

# Diversity in Small Millets Germplasm and Enhancing its Use in Crop Improvement

CL Laxmipathi Gowda<sup>1</sup>, Hari D Upadhyaya<sup>1</sup>, Vudumula G Reddy<sup>1</sup> and Sube Singh<sup>1</sup>

<sup>1</sup>International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru PO, 502324, AP, India Email [c.gowda@cgiar.org](mailto:c.gowda@cgiar.org)

## Abstract

Small millets (finger-, foxtail-, proso-, little-, kodo-, barnyard-millet) are relatively tolerant to stresses in different production systems and are cultivated globally on 18-20 million ha producing 15-18 million tons of grains. ICRISAT genebank conserves 10193 accessions of six small millets from 50 countries. Large variation was observed for several morpho-agronomic traits such as number of basal tillers, days to 50% flowering and maturity. Shannon-Weaver diversity was  $0.530 \pm 0.024$  in finger millet,  $0.491 \pm 0.025$  in foxtail millet,  $0.522 \pm 0.026$  in kodo millet,  $0.525 \pm 0.027$  in little millet,  $0.526 \pm 0.032$  in barnyard millet, and  $0.501 \pm 0.031$  in proso millet. Core collections of finger millet (622 accessions) representing 90% variability and foxtail millet (155 accessions) representing 88% variability of entire collections were developed and evaluated for morpho-agronomic traits. Sixteen accessions flowered significantly early (49-57 days) and 9 accessions ( $2.02-2.15 \text{ t ha}^{-1}$ ) produced higher grain yield than the control cultivar Kalyani (63 days;  $1.92 \text{ t ha}^{-1}$ ) in finger millet. Twenty-three foxtail accessions flowered significantly early (25-40 days) and 19 ( $0.99-1.76 \text{ t ha}^{-1}$ ) produced greater grain yield than control SIA 326 (48 days;  $0.98 \text{ t ha}^{-1}$ ). Cluster analysis of 25 selected early-maturing and high-yielding accessions in foxtail and finger millets indicated that the selected accessions were diverse than control cultivars. Identification of these diverse early-maturing lines would be useful in breeding programs.

## Media summary

Assessment of pattern of diversity and identification of trait specific diverse germplasm for utilization in crop improvement will enhance the genetic potential of small millets.

## Keywords

Characterization, core and composite collection, phenotypic diversity,

## Introduction

Small millets are the hardiest crops, belong to family *Poacea* and include an estimated 8000 species belonging to some 600 genera. Among them, eight small seeded species are used as food crops in different countries globally. These include finger millet [*Eleusine coracana* (L.) Gaertn.], foxtail millet [*Setaria italica* (L.) Beauv.], proso millet (*Panicum miliaceum* L.), little millet (*Panicum sumatrense* Roth. ex Roem. & Schult.), barnyard millet [*Echinochloa crusgalli* (L.) Beauv. & *Echinochloa colona* (L.) Link], kodo millet (*Paspalum scrobiculatum* L.), teff [*Eragrostis tef* (Zucc.)] and fonio millet (*Digitaria exilis* Stapf. & *Digitaria iburua* Stapf.). ICRISAT has the global responsibility of germplasm assembly, characterization, conservation, documentation and distribution of the first six of these crops, and hence this paper confines to these six small millets. The small millets are adapted to varied agroclimatic regions, which can be grown in lands almost at sea level to about 3200 m.a.s.l. Their use as food, feed and fodder make them important for food security. Their grains are rich sources of calcium, iron, zinc, beta-carotene, and high quality proteins, contributing significantly in reducing malnutrition that affects nearly half of the world's population, particularly in developing countries of Africa and Asia.

The stover serves as quality fodder for cattle. Small millets can be stored for long periods without insect damage and provide succor during famine. Considering their nutritive values it would be appropriate to call them nutritious millets. Small millets are early maturing, water-use efficient and input responsive crops. The six small millet crops are together cultivated on 18-20 m ha with a production of 15-18 m tons (Prasada Rao and de Wet 1997). ICRISAT Genebank (Patancheru, India) conserves 10,193 accessions of six millets (finger millet 5949, kodo millet 658, foxtail millet 1535, little millet 466, proso millet 842 and barnyard millet 743) from 50 countries.

### **Characterization**

Small millet germplasm accessions were characterized in batches as and when they were assembled over the years (1974-2007) at ICRISAT, Patancheru, India, which is located at 18°N and 78°E, at an altitude of 545 m.a.s.l. and about 600 km from the sea. Annual rainfall is about 750 mm, most of which occurs during June to September. The germplasm accessions were sown on red soils (alfisols), each accession occupying single row of 4 m length with a spacing of 60 x 10 cm. A basal dose of 20 kg nitrogen and 50 kg phosphorus ha<sup>-1</sup>; and 45 kg nitrogen ha<sup>-1</sup> was applied as top dressing. In all the years, sowings were done towards end of July (20-31 July). Irrigation and hand weeding were done when necessary. Crop was reasonably free from any disease or insect damage and no chemical sprays were applied. Data was recorded on qualitative (discrete classes) and quantitative (continuous variation) traits and for taxonomic classification. However, the set of descriptors varied in barnyard millet (23) kodo millet (23), proso millet (26), finger millet (25), foxtail millet (28) and little millet (28). Ten descriptors that were common between them were: days to 50% flowering, basal tiller number, plant height (cm), flag leaf blade length (mm), flag leaf blade width (mm), flag leaf sheath length (mm), peduncle length (mm), panicle exertion (mm), inflorescence length (mm) and seed size. During field evaluation, accessions were also classified into botanical races. Large range variation was observed for morpho-agronomic traits. Number of basal tillers ranged 1-70 in finger millet, 1-80 in foxtail millet, 1-44 in barnyard millet, 2-48 in kodo millet, 3-46 in little millet and 1-32 proso millet. Similarly range for days to 50% flowering was 50-120 in finger millet, 32-135 in foxtail millet, 27-90 in barnyard millet, 51-112 in kodo millet, 39-138 in little millet, and 26-50 in proso millet. Average basal tiller numbers ranged from 3.9 in proso millet to 15.2 in kodo millet.

### **Diversity**

Assessment and utilization of genetic variability is essential in plant breeding. Understanding the pattern of diversity and the genetic structure of gene pools is critical for effective management and use of germplasm resources. Progress in plant breeding depends on identification of new sources of genetic variation for beneficial traits, in such a way that a combination of alleles produces progenies with superior performance. Pattern of diversity was assessed in all six small millets. The Shannon and Weaver (1949) diversity index ( $H'$ ) was calculated to compare the phenotypic diversity among characters in all the crop species, separately. The index is used in genetic studies as a convenient measure for both allelic richness and evenness. However, because of log transformation, it is not readily interpretable in the genetic terms. A low  $H'$  indicates an extremely unbalanced frequency classes for an individual trait and a lack of genetic diversity. The maximum  $H'$  for qualitative traits was in little millet (0.545±0.059) followed by proso millet (0.477±0.054), finger millet (0.469±0.084), kodo millet (0.445±0.046), foxtail millet (0.408±0.052), and barnyard millet (0.379±0.067). The maximum  $H'$  for quantitative traits was in barnyard millet (0.599±0.007) followed by foxtail millet (0.588±0.016), kodo millet (0.574±0.020), finger millet (0.535±0.042), proso millet (0.517±0.038), and little millet (0.514±0.027). The overall (qualitative and quantitative) Shannon-Weaver diversity was 0.530±0.024 in finger millet 0.491±0.025 in foxtail millet, 0.522±0.026 in kodo millet, 0.525±0.027 in little millet, 0.526±0.032 in barnyard millet, and 0.501±0.031 in proso millet. Similarly, diversity matrix (Johns et al., 1997) was calculated to identify most diverse accessions in finger millet and foxtail millet

collections. Maximum diversity in finger millet was 0.460 between IE 4443 (a landrace from Cameroon) and IE 6546 (a landrace from Nigeria). Maximum diversity (0.544) in foxtail millet was between ISe 1065 (race Indica subrace glabra) from Tamil Nadu, India and ISe 189 (race Indica subrace Moharia) from Syria.

### **Developing core and mini-core collections for enhanced utilization of germplasm**

Potential threat of loss of plant biodiversity during twentieth century and its importance led to the collection and conservation of germplasm in ex-situ genebanks. However, adequate efforts were not made to characterize and evaluate these resources for their utilization in breeding programs. Plant breeders prefer to work with their own lines rather than exotic materials. This has resulted in narrowing the genetic base of cultivated crops, which needs to be broadened through greater use of variability conserved in genebanks. One of the reasons for limited use of germplasm by plant breeders is the lack of information on traits of economic importance, which often shows high genotype x environment interactions and requires replicated multi-location evaluations. This is a very costly and resource-demanding task owing to the large size of the germplasm collections. One of the approaches is to develop core collections. ICRISAT scientists have developed of finger millet core consisting of 622 accessions (Upadhyaya et al. 2006a) and foxtail millet core consisting of 155 accessions, which are about 10% of the entire collection, but represent almost full diversity (90% in finger millet and 88% in foxtail millet) of the species.

Finger millet core collection (622 accessions) along with three control cultivars was evaluated in a replicated trial during 2004 rainy season. Data was recorded for five qualitative and 15 quantitative traits. The data analysis indicated significant genotypic variance for several traits including grain yield and days to flowering. Twenty-five accessions that were better or similar to control cultivars for grain yield and early maturity were identified. Sixteen accessions flowered significantly early (49-57 days) and 9 accessions ( $2.02-2.15 \text{ t ha}^{-1}$ ) produced higher grain yield than the control cultivar Kalyani (63 days;  $1.92 \text{ t ha}^{-1}$ ) in finger millet. Cluster analysis based on first five principal components indicated that the selected accessions were diverse. Similarly, core set of foxtail millet germplasm (155 accessions) along with control cultivars was evaluated during 2005 rainy season. Twenty-five foxtail millet accessions with better grain yield and early maturity were identified. Twenty-three accessions flowered significantly early (25-40 days) and 19 ( $0.99-1.76 \text{ t ha}^{-1}$ ) produced greater grain yield than control SIA 326 (48 days;  $0.98 \text{ t ha}^{-1}$ ). Cluster analysis based on first five principal components indicated that the selected accessions were diverse than the control cultivars. Identification of these diverse accessions could be useful in developing improved cultivars with broad genetic base in finger millet and foxtail millet.

When the size of the entire collection is very large, even a core collection may become unwieldy for evaluation by breeders. To overcome this, ICRISAT scientists have developed a seminal two-stage strategy to develop a mini-core collection, which consists of 10% of the core collection (and hence only 1% of the entire collection) (Upadhyaya & Ortiz, 2001). This mini-core collection still represents the diversity of the entire core collection. The first stage involves developing a representative core collection (about 10%) from the entire collection using all the available information on origin, geographical distribution, and characterization and evaluation data of accessions. The second stage involves evaluation of the core collection for various morphological, agronomic, and quality traits, and selecting a further subset of about 10% accessions from the core collection. Standard clustering procedure should be used to separate groups of similar accessions at both stages. Finger millet mini core collections (65 accessions) was developed using this strategy.

### **Establishing and genotyping composite collection**

With the support from the Generation Challenge Programme, ICRISAT scientists have developed a composite collection of finger millet consisting of 1000 accessions (Upadhyaya *et al.*, 2005), and foxtail millet consisting of 500 accessions (Upadhyaya *et al.*, 2006b). These composite collections were profiled using 20 SSR (Simple Sequence Repeats) markers. Allelic data on 959 finger millet accessions and 20 markers based on quality index was used for statistical analysis. A total of 231 (121 common and 110 rare) alleles were detected in the composite collection. Gene diversity varied from 0.200 to 0.850. Group specific unique alleles observed among the races were: 37 in *Vulgaris*, 5 in *Plana*, 4 in *Africana* and 2 in *Compacta*, and region wise, 29 in East Africa, 12 in South Asia, 11 in Southern Africa, and one each in Central Africa and Europe. A reference set consisting of 300 genetically most diverse accessions was established. The reference set had 206 (89.2%) of the 231 alleles detected in the composite collection, and showed high gene diversity (0.307 to 0.852). Similarly, allelic data on 452 foxtail millet accessions and 19 markers based on quality index was used for statistical analysis. A total of 362 (192 common and 170 rare) alleles were detected in the composite collection. Gene diversity in foxtail millet varied from 0.116 to 0.924. The polymorphism information content (PIC) ranged between 0.114 and 0.919.

The core and mini core collections, and reference sets would be useful in functional and comparative genomics, in mapping and cloning gene(s), and in applied plant breeding to diversify the genetic base of the breeding populations to develop broad-based elite breeding lines/cultivars with high yield and enhanced adaptation to diverse environments.

## Reference

Johns MA, Skroch PW, Nienhuis J, Hinrichson P, Bascur G, Munoz-Schick C. 1997. Gene pool classification of common bean landraces from Chile based on RAPD and morphological data. *Crop Sci.* 37:605-613.

Prasada Rao KE, de Wet JMG. 1997. Small Millets. In D. Fuccillo, L. Sears and P. Stapleton (eds.). *Biodiversity in Trust: Conservation and Use of Plant Genetic Resources in CGIAR Centres*. Cambridge Univ. Press, UK. p 259-272

Shannon CE, Weaver W. 1949. *The mathematical theory of communication*. Univ. of Illinois Press, Urbana, USA. pp 144

Upadhyaya HD, Gowda CLL, Pundir RPS, Reddy V G, Singh S. 2006a. Development of core subset of finger millet germplasm using geographical origin and data on 14 morpho-agronomic traits. *Genetic Resources and Crop Evolution*, 53:679-685.

Upadhyaya HD, Ortiz R. 2001. A mini core subset for capturing diversity and promoting utilization of chickpea genetic resources. *Theoretical and Applied Genetics*, 102: 1292-1298.

Upadhyaya HD, Pundir RPS, Hash CT, Hoisington D, Chandra S, Gowda CLL, Singh S, Gopal Reddy V. 2005. Genotyping Finger Millet Germplasm – Developing Composite Collection. Generation Challenge Program Review Meeting, September 2005, Rome, Italy.

Upadhyaya HD, Varshney RK, Hash CT, Hoisington DA, Gowda, CLL, Reddy VG, Chandra S, Lalitha N, Bharathi A. 2006b. Development of composite collection and genotyping of foxtail millet (*Setaria italica* (L.) Beauv.) composite collection, Generation Challenge Program Annual Research Meeting, September 2006, Sao Paulo, Brazil.