Preserving Genetic Resources

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Abstract

The mission of the U.S. National Plant Germplasm System (NPGS) is to effectively collect, document, preserve, evaluate, enhance, and distribute plant genetic resources for continued improvement in the quality and production of economic crops important to U.S. and world agriculture. Plant genetic resources in the NPGS are made freely available to all bona fide users for the benefit of humankind. The active collection is maintained and distributed by 19 national repositories, and the base collection is preserved at the National Seed Storage Laboratory (NSSL), U.S. Department of Agriculture, Fort Collins, Colorado. The NPGS collections include 40,477 sorghum and 1,507 pearl millet accessions. Of the 20,169 sorghum accessions in the base collection at NSSL, 80% are in conventional storage at about -18°C and 20% are in cryostorage in vapor phase above liquid nitrogen at about -160°C; the pearl millet collection is in conventional storage.

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) located at Patancheru, near Hyderabad, India, has assembled a collection of 35,643 sorghum and 21,195 pearl millet accessions, both ICRISAT mandate crops. All these accessions are maintained and preserved in aluminum cans in the medium-term storage facility at about 4°C and 20% relative humidity. Freshly rejuvenated accessions with at least 90% viability and about 5% seed moisture content are being placed in moisture proof aluminum foil packets that are vacuum sealed and stored in long-term storage at -20°C. For these crops, 17% of the sorghum collection and 23% of the pearl millet collection have been transferred to long-term storage.

Landraces and wild relatives of crops from centers of diversity have been rich sources of resistance to new pathogens, insect pests, and other stresses, as well as sources of traits to improve food and fiber quality, animal feed, and industrial products. But, as farmers in centers of diversity switch to new stress-tolerant, higher yielding cultivars, these valuable sources of useful genes will be lost forever unless they have been collected and preserved *ex situ* in gene banks.

No country has all the plant genetic resources required to develop and maintain a high level of agricultural productivity. The U.S. has an extremely limited number of native agricultural crop species of economic importance. As with many countries, our exceptionally productive agricultural systems were founded on introduced plant genetic resources, including sorghum [*Sorghum bicolor* (L.)

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Moench] and pearl millet [*Pennisetum glaucum* (L.) R. Br.].

Mann et al. (1983) hypothesized that sorghum probably originated and was subjected to domestication more than 5,000 years ago in northeastern Africa. More recently, Wendorf et al. (1992) reported that carbonized seeds of sorghum, evacuated at Nabta Playa near the Egyptian-Sudanese border, appear to be about 8,000 years old.

Duncan et al. (1991) summarized known introductions of sorghum into the U.S., including broomcorn by Benjamin Franklin in 1725, 'Chinese Amber' sweet sorgo in 1851, milo in 1879, 'Blackhull Kafir' in 1886, 'Feterita' in 1906, and 'Hegari' in 1908. They indicate that the first mention of the value of a guinea kafir corn from West Africa occurred at the Philadelphia Agricultural Society in 1810. As the value of sorghum in low rainfall areas of the Great Plains was recognized, breeders in state agricultural experiment stations and in the Agricultural Research Service began sorghum improvement in the 1920s. Introduction of additional materials was important to their programs, and by 1957, more than 13,000 accessions (many of which were landrace collections from Africa) had been introduced (Duncan et al., 1991).

Pearl millet has been used in Central Africa for many centuries by nomads and hunters (Rachie and Majmudar, 1980). Ball (1903) reported that pearl millet has been "known in cultivation as a forage or cereal crop for at least 3,000 years" in India, Arabia, and Africa. He indicates that pearl millet was widely cultivated in the southeastern U.S. by 1873, and he speculates that it probably arrived along with sorghum in the early 1850s. Extensive research by Burton (1980, 1995) and Hanna et al. (1987) at Tifton, Georgia, has concentrated on developing improved pearl millet cultivars for forage production, but their parental lines also have been used in hybrids developed for grain production. More recently, breeding research programs at Tifton; Hays, Kansas; and Lincoln, Nebraska, have included grain types (Hanna, 1995; Stegmeier, 1994; Rajewski and Andrews, 1995).

The Organic Act of 1862, establishing the Department of Agriculture, directed the first Commissioner of Agriculture, Isaac Newton, "to collect, as he may be able, new and valuable seeds and plants; to test, by cultivation the value of such of them as may require such tests; to propagate such as may be worthy of propagation, and to distribute them among agriculturists." In 1898, the Seed and Plant Introduction Section, which later became the Plant Introduction Office, was established to manage plant explorations and introductions. Before the late 1940s, introductions were sent directly to interested scientists without any requirement that they be maintained. Adequate preservation methodologies and facilities were not available, and many accessions were lost.

The U.S. National Plant Germplasm System

Ex situ preservation of plant genetic resources is extremely important to U.S. agriculture. The Research and Marketing Act of 1946 (Public Law 733) authorized the creation of four Regional Plant Introduction Stations (Ames, Iowa; Pullman,

Washington; Geneva, New York; and Griffin, Georgia) with the mission to acquire, maintain, evaluate, and distribute germplasm to scientists to be used for crop improvement. The Inter-Regional Potato Introduction Station at Sturgeon Bay, Wisconsin, was established in 1947. National Clonal Germplasm Repositories were established in the mid-1980s to provide more systematic maintenance of vegetatively propagated germplasm. The National Small Grains Collection (NSGC), now in Aberdeen, Idaho, began in 1894 as a breeders' collection in Beltsville, Maryland. These repositories grow and maintain the active collections and distribute samples to scientists worldwide. The National Seed Storage Laboratory (NSSL), Fort Collins, Colorado, was dedicated in 1958 as a long-term storage facility to preserve the base collection for backup of the active collections. These units have been integrated into the National Plant Germplasm System (NPGS) (ARS Information Service, 1990; Shands et al., 1989).

The mission of the NPGS is "to effectively collect, document, preserve, evaluate, enhance, and distribute plant genetic resources for continued improvement in the quality and production of economic crops important to U.S. and world agriculture. This is achieved through a coordinated effort by the U.S. Department of Agriculture in cooperation with other public and private U.S. and international organizations. Plant genetic resources in the NPGS are made freely available to all *bona fide* users for the benefit of humankind."

New acquisitions must be increased, characterized, and preserved as part of the active collections. Each repository conducts a systematic evaluation program to obtain specific information on disease and insect resistance, nutritional quality, agronomic and physiological attributes, and other traits of interest. Information on the collection and characterization (passport data) and evaluation data are entered in the Germplasm Resources Information Network (GRIN) database. When requested, samples are distributed to scientists worldwide at no cost for use in crop improvement and basic research. Research relating to improved methods of collection, regeneration, propagation, preservation, evaluation, and distribution is conducted, and the results are published.

The National Germplasm Resources Laboratory (NGRL), located at the Beltsville Agricultural Research Center (BARC) in Beltsville, Maryland, is responsible for a number of activities that support the entire NPGS. The Plant Exchange Office (PEO), the Germplasm Resources Information Network Database Management Unit (GRIN/DBMU), and the Plant Germplasm Quarantine Office (PGQO) are components of the NGRL.

The Plant Exchange Office coordinates the acquisition and exchange of plant germplasm, documents passport data and descriptive information for newly acquired material, assigns unique Plant Introduction (PI) numbers, and serves as a liaison on quarantine matters. Strategies are developed for increasing the genetic diversity of U.S. collections. Based on these strategies, gaps in current germplasm collections are identified and communicated to the appropriate Crop Germplasm Committee (CGC) or to other

crop specialists for their concurrence. The NGRL facilitates the activities of the CGCs. The public and private scientists on these committees represent the germplasm user community for a particular crop or group of crops. These committees provide crop-specific expert guidance on germplasm needs, collection gaps, descriptors, documentation, regeneration, evaluation, and research goals to various components of the NPGS.

The GRIN is the computerized database for the NPGS. Information in GRIN is available to any plant scientist or researcher worldwide through a variety of avenues: direct connection to the database, PC GRIN, World Wide Web (http://www.ars-grin.gov), or contact with the curator for the active collection of the crop of interest. GRIN contains data on taxonomy, origin, evaluation, and characterization for plant germplasm preserved in the NPGS.

All plant germplasm entering the NPGS from outside the U.S. must comply with federal quarantine regulations, which are designed to facilitate the exchange of plant germplasm while limiting/ preventing the movement of pathogens. Regulations are written, interpreted, and enforced by the USDA Animal and Plant Health Inspection Service (APHIS).

Although the ARS components of the NPGS are administered by the area director for the geographic location of that component, the Associate Deputy Administrator for Genetic Resources and the National Program Leader for Plant Germplasm on the national program staff provide leadership and coordinate activities for the NPGS. They also provide administrative support to the various advisory councils and committees for plant genetic resources.

The NPGS maintains one of the largest *ex situ* collections of plant genetic resources in the world. A detailed report of the NPGS history, policies, and architecture is given in *Plant Breeding Reviews* (ed. by J. Janick, 1989). Since 1898, about 575,000 accessions with real or potential economic importance to U.S. agriculture have been acquired through the former Plant Introduction Office. Many of these are among the more than 433,000 accessions, representing over 9,000 plant species, that are now preserved in the NPGS. Between 1986 and 1995, the NPGS distributed an average of 161,358 samples each year to U.S. public scientists (64%), U.S. private industry scientists (13%), foreign public scientists (8%), foreign private industry scientists (13%), and international centers and USAID (2%).

The principal mission of NSSL is to preserve the base collection of the NPGS and conduct research to develop new and improved technologies for the preservation of seed and other plant propagules. NSSL also provides long-term storage for plant materials not in the NPGS that are not to be distributed: 1) voucher samples of cultivars and parental lines licensed by the U.S. Plant Variety Protection Office, 2) accessions of endangered species maintained by botanical gardens, 3) quarantined samples queued for regeneration under APHIS inspection, and 4) security backup materials from international centers and other gene banks.

Physical facilities of NSSL were modernized and expanded fourfold in 1992.

High security storage vaults have the capacity to provide protection from natural disasters, including floods, tornadoes, fires, and earthquakes, for nearly one and a half million samples. The insulated walls, ceiling, and floor of the cold vault environmental chambers are 15.2 cm (6") thick, and movable shelves increase capacity. Energy requirements are much less with 15.2 cm insulation and movable shelves (Walters et al., 1997).

Minimizing genetic change during *ex situ* preservation is paramount to retain as much genetic variation as possible for future use (Crossa et al., 1994). A key first step to minimize genetic change is to preserve the initial regenerated sample in the base collection. This regeneration should be done with an appropriate number of plants, with the required pollen control, and under optimum growing conditions to produce high quality seed. Careful processing and drying are required to maintain high viability. Storage of dry, high quality seed at sub-zero temperatures can extend viability for many years before a second regeneration of the base collection is necessary. When continuing demand on the active collection occurs, seed from the base collection should be used for every second or third regeneration.

Scientists in the Plant Germplasm Preservation Research Unit (PGPRU) at NSSL focus on the development of new and improved technologies for the long-term preservation of all forms of plant germplasm. This research is expected to increase the number of species that can be stored at NSSL, the longevity of the various accessions, and the efficiency of viability testing of accessions. Longer storage periods and reduced number of field and/or greenhouse regeneration cycles will result in lower costs and greater genetic integrity of the germplasm.

Preservation of Orthodox Seeds

The technologies for preserving orthodox seeds are well understood. Seeds should be dried and stored at a low temperature (Justice and Bass, 1978; Roos, 1986, 1989). Research by Justice and Bass (1978), Bass (1980), and Bass and Stanwood (1978) showed that reducing the storage temperature from 5°C to sub-zero temperatures increased seed longevity from less than 10 years for some species to several decades for most species.

The ultra-low temperature of liquid nitrogen (LN) used in cryogenic storage should extend seed longevity (Stanwood, 1980, 1985; Stanwood and Bass, 1981). Stanwood and Sowa (1995) reported that after 10 years of storage, oxygen uptake rates and average seedling root lengths were greater for onion samples stored in the vapor phase above LN (approximately -160°C), compared to samples stored at -18°C. Germination percent did not change during 10 years of storage at either of these temperatures. However, major differences in germination were observed between 5°C and the sub-zero temperatures.

Although seed drying extends longevity, there are limits to the beneficial effects, and the optimum moisture content varies with the chemical composition of the seed (Vertucci and Roos, 1990, 1993; Vertucci et al., 1994; Walters-Vertucci and Roos (1996); Ellis et al., 1989, 1990). Drying seeds beyond a critical moisture

content can result in accelerated deterioration at above zero temperatures. Using basic thermodynamic principles, scientists at the NSSL (Vertucci, 1989; Vertucci and Roos, 1990, 1993; Vertucci et al., 1994) have established that, contrary to the viability equations (Ellis and Roberts, 1980; Ellis et al., 1989), the effects of storage temperature and water content of seeds are not independent. Consequently, the optimum water content for seed storage varies with both the species and the storage temperature. The thermodynamic principles used by Vertucci and Roos (1990, 1993) and Vertucci et al. (1994) can be used to predict optimum moisture levels for all orthodox seeds at all storage temperatures. Equilibration at about 25% RH at a specified temperature provides the optimum seed moisture for storage at that temperature for all orthodox seeds studied. Seed moisture at equilibrium will be less in seed with a greater lipid content (Walters-Vertucci and Roos, 1996). The procedure of drying to equilibrium at an appropriate relative humidity (RH) and temperature eliminates the requirement of determining the moisture content of each accession and saves processing time. When high quality seed is dried to the optimum moisture content and stored at sub-zero temperatures, longevity of several decades can be expected (Walters et al., 1997).

Preservation of Sorghum and Pearl Millet in the NPGS

The NPGS active collections of sorghum and pearl millet are maintained and distributed by staff of the Plant Genetic Resources Conservation Unit (PGRCU) at Griffin, Georgia, in cooperation with the sorghum curator at the Tropical Agricultural Research Station (TARS) in Mayaguez, Puerto Rico, and the pearl millet curator at Tifton, Georgia. Accessions of both crops are regenerated at the TARS. About 100 plants are established and selfed by bagging at least one panicle per plant. Panicles are harvested and equal seed quantities from all selfed panicles are bulked. These bulked seed samples are then divided, with part staying in the active collection and the other part deposited in the NSSL base collection. Sorghum and pearl millet samples at the Griffin PGRCU are maintained in cold vaults at about 5°C and 25% RH.

Seed samples received at NSSL are dried initially to equilibrium at about 10°C and 30% RH, to obtain near optimum seed moisture for long-term storage. At this temperature, the dehumidifier seldom runs to achieve 30% RH, whereas at 15°C the RH drops below the desired 35% for this temperature because of the naturally low RH of the ambient air at Fort Collins. Either of these combinations gives the same seed moisture as 5°C and 25% RH (Vertucci and Roos, 1993). Seed quality is normally evaluated by germination tests with four replications of 50 seeds each, using standard Association of Official Seed Analysts procedures. For cryopreservation, two replications of 50 seeds are tested as usual (control), and two replications are stored for 24 hours over LN before the germination tests are conducted. As the seed counts and germination tests are being conducted, a final equilibration is done at about 5°C and 25% RH (seed moisture of sorghum samples stored at NSSL ranges from 6 to 9%,

depending on lipid content). These equilibrium procedures are predicted to result in seed moistures corresponding to those obtained with 20% RH at -18°C.

After seed counts have been made, samples are packaged in moisture-resistant aluminum foil envelopes and stored in the cold vault at about -18°C or placed in polyolefm tubes and stored in the vapor phase above LN at about -160°C in cryotanks. Samples that are substandard for germination (below 65%, or LN-treated samples that deviate from the control by 10% or more) or are substandard for seed number (below 1,000 seeds) are stored in cold vaults while the accessions are queued for regeneration. Operating costs at NSSL to maintain samples at -18°C are estimated to be about $.04 per sample per year and about $0.14 per sample in cryotanks.

The seed quality data for accessions in the NPGS active and base collections are entered in GRIN. Viability is monitored periodically at NSSL depending on initial viability (about every 15 years, except for accessions with poorer initial quality that are tested more often). Substandard samples of sorghum and pearl millet are identified for regeneration by the Mayaguez TARS.

The NPGS collections include 40,477 accessions of sorghum (Table 1). Of the 20,169 accessions now in the base collection at NSSL, 80% are in conventional storage and 20% are in cryostorage. Seed quality is fair, with 79% of the accessions having samples with germination above 64%. The NPGS collections include 1,507 accessions of pearl millet and related species (Table 2). Only 102 *Pennisetum* accessions are backed up in the NSSL base collection. Seed quality is fair, with 79% of the accessions having samples with germination above 64%. Countries of origin of NPGS accessions, as listed in GRIN, are shown in Table 3.

U.S. quarantine regulations require that sorghum and pearl millet accessions from Africa and Asia be grown under controlled conditions and inspected by the Animal Plant and Animal Health Inspection Service (APHIS). In the past seven years, 10,893 quarantined sorghum introductions have been regenerated in St. Croix, Virgin Islands, inspected, and added to the NPGS sorghum collection, with only 278 remaining in quarantine for regeneration. Pearl millet accessions have been regenerated under quarantine restrictions in greenhouses in Tifton, but 724 introductions are backlogged. Authorization to re-

Table 1. Status of *Sorghum* accessions in NPGS.

	Number	Per Cent
Plant variety protection voucher samples	48	
Accessions in quarantine status	278	
Accessions in NPGS	40,477	
Accessions in NSSL base collection	20,169	50
Tested for germination	19,803	99
85 to 100%	9,131	46
65 to 84%	6,479	33
1 to 64%	4,193	21

Table 2. Status of *Pennisetum* accessions in NPGS.

	Number	Per Cent
Plant variety protection voucher samples	5	
Accessions in quarantine status	724	
Accessions in NPGS	1,507	
Accessions in NSSL base collection	102	7
Tested for germination	77	77
85 to 100%	32	42
65 to 84%	29	37
1 to 64%	16	21

Table 3. Source of *Sorghum* and *Pennisetum* accessions in the NPGS and ICRISAT.

	NPGS		ICRISAT	
Country	*Sorghum*	*Pennisetum*	Sorghum	*Pennisetum*
Africa	3			
Algeria	41	52	23	5
Angola			44	
Benin	417		199	46
Botswana	153	2	219	82
Burkina Faso	334	117	549	868
Burundi	154	8	140	
Cameroon	229	41	2486	998
Cape Verde Islands			1	2
Central African Republic	4		249	156
Chad	111		192	136
Congo			1	8
Cote D'Ivoire	1		7	
Egypt	16	1	35	
Ethiopia	7080	42	4401	2
French Equatorial Africa	5			
Gambia	67		57	15
Ghana	46	9	147	283
Guinea	1			
Kenya	723	50	988	98
Lesotho	10	12	271	4
Liberia	3			
Libya	22			
Madagascar	10		14	
Malawi	550	15	423	312
Mali	1016	18	701	1178
Mauritania	15		9	37
Morocco	1	4	27	4
Mozambique	23	13	48	33
Namibia			182	1126
Niger	515	37	412	1270
Nigeria	460	188	1672	1917
Rwanda	86		291	
Senegal	347	4	241	415

(Continued on Next Page)

Table 3. Source of *Sorghum* and *Pennisetum* accessions in the NPGS and ICRISAT, continued.

	NPGS		ICRISAT	
Country	*Sorghum*	*Pennisetum*	Sorghum	*Pennisetum*
Sierra Leone	26		108	60
Somalia	102		446	4
South Africa	807	65	935	162
Sudan	3813	31	2494	614
Swaziland	11		202	
Tanzania	86		718	503
Togo	565	23	294	515
Tunisia		6		
Uganda	682	7	1759	124
Zaire	40	22	52	11
Zambia	580	31	341	157
Zimbabwe	1237	385	1607	1386
Africa sub-total	20,392	1,183	22,985	12,531
Afghanistan	10	3	5	
Bangladesh		1	9	
China	1122	5	380	
India	1083	120	6106	7775
Indonesia	20		33	
Iraq	5		3	
Iran	420	3	7	
Kazakhstan	3			
Korea	40		78	1
Lebanon	32		360	108
Nepal	3		8	
Oman	54	7		
Pakistan	24	15	70	160
Saudi Arabia	23	3	22	
Turkey	111		50	2
Yemen	4635	62	2130	290
Asia, other	83	2	242	2
Asia sub-total	7668	221	9503	8338
Oceania sub-total	239	9	64	8
Caribbean sub-total	134	0	84	0
Europe sub-total	501	25	526	61
North America sub-total	2642	58	2226	245
South America sub-total	132	11	192	2
Unknown	8769		63	10
Total	40,477	1507	35,643	21,195

generate these accessions in St. Croix has been requested.

A total of 1,596 sorghum landrace collections have been submitted for conversion from photoperiod-sensitive to adapted insensitive versions in the TAES-USDA Sorghum Conversion Program, and 623 fully converted lines have been released and are available in the NPGS sorghum collection (Duncan et al., 1995; Rosenow et al., 1995; J.A. Dahlberg, personal communication). Most accessions available from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, are in the NPGS sorghum collection.

The International Crops Research Institute for the Semi-Arid Tropics

Within the Consultative Group for International Agricultural Research (CGIAR), ICRISAT has responsibility for the world sorghum and pearl millet collections, as well as their wild relatives. The policy of the CGIAR centers is to conserve, maintain, improve, and distribute germplasm world-wide for use in agricultural research and development. In 1983, the FAO member countries adopted the International Undertaking on Plant Genetic Resources and established the Commission on Plant Genetic Resources. Under the framework of the International Undertaking, a Global System for Plant Genetic Resources has been proposed. As part of this system, the FAO International Network of *Ex Situ* Collections aims to ensure safe conservation and to provide an equitable means whereby all countries have access to plant genetic resources to enhance their agricultural stability, productivity, and well-being, while they share equally and fairly in the benefits accruing from the utilization of such resources. Under this agreement, ICRISAT has designated 80% of the sorghum collection and 98% of the pearl millet collection to the auspices of the FAO/CGIAR agreement, where these collections will be held in trust for the benefit of humankind. This agreement covers collections held by ICRISAT prior to December 1993 when the Convention of Biological Diversity, which affirmed the sovereign rights of national governments over their national resources, came into effect. The agreement states that ICRISAT's designated germplasm will continue to be readily available to all, since ICRISAT will not claim ownership nor apply intellectual property protection to the germplasm they hold in trust and will ensure that recipients will not apply for intellectual property rights to the germplasm. Germplasm acquired after December 1993 will be subject to conditions imposed by the source country and may be very specific for each accession until a more global system for plant genetic resources can be developed. To meet their responsibilities, ICRISAT requires every recipient of designated germplasm to sign a material transfer agreement. The availability and status of germplasm acquired by ICRISAT after December 1993 will depend on individual agreements.

Chapter 14G of Agenda 21 of the United Nations Conference on the Environment and Diversity (UNCED) recommends that all gene banks duplicate their collections of germplasm for safety. ICRISAT's agreement with FAO also requires a safety duplication. The designation of sites for safety duplication and storage is being investigated for both sor-

ghum and pearl millet. Once sites are identified, an agreement will be arranged to facilitate transfer and ensure safe storage for the duplicate collection. At this time, various options are being investigated, including an arrangement with NSSL, regional gene banks, other CGIAR Centers, and specific national gene banks.

The first major effort to assemble a world collection of sorghum was made in the 1960s by the Rockefeller Foundation in the Indian Agricultural Research Program (House, 1980, 1985; Murty et al., 1967; Rockefeller Foundation, 1970). A total of 16,138 accessions were assembled from different countries, and International Sorghum (IS) numbers were assigned to them. In 1976, ICRISAT was given the responsibility to add sorghum germplasm to the world collection in accordance with the recommendation made by the Advisory Committee on Sorghum and Millet Germplasm sponsored by the International Board for Plant Genetic Resources (now IPGRI, the International Plant Genetic Resources Institute) (IBPGR, 1976; Mengesha and Prasada Rao, 1982). At present, ICRISAT Asia Center (IAC) is a major repository for the world sorghum and pearl millet germplasm collection, with a total of 35,643 sorghum accessions from 90 countries and 21,195 pearl millet accessions from 48 countries. The existing collections of these two crops conserved at ICRISAT have been estimated to represent about 80% of the variability present in the crop. Despite this, germplasm still remains to be collected from a number of specific areas where a high degree of genetic diversity still exists in either the crop or its wild relatives. In the future, collection activities will be targeted to fill these gaps for both ICRISAT and the specific country. These collections will occur in cooperation with the specific country and will be based on a clearly defined agreement on the collection and receipt of the germplasm.

Greater research effort will be expended on assessing the adequacy of the existing collections both within national government programs and at ICRISAT, eliminating redundancy, and characterizing the degree of diversity. These assessments will be conducted in cooperation with specific countries and will hopefully involve a repatriation of collections held by ICRISAT and other gene banks, in-country evaluations using descriptors of value to the national programs as well as ICRISAT, and an opportunity to enhance the training of national scientists to assume responsibility for their genetic resources and to benefit more directly from their use. This increased national control over their own natural resources is affirmed by the Convention of Biological Diversity and will be an objective of any global genetic resource system. In the future, research emphasis will use *in situ* conservation of both the crop and the wild relatives in their natural habitats.

The accessions of both sorghum and pearl millet held by ICRISAT are listed in Table 3 according to their origins. These collections contain material donated by governments and private institutions over the past two decades or collected by ICRISAT in cooperation with national genetic resource programs in various countries. Thus, 90% of the sorghum collection and 98% of the pearl millet collection have come from developing countries in the semi-arid tropics. About 49% of the sor-

ghum collection is from five countries: India, Ethiopia, Sudan, Cameroon, and Yemen. Thirty-seven percent of the accessions in the pearl millet collection are from India. Within Africa, 33% of the accessions are from West Africa, while 18% are from Southern Africa. Besides India, the other main contributors to the pearl millet collection are Nigeria, Zimbabwe, Niger, Mali, and Namibia.

ICRISAT has maintained and continues to conserve 473 wild sorghum accessions from 23 taxa and 688 accessions from 38 taxa for the wild relatives of pearl millet. The sorghum accessions have been screened for downy mildew and shoot fly resistance at IAC. Some of the accessions of diploid wild races of *S. verticilliflorum, S. virgatum*, and *S. arundinaceum* were collected in their natural habitats in Sudan, near the Ethiopian border where sorghum is considered to have been domesticated (Doggett, 1970; de Wet, 1976). Harlan and de Wet's collection of wild sorghum, consisting of 188 accessions from the early 1960s, was obtained from Mayaguez, Puerto Rico, U.S.A., in 1979. A collaborative effort with the Department of Primary Industries, Central Region, Queensland, Australia, has resulted in the recent collection of 162 samples of wild sorghum belonging to the sections *Parasorghum, Stiposorghum, Heterosorghum*, and *Chaetosorghum*. The Australian collection, after being released from plant quarantine at IAC, will be added to ICRISAT's collection and will bring the total number of wild sorghum accessions at IAC to 635 (Prasada Rao et al., 1995). Fifty-five percent of the collection of wild relatives of pearl millet consists of 382 accessions of ssp. *monodii* from 13 countries. The other species represented include *P. pedicellatum* (132 accessions), *P. polystachyon* (79 accessions), *P. orientale* (20 accessions), and *P. purpureum* (16 accessions).

A separately-maintained genetic stock collection includes accessions identified as sources of resistance (to major diseases, insect pests, and *Striga*), stocks with genes for specific morphological and agronomic characteristics, and cytoplasmic male-sterile lines (Prasada Rao and Mengesha, 1988). For pearl millet, four trait-specific gene pool populations have been developed at ICRISAT. These gene pools include an early maturity gene pool (EGP) with 1,143 accessions from 24 countries, a high-tillering gene pool (HTGP) with 1,093 accessions from 28 countries, a large-grain gene pool (LGGP) with 887 accessions from 19 countries, and a large-panicle gene pool with 804 accessions from 22 countries. These all have been random-mated at least four times.

All sorghum collections are regenerated during the post-rainy season at ICRISAT by selfing about 20 representative panicles from each line. Seeds harvested in equal quantities from these panicles are mixed. Landraces of pearl millet are maintained using cluster bagging. This method involves planting 120 plants of each accession. At the time of panicle emergence, five panicles, one from each of five adjoining plants, are clustered into a bag. At harvest, each accession will have 24 of these cluster bags, and an equal quantity of seed will be bulked from each panicle to reconstitute the accession. Breeding lines and genetic stocks are maintained with sibbing and selfing. The cluster bag technique has been used for a

number of years, but phenotypic evidence indicates that the technique may be inadequate to maintain the variability of an original accession. Thus, studies have been initiated at ICRISAT to evaluate the adequacy of this procedure. At harvest, the moisture content of the seeds of both sorghum and pearl millet is about 8-10%, so no additional drying is needed for medium-term storage. A bulk sample of about 500 g of each sorghum and pearl millet accession is preserved in an aluminum can in the medium-term storage facility (at about 4°C and 20% relative humidity). An initial moisture test is conducted on the seeds in medium-term storage, and any of those with viabilities above 95% are dried to 6-7% moisture content in a dryer at 15°C and 15% RH. These accessions are placed in moisture proof aluminum foil packets that are vacuum sealed and stored in long-term storage (-20°C). The exact requirements for the initial viability test to move an accession into long-term storage has been changed to 90% as of 1996. Specifically, 17% of the sorghum collection and 23% of the pearl millet collection have been put into long-term storage (Tables 4 and 5). A higher percentage of the two collections has been tested for viability, 44% for sorghum and 31% for pearl millet. Of those accessions tested for both crops, about 90% had viabilities of 85-100%.

Another important aspect of the ICRISAT genetic resource effort is the distribution of samples of requested accessions. Across all mandate crops since 1992, about 49% of the requests have come from within ICRISAT or other CGIAR Centers; about 43% come from national program scientists in developing countries, and about 1% come from scientists in developed countries. The majority of the germplasm sent from ICRISAT goes to international and national programs; only 2% of all requests have been from the private sector, and none of these were from developed countries. This dis-

Table 4. Status of *Sorghum* Accessions at ICRISAT.

	Number	Per Cent
Accessions in ICRISAT	35,643	
Accessions in long-term storage	5,955	17
Tested for germination	15,574	44
85 to 100%	14,080	90
51 to 84%	1,360	9
1 to 50%	134	1

Table 5. Status of *Pennisetum* accessions at ICRISAT.

	Number	Per Cent
Accessions in ICRISAT	21,195	
Accessions in long-term storage	4,866	23
Tested for germination	6,579	32
85 to 100%	5,934	90
51 to 84%	572	9
1 to 50%	73	1

tribution of requests may change in the future if the role of the private sector increases in the area of basic germplasm enhancement. At this time, research is being conducted primarily by public programs.

The greater utilization of the genetic diversity in developing sustainable solutions to basic crop constraints or enhancing productivity will be critical in the future. Increasing both the utilization of the sorghum and pearl millet collection and access to the information available on the collections will greatly contribute to this effort. ICRISAT has been involved with a CGIAR System-Wide Research Program (SGRP) to enhance the availability of the databases on the collections. The SINGER (System-wide Information Network for Genetic Resources) Program has resulted in the consolidation and entry of passport and a limited amount of characterization data for the sorghum and pearl millet collections. The primary objective of SINGER has been to develop an effective way to link the databases of the CGIAR Centers and allow searching across the Centers through a common user interface for data on specific collections, their transfer to collaborators, and access to the characterization data. SINGER will result in a more compatible and user-friendly format for managing these data at ICRISAT. This objective is being addressed, and the databases for these two collections should be available through a number of different means. This effort also will depend on a continuing effort to collect characterization data at ICRISAT or from specific country evaluations, and to consolidate information about specific accessions. The databases will need to be continually updated by both ICRISAT scientists and other users in the scientific community. This ability to easily and rapidly share information on the potential benefit of an accession is critical to the greater utilization of sorghum and pearl millet germplasm. These databases also will need to be compatible with other information systems, such as GIS databases, and other germplasm databases, such as GRIN or plant genome databases that have information for sorghum and pearl millet.

Core Subsets

When a scientist determines that genetic variation for a desired trait is inadequate in the available germplasm, new accessions are needed that will provide the highest probability of identifying useful source materials with minimum screening. Sometimes this can be achieved by obtaining accessions from an area where the problem has been endemic for many years, e.g., low soil pH. A list of candidate accessions often can be generated when appropriate information is in the database.

In other cases, especially for new pathogen strains or insect biotypes, searching database information is of little or no value. When the scientist must search within the crop collection for the desired trait, an initial screening of a diverse but smaller subset may reduce time and costs. The idea of developing such a subset was proposed by Frankel (1984) and further developed by Brown (1989a, b; 1995). They suggest that "a core collection consists of a limited set of accessions derived from an existing germplasm collection, chosen to represent the genetic spectrum of the whole collection. The

core should include as much as possible of its genetic diversity." The core subset is suggested to be about 10% of the crop collection, but may vary from 5% for very large collections to 50% or more for very small collections, with about 3,000 suggested as a maximum number.

Brown (1989a) recommended stratified sampling methods when establishing core collections. Grouping begins with taxonomic affinity (e.g., species, subspecies, cytological races). Accessions within each taxon can then be assigned to strata based on ecogeographic zones and genetic characteristics (e.g., ploidy level, photoperiod response, races). In some crops, country of origin (or region of adjacent countries) may be the only available means for developing preliminary groups.

Development of a useful core subset may involve the following steps: 1) assembling and reviewing passport data and other information for establishing nonoverlapping groups, 2) assigning accessions to appropriate groups, 3) choosing accessions for the preliminary core subset from each group, and 4) collecting data on phenotypic and genetic traits for accessions in the preliminary core and using multivariate analytical methods to construct clusters and dendrograms to elucidate systematic and statistical genetic relations for further refinement of the core subset.

Proportional sampling within each group may provide a more representative sample of the total genetic diversity in the core subset than would a completely random sampling from the crop collection. Once the number needed from each group has been determined, accessions for the core subset are usually chosen randomly within each group. However, some curators are choosing accessions with more desirable agronomic traits within each group. Clusters generated by multivariate analyses of morphological traits and molecular data may provide a better understanding of patterns of genetic divergence and diversity and will often identify ecogeographic regions that have not been adequately sampled, especially when the origin of each accession in the core is plotted geographically. This information may be valuable in planning future acquisitions.

The core collection concept has gained wide acceptance and core collections are being developed in many countries (Hodgkin et al., 1995; Knupffer and van Hintum, 1995). The NPGS is developing a core subset for each of the major crop collections (Erskine and Muehlbauer, 1991; Holbrook et al., 1993; Diwan et al., 1994).

A sorghum core collection has been established at ICRISAT (Prasada Rao and Ramanatha Rao, 1995) by stratifying the total world collection geographically and taxonomically into subgroups. Accessions in each subgroup were then clustered into closely related groups based on characterization data, using principle components analysis. Representative accessions from each cluster were drawn in proportion to the total number of accessions present in that subgroup to form a sorghum core collection of 3,475 accessions (approximately 10% of the total world collection). Designation of this core subset will not affect the conservation of the total world collection of sorghum germplasm at ICRISAT and at other centers where duplicate sets are conserved.

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