Pearl millet germplasm collection, conservation, characterization and utilization in crop improvement

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1. Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important food and forage crop in Africa and Asia, and forage in Americas. It is primarily grown for grain, but is also valued for fodder (both stover and green fodder) and poultry feed. Pearl millet grains have protein ranging from 5.8 to 20.9%. Traditionally, pearl millet grains are usually used in the preparation of conventional foods such as unleavened flat breads (chapati), fermented breads (Kisra, injera, dosa etc), porridge, mudde or dumpling, biscuits, snacks, malt and opaque beer. It is probably the world's hardiest crop and has great potential because of its suitability to the extreme limits of agriculture. Pearl millet is mainly cultivated in Niger, Nigeria, Burkina Faso, Togo, Ghana, Mali, Senegal, Central African Republic, Cameroon, Sudan, Botswana, Namibia, Zambia, Zimbabwe and South Africa in Africa and India, Pakistan and Yemen in Asia. Interest in pearl millet is increasing because it grows well in poor dry land and sandy soils with few inputs, has a high water use efficiency and can be grown in more than one season with low production costs. The future importance of pearl millet is expected to increase under various climate change scenarios (Lane et al., 2007). The success in crop improvement programs depends largely on the extent of genetic variability available to the researchers. Therefore, to ensure the availability of wide genetic base, collection and assembly, conservation and maintenance, characterization and evaluation, documentation and distribution of pearl millet genetic resources is very important.

2. Origin and Domestication

Considering the diversity and present distribution, Harlan (1971) and Harlan et al. (1975) suggested a defused belt stretching from western Sudan to Senegal as the center of origin for

pearl millet. Though, some researchers reported multiple domestications for pearl millet, it is believed that pearl millet was domesticated some 4000 years ago at its place of origin. From there it reached eastern Africa and then spread to India some 3000 years ago and to southern Africa 2000 years ago (Brunken et al. 1977).

3. Genetic erosion

Pearl millet is endowed with enormous genetic variability for various morphological traits, yield components, adaptation and quality traits. The genetic variability accumulated over centuries is gradually getting eroded, mainly due to replacement of landraces by improved cultivars, natural catastrophes (droughts, floods, fire hazards etc.), industrialization, human settlements, over grazing, destruction of plant habitats for irrigation projects, dams etc.. Realizing the threat to genetic diversity of its mandate crops, ICRISAT responded by establishing a Genetic Resources Unit in 1979 with the objectives of germplasm assembly, conservation and maintenance, characterization and evaluation, documentation and distribution of its mandate crops (sorghum, pearl millet, chickpea, pigeonpea and groundnut), their wild relatives and six small millets (finger millet, foxtail millet, barnyard millet, kodo millet, little millet and proso millet) germplasm for present and future utilization in crop improvement. Different activities of genetic resources are presented in Figure 1 (Kameshwara Rao and Bramel 2000).

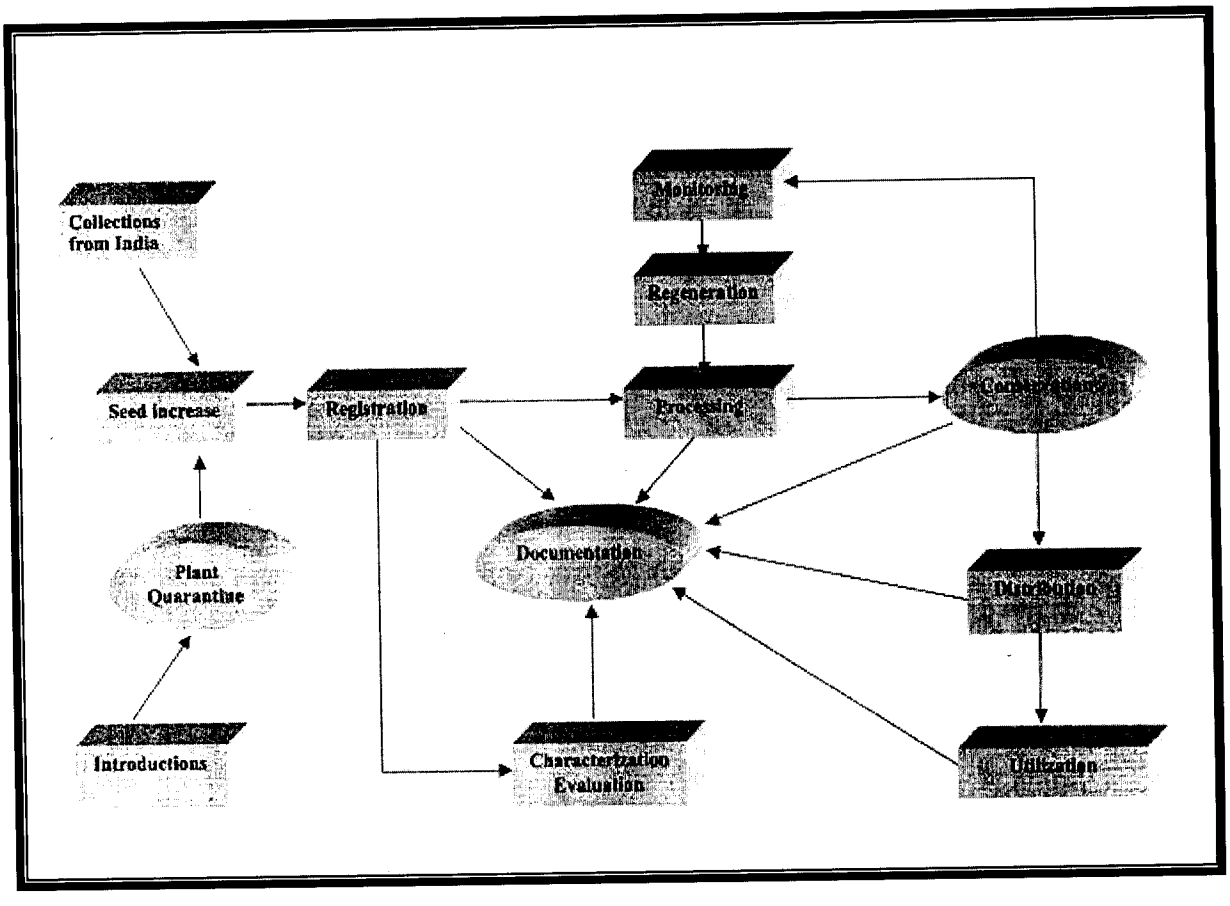


Figure 1. *Operational flow chart showing different activities of genebank*

4. Germplasm Assembly

Assembly of germplasm is the most important among all activities of genetic resources. Pearl millet germplasm was assembled by i) introducing the material that was already gathered at various places across the world and ii) launching germplasm collection missions in priority areas.

4.1 Introduction of germplasm

After assuming the responsibility of serving as world repository for the germplasm of its mandate crops, ICRISAT had made concerted efforts to introduce the pearl millet germplasm

that was assembled at different national and international institutes, universities, NARS etc. A total of 65 organizations contributed 10, 764 accessions including those contributed by different disciplines at ICRISAT, in different years. The major donors of pearl millet germplasm (>200 accessions) include: Institute Francáis dé Recherché Scientifique pour le development en Cooperation (ORSTOM), French organization (2,178); Rockefeller Foundation, New Delhi, India (2,022); IBPGR, Rome, Italy (974); Institut D Economie Rurale (IER), Bamako, Mali (499); Ministry of Agriculture, Sudan (482); Ahmadu-Bello Univ-IAR, Samaru, Nigeria (239); USDA, USA (237); PKV, Rahuri, Maharashtra, India (234) and AICPIP, Kanpur, Uttar Pradesh, India (207).

4.2. Germplasm collection

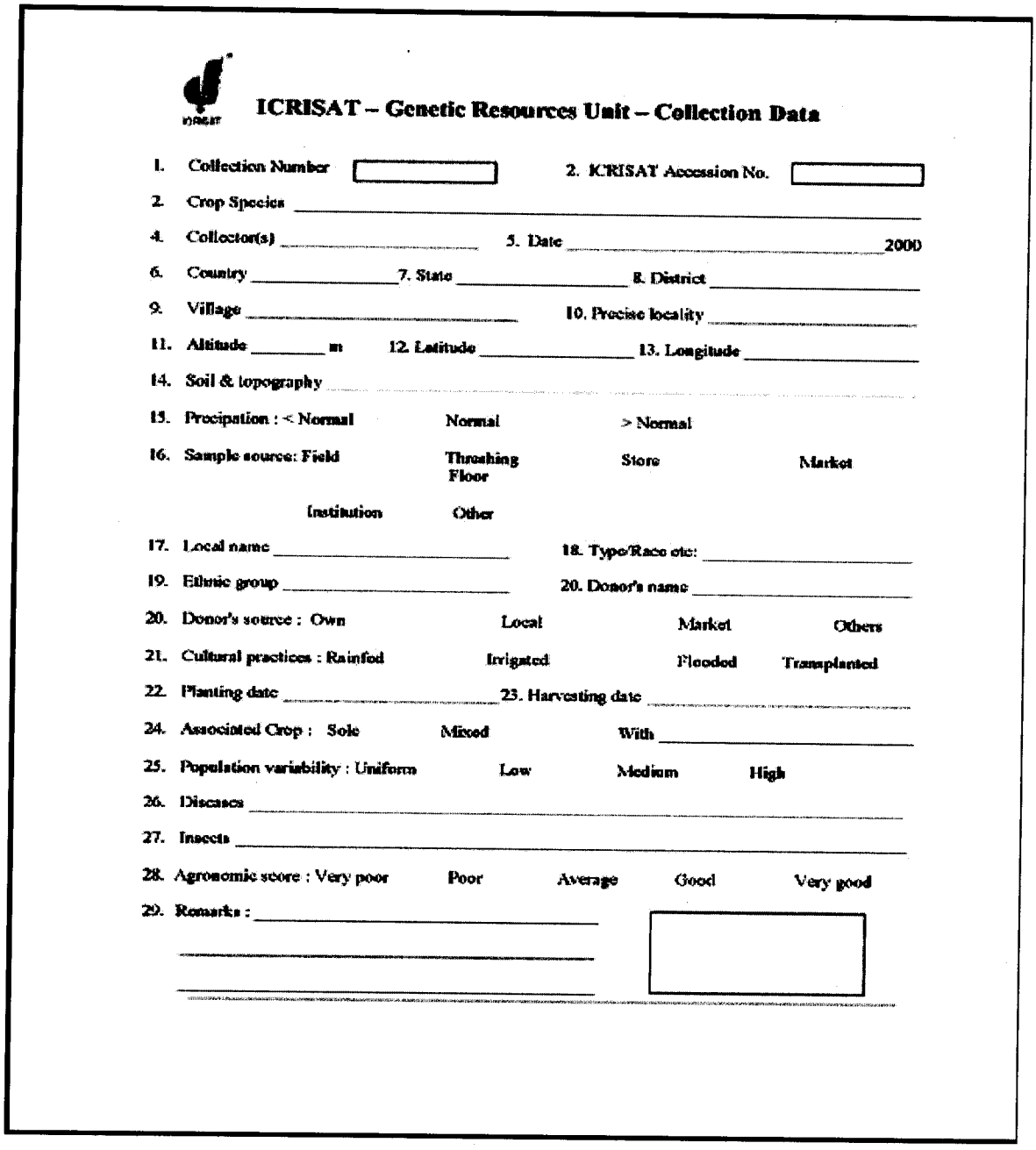
Collecting germplasm is expensive. Therefore, it is important to review the past collections of the targeted crop before embarking on a collection trip. If the targeted area was already explored then efforts should be made to obtain the collected samples. Most of the early collections have lost their genetic identity because of poor maintenance and that was the reason Harlan (1973) suggested fresh systematic collection of pearl millet germplasm. In response, ICRISAT planned for systematic collection of pearl millet germplasm in different countries as per the priority areas identified in collaboration mainly with International Plant Genetic Resources Institute (IPGRI), Rome, Italy, National Agricultural Systems (NARS), universities and non-governmental organizations. Mostly, the germplasm collecting team consisted a germplasm scientist from ICRISAT, representative from collaborating organization and a local expert having knowledge of targeted region.

4.2.1. Germplasm collection kit

Important items of the germplasm collection kit include: collection data sheets, import permit (required if the collection is outside India), Geographic Positioning System (GPS), road map, camera, hand lens, secateurs, cloth bags, harvesting bags and seed envelops, first aid kit and emergency medicines, and vehicle repair kit and important spare parts.

4.2.2. Sampling procedures

Mostly, the timing of collection mission coincided with the harvesting time. Sampling strategy depends on the crop diversity in the targeted area, altitude, cropping systems, ethnic groups and communities in the region etc. About 5-cm portion of a panicle from about 50-100 plants was taken to constitute a sample. Sometimes, samples especially interesting to breeders were also taken for immediate use in crop improvement. At the time of taking samples, information on farmers' name, important plant characteristics, history, source, biological status of sample and precise location, province, country, geographic coordinates, altitude of collection site, donor institute name, donor country etc. was also collected on collection data sheets (Fig. 2). An alpha numeric number comprising of two or three alphabets abbreviating the names of collectors followed by continuous numeric number was assigned to each sample. Herbarium of mandate crop wild relatives was prepared. Collected samples were dried, threshed and clean seed was shared among the collaborating organizations. So far, ICRISAT has launched 212 collection missions for all its mandate crops and could collect 10,830 pearl millet samples during 76 collection missions in 28 countries.



ICRISAT – Genetic Resources Unit – Collection Data

1. Collection Number ☐ 2. ICRISAT Accession No. ☐
2. Crop Species _____
4. Collector(s) _____ 5. Date _____ 2000
6. Country _____ 7. State _____ 8. District _____
9. Village _____ 10. Precise locality _____
11. Altitude _____ m 12. Latitude _____ 13. Longitude _____
14. Soil & topography _____
15. Precipation : < Normal Normal > Normal
16. Sample source: Field Threshing Floor Store Market
Institution Other
17. Local name _____ 18. Type/Race etc: _____
19. Ethnic group _____ 20. Donor's name _____
20. Donor's source : Own Local Market Others
21. Cultural practices : Rainfed Irrigated Flooded Transplanted
22. Planting date _____ 23. Harvesting date _____
24. Associated Crop : Sole Mixed With _____
25. Population variability : Uniform Low Medium High
26. Diseases _____
27. Insects _____
28. Agronomic score : Very poor Poor Average Good Very good
29. Remarks : _____

Figure 2. *Format of germplasm data collection sheet*

4.2.3. Quarantine procedures for acquisition

Donations and collections from outside India are subjected to regulations of Indian Plant Quarantine. To introduce the germplasm samples, Indian Plant Quarantine Authority, National

Bureau of Plant Genetic Resources (NBPGR) will issue an Import Permit. Import Permit generally gives the name of the person granted permission to import, the sender's name, the port of entry, the quantity of material allowed and additional declaration if any. A Phytosanitary Certificate issued by the National Plant Quarantine Services of the country in which the germplasm was collected is an important document containing the information on the health of the seed, treatment, additional declarations required by the government of India and a description of the consignment. Import Permit and the phytosanitory certificate should accompany the seed material arriving in India. All consignments should be addressed to the Director, NBPGR, Pusa Campus, New Delhi 110 012. Director, NBPGR sends the same to the Officer-in-charge, NBPGR regional station, Rajendranagar, Hyderabad, Andhra Pradesh 500 030 for necessary processing. NBPGR Regional Station conducts seed health tests, gives mandatory seed treatments as per guidelines and releases the consignments for growing in the Post-Entry Quarantine Isolation Area (PEQIA) or green house at ICRISAT, Patancheru, India, for surveillance from sowing until harvest to detect and avoid the introduction of exotic pests and pathogens. The pearl millet seeds are harvested from plants that are visibly free from downy mildew, smut and ergot disease and released to the ICRISAT (Chakrabarty et al. 2005)

4.2.4. Integrating germplasm accessions

On receipt of samples at ICRISAT genebank, each sample is assigned an IP (International *Pennisetum*) number if the seed quantity is above critical level (>50 g) and the passport information is adequate (at least identity and country of origin). This number is assigned only once to any sample and not assigned to any other sample. After registration, the operational sequence to integrate an accession into the genebank involves cleaning, moisture determination,

drying, viability testing and packing as per recommended international standards. By the end of year 2007, the R S Paroda Genebank at ICRISAT had registered 21,594 accessions of pearl millet germplasm from 51 countries, including 750 accessions of wild relatives (Table 1). This is the largest collection of pearl millet germplasm assembled at any one place in the world. Mapping of 15,980 landraces and 661 wild accessions for which geographic coordinates data are available, revealed extensive collection of pearl millet germplasm in the primary and secondary centers of diversity. Wild relatives germplasm was collected mostly in the northern part of the defused belt of pearl millet origin and in India, which is the secondary center of diversity for pearl millet (Fig. 3). Although the collection is impressive, exploration for pearl millet germplasm cannot be considered as complete. There is a need to identify geographical gaps, hot spots for trait-specific germplasm etc. for future explorations in order to make the pearl millet germplasm collection nearly complete.

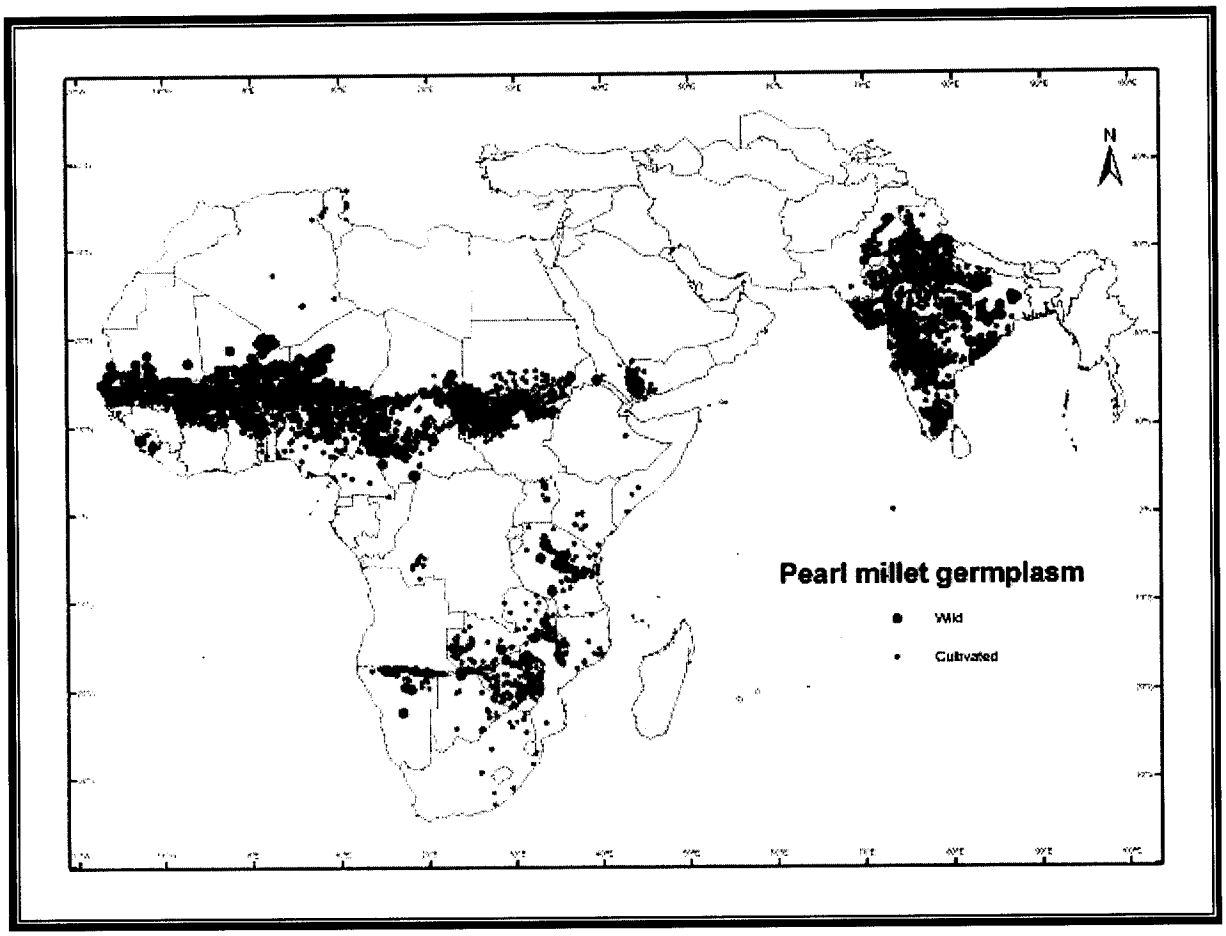


Figure 3. Geographical distribution of cultivated and wild relatives germplasm in the world collection of pearl millet.

Table 1. Geographical distribution of pearl millet germplasm in the world collection at ICRISAT genebank (1 Jan. 2008)

Country	Cultivated	Wild	Total
Algeria	5		5
Australia	8		8
Benin	46		46
Botswana	82		82
Brazil	2		2
Burkina Faso	860	7	867
C. African Rep.	142	10	152
Cameroon	911	85	996
Cape Verde	2		2
Chad	97	37	134
Congo	8		8
Ethiopia	2	1	3
France	11		11
Gambia	15		15
Germany	3		3
Ghana	283		283
ICRISAT	1367	3	1370
India	6468	142	6610
Kenya	98	1	99
Korea	1		1
Lebanon	108		108
Lesotho		4	4
Malawi	298	12	310
Maldives	1		1
Mali	1048	109	1157
Mauritania	6	31	37
Mexico	10	1	11
Morocco	4		4
Mozambique	31	2	33
Myanmar	10		10
Namibia	1118	10	1128
Niger	1132	176	1308
Nigeria	2065	9	2074
Pakistan	168	2	170
Russia & CISs	15		15
Senegal	393	12	405
Sierra Leone	59	1	60
Somalia	4		4
South Africa	162	3	165
Sri Lanka		2	2
Sudan	587	27	614
Tanzania	478	25	503
Togo	520		520
Tunisia	6		6
Turkey	2		2
Uganda	118	1	119
United Kingdom	31	1	32
USA	219	10	229
Yemen	290	3	293
Zaire	11	3	14
Zambia	155	7	162
Zimbabwe	1384	13	1397
Total	**20844**	**750**	**21594**

4.2.5. Sample sources in the collection

Generally, the sources of germplasm include farmer's fields, farmer's stores, threshing yards, commercial markets, institutions and in wild. Primary aim during the collection mission is to collect field samples. In some areas, launching of collection mission does not coincide with the maturity of the crop in fields, then the samples are collected from other sources. Summary of ICRISAT pearl millet germplasm collection for sample source reveals 10,201 accessions from different institutions, 6,537 accessions from farmers field, 1,681 accessions from commercial markets, 1,357 accessions from farm stores, 479 accessions from threshing floors and 750 accessions as wild. A total of 589 accessions have no information on sample source.

4.2.6. Biological status of the collection

The collecting team can better decide biological status of the accessions. Therefore, the recording of sample related information while taking the sample is very important. Mostly, the samples received from farmers are landraces. Biological status of ICRISAT pearl millet germplasm accessions indicates 18,447 landraces, 2,268 breeding materials, 129 advanced cultivars and 750 wild accessions in the collection.

5. Conservation and maintenance

Under ambient conditions, pearl millet seeds loose viability rapidly necessitating their frequent rejuvenation, which is highly expensive and involves the risk of losing of accession integrity due to out-crossing, selection pressure, possibility of mechanical mixture etc. Loss of viability also leads to accumulation of genetic damage in the surviving seeds causing genetic drifts in heterogeneous germplasm accessions due to differential survival of the constituent genotypes

and selection pressure (Kameshwara Rao and Bramel 2000). It is established that temperature and moisture content of the seeds influence seed deterioration during storage, and longevity of seeds can be dramatically improved by controlling these factors. To maximize the longevity and preserve the genetic integrity of accessions, pre-dried seeds are packed in moisture-proof containers and stored in chambers with controlled environment.

5.1 Facilities at ICRISAT genebank for germplasm conservation and maintenance

5.1.1. Short-term store

It is maintained at 18–20°C and 30–40% RH for temporary holding of seeds while they are dried and prepared for subsequent transfer to drying room, medium- and long-term storage.

5.1.2. Medium-term store

It is maintained at 4°C and 20–30% RH for holding active collection. Rust-proof aluminum cans with screw caps and rubber gaskets are used to store about 400 g of pearl millet seeds. Active collection comprises all accessions (21,594) and the seed is available for immediate utilization and distribution. Viability of the conserved seeds can be maintained above 85% for 15-20 years.

5.1.3. Long-term store

It is maintained at –20°C and stores base collection. Re-sealable aluminum foil packets are used to pack about 75 g of seed in base collection or long-term storage. Of the 21,594 accessions, only 17,670 are preserved in long-term storage and the remaining accessions would be transferred in the near future. Seeds are not distributed from base collection. Under long-term storage, viability of conserved seeds can be maintained above 85% for about 50 years.

5.1.4. Seed drying room

Seed drying involves reduction of moisture content to the recommended levels for storage. Seed was dried after thorough cleaning in seed drying room. A seed-drying room and two drying cabinets are operated at 15°C and 15% RH to dry the seed for medium-and-long term storage to a desired level of about 7-9% and 4-5% moisture content, respectively. For drying, seeds are packed in muslin cloth bags and kept in open racks of drying room and the seed moisture content is tested at regular intervals till the seeds are dried to the required moisture level.

5.1.5. Seed biology lab

A seed laboratory is available for conducting germination tests, seed research and cytological work. Monitoring seed viability of active collection carried out at 5-year interval and those conserved as base collection at 10-year intervals. The top of paper method is used to test the viability of pearl millet seeds (Kameshwara Rao and Bramel 2000), which involves germinating pearl millet seeds on top of moist paper (Whatmen Grade 181) in petri dishes of 9 cm size. Healthy seedlings are counted.

5.1.6. Greenhouse

An air-cooled greenhouse with an area of 402 m^2 for regeneration and maintaining wild species of all mandate crops is available.

5.1.7. Field genebank

Wild relatives of pearl millet are regenerated in field genebank. Wild species (*P. clandestinum*,

P. macrostachyum, P. macrourum, P. lanatum, P. squamulatum, and *P. purpureum*) that do not produce seed or produce very less seed are maintained as live plants in the field genebank. Initially, the seeds are germinated in paper cups and seedlings are transplanted after 15-20 days in the field genebank. Perennial species are pruned upto 30 cm from ground level during rainy season to avoid mixing with adjacent accessions.

5.1.8. Field

Sufficient precision field space is available on ICRISAT campus at Patancheru for regeneration, characterization and evaluation of pearl millet germplasm.

The storage chambers are constructed on a modular principle with prefabricated panels and have mobile shelving, each capable of accommodating about 20,000 seed accessions. The genebank has a standby generator to cope with long periods of power failure. Each medium- and long-term storage room has standby refrigeration and dehumidification systems. In addition, audible and visual electronic alarms and fire warning systems help maintain the desired conditions and safeguard the germplasm against fire hazards (Kameshwara Rao and Bramel 2000). Management of seed collections requires that germplasm accessions be maintained with a high proportion (>85%) of viable seeds. This involves storage under optimal conditions, periodic monitoring of seed viability and quantity and regenerating them when the situation warrants.

5.2. Seed health

Viability of germplasm conserved in the medium term store is tested at 5-year intervals, whereas those conserved in long-term store at 10-years intervals. Germination tests of conserved pearl millet germplasm conducted over 25 years indicated 97 percent of the total accessions had

satisfactory levels of viability (>85%). Seeds processed for conservation are tested for seedborne fungi before transferring to medium-term and long-term stores. Common seed health tests include, visual examination under magnifying lens (2x) or under low-power stereo-binocular microscope to detect sclerotia, smut balls, fungal spores and other fructifications. Blotter tests and or Agar tests are conducted to identify seedborne fungi. If the percentage of seeds infected by one or more of the fungi like *Alternaria, Aspergillus, Cladosporium, Curvularia, Penicillium, Fusarium, Macrophomina, Phoma and Rhizopus* spp. is >25%, then the seeds are considered unsuitable for conservation (Kameshwara Rao and Bramel 2000).

5.3. Germplasm regeneration

Regeneration of accessions is required when the seed viability and or the seed quantity in active collection is below the critical level (<1/4 of total quantity and/or <85% viability). Pearl millet germplasm regeneration is carried out during the postrainy season at ICRISAT farm, Patancheru. To minimize the genetic drifts, a samples size of about 160 plants for each accession are grown in 4 rows of 4 m long each with a spacing of 75 cm between rows. The seed is sown using tractor mounted four-cone planter. The crop is thinned 15-20 days after sowing to give approximately 10 cm spacing between plants within a row. Irrigation is provided at regular intervals. Fertilizers are applied at the rate of 100 kg N ha^{-1} and 40 kg P_2O_5 ha^{-1}.

Being a cross-pollinating crop, regeneration of pearl millet germplasm accessions is expensive and involves the risk of losing accession integrity due to foreign pollen, mechanical mixtures, genetic drifts, genetic shifts and mutations. During regeneration, the genetic integrity is maintained by cluster bagging method of pollination control for landraces, selfing for genetic stocks and sibbing for male sterile lines (Rai et al.1997). In cluster bagging method, panicles

from 2-4 adjacent plants in a row are covered in one parchment paper bag. This allows some cross-pollination between plants of the same accession to overcome the problem of inbreeding depression caused if individual plants are selfed. Cluster bagging method is cost-effective to regenerate a large number of accessions in a season.

6. Characterization and evaluation

Germplasm collection is of little value unless it is characterized, evaluated and documented properly to enhance its utilization in crop improvement. Characterization refers to the recording of distinctly identifiable morphological characteristics, which are highly heritable and expressed in all environments (for e.g. Flower color, stem color) and do not show genotypic by environment interactions. Therefore, accessions do not require characterization more than once, unless it is for checking accession integrity. Characterization is distinguishable from preliminary evaluation, which is recording of important agronomic characters, and is done mostly simultaneously with characterization. In addition, crop specialists working in various disciplines of ICRISAT are involved in further evaluation, which refers recording of observations on reaction of accessions to biotic and abiotic stresses. Systematic characterization and evaluation of germplasm will provide the basis for:

- estimation of the extent of variation within the accession/species.
- classification of the germplasm accessions
- identification of new/useful traits in the crop species
- identification and development of interrelationship among geographical and climatic groups of cultivars, and
- identification of obvious duplicate accessions.

To accomplish these objectives, a multi-disciplinary approach is followed at ICRISAT and the data generated in various disciplines are summarized and maintained in the pearl millet germplasm characterization database.

The world collection of pearl millet germplasm maintained at ICRISAT was characterized in batches of 500-1000 every year at ICRISAT farm, Patancheru, in alfisols during the rainy (June-October) and postrainy (November-March) seasons from 1974 through 2007. Germplasm accessions were grown in an augmented block design using systematic checks, repeated for every block of 20 test accessions. Each accession was sown in two 4-m long rows with 75 cm spacing between rows. The seed was sown using tractor-mounted four-cone planter. The crop was thinned 15-20 days after sowing to give approximately 10-cm spacing between plants within a row. Life saving irrigations were provided in the rainy season, and in the postrainy season irrigation was provided at regular intervals. Fertilizers were applied at the rate of 100 kg N ha^{-1} and 40 kg P_2O_5 ha^{-1}.

By the end of 2007, all cultivated accessions were characterized and evaluated for 23 morphoagronomic characters following the pearl millet descriptors (IBPGR and ICRISAT 1993). Time to 50% flowering, plant height, panicle length and thickness were recorded during both rainy and postrainy seasons, whereas number of nodal, productive and total tillers, panicle exsertion, synchrony of panicle maturity, panicle shape, spikelet density, bristle length, grain yield potential, fodder yield potential and overall plant aspect was recorded only during the rainy season. Synchrony of panicle maturity, spikelet density, bristle length, grain yield potential, fodder yield potential and overall plant aspect was visually scored on a 1 to 9 scale, where 1 is most undesirable and 9 most desirable. Observations on grain characters, such as 1000-seed mass, seed shape, seed color and endosperm texture was recorded during the postrainy season

after harvesting.

In addition to the regular characterization, evaluation and further evaluation at ICRISAT center, sets of selected pearl millet germplasm are also evaluated for important agronomic characteristics at different locations in India and several other countries in Africa to realize the true potential of the accessions and to facilitate the selection of genotypes by researchers. Germplasm catalogs have been prepared using the multilocation evaluation data. Full potential of germplasm can be known only when the accessions are characterized and or evaluated at or near the place of its origin during suitable season.

7. Diversity in the collection

Large phenotypic diversity in collection has been observed for several characters. There are accessions in the collection, which can flower as early as in 33 days and as late as in 159 days in the rainy season. Similarly, plant height ranges from 30 cm to 490 cm with a mean of 246.0±0.46 Total tillers per plant varies from 1 to 35 (Table 2).

Table 2. Range of variation for important agronomic characters of pearl millet germplasm evaluated at ICRISAT in rainy (R) and postrainy (PR) season

Character	Minimum	Maximum	Mean	Variance
Days to flower-R	33.00	159.00	72.81+0.17	569.75
Days to flower-PR	32.00	138.00	71.37+0.08	123.47
Plant height (cm)-R	30.00	490.00	246.20+0.46	4427.63
Plant height (cm)-PR	25.00	425.00	160.10+0.25	1311.57
Total tillers per plant (no.)-R	1.00	35.00	2.69+0.01	3.13
Productive tillers per plant (no.)-R	1.00	19.00	2.11+0.01	1.28
Panicle exertion (cm)-R	-45.00	29.00	3.74+0.05	43.34
Panicle length (cm)-R	5.00	135.00	28.22+0.07	112.90
Panicle length (cm)-PR	4.00	125.00	25.44+0.07	109.62
Panicle width (mm)-R	8.00	58.00	24.00+0.03	22.75
Panicle width (mm)-PR	9.00	61.00	22.90+0.04	25.73
1000-Seed mass (g)_PR	1.50	21.25	8.53+0.02	4.98

Panicle size and shape are the other important traits for which diversity was very high. (Fig. 4). 1000-seed mass varied from 1.50-21.25 g. Distribution of different classes in the qualitative traits has indicated occurrence of nine panicle shapes, five seed shapes and ten seed colors in the entire collection (Table 3). Accessions having candle-shaped panicles occurred in maximum frequency (11,132 accessions) followed by cylindrical (4,960) and lanceolate shape (2,564). A total of 2,721 accessions produced compact panicles. Only 92 accessions produced long bristles, a trait considered useful against bird damage. Among the seed colors, accessions having grey (10,473 accessions) and grey brown (6,687 accessions) color seeds are the predominant in the collection. However, accessions producing seeds of light colors such as ivory (174) and cream (760) and yellow (571) were also found in the collection (Fig. 4). Seeds of 2,681 accessions produced almost corneous seeds. In the entire collection, only 141 accessions for fodder yield potential and six accessions for seed yield potential scored maximum ratings (score 9) (Table 3).

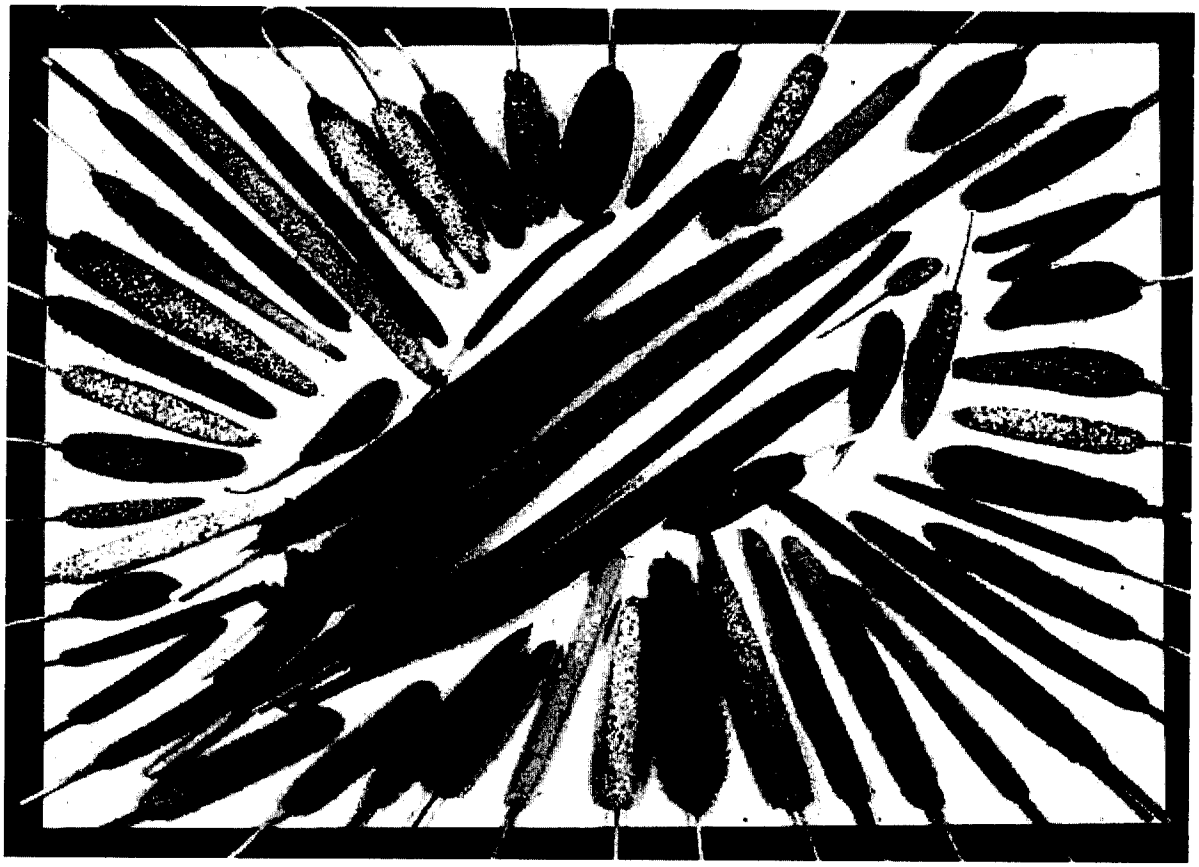


Figure 4. Diversity for panicle size, shape and color in pearl millet germplasm collection

Table 3. Frequency distribution of different qualitative traits in the world collection of pearl millet at ICRISAT genebank, Patancheru, India

Character	No. of accessions[1]
Panicle shape	
Cylindrical	4969 (23.85)
Conical	1354 (6.5)
Spindle	372 (1.79)
Club	62 (0.3)
Candle	11132 (53.43)
Dumb-bell	273 (1.31)
Lanceolate	2564 (12.31)
Oblanceolate	60 (0.29)
Globose	50 (0.24)
Spiklet density	
Loose	729 (3.5)
Intermediate	17386 (83.4)
Compact	2721 (13.1)
Bristle length	
Short	20004 (96.0)
Medium	740 (3.6)
Long	92 (0.4)
Seed shape	
Obovate	3594 (17.25)
Oblanceolate	3488 (16.74)
Elliptical	4937 (23.69)
Hexagonal	3397 (16.3)
Globular	5420 (26.01)
Seed color	
Ivory	174 (0.84)
Cream	760 (3.65)
Yellow	571 (2.74)
Grey	10473 (50.26)
Deep grey	1415 (6.79)
Grey brown	6687 (32.09)
Brown	510 (2.45)
Purple	181 (0.87)
Purplish black	29 (0.14)
Mixture of White and grey	36 (0.17)
Endosperm texture	
Mostly corneous	2681 (12.9)
Partly corneous	13296 (63.8)
Mostly starchy	4859 (23.3)

Contd...

Character	No. of accessions[1]
Fodder yield potential	
1	3 (0.01)
2	54 (0.26)
3	280 (1.34)
4	968 (4.65)
5	2974 (14.27)
6	6320 (30.33)
7	7955 (38.18)
8	2141 (10.28)
9	141 (0.68)
Seed yield potential	
1	2 (0.01)
2	67 (0.32)
3	400 (1.92)
4	1867 (8.96)
5	6438 (30.9)
6	9053 (43.45)
7	2815 (13.51)
8	188 (0.9)
9	6 (0.03)
Overall plant aspect	
1	17 (0.08)
2	116 (0.56)
3	717 (3.44)
4	2591 (12.44)
5	7650 (36.72)
6	8196 (39.34)
7	1439 (6.91)
8	110 (0.53)

[1] Figures in parenthesis are percentage representation in the collection

Considerable variability for several other traits like sweet stalks (Appa Rao et al. 1981), leaf traits (Appa Rao et al. 1984; Appa Rao et al. 1987; Appa Rao et al. 1995), seed protein content (Mengesha et al. 1990), brown midrib (Gupta 1993), dwarfism (Appa Rao et al. 1986), sources of cytoplasmic-nuclear male sterility (Appa Rao et al. 1989; Rai 1995) and yellow endosperm (Hash et al. 1997) were also observed in the collection. Germplasm screening (further evaluation) efforts by different disciplines has resulted in the identification/development of

several promising/resistant sources against downy mildew (54), rust (325) (Singh 1990; Singh et al. 1990), smut (397) (Thakur et al. 1992) and ergot (283) (Thakur et al. 1993) (Table 4).

Table 4. Status of pearl millet germplasm screening for various biotic and abiotic stresses and traits of importance at ICRISAT, Patancheru, India

Stress/Trait	No. of accessions screened	No. of promising accs. identified
Cultivated		
Downy mildew resistance	3164	54
Smut resistance	1747	397
Ergot resistance	2752	283
Rust	2229	332
Drought tolerance	115	7
High seed protein content (>15%)	1270	260
Sweet stalks	892	16
Male sterility	17000	50
Wild		
Downy mildew resistance	534	220

The Shannon-Weaver (Shannon and Weaver 1949) diversity index (H′) was calculated to compare the diversity among germplasm accessions for various traits. Phenotypic diversity (H′) ranged from 0.427 (total tillers) to 0.632 (plant height in postrainy season) for quantitative traits. Among qualitative traits, diversity was maximum for endosperm texture (H′=0.772) and minimum for bristle length (H′=0.443). Diversity for qualitative traits was higher (H′=0.610±0.031) than that of for quantitative traits (H′=0.573±0.021). Averaged over all traits, the diversity index was 0.588±0.018 (Upadhyaya et al. 2008) (Table 5).

Table 5. Shannon-Weaver diversity index (H') for different characters of pearl millet germplasm assembled at ICRISAT genebank

Character	Diversity index (H')
Quantitative characters	
Days to flower-R	0.580
Days to flower-PR	0.615
Plant height (cm)-R	0.627
Plant height (cm)-PR	0.632
Total tillers (No.)	0.427
Productive tillers (No.)	0.431
Panicle exertion (cm)	0.601
Panicle length (cm)-R	0.555
Panicle length (cm)-PR	0.551
Panicle width (mm)-R	0.606
Panicle width (mm)-PR	0.631
1000-Seed weight (g)	0.613
Mean over quantitative traits	0.573±0.021
Qualitative characters	
Panicle shape	0.560
Spikelet density	0.632
Bristle length	0.443
Seed shape	0.690
Seed color	0.567
Endosperm texture	0.772
Fodder yield potential	0.648
Seed yield potential	0.587
Overall plant aspect	0.589
Mean over qualitative traits	0.610±0.031
Mean over all traits	0.588±0.018

8. Germplasm distribution

In line with the policy of the Consultative Group on International Agricultural Research (CGIAR) on plant genetic resources, ICRISAT has been distributing seeds of pearl millet germplasm accessions free to all bona-fide users who sign the Standard Material Transfer Agreement (SMTA) that prevent the recipients from claiming intellectual property rights. ICRISAT genebank has annually distributed several thousands of samples in most years. Since

1975, ICRISAT provided 52,199 samples to researchers working in different disciplines at ICRISAT, 60,268 samples to scientists in India and 30,646 samples in 79 other countries.

9. Germplasm repatriation

In certain situations, in national programs, germplasm materials of crops get lost due to lack of facilities, poor maintenance, political strife or natural disasters were repatriated to make the local germplasm available to the researchers. In this process, ICRISAT genebank has provided 922 accessions to Cameroon and 7,189 accessions to India for preservation and utilization.

10. Documentation

For each crop, passport, characterization, inventory and distribution databases are being maintained. Data from characterization and evaluation are stored in characterization database. Data from further evaluation done by different disciplines of ICRISAT and that of multilocation evaluations are provided to the genebank and added to the characterization database. Information related to the conservation of germplasm is stored in inventory database. Computerization of data was started in 1980 using the ICRISAT Data Management and Retrieval System (IDMRS), software developed at ICRISAT. Then, System 1032 was used and now all databases are maintained using Genebank Information Management System (GIMS), a system developed with MS SQL server as back end and MS visual basic as front end, for faster and more efficient data management. Passport and characterization databases can be accessed through http://SINGER.grinfo.net. Germplasm catalogs were prepared using multilocation evaluation data.

11. Germplasm utilization and impact

With the establishment of genebank at ICRISAT, the range of pearl millet germplasm available to the researchers has increased vastly. However, lack of characterization data, restricted access, inadequate linkages between genebanks and users are the major bottlenecks to the increased use of genetic resources. The lack of information on large accessions, particularly on traits of economic importance, which shows genotype by environment interaction, is the main reason for low use of germplasm. Efforts were made in the past several years to overcome some of these bottlenecks and enhance the utilization of pearl millet germplasm. Sets of selected germplasm accessions were evaluated at different locations in India and several other countries in Africa. Field days were organized and invited pearl millet scientists facilitating the selection of material for use in crop improvement programs. Four trait-specific genepools (early maturing, high tillering, large panicle and large grain) were developed (Appa Rao et al. 1998). However, it met limited success.

To further enhance the utilization of pearl millet germplasm, core collection consisting about 10% of accessions representing the diversity of entire collection was developed (Bhattachrjee et al. 2007). After eliminating uncharacterised accessions, a total of 16,063 accessions were stratified by country of origin and clusters were made in each country based on 11 quantitative characters. From each cluster, 10% of accessions were selected proportionately to form a core consisting 1593 accessions (Bhattachrjee et al. 2007). The same core was augmented by picking 10% accessions (501 accessions) from 4717 newly added germplasm and constituted a final core with 2094 accessions. A comparison of mean data using Newman-Keuls test, variance using Levene's test, and distribution using χ^2 test indicated that the variation in the entire collection of 20 766 was preserved in the revised core collection (Newman 1939; Keuls

1952; Levene 1960; Upadhyaya et al. 2008). The revised core collection was observed to be more valuable than the original core as it has sources of resistance for important diseases, such as downy mildew. The final core collection has been evaluated for important morphoagronomic traits and a mini core collection (10% of core or 1% of entire collection) will be selected following Upadhyaya and Ortiz (2001). This mini core will provide easy access to the pearl millet collection through replicated multilocational evaluation to identify parents useful for crop improvement programs.

Pearl millet wild relatives are promising for traits such as high biomass, large seeds, drought tolerance, male sterility, apomixes etc. There have been a few targeted attempts and the results have been encouraging. For example, the use of *P. glaucum* subsp. *monodii* led to the identification of new sources of cytoplasmic-nuclear male sterility (CMS) (Rai and Rao 1996). Hanna (1992) has summarized the use of this wild relative for new sources of resistance to leaf diseases, that of *P. purpureum* for sources of forage traits, and stiff stalk and restorer genes of the A_1 CMS system, and that of *P. squamulatum* Fresen for apomictic gene (Rai et al. 1997). However, there has been a general lack of interest in using wild species because of the large amount of genetic variability already available in pearl millet landraces.

The greatest impact is conserving the vanishing pearl millet germplasm and making these available for crop improvement. Besides the utilization of germplasm in ongoing research at different research institutes, universities, NARS etc., several pearl millet germplasm accessions from ICRISAT genebank have gone into the release of varieties in different countries. The impacts of such releases require quantification in different countries. In general, Indian pearl millet landraces have mainly contributed for earliness, high tillering, high harvest index and local adaptation, whereas African material has been a good source of high head volume, large seed

size and disease resistance (Anand Kumar and Appa Rao 1987).

11.1. Examples of germplasm utilization

- The most dramatic example of direct use of this landrace is the development of ICTP 8203, a large-seeded and high-yielding open-pollinated variety bred at ICRISAT, Patancheru, by selection within a large-seeded *iniadi* landrace from northern Togo (Rai et al. 1990). This variety was released as MP 124 in Maharashtra and Andhra Pradesh, and as PCB 138 in Punjab states of India in 1988. The same variety was released as Okashana 1 in Namibia during 1990 and as Nyankhombo (ICMV 88908) in Malawi in 1996. Direct selection within the same landrace led to the development of a large-seeded and downy mildew resistant male-sterile line ICMA 88004, a seed parent of an early-maturing hybrid (ICMH 356) released in India in 1993 (Rai et al. 1995).
- Okashana 2, a variety derived from a Zimbabwe local landrace IP 16504 (SDGP 1514) crossed with ICMV 87901 and ICMV 88908 was released in Namibia during 1998 (Obilana et al. 1997).
- IKMP 3, a variety released in Burkina Faso was developed from selection within the landrace IP 11381 (CVP 417) from Burkina Faso.
- IKMP 5, a variety released in Burkina Faso was developed from selection within the landrace IP 11317 (CVP 170) from Burkina Faso.
- An open-pollinated variety ICMV-IS 88102 was developed from selection within the landrace IP 6426 from Mali was released in Burkina Faso in 1993 and also released as Benkadi Nio in Mali during 1994.
- Kangara (SDMV 92040) a variety released in Namibia in 1998 was derived from two

landraces IP 17527 and IP 17531 and S2 progenies of SADC white grain composite. The same variety was also released as PMV 3 in Zimbabwe during 1998 (Obilana et al. 1997).

- Fifteen accessions identified as salinity tolerant are very useful in developing varieties suitable for saline areas. Two of the salinity tolerant accessions IP 22269 (high tillering genepool developed at ICRISAT) and IP 6098, a landrace from Niger was found as the most promising dual-purpose types with salinity tolerance, suitable for saline areas.
- IP 17672, a landrace from Togo, identified for its high level of grain iron (134 ppm) and zinc (82 ppm) content is very useful in improving the heath of poor.

12. Conclusions

The world collection of pearl millet germplasm conserved at ICRISAT genebank has fair representation from all pearl millet growing areas and the range of available pearl millet germplasm has increased considerably over a period of time. However, it needs summarization of collection and identification of gaps in the collection using GIS tools. In addition, it is also important to identify the areas of occurrence of trait-specific germplasm, particularly abiotic and biotic resistance sources. Characterization and evaluation of germplasm helped in identifying several useful and new genotypes for utilization in crop improvement programs. Since 1975, ICRISAT has provided several thousands of samples to researchers working in different disciplines at ICRISAT and to scientists in different countries. Enhanced utilization of pearl millet germplasm is critical in developing high yielding and highly adaptable cultivars suitable for different agro-climatic conditions in different countries.

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