Establishment of a pearl millet [Pennisetum glaucum (L.) R. Br.] core collection based on geographical distribution and quantitative traits

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Abstract ICRISAT conserves a large collection of pearl millet [Pennisetum glaucum L. R. (Br.)] comprising of 21,392 accessions. This includes landraces, cultivars, genetic stocks, breeding lines, and wild relatives from 50 countries. However, only a small fraction of this huge collection has been exhaustively used in the pearl millet improvement program. The objective of our research was to develop a core collection of pearl millet to enhance utilization of genetic resources in improvement programs and simplify their management. For this purpose, accessions were initially stratified according to geographical distribution followed by hierarchical clustering on 11 quantitative traits using Ward's method. This resulted in 25 distinct groups. Approximately 10% accessions were then randomly selected from each of these 25 distinct groups to form a core collection of 1,600 accessions. Different statistical methods like comparison of mean using

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Newman–Keuls test, variance using Levene's test, frequency distributions using Chi-square test, and Wilcoxon's rank-sum non-parametric test for the traits validated that the variation present in entire collection had been preserved in the core collection. The important phenotypic correlations among different traits that may be under the control of co-adapted gene complexes were also preserved in the core collection. The diversity represented in the core collection will therefore, be a guideline to breeders for a wider use of the pearl millet genetic resources available in the genebank.

Keywords Pennisetum glaucum Core collection · Genetic resources · Landraces · Phenotypic diversity

Introduction

Pearl millet (Pennisetum glaucum (L) R. Br.) is the sixth most important cereal, primarily grown for grain production in the arid and semi-arid tropical areas of Africa and Asia (Khairwal et al. [1999\)](#page-9-0). It has been used as a cereal crop for nearly 3,000 years in Africa and parts of Near East, and is grown in over 40 countries, predominantly in Asia and Africa. It is cultivated in 26 million ha in many countries of Africa namely Senegal, Mali, Burkina Faso, Niger, Nigeria, Chad, Sudan, and a few countries of Asia, particularly India. It is also

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grown in some parts of America and Australia, mainly as a forage and/or mulch component of minimum tillage-based cropping systems (FAO-STAT [2005](#page-9-0)). India is the largest producer of this crop, both in terms of area (9.1 m ha) and production (7.3 m t), with an average productivity of 780 kg ha⁻¹ during the last 5 years. Nutritionally, pearl millet is a richer source of protein, calcium, phosphorus and iron in comparison to other important cereals like sorghum, maize, rice and wheat (Khairwal et al. [1999\)](#page-9-0).

Being allogamous in nature, pearl millet accessions are highly heterogeneous reflecting high variability within and among the accessions. Protogyny and time lag between stigma emergence and anther dehiscence favor complete crosspollination leading to greatest morphological diversity. The plant features include a diverse range of plant height, time to flowering, tillering, stem thickness, fodder and grain quality, high growth rate, and adaptability to varied agroecological environments. Therefore, this wide variability maintained by the landraces can be utilized for further improvement of the crop in enhancing the genetic potential for yield and also in alleviating the biotic and abiotic stresses.

International germplasm collections play a very important role in securing genetic diversity and promoting its use. Therefore, in last few decades, emphasis has been given for preserving crop germplasm that resulted in assemblage of large collections in national and international genebanks. Similarly, the germplasm collection at the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) has in excess of 21,000 accessions of pearl millet. This germplasm is the world's most genetically diverse collection of pearl millet from 50 countries. The collection includes landraces collected from most of the pearl millet growing eco-systems and considerable number of wild relatives of the genus Pennisetum (750 accessions). However, the available diversity has not been adequately utilized in pearl millet improvement. The huge size of the germplasm collection has hindered the increased utilization of this diversity due to lack of proper evaluation data. Low use of germplasm has been also reported in other crops like wheat (Dalrymple [1986\)](#page-9-0); spring barley (Vellve [1992](#page-10-0)); maize

(Dowswell et al. [1996](#page-9-0)); groundnut (Jiang and Duan [1998](#page-9-0)); chickpea and pigeonpea (Shiv Kumar et al. [2004\)](#page-9-0); and chickpea (Upadhyaya et al. [2006\)](#page-10-0).

Like any other major crop, there are gaps in pearl millet collection and efforts are being made to collect or assemble new germplasm. In last few years, additional accessions have been acquired from six countries and at present ICRISAT gene bank hold 21,594 accessions (20,844 cultivated and 750 wild). The germplasm collections were acquired at different times and were characterized or evaluated as and when these became available. This resulted in characterization of the collection in batches of 1,000–2,000 accessions at a time for the morphological (qualitative and quantitative) traits. However, for practical plant breeding research, evaluation often requires replicated trials and agronomic traits often display genotype \times environment $(G \times E)$ interaction. This necessitates reduction of entire collection to a manageable size that can be easily evaluated to generate good data and enhance utilization. Recognizing this, Frankel [\(1984](#page-9-0)) suggested the core collection approach for enhancing the management of a large germplasm collection, facilitating the use and study of the conserved germplasm. This implies that collection would be pruned to a manageable sample, called core collection, representing the rich genetic diversity of the crop with minimum redundancy within the sample. The core collection could further serve as a working collection and also as a guide for efficient utilization of the entire collection (Tohme et al. [1995](#page-10-0); Brown [1989b\)](#page-9-0). This core collection could be extensively evaluated, and the accessions that are not included in the core collection would be designated as reserve collection (Frankel [1984](#page-9-0)).

Frankel and Brown [\(1984](#page-9-0)) and Brown ([1989a,](#page-9-0) [b\)](#page-9-0) suggested that a core collection could be established using information on the country of origin and morpho-agronomic characteristics of the accessions. Further, Brown ([1989a\)](#page-9-0) suggested that the issues to be considered while developing the core are the size, the sampling strategy, the grouping within the collection, and the number of accessions to be included in the core from each group. Using the sampling theory of selectively neutral alleles, Brown ([1989a](#page-9-0)) therefore, suggested that about 10% sample size of the entire collection with an upper limit of 3,000 per species would effectively retain about 70% of the alleles in the sample. However, Crossa ([1989\)](#page-9-0) suggested a slightly different approach for cross-pollinated crops. They proposed the use of probability models and determined optimal sample sizes with 95% probability of including at least one copy of alleles with a given frequency. For example, if there are 50 loci with four alleles each, 156 individuals are required to retain at least one copy of alleles with 95% probability and with a frequency of 0.05. Although pearl millet is highly cross-pollinated crop, however, with lack of information available on number of loci and alleles per locus, this method could not be used in developing the core collection. Therefore, the hierarchical clustering suggested by Brown ([1989b](#page-9-0)) was followed in which grouping starts with taxonomy (species, subspecies, and races) followed by grouping based on major geographical strata (country of origin, state), climate, or agro-ecological regions. The clustering within these broad geographical groups sort accessions into different clusters and the number of accessions can then be selected from each cluster depending on the strategy used. A good core therefore, would represent maximum genetic diversity with no genotypically redundant entries.

Ever since the concept on core collection is developed, a number of core collections have already been established for many crop species including perennial Glycine (Brown et al. [1987\)](#page-9-0); peanut (Holbrook et al. [1993;](#page-9-0) Upadhyaya et al. [2003\)](#page-10-0); perennial medicago species (Diwan et al. [1994;](#page-9-0) Basigulp et al. [1995](#page-8-0)); sorghum (Prasada Rao and Ramanath Rao [1995;](#page-9-0) Grenier et al. [2001\)](#page-9-0); common bean (Tohme et al. [1995\)](#page-10-0); okra (Mahajan et al. [1996\)](#page-9-0); quinoa (Ortiz et al. [1998\)](#page-9-0); Caribbean maize (Taba et al. [1998\)](#page-10-0); alfalfa (Skinner et al. [1999\)](#page-10-0); sweetpotato (Huaman et al. [1999\)](#page-9-0); potato (Huaman et al. [2000\)](#page-9-0); chickpea (Upadhyaya et al. [2001\)](#page-10-0); Uruguayan maize (Malosetti and Abadie [2001\)](#page-9-0) and pigeonpea (Reddy et al. [2005](#page-9-0)). The objective of the present study was to develop a pearl millet core collection using data on geographical origin and quantitative traits of 16,063 well-characterized cultivated accessions.

Materials and methods

Plant material

ICRISAT genebank holds 21,392 pearl millet accessions (20,642 cultivated and 750 wild) from 50 countries comprising of mostly landraces, breeding lines, and improved selections from landraces. These accessions are held in trust following the agreement signed in 1994 with the Food and Agriculture Organization of the United Nations (FAO). In the present study, 16,063 cultivated accessions (including landraces, breeding stocks, and advanced lines) from 25 countries that have data on quantitative traits were included. The accessions that do not have adequate characterization data were not included in the study. Of the 16,063 accessions, information on countries of origin was available for all the accessions. The characterization data consisted of data on 11 quantitative traits during rainy (June–October) and post-rainy (November–March) seasons. The data in two seasons were treated separately due to the large variation for the quantitative traits during these two seasons. These two seasons are characteristics of semi-arid tropical areas defined by the day-length and monsoons resulting in considerable difference in the expression of quantitative characters like days to 50% flowering, plant height, spike length, and spike thickness (Appa Rao et al. [1986\)](#page-8-0). Data for all 11 quantitative traits were available in 13,321 accessions (Table [1](#page-3-0)). Data for days to 50% flowering, plant height, spike length, and spike thickness were available on approximately 15,775 accessions and approximately 15,850 accessions during post-rainy and rainy seasons, respectively (Table [1](#page-3-0)). Data on number of productive tillers and spike exertion were recorded only during rainy seasons owing to their better expression and on 1,000-grain weight during post-rainy seasons owing to quality factors. Data were available on 15,848, 15,393, and 16,059 accessions for number of productive tillers, spike exertion, and 1,000-grain weight, respectively.

Establishment of core collection

A flowchart of the methodology used in the establishment of core collection is schematically

Traits	Number of accessions	Description				
Days to 50% flowering $(rainy)$	15,920	Number of days from field emergence.				
Days to 50% flowering (<i>post-rainy</i>)	15,775	to 50% of the plants flower				
Plant height (rainy)	15,889	Height of the plant from ground level to the tip				
Plant height (<i>post-rainy</i>)	15.776	of the spike at dough stage				
Number of productive tillers (rainy)	15,848	Number of spikes that bear panicles at dough stage				
Spike exertion (rainy)	15,393	Length between ligule of flag leaf and base of spike of primary tiller, at dough stage				
Spike length (rainy)	15,870	Length of spike measured at dough stage				
Spike length (post-rainy)	15,774					
Spike thickness (rainy)	15,871	Diameter of the spike, measured at maximum				
Spike thickness (post-rainy)	15,776	thickness, excluding bristles				
1,000-grain weight $(post-rainy)$	16,059	Thousand grain weight in grams at 12% moisture level				

Table 1 Quantitative traits used to establish the pearl millet core collection and information on number of accessions in which characterization data was recorded

presented in Fig. 1. The entire pearl millet collection consisting of 16,063 accessions was first stratified into 25 groups based on their country of

Fig. 1 Schematic representation of establishing a core collection from entire germplasm collection of pearl millet

origin (Table [2](#page-4-0)). The data on 11 traits in each group was standardized using the range of each variable to eliminate scale differences (Milligan and Cooper [1985\)](#page-9-0). A hierarchical cluster analysis was performed on the standardized quantitative data, using Ward's minimum variance method (Ward [1963](#page-10-0)) with an R^2 (squared multiple correlation) of 0.70 for grouping the accessions. The Ward's ([1963\)](#page-10-0) method was used as per the PROC-CLUSTER program in SAS (SAS Institute [1989](#page-9-0)). This method first computes a matrix based on Euclidean distances among group means and produces a dendrogram depicting successive fusion of individuals, and conclude at the stage in which all the individuals of the same group form a cluster (Romesburg [1984\)](#page-9-0). The number of groups depends on the size of collection, the intended size of the core and the dissimilarity of the groups at the lowest level of sorting. Following the proportional sampling procedure, approximately, 10% of the accessions were randomly selected from each cluster (Brown [1989b\)](#page-9-0) to be included in the core subset. The proportional allocation incorporates the alleles even with lower variance. At least one accession was included from those clusters that had less than 10 accessions.

Validation of the core selection method

Means of the entire and core collection were compared using Newman–Keuls procedure Table 2 Pearl millet germplasm accessions based on ecological regions and country

of origin.

(Newman [1939](#page-9-0); Keuls [1952](#page-9-0)) for the 11 traits. Levene's homogeneity test for variances was used to compare the core and entire collection (Levene [1960\)](#page-9-0). The frequency distributions observed for country of origin in the selected sample was compared to the expected frequency distribution from the entire collection using the chi-square test (Spagnoletti-Zeuli and Qualset [1993](#page-10-0)). The frequency deviations from the entire collection for these traits in the core sample were calculated. The deviations for country of origin were also compared by chi-square analysis. The Wilcoxon ([1945\)](#page-10-0) rank-sum non-parametric test was performed with the SAS NPAR1-WAY procedure (SAS Institute [1989](#page-9-0)), to determine if the core collection represented the entire germplasm collection for each of the 11 traits. The phenotypic correlation between different traits in core and entire collection were estimated separately to determine whether the associations which maybe under the same genetic control were conserved in the core. Phenotypic diversity was estimated by the Shannon–Weaver diversity index (SDI) (Shannon and Weaver [1949](#page-9-0)) as follows:

$$
SDI = \left(-\sum_{i=1}^{n} P_i \times \log_e P_i\right) / \log_e n
$$

where, $n =$ number of phenotypic classes for a trait, P_i = proportion of the total number of entries in the ith class.

Results and discussion

The procedure used to establish the pearl millet core collection resulted in the selection of 1,600 accessions from the entire ICRISAT germplasm collection. The composition of the core collection reflected the predominance of accessions from India and North-West Africa, both representing dry semi-arid tropical ecology (Table 2). According to Harlan and de Wet ([1971\)](#page-9-0), the greatest morphological diversity in pearl millet occurred in the dry semi-arid tropical region of North-West Africa and the entire collection accounted for 3,801 (23.7%) accessions with 399 accessions (24.9%) in the core subset. India, which is considered as the secondary center of diversity for pearl millet, accounted for 5,373 accessions (33.45%) in the entire collection and 522 accessions (32.63%) in the core collection (Table [2\)](#page-4-0). The germplasm from some of the countries like Yemen, Chad, and Sierra Leone appears to be under-represented in the collection held at ICRI-SAT genebank and consecutively in the core collection. Overall, the geographical distribution of the entire pearl millet landrace collection showed a disparity in the collection from different ecological zones. However, this represented a wide distribution of pearl millet growing areas over the world. The unequal representation of landraces from different countries may be the result of use of improved varieties instead of landraces according to farmer's needs in the past. The poor representation of some countries could also be attributed to the difficulties in collection from the fields, the political situations in the prospected areas, the socio-economic conditions of the farmers, or even the countries with civil wars. The entire landrace collection, however, fits the evolutionary patterns, nearly representing the geographical distribution and ecological zones of the crop. Further, there was a wide range of variation captured in the entire collection for all the quantitative traits based on their country of origin.

Significant differences among means of the entire collection and core collection were recorded only for plant height (rainy) (Table 3). For four traits, variances of the entire collection and core subset were homogeneous. Of the remaining seven traits, variances in the core subset were higher, except number of productive tillers, indicating that the core captured greater variation. The range for most of the characters was retained in the core sample (Table 3). Between 85% and 100% of the range of the entire collection was included in the core for plant height *(rainy* and *post-rainy)*, days to flowering (rainy and post-rainy), spike length (rainy and post-rainy) and spike thickness (rainy). For the remaining five traits, the range was between 73% and 83%. Thus, the selected core collection is representative of the entire collection.

The analysis of deviation of frequency distribution indicated homogeneity among the entire collection and the core subset for country of origin. However, some countries were represented proportionately more (Nigeria, Namibia) and some less (India, Togo, and Zimbabwe) in the core sample. The percentages of accessions selected from different countries ranged from 0.38% for Yemen to 32.63% for India, in the core

Traits	Entire collection		Core collection					
	Mean	Range	Variance Mean		N-K test Range			Variance Levene's test
Days to flowering (R)	76.0 ± 0.20	$33 - 159$	550.8	75.2 ± 0.58 NS		$33 - 157$	531.1	NS
Days to flowering (PR)	71.0 ± 0.10	$32 - 138$	140.9	71.0 ± 0.31 NS		$32 - 125$	148.7	**
Plant height (R)	245.0 ± 0.50	30–480	4.163.0	243.4 ± 1.66 **		$30 - 450$	4426.8	**
Plant height (PR)	160.0 ± 0.30	25–425	1,434.3	159.0 ± 1.10 NS		$25 - 365$	1579.6	**
Number of productive tillers	3.0 ± 0.01	$1 - 19$	3.3	2.7 ± 0.04 NS		$1 - 15$	2.8	**
Spike exertion	$3.6 \pm 0.10 - 45 - 29$		45.1	3.2 ± 0.17 NS		$-32-22$	48.9	**
Spike length (R)	28.2 ± 0.10	$5 - 120$	119.1	28.3 ± 0.29 NS		$6 - 120$	135.8	**
Spike length (PR)	25.7 ± 0.10	$4 - 125$	120.4	25.7 ± 0.28 NS		$5 - 115$	128.0	**
Spike thickness (R)	24.0 ± 0.03	$8 - 58$	25.3	23.8 ± 0.12 NS		$10 - 55$	24.6	NS
Spike thickness (PR)	23.3 ± 0.04	$9 - 61$	28.3	23.2 ± 0.14 NS		$10 - 52$	28.8	NS
1,000-grain weight	8.6 ± 0.02 1.5-21.3		5.6	8.6 ± 0.06 NS		$2.9 - 19.3$	5.6	NS.

Table 3 Mean, range, and variance for 11 quantitative traits in the entire pearl millet collection and its core subset

R, Rainy season; PR, Post-rainy season; N-K test, Newman–Keuls procedure to compare means; NS, Non significant at $P = 0.05$

** Significant at $P = 0.05$

collection (Table 4). The proportional sampling strategy suggested by Brown [\(1989b\)](#page-9-0), used for selecting the core sample, showed significant similarity in the frequency distribution for countries of origin. This was evident for most of the countries that were represented more or less in the entire and the core collection. For quantitative traits, the analysis of frequency distribution indicated homogeneity of distribution among the entire and core collection (data not shown). The Wilcoxon's rank-sum test also indicated that all the characters except spike exertion ($P = 0.011$) have similar distribution in both core and entire collection (data not shown).

The Shannon–Weaver diversity indices calculated over all the 11 traits indicated similar pattern of diversity among entire collection and core collection (Table 5). No significant differences were observed in the diversity estimates for each of the traits in both entire as well as core collection. Significantly less diversity was observed for number of productive tillers in both the collections. A proper sampling strategy used in developing a core collection should consider

Table 5 Shannon–Weaver diversity indices for 11 quantitative traits in the entire and core collection of pearl millet

Traits	Entire Collection	Core Collection		
Days to flowering (R)	0.59	0.60		
Days to flowering (PR)	0.60	0.61		
Plant height (R)	0.63	0.63		
Plant height (PR)	0.63	0.62		
Number of productive tillers	0.41	0.44		
Spike exertion	0.59	0.58		
Spike length (R)	0.56	0.55		
Spike length (PR)	0.56	0.57		
Spike thickness (R)	0.61	0.62		
Spike thickness (PR)	0.58	0.62		
$1,000$ grain weight	0.62	0.62		
Mean \pm SE	0.58 ± 0.097	0.59 ± 0.056		

R, Rainy Season; PR, Post-rainy Season

rainy); TGW, 1000-grain weight \mathbb{H} ă \mathbf{r} , \mathbf{H} y), \mathbf{H}

Figures in parenthesis: phenotypic correlation coefficients for the core sample

* $P < 0.05$
** $P < 0.01$ $P < 0.05$

the conservation of phenotypic associations arising from co-adapted gene complexes (Ortiz et al. [1998\)](#page-9-0). The established core collection conserves the phenotypic correlations among the traits as observed for the entire collection (Table 6). This clearly indicates that most of the co-adapted gene complexes governing these traits were properly sampled. In the present study, strong associations were observed among some of the traits like days to 50% flowering (rainy) and plant height (rainy) $(r = 0.550)$ in the entire collection and 0.529 in the core sample); spike length (rainy) and spike length (*post-rainy*) ($r = 0.772$ in the entire and 0.766 in the core collection); spike thickness (rainy) and spike thickness (post-rainy) $(r = 0.585$ in the entire collection and 0.594 in core sample) indicating that in future evaluation of germplasm fewer traits may be taken into consideration during rainy or post-rainy seasons, making it less laborious in regenerating the material. For other traits, correlations were low in both entire and core collection.

The core subset was developed considering 11 quantitative traits, which are influenced by $G \times E$ interaction, however, the data from two seasons were treated separately to nullify the effect of $G \times E$ interaction to some extent with an assumption that the effect of rainy and post-rainy seasons in different years were similar. Thus, the proportional sampling strategy used in the present study was found effective in establishing the core sample that represents the world variation of the entire pearl millet collection. There were no significant differences in the mean and range for the characters studied and frequency distributions were not affected due to proportional sampling. The core also retained overall phenotypic diversity present in the entire collection. Similar results were also obtained in other studies when proportional sampling strategy was adopted for selecting samples from each cluster to constitute the core collection (Erskine and Muehlbeur [1991;](#page-9-0) Schoen and Brown [1993](#page-9-0); Bataillon et al. [1996;](#page-9-0) Ortiz et al. [1999\)](#page-9-0). The established pearl millet core collection, therefore, can be used very efficiently as a starting point for further improvement programs, involving research on screening the germplasm collection for sources of desirable traits as well as photoperiod sensitivity, disease resistance, drought tolerance, and adaptation to saline or alkaline environments. For example, in diseases, like downy mildew (one of the most destructive diseases of pearl millet), the information on amount of variability present in the germplasm is very limited (only 4,727 cultivated accessions have been screened so far) and drought, a major abiotic stress, only 115 cultivated accessions have been screened, and it will take at least another few years to examine the entire germplasm collection for specific traits of interest. In such a case, the core collection would allow quick identification of new sources of alleles for resistance owing to the conservation of genetic variability in these accessions for most of the characters. The core subset will also help in tackling new constraints that may arise because of onset of new diseases and pests. The core being representative of the entire collection and seeds of the core subset being available, resistance sources for new constraints could be rapidly identified. Furthermore, additional sources of resistance can be found in the reserve collection by selectively examining the clusters from which the core accessions have been identified. The core will also provide a guideline to the curator while acquiring new accessions in the genebank collection and it should be revised periodically as and when additional accessions and information becomes available.

The list of pearl millet entries included in the core collection with details on country of origin, IP number, and the cluster composition are available on diskette from the corresponding author.

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