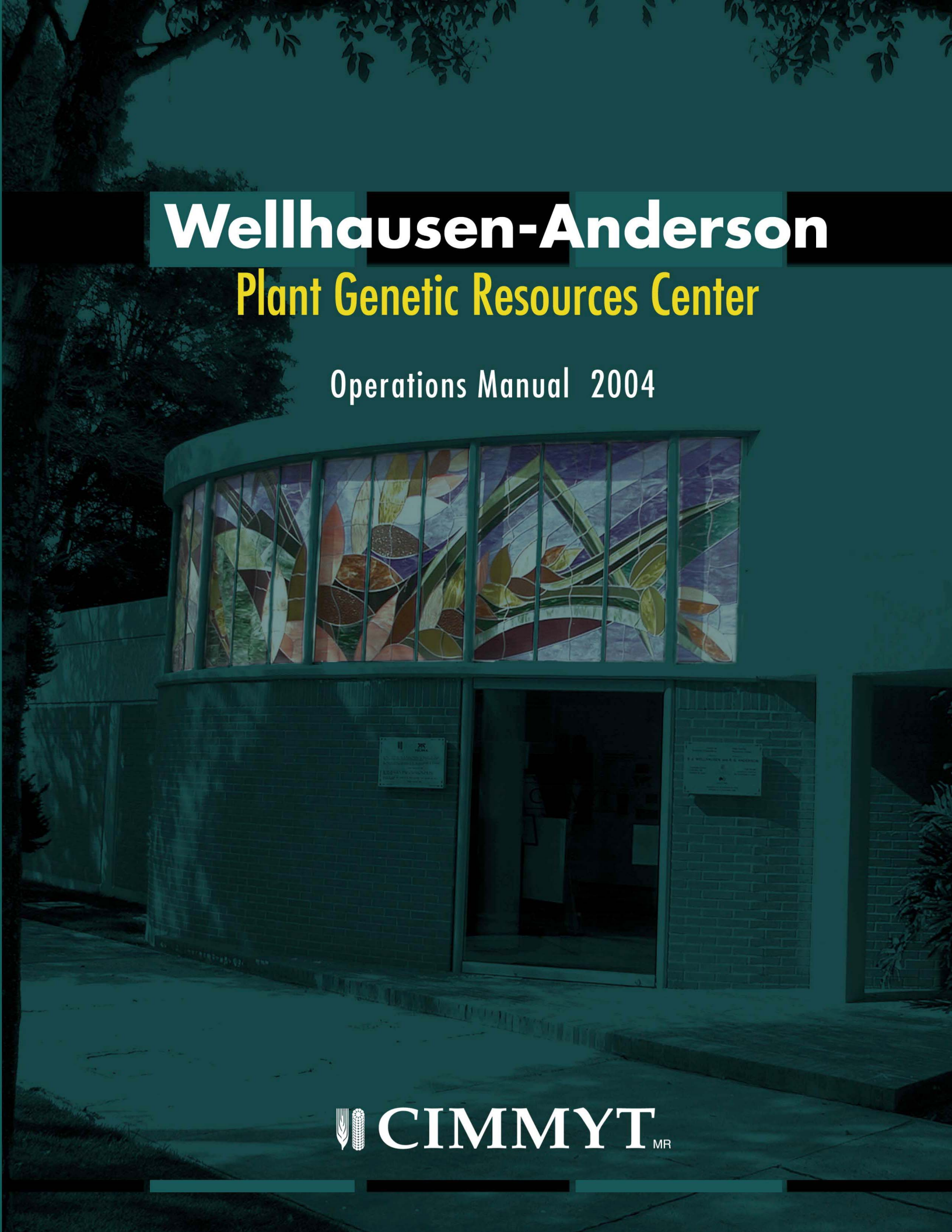

# Wellhausen-Anderson

## Plant Genetic Resources Center

Operations Manual 2004

CIMMYT MR

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# Contents

# Preface

In 2004, CIMMYT restructured its research programs into six new global and ecoregional programs. One of these, the Genetic Resources Program, is now home to the maize and wheat germplasm collections in CIMMYT's gene bank. This new organizational structure indicates the high importance and visibility that CIMMYT places on our role as custodians of maize, wheat, and related species genetic resources.

One of the first priorities of the program was to update the operations manual for the gene bank. The result of this effort is this publication, the *Wellhausen-Anderson Genetic Resources Center Operations Manual*. Many staff contributed to this version that was ultimately assembled and edited by Suketoshi Taba, maize germplasm collection manager, Maarten van Ginkel, wheat germplasm collection manager, David Poland, senior writer/editor, and Dave Hoisington, Genetic Resources Program Director.

The policies and procedures outlined in the manual represent those currently being used in the introduction, evaluation, maintenance, regeneration, and distribution of genetic resources at CIMMYT. By following these procedures, CIMMYT ensures that the genetic resources entrusted to it in its germplasm collections are available to the world and that they maintain their genetic integrity while under CIMMYT's custodianship.

CIMMYT will continue to evaluate and update these policies and procedures. This is especially critical as new legal requirements come into force, such as the International Treaty of Plant Genetic Resources for Food and Agriculture, and other aspects of gene bank management come to the fore, such as requirements for monitoring the presence of transgenes.

# I. Background

## A. Description of Maize and Related Species

### A.1. Importance of maize for global food security and research

According to an ancient Indian legend, maize was "the food of the gods that created the Earth." It is scarcely less important today, as along with wheat and rice, it is one of the world's three most important cereals. Maize originated in southern Mexico and was first domesticated more than 6,000 years ago. Its cultural significance to the region is similar to that of rice to Asia and wheat and barley to the Middle East. Maize exists only as a cultivated crop, because the seeds cannot be separated from the cob without human intervention.

By 2020, the demand for maize in developing countries is projected to surpass the demand for both wheat and rice. This is reflected in a 50% increase in global maize demand from 558 million tons in 1995 to a projected 837 million tons in 2020. In the developing world alone, maize demand will increase from 282 million tons in 1995 to a projected 504 million tons in 2020.

Approximately 140 million hectares of maize is cultivated globally. The main producers are the USA, China, and Brazil, followed by Argentina, South Africa, and the EU. Approximately 96 million hectares are grown in developing countries with four countries (China, Brazil, Mexico, and India) accounting for more than 50% of the total.

Although used primarily for animal feed (78%), mainly for cattle, pigs, and poultry, 13% is used as food for humans, where its applications are diverse. It is eaten, for example, as corn on the cob or polenta, or in processed forms such as oil, starch, sweeteners, and flour. Such is its versatility that its derivatives can also be found in drugs like aspirin and antibiotics, in cosmetics and soaps, and in a broad range of industrial products.

Maize has been a major focus of genetic and biotechnology research for several reasons. The presence of hybrid technology and its commercial importance enables the private sector to capitalize on the sale of hybrid seed and derive benefits from their investments in research and development.

In addition to commercial benefits, maize also offers a number of significant scientific advantages. Classical genetic studies have evolved to the point that the collection of known loci and genetic/cytogenetic stocks are enormous. This, coupled with the ease with which many molecular studies—both genetic and biological—can be accomplished has lead to a wealth of investigations and will ultimately lead to an in-depth understanding of the maize genome. Efforts to develop the tools and techniques for expanded identification of genes and gene functions via modern genomics promise to maintain the position of maize as a lead genetic organism, while providing powerful approaches for enhancing maize productivity.

### A.2. Origin of maize

Maize is believed to have originated in southern Mexico. Many studies have been made on maize evolution and domestication using archaeological evidence, maize landrace diversity, the natural habitats of the close relatives of teosinte and *Tripsacum*, maize culture in Mexico and Guatemala, and molecular genetics and evolution. Maize was domesticated more than 6,000 years ago in Tehuacan, Puebla, Mexico and became the dietary staple about 3,500 years ago. The Tehuacan cob specimens, by new measurement, date back 5,500 years without showing introgression of teosinte characteristics, but with the pistillate spikelets below and the staminate spikelets at the tip of the ear (a bisexual condition). Later Tehuacan specimens indicate that about

3,000 years ago there was an explosive change in cob size. The specimens of Guila Naquitz cave, about 5 km from Mitla, Oaxaca, date back even further, 6,250 years. The cobs of Guila Naquitz cave indicate maize x teosinte hybridization by its indurated rachis.

The emergence of maize remains scientifically controversial, especially the roles played by teosinte and *Tripsacum* in maize evolution and domestication. A recent study of the molecular phylogeny based on the diversity of maize landraces and teosinte using simple sequence repeats (SSR) suggests a single domestication of maize, possibly from the race Balsas teosinte (*Zea mays L. subsp. parviglumis* Iltis and Doebley).

**A.2.1. Origin of teosinte**

Teosinte is believed to have originated in Mexico and Guatemala. The in-situ populations of teosinte are found on the Central Plateau and western escarpment of Mexico/Guatemala, in a seasonally dry, subtropical zone between 500 and 2,200 m with summer rains. Annual teosinte populations are Nobogame, Durango, Central Plateau, Chalco, Balsas, and Oaxaca in Mexico, and Huehuetenango and Guatemala in Guatemala. These populations are classified among the teosinte races by Wilkes (1967, 2004). Perennial teosinte has a tetraploid and a diploid species. *Zea perennis* (4n) is considered an autotetraploid derived from *Zea diploperennis* (2n). *Zea diploperennisis and Zea perennis* are located respectively in the Sierra de Manantlan, and Ciudad Guzman, Jalisco, Mexico. The annual teosinte races Guatemala and Huehuetenango are located in the Departments of Jutiapa, Jalapa, and Chiquimula, and Department of Huehuetenango, northwestern Guatemala. Iltis and Benz (2000) reported a teosinte population (*Zea nicaraguensis* Iltis & Benz) that is differentiated from *Zea luxurians* and grown in Chinandega, Nicaragua. Teosinte can hybridize with maize although some teosinte races show cross incompatibility with normal maize genotypes.

**A.2.2. Origin of *Tripsacum***

Mexico and Guatemala are the center of diversity of *Tripsacum*. *Tripsacum* species are widely distributed in the Americas and are numerous in Mexico. The accumulated information on maize-*Tripsacum* hybrids and their derivatives indicate that the respective genetic architecture of maize and *Tripsacum*, although different, are more similar than their karyotypes would suggest. Maize and *Tripsacum* diverged long before domestication of maize. Many genes have homologous counterparts, but the blocks of linked maize genes are spread over many chromosomes.

**Table1. CIMMYT gene bank conserves samples of teosinte races from Mexico, Guatemala, and Nicaragua**

| Formal species and subspecies | Evolutionary groupings *in Zea* and race name | Country |
|---|---|---|
| Section: Euchlaena (Schrader) Kuntze in *Von Post and Kuntze Lexicon*, 599 (1904) | | |
| *Zea mays subsp. mexicana* (Schrader) Iltis | *Zea mexicana* (Schrader) Kuntze Race Nobogame | Mexico |
| *Zea mays subsp. mexicana* (Schrader) Iltis | *Zea mexicana* (Schrader) Kuntze Race Central Plateau | Mexico |
| *Zea mays subsp. mexicana* (Schrader) Iltis | *Zea mexicana* (Schrader) Kuntze Race Chalco | Mexico |
| *Zea mays subsp. parviglumis* Iltis and Doebly | *Zea mexicana* (Schrader) Kuntze Race Balsas | Mexico |
| *Zea Mays subsp. huehuetenangsis* Doebly | *Zea mexicana* (Schrader) Kuntze Race Huehuetenango | Guatemala |
| Section: Luxuriantes (Durieu) *Bull Soc. Acclimat.* **19**:581 (1872) | | |
| *Zea luxurians* (Durieu and Ascherson) Bird | *Zea luxurians* (Durieu and Ascherson) Bird | Guatemala |
| *Zea perennis* (Hitchcock) Reeves and Mangelsdorf | *Zea perennis* (Hitchcock) Reeves & Mangelsdorf | Mexico |
| *Zea diploperennis* Iltis, Doebley and Guzmán | *Zea diploperennis* Iltis, Doebley & Guzman | Mexico |
| *Zea nicaraguensis* Iltis and Benz. | | Nicaragua |

Reference sources: Wilkes (1967, 2004), Iltis (1972), Bird (1978), Doebley (1980, 1990), Doebley and Iltis (1980), Iltis and Doebley (1980), Sánchez and Ordaz (1987), and Iltis and Benz (2000).

All taxa of *Tripsacum* are perenial. Diploid (2n=36) plants are sexual, while higher ploidy levels (triploids, tetraploids, and pentaploids) are generally apomictic, with fleshy rhizomes. *T. zopilotense*, the small, narrow-leaved, xeric-adapted species from the Canon del Zopilote in Guerrro, Mexico lacks rhizomes. Plants can be recovered from *Zea* x *Tripsacum* hybrids through backcrossing, suggesting a possible gene flow between the two genera.

*Tripsacum andersonii*, guatemala grass, has 64 chromosomes. It was discovered in Guatemala and spread to South America to feed guinea pigs. Results of molecular marker experiments at CIMMYT indicate that *T. andersonii* was formed by two hybridization events: first between *T. latifolium* (2x) x *T. maizar* (2x) that formed *T. latifolium* (3x=54 chromosomes) and the subsequent second hybridization between *T. latifolium* (3x=54 chr) x *Zea luxurians* (2n=20) led to the formation of *T. andersoni.*

## A.3. Centers of diversity

Domestication and evolution of maize in southern Mexico and Guatemala (Mesoamerica) and the spread of maize to North and South America as a staple food crop generated large landrace diversity first encountered when collections began in earnest during the last 60 years. Goodman (1978), in considering maize in the Americas, distinguished six large racial complexes of economic importance: the Mexican dents, the Corn Belt Dents, the Tusons (Caribbean dent), the Caribbean flints, the Northern Flints and Flours, and the Cateto or

**Table 2. CIMMYT field germplasm bank conserves clones of *Tripsacum* from Mexico, Central America, South America, and USA**

| Species | ID | No. of Chromosomes | Ploidy level | Reproduction | Country |
|---|---|---|---|---|---|
| *Tripsacum australe* var australe | TAA | 36 | Diploid | Sexual | Peru, Colombia |
| | | 72 | Tetraploid | Apomictic | |
| *Tripsacum andersonii* | TAD | 54 | Hybrid | Sexual | Venezuela, Colombia, Peru, Brazil, Mexico, Honduras, Belize |
| *Tripsacum bravum* (Gray) | TBV | 72 | Tetraploid | Apomictic | Mexico |
| | 1 | | Hexaploid | Apomictic | |
| *Tripsacum cundinamarca* | TCD | 36 | Diploid | Sexual | Colombia |
| *Tripsacum dactiloides* (L.) L. | TDD | 72 | Tetraploid | Apomictic | USA |
| *Tripsacum dactiloides* var. hispidum(H) | TDH | 72 | Tetraploid | Apomictic | Mexico |
| *Tripsacum dactiloides* var. mexicana | TDM | 72 | Tetraploid | Apomictic | Mexico |
| *Tripsacum intermedium* | TIT | 72 | Tetraploid | Apomictic | Mexico, Honduras |
| | | 90 | Pentaploid | Apomictic | |
| *Tripsacum jalapense* | TJL | 72 | Tetraploid | Apomictic | Mexico |
| *Tripsacum lanceolatum* | TLC | 72 | Tetraploid | Apomictic | Mexico, USA |
| *Tripsacum latifolium* (Hitchc) | TLT | 54 | Triploid | Apomictic | Mexico |
| | | 72 | Tetraploid | Apomictic | |
| *Tripsacum dactiloides* var. meridionale | TMR | 54 | Triploid | Apomictic | Colombia |
| | | 14 | Diploid | Sexual | Venezuela |
| *Tripsacum maizar* (Hern. and Rand.) | TMZ | 72 | Tetraploid | Apomictic | Mexico |
| | | 3 | Triploid | Apomictic | |
| *Tripsacum pilosum* (Scribner and M) | TPL | 36 | Diploid | Sexual | Mexico, Belize |
| | | 4 | Tetraploid | Apomictic | |
| | | 2 | Triploid | Apomictic | |
| *Tripsacum peruvianum* | TPR | 90 | Pentaploid | Apomictic | Peru, Venezuela, Ecuador |
| *Tripsacum zopilotensis* (Herna. And R.) | TZP | 36 | Diploid | Sexual | Mexico |
| No name | | 72 | Tetraploid | Sexual | |

Argentine Flints. In addition, the Andean Complex and Amazonian Coroico types are the other groups of maize races known to contain local racial diversity. Races of maize and their interrelationships are further described by Goodman and Brown (1988).

CIMMYT preserves maize germplasm accessions from 64 countries (19 in Latin America, 19 in the Caribbean, 11 in Africa, 10 in Asia, 3 in Europe, and 2 in Oceania). The CIMMYT germplasm bank preserves 329 classes of landraces, some of them are identified with race classifications and others by local common names.

## B. Description of Wheat and Related Species

### B.1. Importance of wheat for global food security and research

Of the major global cereal staples, wheat is the most widely grown, ranging from sea-level to 4,000 masl, and from the equator to Norway in the north, and to southern Chile in the south.

Unlike maize, more than 90% of wheat is directly consumed by humans, with little used for livestock feed or other purposes. Scarcely a person on planet does not eat a wheat product at least once a week, with some consuming wheat three times a day, providing half, or more, of all calories consumed. Wheat is a major staple and calorie source for more than half of the world population, and is expected to remain so in the medium to long term. About 90% of the wheat produced is common wheat or 'bread' wheat (*triticum aestivum*), used for diverse leavened breads (e.g., pan-type, steamed) and flat breads (e.g., chapattis, Arabic flat bread, tortillas), noodles, biscuits (cookies), and other baked products. The remaining 10% is durum wheat (*T. durum or T. turgidum*), which is consumed as semolina (coarse grits), pasta, couscous, bulgur, and local flat breads. One of the fastest growing product categories for common wheat is instant flour noodles in East and Southeast Asia. Alternate uses such as for starch and glutens are expected to increase.

Projections for 2020 are that wheat area, currently about 210 million hectares, will not increase but may actually decrease somewhat as yields continue to rise. Of this total, about half the area is located in developing countries. This change in area planted can be attributed to a portion of developing country farmers moving into crops with a greater rate of financial return or leaving the farm to find gainful employment in higher paying jobs. China, today, is a significant case in point.

Presently global production is 560 million tons annually, almost half being produced in developing countries. Average yield, now at 2.7 t/ha, will need to increase by at least 2% annually to meet higher demand. Both agronomic and genetic improvements are expected to continue to play key and equal roles in meeting this challenge.

The major wheat producers are China, Europe taken as a whole, India, USA, Australia, and Canada. Among developing countries, China, India, and Pakistan plant 60% of the wheat area. The major exporters of wheat are the USA, European nations, Australia, and Canada, with Kazakhstan also emerging as a potentially major future exporter. Many small developing countries usually import wheat. Depending on trends towards agricultural and income diversification, some larger producers, such as China, may return to requiring importation of considerable amounts of wheat. Part of this can be traced to an economic paradigm shift from the older aim of 'self-sufficiency to the more recently annunciated aim of 'self-reliance' in this age of globalization.

Wheat, despite its genetic complexity, presents considerable potential for the use of molecular markers in the breeding process, with some programs already using such markers routinely, including CIMMYT's breeding program. In wheat, molecular relationships tend to hold better across diverse populations and this consistency increases their reliability and wider application. Work on transgenic wheat has been limited globally, with no known transgenic wheat being commercially released and grown.

Due to its polyploid nature, wheats can survive the absence, addition, or substitution of entire chromosomes and translocations of parts of chromosomes. This has allowed the development of unique sets of genetic stocks since the middle of the 20th century. These genetic stocks have allowed the location and manipulation of genes responsible for minor and major traits through the use of biotechnological and cytogenetic tools. With recent crosses involving modern commercial durum wheats (tetraploid) and *Aegilops tauschii* (diploid), common wheat (hexaploid) can be reconstructed into what are essentially 'remixes' of wheat, which are known under various names. These remix wheats have greatly expanded potential diversity in wheat.

Genetic diversity is critical to enhancing and stabilizing yield potential as well as sustaining that potential through new sources of resistances and tolerances to biotic and abiotic stresses. Thus, genetic resources are fundamental to sustaining wheat production in the future.

Wheat cultivars up to the 1950s were an assembly of gene combinations pyramided over the last century by breeders using, in most cases, well-adapted cultivars from within their own region with rare imports from outside (e.g., introduction in the early 1900s of Japanese semi-dwarf wheat into Strampelli's program in Italy; 1920s the introduction of Strampelli's wheats into Argentina; introduction of two dwarfing genes originating from the Japanese cultivar NORIN 10 into the Mexican Office of Special Studies program led by Norman E. Borlaug in the early 1950s, via cross progeny made by Vergil Vogel at the Washington State University in Pullman, Washington, USA).

The advance of international agriculture since the mid 20th century enormously expanded the global availability of germplasm from more diverse sources, thus significantly changing patterns of cross hybridization and cultivar release and distribution. The Green Revolution showed how use of introduced germplasm either directly or in crosses could provide significant increases in genetic diversity expressed in yield and disease resistance. By the early 1970s a rapid release of cultivars to farmers in particular in developing countries occurred that were derived directly or indirectly from external breeding programs. This spread of new cultivars was associated with an equally rapid replacement of the local cultivars, which resulted in a large and concerted effort to collect remaining local germplasm.

Gene bank *modus operandi* sufficed until the recent past to 'collect and cool' such latent diversity. Now that, especially in the case of wheat, the collection of most important local landraces has apparently been achieved, modern ways need to be explored to identify and unlock the genetic diversity held in these sub-zero genebank storage rooms and in in situ collections. In particular where the number of accessions held is high, such as in the CIMMYT wheat collection, biotechnological tools in concert with GIS approaches will be needed to locate the rare gems within the collections.

CIMMYT's major objectives include increasing farm-level productivity while safe-guarding against genetic vulnerability, and through this, enhancing livehoods of the resource poor. The preservation, documentation, evaluation, enhancement, and easy accessibility of genetic resources are central to those ends.

## B.2. Origin of wheat

Genetic analyses prove beyond a doubt that wheat is a very young crop with paradoxically, one of the broadest and one of the narrowest of foundations. Still it is the most widely grown crops on the Earth. About 10-12,000 years ago, two diploid grasses (one each from the *Sitopsis* group and the *Triticum uratu* species) crossed and formed two of the three legs of modern wheat. Such crosses between distinct grasses are very rare and tend to lead to infertile progeny. The two parents of the resulting tetraploid wheat are from somewhat related species but not overly close. The progeny is generally sterile. Occasionally and spontaneously chromosomes in young progeny from a cross between these two grasses double thus leading to a fertile descendant (with twice the chromosome number of either of its parents: 2x14 = 28 a tetraploid), which can produce fertile offspring of its own. Years pass and fortunately a fertile progeny of yet another cross between two grass individuals from within each of the *Sitopsis* genus and the *Triticum uratu* species results in fertile progeny (known as *Triticum dicoccoides*). Thus early wheat was introduced to domestic life and itself domesticated.

Three problems were directly noted by early humans: the seeds of the spike separated even before the plant fully matured and fell straight to the ground, a prey for birds and rodents. The seed was also fused to the outer leaves (a.k.a. glumes, not unlike in the case of barley) and was difficult to separate from the glumes during threshing. Finally, while a sufficient number of seeds were planted the following year to produce a good stand of plants, only a proportion actually germinated and formed plants, which for wild grasses is a good risk avoidance strategy, but is undesirable in cultivated crops.

Domestication is essentially humans looking out for their favored plants and dealing with such threats as dry conditions (e.g., by building irrigation works in lower Egypt), competition by other grasses (i.e., through weeding), or threshing in the safety of a compound. Within one thousand years, according to some estimates, minor deviations or mutations of the spikes within the young wheat crop were noted where the seeds did not separate on their own (i.e., non-brittle rachis), seeds that did not adhere to the glumes (i.e., free-threshing), and seeds that germinated pretty much all at the same time (i.e., uniform dormancy). Thus the young wheat was transformed during domestication to essentially a distinct species known as *Triticum dicoccum* or emmer wheat.

The young emmer wheat was popular and spread from its ancestral region, the northern Fertile Crescent, to the southwest and east. Over time it evolved to what we now know as *Triticum durum* or *Triticum turgidum*, our modern durum wheat used to make pasta products. About 8,000 years ago, in what is now Iran, a third grass known in some areas as goat grass (*Aegilops tauschii*) crossed with the young *Triticum dicoccum* crop to produce the fertile progeny now known as *Triticum aestivum*, or common bread wheat. Domestication continued and through the appearance of, for example, spelt wheats, modern bread wheat emerged alongside durum wheat. Wheat then has a broad base in that three distinct grasses intercrossed to form its early progenitors, though in some terms also a narrow base in that modern wheats likely trace back to just very few individual plants that contributed their genes within each of these three grasses.

## B.3. Centers of diversity

Wheat originated in various steps in the Fertile Crescent, as described in the section on 'Origin.' From there it move rapidly south, though initially not far beyond Ethiopia, at which point its spread southward slowed considerably (wheat was not introduced into South Africa until the mid 17$^{th}$ century.); east (to India and China) and west/northwest (to the Mediterranean region and Europe). By 4000-2000 B.C., these regions had acquired wheat introductions, represented by various diploid, tetraploid and hexaploid forms. Only in the early and mid 16$^{th}$ century was wheat introduced to the Americas, and in the late 18$^{th}$ century to Australia.

Some examples of centers of diversity of early wheat relatives are

- *T. monococcum,* still grown for animal feed in the mountainous regions of Turkey, Yugoslavia, southern Italy and Daghestan (Central Asia);
- *T. dicoccoides* and various other early tetraploids, found in Ethiopia, India, Iran, Transcaucasia, the Mediterranean basin, eastern Turkey, and the Balkans;
- *Ae. tauschi,* grown in the Caucasus, Trancaucasia, Central Asia, Afghanistan, China (Himalaya), India (Kashmir), Pakistan, Iran, Iraq, and eastern Turkey; and
- *T. aestivum* early relatives such as *T. aestivum* ssp. *sphaerococcum* and others, still grown in parts of India, Pakistan, Afghanistan (Hindu-Kush region) and Trancaucasia.

**Table 3. Domesticated and cultivated wheats and their relatives held in the CIMMYT wheat collection. Notation modified from Kimber and Sears (1987), Feldman (2001) and Gill and Friebe (2002)**

| (Sub)species | Common name | Genome |
|---|---|---|
| *Triticum monococcum*s pp | | |
| *spp. aegilopoidess* pp | Wild einkorn | $A^M$ |
| spp. *monococcum* | Cultivated einkorn or small spelt | $A^M$ |
| *T. urartu* | - (wild form) | A |
| *T. boeoticum* | - (wild form) | A |
| *T. timopheevii* | Timopheevii | $A^TG$ |
| *T. turgidum*spp | | |
| *spp. dicoccoides* | Wild emmer | AB |
| spp. *dicoccon* (syn. *dicoccum*) | Cultivated emmer | AB |
| spp. *durum* | Macaroni or hard wheat | AB |
| spp. *polonicum* | Polish wheat | AB |
| spp. *carthlicum* | Persian wheat | AB |
| *T. aestivum* | | |
| spp. *spelta* | Dinkel or large spelt | ABD |
| spp. *aestivum* | Common or bread wheat | ABD |
| spp. *compactum* | Club wheat | ABD |
| spp. *sphaerococcum* | Indian dwarf or shot wheat | ABD |

**Table 4. *Aegilops* species held in the CIMMYT wheat collection. Notation modified from Kimber and Sears (1987), Feldman (2001) and Gill and Friebe (2002)**

| (Sub)species | Genome |
|---|---|
| *Aegilops bicornis* | $S^b$ |
| *Ae. biuncialis* | $\underline{U}\underline{M}$ ($UM^o$) |
| *Ae. caudata* | C |
| *Ae. columnaris* | $\underline{U}\underline{M}$ ($UX^{co}$) |
| *Ae. comosa* | M |
| *Ae. crassa* | $\underline{D^{c1}}\underline{M^c}$ ($D^{c1}X^c$) |
| *Ae. cylindrica* | $D^cC^c$ |
| *Ae. geniculata* | $\underline{U}\underline{M}$ ($UM^o$) |
| *Ae. heldreichii* | - |
| *Ae. juvenalis* | $\underline{DMU}$ ($D^cX^cU^j$) |
| *Ae. kotschyi* | $\underline{U}\underline{S}$ ($US^1$) |
| *Ae. ligustica* | - |
| *Ae. longissima* | $S^1$ |
| *Ae. markgrafii* | - |
| *Ae. meyeri* | - |
| *Ae. mutica* | T |
| *Ae. neglecta* | $\underline{U}\underline{M}$ ($UX^n$) |
| *Ae. ovata* | - |
| *Ae. peregrina* | $\underline{U}\underline{S}$ ($US^1$) |
| *Ae. persica* | - |
| *Ae. seansu* | - |
| *Ae. searsii* | $S^s$ |
| *Ae. sharonensis* | $S^{sh}$ |
| *Ae. speltoides* | S |
| *Ae. squarrosa* (syn. *tauschii)* | D |
| *Ae. strangulata* | - |
| *Ae. triaristata* | - |
| *Ae. triuncialis* | $\underline{U}\underline{C}^t$ |
| *Ae. typica* | - |
| *Ae. umbellulata* | U |
| *Ae. uniaristata* | N |
| *Ae. variabilis* | - |
| *Ae. ventricosa* | $D^vN^v$ |

Underlined genomes are modified at the polyploidy level. Those in brackets were deduced from DNA analysis.

# C. History of CIMMYT's Gene Bank

## C.1. Maize

The current holdings of CIMMYT's maize germplasm collection can be traced to samples of landraces collected and regenerated from 1943 to 1959 by the Office of Special Studies, a research unit operated jointly by the Rockefeller Foundation and the Mexican Ministry of Agriculture. Collections were made initially to assemble the raw material for breeding improved maize in Mexico. Later, these were broadened to include some 2,000 farmer landraces as an endowment to humanity and to guard against the day they would be replaced by improved maize. Soon thereafter, more than 11,000 samples were obtained throughout the Americas under a project supported by the U.S. National Academy of Science-National Research Council. These were stored in regional germplasm banks in Brazil, Columbia, Mexico, Peru and the U.S. Regional Plant Introduction Station.

Two research initiatives were started following the closing of the Office of Special Studies in 1959: the National Institute of Agricultural Research (INIA) formed by Mexico, and the Inter-American Maize and Wheat Programs launched by the Rockefeller Foundation to extend the accomplishments of the Office of Special Studies beyond the borders of Mexico. Under this new arrangement, the maize collections gathered previously entered the patrimony of INIA.

The Inter-American Maize Program assisted INIA in regenerating the newly received collections. In doing so, the program retained an extra set of the renewed seed, storing it in a refrigerated facility in Chapingo, Mexico. This material, augmented by more than 600 new entries from collecting missions in the Caribbean, Mexico, and Central America, formed the inaugural holdings of the CIMMYT maize germplasm collection.

After CIMMYT was established in 1966, several other sets of maize collections were added: backup samples from the Andean and Central American regions held temporarily at the U.S. National Seed Storage Laboratory in Fort Collins, Colorado, USA; collections originally stored in the Brazilian gene bank; collections from CIMMYT missions in the Andean region in the late 1960s; and part of the collections from Brazil and Uruguay collected with support from the International Board of Plant Genetic Resources (IBPGR).

Following the completion of CIMMYT's facilities in El Batán, Mexico in 1971, including suitable facilities for long-term seed storage, all samples in the Chapingo gene bank were transferred to CIMMYT headquarters. Dr. Mario Gutiérrez, head of the CIMMYT maize gene bank from 1976 to 1996, supervised the transfer and initial organization at El Batán. By 1973, CIMMYT had sent some 470 shipments of nearly 15,000 samples to researchers in 80 countries. More than 8,000 accessions had been regenerated and basic information recorded on them.

Additional collections in Latin America and parts of Africa, Asia, and Europe were made in 1974 with sponsorship by the IBPGR, and some 14,500 samples were obtained. After Dr. Gutiérrez left CIMMYT in 1976, a maize scientist was assigned part-time responsibility for the gene bank. The limited remaining staff regenerated nearly 1,000 accessions, and met the seed requests to the bank.

In 1984, following criticism regarding CIMMYT's management of the genetic resources under its care, a –15˚C cold storage room was built, doubling the life of seed placed there. Each gene bank accession was subsequently divided, one portion earmarked for long-term storage as a "base collection" in the new facility, and the remainder was kept in the intermediate storage or "active" chamber. This dual system continues today.

In 1986, Dr. Suketoshi Taba was appointed fulltime curator of the maize germplasm collection. Dr. Garrison Wilkes was brought in to help assess the condition of the gene bank and to organize the wealth of handwritten information on the accessions into a computer database. Later the same year, CIMMYT's Board of Trustees endorsed a proposal that CIMMYT should continue to collect, conserve, document, and evaluate specific parts of the global collection of maize germplasm. Discussions with IBPGR led to CIMMYT's acceptance of responsibility for maintaining a base collection of landraces of maize native to the Western Hemisphere.

### C.2. Wheat

From its inception in 1966 to 1981, CIMMYT operated a relatively small germplasm cold storage facility for conserving small amounts of certain wheat genetic materials, especially those used in or resulting from its own wheat improvement programs. The first actual gene bank of limited capacity became operational in 1981. The first head of the wheat collection was Ayla Sencer, taking up her position in 1982. In 1989 she was succeeded by Bent Skovmand, who led the management of the CIMMYT wheat collection until 2003, widely expanding the number of accessions held in the gene bank. In 2003 Maarten van Ginkel took over the leadership of the wheat collection, with the specific objective of increasing the utilization of the many collected genetic resources.

### C.3. Wellhausen-Anderson Plant Genetic Resources Center

In September 1996, CIMMYT inaugurated the Wellhausen-Anderson Plant Genetic Resources Center, built to replace the outdated 25-year-old gene bank and seed distribution facility. Funded in part by the Japanese Government, the state-of-the-art facility was named in honor of two visionaries in the arena of recognizing and applying the power of crop genetic resources. Edwin J. Wellhausen, as a staff member of the Office of Special Studies in the 1940-50s, coordinated and participated in the systematic collection and preservation of native Mesoamerican maize germplasm. He later served as CIMMYT's first director general. Glen Anderson was a talented wheat scientist, teacher, research administrator, and especially an inspiring leader who helped spark the Green Revolution.

## D. International Laws/Agreements that Affect Genetic Resources

There are a number of international and national laws that address issues related to plant genetic resources for food and agriculture (PGRFA). A detailed description of these can be found in *International Law of Relevance to Plant Genetics Resources: A Practical Review for Scientists and Other Professionals Working with Plant Genetic Resources* (S. Brandon, editor, Issues in Genetic Resources No. 10, March 2004, International Plant Genetic Resources Institute, Rome, Italy). Treaties and conventions of major relevance to the operations of CIMMYT's gene bank are outlined briefly below.

- ***International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA)*** was adopted by the FAO Conference in 2001. It came into force in June 2004 following ratification by the 40th country. It is legally binding for all countries that ratify. Countries that ratify are required to bring national laws and regulations into conformity with the Treaty. CGIAR Centers, including CIMMYT, will likely sign agreements with the Treaty's Governing Body in order to adhere to the ITPGRFA. The ITPGRFA covers all PGRFA and addresses conservation, use, international cooperation, technical assistance and farmers' rights. It established a multilateral system for select crops. (Details at www.fao.org/ag/cgrfa/itpgr.htm). The following crops, managed in CIMMYT's gene bank, are included:

| | |
|---|---|
| Barley | *Hordeum* |
| Triticale | *Triticosecale* |
| Wheat | *Triticum* et al., including *Agropyron*, *Elymus* and *Secale* |
| Maize | *Zea*, excluding *Zea perennis*, *Zea diploperennis* and *Zea luxurians* |
| Tripsacum | *Tripsacum luxum* |

- ***Convention of Biological Diversity (CBD)*** is legally binding for all countries that have ratified (168 as of October 2004). All ratifying countries must adopt appropriate legislation and regulations and/or bring those existing into harmony with the Convention. It covers all biodiversity and provides general principles for access and benefit-sharing of materials accessed after the coming into force of the CBD and not covered by the ITPGRFA (i.e., non-multilateral and non-CGIAR PGRFA). (Details at www.biodiv.org)

- ***International Plant Protection Convention*** is a legally binding document for the 113 countries that are party to the Convention. It addresses phytosanitary issues involving the transfer of plants and animals, including PGRFA. (Details at www.ippc.int)

- ***FAO Global Plan of Action*** was adopted in 1996 by the 4th International Technical Conference on PGRFA involving 150 countries. It is legally non-binding like the International Undertaking (description follows) and serves as a framework, guide and catalyst for PGRFA. It addresses all PGRFA and contains specific references to in situ and ex situ conservation, institutions, and capacity building. It is referenced in the ITPGRFA. (Details at www.fao.org/ag/AGP/AGPS/GpaEN/gpatoc.htm)

- ***FAO-CGIAR Agreements*** were signed in 1994 by the then 11 CGIAR Centers, including CIMMYT, that have ex situ collections. The agreements apply to the management, availability and transfer of designated germplasm in the Centers' gene banks. All such "in-trust" accessions are distributed under a common, agreed Material Transfer Agreement (MTA). These agreements were foreseen as interim pending the ratification of the ITPGRFA.

- ***International Undertaking on Plant Genetic Resources (IU)*** was adopted by the FAO in 1983 and 113 countries agreed to adhere to the IU. All provisions are voluntary because the IU is a non-binding agreement. The IU covers all PGRFA and addresses the exploration, preservation, evaluation, and dissemination of PGRFA. The FAO-CGIAR "in trust" agreements refer to the IU. The IU has been renegotiated resulting in the ITPGRFA and thus has been superceded by this treaty. (Details at www.fao.org/ag/cgrfa/IU.htm)

# II. Germplasm Bank Operations

## A. Physical Infrastructure

The Wellhausen-Anderson Genetic Resources Center (GRC) was inaugurated in September 1996. This state-of-the-art complex houses CIMMYT's operations in maize and wheat international nurseries and germplasm collections. The gene bank is a two-floor structure constructed using reinforced concrete walls. On the main floor is a chamber maintained at –3˚C and 25-30% relative humidity (RH) that contains the "active" maize and wheat collections. Seed here has an average shelf life of approximately 30 years for maize, and 30-50 years for wheat.

On the lower level is an equivalent chamber maintained at –18˚C. Similar rows of movable shelving are used to store the base and black-box collections. Seed stored in this chamber has a shelf life of approximately 60 years for maize and for wheat. Access to the storage chambers is through a multi-locked entry system. Entry into the hallway leading to the main germplasm bank entrance is through a glass door that is opened via an electronic key card. Only authorized personnel have such a key card.

Access to the gene bank anti-chamber is through a steel and aluminum door with a numeric coded lock. Again, only authorized personnel have the code necessary to enter the bank. Inside the anti-chamber are stairs and a freight elevator leading to the lower floor and storage room. Access into each storage chamber is via a sliding steel and aluminum, thermal insulated door, again with a numeric coded lock.

Temperature and RH is monitored via remote sensing devices in several locations in both chambers. Germplasm bank staff monitor these daily for any fluctuations. Alarms are installed to indicate when either chamber deviates from the set point. A diesel generator provides 24/7 automatic dedicated backup power to the gene bank lighting, air conditioning, and access locks during power outages.

The GRC complex also houses areas for seed preparation, short-term storage, seed drying, and germination testing. For the growth of plants for either observation and/or regeneration, the GRC has access to greenhouses, net-houses, and field space in CIMMYT's El Batan station. Additional field space is available at other CIMMYT research stations in Mexico: Cd. Obregon (wheat), Toluca (wheat), Aqua Fria (maize) and Tlaltizapán (maize). Each of these sites provides appropriate climatic conditions for the regeneration of most maize and wheat accessions held in the gene bank. Certain varieties, especially of maize, require regeneration outside Mexico and these are done in collaboration with national programs in the appropriate country (usually close to the point of original collection).

## B. New Introductions/Accessions to the Maize and Wheat Collections

Introducing new materials into the gene bank is perhaps the most critical step in bank operations. Following are the guiding principles for new introductions into the maize and wheat germplasm collections.

### B.1. Maize: guiding principles for new introductions

**B.1.1. The CIMMYT maize collection holds representative Latin American maize landrace diversity as a core of maize genetic resources in collaboration with the NARS gene banks.**

**B.1.2. CIMMYT will continue to cooperate with the national partners in Latin American and in other regions of the world to collect, regenerate, and preserve landrace diversity.**

**B.1.3. Several systematic collection missions for maize germplasm in the Western Hemisphere were conducted during the past 60 years. CIMMYT will continue to conserve these samples as they represent a major and valuable part of original maize diversity.**

**B.1.4. There is need for collection of intra-racial diversity of locally important maize races for conserving them ex situ and in situ in parts of Mexico, Central America, and the Andean and Amazonic regions of the South America, and in other continents.**

**B.1.5. There is a need to identify and conserve diversity in landraces from Africa and Asia because they are likely to contain unique tropical, subtropical, and highland germplasm adapted to unique climatic and edaphic conditions.**

**B.1.6. There is a continuing need to conserve enhanced germplasm, germplasm with useful traits, obsolete but unique varieties, and lines used in genetic and genomic research.**

## B.2. Wheat: guiding principles for new introductions

**B.2.1. Through the introduction of new wheat accessions, the gene bank works to produce representative samples of diverse alleles in the three wheat genomes, that are available for long-term storage and distribution, as feasible.**

**B.2.2. Undertake collection expeditions where previously no collections have taken place or where additional genetic diversity of interest is expected to reside based on GIS or other information regarding prevailing climate and soil conditions.**

**B.2.3. Acquire critical germplasm, such as cultivars from around the world released by breeders in the 20th century that are now obsolete.**

**B.2.4. Maintain collections of selected germplasm representative of all significant germplasm pools.**

**B.2.5. The type of wheat genotypes included in the wheat collection include:**

- Wheat's diploid and tetraploid undomesticated ancestor species
- Primitive domesticated wheats
- Landraces
- Commercial wheat cultivars from around the world
- Advanced lines bred by CIMMYT breeders over the course of its history
- Genetic stocks (e.g., cytogenetic stocks, deletions, point mutations, –mono- and polysomic series, translocations, mapping populations)
- Miscellaneous stocks of rye and other related grasses
- DNA of selected entries
- Barley germplasm is stored only as a working collection for the joint ICARDA-CIMMYT Barley Program.

## B.3. Introduction of new materials

**B.3.1. Sample registration**

New accessions are introduced into the gene bank with passport documentation after clearing quarantine regulations of Mexico as necessary. Upon receipt, a gene bank identification or accession ID (e.g., CIMMYTMA-000001 for maize, or codes starting with CWI/BW/DW followed by sequential numbers for wheat species and landraces, bread wheat advanced lines, or durum wheat advanced lines, respectively) is assigned to the new introductions/accessions. Passport data are registered into the database for each new accession. Throughout its existence in the gene bank and when shared with others the accession ID links the accession to the gene bank's central database. The ID number is also associated with passport, characterization and/or evaluation data, which can be stored, searched, and retrieved.

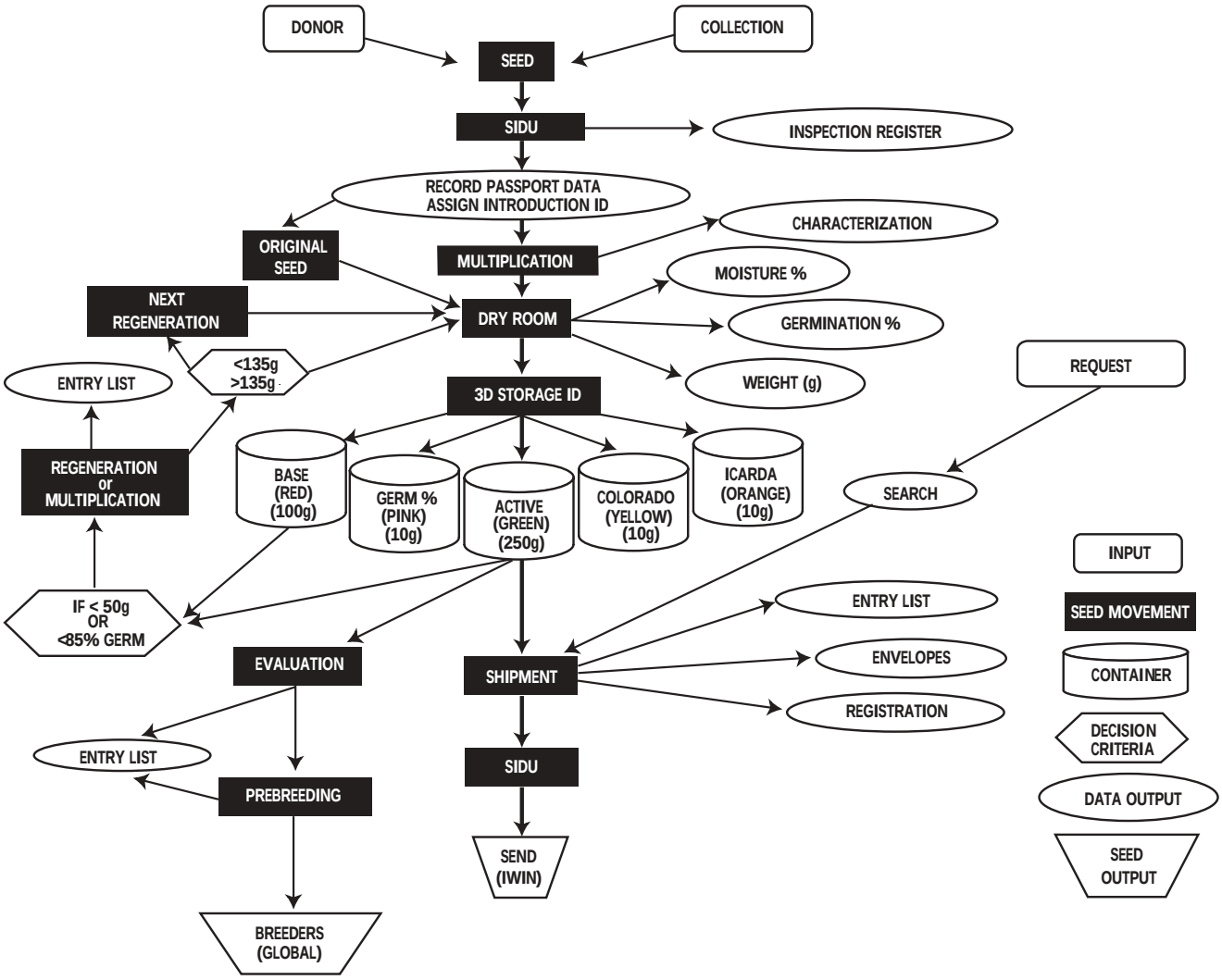


Figure 1. Gene bank operation (wheat). **Small samples of seed are received and enter the germplasm bank (top of the flowchart). It is then checked for seed health by the Seed Inspection & Distribution Unit (SIDU), its passport data is registered in the database and it is assigned an accession ID. The seed is then multiplied to have sufficient seed to store and satisfy outside requests. The seed is dried to a low moisture level to increase its longevity, and five sub-samples are assigned 3D storage IDs that record the physical location of the seed in the gene bank. The five sub-samples are stored (i) in the active collection to satisfy client requests, (ii) in the very long-term storage area of the base collection; (iii) maintained for later germination tests, (iv) shipped as back-up seed to the National Center for Genetic Resources Preservation (NCGRP) in Fort Collins, Colorado, USA, and (v) shipped as back-up seed to ICARDA in Syria. After seed requests arrive and are documented, desired seed is taken from the active collection and processed for shipment. Finally the seed is sent to the requesting collaborator through the International Wheat Information Network (IWIN). Evaluation for specific traits and pre-breeding activities enhance the usefulness of the products that we make available to breeders worldwide. Regeneration takes place as seed quantity or germination percentage drop below set limits. At all levels data is generated and stored in a central database.**

**B.3.2. Seed health procedures**

All new maize and wheat germplasm (landrace collections, breeder lines, breeder populations, gene pools, genetic materials, and related species) are sent to the CIMMYT Seed Inspection and Distribution Unit (SIDU), where they are inspected following Mexican quarantine regulations in the seed laboratory and greenhouse. The SIDU works under its own operational procedures (Mezzalama et al., 2001).

**B.3.3. Seed cleaning and seed drying**

After SIDU's inspection and clearance, seed is cleaned and dried in a drying room at $10^0$C and 25% RH to

- a seed moisture of 6-8% for maize
- a seed moisture of 5-7% for wheat

in equilibrium with the drying conditions. This normally takes 6-8 weeks.

**B.3.4. Seed moisture content, seed viability, and reference seed samples**

After seed drying, seed moisture content is measured to determine if it has reached the required reduced-moisture level. Subsequently, initial seed viability is tested. Initial seed germination must be more than 90%. Information on the moisture content, germination ability and seed weight, are registered in the central database. The amount of the seed available is measured before seed packaging and storage. For potential future identification purposes, a sample of 50-200 seeds from the original sample is packaged for storage as the reference sample. Landrace collections often include segregating seed textures, colors, and shapes. The reference samples are useful for rechecking the accession identity after regeneration. These reference samples are maintained in the base collection.

**B.3.5. Base collection (long-term): seed packaging and seed storage**

Seed is packaged in a laminated aluminum foil packet that can contain about

- 1 kg (1,000-2,500 seeds) normal maize seeds
- 100 grams (about 3,000) wheat seeds.

The packet for maize is 7.0-7.5 cm (depth) x 10 cm (width) x 15-16 cm (height). The packet for wheat is 6 cm (depth) x 26.5 cm (height) x 8.9 cm (width). The packages are hermetically sealed.

- Two packets are prepared for the base collection for maize at regeneration
- One packet is prepared for the base collection of wheat. For wheat, the laminated aluminum foil packets are packaged into boxes, 75 packets to a box. Boxes are coded and information, including where the boxes are shelved within the storage facility, is entered into the central data base.

**B.3.6. Active collection: seed packaging and storage**

- For the active maize collection (where seed is obtained to respond to outside requests), one-gallon plastic airtight containers holding 2-3 kg (5,000-10,000 seeds) are used for storage.
- For the active wheat collection, laminated foil packets holding 250 grams (about 7,000 seeds) are used. The foil packets are packaged 40 packets to a box. Boxes are coded and information, including where the boxes are shelved within the storage facility, is stored in the central data base.

All seed processing operations entail careful handling of the seed packets and containers. Seed weight (1000 test weight) and initial seed amount stored in the active and base collections are recorded. During seed processing, seed characteristics are checked against the passport data to insure the correct accession identity by seed texture, color, and shape. No fungicide and insecticides are used for seed storage, except in certain cases where the incoming seeds underwent seed treatment as required by quarantine regulations.

**B.3.7 Introduction nursery and quarantine inspection**

Following the application of proper seed health procedures, new maize introductions are planted in the introduction nursery at CIMMYT's Tlaltizapan (tropical and subtropical maize materials) or El Batan (highland and temperate maize materials) stations. In the case of wheat, new introductions are planted in a screenhouse at El Batan. CIMMYT seed health specialists and Mexican quarantine authorities inspect incidence of diseases and other traits during the growing period and harvest in the nursery. Careful hand-pollination is conducted for maize to increase the introductions as required. Wheats will naturally self-pollinate, and little, if any, pollen movement occurs within a screenhouse.

Further increase or regeneration, if needed, is performed by planting accessions in regeneration nurseries to obtain enough quality seeds for storage in the gene bank.

- For maize, these procedures are conducted at CIMMYT's Tlaltizapan (tropical and subtropical maize materials) or El Batan (highland and temperate maize materials) stations.
- For spring wheat accessions, these procedures are conducted in Mexicali, an essentially disease-free location in the northwest Mexico.
- For winter wheat accessions, these procedures are conducted at the CIMMYT highland Toluca station (for more details, see section D, "Regeneration of Introductions/Accessions," p. 15).

The plot size for the introductions in the introduction nursery depends on the amount of seed available and the required quarantine inspection. Any risk factors that exist in the introductions—such as presence of diseased seed or a transgene—would require pre-screening for characterization or purification, or elimination before and after planting in the nursery. Introduction nursery blocks are isolated from the other nursery blocks in the stations.

**B.3.8 Reintroduction of the seed accessions into the gene bank after regeneration and seed increase**
The accessions regenerated at CIMMYT stations and by the cooperators outside CIMMYT such as the case for maize in Andean regions of South America go through the plant health unit procedures for reintroduction of the seed accessions into the gene bank. Stringent inspection is applied to the germplasm samples coming from risk prone region(s) or site(s) in terms of transgenes and epidemic diseases. The same reintroduction procedure is applied to the seed lots increased in the introduction nurseries.

Characterization data is taken both in the introduction and regeneration nurseries. Field books are printed separately for the new introductions and the accession regeneration. The seed health clearance of all incoming seed accessions from regeneration and seed increase are required each time and recorded in the gene bank database system. The SIDU also regularly monitors the germplasm bank working areas for the presence of disease spores. Seed health inspection at entry point to the gene bank reduces the need of seed health inspection when they are requested by the users.

## C. Seed Viability and Germination Tests

The CIMMYT gene bank maintains seed viability as high as possible during seed storage. The initial germination test of seed lots of new introductions or seed increased in the introduction and regeneration nurseries is conducted *after* the seed is dried to the optimal moisture content (6-8% for maize; 5-7% for wheat).

The initial germination test of seed accessions must exceed 90% germination to be stored in the gene bank. All new introductions stored in the gene bank will become new storage units, with the same bank identification number that was given during sample registration. Regeneration may be repeated two to three times to produce sufficient quality seed.

In the course of seed preservation for the active collection, if seed viability of the accessions drops below 85%, or the number of seeds falls below 1,500, the accession is regenerated or multiplied. The first monitoring of seed viability is conducted after ten years of storage in the active collection; then, after every five years as recommended by the standard germplasm bank operation guidelines (FAO/IPGRI 1994).

CIMMYT follows the ISTA rule to count the seeds that have normal and abnormal germination after four days and seven days to determine percent of germination.

- For maize, absorbent paper is used for the germination tests.
- For teosinte, absorbent paper is used for the germination tests. The seeds can be pretreated to break dormancy with 20% hydrogen peroxide ($H_2O_2$) solution for 24 hours before germination. Some teosinte accessions can germinate without seed treatment.
- For *Tripsacum*, the embryo is separated from rachis and germinated in asceptic conditions using N6 media without hormones.
- For wheat, petri dishes lined with sterile filter paper are used for germination tests.

The rolled wet paper with (maize) seeds, or petri dishes with (wheat) seeds, is/are placed inside a germinator at 25°C and 100% RH with a 12/12 hours dark/light regime. Normally, two sets of 50 seeds each are tested for monitoring seed viability of each accession. If the variability between the two replications is high, another germination test is conducted with either 50 or 100 seeds.

## D. Regeneration of Introductions/Accessions

Multiplication and regeneration are two of the most important functions of a gene bank because the long-term viability of seed is very much dependent on the quality of the seed being placed in storage. It is ultimately the objective of the gene bank to provide the allele that was successfully sampled in a distant farmer's field during a collection trip or that is present in a wheat relative, when a small seed sample is provided to the person requesting such genetic diversity. Rather than globally common alleles, interest is in those alleles that are regionally common due to local adaptation conditions (e.g., abiotic or biotic stresses, consumer end-use preference) or that are globally rare (e.g., alleles providing stability in low numbers to a dynamic system). Therefore, during this process of seed regeneration care must be taken to avoid genetic drift resulting in allele loss, as well as mechanical mixtures and other handling errors. The dimensions (i.e., number of seeds and measures of genetic diversity) of the original sample, of the sub-sample to be multiplied, of the newly multiplied sample to be stored for humanity, and of the sample sent out addressing future requests must be carefully considered (Wang et al., 2004). The chance of the loss of rare alleles can be calculated through simulation routines and provide guidelines as to the preferred multiplication procedures and seed numbers required.

It is clear that any round of seed multiplication carries with it a chance of losing rare alleles. It is therefore paramount that seed storage conditions are such that seed needs be multiplied as few times as possible. This is best obtained by lowering the seed moisture content to the recommended values and maintaining the seed at a low temperature, where respiration is reduced dramatically, but further reduction in temperature adds little to raising the odds for proper conservation while costs increase greatly.

### D.1. Maize regeneration

Latin American maize landraces are adapted to the tropical, subtropical, and highland growing conditions. Regeneration of maize germplasm accessions should take place where they can adapt and reproduce themselves in a cost effective manner, while maintaining the original genetic integrity of the populations. Artificially controlled pollination and proper seed management for ex situ conservation is the key to proper gene bank operations. Following are the protocols for planting, pollinating and harvesting accessions of maize at CIMMYT.

- Tropical and subtropical maize accessions are regenerated at CIMMYT's Tlaltizapan experiment station, Morelos, Mexico (940 masl). The maize growing seasons start in October (cycle A) and April (cycle B). Highland maize accessions are regenerated at CIMMYT's El Batan station (2,300 masl).
- Andean highland and some of the Central American highland accessions are not well adapted to the El Batan highland station. They are regenerated in collaboration with the national gene banks, following the same regeneration protocol followed by CIMMYT.

- The seed for the accession are prepared from the base collection. The accessions are grouped by maturity to facilitate pollination.
- For regeneration, seed packets containing 512 seeds each are prepared and transported to the appropriate field site. They are planted in the regeneration nursery (1-2 ha in block size) that is isolated from other nursery blocks in the station. Each packet is sown in 16, rows each 5 meters long. Two seeds are planted in each of 16 hills in 5 meter rows and later thinned to establish 256 plants per plot (60 $m^2$).
- Field plot management follows the normal maize growing practice of the station.
- Chain crosses (most often), paired crosses (as required) or selfing (inbreds) are used for the controlled pollination in all regenerations. Artificially controlled pollination is used to avoid contamination by other pollen sources at anthesis and silking. The ear shoot of each plant is covered with a shoot bag (glassine) before the silks emerge and a tassel bag (pollination bag) is placed on the male flower (tassel) to collect pollen the day before pollination.
- CIMMYT's maize pathologist inspects the plant health in the regeneration blocks. Clean ears are harvested to insure seed quality for seed health inspection and seed longevity. Harvested ears are inspected individually and diseased kernels or undesirable kernels such as those not well filled or broken are removed on the cob before and after shelling. Ear rots often reduce the number of quality ears at harvest.
- After harvest, individual ears are shelled into a paper envelope, cleaned, and inspected for accession identification. For the base collection, two sets of a balanced seed bulks of 50 seeds (about 1.5-2 kg) each are prepared. For the active collection, another balanced seed bulk is prepared containing about 100 seeds (3 kg). One seed bulk of the base collection is used for safety duplicate storage at NCGRP, Fort Collins, Colorado, USA.
- Every cycle, the regenerated seed samples of the accessions are sent to the seed health unit (70 seeds per accession) prior to introduction of the accessions into the gene bank.
- Regeneration of the accessions is repeated in order to obtain enough sample size and quality ears. The seeds of initial and repeated regeneration plantings are combined to represent the regeneration of the accession.
- The bank identification numbers and the field plot numbers of the accessions under regeneration are placed on the seed envelopes, cloth bags, and the field labels to make sure their identity.

The standard practices for maintenance of genetic integrity of germplasm collection accessions involve proper sample size, a system of artificial pollination, and accession identification.

**D.1.1. Proper sample size**

A proper sample size must be employed in order to avoid loss of genetic diversity from generation to generation due to population bottlenecks. An optimum sample size for regenerating maize landrace accessions (panmictic populations) is determined by the frequencies of the rare alleles present in the accession. Based on statistical data, CIMMYT has determined that the proper sample size is based on producing 100 or more ears for regenerating landrace accessions (Crossa, 1989; Crossa et al., 1994; Wang et. al., 2004).

**D.1.2. Artificial pollination**

Artificial pollination control is made either by plant to plant crosses (dioecious mode) or by chain crosses (monoecious mode) within the accession. The appropriate mating system is used for maintaining effective population size. Plant to plant crosses require twice as much land as chain crosses to produce the same number of ears. Usually, chain crosses are used to regenerate a large number of accessions. Inbred lines are selfed or sib-mated. Throughout the regeneration cycle, it is important to maintain an equal effective population size to avoid genetic drift, inbreeding, and associated loss of alleles. Contamination by other germplasm or alien pollen sources must also be avoided.

**D.1.3. Accession identification**

The regeneration of an accession not only provides the ex situ gene bank manager with quality seeds for storage and distribution, but also with an opportunity to ensure the accession identity. Seed color and texture, ear and grain types, and maturity and race classification are rechecked against the original records of the accession in the passport data, which are printed in the regeneration field book.

The regeneration field book is used to register data on the number of plants germinated and established, the number of plants pollinated and harvested, agro-morphological plant and ear traits, the amount of seeds produced for the active and base collection, date of seed storage, initial germination percentage, seed moisture percent at seed storage, previous regeneration site and plot number, and date of the previous regeneration. All data are kept in the regeneration dataset in the bank database system.

## D.2. Teosinte regeneration

For regenerating or seed-increase of teosinte accessions, 100-150 seeds of each accession are germinated in an environment chamber and germinating seeds are planted in plastic pots to flower during the off-season of maize, between cycles A and B at the Tlaltizapan station. A pot contains 6-10 plants. The accessions are grown in the plastic pots and are located 200 meters or more apart from maize or other plantings, along the edge of the station. At maturity, the seeds are collected from the spikes and non-fertilized seeds are removed from the seed lots before drying. The seeds are dried at 10˚C and 25% RH for 1 month before processing for the active and base collections.

## D.3. *Tripsacum* regeneration

A field germplasm bank at the Tlaltizapan station maintains *Tripsacum* clones collected in Mexico, Central and South America, and the USA (Berthaud et al. 1997). The blocks of the field germplasm bank are separated from the rest of the blocks in the station. Most of the accessions are represented by two clones. For collecting seeds, sets of several spikes are bagged in each clone and the seed lots collected are cleaned of unfertilized seeds for conservation. It is often difficult to obtain enough seeds because each spike will have only a few seeds and also because the seed samples may contain hybrid seeds. Clonal propagation is an alternative means of distribution. Some *Tripsacum* species do not flower, although the majority do during the summer months. In the field germplasm bank, maize-T*ripsacum* hybrids do not occur.

## D.4. Wheat regeneration

CIMMYT multiplies wheat seed in a disease free location or within a semi-contained screenhouse, to obtain healthy seed prior to cooled storage. The latter facility expedites the production of quality seed for medium- and long-term storage. Depending on whether the accession is a spring (non-vernalization requiring) or winter (vernalization requiring) habit genotype, or a domesticated or wild relative they are regenerated in different locations.

- Mexicali is located in the northeast of Mexico, where Karnal bunt (KB), a quarantinable disease does not occur. Here, domesticated spring wheats are regenerated. During flowering time, when in theory KB spores could infect the florets, fungicides are applied at intervals to cover all accessions, even if they differ in flowering date. About 10-15,000 entries are regenerated here annually.
- Toluca is located in the Mexican central highlands at 2,640 masl, and in winter experiences at least 8 weeks of temperatures below 4˚C at night. Generally these conditions are sufficient to vernalize most facultative and winter wheats. Winter wheats are multiplied here, if their day length requirement is not too long. They are also treated with fungicides as described above. Several hundred to several thousand entries are regenerated here annually.
- Screenhouses at El Batan, CIMMYT, HQ. This location is also located in the Mexican central highlands, though 400 meters lower than Toluca. The screenhouses are equipped with special high-intensity lighting, to allow the long day-length required by day-length sensitive wheats to be satisfied. Several hundred entries are regenerated here annually.

An important consideration in seed multiplication and regeneration is the amount of seed to be planted. The number of seeds planted or the actual number of spikes or plants harvested depends on two factors: homogeneity or heterogeneity of the accessions, and size of the seed sample originally received.

Initially, upon receipt of a seed sample from outside the gene bank, 25-50 seeds are planted. If an accession is visually judged homogeneous, one basic unit of seed is produced for storage (split between the base and active collection). Given the self-pollinating nature of wheat, many advanced lines and commercial cultivars show little, if any, visual heterogeneity. However, if heterogeneity is noted (e.g., often the case with landraces), the original sample is stored as is, but during the initial multiplication phase separate sub-samples (i.e., individual spikes from distinct plants) are taken to represent the diversity observed. In the database such sub-samples are handled separately to avoid remixing, but noted as deriving from the original sample received, linked through their original accession ID.

Presently, the number of sub-samples taken from heterogeneous introductions may vary from 2 to 100, depending on the visual diversity noted. Landraces may, upon planting under conditions outside their area of origin, display a wide array of diversity. If there are doubts as to the number of sub-samples to produce, we prefer to err on the high side to ensure maintenance of all potential useful diversity.

Once major and minor wheat cultivars and advanced lines of interest from around the world are stored in the gene bank, no active search is made to re-acquire seed of the same genotypes from outside for storage. However, if cultivars from one country are released in another country under the same, similar, or totally different name (e.g., Mexican cultivar Pavon 76 released in Ethiopia under the name Pavon; Indian cultivar HD2172 released as Debeira in the Sudan) seed of both releases will be sought and stored. The reason is until precise molecular studies can be conducted, one cannot be sure whether such accessions are totally identical. There may be various reasons why in fact they are (somewhat) different, which include, labeling error, planting error, outcrossing, genetic drift, and mutations. Such seemingly identical accessions are stored under distinct introduction IDs, so that distinct passport data related to their physical origin is maintained by the unique introduction ID. The total number of such potential 'duplicates' held in the CIMMYT germplasm bank is probably less than 2,000 accessions.

## E. Evaluation of Maize and Wheat Accessions

Objectives of phenotypic evaluation are to document the extent of diversity of phenotypic traits and to choose representative subsets (core subsets) of the stratified groups in the maize and wheat landrace collections.

For phenotypic evaluation of landraces, we have taken the approach of stratifying them by race groups and geographic origins. However, in the future we plan to introduce GIS-based criteria to better stratify our collection. This would allow the linkage of accession data to environmental and edaphic data at the site of the original collection.

The traits or characteristics that need to be evaluated are demand-driven and decided in discussions with the breeders, including those at CIMMYT HQ and regional offices, as well as breeders from partner organizations. Evaluation, therefore, is carried out for traits for which breeding programs lack variability or lack durable forms of diversity. In the case of wheat, the following traits have been identified as high priority:

- yield potential through expanded diversity in yield components, and physiological processes;
- resistance to diseases and pests, of a more durable nature;
- tolerance to such abiotic stresses as drought, heat, cold, acid soils, salinity, waterlogging, particulate and ozone air pollution, micro-nutrient excess or deficiency;
- improved and diversified quality (including micronutrients) for a wider range of end-use products more representative of our clientele, including future alternative uses, such as bio-degradable products and bio-pharmaceuticals;

- adaptation to more sustainable production practices, such as conservation tillage (e.g., zero/minimum tillage, plant residue retention); and
- genetic diversity to study underlying processes in physiological pathways.

Evaluation can be carried out by gene bank personnel, by fellow CIMMYT scientists expert in their disciplines either within or outside Mexico, or by colleagues outside of CIMMYT working with national wheat improvement programs in public or private settings either in developing or developed countries. An example of a combination of joint collaborators is the request by bread wheat breeders to evaluate gene bank accessions for sources of resistance to the powdery mildew (*Erysiphe graminis*). With powdery mildew not endemic in Mexico in our research fields, we turned to colleagues in China, France, South Africa, the UK and Uruguay, to help us evaluate and acquire new resistances.

Ex situ evaluation is conducted at CIMMYT experiment stations and in situ evaluation is done in collaboration with cooperators at sites nearest to where the germplasm accessions were collected.

For maize, evaluation is conducted as two replications of two 5 meters rows per plot for each entry, with 32 plants arranged in alpha lattice design. The trial is evaluated at one or several locations.

For wheat, plot size and experimental design depend on whether disease resistance traits are measured, which require only small plots, or abiotic stresses, such as drought and heat response, which require large plots with replications, properly managed for the respective stress.

Morph-agronomic data include

- For maize: plant height, ear height, days to silking, days to male flowering, leaf senescence, root and shoot lodgings, ear length, ear diameter, ears per plant, ear rot%, ears per plant, ear quality rating, kernel width, kernel depth, kernel row number, grain moisture % at harvest, grain shelling percent, grain yield, and agronomic rating and other observations such as leaf diseases, insect damage, and adaptation to the environment are taken in the trial.
- For wheat: growth habit, plant height, heading date, flowering date, maturity date, spike length, spikelets/spike, florets/spikelet, awnlessness, peduncle length, stem thickness, leaf thickness, leaf rolling, pubescence, agronomic score, tillers per $m^2$, grain yield per per $m^2$, thousand kernel weight, grain color and appearance, disease ratings, etc.

In maize, a multivariate cluster analysis is performed using the agro-morphological traits, except grain yield, ear quality rating, ear rot percent, and root and stalk lodgings. Core subsets (20% of the trial entries) are chosen in each accession cluster using the selection index constructed with seed moisture percent, root and stalk lodging, and yield (Taba et al. 2003).

In wheat, analyses including GIS information on the original collection sites are carried out that may allow the identification of those geographic locations that have a higher frequency of useful germplasm for the trait being studied. As more information is acquired, analyses similar to those carried out for maize can be conducted to determine core subsets.

Based on preliminary data, two core wheat subsets have been formed in an attempt to capture the diversity held in thousands of lines into sets of just a few hundred accessions. It is only through such reductions that one can hope to encourage colleagues around the world to study these sets for traits of relevance to them. The two core sub-sets are:

- Species core subset: 150 accessions, representing 10 distinct sources and combinations of genomes A, B, and D.
- Landraces core subset: 500 landraces from 53 countries, representing putative genetic diversity based on preliminary origin and geographic (GIS) considerations.

As these two core subsets are studied more widely, especially in regard to their molecular and GIS structure, we will be able to return to the complete collection for further sub-sampling of clusters either on a genetic or GIS basis, and to home in on diversity of the greatest promise. While some may claim that developing imperfect core subsets at this stage is premature, it allows a more focused initial search towards the development of subsequent improved subsets based on more rigorous data.

## F. Shipments

### F.1. Maize

The designated germplasm accessions (all maize landrace accessions, teosinte and *Tripsacum* in Annex-1 in ITPGRFA, and obsolete varieties and breeding populations) in the in-trust collection under CIMMYT-FAO agreement (1994) are distributed upon request to all bona-fide users with the interim material transfer agreement (MTA) pursuant to the International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA). The germplasm accessions from CIMMYT research products are distributed with the MTA for non-designated germplasm to all bona-fide users.

- Standard shipments for maize are 50-100 seeds; for teosinte 15-20 seeds; and for *Tripsacum,* 5-15 seeds.
- When the bank manager receives the seed requests, he/she will respond to the requester if the seed accessions are available, or suggest the best suitable seed accessions in the collection and relevant MTA to be used.
- The list of the seed accessions is prepared with the accession and seed identification data: ID number, race name or common name, seed quantity and origin and plot number, designated or non-designated, and observation.
- The Seed Inspection and Distribution Unit (SIDU) receives the seed packages, seed list, and the formal seed request from the gene bank and examines the health status of the seed. If required, the standard seed health procedure is applied to the sample accessions before shipment. All seed shipments of gene bank accessions are accompanied by the necessary documentation, as required by the recipients (import permit, phytosanitary certificate, MTA, list of the accessions with key identifiers of the accession and seed origin, etc).
- The seed samples are, for the most part, prepared from the active collection and have the corresponding bank accession number and a serial number labeled on the seed envelope. The seed samples can be prepared from the base collection when the requested accessions are only preserved there and there is sufficient seed stock.
- Accessions under regeneration may not be immediately available, and requests may have to wait until the accessions have been reintroduced.

### F.2. Wheat

Most seed requests arrive at the gene bank through email, with a decreasing proportion by postal mail. Plans for the future call for a fully searchable website with all relevant data posted for the wheat germplasm collection. The user will be informed within 24-hours of the receipt of the request about how the request will be processed, including a projected time-line leading to final shipment. The aim is for nearly all requests to be honored, with a seed shipment arriving in the country of designation one month prior to the upcoming planting cycle. In some cases, time will be too short to achieve this aim, but seed will be shipped as soon as feasible. If the seed amount requested is larger than average, an internal round of seed multiplication will be carried out, which will delay the procedure by about seven months for spring-habit genotypes and 12 months for winter-habit genotypes. If the request is not for seed but rather for information about DNA samples, then the turn-around time can be reduced to a matter of days or weeks.

Present and future seed requests can be divided into two categories:

- Genotype-specific seed requests: These requests indicate the specific cultivar name or cross description. Such requests can be readily fulfilled, seed availability permitting. Further automation of the process is foreseen for the future.
- Information-specific seed request: These requests indicate the traits or origins of the genotypes requested (e.g., drought tolerant germplasm; all spring wheat accessions from Afghanistan). Here the gene bank manager will need to conduct an internal search based on the data available relevant to the information provided. Subsequent correspondence with the requester may be needed to narrow down or broaden the accessions proposed for final shipment. As data is increasingly posted on the CIMMYT website, users should be able to search for genotype data on accessions that most closely resemble those being sought.

Generally, the more specific a request is, either detailing the genotype or the trait(s) sought, the quicker the response.

Once the identity of the genotype to be retrieved is determined based on the seed request, a small amount of the seed (i.e., 50-100 seeds) is obtained from the active collection. When this occurs, the database system will register the reduction in seed stock. In special cases, such as segregating populations or with landraces that are expected to be highly heterogeneous, larger seed amounts can be requested and sent, seed availability permiting. However, an intervening round of seed multiplication may be needed.

The seed is treated with a fungicidal/insecticidal mixture just prior to shipment in order to strictly adhere to official regulations of national governments regarding seed imports of recipient cooperators. The seed held in the gene bank is, however, already guaranteed free of disease. During multiplication, fungicides are applied and the seed is subsequently checked by the SIDU for any form of infection or contamination, prior to it being stored in the germplasm collection. The SIDU also regularly monitors the gene bank working areas for the presence of disease spores. All national regulations from cooperating countries are held by CIMMYT in electronic or hard copy form, and are consulted prior to seed shipment. Particular efforts are made to remain abreast of new developments in this regard with our cooperating countries.

We encourage cooperators and recipients of seed from our gene bank to share relevant data that may be collected on it in the future, plus any information on its use, such as the introgression of traits into new cultivars through breeding that are later made available to farmers.

## G. Back-up collections

### G.1. Maize

Historically, duplicate samples of maize landrace collections of NAS-NRC in Latin America during the 1940s-1950s were sent to NSSL, now USDA's National Center for Genetic Resources Preservation (NCGRP) at Fort Collins, Colorado, USA. Since the mid-1960s, these original samples have been regenerated at CIMMYT and have become the core of the CIMMYT maize germplasm collection. Most of the samples have now been duplicated at NCGRP, as recommended by the ex situ conservation network of FAO.

CIMMYT maintains a safety duplicate arrangement (MOU) with NCGRP in a cooperative manner to back-up its maize germplasm collection. The NCGRP conserves duplicate samples of 82% of the CIMMYT maize collection (24,450 accessions) at -20˚C, under long-term seed storage, using CIMMYT gene bank identification numbers. Upon regeneration of accessions, CIMMYT sends the NCGRP 1-2 kg seed samples to serve as a back-up. None of the seeds are distributed by NCGRP.

CIMMYT also coordinates the maize germplasm conservation network for Latin American national gene banks. The network project has regenerated about 10,000 accessions and duplicated them at CIMMYT and NCGRP. The cooperative national gene banks, meanwhile, conserve their own national accessions.

### G.2. Wheat

CIMMYT has agreements with NCGRP in Fort Collins, Colorado, USA, and ICARDA in Syria to send these two parties black-box, back-up seed shipments of CIMMYT wheat and wheat-related accessions held in the CIMMYT gene bank. Likewise CIMMYT holds a black-box shipment from ICARDA. The rationale is that materials are thus duplicated in several sites around the world, and in case an environmental disaster or other circumstances cause accessions to be lost or destroyed, they can be replenished from the back-up location. The system is based on so-called black-box collections, meaning that the recipient should not open the boxes or seed packages, but only accept responsibility to store them well in their long-term base storage area. Lists of the accessions are provided to the recipients. The recipient is prohibited from fulfilling any seed requests from the black-box accessions, since they are not officially part of their collection, but only kept there for security reasons. Related seed requests should be channeled to the sender of the black-box shipment.

## H. Data Management

### H.1. Maize

The datasets of passport, regeneration (characterization), evaluation (core subsets), seed monitoring (seed amounts, germination, storage address), and seed shipment are incorporated into the current maize gene bank information database system (MZBANK). A new CIMMYT gene bank database system is under development.

### H.2. Wheat

The management process of the data held by the wheat gene bank, as well as other information residing with outside cooperators, is currently undergoing review and reorganization. This is primarily driven by the need to provide internal and external web-service access to the accessions held in the bank, based on information associated with it through the unique accession ID coding.

Presently, the Wheat Germplasm Bank System (WGBS) is being used for data management. This is a component of the IWIS software system that electronically manages data (i.e., passport, characterization, evaluation, and logistical information) in the germplasm bank collection. WGBS can also generate field books and data reports. This system will soon be upgraded, or integrated with another database management system, or CIMMYT may choose to develop wholly new software to carry out the task.

The future system will address the following five processing areas within gene bank operations that generate data:

- Introduction of new accessions, registration of related passport data, assignment of ACCID and storage location ID;
- Monitoring and updating storage dates, seed viability, and seed amounts;
- Storage of characterization and evaluation data, including germination tests;
- Web-enabled searches by users, generation of associated seed request and entry lists, seed shipment processing; and
- Regeneration information as needed.

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