different genotypes. For measuring combining ability potential, the tester genotypes--which can be inbreds, single cross hybrids, or populations--need to be genetically distant from the collection being examined. Biochemical gene markers provide additional information for identifying genotypes that are distinct from the genotypes to be assessed. These would be

expected to provide the best test of crossing ability (Moll et al. 1961). If genotypes are too genetically distant, however, the association between genetic distance and combining ability begins to disintegrate (Moll 1965).

Acceleration of backcrossing programs--Biochemical gene marker data could facilitate the backcrossing of exotic germplasm into adapted lines in two ways. First, if a minimum of exotic introgression is required, monitoring of individual plants during the backcross program will allow only those individuals with the greatest amount of recurrent parent background to be continued through the program, thereby decreasing the number of generations necessary for recovery. Second, if more exotic germplasm is to be integrated, then assaying gene markers can be employed to monitor the incorporation of rare alleles from the exotic donor parent into adapted material. Thus, the unique genetic contribution of the exotic material can be maintained during backcrossing. Previous studies have shown that exotic germplasm does contain alleles useful for the improvement of agronomic traits (Goodman 1985). Use of such material in a backcrossing program permits useful genes from exotic material with poor per se performance to be introduced into adapted materials, in which their contribution to agronomic performance can be accurately assessed.

Tagging agronomically useful genes--Because it is possible to saturate the maize genome with RFLP gene markers, there are opportunities to establish linkages between gene markers and regions of the genome (quantitative trait loci or QTLs) that affect agronomically important traits (Edwards et al. 1987). Regions of the genome contributing to an agronomic trait of interest, such as disease resistance, stress tolerance, or yield, would first be identified by analysis of marker and trait segregation in the F_1 population. Once linkages are established between specific marker loci and QTLs contributing to complex traits, collections could then be screened for novel genetic variants of agronomic importance, using these specific biochemical markers. Detailed field and laboratory analysis could then be focused on the collections chosen.

Conclusion

Maize genetic diversity can be assessed both in the field and laboratory. A variety of biochemical gene marker technologies offer an environmentally independent means of quantifying maize genetic diversity in detail. A point of fundamental importance is that genotypic, not phenotypic, diversity can be characterized and compared between collections.

Allozyme technology is well established and relatively inexpensive, but its use is limited by the relatively few marked loci. Two-dimensional protein electrophoresis and endosperm storage protein analysis offer potential for further characterizing diversity, but their application is still in the experimental phase. RFLP gene marker technology is developing rapidly and can be used in measuring maize genetic diversity. Large numbers of polymorphic RFLP loci have been mapped, and a saturated maize genetic map will be available in a matter of months. As RFLPs become more numerous, specific probes linked to agronomically important traits will be identified. Technological advances should make the use of RFLPs more economical. RFLPs may then provide germplasm banks with a powerful tool for evaluating and utilizing their genetic resources more efficiently.

For maximum effectiveness germplasm banks must ensure that their collections are representative and well documented and maintained. Biochemical marker technology will be of no use unless germplasm banks first look to their primary responsibilities of collecting, maintaining,

and cataloging genetic diversity. It is also important to note that the use of biochemical gene markers to quantify genetic diversity can only supplement and not substitute for current collection and breeding practices.

Acknowledgements

The authors wish to acknowledge the helpful comments made by Drs. A. Cavalieri, R. Fincher, and D. Grant on a preliminary version of this paper. We also thank Nancy Wiig for providing technical assistance.

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Table 1. Summary of the known chromosomal locations of enzyme loci in maize

Symbol	Name	Location	Routinely used
Acp1	acid phosphatase-1	9	*
Acp4	acid phosphatase-4	1L	
Acol	aconitase-1	Probably 4S	*
Adh1	alcohol dehydrogenase-1	1L	*
Adh2	alcohol dehydrogenase-2	4S	
Amp1	aminopeptidase-1	1L	*
Amp2	aminopeptidase-2	1L	
Amp3	aminopeptidase-3	5S	*
Amy2	amylase-2	5S	
Cat1	catalase-1	5S	*
Cat2	catalase-2	1S	
Cx1	catechol oxidase	10L	
Dia1	diaphorase-1	2S	*
Dia2	diaphorase-2	1L	*
E1	esterase-1	7	
E3	esterase-3	3	
E4	esterase-4	3S	
E8	esterase-8	3S	*
E16	esterase-16	7	
Enp1	endopeptidase	6L	*
Glu1	Beta-glucosidase	10L	*
Gdh1	glutamate dehydrogenase-1	1L	
Gdh2	glutamate dehydrogenase-2	10	
Got1	glutamic-oxaloacetic transaminase-1	3L	*
Got2	glutamic-oxaloacetic transaminase-2	5L	*
Got3	glutamic-oxaloacetic transaminase-3	5S	*
Hex1	hexokinase-1	3S	
Hex2	hexokinase-2	6L	*
Idh1	isocitrate dehydrogenase-1	8	*
Idh2	isocitrate dehydrogenase-2	6L	*
Mdh1	malate dehydrogenase-1	8	*
Mdh2	malate dehydrogenase-2	6L	*
Mdh3	malate dehydrogenase-3	3L	*
Mdh4	malate dehydrogenase-4	1L	*
Mdh5	malate dehydrogenase-5	5S	*
Me1	malic enzyme	3L	*
Mmm	modifier of mitochrondrial MDHs	1L	*
Pgd1	6 -phosphogluconate dehydrogenase-1	6L	*
Pgd2	6 -phosphogluconate dehydrogenase-2	3L	*
Pgm1	phosphoglucomutase-1	1L	*
Pgm2	phosphoglucomutase-2	5S	*

Continued on next page

Table 1. Continued

Symbol	Name	Location	Routinely used
Phi1	phosphohexose isomerase	1L	*
Px1	peroxidase-1	2	
Px3	peroxidase-3	7	
Sad1	shikimate dehydrogenase-1	10	*
Tpi1	triose phosphate isomerase-1	?	*
Tpi2	triose phosphate isomerase-2	?	*
Tpi3	triose phosphate isomerase-3	Probably 8L	*
Tpi4	triose phosphate isomerase-4	3L	*
Tpi5	triose phosphate isomerase-5	?	*

Source: Goodman and Stuber 1983; Kahler 1985; Wendel et al. 1985.

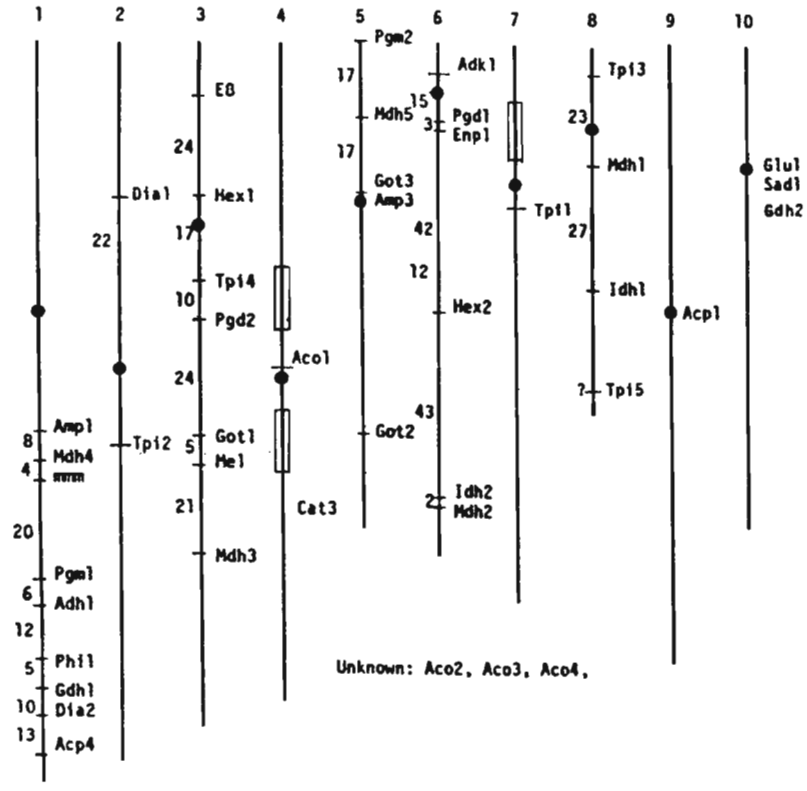

Figure 1. Boxes on chromosomes 4 and 7 represent regions of zein polypeptide coding. Isozyme data from Stuber et al. (In press), Zein data from Soave and Salamini (1983).

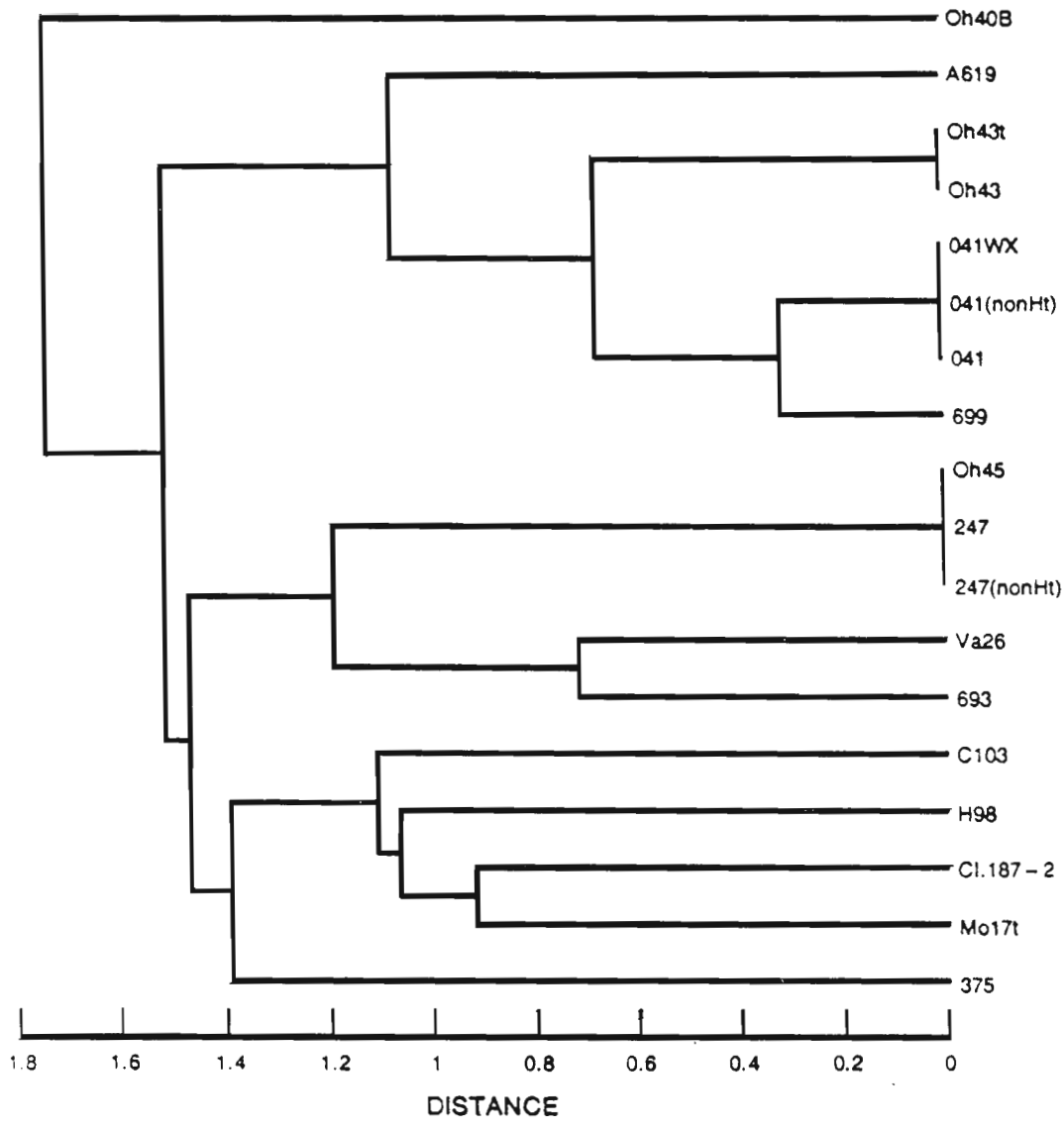

Figure 2. Associations between lines on the basis of the first three principal coordinates from ultivariate analysis of allozymic data.

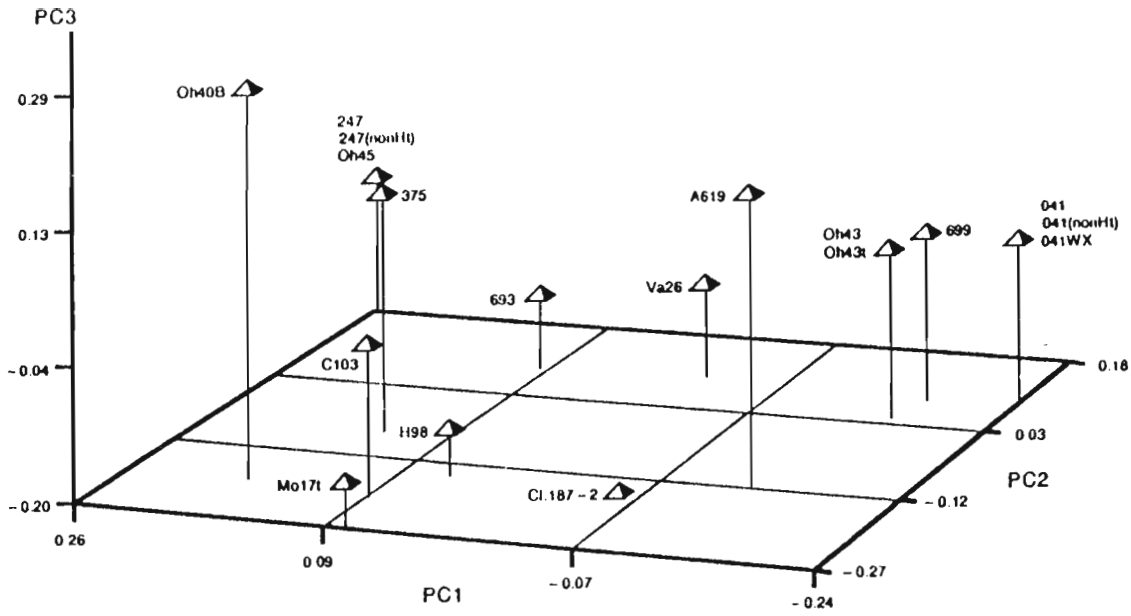

Figure 3. Dendrogram showing associations between lines following cluster analysis of allozymic data.

US Maize Germplasm: Origins, Limitations, and Alternatives

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Abstract

US Corn Belt Dents arose from a germplasm pool consisting of about 75-80% Southern Dent and 20-25% Northern Flint material. Most current hybrids are derived from a few inbred lines (A632, B14, B37, B73, and their derivatives) of Stiff Stalk Synthetic origin crossed with a few lines (C103, Mo17, Oh43, and their derivatives) of largely Lancaster origin. In addition, at least half of the popular midwestern hybrids are not unique but have the same pedigrees as various other competitive hybrids.

In an attempt to greatly broaden the germplasm base of US hybrid maize, we are attempting to derive temperate adapted lines of mainly tropical origin. So far, we have been more successful in extracting promising materials from tropical hybrid intercrosses than from synthetics, which have no history of inbreeding. Nevertheless, using hybrids and synthetics to derive lines causes major problems: the loss of heterotic patterns and difficulty in identifying especially useful source populations. To facilitate identification of source populations, the available typical race collections were evaluated under daylength neutral conditions. We have selected 40 of the most promising collections (from an initial set of some 1,300 and a tested set of about 400) and are now attempting to convert them to daylength neutrality via backcrossing.

If germplasm banks are to play a consequential role in future maize genetics and breeding, they must have programs of evaluation and prebreeding in addition to their maintenance and distribution activities. Isozyme and chromosome knob studies have demonstrated that in evaluating germplasm for rare traits (such as insect and disease resistance) in replicated trials, it is essential to examine individual collections, since rare alleles are usually found at high frequencies in only a few accessions. These alleles would consititute, at best, miniscule components of racial or other composites and would have to be reisolated from synthetics for effective evaluation.

Maize germplasm banks have tended to procrastinate in the regeneration of "difficult" collections. They have concentrated instead on the better adapted materials that are easier to maintain and waited for a "more appropriate location" to regenerate the difficult accessions. This problem can be avoided through cooperation among banks. Accessions that are difficult to regenerate at one site may not be at others. There is also clearly a need for an appropriately equipped, high elevation, frost free experiment station where the many accessions that are being lost in maize germplasm banks throughout Latin America can be successfully increased.

Since the late 1960s, we have been working with Latin American maize races, using accessions provided by CIMMYT, the Colombian Institute of Agriculture and Livestock Research (ICA), Mexico's National Institute of Forestry, Agriculture, and Livestock Research (INIFAP), the Cooperative Program for Maize Research (PCIM) in Peru, the National Seed Storage Laboratory (NSSL) in the USA, and W.L. Brown of Pionner Hi-Bred International (USA). By using winter nurseries in Florida, we have been able to maintain most of the 1,500 or so accessions (including some duplicates) representing the available typical accessions described in most of the various

bulletins on the maize races (Wellhausen et al. 1952, 1957; Hatheway 1957; Roberts et al. 1957; Brieger et al. 1958; Brown 1960; Ramirez et al. 1960; Grobman et al. 1961; Timothy et al. 1961, 1963; Grant et al. 1963; Paterniani and Goodman 1977).

During the mid-1970s, we began to study the isozyme genetics of maize, with the goal of comparing the races of maize on the basis of isozyme allele frequencies. We have now completed analyses of the races found north of the Isthmus of Panama (Doebley et al. 1985; Bretting et al. 1987, in preparation), including the maize of the USA (Doebley et al. 1983, 1986, 1988), and have some additional data for South American maize (Goodman and Stuber 1983b). A summary of the genetics of the isozymes has been completed (Goodman and Stuber 1983a), and summaries of the laboratory techniques used have been published (Cardy et al. 1983; Stuber et al., in press) and will not be repeated here.

Origins of US Maize

The use of isozyme allele frequencies to trace the origin of US maize is illustrated in Figure 1. The Mexican materials used came largely from INIFAP and CIMMYT and the US materials from the North Central Plant Introduction Station at Ames, Iowa. All of these materials were individual accessions from germplasm banks, without which the studies would not have been possible. The points in the figure represent population means or centroids. The figure indicates the relative isolation of US Northern Flints and Flours from Mexican maize races and the similarity of US Southern Dents to the maize races of southern Mexico (Doebley et al. 1988). Similar techniques have helped estimate the heritage of modern Corn Belt Dents, which were shown in studies by Anderson and Brown (1952) to have been derived from hybrids between the Northern Flints and Southern Dents. Figure 2 shows a plot, based upon isozyme allele frequencies, of the Corn Belt Dents, Southern Dents, and Northern Flints along the axis joining the Northern Flint means with the Southern Dent means. Clearly, the Corn Belt Dents are isoenzymatically much more closely related to the Southern Dents than to the Northern Flints. They appear to consist, on the average, of about 25% Northern Flint germplasm and 75% Southern Dent germplasm.

Limited US Maize Germplasm Base

Prior to our isozyme work, we were concerned about the narrowness of the US maize germplasm base, as were many others (Wellhausen 1965; Committee on Genetic Vulnerability of Major Crops 1972; Brown 1975). Surveys of inbred line use suggested that much US maize consisted of derivatives of Stiff Stalk Synthetic lines (A632, B14A, B37, B73) crossed with lines of the Lancaster family (C103, LH38, Mo17, Oh43). Recently, extensive biochemical surveys by J.S.C. Smith (1988) has shown that a great amount of pedigree duplication exists in US hybrid maize and that its genetic base is perhaps even narrower than previously thought. This is especially true among products of the second-tier companies, some of which appear to be closely mimicking widely sold hybrids produced by larger companies rather than specializing in regionally adapted (and therefore, hopefully, regionally superior) hybrids.

It has been known for many years that the number of open-pollinated varieties contributing to our current stock of inbred lines in the US is limited (Committee on Genetic Vulnerability of Major Crops 1972; Baker 1984), consisting largely of Reid and Lancaster varieties, with lesser contributions by Krug, Midland, Leaming, Pride of Saline, and a handful of others (Anderson and Brown 1952; Wallace and Brown 1956; Baker 1984, 1986). Although various surveys have measured the number of lines in use (Sprague 1971; Zuber 1975; Zuber and Darrah 1980;

Duvick 1984; Baker 1984; Goodman 1985a; Darrah and Zuber 1986), it was only with the advent of isozyme fingerprinting that diversity among lines (Goodman and Stuber 1980; Stuber and Goodman 1983; Smith et al. 1985a, b) and diversity among hybrids (Cardy and Kannenburg 1982; Smith 1984; Smith 1988; Smith and Orman 1988) could be objectively measured. By 1985 it had been demonstrated that little exotic germplasm was represented in current US hybrids (Goodman 1985a) and that considerable similarity existed among them (Smith 1984). However, it was hoped that the US germplasm base had become less narrow during the 1970s and that the breeding materials under development represented a broader germplasm base (Duvick 1984).

Unfortunately, it now appears that the US germplasm base, as represented by widely sold midwestern hybrids, is even narrower than had been perceived earlier (Smith 1988). Roughly 50% of the widely sold Corn Belt hybrids differ minimally from other hybrids that are only cosmetically different or even identical (Table 1). Clearly, a number of companies that rely heavily on foundation seed firms for a large portion of their lines or hybrids are marketing hybrids that are similar or identical to those produced by their competitors, who rely on the same sources of inbred lines. That some of these hybrids are very similar, if not identical, to those sold by companies with intensive breeding programs and supposedly unique hybrids and pedigrees indicates a still further narrowing of the employable maize germplasm base (Table 2). According to Smith (1988) the biochemical evidence indicates much stronger reliance on lines such as A632, B37, B73, Mo17, Oh43, and their derivatives than the survey data of Darrah and Zuber (1986) and Zuber and Darrah (1980) would suggest. Baker (1984) earlier pointed out similar implications, based upon independently acquired survey data. Although a reasonably wide array of hybrids is available, the numbers of unrelated hybrids that share no inbred lines in common are apparently few and currently undocumentable. There appear to be virtually no Corn Belt hybrids without either a Stiff Stalk Synthetic line (A632, B14A, B37, B73, N28, etc.) or a Lancaster line (C103, LH38, Mo17, Oh43, etc.). Most midwestern hybrids appear to have one line from each family (Baker 1984; Smith 1988).

Use of Synthetics

It would appear prudent to make a reasonable effort to broaden the US germplasm base by utilizing elite exotic germplasm. This idea is hardly novel (Anderson 1944; Brown 1953, 1975; Kramer and Ullstrup 1959; Eberhart 1971; Hallauer and Sears 1972; Nelson 1972; Lonnquist 1974; Hallauer 1978, 1980; Gerrish 1983; Wellhausen 1965), even among the *maiceros* at North Carolina State University (Thompson 1973; Stuber 1978, 1986). Stuber (1978, 1986) has probably made as large an effort in this area, if not a larger one, than we have, mostly using US x exotic synthetics, and his results appear to be promising (Stuber, unpublished data).

In addition, my predecessor at North Carolina State, Dr. D.L. Thompson, had a major program of attempting to adapt several tropical and semitropical synthetics (from CIMMYT and elsewhere) to temperate regions through a recurrent backcrossing regimen. Although we are continuing to work with a number of derivatives of these synthetics (Florida Synthetic, Amarillo Dentado-2, Tuxpeño, Cogollero, Ibadan B, ETO Blanco, and Antigua x Veracruz 181), testcrosses of adapted lines based upon high percentages (50%, 75%, or greater) of exotic germplasm from such synthetics have been somewhat disappointing, especially in the case of the synthetics Blanco Cristalino-1, Blanco Subtropical, Mic-ETO, Amarillo Cristalino-1, Amarillo Bajío, IDRN, and Ibadan A.

Tropical Hybrid Derivatives

In contrast, the results of testcrosses of adapted lines derived from intercrosses of tropical hybrids have been much more promising (Goodman 1985a; Holley and Goodman, in press). Some first cycle inbred lines derived from intercrosses of tropical and subtropical hybrids performed well in hybrid combinations (Table 3) with US testers, but these first cycle inbreds performed poorly as lines, perhaps because the tropical hybrids themselves were often based upon S_3 or S_4 (possibly even S_2) bulk increases rather than on completely inbred lines or because the temperate environment is an exceedingly severe challenge for tropical germplasm. We are currently testing second cycle lines from intercrosses of random first cycle lines. We are also developing second cycle lines derived from intercrosses of selected, first cycle, temperate adapted lines of 100% tropical and semitropical origin. Our preliminary data suggest that some of the second cycle lines meet or exceed the minimum criteria for commercial use in hybrid production. Whether those particular lines also perform adequately in hybrid combinations is currently under study. A few such comparisons are shown in Table 4. The results seem reasonably promising.

Loss of Heterotic Patterns

One unsatisfactory aspect of working with derivatives of tropical and subtropical hybrids and composites is that in many cases these materials have been derived from very wide backgrounds, as most tropical and subtropical hybrids are double crosses. Often, each line in the double cross arises from a different race, racial hybrid, or synthetic. Single-race hybrids, such as INIFAP's H507, or even single-synthetic hybrids, such as KU2301 from Thailand, are rare. Often, the synthetics have an even more heterogeneous background, so much so that component percentages, or even the identity of some of the components, are unknown (or perhaps equally troublesome, extinct). Thus, identifying truly useful heterotic combinations among such materials is a bit frustrating.

Excellent hybrids might have been developed from a composite of all US midwestern open-pollinated maize varieties, but it is doubtful that such hybrids could equal today's Reid x Lancaster combination. Indeed, even now companies that have developed inbreds from Reid x Lancaster single crosses (such as selfs from their competitors' best hybrids) often end up with attractive, even high yielding, inbred lines that lack sufficient heterosis for commercial use (Forster, personal communication).

Evaluation of Racial Accessions

Therefore, we have been very interested in evaluating racial accessions held in germplasm banks (Goodman 1985b; Jones 1987). Over ten years ago, Mario Gutiérrez (1974) at CIMMYT demonstrated that certain bank accessions are competitive with commercial materials. These superior accessions comprise a very small fraction of bank holdings, usually well under 5%, and they are often overlooked among the thousands of other bank accessions that generally leave maize breeders with an unfavorable impression.

Assuming that no one had ever attempted to evaluate agronomically the entire spectrum of Latin American maize germplasm bank accessions, Fernando Castillo (now at the Postragraduate College, Montecillo, Mexico) compared a set of some 400 accessions culled from a set of about 1,500 (Goodman 1983) representing the so-called typical collections of the 250 or so Latin

American races of maize. Using midwestern and southern US hybrids and commercial tropical and semitropical hybrids as checks, he conducted yield trials under the daylength neutral conditions of fall in Florida and Texas (Castillo and Goodman, in review). The test conditions clearly favored accessions from the lowlands and low-intermediate elevations, as did the criteria employed to cull the 400 accessions from the 1,500. Nevertheless, the results are not only informative but in a few cases quite surprising (Table 5).

Most of the outstanding races and collections (Tables 6, 7, and 8) represented widely used breeding materials, such as the Cuban Flints, Tusons, Tuxpeños, various Coastal Tropical Flints (Costeños, Cateto Nortista), Brazilian Dents, Catetos, Perlas, etc. The excellent performance of lowland Ecuadorean commercial races was quite a surprise, as these have not been especially renowned among breeders of tropical maize. Equally surprising was the almost total susceptibility and dramatic yield reduction observed among midwestern and even central and southern US hybrids when infected with both southern rust (*Puccinea polysora*) and southern leaf blight (*Bipolaris maydis*) in the fall of 1984 in Texas. Both the tropical hybrids and many of the individual accessions had excellent resistance to both diseases.

Conversion to Daylength Neutrality

We are presently converting some 40 of the better accessions (Table 9) identified on the basis of Castillo's experiments to daylength neutrality via a recurrent backcrossing scheme. We are now at the BC_1F_1 = 75% exotic level and plan to continue the conversion scheme, using Mo44 as the donor parent and the individual accessions as the recurrent parents. We plan to begin testing each accession for combining ability with Stiff Stalk materials (A632 x B73) and with Lancaster materials (Mo17 x Oh43E) during the next several years. Hopefully, similar and more extensive projects will be conducted with the elite materials being identified by the Latin American Maize Project (LAMP) now in progress in 10 Latin American countries under the leadership of Quentin Jones and coordination of Ricardo Sevilla (Jones 1987).

Defense of Germplasm Banks

If our germplasm banks are ever to be of more than academic interest, then there is a desperate need for publicly available evaluation data. The majority of our maize germplasm banks function more as seed morgues than seed banks. Many are run as semiprivate subsidiaries to national breeding programs. The number of collections being regenerated annually is often less than the number being acquired or becoming inviable (or both). Thousands, or even tens of thousands, of collections have been acquired in the past 25 years without any rational plans for permanent storage, regeneration, evaluation, or active distribution. We have had core collections since the 1950s (known then, and still known, as typical racial accessions), but our system of regeneration, evaluation, and distribution is nothing short of embarrassing. The costs of doing the job right are small, especially in relation to the costs of not doing so.

All of us recognize that a relatively homogeneous gene pool poses several major problems: 1) the danger that a new pathogen or insect biotype might develop that is particularly adapted to a prominent genotype, as exemplified by the southern corn leaf blight epidemic in the USA during the early 1970s (Sprague 1971); 2) limited potential for genetic gains due to a slow reduction in the amount of genetic variation, which locks breeders into unfavorable linkages of plant characteristics and a reduction in the rate of agronomic improvement; 3) lack of flexibility in seeking alternate, efficient uses of the crop (i.e., ethanol production, industrial and food starches,

sugars, biodegradable plastics, etc.). In most cases these problems cannot be addressed by simply placing seed from a wide range of germplasm types in the freezer, since a long time (5-50 years) is needed to incorporate traits of interest into commercially acceptable materials. Generally, breeders lack even the most basic plant descriptors that would allow them to identify source material for a trait of interest. In addition, adaptation problems frequently make evaluation of many germplasm groups impossible.

I have bored you with the details of programs of evaluation, daylength conversion, and utilization of exotic germplasm because the existence of germplasm banks depends upon widespread utilization of their holdings. Unless some demonstrable successes can be achieved with our germplasm collections, we will find it increasingly difficult to preserve them. Ultimately, the pursuit of academic studies, such as those of McClintock et al. (1981) or our own isozyme studies, are probably insufficient justification for the costs of maintaining germplasm collections.

Requirements for Maintaining Individual Accessions

Such studies have, however, provided vital, basic biological information. They have been of more than academic interest, because they have demonstrated once more why we must maintain collections individually rather than in composites. Composite collections have considerable value in screening large sets of germplasm for adaptation and with certain types of breeding procedures. However, isozyme and chromosome knob studies have demonstrated the nonrandom distribution of genetic variation among accessions. Specifically, rare alleles present at frequencies of less than 1% (often much less) are commonly encountered at high percentages (greater than 50%) in one or two individual accessions. Thus, only in the individual accession, can a pathologist or entomologist effectively identify rare, useful alleles for pest resistance. Such alleles disappear in composites, and large numbers (thousands) of lines derived from a composite have to be screened to reisolate the desired allele. In almost any procedure for identifying particular major genes, for which replicated trials are necessary to identify genotypes of interest, the use of individual accessions is not only preferred but is virtually a prerequisite.

Regeneration difficulties

In the USA we are faced with the problem of having many accessions of maize (and other crops) that show great potential but cannot readily be maintained or evaluated due to the lack of appropriate experimental facilities and personnel in short day environments. This is leading slowly to the loss of that material. Similar problems exist in maize germplasm banks elsewhere: for example, Bolivian collections are very difficult to maintain in Colombia, as are Guatemalan collections in Mexico. Lack of planning for regeneration of "difficult" sets of accessions will inevitably lead to their loss, and where breeding programs have been successful, the loss of materials in germplasm banks means their extinction, since successful breeding almost inevitably leads to the loss of germplasm. The USA probably has less than 1% of the open-pollinated maize varieties that existed a century ago. It is hardly possible that the 99% loss did not include valuable genes for disease and insect resistance, alternative uses, and even an occasional "yield" allele. The raw material of molecular genetics lies today in our germplasm banks. Unfortunately, this material is often poorly labelled, and sometimes it is barely viable. Hopefully, the renaissance of the maize germplasm bank at CIMMYT is an indication that the future of maize germplasm is not slow decay in what Garrison Wilkes calls "depositories" and what I am more inclined to call "morgues."

Acknowledgments

Paper No. 11555 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, North Carolina, 27695-7601. This investigation was supported in part by a grant from the National Institute of General Medical Sciences of the USA and a grant from Pioneer Hi-Bred International.

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Table 1. Fraction of US Corn Belt hybrids surveyed that had unique pedigrees, as determined by isozyme analyses

Gold Harvest	4/6
Jacques	2/6
Northrup King	1/2
Pioneer	15/17
Stauffer	0/6
Asgrow	0/2
Cargill	5/5
Dekalb-Pf	7/13
Funks	8/11
Garst	0/10
Others	3/20
Total	45/98

Source: Adapted from Smith 1988.

Table 2. Five groups of widely distributed hybrids that are virtually indistinguishable by isozyme analyses

B73 x LH51	B73 x LH38	B73 x Mol17	A632 x LH38	LH74 x LH51
DF20 x LH51	B73 x LH98	FR27 x FR303	A632 x LH98	Funks 64425
Dekalb-Pf 656	Asgrow RX6882	FR141 X FR303	Dekalb-Pf 484	Garst 8555
Dekalb-Pf 636	Dekalb-PF T1100	Dekalb-Pf 524	Dekalb-Pf 556	Jacques 7700
Funks 64500	Garst 8520	Garst 8333	Garst 8711	Stauffer 5340
Garst 8344	Garst 8538	Jacques 8100	Pioneer 3780	Six others
Garst 8345	Pioneer 3541		Jacques 4700	
Garst 8388	Stauffer 6595		Stauffer 5260	
Golden Harvest H2572			Two others	
Golden Harvest H2604				
Jacques 7900				
Northrup King 9540				
Stauffer 7751				
Stauffer 7759				
Eight others				

Source: Smith 1988.

Table 3. Testcross performance of selected lines derived from tropical and subtropical hybrids using Stiff Stalk Synthetic (Exp. 1: A632 x B73) and Lancaster [Exp. 2: Mo17 x (H95 x H99[3])] testers. Average for three replications, three locations in North Carolina, and two years' data

Pedigree	Yield (Mg/ha)		Grain moisture		Erect plants(%)		Days to pollen shed		Ear height (cm)		Select[a]	
	Exp.1	Exp.2	Exp.1	Exp. 2	Exp.1	Exp.2	Exp.1	Exp.2	Exp.1	Exp.2	Exp.1	Exp.2
(X105A x H5)-4	8.03	7.48	.168	.170	88	88	70	70	112	99	133	121
(X105A x H5)-5	8.18	7.51	.163	.172	93	90	70	70	109	97	142	123
(X105A x X306B)-2	7.37	7.17	.172	.184	97	97	71	71	108	101	129	120
(X105A x X306B)-3	7.67	7.65	.181	.191	95	95	71	71	108	100	128	121
H101-1	7.93	7.14	.169	.172	92	93	68	70	108	102	133	122
(X105A x Ag155)-2	7.71	7.11	.166	.174	94	91	71	70	104	93	133	118
(X105A x Ag155)-3	7.64	7.69	.164	.170	92	89	70	69	111	99	132	126
(X105A x Ag155)-4	8.15	7.37	.160	.172	92	93	69	69	110	95	142	123
(X304A x Ag504)-1	7.85	7.24	.173	.175	94	93	70	70	108	100	132	120
Pioneer Brand 3369	7.46	7.35	.168	.169	93	92	66	66	97	91	126	125
Pioneer Brand 3165	8.60	8.35	.183	.189	95	93	71	71	99	96	140	131
Pioneer Brand 3055	8.86	9.08	.203	.201	92	93	72	71	107	106	132	136
Pioneer Brand 3389	7.57	7.05	.168	.168	97	97	66	67	92	90	134	126
Pioneer Brand 3358	7.28	7.20	.167	.167	95	99	67	67	91	90	127	131
USS 9001	7.33	6.99	.172	.173	91	94	67	66	86	87	121	119
DeKalb 789	7.82	7.97	.200	.200	96	95	69	69	96	91	121	121
B73 x Mo17	7.61	7.73	.161	.164	84	90	66	66	101	95	124	130
LSD(.05)	.64	.60	.007	.008	10	7	2	2	5	5	16	14

Source: Adapted from Holley and Goodman 1988.

[a]Select = yield (Mg/ha).0628 + percent erect plants - 500 (grain moisture / total grain weight)

Table 4. THROPY (tropical hybrid lines cycle 2), (B73 x Mo17) testcrosses. Average for three locations, 1987 (Clayton, Lewiston, and Plymouth, North Carolina), except tassel date and silk-tassel nick, which were from Clayton only

Pedigree	Source	Grain yield (t/ha)	Percent moisture	Ears/plant x 100	%lodging Root	%lodging Stalk	Stand (%)	Ear ht. (cm)	Tassel Days	Tassel Nick	Sel.[a]
(105.N5) x 155 F2S4 x (B73 x Mo17)	7B46 - 2(85)	7.41	18.9	95	0	5	93	111	71	0.0	119
(105.5) x (155.504) F2S4 x (B73 x Mo17)	8007 - 1(85)	7.18	18.8	98	1	2	92	124	73	0.0	119
(105.N5) 155 F2S4 x (B73 x Mo17)	7846 - 1(85)	8.05	19.0	99	0	1	92	116	71	0.0	133
(105.N5) x 155 F2S4 x (B73 x Mo17)	7848 - 1(85)	7.76	18.9	97	1	3	91	111	71	0.3	126
(105.N5) x 155 F2S4 x (B73 x Mo17)	7873 - 1(85)	7.40	18.4	93	0	7	101	120	71	0.0	118
(105.N5) x 101 F2S4 x (B73 x Mo17)	7962 - 2(85)	7.26	18.2	94	1	6	98	118	72	0.3	118
Pioneer 3165	Rec. 1987	8.83	19.8	93	2	6	101	109	72	0.0	135
Dekalb 689	Rec. 1986	8.29	17.9	95	0	5	99	108	72	-.3	138
B73Ht x Mo17Ht	Rec. 1987	7.25	17.5	92	0	8	102	100	70	0.7	120
Experiment means		7.13	18.7	92	1	7	97	115			112
LSD .05 (entry x location)		.84	1.1	7	2	7	6	10			17
LSD .01 (entry x location)		1.12	1.5	10	3	9	8	13			23
C.V., % (entry x location)		7	3.6	5	151	55	4	5			10
(105.N5) x 155 F2S4 x (B73 x Mo17)	7865 - 1(85)	7.16	18.2	97	0	4	102	118	61	0.8	120
(105.5) x (155.504) F2S4 x (B73 x Mo17)	8020 - 2(85)	7.64	18.8	95	0	4	108	118	63	1.3	123
(105.N5) x 155 F2S4 (B73 x Mo17)	7876 - 1(85)	7.54	18.1	93	0	7	101	127	62	0.0	122
Pioneer 3165	Rec. 1987	8.54	19.5	93	2	5	104	111	64	-.3	132
Dekalb 689	Rec. 1986	7.99	17.5	95	0	5	99	110	62	0.2	134
B73Ht x Mo17Ht	Rec. 1987	7.35	16.9	94	0	6	99	102	59	0.7	126
Experiment Means		6.83	18.5	92	1	7	99	118			108
LSD .05 (entry x location)		0.90	1.0	8	2	8	5	9			21
LSD .01 (entry x location)		1.20	1.3	10	2	10	7	11			28
C.V., % (entry x location)		8	3.2	5	133	62	3	4			12
105 x (306.H5) F2S4 x (B73 x Mo17)	7995 - 1(85)	7.58	18.4	94	1	5	107	115	63	1.3	123
(105.5) x (155.504) F2S4 x (B73 x Mo17)	8020 - 1(85)	7.29	18.0	92	1	7	102	111	63	1.3	119
(105.H5) x 155 F2S4 x (B73 x Mo17)	7872 - 3(85)	7.03	17.8	90	1	9	106	114	61	0.0	112
P.105 x Ag. 155 F2S4 x (B73 x Mo17)	7811 - 2(85)	7.08	19.2	93	2	4	100	116	63	1.0	112
Pioneer 3165	Rec. 1987	8.14	20.4	92	2	6	102	110	64	1.0	121
Dekalb 689	Rec. 1986	6.87	18.4	88	0	12	102	109	62	0.3	106
B73Ht x Mo17Ht	Rec. 1987	7.81	17.2	99	0	1	100	103	59	1.0	138

Continued on next page

Table 4. Continued

Pedigree	Source	Grain yield (t/ha)	Percent moisture	Ears/plant x 100	%lodging Root	%lodging Stalk	Stand (%)	Ear ht. (cm)	Tassel Days	Tassel Nick	Sel.*
Experiment means		6.56	18.5	90	1	8	100	112			102
LDS .05 (entry x location)		1.06	1.2	9	2	8	5	6			23
LSD .01 (entry x location)		1.41	1.6	11	3	11	7	8			31
C.V., % (entry x location)		10	3.9	6	116	60	3	3			14
(105.H5) x 155) F2S4 x (B73 x Mo17)	7842 - 1(85)	6.88	18.1	95	0	5	108	111	61	0.7	114
(105.5) x (155.504) F2S4 x (B73 x Mo17)	8006 - 2(85)	7.24	18.9	93	1	7	96	114	63	0.7	114
155 x (304.101) F2S4 x (B73 x Mo17)	7905 - 1(85)	7.06	18.6	96	0	4	100	109	62	0.3	116
(105.H5) x 101 F2S4 x (B73 x Mo17)	7948 - 2(85)	7.07	18.0	93	1	6	98	108	61	1.0	115
(105.H5) x 155 F2S4 x (B73 x Mo17)	7844 - 1(85)	6.84	18.0	97	0	2	101	111	62	0.7	116
Pioneer 3165	Rec. 1987	7.92	19.6	91	2	7	105	108	63	0.3	119
Dekalb 689	Rec. 1986	6.91	17.8	90	0	11	102	106	63	0.0	111
B73Ht x Mo17Ht	Rec. 1987	7.10	16.9	90	0	9	100	101	59	0.7	119
Experiment means		6.50	18.4	88	1	11	102	111			99
LSD .05 (entry x location)		1.02	1.0	10	2	10	5	6			22
LSD .01 (entry x location)		1.35	1.3	13	3	13	7	8			30
C.V., % (entry x location)		10	3.3	7	132	53	3	3			14
(105.H5) x 101 F2S4 x (B73 x Mo17)	7950 - 1(85)	7.55	18.0	98	1	1	99	120	63	-.1	128
(105.H5) x 101 F2S4 x (B73 x Mo17)	7969 - 1(85)	7.34	17.2	96	0	4	98	122	62	0.7	127
Pioneer 3165	Rec. 1987	8.40	17.9	95	1	3	102	109	63	0.7	139
Dekalb 689	Rec. 1986	8.44	17.2	97	1	2	102	111	62	-.1	147
B73Ht x Mo17Ht	Rec. 1987	7.39	16.2	98	0	2	100	103	59	0.7	135
Experiment means		6.91	17.4	93	1	6	100	117			116
LSD .05 (entry x location)		0.91	1.0	9	2	8	6	9			20
LSD .01 (entry x location)		1.21	1.3	12	3	11	9	12			26
C.V., % (entry x location)		8	3.5	6	124	80	4	5			10

*Sel. = yield in bu/acre + % erect plants - 5 (% moisture).

Table 5. Numbers of agronomically promising races and collections arranged by maturity groupings

	Short-day maturity				
	Northern US Corn Belt	Central US Corn Belt	Southern US Corn Belt	Deep South	Total
After screening, before replicated trials					
Number of races	25	70	62	37	130
Number of collections	50	107	167	70	394
After replicated yield trials					
Number of races	4	13	28	7	41
Number of collections	9	20	46	8	83

Source:After screening--Goodman (1983); after replicated yield trials--Castillo and Goodman (in review).

Table 6. Means for traits of Latin American maize with early, short day maturities equivalent to central US Corn Belt materials, Weslaco, 1984

	Source	Grain yield (t/ha)	Ear quality*	Ears/plant x 100	Nick (gdu)	Male flowering (gdu)	Percent grain moisture	Ear ht. (cm)	Plant ht. (cm)	Erect plants (%)	Leaf blight*	Rust*
507	Pioneer	10.28	8.5	97	46.4	1,358	28.9	113	215	97.3	6.8	6.0
1J100	Pioneer	10.17	8.5	95	61.3	1,319	25.1	105	185	93.0	6.5	6.0
X304C	Pioneer	10.08	9.0	88	43.2	1,337	29.8	105	200	95.1	8.0	6.0
3214 (YOMO3)	Pioneer	10.07	9.0	99	59.9	1,369	28.9	120	210	75.4	8.0	6.5
Amarillo Salvadoreño	Comp. CIMMYT	7.15	8.5	93	60.3	1,320	24.9	110	205	84.9	6.5	7.0
Costeño	ATL 328 ICA	6.97	8.5	92	47.0	1,357	28.0	110	195	86.7	6.5	6.5
3055	Pioneer	6.69	8.3	99	34.6	1,328	20.0	98	193	79.5	4.8	2.8
Dente Branco R.G.	RGS XI CIMMYT	6.69	7.5	97	101.6	1,404	24.1	140	220	62.3	5.0	4.5
Costeño	ATL 329 FT C	6.62	8.0	96	67.0	1,337	26.9	100	190	90.1	8.0	6.5
Cubano Dentado	BOV 585 WLB	6.58	8.0	91	51.8	1,441	24.2	115	225	83.1	7.0	6.5
Costeño	ATL 314 FT C	6.27	8.0	94	54.9	1,337	25.1	125	190	78.3	7.0	7.5
Tusón	BAI III CIMMYT	6.18	8.0	88	64.5	1,441	24.4	130	210	70.9	7.0	7.5
Dente Branco R.G.	RGS XII CIMMYT	5.79	6.5	93	100.9	1,392	29.5	130	230	62.1	4.0	3.0
Cateto Assis Brasil	RGS XIV CIMMYT	5.77	8.0	94	61.5	1,392	22.0	120	205	63.6	7.0	4.0
Dente R.G. Rugoso	RGS I CIMMYT	5.69	7.5	95	50.5	1,417	29.1	130	225	57.6	5.0	4.5
B73 X Mo17		5.66	7.5	98	13.4	1,320	16.8	90	190	76.4	3.0	4.0
Early Caribbean	MAR 9 WLB	5.35	7.5	85	24.7	1,304	23.1	85	170	66.6	5.0	5.5
Cateto	CEI CIMMYT	5.33	8.5	98	50.0	1,506	26.1	125	220	67.9	7.0	6.5
Early Caribbean	MAR 4 WLB	5.28	7.5	85	47.0	1,357	24.0	100	170	77.0	2.5	4.5
Dente Branco R.G.	SC II CIMMYT	5.20	7.5	98	84.8	1,319	24.1	110	205	32.9	4.0	5.0
Celaya	GTO 69 CIMMYT	5.12	7.5	84	63.6	1,455	29.2	135	210	65.7	4.0	4.5
Cateto Sulino Escuro	URG V-A CIMMYT	4.97	8.5	91	67.0	1,337	22.3	105	180	74.8	5.5	4.0
Perola	BOV 437 WLB	4.94	7.5	92	65.0	1,454	23.6	130	210	74.4	5.5	5.0
Dzit Bacal	GUA 322 INIA	4.93	8.0	81	101.6	1,404	23.0	130	220	63.1	6.0	7.5
Hickory King	RGS IX CIMMYT	4.93	8.0	79	69.3	1,347	23.0	115	210	61.5	4.0	3.5
Comiteco	GUA 418 INIA	4.89	7.0	93	50.6	1,493	28.8	135	245	61.9	7.0	5.0
Bofo	NAY 191 CIMMYT	4.88	8.0	83	50.4	1,417	26.8	135	220	65.3	4.0	4.0
Cristal Semidentado	PAG III CIMMYT	4.88	7.5	82	50.1	1,404	22.5	130	215	68.3	4.5	3.5
Cateto Sulino	ARG II CIMMYT	4.78	7.5	93	42.4	1,338	19.5	110	195	46.1	3.0	4.0
Celaya	GTO 84 INIA	4.72	6.5	87	51.1	1,480	27.9	140	225	65.9	5.5	4.5
Coroico Blanco	BOV 1060 Zuber	4.68	7.0	90	57.0	1,347	22.1	115	190	77.6	6.5	5.5
Caingang	SP XIII CIMMYT	4.67	7.0	86	50.8	1,429	22.0	120	205	67.3	6.5	6.0
Four entries omitted												
3165	Pioneer	4.48	8.0	97	44.9	1,347	17.1	80	185	76.3	3.0	3.0
77 entries omitted												
Tukey's HSD (0.05 prob. n = 2)		1.96	4.0	46	129.1	120	11.8	58	82	54.8	3.9	3.6
(n1 = 2, n2 = 4)		1.70	3.4	40	111.8	104	10.2	50	71	47.4	3.4	3.1

*9 = best, 1 = worst.

Table 7. Means for traits of Latin American maize populations with intermediate, short day maturities equivalent to southern US Corn Belt Materials, Weslaco, Texas, 1984

Race or hybrid	Source	Grain yield (t/ha)	Ear quality*	Ears/plant x 100	Nick (gdu)	Male flowering (gdu)	Percent grain moisture	Ear ht. (cm)	Plant ht. (cm)	Erect plants (%)	Leaf blight*	Rust*
3214 (YOMO3)	Pioneer	10.05	8.8	99	56.3	1,378	27.6	135	207	65.9	8.0	6.8
507	Pioneer	9.93	9.0	100	52.2	1,454	32.9	120	215	91.0	5.5	7.0
X304C	Pioneer	9.28	8.8	99	41.1	1,354	28.6	112	207	90.5	8.0	6.5
Cubano Amarillo Duro	ECU 326 ICA	8.13	8.0	100	39.0	1,441	25.4	140	235	70.6	7.0	5.5
Cubano Amarillo Duro	ECU 904 ICA	8.01	8.5	96	50.4	1,417	26.1	140	220	90.1	7.0	6.5
Tuxpeño	VEN 598 ICA	7.40	8.5	95	52.2	1,454	29.1	140	240	79.4	7.0	6.5
Cubano Cateto	ECU 339 ICA	7.37	8.5	94	50.4	1,417	24.5	135	220	92.9	7.0	6.5
Tuxpeño	VEN 767 ICA	7.29	8.0	89	51.1	1,480	24.5	150	235	70.7	9.0	7.5
Cubano Tusón	ECU 542 ICA	7.15	8.0	70	52.2	1,454	25.1	125	225	79.2	7.0	6.0
Cubano Amarillo Duro	ECU 770 ICA	7.11	8.5	93	64.5	1,441	24.6	140	235	66.3	6.5	6.0
Cuban Flint	CUB 63 CIMMYT	7.01	9.0	95	75.9	1,328	25.3	140	220	76.7	8.0	6.0
Común	VEN 897 ICA	6.99	8.0	86	12.8	1,519	35.8	165	250	77.9	8.0	7.0
Perla	LIM 13 FT C	6.98	8.0	91	63.3	1,480	24.7	170	265	75.7	7.5	6.0
Chandelle	VEN 460 ICA	6.94	8.0	95	25.0	1,519	34.1	130	220	87.5	8.0	7.0
Cubano Tusón	ECU 660 ICA	6.84	7.5	90	64.1	1,429	25.7	125	225	77.4	7.0	5.5
Tusón	CUB 57 CIMMYT	6.74	8.0	86	48.9	1,380	25.3	125	215	73.0	7.0	7.0
Tusón	CUB 62 CIMMYT	6.74	8.0	90	49.1	1,392	29.6	135	230	84.0	8.0	6.5
Cubano Tusón	ECU 659 ICA	6.69	8.5	85	89.5	1,441	26.1	140	230	64.7	6.5	6.5
Costeño	VEN 453 ICA	6.61	7.5	92	39.4	1,454	24.6	135	220	53.4	7.0	6.0
Yungueño	BOV 747 ICA	6.57	8.0	88	39.0	1,441	21.2	145	235	68.8	8.0	3.0
Dente R.G. Liso	RGS V CIMMYT	6.55	8.0	91	63.4	1,493	30.4	125	220	78.0	6.5	6.5
Cubano Amarillo Duro	ECU 653 ICA	6.55	8.0	83	49.4	1,404	23.0	140	230	81.5	7.5	5.5
Canilla (Puya Grande?)	VEN 981 ICA	6.40	9.0	97	76.9	1,429	22.6	125	190	64.6	8.0	6.5
Cubano Cateto	ECU 330 ICA	6.40	8.0	88	76.1	1,480	27.9	160	235	86.0	7.5	6.5
3055	Pioneer	6.38	7.8	100	43.3	1,323	19.4	90	203	78.0	5.4	3.3
Tuxpeño	OAX 9 INIA	6.34	7.0	85	50.6	1,493	28.7	170	260	77.4	7.0	4.0
Tepecintle	GUA 597 CIMMYT	6.34	8.0	94	38.4	1,493	27.8	145	225	83.0	7.0	7.0
Puya	MAF 322 ICA	6.34	8.0	89	75.5	1,480	30.3	155	215	74.4	7.0	6.5
Cuban Flint	CUB 65 CIMMYT	6.32	7.5	95	49.4	1,404	23.5	100	210	87.3	4.0	4.5
Costeño	VEN 859 ICA	6.30	8.0	96	51.1	1,480	26.4	165	235	54.8	8.0	6.5
Gallina	ECU 929 ICA	6.23	7.5	97	50.8	1,581	29.4	170	260	84.0	7.0	8.0
Tuxpeño	VEN 414 ICA	6.21	7.5	95	64.5	1,441	25.0	135	230	72.9	7.0	7.0
Cubano Amarillo Duro	ECU 975 FT C	6.21	7.5	78	62.7	1,531	27.5	130	230	81.1	7.5	4.5
Cubano Amarillo Duro	ECU 370 ICA	6.20	8.0	83	51.8	1,441	22.3	135	225	73.1	6.0	6.5
St. Croix	IVC 2 WLB	6.19	8.0	92	47.0	1,357	23.2	125	195	51.6	7.0	6.5
Puya	SAN 349 WLB	6.14	8.0	87	38.0	1,429	27.8	135	225	92.0	7.0	7.0
20 entries ommited												
B73 x Mo17		5.28	7.2	97	21.5	1,325	16.3	110	190	69.7	4.0	3.8
36 entries omitted												
3165	Pioneer	4.26	7.2	100	51.8	1,345	17.2	72	182	75.9	4.0	2.8
79 entries omitted												
Tukey's HSD (0.05 prob., n = 2)		2.20	2.7	38	104.9	119	9.4	77	63	52.7	3.3	3.6
(n1 = 2, n2 = 6)		1.80	2.2	31	85.6	97	7.7	63	51	43.0	2.7	2.9

*9 = best 1 = worst

Table 8. Means for traits of Latin American maize populations with late, short day maturities equivalent to Deep South US materials, Weslaco, Texas, 1984

Race or hybrid	Source	Grain yield (t/ha)	Ear quality[a]	Ears/plant x 100	Nick (gdu)	Male flowering (gdu)	Percent grain moisture	Ear ht. (cm)	Plant ht. (cm)	Erect plants (%)	Leaf blight[a]	Rust[a]
3214 (YOMO3)	Pioneer	10.54	9.0	99	50.4	1,417	28.0	115	225	78.2	7.8	7.0
X304C	Pioneer	9.20	9.0	96	52.0	1,352	27.5	113	220	83.7	8.3	6.8
Olotón	GUA 383 CIMMYT	7.51	7.0	83	50.9	1,569	31.3	140	235	73.9	7.5	6.0
Chandelle	VEN 352 ICA	7.47	9.0	95	37.6	1,556	31.4	155	250	70.9	7.5	6.5
Costeño	MAG 350 FT C[a]	7.07	8.0	94	100.8	1,607	40.2	125	215	87.7	6.5	7.0
Chandelle	CUB 68 CIMMYT	6.97	8.0	99	76.7	1,467	29.0	145	215	75.0	8.0	6.0
Tehua	CHS 159 CIMMYT	6.97	7.5	86	135.3	1,811	58.8	180	275	84.5	7.0	7.0
Costeño	COR 320 FT C[a]	6.72	7.5	95	75.8	1,607	40.2	145	225	80.6	7.5	6.0
Carlaco	VEN 408 ICA	6.61	8.0	91	75.6	1,506	30.5	100	200	82.9	7.5	6.5
Chandelle	VEN 489 ICA	6.27	8.0	89	63.1	1,607	33.6	140	215	77.9	6.5	7.0
3055	Pioneer	6.10	8.0	97	58.8	1,333	19.6	90	193	82.5	5.3	3.3
Tuxpeño	ECU 942 ICA	6.09	7.5	89	76.1	1,581	30.8	140	230	74.5	8.0	7.0
Comiteco	CHS 86 INIA	5.87	8.0	83	113.8	1,619	46.4	135	245	39.4	7.0	5.5
Tuxpeño	GUA 456 CIMMYT	5.87	7.5	92	101.0	1,632	36.5	160	235	57.0	7.0	7.0
Puya Grande	VEN 495 ICA	5.87	8.0	88	63.3	1,607	26.7	150	230	41.3	7.0	5.5
Cubano Amarillo Duro	ECU 327 FT C	5.74	7.5	84	126.7	1,632	36.8	165	240	78.1	8.0	7.5
Costeño	BOL 375 FT C[a]	5.72	7.0	92	102.1	1,670	40.4	140	220	78.3	7.0	6.0
Costeño	VEN 847 FT C	5.57	7.5	85	62.7	1,519	29.7	130	220	87.2	7.5	7.5
B73 x Mo17		5.48	7.3	99	54.8	1,320	16.9	93	185	89.2	4.0	4.0
Cateto Nortista	GIN I CIMMYT	5.34	8.0	92	75.8	1,607	31.8	145	225	83.3	8.0	7.5
Tehua	CHS 29 CIMMYT	5.27	6.5	84	158.6	1,746	46.1	175	270	89.1	7.0	6.0
Chandelle	VEN 432 ICA	5.22	7.5	90	101.0	1,632	35.2	140	235	81.8	6.5	7.5
Chandelle	VEN 717 FT C	5.22	8.0	92	101.3	1,619	36.0	145	225	82.0	7.5	5.5
Comun	VAL 390 ICA	5.22	7.0	78	138.7	1,519	31.4	150	235	80.6	4.5	7.0
Puya	SAN 346 ICA	5.17	8.0	83	63.1	1,556	27.8	135	230	83.2	6.0	7.5
Haitian Yellow	HTI 13 WLB	5.12	8.0	80	77.5	1,733	36.4	125	220	73.9	7.5	7.0
Cateto	MG II CIMMYT	4.99	8.0	87	50.9	1,569	28.1	150	235	72.3	7.0	6.0
Carlaco	VEN 631 FT C	4.92	7.0	96	101.3	1,619	32.2	120	200	76.6	6.5	7.5
Cravo Paulista	SP II CIMMYT	4.84	7.5	82	63.5	1,569	34.0	140	235	42.7	6.5	7.5
Cateto	BOV 1083 ICA	4.69	8.0	94	101.1	1,645	28.0	150	225	45.3	6.5	6.0
Haitian Yellow	HTI 14 WLB	4.62	7.5	81	90.4	1,720	33.5	115	230	93.9	6.5	7.0
3165	Pioneer	4.58	8.0	98	47.5	1,363	17.0	78	190	91.2	3.0	2.8
43 entries omitted												
Tukey's HSD (0.05 prob., n = 2)		1.85	2.3	34	119.5	101	14.0	64	51	46.0	3.2	3.8
(n1 = 2, n2 = 4)		1.61	2.0	30	103.5	88	12.1	55	44	39.8	2.8	3.3

[a]9 = best, 1 = worst

Table 9. Accessions undergoing conversion to daylength neutrality

NAL-TEL ATB	GUA	110	Puya	MAG	322
Oloton	GUA	383	Puya	SAN	349
Tepecintle	GUA	597	Cubano Amarillo D	ECU	326
Dzit Bacal	GUA	131	Cubano Amarillo D	ECU	327
Amarillo Salva	COMP		Cubano Amarillo D	ECU	653
Early Caribbean	MAR	9	Cubano Amarillo D	ECU	770
Cuban Flint	CUB	63	Cubano Amarillo D	ECU	904
Tusón	CUB	57	Cubano Tusón	ECU	542
Tusón	CUB	62	Cubano Tusón	ECU	660
Chandelle	CUB	68	Cubano Cateto	ECU	339
Cariaco	VEN	408	Tuxpeño	ECU	942
Canilla (P.G.)	VEN	981	Perla	LIM	13
Chandelle	VEN	352	Morado	BOV	402
Tuxpeño	VEN	598	Cubano Dentado	BOV	585
Tuxpeño	VEN	767	Coroico Blanco	BOV	582
Costeño	ATL	314	Cateto	CE	I
Costeño	ATL	328	Cateto	DES	I
Costeño	ATL	329	Cateto	MG	II
Costeño	MAG	350	Cateto Nortist	GIN	I
Negrito	MAG	321	Tusón	BAI	III

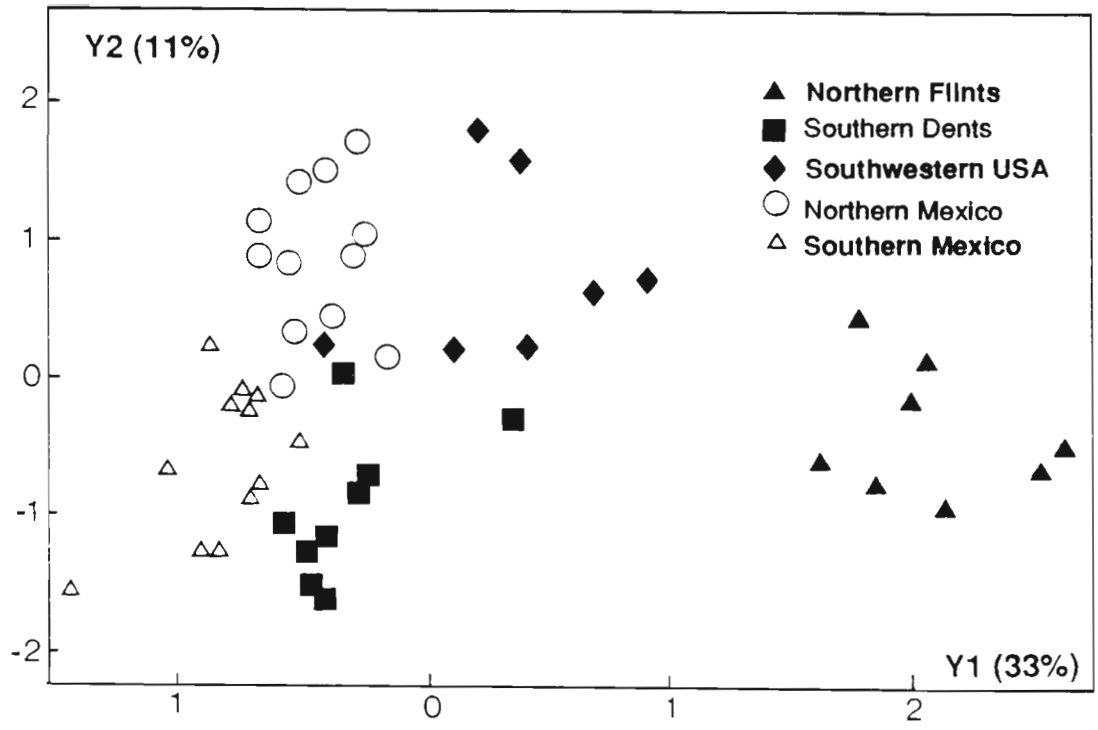

Figure 1. Principal components analysis of isozyme allele frequencies for Mexican and US maize populations. The first two components of the analysis are based on the covariance matrix. Adapted from Doebley et al. 1988.

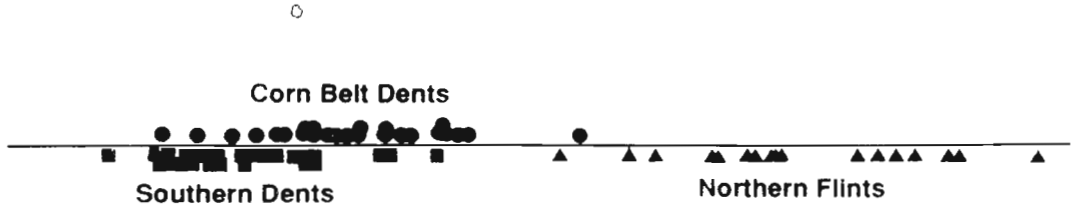

Figure 2. Projection of isozyme allele frequencies of samples of Northern Flints, Southern Dents, and Corn Belt Dents onto the axis joining the menas (centroids) of the Northern Flints and Southern Dents. Means of the three races are indicated by vertical lines. Adapted from Doebley et al. 1988.

Theory and Practice in Determining Sample Size for Conservation of Maize Germplasm

J. Crossa, Biometrician, CIMMYT

Abstract

This paper examines the use of probability models for choosing a sample size in the regeneration of seed stocks, uses population genetics theory in considering the genetic consequences of random drift in small populations, and describes some practical options in maize seed regeneration, based on the theoretical results.

The CIMMYT maize germplasm bank preserves and has regenerated landrace collections from the Americas, which represent a major part of the genetic variability of maize. The bank is well advanced in regenerating materials from the lowland tropics of Latin America but has made less progress with germplasm from the highlands of Central America (Costa Rica and Guatemala) and the Andean Zone (Taba 1988).

To preserve the genetic variability of maize in seed regeneration, it is important that sampling be done efficiently and that the populations be of sufficient size to maintain as much genetic diversity as is practicable. Large samples are expensive and difficult to manage, but if the samples are too small, valuable genes may be lost through random changes in gene frequencies (random genetic drift). In regenerating seed it is important for bank managers to know 1) how large a sample of a bank accession is needed to obtain one or more particularly rare alleles with a certain probability and 2) how this sample size will affect the genetic integrity of the collection in terms of changes in allele frequencies and inbreeding depression.

The purposes of this study were to 1) examine the use of probability models for choosing a practical sample size in the regeneration of seed stocks, 2) use population genetics theory in considering the genetic consequences of random drift in small populations, and 3) describe some practical options in maize seed regeneration, based on the theoretical results. Since the first two items are covered in Crossa (1989), a brief summary of the results is given here, and the remainder of the paper concentrates on the practical options.

Probability Models in Seed Regeneration

Using probability theory, the germplasm bank manager can address various issues that arise in regenerating accessions, including: 1) the number of desired rare genotypes (alleles) in the sample, 2) expected number of parents represented in the sample, 3) number of alleles represented in the sample, and 4) expected number of neutral alleles in the sample. In another publication Crossa (1989) derived probability formulas that had been applied in similar contexts by other authors and proposed some new interpretations and applications of these formulas. It was concluded that the size of the sample depends more on the frequency of the rare allele or alleles than on their number. A sample size of 100 will preserve rare genotypes (or alleles) occurring in a proportion of about 0.05 with a probability of about 95%. Genotypes appearing at frequencies of more than 10% can be recovered with a sample size of 40. However, if the frequency drops below 0.05, larger sample sizes are required to maintain a high probability of including some of the rare individuals in the sample.

Bulking equal quantities of seed from each maize ear and then taking a sample from the bulk is appropriate only for large seed samples. If this procedure is applied to small samples, some ears included in the bulk will be lost in the sampling process. Suppose, for example, that a balanced composite of seeds is taken from each of 100 ears and bulked. If 100 seeds are taken from the bulk, it is expected that, on the average, about 37 parents will not be represented in the sample.

For two, three, and four alleles at a locus, the sample size required to include at least one copy of each allele with 95% probability depends more on the frequency of the rare genes than on the number of rare alleles. It is expected that sample sizes of 300 to 400 will retain two, three, or four alleles even when some of them occur at a frequency of 1%. For 100 independent loci with the desired allele at a frequency of 5% in each locus, there is a 99% probability that a sample of 179 lines will include the favorable allele at each of the 100 loci in at least one line.

Random Genetic Drift in Seed Regeneration

Population genetics theory is useful in determining how allele frequency is affected by changes in population size. Unpredictable changes in gene frequency caused by sampling errors in small populations (random genetic drift) leads to continuous fixation and loss of alleles and reduces the proportion of heterozygous individuals in the population.

In a large mating population of N individuals, the reduced number of progenitors whose offspring will constitute the next generation is referred to as the effective population size (Ne). In general, factors such as variation in the sex ratio, number of breeding individuals at different times, type of reproduction, and number of offspring per family reduce the actual size of the breeding population ($Ne < N$). It has been shown that equalizing the reproductive output among families to two progeny doubles the effective population size (Gale and Laurence 1984; Crossa 1989). This case is of much interest in the regeneration of seed stored in germplasm banks. According to Frankel and Soule (1981), this method of minimizing random genetic drift is as good as some special mating systems, such as circular half-sib or maximum avoidance of inbreeding. An ideal system for seed regeneration should 1) equalize the number of offspring among families to two and 2) avoid small populations in any regeneration cycle.

Bottlenecks--A founder or bottleneck effect is a sampling error that occurs when a population is reduced to a small number of individuals, a situation that can alter allelic frequencies from those in the original population. Rare alleles occurring at low frequencies have a high probability of being lost as a result of a bottleneck, although the average heterozygosity is influenced more by the rate of population growth after the bottleneck occurs than by the size of the bottleneck.

Theoretical studies on the effect of bottlenecks on average heterozygosity and on the loss of alleles showed that the amount of reduction in heterozygosity per locus depends on two factors: 1) the size of the bottleneck and 2) the rate of population growth after the founder effect. On the other hand, the loss in the average number of alleles per locus is highly affected by bottleneck size but not so much by the rate of population growth (Nei et al. 1975).

It is very common for maize germplasm banks to start with collections of less than 10 ears. Let us assume that these ears are pollinated by a very large (infinite) number of plants. The Ne for unequal number of sexes is twice the harmonic mean of the number of males (Nm) and of females (Nf); that is, $1/Ne = (1/4Nm) + (1/4Nf)$ and depends much more on the sex that is less numerous. Thus, with the limited number of 10 females and a very large number of males, the

effective size is 4Nf or Ne = 40. This may represent a severe bottleneck, possibly causing some rare alleles with a frequency of 0.05 or less to be lost. One can expect that for a certain level of polymorphism: 1) the mean number of alleles in the sample of 10 will be about half that of the original population and 2) the loss of alleles in subsequent cycles of regeneration is reduced if the population grows rapidly. Therefore it is important in subsequent cycles to increase the population size as much as possible to prevent a large additional reduction in heterozygosity and a further increase in the rate at which desirable alleles are lost.

The proportion of unfixed alleles that becomes fixed or lost as a result of random genetic drift (F = rate of inbreeding), expressed as a function of the effective population size (Ne) and number of alleles (x), is calculated as follows:

$$F = x(x - 1)/4Ne \text{ (Kimura 1955)}$$

If the initial collection consisted of only 10 ears (Ne = 40) with two alleles per locus, the rate of inbreeding is 1.25%, and with three alleles per locus, F = 3.75%. Both of these F values are higher than the 1% suggested by Frankel and Soule (1981) as a general rule for conservation genetics.

Practical Considerations in Maize Seed Regeneration

Because of limited resources, ideal procedures may not be practical. Suppose, for example, that we have a collection of 100 maize ears. The ideal system for regenerating this collections would be as follows:

1. Take at random one kernel from each ear and put it in a packet; repeat until 8 to 10 packets are completed.

2. Plant as many blocks as there are completed seed packets; each block will have 100 plants (one from each original maize ear).

3. Make 50 random paired crosses within a block using each plant only as a male or female but not both.

4. Because of germination problems, not all of the plants within a block will produce progeny. Discard blocks that do not complete 50 paired crosses.

5. Equalize the number of progeny per original family to two by harvesting only two blocks in which all the crosses were made.

6. From the 100 ears collected (50 from each block), take at random one kernel and put it in a packet; repeat until 8 to 10 packets are completed. These will be used for the next cycle of regeneration.

With this procedure Ne = 200. It is similar to the biparental mating scheme recommended by Gale and Lawrence (1984). However, this ideal procedure is highly impractical and very costly, requiring extensive use of land, labor and management resources. More practical and less expensive alternatives can be found by modifying the procedure outlined above. Some of these options are described below.

Option 1--Similar to the ideal system, but only two blocks (100 seeds each) are planted, so that less land is required. However, seed mortality may restrict the total number of harvested ears to less than 100.

Option 2--Similar to the ideal procedure, but only three blocks are planted with 100 seeds each. That is, the number of progeny per family is equalized to three. This procedure has two advantages: (1) it requires less field space than the ideal system, and (2) if 100 ears can be collected from only two blocks, the number of progeny per family is still equalized to two. If three blocks are harvested, the effective size of the population is Ne = (3N - 1)/(3 - 1) = 150.

Option 3--Similar to the ideal system, but only four blocks are planted with 100 seeds each. Complete as many paired plant crosses as possible in each block. Harvest all usable ears from the block that has the most ears, and complete 100 ears by harvesting the remainder from another block. Take at random one kernel from each ear, and put it in a packet. Repeat this procedure at least four times for the next cycle of regeneration. Option 3 requires more field space than options 1 and 2.

Option 4--Produce a reduced bulk of 200 seeds by taking at random two kernels from each of 100 ears. Put the 200 seeds in a packet. Repeat the procedure, so that there are two packets of 200 seeds each. Plant the two packets of seed, each in a separate block. Make all possible paired crosses within each block. Harvest all usable ears from one block and, if necessary, complete the 100 ears required by harvesting the rest from another block. Finally, produce two different bulks of 200 seeds each, using the same procedure described above. The probability of crossing plants from the same ear in one block is given by the following equation:

$$\frac{i - 1}{(i * n) - 1} \qquad (1)$$

where n is the number of original ears, and i is the number of kernels taken from each ear. For example, for n = 100 and i = 2, the probability of mating two plants originating from the same ear is very low (0.005). This alternative requires the same amount of land as option 3 but less work since the seed is bulked.

Option 5--Similar to option 4, but to allow for seedling mortality and to save field space, each of the two blocks is planted with two kernels per hill and later thinned to one. All possible paired plant crosses are made within each of the two blocks with 100 plants. This requires half of the field space used with options 3 and 4.

In summary, all the options are modifications of the optimum system and have the advantage of requiring much less land and work while levelling out the number of progeny per family. Option 4 requires just as much field space as option 3 but less work. Option 5 requires half the amount of land needed for options 3 and 4.

Option 6--To regenerate an accession, make a balanced composite of 600 seeds of a given number of ears (usually about 100). Plant 512 seeds in 256 hills (2 seeds per hill), and make about 200 chain crosses. Field experience has shown that, to obtain about 100 good ears for storage, twice that many plants have to be pollinated. Some of these may be lost or not fully

developed or well pollinated, and others may be damaged by disease or insects. It is advisable to plant two sets of 512 seeds and to make paired crosses between sets.

With all of the options outlined above, there is a chance that some original families may not be represented, because of seed mortality during storage or establishment problems. Moreover, with option 5 materials representing a given family may be lost by chance during thinning. On the other hand, some families may be represented more than twice. Therefore, in practice one is not very likely to obtain exactly two progenies of each family or an equal number of families over various cycles of regeneration.

Optimum conditions for growing and drying the seed and good facilities for long term seed storage will ensure that the variability and genetic integrity of every accession are maintained. Monitoring the seed viability of collections in storage is an important means of determining whether or not to regenerate an accession (Ellis and Roberts 1984). The results of a viability test are also useful in deciding which is the appropriate option for seed regeneration. For example, one would have to compensate for a decline in germination percentage by planting more blocks in the field or multiple seeds per hill.

Regardless of the option one chooses, different pollination procedures can be used within each block. One is ***paired crossing***, which is carried out by making plant-to-plant crosses between rows within a block, using each plant as male or female but not both. A second possibility, ***chain crossing***, involves crossing plant 1 with plant 2, plant 2 with plant 3, and so on up to plant *n* crossed with plant 1. This mating scheme takes into consideration cytoplasmic factors and requires about the same amount of field work as paired crossing. However, it does not allow for generation of unrelated families, because one plant is used as a male in one cross and as a female in another cross. This generates a chain of *n* half-sib families; that is, ear i and i + 1 are half sibs, as are ear i + 1, i + 2, and so on. A third procedure, ***bulk pollination***, is sometimes used in seed increase and regeneration and produces bulk half-sib families. However, as pointed out above, the main limitation of this approach is that effective pollen may be obtained from only a few plants. In practice only a reduced number of plants in a block are available for crossing. In cases where plants are used both as males and females, regardless of the pollinations system, the sex ratio does not influence the effective population size since Nm = Nf. Thus, the effective population size is equal to the real size of the population (Ne = N).

Regeneration of Small Original Collections

For regenerating an original collection that consists of only a few ears, it is important to increase the population size to 100 or more for the next regeneration cycle. Suppose, for example, that a collection of 10 ears is to be regenerated. Take at random 30 kernels from each ear, and plant the 300 seeds in an isolated block. Make as many random paired crosses as possible and harvest all usable ears. For n = 10 and i = 30, the probability of mating plants from the same ear is 10% (see equation 1). Suppose that 100 ears are harvested and option 4 is chosen for the next regeneration cycle. Produce a bulk of 200 seed by taking at random two kernels from each of the 100 ears and put them into a packet. Repeat this procedure until four or more packets are completed.

Summary of Recommendations

Two important aims of any seed regeneration procedure are to 1) control and equalize the number of progeny per family and 2) use the same number of breeding individuals over different cycles of regeneration. For large original collections, maintenance regeneration has first priority and can be performed using any of the practical options outlined above. With small original collections (30 ears or less), the first priority must be to increase population size to 100 or more ears before storage. The second priority is to carry out maintenance regeneration after storage.

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Country Reports

Of the 27 national program representatives participating in the workshop, 22 gave reports on their maize germplasm banks and on activities related to maize genetic resources. Presented below are brief summaries of those reports, emphasizing primarily the composition of national maize bank holdings, collection activities, and the availability and management of information about bank accessions. For some countries a few other items of information are included that are not readily available elsewhere or that provide good examples of a particular activity or policy.

Argentina

Marcelo E. Ferrer and Julio Safont Lis

Germplasm holdings--The maize germplasm bank at Pergamino also manages an active collection containing 3,444 entries, which can be classified into various groups. First are the original or primary samples representing distinct races that are native to Argentina, some of which have been regenerated. These materials comprise the following groups organized according to their age and origin:

- 113 entries of local flint varieties (races) from the pampas collected during 1951-1962
- 80 entries of various races collected in the northeast during 1955-1968
- 1,737 entries, representing diverse races and types, collected in 1977, 1978, and 1981 during nine expeditions covering most of the country (northwest, northeast, central, and southeast) with assistance from IBPGR (Table 1)

All of this material has been characterized by race. In 1986, again with IBPGR support, a tenth collection trip was made in the southwest Patagonian region, and the 185 materials collected are in the process of being characterized. Also included in this first group are 86 duplicates provided by CIMMYT in 1987 of collections of various races collected in Argentina during the 1950s.

The second group, with 859 entries, consists of duplicates of collections of races native to Uruguay. The third group, with 38 entries, includes racial composites made up of Argentine races recollected since 1977. The fourth group (150 entries) contains duplicates of populations, pools, and synthetics from Paraná, Argentina. The fifth group (171 entries) includes duplicates of inbred lines from Paraná. And the sixth group (25 entries) is made up of duplicates of materials from Paraná having certain genetic characters and cytoplasmic sterility.

Conserving materials in the maize improvement program at Pergamino is not the responsibility of the germplasm bank. All of these materials make up a working collection that is maintained by the breeders; part of it is kept under refrigeration.

Data storage--Our listing of bank materials at Pergamino, based on passport data on the Argentine local materials, includes 2,115 entries, each of which is assigned a unique number for identification. Numbers have not yet been assigned to advanced cultivars. Materials normally receive a number as soon as seed is received. Information on the germplasm is stored using the GDM data management system. The information is processed on two North Star Horizon microcomputers with 58 K bytes of memory and storage on diskettes, each with a capacity of 360 K bytes.

Evaluation--So far, 2,115 entries have been evaluated (at least the original sample), giving an estimated 1,200,000 items of data, only part of which has been entered in the computer. On the basis of this information, our first genetic resources catalog was published in 1979 and included passport data on 1,786 original samples of populations from the pampas, the northeast, and the northwest of Argentina, which had been collected up to 1978. Also presented were characterization and primary evaluation data on populations from the pampas and northeast collected up to 1967. In 1983 a second catalog was published to provide information from the characterization and primary evaluation of part of the samples collected in 1977 and 1978 (918 out of a total of 1,591) along with 116 collected in 1981, with a limited number of descriptors.

The 859 duplicates from Uruguay possess a distinct identifier and are registered at the bank in Pergamino on the basis of their passport data. The identifier is included in the catalog on Uruguay's genetic resources, which was published at Pergamino in 1983 and includes, together with the passport data, characterization and evaluation data. At Pergamino we also published recently catalogs for the banks in Bolivia, Chile, and Paraguay containing information provided by the persons directly responsible for these collections.

In addition to characterization and agronomic evaluation, we have conducted preliminary cytological studies to establish the constitution of chromosome knobs and other components of the native races of Argentina with elite representatives of original samples collected between 1951 and 1967. These were the only materials available until 1975, when these studies were suspended. The studies included 29 samples corresponding to 11 races or recognized types (4 from the pampas and periphery and 7 from the northeast), with five plants of each sample.

Bolivia

Gonzalo Avila

Germplasm holdings--Bolivia's maize germplasm collection, which is being conserved at the Pairumani Center for Plant Breeding Research, was obtained during 17 collection missions covering the entire country under a cooperative arrangement with IBPGR during the late 1970s and early 1980s. The bank currently contains 1,024 samples, for 884 of which we have 1-4 kg of seed. Duplicates of our materials are stored at the US National Seed Storage Laboratory at Fort Collins, Colorado. The great majority of the samples collected are of local varieties grown between 200 and 3,400 m above sea level (masl), and a few are varieties introduced within the last 40 years. Among the latter the most widely distributed was the race Cubano Amarillo.

Characterization--Passport data are available for about 94% of the collection. About 41% has been characterized using 60 descriptors, which were originally discussed within a program organized for the Southern Cone region by the Inter-American Institute for Agricultural Cooperation (IICA) and Inter-American Development Bank and were later presented upon request to IBPGR. The results of characterization are contained in a series of catalogues published jointly by the countries of the Southern Cone and Peru in cooperation with the Cooperative Program for Agricultural Research in the Southern Cone (PROCISUR) and Argentina's National Institute of Agricultural and Livestock Technology (INTA). Currently, 25% of the collection is being characterized, leaving 34% still to undergo this process.

Brazil

Jairo Silva

Germplasm holdings--The first effort to collect and describe Brazilian maize varieties and landraces was made in 1937 by F.G. Brieger and a few associates at the University of Sao Paulo, College of Agriculture Luiz de Queiroz, Genetics Institute, in Piracicaba. Through several international cooperative projects, a germplasm bank was established there and remained in operation until 1975. Bank holdings, which consisted of 145 accessions from Brazil (mostly composites) and 138 from CIMMYT, were then transferred to the National Research Center for Maize and Sorghum at Sete Lagoas, where they remain. Before 1984 seven collection missions were made by the National Center for Genetic Resources (CENARGEN), resulting in the addition of 772 accessions. More than 322 accessions were introduced into the country. From 1984 to 1987, another 200 accessions were added, and some 400 were introduced. Early in 1987 we received the first 577 accessions of Brazilian material being conserved in CIMMYT's germplasm bank. Altogether, the Brazilian bank now holds more than 2,500 accessions, 2,000 of which are varieties and landraces, 200 composites, and around 300 indigenous materials. At least six more collection missions are planned for 1988 and 1989 in the northeastern and northern areas of the country.

Evaluation--Six hundred accessions were characterized at Sete Lagoas from 1980 to 1984 and the results published in a catalog.

Chile

Orlando Paratori B.

Germplasm holdings--The maize program of the Institute for Agriculture and Livestock Research currently maintains a collection of 914 samples of indigenous maize. Of these 536 accessions were collected across the country under a cooperative arrangement with IBPGR, which financed this work. The material was collected during 1981-1982 and has been classified, characterized, and regenerated at La Platina Experiment Station.

Another 263 accessions were collected in 1955 but suffered a certain degree of genetic erosion as a result of maintenance problems. This germplasm consists of the following material: 204 collections from the northern and central areas of the country (regenerated during the 1984-85 season), plus 59 early maturing collections (regenerated in 1985-86). This material has been evaluated, classified, and multiplied according to methodologies developed through germplasm workshops sponsored by IBPGR. As with the 536 accessions mentioned above, 12 descriptors pertaining to the origin and identification of the samples, 17 for the ear and grain, and 22 for the plant and tassel were used.

The remaining accessions include 56 samples obtained from the Colombian germplasm bank and 59 Chilean maize accessions obtained from the CIMMYT bank. The former were characterized and classified in the 1987-88 season, and the latter, received in 1987, have not yet been classified.

Data storage--Passport and characterization information for the 536 accessions collected in cooperation with IBPGR is currently stored at the Pergamino computer center in Argentina, but it will soon be stored in the Computer Services Unit at La Platina. To these data will be added information on the 263 accessions characterized during 1985-1986. Information from evaluations

of 759 accessions conducted during 1986-1987 under the Latin American Maize Project (LAMP) is also being kept at La Platina.

China

Cui Cong-shu

Facilities--The Institute of Crop Germplasm Resources has two germplasm banks. Bank no. 1, which was established in 1984, has two cold rooms, a large one for medium term storage and a smaller one for long term storage. This facility can accommodate 50,000 to 80,000 accessions in long term storage. Construction of bank no. 2 was completed in 1986 and was financed in part by the Rockefeller Foundation and IBPGR. It has two cold rooms with a capacity of 400,000 accessions for long term storage.

Germplasm holdings--Maize is a major cereal crop in China, ranking third after rice and wheat, both in area grown and production. Both dent and flint types are grown. About 11,000 accessions of eight subspecies have been collected in China, most of which are domestic landraces. Some materials have been introduced as well. The bank currently stores 5,000 accessions of maize and expects to have 11,000 by 1990.

Colombia

Carlos Díaz Amaris, Maria Elena Botero E., and Fernando Arboleda Rivera

Germplasm holdings--In 1950 Colombia's Ministry of Agriculture and the National Research Council of the USA, in cooperation with the Rockefeller Foundation, initiated the collection and classification of the Colombian maize races. This activity was extended to include Venezuela, Ecuador, Peru, Bolivia, and Chile, making the Colombian maize germplasm bank one of the world's best sources of germplasm for maize improvement, particularly in the tropics and subtropics of Latin America. As a result of those collection efforts, the bank now holds, after 37 years of operation, 5,044 registered and classified collections of 123 races originating in some 30 countries (Table 2). In Colombia alone 2,089 samples have been collected in all of the country's ecological zones, and the following races have been classified: Pollo, Pira, Pira Naranja, Clavo, Güirua, Cariaco, Andaquí, Imbricado, Sabanero, Cabuya, Montaña, Capio, Amagaceño, Común, Yucatán, Cacao, Costeño, Negrito, Puya, Puya Grande, Chococeño, Dulce, and Harinoso Dentado.

Characterization--Of the 29 countries represented by the Colombian bank's holdings, those for which the characterization is most advanced include: Bolivia, Peru, Ecuador, and Venezuela. Accessions from some countries, such as Mexico and the 15 Caribbean nations, have not yet been characterized.

Future plans--In the coming years, we intend to work toward the following objectives: 1) regenerate 2,946 collections obtained from abroad, 2) characterize the 1,430 collections from outside Colombia for which we still have no data, 3) evaluate those collections for yield potential and other agronomic and physiological characteristics, 4) achieve more dynamic germplasm exchange with other banks, and 5) reorganize the bank in such a way that the nomenclature corresponds to zones of adaptation.

Ecuador

Raúl Castillo Torres

Germplasm holdings--Bank entries include local varieties, introductions, and basic populations or genetic pools. Of the local materials, there are 40 accessions, mostly from the highlands of Ecuador. These materials constitute a basic collection and are kept in a refrigerated chamber. Some 330 introductions from CIMMYT, most of them Ecuadorian germplasm, have been regenerated in Ecuador, and a small part of the seed is maintained by the maize program at the Santa Catalina experiment station of the National Institute of Agriculture and Livestock Research (INIAP). The maize program is also working with eight populations, which have been classified according to grain texture and color and maturity. The pools are as follows: harinoso blanco, harinoso amarillo, morocho blanco, and morocho amarillo, for each of which there is an early and late maturing version. Of these pools 75% have characteristics of maize used for human consumption. The important point is that all of the pools are made up of a wide range of materials from the Andean countries and from CIMMYT.

Storage of data--A database for these materials is not currently available, but we do have storage cards, containing mainly passport data, for the 40 entries in the bank's collection of basic germplasm. Data from evaluations of entries from CIMMYT have been sent to the Center's headquarters in Mexico.

Egypt

H.Y. Shehata

Germplasm holdings--Germplasm collection was begun in 1961, with the acquisition of Egyptian landraces from various provinces and districts. A few of these collections were examined in cooperation with an Italian germplasm mission that visited Egypt in 1982. The white endosperm stocks were introduced first from the USA in 1953 (we now have a total of 116 US introductions) and later from India (59), the USSR, Morocco, Kenya, Pakistan, Czechoslovakia, and CIMMYT (92). The exotic, yellow introductions were obtained from the USA, India, Morocco, Spain, the USSR, and some other countries, along with 100 from CIMMYT.

The Egyptian national program's germplasm accessions include the following: 187 locally developed and imported inbred lines (152 white and 35 yellow endosperm); 307 local collections (including 5 obsolete cultivars and 5 composites developed from the local collections); 295 exotic, white endosperm varieties, composites, and populations; and 433 exotic, yellow introductions. The collection does not include the parental inbreds of released hybrids, recently imported inbreds and populations, and some breeder's matertials.

Hungary

J. Pintér and G. Hadi

Germplasm holdings--A large number of germplasm collections are held at Hungary's Tápiószele Gene Bank, research institutes, and universities and provide a genetic resource for the medium and long term improvement of inbred lines. Provided below is a brief review of the genetic background of the populations (adapted and nonadapted) that are maintained, developed, and improved at Martonvásár.

Altogether 13 populations of the Food and Agriculture Organization (FAO), belonging to the 100-600 maturity group, are improved through recurrent selection. These populations are composed of Hungarian bred varieties; synthetic varieties developed from US and European flint lines and populations; and populations related to Iowa Stiff Stalk, Lancaster based populations, and varieties developed in Chile. Mass selection for early flowering, adaptability, and density tolerance is performed in these 12 populations, using the Troyer and Brown method. Another 12 gene pools are composed of European, very early and late flint and dent populations; pools developed from varieties that had previously been used extensively; and gene pools developed for yield components or other special purposes.

India

Bhag Singh and R.S. Paroda

Collection--The National Bureau of Plant Genetic Resources has placed major emphasis on collecting local maize germplasm. Tremendous genetic variability exists in India, and local materials show a high level of adaptation to environmental stress, making them a potentially valuable source of genes for several desirable traits. Moreover, a number of landraces are early to very early maturing, and we thus expect them to be suited to the cropping systems in different agroclimatic zones. A great deal of variability (with respect to ear and kernel size, type, shape, and color as well as maturity and plant type) has been collected over the past two years: in all 1,220 landraces through 24 multicrop and crop specific missions (Table 3).

Some of the most notable characteristics were observed in landraces from the Khasi and Garo Hills, materials that represent the most primitive as well as highly evolved types. We noted that 'Khasi Riewadem', which produces the largest ears of any Indian landrace, is still being preserved in its pure form among the Khasi tribes. Apart from being highly productive, this type has attractive, creamy white or yellow flinty grains that are highly preferred in the area where it is produced. Primitive types collected from the Garo Hills are called 'Merakhu babret' (Singh 1986).

A number of primitive maize cultivars possess such characteristics as popping type kernels, marked striations in the kernels, small ears, and prolificacy. Primitive materials showing these traits were collected in tribal areas of the Garo Hills, where they are grown in *Jhum* or shifting cultivation as a mixed crop with cotton, millets, chilies, beans, tapioca, and other crops. This race was reported about 20 years ago from Sikkim and is thought to possess desirable attributes (reproductive efficiency and defense mechanisms) that enable it to survive variable weather and insect pest and disease attack (Sachan 1986). A number of accessions invariably possessing eight kernel rows were obtained from this area as well. In 1987 additional maize variability was collected through 13 exploration trips, in which 308 accessions were obtained.

Evaluation--The Bureau has designated regional stations, including its headquarters, for maintenance, multiplication, and multilocation evaluation of seed of each crop. In the case of maize, major responsibility for seed multiplication rests with the Bureau's headquarters at Delhi. Part of the collection is retained by the collector at regional stations for multiplication of landraces belonging to particular regions. It is also grown for maintenance and multiplication in the first year at Delhi. Subsequently, these working collections are passed on to the regional stations, Shillong and Bhowali, for preliminary evaluation and maintenance.

Japan

Minoru Yamada

Introduction of maize--Maize was introduced into Japan in two stages, first about 400 years ago and again during the Meiji Era more than 100 years ago. The Caribbean flint type was first introduced by the Portuguese. During the Meiji Era North American flint and dent types were introduced. Landraces that are well adapted in Japan are considered to be derived from the materials introduced in these two stages.

Collection--Both national and regional organizations have collected maize germplasm. From 1955 to 1968, collection of maize (mainly flint type landraces) was carried out by the National Institute of Agricultural Sciences (reorganized as the National Institute of Agrobiological Resources, NIAR, in 1983) at more than 700 sites. Expeditions at the foot of Mount Fuji and in Shikoku and Kyushu were conducted from 1955 to 1960 and those in northern Kanto and southern and northern Tohoku from 1965 to 1968.The materials collected in northern Kanto and northern Tohoku included some of the landraces derived from the North American flint type introduced to Hokkaido during the Meiji Era. These materials are characterized by a typical ear type with eight rows of brown kernels.

Landraces collected by national organizations are preserved at the NIAR Seed Storage Laboratory at Tsukuba for use in breeding and genetics. A large number of materials collected at Mount Fuji and in Shikoku and Kyushu were lost, because seed storage facilities were not available at that time.

The regional maize breeding stations continuously collect local landraces. A total of 412 landraces have been used in breeding, including 391 of the flint type, 9 sugary, 6 pop, 3 dent, 2 waxy, and 1 unknown. These materials are preserved at each of the stations and/or at the NIAR Germplasm Storage Center.

Evaluation--Materials collected in the 1950s and 1960s were planted in the field at the Division of Genetics at Hiratsuka, Kanagawa, NIAS, and at three other experiment stations for evaluation. Seventy-one botanical and agronomic characteristics were observed, although information on some of these for the materials from northern Kanto and southern and northern Tohoku is not available. In addition, cytological studies of chromosome knobs and supernumerary chromosomes in pollen mother cells were done on materials collected at the foot of Mount Fuji and in Shikoku and Kyushu. The chromosome constitution of these materials was commonly 3L (long arm of the third chromosome and so on). 5L, 6L, 7L, and 8L, and only some of the materials from Mount Fuji showed the presence of supernumerary chromosomes. Based on these observations, the Japanese flint landraces were classified as Caribbean flint types.

Korea

B.H. Choe, H.B. Lee, J.S. Park, W.S. Ahn, and K.Y. Park

Collection--Maize collection has been confined to local landraces, since its primary objective is to prevent genetic erosion of these materials. Not much attention has been paid to maize from other countries or from our own breeding programs, although some of the introduced lines are kept in our storage facilities. Over 70% of our bank holdings consist of Korean landraces (Table 4), all of which are flint types.

Our first effort to collect landraces was initiated by the Crop Experiment Station in 1962, and a total of 26 landraces were collected. These were compared on the basis of a few characteristics with introduced hybrids, and since no useful lines were found among the collections, they were discarded with no further investigation.

More effective collection has been done recently in two phases. During the first phase (1977-1981), 450 local materials were collected from all over the country in cooperation with the Crop Experiment Station. More heterogeneous plant types were found in areas where maize was grown on a small scale. During the second phase of collection (1984-1985), which was conducted with funding from IBPGR, areas of comparatively large scale production were deliberately omitted, and collecting was concentrated in the central to southern regions of the country. A total of 354 accessions were collected through correspondence and visits (as unshelled ears to avoid mixture of seeds of different types) from fields, villages, and markets. Collected ears were measured and weighed before planting. During this phase it was found that the area planted to native maize lines was being reduced and that to foreign hybrids was increasing. Collection and evaluation of landraces and of exotic inbred lines has been a continuous activity of the Crop Experiment Station since 1983.

Mexico

Francisco Cárdenas R. and Juan Manuel Hernández

Collection--The bank currently contains 9,988 accessions of materials collected in Mexico. During 1943-1954 in all states of the country, 3,848 materials were collected and from 1955 to 1966 only 330. In 1968, since some regions had not yet been explored (being previously inaccessible) and because 808 of the original accessions no longer existed, it was decided to recollect throughout the country. As a result, 4,756 samples were obtained during 1968-1978 and another 1,054 from 1979 to 1987.

In addition to those materials, the bank holds 1,539 introduced materials: 1,148 from Central America, 249 from the Caribbean, 58 from the Andean region, 28 from the Southern Cone, and 56 from other locations. The current state of these materials in terms of viability is not known, although we suppose that it is not good since the majority have not been regenerated in more than 30 years.

Our database includes passport information on 9,369 accessions, or 94% of the total, and we are in the process of compiling data on the remaining 6%.

Characterization--Preliminary characterization includes observation of some morphological traits of the plant, ear, and kernel as well as important agronomic characteristics. We currently have information from preliminary characterization of 60 accessions.

Collections are grouped by race on the basis of more detailed observations on the morphological characters of the tassel, spikelet, glume, ears, grain, and cob as well as vegetative and physiological traits of the plant, together with information from studies of chromosome morphology. Recently, allozymes have been employed in studies of this type. Wellhausen and his collaborators described 25 races and 7 less well defined groups. In subsequent work Hernández X. and Alanis defined 5 more races and shortly afterwards Ortega Pazcka defined another 5.

The classification of Mexican maize races reported by Wellhausen et al. (1951) and Hernández and Alanis (1970) has been reviewed, with the aim of regrouping these races and investigating their potential use in breeding. The typical race collections listed in Table 5 were grouped according to taxonomic parameters and adaptation to different environments--the lowland tropics, the highland valleys, and the Bajío (a transitional area). In addition, each of the typical race collections was topcrossed to three testers--a single cross for the highlands and Bajío and a double cross for the tropics.

In Table 6 the races are classified according to measurements of 12 characters: ear weight per plant, ear diameter, ear length, cob diameter, plant height, number of leaves, days to silk, kernel row number, grain width, grain thickness, grain length, and yield per plot. The taxonomic analysis method of Abou-El Fittouh et al. (1969) and Cervantes (1976, 1982) was used to determine dissimilarity between pairs of different races by the mean euclidian distance and the complement of the correlation coefficient, considering the genotype and genotype x environment effects of 12 characters in eight environments at seven locations. The locations were as follows: Iguala, Guerrero (two years); Chapingo, Edo. de México; Pabellón, Aguascalientes; Zapopan, Jalisco; Río Bravo, Tamaulipas; Coaxtla, Veracruz; and Roque, Guanajuato.

Among typical collections of the races, Gto 265, Coah 21, Oax 298, Nay 189, Jal 631, and Zac 180 were well adapted to the Bajío area and the highlands and Tams 125, Nay 54, Sin 7, and Chis 224 to the tropical environments. Coa 25, Jal 63, Son 155, Nay 191, Jal 78, Son 114, and Oax 221 seemed to do well across those environments. Of the top crosses with three testers, top ranking collections from each group are listed in Table 7.

Morocco

M. Sali Belaid

Collection--The National Institute of Agricultural Research maintains in its maize improvement program varieties collected in three different stages from the country's principal maize production zones. The first collection expedition took place in 1960-1961, the second in 1979, and the third during 1985 in cooperation with CIMMYT. In addition, the maize improvement program has periodically introduced a series of local varieties, synthetic varieties, and pure lines from many sources, including the USA, France, Argentina, Peru, Spain, Italy, Portugal, Turkey, Hungary, Mexico, CIMMYT, and FAO.

Nepal

M.P. Upadhyay

Collection and evaluation--In 1971 the Department of Agriculture, with assistance from the US Agency for International Development (USAID), collected a total of 302 maize landraces from 50 districts of the country. These materials were evaluated in unreplicated trials in three distinct environments--Kakani (2,010 masl), Khumaltar (1,212 masl), and Rampur (228 masl)--to collect preliminary information about their earliness, plant performance, yield potential, and disease resistance. Most of the local landraces were earlier than the recommended improved varieties. The ratio of grain colors was 3 (white): 2 (yellow): 1 (other). Flint and semiflint types predominated over dent and semidents. Almost all of the materials were found to be susceptible to *Puccinia sorghi*.

Another effort to collect landraces was made by the national maize development program in 1982. Altogether, 38 specimens from 15 districts were evaluated under high fertility at Khumaltar in 1984. These landraces were typically tall, early maturing, low yielding, and susceptible to *P. sorghi*. To a lesser extent, they tended to have open ear tips and problems with ear rot. They were more susceptible to stalk lodging than to root lodging. Their grain type and color followed much the same pattern described above.

Paraguay

Mercedes Alvarez

The germplasm held in Paraguay consists of 94 collections made by the US National Research Council (NRC) and 448 viable collections made with IBPGR funding in 1979-80 and 1987 by the Ministry of Agriculture and Animal Husbandry of Paraguay. The racial composition of these materials is indicated in Table 8.

Peru

Ricardo Sevilla

Germplasm holdings--The maize germplasm bank of the Cooperative Maize Research Program at La Molina National Agricultural University tries to conserve all of the genetic diversity of maize in Peru for use in maize improvement. The germplasm bank conserves only samples of seed obtained from farmers' fields in all parts of Peru. In general, a sample consists of 10-20 ears collected in the same field or from a farmer's stored grain. In areas where small scale farmers predominate, fewer ears are collected (two or three from each farmer), and these are afterwards gathered in a single sample of 15-20 ears if they belong to the same race and were collected at the same location. During the first years of collection, some samples were obtained as shelled grain and in markets.

Of the 3,680 collections held by the bank, we have viable seed for only 2,676. The seed that has lost its ability to germinate can only be regenerated by nonconventional methods. The number of collections regenerated by the bank is shown in Table 9. Efficiency of regeneration is measured according to the number of collections producing 100 pollinated ears at each location.

Data storage--Four types of information about the accessions are generated: passport, characterization, inventory, and evaluation data. Passport data are available for all 3,680 of the bank's collections and have been published in the Catalogue of Maize Genetic Resources of South America. The amount of passport data available varies among descriptors, of which there are 13. For some we have data on 100% of the collection and for others only about 16%. There are 46 descriptors in the characterization file. We do not have information for all of these on all collections, varying from about 9 to 86% of the collections. The seed inventory indicates as many as four distinct seed origins for each collection. Of the total number of collections, 3,270 or 89% have information on the quantity of seed in the inventory. Currently, 2,344 collections are being evaluated in the Latin American Maize Project (LAMP)

The Philippines

Nestor C. Altoveros

Germplasm holdings--Before 1976 maize germplasm in the Philippines was held as working collections by maize breeders of the University of the Philippines at Los Baños (UPLB), the

Bureau of Plant Industry, and a few other agricultural universities. Systematic collection of maize germplasm was then undertaken by the National Plant Genetic Resources Laboratory (NPGRL) of the Institute of Plant Breeding (IPB) at UPLB, beginning with the acquisition of 552 viable accessions from maize breeders at UPLB. The NPGRL currently holds 1,813 accessions, of which 557 are Philippine landraces, 223 are Philippine breeding lines, and 1,033 are introductions.

Data storage--Passport data on the collections were originally stored in the mainframe computer at UPLB in 1981. In 1986 these data were transferred to a microcomputer (DBASE III-plus version 1.1), together with the data generated so far on morphological and agronomic traits.

Distribution--The NPGRL maintains national base and active collections of maize germplasm and follows a policy of free availability of materials to maize researchers in the Philippines and other countries. Germplasm is sent out upon receipt of an import permit. Seeds are submitted to the Plant Quarantine Office of the Bureau of Plant Industry for preshipment treatment, as specified by the plant quarantine authority in the country of destination. Materials that pass through quarantine are sent to the requesting parties by air mail. A total of 1,176 samples were sent out in 1986 and 1987, mostly to local maize researchers.

Portugal

R.M. Farias

Collection--Maize breeders began collecting local germplasm during the 1940s and 1950s in Portugal, where there is still a wealth of genetic variability in farmers' fields. Most of the material collected then has been lost, although some is still being used in the breeding program. Since 1977 several systematic collecting missions have been carried out, with support from FAO and IBPGR, throughout mainland Portugal, the Azores, and Madeira. The germplasm currently in storage consists of local cultivars, or open-pollinated local varieties (618 from mainland Portugal, 88 from the Azores, and 72 from Madeira), some accessions of local cultivars from outside Portugal (43 from Yemen and 94 from Morocco), as well as breeders' materials (1,000 inbred lines).

Data storage--Information on our accessions is stored in an IBM Personal Computer (using DBASE III-Plus). We use 78 descriptors for passport, characterization, and preliminary evaluation data.

Thailand

Apmol Senanarong

Introduction of maize--The Portuguese, the first Western traders to reach Thailand, are thought to have introduced maize into the country upon or shortly after their first contact with the country in 1601.

Collection--Systematic collection and evaluation of maize germplasm were started in 1950 by the national maize improvement program with assistance from the United States Operation Mission. A number of lines, varieties, and hybrids from North, Central, and South American and Asian countries were introduced and evaluated. In 1954 the two best performing varieties from Central America, C-110 (Tiquisate Golden Flint) and C-111, were released for commercial production.

The native maize type, which was grown in Thailand long before the introduction of exotic germplasm in 1950, is white, glutinous or waxy maize (wx). Though found in all parts of the country, waxy maize is planted to only a small area and used as a vegetable. Farmers have customarily selected and kept their own seed for future planting. This practice has brought about inbreeding depression, as indicated by small plants and ears. Nonetheless, because of intensive visual selection by farmers, their varieties showed desirable characters as well, such as adaptability, drought resistance, earliness, and good cooking quality. There is some variation among locations in the characteristics of varieties.

In 1983-1984, with funding from IBPGR, an effort was made to collect the native landraces of waxy maize. A total of 168 accessions were collected and characterized and are now conserved in the cold room at the Chiang Mai Field Crops Research Center for further evaluation and utilization. Duplicate samples have been stored in the long term Asiatic maize collection of the National Gene Bank of Thailand.

Data storage--Characterization data are recorded in nursery notebooks; the characteristics recorded are similar to IBPGR's maize descriptors, including kernel type, endosperm color, days to 50% silking, plant height, ear height, days to harvest, disease ratings, stalk and root lodging, and so forth. Passport data have seldom been recorded for introduced breeding materials.

USA

R.L. Clark and Mark J. Millard

Germplasm holdings--There are currently 6,044 accessions in the collection at the North Central Regional Plant Introduction Station, Ames, Iowa. Many of these are landraces (800), but there are also 337 inbreds from various countries, including 66 from the USA.

Our plans call for continued acquisition of maize germplasm from Mexico, Colombia, and Peru. We project a final collection size of just over 20,000 accessions by the time introduction of the Latin American materials has been completed.

Data storage--All information on our collection, including passport, observation, inventory, literature, germination, and regeneration data, is stored in the database for the Germplasm Resources Information Network (GRIN), which is kept on a computer at the National Agricultural Library, Beltsville, Maryland. We access the GRIN data and add to or modify it by means of a national telephone system called Telenet. We use both IBM compatible PCs and Hewlett-Packard "dumb" terminals. We have two four-port multiplexor units as the interface between our PCs or terminals and the Telenet link to GRIN. Much local data management is done on the PCs, so that more efficient use can be made of the Telenet and GRIN hardware. All data are verified by the double-entry process.

The GRIN database is freely accessible to the public. All you need to get on line is to obtain a user identification and a password by contacting: Jimmie Mowder, GRIN/Database Management Unit, USDA-ARS-BA-PSI-GLS-DBMU, BARC-West, Bldg. 001, Beltsville, MD 20705 (phone: 301-344-1666). You must also have a computer terminal or PC, plus a modem and a dedicated telephone line. Mr. Mowder can help you determine whether the hardware you have now is appropriate.

Utilization--Many accessions have contributed specific traits having high commercial value. For example, PI 251934 contributed earliness to breeding programs in the 1960s, leading to the release of Trojan TX68, and PI 217413 (Zapalote Chico) has been widely used as a source of the Ht gene for resistance to northern leaf blight. High lysine in maize has been obtained in opaque endosperm accessions such as PI 218130, 218135, 218136, and 218410, all of which are from the southwestern USA. Additional traits derived from our maize germplasm by breeders include resistance to southern leaf blight, smut, rust, northern leaf blight (in addition to the Ht gene), European corn borer, corn earworm, heat, drought, and flooding, along with other useful characters such as earliness, standability, ability to emerge from deep planting, multiple ears, and cold germination and emergence.

Venezuela

Arnoldo Bejarano M.

Germplasm holdings--The bank's entries currently include 60 local varieties, 154 introduced varieties, 45 improved varieties, and 630 original collections. With assistance from FAO, maize germplasm is now being collected to the south of the Río Orinoco. In addition, we expect to repatriate the original collections of the native races being conserved in germplasm banks at Medellín, Colombia, and at CIMMYT.

Data storage--A computerized system is currently being implemented for recording passport, evaluation, and characterization data. We are also updating the passport data on all of our collections.

Yugoslavia

Gordana Radovic

Introduction of maize--The first maize types brought to Europe by Columbus are thought to have been flints from the Caribbean Islands and West Indies. Through Spain and Italy, these types spread to other Mediterranean countries, where they were grown on a limited scale as a garden crop (Pavlicic and Trifunovic 1966). Maize reached what is now Yugoslavia by the middle of the 16th century. Afterwards, maize genotypes from Mexico's central plateau and from the Andes were introduced and crossed with the existing varieties. The resulting increase in variability improved the adaptability of the germplasm to diverse soil and climatic conditions. Domestic maize biotypes were developed, which in the process of evolution differentiated into very early and medium maturity flints.

In the 18th century, flints of different genetic origin were carried over from Canada and New England to northern France and from there spread through Central Europe to Croatia and Slovenia (Brandolini 1968). These materials were adapted to cooler growing conditions.

During the last decades of the 19th century and at the beginning of the 20th century, dent maize types were introduced into certain parts of Yugoslavia. Originating in the US Corn Belt through natural crossing of northern flint and southern dent types, they were the most productive maize varieties introduced into Europe and rapidly expanded to the Balkans. Natural crossing of the American dents with the flints already grown at that time resulted in the development of the European dent type, which completely replaced the flint types in the optimal maize growing regions. This was the last large scale natural hybridization to take place in the evolution of maize varieties in Europe (Trifunovic 1978).

The pathways of maize introduction are indicated by linguistic evidence. The local term *kukuruz* in Serbo-Croat comes from the Turkish word *kukuru*, meaning grain. In the eastern part of Yugoslavia (Serbia), maize was brought by Turkish conquerors, reached the Sava and Danube Rivers, and followed the Danube River Valley in Central Europe. In Montenegro in the southern part of Yugoslavia, the local term *rumetin* derives from the Italian *frumento* or *kolomboch*, resembling the name Columbus. At the border with Bulgaria, maize is called *Tsarevica* from the word *tsar* for the Turkish emperor.

Maize production in Yugoslavia went through several stages (Trifunovic 1986). Introduction of source types was followed by spontaneous adaptation, crossing, and differentiation into ecotypes, a process that lasted more than two centuries. The next period was characterized by human influence through selection and the beginning of planned selection of more productive genotypes.

The maize germplasm bank--Systematic collection of local cultivars began in 1962, and by 1965 about 1,000 samples had been collected. All institutions that had begun maize breeding programs took part in this activity. But the Maize Research Institute at Zemun Polje worked most intensively with local cultivars, continued collecting materials, and eventually became responsible for the maize germplasm bank, which contains 700 introduced varieties (local materials and synthetics), 1,500 inbred lines (local and foreign), and 2,000 populations collected in all parts of Yugoslavia.

Classification of populations--All of the Yugoslav populations were classified into 18 races or groups according to the phenological and morphological characteristics described by Anderson and Cutler (1942). The most important flint types are Montenegrin, Macedonian, Mediterranean, eight-row maize of the Northeastern American type, derived flints, small kernelled flints, Rumanian, and large eared flints. These materials are characterized by great diversity, because of the earlier arrival of flints in the country and because various original flint genotypes were introduced in different regions. According to Brandolini (1968), flint maize types in southern Europe are more differentiated than dent types, especially in Italy, followed by those grown in Yugoslavia. The importance of flint types for breeding is that they are a source of earliness and resistance to low temperatures at the early growth stages.

Semiflint types grown in Yugoslavia were either introduced as such or resulted from the natural hybridization of flint and dent types. The following races are of this type: Bosnian Early Dents, Kosmet Semiflints, and Moravac White Semidents. The semiflints and semidents are evolutionarily the youngest types, developed through crossing of all existing varieties.

Dent varieties were the last to be introduced and the first to be replaced by hybrids. This may account for the significantly lower variability of dent varieties compared with flint varieties in Yugoslavia. All dents are classified into several groups: eight rowed soft dents, dent types of the US Corn Belt dents, dent types of the southern areas of the USA, derived dents, and Serbian dents.

Although Anderson's natural classification has several advantages over earlier methods, it is somewhat intuitive and can be applied only when the overall polymorphism of the species is known. More recently, numerical taxonomy methods, which give more objective results, have been used for classification. This research is still in progress, but initial findings indicate a smaller distance among some groups, such that they can be placed in the same group.

In other recent studies, differences among local maize populations were studied at the level of isoenzymes. This direct measure of genetic distance is used in studies of the dissimilarities among maize populations. Tucic and Tucic (1983) confirmed the previous classification done according to Anderson and Cutler's method.

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Table 1. Racial composition of entries of local populations collected in Argentina in 1977, 1978, and 1981 with assistance from IBPGR

Race or material	No. of entries
Dulce	11
Avatí Morotí	122
Avatí Morotí Tí	17
Avatí Morotí Mitá	3
Dentado Amarillo	41
Dentado Amarillo Fino	5
Dentado Blanco	253
Dentado Blanco Fino	2
Dentado Blanco Rugoso	3
Cravo	10
Perla	23
Calchaquí	69
Blanco Ocho Hileras	13
Chaucha Blanco	9
Colita	3
Cristalino Colorado	205
Amargo	10
Camelia	10
Catete Oscuro	3
Cristalino Amarillo Anaranjado	21
Socorro	17
Amarillo Ocho Hileras	34
Pisingallo	131
Avatí Pichingá	10
Perlita	66
Pericarpio Cereza	8
Complejo Tropical	76
Venezolano	36
Tuzón	34
Canario de Formosa	35
Chullpi	5
Culli	7
Azul	3
Negro	2
Cuzco	5
Capia Blanco	34
Capia Variegado	10
Capia Rosado	7
Garrapata	17
Marrón	2
Altiplano	7

Continued on next page

Table 1. Continued

Race or material	No. of entries
Amarillo de Ocho	69
Morochito	18
Chaucha Amarillo	6
Difficult to classify	109
Largely contaminated	118
Mixtures	36
Total	1,737

Table 2. Countries of origin of collections in Colombia's maize germplasm bank, 1987

	Races	Number of of collections
Africa	0	2
Argentina	0	12
Bolivia	32	520
Brazil	0	2
Central America and the Caribbean (15 countries*)	0	300
Colombia	23	2,098
Chile	5	99
Ecuador	30	576
India	0	3
Indonesia	0	2
Mexico	0	368
Panama	14	150
Peru	0	222
Thailand	0	2
USA	0	20
Venezuela	19	668
Total	123	5,044

* The countries in this region include Antigua, Barbados, Cuba, Guadalupe, Granada, Trinidad, Haiti, Jamaica, Martinique, Puerto Rico, the Dominican Republic, San Vicente, Santa Lucia, Saint Croix, and Tobago.

Table 3. Collection activities of India's National Bureau of Plant Genetic Resources, 1985-1986

Area of collection	Number of collections	Collector
Himachal Pradesh	464	B.D. Joshi N.K. Guatam
Uttar Pradesh	131	K.C. Pant K.S. Negi
Haryana	38	K.P.S. Chandel
Jammu and Kashmir	95	K.P.S. Chandel
Madhya Pradesh	15	U.C. Srivastava
Meghalaya and Assam	370	R.K. Arora B. Singh B.D. Sharma D.K. Hore
Nagaland	42	B.D. Sharma D.K. Hore
Mizoram	16	R.K. Arora
Sikkim	14	R.K. Arora
Tripura	13	B.D. Sharma D.K. Hore
Other areas	22	
Total	1,220	

Table 4. Composition of maize germplasm in long term storage in Korea

Type	Korea	USA	Japan	China	Others	Total
Flint	3,819	-	5	7	-	3,831
Dent	-	823	-	-	556	1,379
Sweet	-	96	-	1	-	97
Pop	120	74	-	-	-	194
Waxy	325	384	-	1	-	359
Other	-	89	-	-	-	89
Total	4,264	1,116	5	9	556	5,950

Table 5. Mexican maize races

Race	Typical collections
Highlands	
Cónico norteño	Gto. 23, 34, 165
Apachito	Chih. 166, 180, 207
Cristalino de Chihuahua*	Chih. 128, 154, 254
Azul	Chih. 147, 158, 218, 220
Gordo	Chih. 160, 205, 214
Palomero tipo Chihuahua*	Chih. 135, 148, 150
Palomero Toluqueño	Mex. 5, 6, Tlax. 311
Arrocillo	Pue. 91, Ver. 311, 342, 359
Cónico	Mex. 58, 72, 108, Pue. 32
Elotes Cónicos	Pue. 403, 510, Tlax. 521
Cacahuacintle	Mex. 7, 12, Pue. 552
Chalqueño	Hgo. 7. Méx. 37, Pue. 87
Mushito	Mich. 328, 351, 371
Serrano de Jalisco	Jal. 173, 753
Oloton	Chis. 684, 687, 695
Midaltitudes	
Pepitilla	Gro. 3, 335, Mor. 99, 102
Ancho*	Gro. 326, 383, Mor. 46
Dulce	Jal. 78, 300, 304, Zac. 182
Bofo	Dgo. 95, Nay. 191, Jal. 289
Elotes occidentales	Nay. 24, Gto. 1, Zac. 180
Tabloncillo	Jal. 43, 44, 63
Tablilla de Ocho	Zac. 187, Jal. 306, Nay. 185, 189
Celaya	Gto. 36, 69, 101, 265
Zamorano	Jal. 631, Gto. 191, Mich. 5
Jala	Nay. 6, 54, 130
Comitico	Chis. 39, 352, 609
Matozinteco*	Chis. 650, 652, 653
Tehua	Chis. 29, 229, 596
Cascomatepec	Ver. 404, 457
Bolita	Oax. 28, 40, 221
Nal-Tel de Altura*	Chis. 196, Oax. 298, 301
Lowland tropics	
Nal-Tel	Yuc. 7, 148, Camp. 48
Conejo	Gro. 17, 157, 176
Zapalote chico	Oax. 48, 52, Chis. 390, 662
Zapalote Grande	Chis. 224, 236, 521
Raton*	Coah. 25, Tamps, 66, 3, 29
Tuxpeño norteño*	Coah. 21, Chih. 13, 121
Vandeño	Chis. 25, 30, 114
Tuxpeño	Oax. 9, Tamps. 125, Ver. 39, 128
Tepecintle	Chis. 26, 76, 528, Oax. 177
Olotillo	Chis. 81, 440, 562, SLP 108

Continued on next page

Table 5. Continued

Race	Typical collections
Dzit-bacal	Chis. 447, Q. Roo 20, Ver. 96
Chapalote	Sin. 2, 6, 65
Reventador	Nay. 39, Sin. 55, 60
Tabloncillo Perla*	Nay. 12, 16, 41
Onaveño	Son. 105, 114, 155, 184
Harinoso de Ocho	Nay. 24
Elotero de Sinaloa*	Sin. 17
Blandito	Sin. 7, 61, Son. 117
Dulcillo del noroeste	Sin. 25, 33, 34, 79

* Undescribed race, in the process of definition.

Table 6. Classification of Mexican races of maize according to 12 taxonomic parameters

Group 1
Tehua
Olotón
Comiteco
Coscomatepec
Motozinteco
Dzit-Bacal
Tuxpeño
Olotillo
Nal-Tel de Altura

Group 2
Serrano de Jalisco
Apachito
Cristalino de Chihuahua
Gordo
Azul
Cacahuacintle

Group 3
Conejo

Group 4
Elotero de Sinloa
Blandito
Harinoso de Ocho
Tabloncillo Perla
Bofo
Elotes occidentales
Tabloncillo
Onaveño
Zamorano Amarillo

Group 5
Ratón
Celaya
Tuxpeño Norteño
Jala

Group 6
Pepitilla
Chalqueño
Elotes Cónicos
Dulce
Mushito
Cónico Norteño
Cónico
Arrocillo
Palomero Toluqueño
Palomero Tipo Chihuahua

Group 7
Zapalote Grande
Tepecintle
Vandeño
Nal-Tel

Table 7. Results of a preliminary evaluation of top crosses of typical maize race collections with testers for three different environments in Mexico

Collection	Yield (t/ha)	Days to silk
Highlands (Chapingo, Edo. de México, 1986)		
Chis 684 (Oloton)	8.96	102
Jal 631 (Zamorano Amarrillo)	8.93	94
Mex 37 (Chalqueño)	8.72	99
Gto 265 (Celaya)	8.42	102
Ver 404 (Coscomatepec)	8.31	113
Gto 36 (Celaya)	8.27	99
Pue 510 (Elotes Cónicos)	8.11	89
Zac 180 (Elotes Occidentales)	8.10	94
Oax 221 (Bolita)	8.05	92
Coah 25 (Ratón)	7.95	89
Coah 21 (Tuxpeño Norteño)	7.94	101
Gto 34 (Conico Norteño)	7.93	90
Bajío (Río Bravo, Tamaulipas, 1987)		
Tams 125 (Tuxpeño)	4.7	82
Son 155 (Onaveño)	4.6	78
Coah 25 (Ratón)	4.4	75
Tams 29 (Ratón)	4.2	79
Nay 191 (Bofo)	4.1	81
Sin 2 (Chapalote)	4.1	78
Chih 121 (Tuxpeño Norte)	3.9	80
Sin 33 (Dulcillo)	3.9	81
Chis 25 (Vandeño)	3.8	77
Nay 24 (Harinoso de Ocho)	3.8	77
Ver 39 (Tuxpeño)	3.7	85
Sin 55 (Reventador)	3.5	78
Tropics (Río Bravo, Tamaulipas, 1987)		
Tamp 125 (Tuxpeño)	4.9	85
Coah 25 (Ratón)	4.8	80
Sin 7 (Blandito)	4.7	85
Son 114 (Onaveño)	4.7	81
Gto 36 (Celaya)	4.6	88
Coah 21 (Tuxpeño Norteño)	4.5	88
Tamps 29 (Ratón)	4.5	84
Nay 24 (Harinoso de Ocho)	4.3	82
Sin 17 (Elotero de Sinaloa)	4.2	82
Chis 25 (Vandeño)	4.2	83
Sin 55 (Reventador)	4.2	84
Son 155 (Onaveño)	4.1	82
Ver 39 (Tuxpeño)	4.0	90

Table 8. Racial composition of maize accessions in Paraguay's maize germplasm bank

Racial group	Number of accessions
Avati Moroti	139
Avati Mita	16
Avati Ti	26
Avati Guapy	2
Sape Pyta	124
Typi Pyta	88
Sape Moroti	10
Tupi Moroti	66
Pichinga Redondo	47
Pichinga Aristado	18

Note: Avati Moroti, represented by 174 collections, includes collections from Brazil and 114 from Argentina. Of the 86 collections representing Pichinga Redondo, 59 are from Brazil, and of the 19 representing Pichinga Aristado, 13 are from Brazil.

Table 9. Regeneration of maize collections in Peru

Location	No. of collections Planted	>100 ears harvested	Pollination efficiency (%)
1985			
Caraz	171	1	81.5
Junín	193	97	58.7
La Milina	103	19	65.0
Tarapoto	107	5	88.4
1986			
Piura	223	143	76.5
Caraz	150	16	77.5
La Molina	186	73	54.2
Tarapoto	117	18	63.7
Chiquian	297	191	85.5
1987			
La Molina	107	69	61.0
Cuzco	93	49	60.9
Carhuaz	164	73	82.9
Junín	179	73	82.9
Huánuco	199	71	81.5
Total (until March 1988)	2,289	856	74.1
1988			
La Molina	98		
Carhuaz	260		
Cuzco	264		
Junín	244		
Huánuco	300		

Conclusions and Recommendations

Collection, Documentation, and Characterization

1. The descriptor list published by IBPGR in 1980 was accepted, although it was agreed that time and experience would alter some details, necessitating occasional revision. An updated version of the descriptor list will be jointly published by IBPGR and CIMMYT as soon as possible.

2. An obvious way to enhance cooperation would be to encourage the adoption of standardized procedures. CIMMYT might contribute more to global cooperation by taking a leading role in the distribution of information.

3. IBPGR and CIMMYT should examine the possibility of establishing regional short courses in the collection, documentation, and characterization of maize germplasm. The IBPGR courses offered at the University of Birmingham, England, are good, but there is a need for more practical courses that place greater emphasis on the use of field techniques.

4. This requires improvement in general but particularly in relation to collecting missions. Newsletters have proved useful but are not being circulated and used as widely as they should be. *Diversity* magazine is also helpful but expensive.

5. It is strongly recommended that each country designate approximately 10% of its samples as a core collection. These materials should be evaluated more fully, with emphasis on knob chromosome counts and biotechnical studies, such as electrophoresis. The core collection could then provide a focus for evaluation by breeders.

6. Honduras, Nicaragua, and Panama are under collected, and the maize diversity of these countries is poorly represented in international germplasm banks. Amazonia may be a source of material adapted to acid soils and tolerant to aluminum toxicity. Other countries requiring further collection are Angola and Mozambique in Africa, Burma and Laos in Asia, and Albania and Austria in Europe.

Evaluation and Utilization

1. The first step in evaluation is to determine what information is available on the current holdings of germplasm banks. In order for data generated through evaluation to be widely useful, germplasm banks will require adequate documentation facilities, including computerized systems for data acquisition and exchange.

2. Evaluation should be conducted within a network of cooperating institutions, of which the Latin American Maize Project (LAMP) is a good example. Resources should be sought to establish similar networks in other regions. Perhaps, CIMMYT could initially take responsibility for planning a global evaluation network, with regional germplasm banks also playing an active role.

3. Highest priority must be given to yield and to characteristics contributing to yield stability. High priority should also be given to determining the heterotic patterns of maize germplasm.

4. Bank managers must have a thorough knowledge of the germplasm in storage and possess as much data as possible on useful traits. They can encourage germplasm utilization by making this information readily available to breeders. Managers should also take the initiative in prebreeding to help determine which materials might be most valuable to maize breeding programs.

Storage, Regeneration, and Seed Physiology

1. Seed stored in a germplasm bank should have an initial germination percentage greater than 95%. When the germination percentage of stored seed has fallen to 80-85%, regeneration is recommended. How quickly germination percentage declines depends on the genotype, storage conditions, and initial quality of the seed. Sterilized water should be used for germination tests, and these should be standardized, with respect to the paper used, temperature, and so on. To determine vigor cumulative testing of germination percentage is recommended.

2. No fungicide treatment is recommended for stored seed to control field fungi, such as *Fusarium* or *Aspergillus* and *Penicillium*, since storage conditions in a germplasm bank do not permit these pathogens to damage the seed. Moreover, some fungicides might have phytotoxic effects in long term storage.

3. Bank accessions should be systematically classified, based on the viable equation of E.H. Roberts (see: 1986. Quantifying seed deterioration. In *Physiology of Seed Deterioration*, 101-123. CSSA Spec. Pub. no. 11).

4. Accession rescue by osmoconditioning is recommended.

5. Collaboration among germplasm banks is needed for regeneration of cool, long season accessions.

6. Research is needed to determine what occurs with materials stored at low moisture content for long periods.

7. Training is needed on storage, regeneration, and seed physiology.

8. A guide should be prepared providing information on these topics.

Goals to be Reached by the Year 2000

1. A study should be completed on the basic taxonomy, morphology, and cytology of the genus *Tripsacum*, both as independent taxa and in hybrids with maize. Biotechnology work at CIMMYT will be accompanied by a shift in the breeding programs toward a backcross approach. Isozymes and/or RFLP techniques will be used to characterize the germplasm, starting with the core collection.

2. All banks should be computerized and engaged in free exchange of data and germplasm. CIMMYT will be responsible for cooperation among germplasm banks in the Western Hemisphere.

3. A two- to four-month workshop should be held at CIMMYT periodically (perhaps every three years) for managers of germplasm banks. The course should provide hands-on instruction in managing and evaluating collections and cover such techniques as roguing off-types and selecting suitable samples for regeneration.

4. All banks should be linked by electronic mail via satellite. Each bank should at least have a PC comparable to the IBM PC AT and use standardized data formats and data management software. To facilitate the exchange of small shipments of seed, CIMMYT's regional specialists could hand-carry materials from one country to another. Regeneration and evaluation protocols should be established for all banks.

5. A research unit should be established within the CIMMYT bank, and the job description of the Center's bank manager should have a research component. A core collection should be established for distribution, especially to nonbreeders, to meet general germplasm requests. By means of biotechnology techniques, the quality of seed being placed in storage should be evaluated, both for longevity and the presence of seedborne pathogens.

6. CIMMYT should assume more of a leadership role in evaluating and distributing germplasm. It ought to be the focal point for germplasm conservation and research in the Western Hemisphere. IBPGR should pay more attention to the specific needs of "orphan" collections.

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