Conserving and Enhancing Maize Genetic Resources as Global Public Goods– A Perspective from CIMMYT

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ABSTRACT

The growing demands for food, feed, and bio-energy worldwide will require a 2% per annum increase in global maize (*Zea mays* L.) production. Maize is one of the most important staple food crops across the developing world as well as being an important feed crop for global livestock production and the emerging biofuel industry. Maize genotypes can range from 0.5 to 5 m standing height at flowering, mature in 60 to 330 d from planting, produce 1 to 4 ears per plant, 10 to 1800 kernels per ear and yield from 0.5 to 23.5 Mg of grain per hectare. Even greater genetic diversity is present in related species yet surprisingly little of the maize-related biodiversity is present in the current elite breeding pools. Improved methods and tools for germplasm conservation, characterization, and data sharing, as well as for population improvement, gene pool enhancement, and genomics-aided breeding are urgently needed if increases in maize productivity, particularly in the developing world, are to keep pace with predicted increases in demand. Progress in the private sector, particularly with the development of temperate maize cultivars, is far beyond that of the public sector, particularly regarding tropical maize cultivar development. This article provides an overview of progress at the International Maize and Wheat Improvement Center (CIMMYT) with national partners across the developing world. Particular emphasis is given to issues related to the continued development of elite maize breeding material as global public goods, especially regarding the introgression of new variations from genetic resources and the legal and phytosanitary issues related to international exchange of maize germplasm.

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Abbreviations: CGIAR, Consultative Group on International Agriculture; CIMMYT, International Maize and Wheat Improvement Center; CML, CIMMYT elite breeding lines; CRIL, IRRI-CIMMYT Crop Research Informatics Laboratory; ESIM, eigen selection index method; GE, genetically engineered; GEM, Germplasm Enhancement of Maize; HOPE, hierarchical open–ended population enrichment; IITA, International Institute of Tropical Agriculture; IRRI, International Rice Research Institute; IT, International Treaty; LAMP, Latin American Maize Program; MAS, marker-assisted selection; MLM, modified location model; NARS, national agricultural research systems; OPV, open pollinated cultivar; PGRFA, Plant Genetic Resources for Food and Agriculture; QTL, quantitative trait loci; SAGARPA, Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (Mexico); SHL, Seed Health Laboratory; SMTA, Standard Material Transfer Agreement; SNP, single nucleotide polymorphism; SSR, simple sequence repeats; UPGMA, unweighted pair-group method using arithmetic average.

MAIZE (*Zea mays* L.) ranks among the three most important food crops worldwide (FAO, 2009a); however, average global per capita consumption in 2003 was only about 18 kg for maize, substantially below that of wheat (*Triticum aestivum* L.; 67 kg), rice (*Oryza sativa* L.; 54 kg milled equivalent) and potato (*Solanum tuberosum* L.; 32 kg) due to the high proportion of global maize production used for animal feed. At the national level, per capita maize consumption is very high in certain countries such as Lesotho (174 kg), Zambia (133 kg), Malawi (132 kg), Mexico (125 kg), and South Africa (113 kg). Maize productivity at the

national level varies considerably across regions and years (Fig. 1). The average annual growth in maize yield over the past half century has been highest in the U.S. (115 kg ha^{-1}) and lowest in Middle (or Central) Africa (4 kg ha^{-1}) (Table 1). The rate of yield increases in sub-Saharan Africa has been more then three times lower than the global average. In Middle and Eastern Africa, 50% of years had yields below that of the previous year, emphasizing the vulnerability of the crop to yield losses that are primarily due to abiotic and biotic stresses (CIMMYT, 2004; p. 15).

The growth in demand for human consumption of maize in the developing world is predicted to be 1.3% per annum until 2020 (CIMMYT, 2005). Moreover, rising incomes are expected to result in a doubling of consumption of meat across the developing world (Naylor et al., 2005), leading to a predicted growth in demand for feed maize of 2.9% per annum. Hence, there is need for at least a 2% per annum increase in maize production to meet this growth in global human population and shift in dietary preferences. We believe that gains from plant breeding will need to provide a major proportion of these increases. Maize genetic resources enhancement and population improvement are vital to protect the

gains in maize yield made to date, and to continue to drive increases in genetic yield potential.

This article assesses the conservation through use of maize genetic resources for the betterment of this critically important crop, with an emphasis on experience at the International Maize and Wheat Improvement Center (CIMMYT) and national partners across the developing world. CIMMYT's mission is to serve resource-poor farmers in low income countries, especially those in marginal production areas where prevailing pathogens, pests, and abiotic stresses cause large yield losses. Thus, we describe what CIMMYT and other international breeding programs should be doing to conserve and improve tropical maize germplasm.

CONSERVING EX SITU MAIZE GENETIC RESOURCES

Ancient farmers, in what is today's southern Mexico, domesticated maize from teosinte (*Z. mays* ssp. *parviglumis* Iltis & Doebley) (Matsuoka et al., 2002). Maize landraces exhibit significant morphological variation and genetic polymorphism and are grown from sea level to 3800 m. Maize landraces with similar morphological characteristics
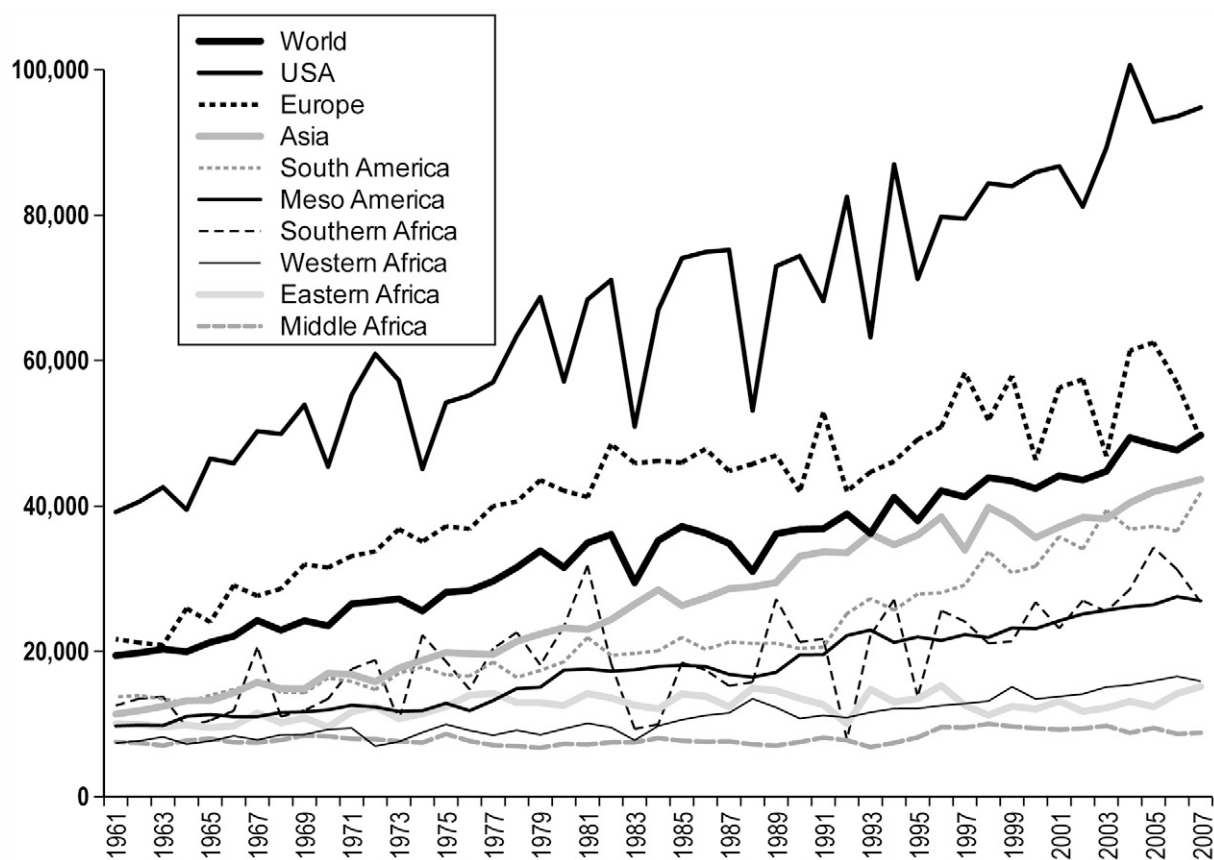

Figure 1. Maize yields (hg ha^{-1}) in the world, the U.S. and across regions (1961–2007). Source: FAO (2009a). Southern Africa mainly due to South Africa (at least 90% of total area during this period) but also adding Botswana, Lesotho, Namibia, and Swaziland; Western Africa includes Benin, Burkina Faso, Cape Verde, Côte d'Ivoire, Gambia, Ghana, Guinea, Guinea-Bissau, Mali, Mauritania, Niger, Nigeria, Senegal, Sierra Leone, and Togo; Eastern Africa considers Burundi, Comoros, Djibouti, Eritrea, Ethiopia, Kenya, Madagascar, Malawi, Mauritius, Mozambique, Réunion, Rwanda, Somalia, United Republic of Tanzania, Uganda, Zambia, and Zimbabwe; Middle Africa refers to Angola, Cameroon, Central African Republic, Chad, Congo, Democratic Republic of Congo, Gabon, Sao Tome, and Principe.

were first classified into 'races' over half a century ago (Anderson and Cutler, 1942; Cutler 1946). Grouping was then refined on the basis of on specific geographical areas, e.g., maize races of Mexico (Wellhausen et al., 1952), Cuba (Hatheway, 1957), Colombia (Roberts et al., 1957), Central America (Wellhausen et al., 1957), Brazil and other eastern South American countries (Brieger et al., 1958), Bolivia (Ramírez et al., 1960), and Peru (Grobman et al., 1961).

The total number of unique New World maize germplasm accessions exceeds 27,000 (GCDT, 2007). Table 2 lists the major maize collections held in genebanks across Latin America and the U.S. Most of these accessions are available from CIMMYT or from the USDA's North Central Regional Plant Introduction Station at Ames, Iowa (NCRPIS). There are about 20,000 accessions in Old World genebanks, which are most likely old farmers' cultivars as well as descendants of widely–distributed, open-pollinated (OPV) or hybrid cultivars. In addition, for the past 75 years, the Maize Genetic Cooperation Stock Center (or USDA-ARS GSZE) in the Department of Crop Sciences of the University of Illinois has conserved and annotated a large maize mutant stock collection (now nearly 80,000 accessions), which is available to maize geneticists worldwide. Genetic data describing all the maize mutant stocks in this collection can be accessed at MaizeGDB, the Maize Genetics and Genomics Database (http://www.maizegdb.org; verified 16 Nov. 2009). More recently, USDA-ARS GSZE has added thousands of "terminal" segregating offspring ensuing from various genomics research projects (GCDT, 2007).

The genebanks at the Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP, Mexico), USDA-ARS, CIMMYT, and Universidad de Guadalajara (Mexico) hold the major collections of teosinte accessions (Table 3). Most of the accessions held by the CIMMYT genebank are landraces (Table 4) but breeding lines, gene pools, populations, and cultivars are also preserved in this ex situ collection. Taba et al. (2003) provide agro-morphological data recorded at CIMMYT experiment stations in Mexico for Mexican dent races, Caribbean flint and dent races, Cateto and related races, and the Brazilian flint and dent races.

The global crop strategy for ex situ conservation and utilization of maize germplasm (GCDT, 2007) describes best practice methods for preservation, documentation, and determination of 'uniqueness' as well as use of the maize and teosinte accessions held in these genebanks. CIMMYT places substantial emphasis on helping establish these best practices in national genebanks and germplasm enhancement programs across the developing world. However, the mainstreaming of maize genetic resources into conventional maize breeding programs remains challenging since historically such efforts have been inconsistent.

Table 1. Maize overall yield gain (%), significant ($P \leq 0.05$) annual maize yield growth (kg ha^{-1}) in respective rows, and percentage of number of years with maize yield gains (vis-à-vis previous year) between 1961 and 2007 in the world, across continents, selected sub-regions and countries. Original data taken from FAO (2009a).

Region	Overall period gain	Annual growth	Years with yield gains
	%	kg ha^{-1}	%
World	156	63	63
U.S.	142	115	65
Pre-transgenic maize (1961–1996)		108	
Post-transgenic maize (1997–2007)		156	
Europe	127	74	59
Asia	284	72	70
South America†	205	56	61
Argentina pre-transgenic maize (1961–1997)		77	
Argentina post-transgenic maize (1998–2007)		187	
Meso America†	177	39	70
Africa†	69	13	56
Southern Africa (South Africa accounts for ≥ 90%)	113	33	65
South Africa pre-transgenic maize (1961–1999)		25	
South Africa post-transgenic maize (2000–2007)		93	
Western Africa	115	18	74
Eastern Africa	51	7	48
Middle Africa	17	4	43

†African and Latin American sub-regions as per Food and Agriculture Organization of the United Nations.

This approach is perhaps one of the greatest differences between the public and private sectors, and particularly between tropical and temperate maize improvement programs. Bridging this gap is one of CIMMYT's primary long-term goals.

EFFICIENT ACCESS TO BENEFICIAL GENETIC VARIATION

Characterizing maize genetic resources and understanding the structure of the diversity they encompass should lead to enhanced utilization for the improvement of this crop. Advances in computational technologies have facilitated the development of powerful tools for numerical taxonomy and multivariate statistical analysis to classify maize genetic resources and define core germplasm subsets (Franco et al., 1998, 1999). However, much remains to be done to convert this knowledge into tangible tools for routine application in breeding programs. Achieving this next step is a primary goal of the Crop Research Informatics Laboratory (CRIL)—a collaborative undertaking between the International Rice Research Institute (IRRI, Los Baños, Philippines) and CIMMYT.

Table 2. Major maize collections in the American continent. Source: GCDT (2007).

Location	Own collection	International seed distribution[‡]	Duplicate stocks from elsewhere	Wild species stocks
Argentina	2400	S-MTA		
Bolivia	1500	N	23	1
Brazil	3200	S-MTA	288	7
Chile	950	S-MTA		
Colombia[†]	1800	N	1800	
Ecuador	1100	S-MTA	168	
Guatemala	900	N		
Mexico[†]	12,000	S-MTA	1800	136 [§]
Paraguay	478	N		
Peru	3000	S-MTA	37	
U.S.- NCGRP[†]	200	S	39,000	354
U.S.- NCRPIS[†]	1300	Y	13,500	238
Uruguay	852	S		
Venezuela	1200	N		
CIMMYT[†]	7311	Y	17,632	308
Total	38,191		57,248	1044

[†]Holds regional or international accessions.

[‡]Y = yes, N = No, S = some; S-MTA = requires a material transfer agreement.

[§]Source: Tabulated data from 2006 survey led by Suketoshi Taba (CIMMYT) among respective gene-bank curators.

Vegetative traits can be included as descriptors for describing and characterizing maize germplasm. However, reproductive traits are among the most appropriate for clustering this germplasm into respective races (Ortiz et al., 2008a,b), or for defining a core subset of maize landraces (Malosetti and Abadie, 2003; Abadie and Beretta, 2005). Such results are due to the high heritabilities of reproductive traits compared to vegetative traits (Goodman and Paterniani, 1969; Sánchez et al., 1993; Ortiz and Sevilla, 1997). Furthermore, reproductive traits need fewer replications for minimizing phenotypic variation than vegetative traits because of their high heritability (Ortiz et al., 2008c). Statistical classification on the basis of reproductive trait data has been shown to provide detailed insight into the relationships among maize landraces as compared to visual racial classification based on ear and kernel traits (Gutiérrez et al., 2003).

A core germplasm collection should represent a large proportion of the diversity in the entire collection with a minimum level of duplication (redundancy). Core collections have significantly expanded the use of crop-related genetic resources in many crops (Hodgkin et al., 1995). Detailed classification of accessions into similar and related groups should be performed before the selection of a core subset because significant amounts of crop diversity are often distributed both between and within such groups, especially in out-crossing species. Representative accessions from each race are selected on the basis of phenotypic diversity in plant and ear traits using multivariate cluster analysis and a selection index (Franco et al., 1998, 2005). This multivariate cluster analysis, based on the Ward method, has been used to define distinct and well separated groups. The clusters were then refined through the application of a modified location model (MLM). This sequential strategy for classifying maize germplasm (whether accessions, cultivars, or inbred lines) makes best use of all available information to group individuals into robust clusters according to their phenotypic similarity. A sample allocation strategy (D method) is then used to determine the effective size of each cluster on the basis of the relative extent of diversity. The D method attempts to capture the maximum amount of diversity from the entire collection in the core collection (Franco et. al., 2005). Table 5 provides a summary of some of the methods used for defining maize core collections. The eigen selection index method (ESIM) (Cerón-Rojas et al., 2006) can also be used for selecting the most appropriate representative genotype from each cluster for inclusion in the core collection. The accessions selected through the ESIM approach maximize the selection response when selecting for all traits included in the index. This selection index can be used with accessions or test-crosses or top crosses and has the advantage that no weighting of traits is required.

A Caribbean maize core subset was defined based on 100 of the original 498 accessions from West Indies, Central, and South America (Taba

Table 3. Teosinte collections in Mexico and the United States. Source: GCDT (2007).

Race or species	Universidad de Guadalajara [†]	INIFAP[†]	CIMMYT	USDA (GRIN)[§]
Balsas	96	135	50	120
Chalco	18	44	45	16
Central Plateau	37	55	18	12
Nobogame	2	4	2	6
Zea diploperennis Iltis, Doebley & Guzmán	2	7	2	14
Zea perennis Iltis, Doebley & Guzmán	1	4	2	10
Huehuetenango			1	7
Zea luxurians (Durieu & Ascherson) Bird			1	21
Zea nicaraguensis Iltis & Benz			1	2
Unknown			40	1
Total accessions	156	249[‡]	162	209

[†]Source: José de Jesús Sánchez Gónzalez (Universidad de Guadalajara, Mexico).

[‡]Includes 100 accessions from Universidad de Guadalajara.

[§]Sources: www.ars-grin.gov and Plant Inventory No. 173, 174, 176, 177, 179, 181 (USDA).

et al., 1998). Non-overlapping clusters were formed and patterns of phenotypic diversity among and within clusters were determined using canonical discriminant analysis along with a selection index based on yield, ear rot, erect plants, and moisture. Representative accessions from each of the clusters were then selected and used to form the Caribbean maize core subset. During 1999 to 2001, the heterotic designations of these 100 accessions were assessed using two testers: CML 287 (A) and CML413 (B). This information was then used to direct further crossing with breeding materials. In addition, enhanced special purpose trait populations were used to broaden some of these gene pools, while some pools underwent improvement cycles without addition of new maize germplasm. At the same time, different selections from Pool 19 were continued separately as Pools 19 and 20. Similarly, Pool 21 was spilt into Pools 21 and 22. In both cases selection decisions were based on performance of progeny generated from crossing S_1 lines with inbred testers A and B, which generated heterotic gene pools of intermediate maturity. Some of these gene pools have now passed through more than 30 improvement cycles. The best three S_2 lines of the Caribbean landraces × CML crosses and the best two S_2 lines of the resultant gene pools have been included in a diallel mating design for developing the Tropical Late Yellow Pools 25 and 26. The heterotic populations were then improved to make new source germplasm for Pools 25 and 26. Pedigrees as well as the performance at Agua Fría and Tlaltizpan (Mexico) of test-cross and top cross progeny compared with that of checks for each gene pool are available as supplemental materials to this article. This and other core subsets from across the primary center of diversity for maize will greatly facilitate the utilization of maize landraces for both breeding and research. The next

important advance in this area will be to use genomic characterization data from DNA markers (Franco et al., 2007) to validate these core subsets within the broader context of diversity across the entire gene pool. Given the highly heterogeneous and highly heterozygous nature of the accessions involved, this remains a major logistical challenge for simple sequence repeat (SSR) marker analysis. CIMMYT scientists are however working on optimizing DNA pooling techniques for single nucleotide polymorphism (SNP) analysis in maize.

Tropical maize germplasm of dent and flint types (heterotic groups A and B, respectively) has accumulated distinct sets of genes (Taba et al., 2004). Maize germplasm accessions can be test-crossed with inbred testers from groups A and B to determine heterotic patterns across core subsets. Subsequently, breeding crosses of core accessions × elite inbred lines can be developed within the A or B heterotic groups. In this way, the core accession is enhanced by incorporating a substantial amount of the background genotype from elite germplasm of the same heterotic group. CIMMYT elite breeding lines (CML) with known combining abilities

Table 4. Maize and wild species accessions held in trust at CIMMYT genebank.

Germplasm type	Number of accessions
Landraces	23,409
Gene pools	554
Improved populations	355
Synthetics and composites	329
Cultivars	1092
CIMMYT maize lines (CML)	513
Teosinte	162
Tripsacum	138
Total	26,552

Table 5. Some methods and descriptors for clustering and defining core subsets of maize germplasm.

Method and descriptors	Reference
The Modified Location Model (MLM) was used to develop a two-stage classification strategy for 3-way data (accession × descriptor × environment) with categorical and continuous descriptors. The MLM model was suggested for classifying genetic resources into homogeneous groups after multi-environment testing to form core subsets.	Franco et al., 1998
Non-overlapping clusters were formed and patterns of phenotypic diversity were determined using canonical discriminant analysis. A selection index including grain yield, and percentages of ear rot, erect plants, and moisture was used to select the upper 20% of the accessions, which represent the phenotypic diversity of the clusters to form the Caribbean maize core subset.	Taba et al., 1998
The Relative Diversity method, which takes into account the morphological diversity of the different groups, combined with the logarithmic strategy produced the best maize core collection of Uruguay, as it retained the highest percentage of the ranges for the quantitative descriptors included in the analysis. A combination of geographic origin and kernel type was regarded as the best classification rule because both are closely related to the distribution of genotypic and spatial diversity, respectively.	Malosetti and Abadie, 2003
Geography data, race group, and kernel type were the main descriptors used for germplasm clustering. The race was very important because this descriptor captures the diversity associated to both cultural practices and use of maize in the Southern Cone of South America.	Abadie and Beretta, 2003
The D allocation method produced samples with significantly more diversity, larger ranges, and higher variances for the quantitative descriptors than other methods, and provided higher variances than the variance among accessions of the whole collection. In the D method, the number of accessions sampled per cluster is proportional to the mean of the Gower's distance between accessions within the cluster.	Franco et al., 2005
The unweighted pair-group method using arithmetic average (UPGMA) with D allocation methods, which maximize distances, produced core subsets with significantly more diversity than the other methods when forming core subsets using molecular markers.	Franco et al., 2007

for tropical hybrid development are used as the recurrent parent (Taba and Chávez, 2007). Progeny from test-crosses with S_2 or S_3 lines are then evaluated using a tester from the opposite heterotic group. In most cases, additional cycles of S_2 or S_3 recurrent selection are needed to remove unfavorable alleles from the breeding population. The best families generated from this approach will be useful for broadening breeding pools and providing synthetic cultivars for public release. Figure 2 shows a germplasm enhancement system for improving maize core subset accessions through crosses with elite breeding materials.

POPULATION IMPROVEMENT FOR MAIZE GERMPLASM ENHANCEMENT

Germplasm enhancement serves to broaden the genetic base of breeding materials and to provide beneficial genetic variation for specific target traits (Ortiz, 2002). During the mid-1970s and throughout the 1980s, CIMMYT bred a range of broad-based maize gene pools with the aim of generating improved populations for the tropical highlands and lowlands, as well as subtropical regions of the developing world (Vasal et al., 1982). The initial gene pools were classified by grain types (dent or flint), maturity (early, intermediate, and late), and grain colors (white and yellow). The goal of these population improvement programs was to develop maize germplasm combining wide adaptation with plant types suitable for enhanced grain production. A modified ear-to-row half-sib selection breeding system (Lonquist, 1964) was used for improving the harvest index and other agronomic traits. These gene pools integrated genetic diversity from many useful germplasm accessions representing a broad genetic base for selection and recombination of various important agronomic traits. This early CIMMYT maize breeding scheme considered both the improvement of general purpose gene pools (or back-up gene pools) aiming at a broad genetic base for recombination, as well as the genetic enhancement of populations for further cultivar development using a modified full-sib selection approach. Superior families from the back-up gene pools were integrated into breeding populations to broaden their genetic base. Full-sib progeny of the advanced breeding populations were then tested in diverse locations by national breeding programs. Experimental cultivars were bred for enhanced performance within and across target environments. Population improvement schemes (S_1 or S_2 recurrent selection, or modified full-sib and half-sib family selection) were used for enhancing host plant resistance to specific leaf pathogens and insect pests, as well as tolerance to drought and low nitrogen in the soil. These were based on special purpose gene pools that been bred for each of the target traits since the mid-1980s. Both line and hybrid developments were initiated at the same time using the same improved gene pools and populations.

Using population improvement for maize germplasm enhancement provides a mechanism for addressing the needs of a broad range of stakeholders; i.e., from direct use by farmers as OPVs to indirect use by breeders who recycle selections from these gene pools in their development of inbred lines. For these reasons, the genetic enhancement of broad-based gene pools has been a major priority for CIMMYT since 1999 (Taba, 2005). The systematic

The Breeding Crosses between the core and elite lines to form S_2 or S_3 lines
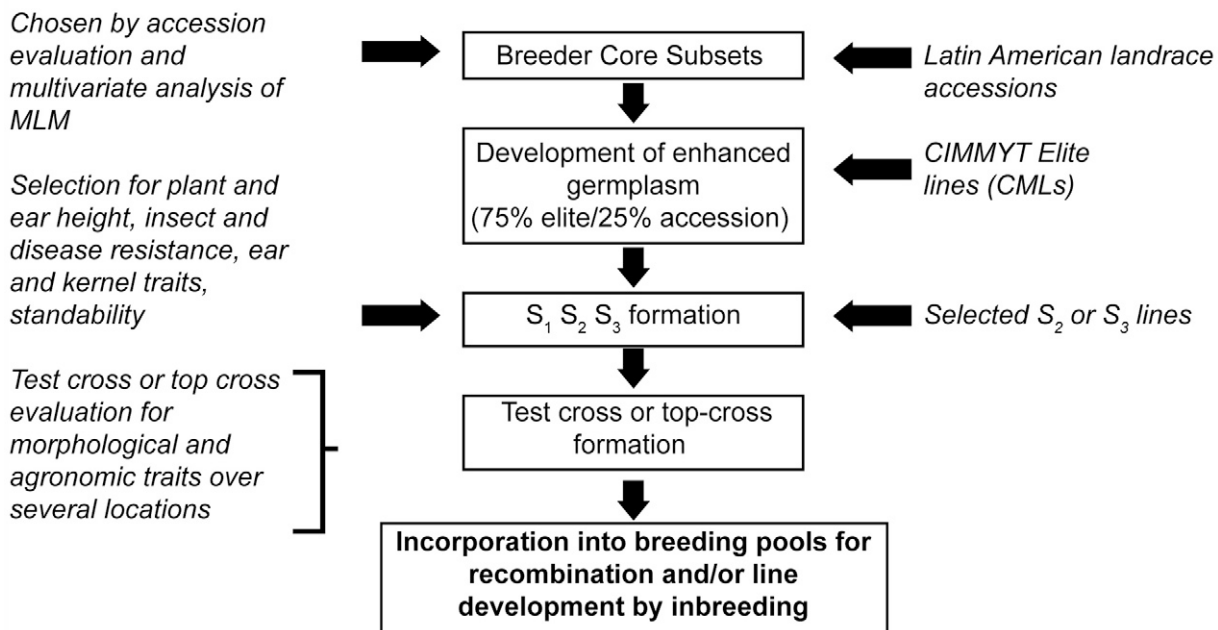

Figure 2. CIMMYT enhancement of maize core subset accessions through crosses with elite breeding materials. Selection criteria and germplasm utilized are indicated in the left and right columns, respectively.

organization of maize germplasm populations based on their adaptation, maturity group, grain color, and heterotic group has allowed broad-based gene exchange, selection, and recombination within distinct target orientated gene pools (Taba and Chávez, 2007). Diverse sources of donor germplasm are very important for enabling trait-based enhancement of these broad-base gene pools while DNA markers are important tools for helping to maintain overall genetic diversity. DNA markers can also be used for monitoring the available genetic diversity in each gene pool, as a guide for their overall management or toward the development of new germplasm enhancement strategies such as whole-genome selection systems.

The Latin American Maize Program (LAMP) and the hierarchical open-ended population enrichment (HOPE) breeding programs are two other examples of genetic broadening in maize. LAMP has assessed national germplasm and facilitated the exchange of genetic resources across Latin America (Salhuana and Pollak, 2006). Whereas HOPE was developed by Kannenberg and Falk (1995) as a mechanism for broadening genetic diversity while maintaining germplasm quality to obtain promising parental inbred lines for production of commercial hybrids. A LAMP core subset has been made available to encourage further use in maize genetic broadening (Taba et al., 1999). The Germplasm Enhancement of Maize (GEM) project has used the Latin American landrace accessions selected by LAMP in crosses with elite temperate maize lines provided by private companies in North America (Salhuana and Pollak, 2006). GEM breeders have arranged the crosses into non–Stiff Stalk and Stiff Stalk heterotic groups. The primary purpose of the GEM project has been to introgress useful genetic diversity from Latin American maize races and other tropical maize donor sources (lines and hybrids) into U.S. maize germplasm to broaden the genetic base of the corn belt hybrids (Balint-Kurti et al., 2006; Goodman, 2005).

CIMMYT Enhancement of Maize Germplasm Core Accessions and Broad-Based Gene Pools

The CIMMYT genebank has been steadily accumulating new accessions with particular emphasis on new collections of Latin American maize races and introduction of new accessions from national genebanks across Latin America. This has resulted in more than doubling the size of the maize collection from 10,000 (in the 1980s) to 20,000 maize holdings in the late 1990s (Taba et al., 2005) to nearly 27,000 accessions today. The aim of this germplasm collecting endeavor was to identify material with a high combining ability for grain yield that could be introduced into the existing gene pools, with a particular emphasis on generating material for producing new hybrid cultivars (Taba, 2005). CIMMYT's maize germplasm enhancement scheme started with assessing the phenotypic performance of each gene pool in a half-sib family. In the most recent variation of this scheme, S_2 lines were top crossed with an inbred tester from the opposite heterotic pool; i.e., the dent gene pool became the A heterotic group and the flint pool became the B heterotic group (Taba and Chávez, 2007). The test-cross or the top cross progeny were then evaluated in breeding stations, and the best 25 to 30% of the lines, selected on the basis of performance data, were inter-crossed to create the next cycle. The inbred tester used was able to identify heterotic patterns of the lines across the gene pool. One or two test-crosses were performed before switching to top crosses with a bulk of the gene pool from the opposite heterotic group (Fig. 3). For both gene pools, the families of breeding crosses and old gene pools were inter-crossed using the same germplasm enhancement scheme as shown in Fig. 3.

Figure 4 shows the main CIMMYT germplasm enhancement gene pools, their target areas, heterotic groups and heterotic partners. The grain types of the highland maize races include floury, Morocho, popcorn, sweet corn, dent, and flint. Dent and flint types are not common in the Andean highland maize races. Morocho could be regarded as a flint type but it has floury maize backgrounds. Both Morocho and floury types are important gene pools for the Andean highlands and to a lesser extent for other highland maize areas. CIMMYT highland white endosperm CML were used in crosses with the core accessions at the initial phase. CIMMYT segregating breeding populations for yellow and white grain types have been used for developing white and yellow gene pools. The two heterotic gene pools are given the suffix of HA (heterotic group A) or HB (heterotic group B). Pedigrees and performance at El Batán (Mexico) of top cross progeny compared with that of entry checks for each gene pool are available as supplemental material to this article.

New germplasm sources have been incorporated into CIMMYT subtropical gene pools to improve their performance. This included accessions from Brazil, Mexico, and the U.S. that were crossed with CML testers, and the progeny were then assessed for their combining ability and key agronomic traits. The S_2 lines from the best crosses were subjected to further selection and progeny from test-crosses with CML testers were evaluated in field trials. The best resultant materials have been incorporated in all pools except for Early White Pools 27 and 28, where the sole focus was on improvement for earliness. S_2 and S_3 lines have been developed in the latest selection cycle and top crossed with the pool from the opposite heterotic pool, leading to the development of Late White and Yellow Gene Pools 31, 32, 33, and 34. These gene pools possess higher levels of genetic diversity than the previous improvement cycles from which they were derived. Pedigrees and performance at Tlaltizapan (Mexico) of progeny from top crosses compared with

Reciprocal recurrent selection (RRS) between the heterotic gene pools

Incorporation of enhanced lines from the breeding crosses into the pools
(<5% in the recombination cycle of the pool)

200 ears

Recombination among the
selected lines (40-60)

selected
40-60 lines (20-30%)

S_1 progeny >200 ears

S_2, S_3 progeny >200 ears

Evaluation of the progeny
at 2-3 sites

Test cross or top cross with
the opposite tester or
heterotic pool

>200 top crosses

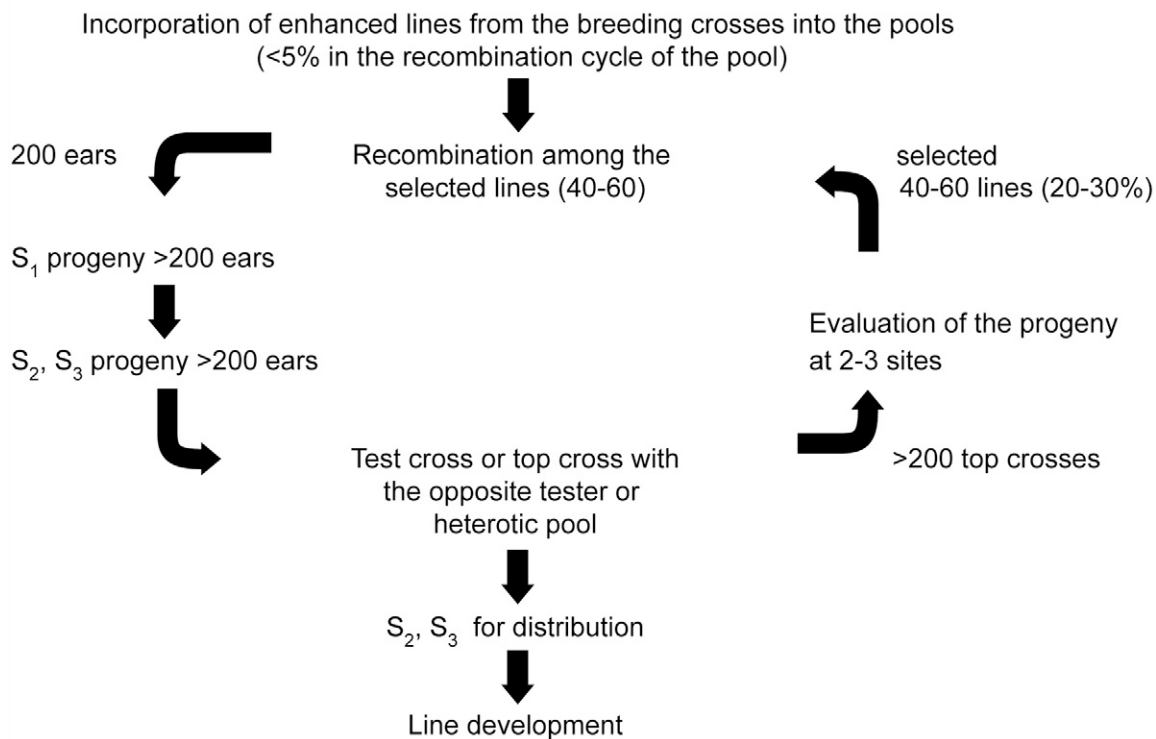S_2, S_3 for distribution

Line development

Figure 3. Reciprocal recurrent selection (RRS) scheme for the improvement of the paired maize gene pools used by CIMMYT. Inter-population improvement between paired A and B gene pools builds on intra-population genetic gains from breeding cross-populations and the gene pools (Fig. 2). Enhanced breeding populations and gene pools become both hybrid-oriented germplasm sources. Progeny tests are conducted in single 5-m row plots at a 66,000 plants ha^{-1} plant density with two replications. The test-crosses or top crosses are arranged in an α-lattice design. The second replication may undergo screening for host plant resistance to ear rots (*Fusarium graminearum* Schwabe or *F. moniliforme* Sheldon) and leaf blights (*Bipolariz maydis* (Y. Nisik. & C. Miyake) Shoemaker or *Exserohilum turcicum* (Pass.) K. J. Leonard & E. G. Suggs).

that of checks for each gene pool are available as supplemental material to this article.

CIMMYT SAFE AND LEGAL SHARING OF MAIZE GENETIC RESOURCES AND INTERMEDIATE PRODUCTS AS GLOBAL PUBLIC GOODS

Maize germplasm bred by CIMMYT remains available for further use by breeders and farmers. Seed bulks of S_2 lines, selected S_2 lines, and the latest cycles of selection from gene pools are shared with maize breeders at CIMMYT, and on request with other institutions including the private sector. On-farm management of maize races can benefit from this genetically enhanced germplasm without altering the traits for which these races are preferred by local farmers and consumers. For example, the Zapalote Chico race from Tehuantepec (Oaxaca, Mexico) has been genetically enhanced for specific agronomic traits by the introgression of early CIMMYT gene pools through a collaborative program with a local breeder (Taba et al., 2006). The resultant trait-enhanced populations as well as their respective landrace genetic background may now

be used in local participatory breeding. Maize breeders developing special purpose gene pools (e.g., with tolerance to drought, nitrogen–use efficiency, and host–plant resistance to insect pests and pathogens) can introgress this germplasm into their current breeding populations.

The Governing Body of the International Treaty (IT) on Plant Genetic Resources for Food and Agriculture (PGRFA) at its First Session held in Madrid (Spain) in June 2006 adopted a Standard Material Transfer Agreement (SMTA) for transfers of PGRFA of crops and forages listed in Annex 1 of this Treaty (FAO, 2006, 2009b). This includes maize *Tripsacum laxum* but excludes *Z. perennis* (A.S. Hitchc.) Reeves & Manglesdorf, *Z. diploperennis* Iltis, Doebley, & Guzman, and *Z. luxurians* (Durieu & Asch.) Bird. CIMMYT, along with other Centers of the Consultative Group on International Agriculture (CGIAR), has been using this SMTA for all relevant seed shipments since 14 January 2007. As agreed by the Governing Body, the SMTA used by the Centers includes a footnote indicating that the provisions of the SMTA refer to Annex 1 materials but that this should not be interpreted as precluding the use of the SMTA for transfers of non-Annex 1 materials. The implementation of the SMTA relies on acceptance of

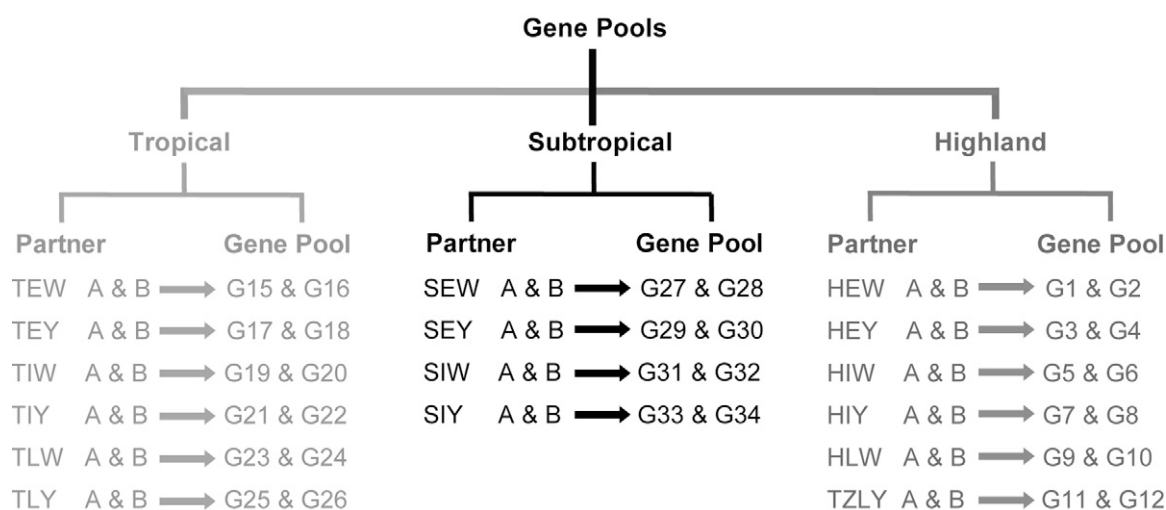## Heterotic Partners of CIMMYT gene pools

Figure 4. Maize gene pools bred at CIMMYT and their heterotic partners according to their maturity, grain color and target agro-ecology. TEW and TEY, TIW and TIY, and TLW and TLY indicate tropical early white and yellow, tropical intermediate white and yellow, and tropical late white and yellow, respectively. SEW and SEY, and SIW and SIY indicate subtropical early white and yellow, and subtropical intermediate white and yellow, respectively. HEW and HEY, HLW and HLY, and IHW and IHY indicate tropical highland early white and yellow, tropical highland late white and yellow, and tropical intermediate highland white and yellow, respectively.

the conditions on breaking of the seal of the box containing the seed (i.e., a shrink–wrap agreement); although a click–wrap option is also available at the CIMMYT website: http://www.cimmyt.org/english/wps/obtain_seed/frmseedrequestmaize.htm; verified 16 Nov. 2009. At its second session, held in Rome (Italy) from 29 October to 2 November 2007, the Governing Body of the IT decided that the CGIAR Centers should also use the SMTA when transferring non–Annex 1 PGRFA (e.g., teosinte).

CIMMYT has been sharing maize genetic resources held in its genebank for many decades (Table 6). In addition, CIMMYT's germplasm improvement programs rely heavily on the free international exchange of improved maize materials. However, the exchange of such germplasm is dependent on all associated institutions and regulating authorities having confidence in the safety of both imported and exported seed. Thus, CIMMYT is fully committed to maintaining international seed health standards in all of its worldwide operations. These standards are dictated at different levels by the International Plant Protection Convention 1997 (https://www.ippc.int/IPP/En/default.jsp; verified 28 Oct. 2009), by the IT-PGRFA, and by various CGIAR operating guidelines.

CIMMYT's internal standard operating procedures reflect its obligation to address both national and international phytosanitary regulations. CIMMYT's Seed Health Laboratory (SHL) has operated since 1998 under the approval of Mexico's Ministry of Agriculture (SAGARPA) (*Norma Oficial Mexicana* 036–FITO–1995), and since April 2007 with accreditation under ISO/IEC 17025:2005 *General Requirements for the Competence of Testing and Calibration Laboratories*. Seed samples are provided

by CIMMYT programs or external collaborators for analyses, and, on completion of the testing process, the seed is "released" and ready to be shipped abroad or planted in CIMMYT experiment stations. When a pathogen of quarantine importance is identified, either in outgoing or incoming material, the SHL is obligated to inform the Mexican phytosanitary authorities, who will indicate the measures to follow to prevent the spread of the pathogen. The majority of pathogens detected on maize seed are saprophytes or minor seed–borne pathogens that do not affect seed physiological quality, such as *Fusarium verticilloides* (Sacc.) Nirenberg, *Aspergillus* spp., *Penicillium* spp. These pathogens are generally well controlled by standard seed treatments applied before seed shipment.

CIMMYT annually distributes a huge amount of maize and wheat seeds, particularly of improved germplasm, to partners across the world. For example, during 2008 a total of 254 shipments were sent from CIMMYT headquarters to 383 collaborators in 80 countries (Table 6). These shipments include international trials focusing on trait enhancements targeted at specific environments, which are sent on request to breeding programs across the world. CIMMYT also provides seed on request from individual genebank accessions (Fig. 5). CIMMYT also distributes a substantial amount of improved maize germplasm directly from its regional operations. For example, seven regional nurseries (a total of 238 entries) were distributed from CIMMYT Kenya to 24 countries during 2008. In addition, special requests for a total of 7827 accessions received from 193 institutions (21.76% private sector) in 78 countries were distributed from CIMMYT offices in Colombia, Ethiopia, India, Kenya, and

Table 6. Number of maize seed shipments from CIMMYT headquarters in 2008.

A. Distribution of international trials (number of trial sets by region)

| | Sector | | | | |
Region	Educational	Government	Private	Grand total	Percentage (%)
Africa	–	37	22	59	23.23
Asia	12	35	2	49	19.29
North America[†]	–	4	–	4	1.57
Central America	17	48	38	103	40.55
South America	–	21	14	35	13.78
Middle East	–	4	–	4	1.57
Grand total	29	149	76	254	100.00

B. Distribution of miscellaneous shipments (as per amount in kg)

| | By sector within region (%) | | | By region within sector (%) | | | |
Region	Educational	Government	Private	Educational	Government	Private	Grand total[§]
Africa	0.27	96.52	3.21	0.12	2.73	1.60	2.53
Asia	26.74	71.40	1.86	19.67	3.32	1.52	4.15
Central America	28.44	41.95	29.61	73.75	6.87	85.55	14.63
North America	0.62	99.26	0.12	4.30	43.77	0.94	39.37
South America	1.50	87.97	10.53	1.18	4.37	9.23	4.44
Middle East	3.51	96.49	0.00	0.06	0.10	0.00	0.10
Europe	0.06	99.79	0.15	0.35	38.83	1.02	34.75
Oceania	82.61	0.00	17.39	0.56	0.00	0.13	0.04
Grand total[‡]	5.64	89.29	5.06				100.00

[†]Including Mexico.

[‡]The values at the bottom of each column indicate the percentages for each sector irrespective of region.

[§]The values at the end of each row indicate the percentages for each region irrespective of sector.

Zimbabwe (Thomas S. Payne, CIMMYT, personal communication, 2009).

MARKER-ASSISTED UTILIZATION OF GENETIC RESOURCES

There has been a wide range of applications of molecular markers in the area of maize genetic diversity analysis including: (i) allele frequency analysis (Reif et al., 2004) and characterization of molecular variation within or between populations (Dubreuil and Charcosset, 1998; Warburton et al., 2002; Reif et al., 2004), (ii) construction of "phylogenetic" trees and determination of genetic distances (Lu and Bernardo, 2001; Warburton et al., 2002; Betrán et al., 2003; Liu et al., 2003; Reif et al., 2004; Xia et al., 2004, 2005) and determination of heterotic groups (Warburton et al., 2002; Xia et al., 2004; 2005), (iii) correlation between the genetic distance and hybrid performance, heterosis, and specific combining ability (Melchinger, 1999; Betrán et al., 2003), and (iv) comparison of genetic diversity among different groups of maize germplasm (Liu et al., 2003; Tarter et al., 2004; Xia et al., 2005). These studies have provided important information for genebank curation, gene identification, and breeding. DNA fingerprinting of cultivars and inbred lines, haplotype analysis, allele mining, gene discovery, and improved understanding of genotype × environment interaction are also important uses of molecular markers in maize genetic enhancement in addition to direct use in marker-assisted selection.

There has been a gradual decline in genetic diversity of the elite temperate maize pool over the past century (Duvick et al., 2004; Reif et al., 2005). Tropical and subtropical inbred lines contain a greater number of alleles and gene diversity than temperate inbred lines, indicating that they may be important source germplasm for temperate breeding programs. However, on average, maize inbred lines capture less than 80% of the alleles observed in the landraces, suggesting that landraces can provide substantial additional genetic diversity for all maize breeding programs. In addition, many alleles in the progenitor species of maize (teosinte) are not present in maize (Vigouroux et al., 2005) providing a third level of genetic variation for breeders. However, all this diversity needs to be structured and understood before it can be fully and efficiently utilized.

More than 2000 quantitative trait loci (QTL) have been reported in maize related to a wide range of agronomically important traits, including yield, yield components, plant morphology and physiology, and biotic and abiotic stress responses. Simple sequence repeats (or microsatellites) are currently the most commonly used markers in maize as they are simple to use and there are many markers available. Simple sequence repeats often have several alleles, making them useful for the study of diversity and for the identification of useful polymorphisms for
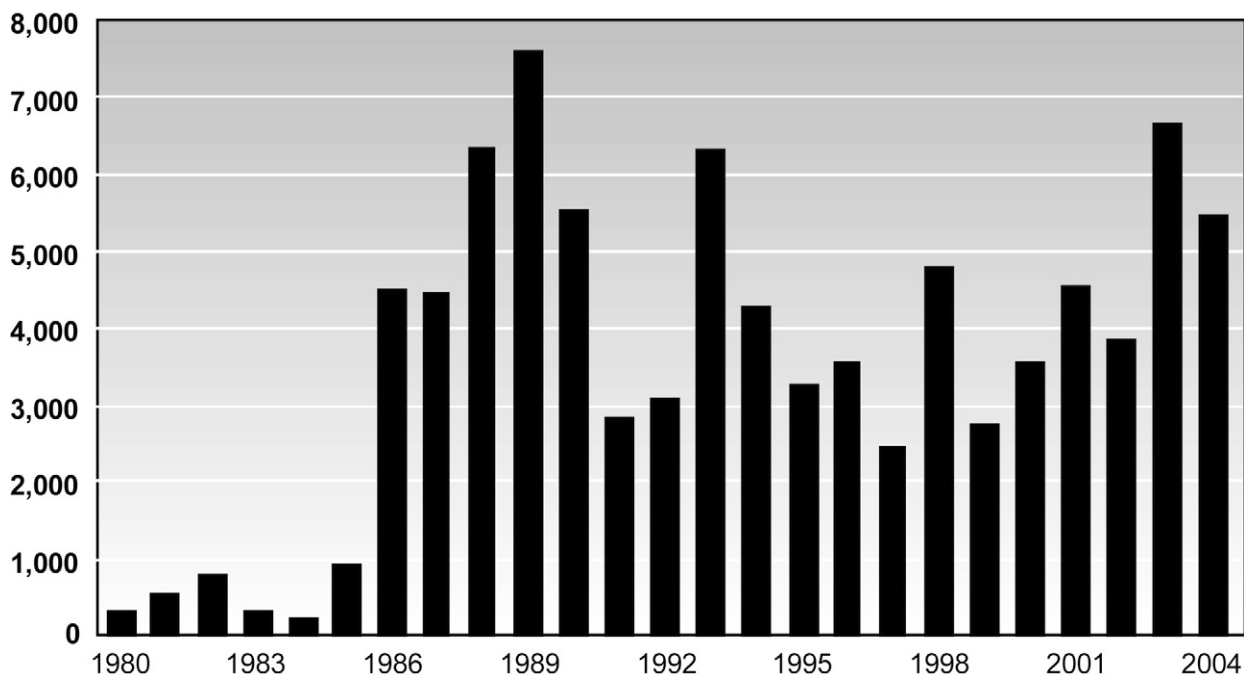
Figure 5. Annual maize seed shipments from CIMMYT genebank since 1980.

breeders. Single nucleotide polymorphisms are the most abundant sequence variations in maize (Tenaillon et al., 2001) and are ideally suited to the generation of high density genetic maps (Cho et al., 1999). Single nucleotide polymorphisms are evenly distributed across the genome, and unlike gel-based markers, the methods of assaying SNP can be more easily automated. A major effort to define SNP in a diverse set of *Zea* species is ongoing through the Panzea project in which over 6000 SNP are ready for use in diversity, mapping, or other applications (www.panzea.org; verified 16 Nov. 2009; Zhao et al., 2006).

Microsatellite markers have been used for estimating genetic structure and diversity in temperate, tropical, and subtropical maize inbred lines from the U.S., Europe, Canada, South Africa, Thailand, CIMMYT, and the International Institute of Tropical Agriculture (IITA, Nigeria) (Liu et al., 2003). On the basis of this analysis, inbred lines could be arranged into five clusters, which aligned with the major breeding groups, plus a set of lines showing evidence of mixed origins. Core subsets of inbred lines were then defined that captured maximum allele richness, which can be further used in maize breeding and genetic research.

Maize is a crop that has high resolution genetic maps and therefore is an ideal choice for development of diversity maps that could provide new information about the consequences of natural selection, domestication, and polyploidy formation. Diversity analysis of individual genes promises to shed new light on crop productivity and evolutionary processes underlying plant domestication (Wang et al., 1999; Buckler et al., 2006; Doebley et al., 2006). This type of information can be readily converted

into tools for molecular breeding through so-called allele mining that may be achieved through re-sequencing or eco-tilling (Comai et al., 2004). Eco-tilling is not being widely used in maize at this time, due to the very high number of sequence differences found between different maize accessions, which make the interpretation of the differences in such a complex system difficult. For re-sequencing, the same methodology used in association mapping may also be used for allele mining of the diverse core subsets of maize being created from breeder's lines, genebank accessions, and wild relatives. Once a gene of interest is positively identified (via association mapping or any other technique) and the sequence determined, the same gene can be re-sequenced (entirely or in part) in all the individuals in the subset. This research approach will allow the identification of candidate allele-specific markers that can be validated in relevant breeding populations.

Knowledge of the genetic diversity and relationships between maize inbred lines is indispensable when designing crossing programs and breeding systems. Microsatellite marker analysis has been used to characterize CIMMYT tropical, sub-tropical, and temperate maize breeding material (Reif et al., 2004) and CIMMYT highland and mid-altitude lines bred in Africa (Legesse et al., 2007), and to compare CIMMYT breeding populations with inbred lines with maize landraces from Mexico (Warburton et al., 2008). A large proportion of the genetic diversity was detected within the maize breeding populations with only a small percentage detected between the populations (Reif et al., 2004). The low genetic diversity between populations could be due to admixture effects as a result of the breeding methods used for developing populations that

disregarded racial complexes. Unique alleles have been detected in all major maize germplasm groups, indicating the presence of a great reservoir of untapped genetic variation for maize genetic enhancement (Warburton et al., 2008). This analysis has laid the foundation for allele mining studies in core germplasm subsets that will lead to the identification of new alleles for important agronomic traits where the underlying genes have been well characterized. Molecular marker analysis may also assist in determining the most appropriate use of inbred lines in the exploitation of heterosis and in the formation of genetically diverse source breeding populations (Legesse et al., 2007). Most recently, new high-throughput genotyping systems using SNP markers have been developed that provide a powerful tool for large-scale maize germplasm evaluation. SNP chips have already been used for characterizing inbred lines from maize breeding programs of Brazil, China, and CIMMYT (Kenya, Mexico, and Zimbabwe) (Lu et al., 2009). Most of the lines that were clustered very closely to each other had been bred for adaptation to similar geographical (environmental) areas. The scale of genetic diversity analysis that is now possible through the use of SNP chips is likely to drive a new level of performance from DNA marker analysis in public sector maize breeding programs, which will facilitate particularly rapid catch-up progress for tropical maize breeders.

Population structure and linkage disequilibrium analysis has also facilitated rapid advances in association mapping in maize (Yu and Buckler, 2006), which has provided a new method for mapping important breeding traits: including maize kernel composition and starch production (Wilson et al., 2004), carotenoid (Palaisa et al., 2003) and maysin contents (Szalma et al., 2005), as well as tolerance to aluminum (Krill et al., 2006). In contrast to linkage mapping, association mapping, or linkage disequilibrium (LD) mapping, relies on surveys of natural variation. Association mapping projects are underway in many plant species. Association mapping seeks statistical correlations between phenotypic variation in a panel of individuals and changes in candidate gene sequences (known as the candidate gene approach) or arbitrary marker polymorphisms (spread throughout the genome, and known as whole-genome scanning). Association mapping will identify alleles associated with wide adaptation, and new alleles of previously identified genes that can be screened in genebank materials and characterized to determine their relative value. New alleles positively affecting important agronomic traits can be backcrossed into elite breeding materials quickly using the perfect markers identified through candidate gene-based screening.

Several genes with important functions in maize are being studied through comprehensive association genetics research using methods such as nested association mapping (Buckler et al., 2009; McMullen et al., 2009). However, marker density has become a constraining factor in this approach, particularly for highly complex traits. Thus, high resolution whole-genome scans (using many thousands of SNP markers) are now being used for association mapping in maize. For example, the major loci affecting oleic acid content in maize kernels have been fine mapped using this approach (Beló et al., 2008), providing a marker ~2 kb from a fatty acid desaturase (*fad2*) gene. The combination of the high LD in maize germplasm with whole genome scans will enable maize germplasm collections to be used as a mapping resource for many agronomic traits. However, the rate-limiting factor in this approach will be the degree to which traits can be effectively subjected to large-scale but precise phenotyping across diverse genotypes tested in replicated multi-location field trials. Association analysis has been coupled with linkage mapping, expression analysis, and mutagenesis to correlate variation at the lycopene epsilon cyclase (*lcyE*) locus with alterations in metabolic activity of α-carotene versus β-carotene branches of the carotenoid pathway (Harjes et al., 2008). Simple polymerase chain reaction (PCR) marker systems have now been developed at CIMMYT for selecting parental combinations with optimal combinations of favorable *lcyE* alleles to enhance the pro-vitamin A levels in maize grain. This is an important target trait for tropical maize breeders as large areas of the developing world have dietary vitamin A deficiency causing eye disease in millions of children while placing many millions of people at risk of other health disorders (Graham et al., 2001, 2007; Pfeiffer and McClafferty, 2007).

Genomics tools have become a routine component of most private-sector maize breeding programs (Fu and Dooner 2002; Niebur et al., 2004; Cooper et al., 2004; Crosbie et al., 2006) where they are used to (i) dissect the genetic structure of relevant maize germplasm to understand gene pools and germplasm (heterotic) groups, (ii) provide insights into allelic content of genetic resources for potential use in breeding, (iii) screen early generation breeding populations to select segregants with desired combinations of marker alleles associated with beneficial traits (to reduce the scale of costly phenotypic evaluations), and (iv) to establish genetic identity (fingerprinting) of their products. Routine integration of molecular markers from introduction of new genetic resources to release of new elite breeding lines in public-sector tropical maize breeding programs is a primary strategic goal for CIMMYT.

CIMMYT researchers are also using selective genotyping (from the two tails of the phenotypic distribution of a population) combined with pooled DNA analysis as a highly cost effective alternative to analysis of the entire population of individual genotypes for genetic mapping (Xu et al., 2008). Selective genotyping can also be used for genome-wide association mapping where it can be integrated with selective phenotyping approaches. With

this goal in mind, over 2000 maize genotypes and breeding lines have been collected by CIMMYT scientists from genetics and breeding programs worldwide. This material represents phenotypic extremes for many traits including tolerance to drought, host plant resistance to pathogens and pests, and grain quality traits. The whole collection has been genotyped using a 1536 SNP chip developed specifically for maize association mapping studies and data from this genotyping will be compared with corresponding phenotype data. This will allow feasibility testing of a simultaneous one-step marker-trait association analysis for a large number of agronomic traits (Xu et al., 2009). Elements of this approach have already been individually validated. For example, kernel hardness and host plant resistance to maize streak virus (MSV) have already been mapped using pooled DNA analysis (Babu et al., 2009). In addition, selective genotyping has been successfully used for genetic mapping of host plant resistance to both head smut [caused by *Sphacelotheca reiliana* (Kuhn) Clinton] and mosaic virus, and for mapping of tolerance to drought using 235 introgression lines developed by recurrent backcrossing (Hao et al., 2009). With the rapid increase in publically available SNP markers, genome-wide association mapping will soon become possible. When this is coupled with selective genotyping, it should provide a dramatic shortcut for discovering functional allelic variation associated with agronomic traits of interest.

OUTLOOK

Crop-related biodiversity is the founding asset of the CGIAR system and continues to be the basic raw material for the breeding programs of CIMMYT and her partners. In this article we have attempted to highlight the 'conservation through use' of the global maize genetic endowment. Some of the research outputs described in this article (e.g., newly collected germplasm accessions and enhanced gene pools, as well as biotechnology-based tools and methods plus computational systems) can be regarded as "intermediate products" for improving access to and utilization of maize genetic resources toward the ultimate goal of releasing new improved cultivars (Crouch et al., 2008). Regular unbiased quantitative analysis of progress in generating intermediate products, and their rate of uptake in end-product development, will be critical to effectively orient future research priorities and identify where critical linkages have become rate-limiting steps for the rest of the pipeline. The emerging strategy of the CGIAR system in this millennium should therefore give emphasis to the development of technology-assisted methods and intermediate germplasm products for the efficient identification of value-added traits and their rapid introgression into agronomically elite widely adapted breeding materials.

Private-sector maize breeding programs have been making substantial investments in the development of genomic and computational tools and now report dramatic increases in the pace and scale of genetic progress in their molecular breeding programs compared to previous conventional approaches. Unfortunately, public-sector breeding programs, particularly those focused on tropical maize, have not been able to make similar advances. Given the substantial estimated increases in demand for maize that will be required to feed the increasing global population, especially in low-income countries, a primary goal of the international community (both public and private) must be to bridge the gap between private-sector temperate maize breeding programs and public-sector tropical maize breeding programs. At CIMMYT we firmly believe that there are win-win synergies to be gained from public-private partnerships in maize breeding. In particular, as described in this article, a huge portion of the largely untapped maize-related biodiversity is harbored in tropical maize landraces and wild species. CIMMYT holds a large proportion of this germplasm for distribution as global public goods. Efficient utilization of this genetic resource will require substantial investments in tropical maize genomics and associated disciplines. Yet the outputs will benefit maize breeders, farmers, and consumers everywhere. However, this will require a mindset change among both the agricultural development donor community and the multinational seed industry.

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