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Diversity of small millets germplasm in genebank at ICRISAT

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The family *Poaceae* includes an estimated 8000 species belonging to some 600 genera. Species of *Poaceae* (=grasses) occur in habitats across all countries that support the growth of flowering plants. Their use as food grains and feed for livestock make them very relevant to human survival. Among them, 35 species belonging to 20 genera are known to have been domesticated. Of these, eight species that have small seeds are used essentially as food crops in their respective agro-ecosystems. 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.). Small millets are also called minor millets, however, considering their nutritive values it would be more appropriate to call them nutritious millets. Of the eight crop species, ICRISAT was requested to assume the responsibility of germplasm assembly, characterization, documentation and conservation of the first six of these crops, and hence this paper confines itself to these six small millets.

Small millets are mainly cultivated in arid, semi-arid or montane zones as rainfed crops in South Asia, China, CIS countries and several countries in Africa. Small millet crops are together cultivated on 18-20 m ha with a production of 15-18 m tons (Prasada Rao *et al.,* 1987). Small millets are often grown under adverse soil and weather conditions compared to other crops. They have always been crops of drought-prone areas, but give reliable harvests, though these could be lower than from other crops. Information about the crops and germplasm related activities with the ICRISAT collections are discussed in this paper.

ABOUT THE CROPS

Finger millet (*Eleusine coracana)*

This is cultivated for human food in 14 countries of Africa and five countries of South Asia on over 3.5 m ha annually (Upadhyaya *et al.,* 2006a). Finger millet was domesticated about 5000 years B.C. in Eastern Africa (possibly Ethiopia) and introduced into India as a crop about 3000 years ago (Hilu *et al.,* 1979). The closest wild relative of finger millet is *Eleusine coracana* subsp. *africana* (Kennedy-O'Byrne) Hilu & de Wet. *Eleusine coracana* subsp. *africana* is a native to Africa. These two taxa (finger millet and subsp. *africana*) are tetraploids (2n=36) with basic chromosome number x=9. These sub-

species hybridise where they are sympatric in Africa and derivatives of such crosses often occur as weeds in cultivated fields.

Finger millet is an annual grass, erect, about 100 cm tall, profusely tillering and producing aerial branches. Plants invariably lodge. The inflorescence is a whorl of 2-8 digitate, straight or curved spikes of 1-32 cm length (Table 1), about 1.3 cm broad, with about 70 spikelets arranged alternatively on the rachis, each containing 4-7 seeds, seed weight being 2-5 g per 1000 seeds. Seeds are nearly globose, reddish-brown to nearly white in colour. Finger millet has a wide range of seasonal adaptation and can be grown in lands almost at sea level (parts of Andhra Pradesh and Tamil Nadu –India) to about 2400 m.a.s.l. in hills of Uttrakhand (India) and Nepal, and similarly at high altitudes in Uganda, Kenya and Ethiopia. On average, finger millet yields one ton ha^{-1} grains, but its reported yield potential is up to 3.7 t ha^{-1} in Ethiopia (Mulatu & Kebebe, 1993), 4.3 t ha^{-1} in Uganda (Odelle, 1993), 4.8 t ha^{-1} in India (Bondale, 1993), and 6.1 t ha^{-1} in Zimbabwe (Mushonga *et al.,* 1993).

Finger millet seeds are consumed in a variety of forms, such as unleavened bread (roti), thin or thick porridge, fermented porridge; and also extensively used in brewing. Finger millet food has high biological value. Seed protein content is about 7.4%, which is comparable to that of rice. However, some lines have as much as 14.2% protein (Iyengar *et al.,* 1945-46). Finger millet seeds are particularly rich in tryptophan, cystine, methionine, and total aromatic amino acids compared to other cereals. The seeds are exceptionally rich in calcium containing about 0.34% in whole seed compared with 0.01 – 0.06% calcium in most cereals (Kurien *et al.,* 1959). The seeds are also rich in iron containing 46 mg kg^{-1} (Serna-Saldivar & Rooney, 1995), which is much higher compared to wheat and rice.

Table 1. Summary of germplasm characterization data and economic traits of small millets

Trait	Finger millet		Foxtail millet		Proso millet		Little millet		Kodo millet		Barnyard millet	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Days to flowering	50-120	80.4	32-135	53.5	26-50	34.5	39-138	58	51-112	77.5	27-90	46.1
Plant height (cm)	30-240	100.7	20-215	110.1	27-133	59.3	50-240	113	30-97	54.8	29-235	83.1
Basal tiller (no.)	1-70	5.2	1-80	7.5	1-32	3.9	3-46	14.6	2-48	15.2	1-44	6.5
Flag leaf blade length (mm)	100-750	358.1	30-520	284.7	29-380	222.3	60-560	247.2	24-440	183.5	50-420	198.3
Flag leaf blade width (mm)	5-20	12.6	5-40	20.2	6-130	19.4	5-160	38.8	5-15	7.7	5-40	18.4
Peduncle length (mm)	18-450	215.4	80-500	299.6	32-400	180.9	60-480	189.6	-	-	20-520	151.3
Exsertion (mm)	10-800	112.9	10-360	162.4	50-320	99.7	80-280	31.1	-	-	50-280	64.8
Inflorescence length (mm)	10-320	93.1	10-390	163.1	34-400	193.1	27-500	282.2	20-160	64.3	13-280	144.7
Seed size (g 1000-seeds-1	2.6			1.86		4.7-7.2						
Special trait	Drought resistant	Highly nutritious			Short duration		High fat				Highly nutritious	
	High iron											
	High calcium											

Foxtail millet (*Setaria italica)*

Foxtail millet was first domesticated in the highlands of Central China; remains of the cultivated form are known from the Yang-Shao period dating back some 5000 years. Comparative morphology suggests that foxtail millet spread to Europe and India as a cereal crop soon after its domestication (Prasada Rao *et al.,* 1987). *Setaria viridis* is a possible ancestor. The cultivated species and the progenitor have 18 somatic chromosomes (2n=18). Three races namely, *moharia, maxima,* and *indica* are recognised. Race *moharia* is common in Europe, Southeast Russia, Afghanistan and Pakistan. Race *maxima* is grown in Eastern China, Japan and Korea and has been introduced into the USA. It also occurs in Nepal, Northern India and Georgia (of the former USSR). Race *indica* is found in remaining parts of India and Sri Lanka.

Foxtail millet is an annual grass and variable in its morphology. Plants range from single-stemmed to highly tillered. The average plant height is little over 1.0 m but can reach 2 m. Panicle length varies from 1-39 cm (Table 1) and diameter from 1.5 to 3 cm. The central rachis is often weak, so panicles droop. By contrast, some varieties in China are single-stemmed and with a height as tall as 150 cm. Leaves are 30 to 35 cm long and 1.5 to 3 cm wide. Panicle length varies from 12 to15 cm and width 4 to 6 cm. Seeds are small (2-3 g 1000 seeds^{-1}) and generally light cream coloured. Seeds are nutritious, with protein, fat and minerals contents comparable to wheat (Mangala Rai, 2002). Foxtail millet is adapted to temperate regions although found in the tropics. It has a broad range of maturity, from 70 to 120 days.

Proso millet (*Panicum miliaceum)*

Domestication of proso millet probably occurred in Manchuria (de Wet, 1986). It was probably introduced into Europe about 3000 years ago. After this date, proso millet was introduced to the Near East and India (Zohary & Hopf, 1988). Presently proso millet is important in Northwest and Northeast China and CIS countries. In India, proso millet is cultivated in Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka, Gujarat, Uttar Pradesh, Maharashtra, Orisa and to a limited extent in foothills of Himalaya. It is cultivated in Bangladesh (Majid *et al.,* 1989) and is of minor importance in drier parts of Sri lanka (Ponnuthurai, 1989). The chromosome number have been reported to be 2n=36, and 2n=72 in some of the Indian races (Bor, 1960).

Proso millet is an annual grass, adapted to temperate and subtropical parts of the world. It is a highly variable crop. Plant height can range from 27 to 133 cm, panicle length 3 to 40 cm (Table 1), seed weight 5 to 9 g 1000-seeds^{-1}. Seeds are generally oval-shaped and of varied colours: white cream, yellow, orange, brown and black. Proso millet accumulates higher dry matter in the reproductive parts as compared to wheat, maize and sorghum. Proso millet has a high water use efficiency (Hulse *et al.,* 1980), though this is not because of drought resistance but due to its short growing season. The plant has a shallow root system and does not grow well under water stress, or under excessive moisture. Crop maturity varies from 60 to 90 days depending on varieties.

Little millet (*Panicum sumatrense)*

Little millet has its progenitor in *Panicum psilopodium.* This is cultivated across India, Nepal and Western Myanmar. The chromosome number of little millet have been reported to be 2n=36 (Bor, 1960). Sub-species *P. psilopodium* is distributed from Sri Lanks to Pakistan and eastward to Indonesia. Of the cultivated *species*, two races, *robusta* and *nana* are recognised. *Robusta* is cultivated in Northwestern Andhra Pradesh and parts of Orissa where it crosses with race *nana*. Plants in race *nana* vary from 60 to 70 cm in height. The inflorescence is 14 to 15 cm long, erect, open, and highly branched. These branches sometime droop at maturity. Plants of race *robusta* are 50 to 240 cm tall. The inflorescence is 3 to 50 cm long (Table 1), open or compact, and highly branched. It is a primarily self-pollinated crop with up to 3.5% cross-pollination.

Kodo millet (*Paspalum scrobiculatum)*

Kodo millet was domesticated in India, almost 3000 years ago. The species is found across the Old World in humid habitats of the tropics and subtropics. The chromosme number is reported to be 2n=40 (Hiremath & Dandin, 1975). Crossing readily occurs between cultivated and weedy races, and seed from hybrids is harvested along with those of the sown crop, racial differentiation is not distinct despite years of cultivation in India (de Wet, 1986). As a wild cereal, it is harvested in West Africa and India. In India, it is widely spread from Tamil Nadu and Kerala in the south to West Bengal and Rajasthan in the north (de Wet, 1986). Kodo is grown to a limited extent in Bangladesh (Majid *et al.,* 1989).

As a commercial crop, kodo millet is grown only in India. Plant height varies from 30 to 97 cm and produces many basal tillers, ranging from 2 to 48. The inflorescence is small, 2 to 16 cm long (Table 1). It matures late compared with other small millets. It is highly self-pollinating; florets generally remain closed during the

flowering period. The grains occur in a hard husk, making debranning difficult. The crop is drought tolerant and often cultivated on marginal soils. Kodo millet grains are consumed as food, though, nutritionally, these are poor. On average, kodo millet grains have 8.3% protein, 1.4% fat, and 2.6% minerals.

Barnyard millet (*Echinochloa crusgalli* and *E. colona*)

Two species of *Echinochloa* are grown as cereals, i) *E. crusgalli* is a native of Eurasia and was domesticated in Japan some 4000 years ago; ii) *E. colona* occurs widely in tropical and subtropical areas and was domesticated in India. Both species are fairly similar and have the same chromosome number (2n=54; hexaploids), but hybrids between them are sterile. *E. colona* differs consistently from *E. crusgalli* in having smaller spikelets with membranaceous rather than chartaceous glumes. Simply put, *E. colona* is awnless whereas *E. crusgalli* has awned glumes. *E. colona* is cultivated in India in Madhya Pradesh, Uttar Pradesh, Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra, Bihar and in the foothills of Himalaya. Plants of *E. crusgalli* are erect, growing up to 235 cm tall (Table 1), leaf blades are linear with a broad round base gradually tapering to a fine point, panicles are 13 to 280 mm long and 5 to 25 mm wide. The spikelet head is windmill-shaped, short and stiff awned. Plants of *E. colona* are reddish-purple or green with a reddish base. Inflorescences are usually erect, rarely drooping and awnless spikes.

Echinochloa species are drought resistant and also capable of withstanding water logging. Generally yields are low: 300-600 kg ha^{-1} of grains, and a straw yield of around 1000 kg ha^{-1}. Active plant breeding research at the agricultural universities in Karnataka, Tamil Nadu and Uttrakhand (India) has resulted in release of a number of varieties, yielding up to 3 tons of grains and 7 tons of fodder per hectare. Grains are nutritious, having 11.6% protein, 5.8% fat and 4.7% minerals (Mangala Rai, 2002).

GERMPLASM ASSEMBLY AT THE ICRISAT GENEBANK

The major donors of small millet germplasm to ICRISAT are listed in Table 2 and have contributed 5977 samples. Subsequent to this, geographical representation in the collection was reviewed and attempts were made to secure new germplasm from hitherto under-represented areas, and 2459 germplasm samples were collected from farmer's fields through 60 missions. Most of these missions were organised for other crops; however, small millets were also collected. Of these, only four missions, one each in India and Zimbabwe, and two in Uganda were organised with greater emphasis of collecting small millet germplasm. The current status of small millet germplasm in the ICRISAT genebank is given in Table 3.

Table 2. Major donors for small millets germplasm to ICRISAT genebank

Donor organization	Number of accessions
ICRISAT, Zimbabwe	1440
Rockefeller Foundation, USA	1255
Hill Crop Improvement Programme, Dolakha, Nepal	733
Royal Botanical Gardens, Kew, UK	634
Tamil Nadu Agricultural University, Kovilpatti	530
University of Illinois, USA	475
National Bureau of Plant Genetic Resources, Regional Station, Akola	469
ICARDA, Syria	441

Table 3.Germplasm assembly of small millets in ICRISAT genebank

Country/Crop	**Finger millet**	**Foxtail millet**	**Proso millet**	**Little millet**	**Kodo millet**	**Barnyard millet**	*Total*
Afghanistan		30	16				46
Australia			2				2
Bangladesh			2				2
Burundi	15						15
Cameroon	8	8		3		1	20
China		60	2				62
Egypt						1	1
Ethiopia	31	1					32
Germany	1		12				13
Hungary		10	10				20
India	1364	978	69	459	656	447	3973
Iran		6	9				15
Iraq			2				2
Italy	7						7
Japan			1			164	165
Kenya	946	9	1				956
Korea, Republic of		52	73				125
Lebanon		33	1				34
Malawi	252	1	1			2	256
Maldives	4						4
Mexico	1	2	13				16
Myanmar		6		2			8
Nepal	780	21	6				807
Nigeria	19						19
Pakistan	1	29	41			11	82
Senegal	5						5
South Africa	1	3					4
Sri Lanka	18	14	2	1	2		37
Syrian Arab Republic		119	35	1		2	157
Taiwan		28					28
Tanzania	42						42

Turkey		26	49				75
Uganda	959						959
Ukraine			4				4
Former USSR		67	121			16	204
UK	14	4	4				22
USA	6	25					31
Other countries	4	3	9			2	18
Unknown	181		358			97	636
Zambia	136						136
Zimbabwe	1154						1154
Total	5949	1535	842	466	658	743	10193

GERMPLASM CHARACTERIZATION

Small millet germplasm accessions were planted in batches over the years (1974-2006) at ICRISAT, Patancheru, India for seed increase and botanical and agronomic evaluation and characterization. ICRISAT-Patancheru is located at 18^0N and 78^0E, at an altitude of 545 m, and about 600 km from the sea. Annual rainfall is about 750 mm, most of which occurs from June to September. Germplasm accessions were sown on red soils (alfisols), on ridges 60 cm apart, each accession occupying a single row of 4 m length, spacing being 60 x 10 cm. Basal doses of 20 kg nitrogen and 50 kg phosphorus ha^{-1} were applied, with 45 kg nitrogen ha^{-1} as top dressing. In all the years, sowings were made towards the end of July (20-31 July). Irrigation and hand weeding were carried out when necessary. The crop was reasonably free from any disease or insect damage and no chemical sprays were applied.

Data was recorded of qualitative (description in discrete classes) and quantitative (continuous variation) traits. However, the set of descriptors differed from one species to another. 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 exsertion (mm), inflorescence length (mm) and seed size. During field evaluation, accessions were also classified into botanical races. A brief summary of characterization data is given in Table 1.

GERMPLASM REGENERATION, CONSERVATION AND SAFETY BACKUP

Regeneration

The need for regeneration of an accession is triggered by one of the three criteria: (1) accessions that had reached a minimum level of seed stock or viability (<75% germination); (2) accessions required for medium-term storage (MTS) and/or long-term storage (LTS); and (3) germplasm repatriation when needed.

Conservation

Seed must be cleaned and dried to a minimal seed moisture content, before storing in cool and dry conditions with regular monitoring of seed health. In the ICRISAT genebank, the seeds of the entire collection are stored in aluminum cans at 4^0C, 20–30% RH for MTS.

The germplasm accessions are also conserved in LTS (-20^{0}C) after packing in vacuum-sealed aluminum foil pouches. Before packing, the seeds are dried to about 5% moisture content in a walk-in-drying room (100 m^3 size; 15^{0}C and 15% RH) facility. By July 2007, 87% of the total germplasm collection had been transferred into the LTS facility.

Recently conducted seed health monitoring of seeds conserved for 10–25 years (MTS) indicated greater than 75% seed viability for the majority of accessions. Accessions with declining seed viability (less than 75% seed germination) are regenerated as a priority and the old stock is replaced with fresh seeds. Most of the accessions showing low germination had been held in MTS for more than 25 years . Seed is also evaluated for diseases and treated with appropriate agrochemicals where possible before regeneration.

Safety back up

ICRISAT's agreement with the Governing Body of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) requires safety duplication preferably at –18°C. We have established a safety backup of 7622 small millet germplasm accessions (4580 finger millet, 1039 foxtail millet, 628 kodo millet, 521 proso millet, 479 barnyard millet and 375 little millet) at the ICRISAT genebank at Niamey, Niger. The remaining accessions will be transferred in due course.

DEVELOPING CORE, MINI-CORE AND COMPOSITE SETS TO ENHANCE UTILIZATION OF GERMPLASM

Developing core collections

One of the reasons that plant breeders are using less basic germplasm in research is the lack of information on traits of economic importance, which often shows high genotype x environment interactions and requires replicated multilocational evaluations. This is a very costly and resource-demanding task owing to the large size of the germplasm collections. To overcome this, our research now focuses on studying the diversity of germplasm collection and developing "core collections", which are about 10% of the entire collection, but represent almost the full diversity of the species. From the germplasm collection in the ICRISAT genebank, we have already developed core collections of sorghum, pearl millet, chickpea, pigeonpea and groundnut and also of finger millet (622 accessions, Upadhyaya *et al.,* 2006a) and foxtail millet (155 accessions, Upadhyaya – unpublished data).

The finger millet core collection (622 accessions), along with three control cultivars, was evaluated in a replicated trial during the 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. We identified 25 accessions which were better or similar to control cultivars for grain yield and early maturity. Cluster analysis on these accessions based on the first five principal components indicated that the selected accessions were diverse (Fig. 1). Similarly, the core set of foxtail millet germplasm accessions (155) along with four control cultivars was evaluated during the 2005 rainy season. Data was recorded on 14 qualitative and 11 quantitative traits. We identified 25 accessions, which were better or similar to control cultivars for grain yield and early maturity. Cluster analysis of these

accessions based on the first five principal components indicated that the selected accessions were more diverse than the control cultivars (Fig. 2). Identification of these superior and diverse accessions could be useful in developing cultivars with broad genetic base, high yield and early maturity in finger millet and foxtail millet.

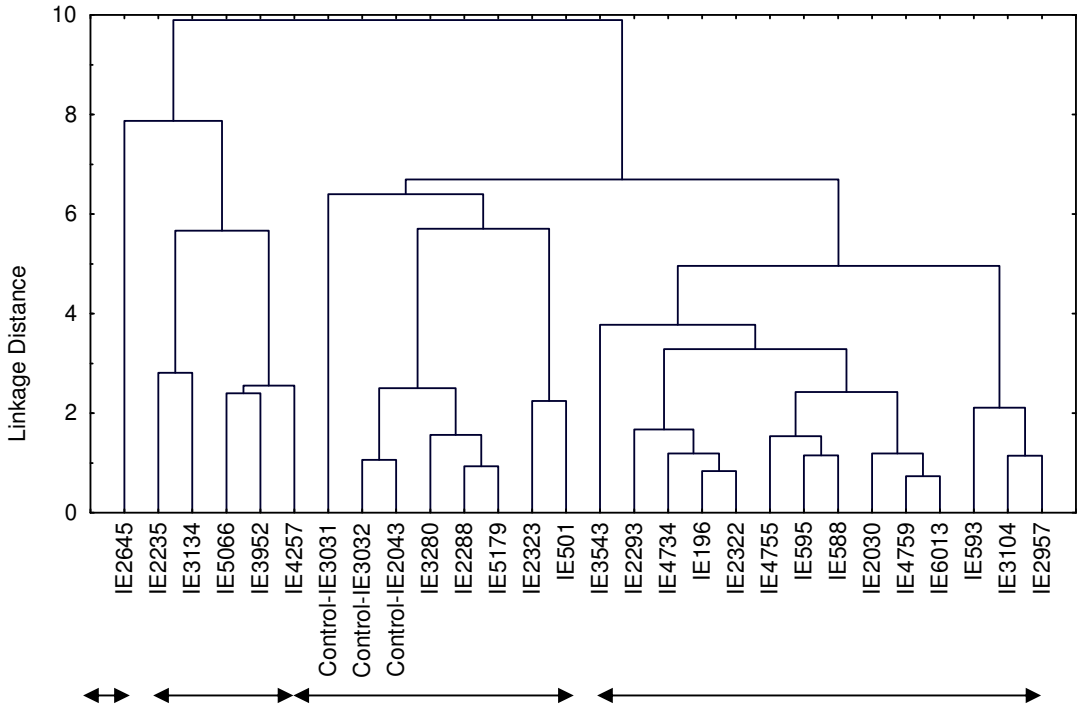

Figure 1. Finger millet dendogram based on first five principle components of 25 accession and three control cultivars

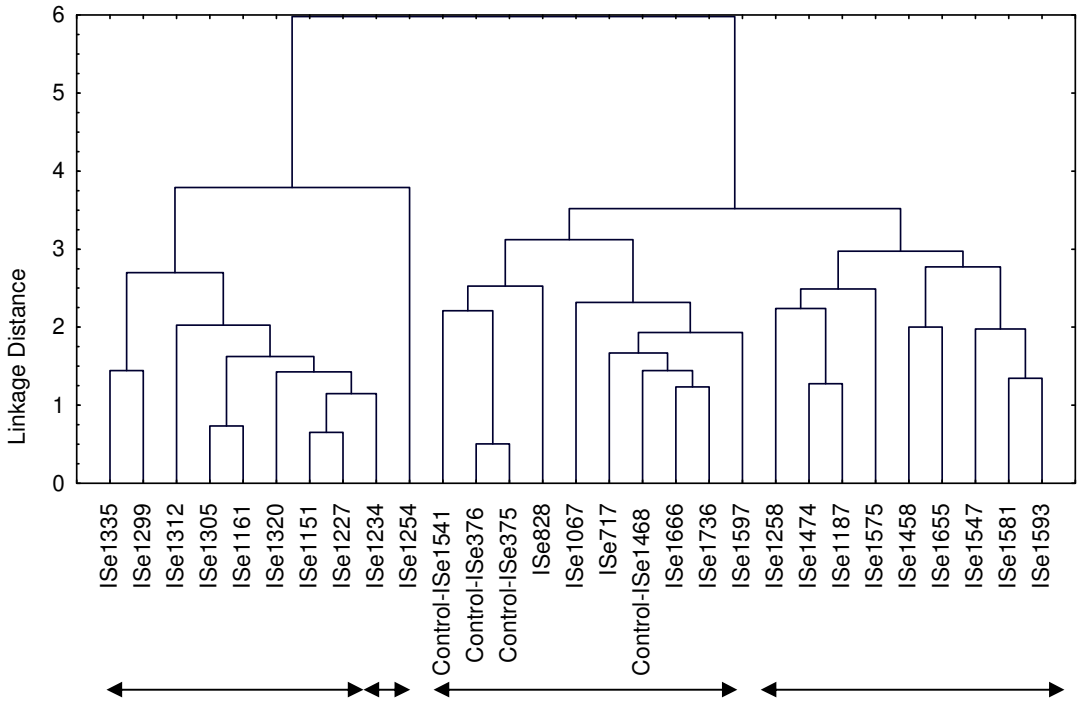

Figure 2. Foxtail millet dendogram based on first five principle components of 25 accession and four control cultivars

Developing mini-core collections

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 developed a seminal two-stage strategy to develop a mini-core collection, which consists of 10% accessions 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, 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. At ICRISAT, we have already developed mini-core collections of chickpea consisting of 211 accessions (Upadhyaya & Ortiz, 2001), groundnut (184 accessions; Upadhyaya *et al.,* 2002), pigeonpea (146 accessions; Upadhyaya *et al.,* 2006b), finger millet (65 accessions), and foxtail millet (46 accessions) (Upadhyaya – unpublished data).

Development of composite collection

With the support of the Generation Challenge Programme, ICRISAT has developed composite collections of finger millet consisting of 1000 accessions (Upadhyaya *et al.,* 2005) and foxtail millet consisting of 500 accessions (Upadhyaya *et al.,* 2006c). These

composite collections will be genotyped using 20 SSR markers. The data generated will be used to define the genetic structure of the global composite collection and to select reference accessions (300 of finger millet and 200 of foxtail millet) representing maximum diversity for the isolation of allelic variants of candidate gene(s) associated with beneficial traits. It is then expected that molecular biologists and plant breeders will have opportunities to use diverse lines in functional and comparative genomics, in mapping and cloning gene(s), and in applied plant breeding to diversify the genetic base of breeding populations, which should lead to the development of broad-based elite breeding lines/cultivars with superior yields and enhanced adaptation to diverse environments.

FUTURE PLANS

In the future, we will examine gaps in germplasm collection and attempt to fill them either by germplasm exchange or through fresh exploration. Focus will be on assessment of the germplasm for usefulness in crop improvement. Core and mini-core subsets of germplasm will be evaluated at diverse locations to identify trait specific diverse parents for various agronomic and nutritional traits. Molecular characterization of mini-core collections to identify trait specific germplasm lines, and reference sets of finger millet and foxtail millet will be enhanced to add value to the germplasm accessions. The reference sets will be evaluated more systematically and data published for the benefit of researchers.

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