Field Crops Research 124 (2011) 459–467

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Identification of trait-specific germplasm and developing a mini core collection for efficient use of foxtail millet genetic resources in crop improvement

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ARTICLE INFO

Article history:
Received 12 May 2011
Received in revised form 11 August 2011
Accepted 12 August 2011

Keywords:
Foxtail millet
Mini core collection
Co-adapted gene complexes
Nutritional traits
Trait-specific germplasm

ABSTRACT

Foxtail millet is an important staple crop in some parts of China, India and Japan, and a potential bioenergy source. The grains are rich source of protein, fiber, minerals and vitamins. We had earlier reported the development of a core collection (155 accessions) of foxtail millet. This study was initiated to identify trait-specific germplasm for agronomic and nutritional traits, and to develop a mini core through multi-locational evaluation of the foxtail millet core collection. One hundred and fifty-five accessions together with five controls (four common and one location-specific control) were evaluated for 21 descriptors at five agro-ecologically diverse locations in India during the 2008 rainy season. The experiment was conducted in an alpha design with three replications at Patancheru, and in an augmented design with one of the five controls repeated after every nine-test entries at other locations. A number of diverse germplasm accessions with agronomically (earliness and high grain yield) and nutritionally (high seed protein, calcium, iron and zinc) superior traits were identified for use in foxtail millet breeding. The hierarchical cluster analysis of data using phenotypic distances resulted in 25 clusters, from each cluster, ~10% or a minimum of one accession was selected to form a mini core, which comprised of 35 accessions. The comparison of mean, variance, frequency distribution, diversity (H') and phenotypic correlations revealed that the mini core indeed captured adequate variability from the core collection. This mini core collection is an ideal pool of diverse germplasm for studying population structure and diversity, and identifying new sources of variation for use in breeding and genomics studies in foxtail millet.

1. Introduction

Foxtail millet (*Setaria italica* (L.) Beauv.), a self pollinating C_4 plant closely related to the bioenergy grasses such as switchgrass (*Panicum virgatum*) and Miscanthus (*Miscanthus x giganteus*) (Doust et al., 2009), has contributed to ancient human civilization in Euroasia (Sakamoto, 1987). Foxtail millet is an important grain crop used as staple food in some parts of China, India and Japan, while it is grown for silage and hay in America, Australia and North Africa (Seetharam et al., 1989; Wanous, 1990). It belongs to the genus *Setaria*, the tribe Paniceae, subfamily Panicoideae, and family Poaceae in the grass family. It originated from *S. virdis* (the green foxtail) (Sakamoto, 1987). Surface sculpture of the lemma and length to breadth ratio of the grain differentiate *S. italica* from *S. virdis* (Nasu et al., 2007). Vavilov (1926) cited East Asia, including China and Japan, as the principal center of diversity for foxtail millet, while Harlan (1975) suggested independent domestication in China and Europe. Furthermore, Li et al. (1995) reported multiple domestication centers: China, Europe and Afghanistan-Lebanon. Prasad Rao et al. (1987, 1993) classified foxtail millet germplasm into three major races (*moharia*, *maxima*, and *indica*) and 10 subraces (*aristata*, *fusiformis* and *glabra* in *moharia*; *compacta*, *spongiosa* and *asamense* in *maxima*; *erecta*, *glabra*, *nana* and *profusa* in *indica*), whereas another report indicates four races (*maxima*, *moharia*, *indica* and *nana*) related to a specific geographic region – race *maxima* from East Asia, *moharia* from Europe, *indica* from South Asia and *nana* from Afghanistan and Lebanon (Li et al., 1995).

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doi:10.1016/j.fcr.2011.08.004

Foxtail millet grains are rich in protein, minerals (calcium, iron, potassium, magnesium, and zinc) and vitamins (Rai, 2002). It is not only widely used as an energy source for pregnant and lactating women, but also for sick people and children, and especially for diabetics. It reduces blood sugar concentration in women diabetics (Sema and Sarita, 2002). The grains have long shelf-life, a preferable attribute (Ravi et al., 2010). It has been suggested to use foxtail millet protein as a food component to fight type 2 diabetes and cardiovascular diseases (Choi et al., 2005). In spite of the foxtail millet being beneficial to health, it remained a neglected crop from the mainstream of crop improvement research compared to cereals such as maize (*Zea mays*), rice (*Oryza sativa*), wheat (*Triticum aestivam*), sorghum (*Sorghum bicolor*), and pearl millet (*Pennisetum glaucum*). Genetic variability studies for the identification of trait-specific germplasm accessions for various agronomic and nutritional traits are lacking in foxtail millet, and are hence seldom used in breeding.

The Patancheru-based ICRISAT genebank in India holds 1535 foxtail millet accessions from 26 countries. Using passport information and data on 23 morphological descriptors on 1474 accessions, Upadhyaya et al. (2008) developed a core collection consisting of 155 foxtail millet accessions. In this core, the accessions from race *indica* were predominant (102 accessions; 65.8%), while those from race *maxima* were 15.5% (24 accessions) and *moharia* were 18.7% (29 accessions). The predominant subraces were *nana* (81 accessions, 52.2%) and *glabra* (16 accessions, 10.3%) in race *indica*; *compacta* (20 accessions, 12.9%) and *spongiosa* (3 accessions, 1.9%) in race *maxima*; and *glabra* (17 accessions, 10.9%) and *aristata* (11 accessions, 7.1%) in race *moharia*, while accessions from other subraces were represented by 0.6–2.0%. Geographically, accessions from South and Southeast Asia were represented by 66.5% (103 accessions), while those from West Asia and East Asia were 12.9% (20 accessions) and 7.1% (11 accessions), respectively. Europe and Africa were each represented by 2.6% (4 accessions), while those from Russia and Commonwealth of Independent States (CIS) countries were only 4.5% (7 accessions).

The aims of the present investigation were to identify genetically diverse and agronomically and nutritionally superior germplasm accessions, and to develop a mini core collection (Upadhyaya and Ortiz, 2001) by evaluating core collection accessions for morpho-agronomic and nutritional traits at agro-ecologically diverse locations in India, for increased use in foxtail millet breeding and genomics.

2. Materials and methods

One hundred and fifty-five accessions of the foxtail millet core collection and five controls were evaluated at five locations in India during the 2008 rainy season. The test locations included Patancheru (17.31°N and 78.45°E), Nandyal (15.29°N and 78.29°E) and Vizianagaram (18.7°N and 83.25°E) in Andhra Pradesh; Mandya (12.33°N and 76.54°E) in Karnataka; and Dholi (24.9°N and 72.1°E) in Bihar, India. The four common controls were ISe 375, ISe 376, ISe 1468 and ISe 1541; and another location-specific control was added in the study. The experiment was conducted in an alpha design with three replications at Patancheru and in an augmented design with one of the five control cultivars repeated after every nine test entries at four other locations. Each plot consisted of 1 row of 4 m. Row-to-row spacing was maintained 30 cm at Mandya, Nandyal and Vizianagaram, 40 cm at Dholi and 60 cm at Patancheru, while plant-to-plant spacing within rows was fixed at 10 cm at all locations. The inorganic fertilizer dose included basal application of 20 kg N and 50 kg P, and a top dressing of 50 kg N ha^{-1} at 25 days after sowing. Standard agronomic practices were followed to raise a good crop. The crop was irrigated as and when necessary. Data on 9 qualitative (plant pigmentation, leaf color, growth habit, culm branching, bristle length, panicle lobbing, inflorescence compactness, lobe compactness and grain color) and 12 quantitative (days to 50% flowering, plant height, basal tillers, flag leaf blade length and width, flag leaf sheath length, peduncle length, panicle exertion, inflorescence length and width, panicle weight, and grain yield) traits were recorded using foxtail millet descriptors (IBPGR, 1985). The number of days to 50% flowering was recorded as the number of days from sowing to the date when 50% plants had started flowering. Data on plant pigmentation and growth habit were recorded on a plot basis and after completion of flowering. Data on plant height, basal tillers, flag leaf blade length and width, flag leaf sheath length, peduncle length, panicle exertion, inflorescence length and width, and panicle weight were recorded on five randomly selected plants. Panicle exertion was measured as the length of exposed peduncle from the flag leaf to the base of the panicle. Panicle length and width were measured at maturity as the maximum length from the base to the tip of the panicle, and maximum width in its natural position. A random well cleaned grain sample from each plot was used to record the observation on grain protein, calcium (Ca), iron (Fe) and zinc (Zn) concentrations in core collection accessions evaluated at Patancheru. Grain samples were powdered and digested using the tri-acid mixture and Ca, Fe, Zn in the digests were determined by atomic absorption spectrometer (Sahrawat et al., 2002), while protein was determined in the digests using an Autoanalyzer (Singh and Jambunathan, 1980).

The Residual Maximum Likelihood (REML) (Patterson and Thompson, 1971) was used to analyze data of 12 quantitative traits for individual location using GenStat 13 (http://www.vsni.co.uk). In the pooled (meta) analysis genotypes were considered as random and locations (environments) as fixed (DerSimonian and Laird, 1986; Hardy and Thompson, 1996; Whitehead, 2002). Variance components due to genotype (σ^2g) and its standard errors (σ^2e) were estimated for individual environments and for pooled analysis. The best linear unbiased predictors (BLUPs) (Schönfeld and Werner, 1986) for pooled analysis were worked out for all 16 traits. The top 21 accessions that were superior to the best control for each of the two agronomic (earliness and higher grain yield) and four nutritional (Ca, Fe, Zn, protein content) traits were identified. Further, data on 16 agronomic and quality traits including those mentioned above on 26 selected accessions (with ≥3 agronomic and quality trait combinations) were used for principal component analysis (Hotelling, 1933). The cluster analysis was performed following Ward (1963) using scores of the first five PCs.

A Gower (1985) dissimilarity matrix was created for 155 accessions using 9 qualitative (morphological) and 12 quantitative traits. Data on qualitative traits was transformed to a numerical scale (IBPGR, 1985) to calculate the dissimilarity matrix, which was subjected to hierarchical cluster algorithm (Ward, 1963) at an R^2 (squared multiple correlation value) of 0.75. This method optimizes an objective function because it minimizes the sum of squares between groups. A proportional sampling strategy of selecting the accessions was used, and ~10% of the accessions or a minimum of one accession from each cluster was randomly selected to form a mini core collection.

The 155 accessions from 23 countries were grouped into 7 regions: Africa, East Asia, South and Southeast Asia, West Asia, America, Europe and CIS countries. The origin of accessions in the eighth group was unknown. Frequency distribution of accessions among geographic regions and countries within region, races, subraces within race or qualitative traits in the core and mini core collections were tested by χ^2. Yates (1934) correction was applied if accessions for a given class in the core collection were less than five. Means for the core and mini core collections were compared by the Newman–Keuls procedure (Newman, 1939; Keuls, 1952). Homogeneity of variances was tested by Levene's test (Levene, 1960).

The coincidence rate (CR%) and variable rate (VR%) were calculated to compare the representation of accessions in core and mini core collections (Hu et al., 2000). Shannon and Weaver (1949) diversity index (H′) was used to measure and compare the phenotypic diversity for each trait in core and mini core collections. Phenotypic correlations among 12 quantitative traits in the core and mini core collections were estimated separately to determine whether associations, which may be under the same genetic control, were conserved in the mini core collection (Ortiz et al., 1998).

3. Results and discussion

In pooled analysis, both genotypic (except for inflorescence width and grain yield) and genotype × environment interaction variances were significant for all the traits (Table 1). Highly significant ($P < 0.001$) Wald (1943) statistics revealed that the environments differed significantly. The genotypic variances for most of the traits were significant at Patancheru (except for basal tillers), Dholi (except for grain yield), Mandya (panicle weight) and Vizianagaram (except for plant height, inflorescence width and grain yield), while at Nandyal the significant variances were noted only for basal tillers, panicle exertion, and inflorescence width and panicle weight (Table 1).

3.1. Identification of trait-specific accessions

The evaluation of the core collection resulted in the identification of the top 21 accessions that recorded higher grain yield (>980 kg ha^{-1}) compared to the best control, ISe 1468 (979 kg ha^{-1}), of which 12 had grain yield >1000 kg ha^{-1} (Table 2). The mean grain yield of these accessions (1026 kg ha^{-1}) was 19.3% higher than the mean grain yield of control cultivars (860 kg ha^{-1}), indicating higher yield potential of the identified accessions. The core collection had a wide range of variation for days to 50% flowering (36–78 days) from which the identified 21 accessions flowered from 36 to 43 days, on average 16 days earlier than the mean of the control cultivars (56 days). ISe# 1312, 1151, 1227, 1201, 1234, 1335, 1286, 1161, 1320 and 1647 flowered in ≤40 days; and are the best sources for early maturity.

A three-fold difference in grain calcium (Ca) concentration (90.3–288.7 mg kg^{-1}) was noted among the core collection accessions. The 21 accessions identified in this study had on average 63% higher Ca (204.4 mg kg^{-1}) than the mean of control cultivars (125.4 mg kg^{-1}). ISe# 1227, 1181, 1059, 1419, 827, 751, 1474 and 1685 had ≥200 mg kg^{-1} Ca compared to 152.8 mg kg^{-1} in the best control cultivar, ISe 1468 (Table 2). For grain iron (Fe), wide variations (24.1–68.0 mg kg^{-1}) were noted among core collection accessions. The mean Fe concentration of the 21 identified accessions was 61.4 mg kg^{-1}, 36% higher grain Fe compared to the mean of control cultivars (45.0 mg kg^{-1}). ISe# 1151, 1286, 1400, 1305, 1332, 1059, 1581 and 1320 had ≥62 mg kg^{-1} Fe. The grain zinc (Zn) concentration in core collection ranged from 33.6 to 74.2 mg kg^{-1}. The mean Zn concentration of the identified accessions was 57.2 mg kg^{-1}, 26% higher grain Zn compared to the mean of control cultivars (45.4 mg kg^{-1}). Accessions ISe 1286 (74.2 mg kg^{-1}), ISe 748 (60.1 mg kg^{-1}) and ISe 1387 (59.3 mg kg^{-1}) were the best for grain Zn concentration. The grain protein in the core collection ranged from 10.7 to 18.5%. The mean grain protein of the 21 identified accessions was 16.4%, 23.3% higher than mean of the control cultivars (13.3%). Although none of the accessions were significantly superior for protein compared to the best control cultivar ISe 1541 (grain protein 17.2%), accessions ISe# 1312, 1227, 1789, 1254 and 1541 had similar or relatively higher grain protein than ISe 1541 (Table 2).

The search for accessions with more than three desirable trait combinations resulted in the identification of 26 accessions (Table 3). ISe# 1767, 1808 and 1820 were identified as high yielding (~1000 kg ha^{-1}) with greater grain Fe, Zn and/or Ca concentrations compared to the controls. ISe# 1151, 1227 and 1312 were earliest to flower (36–37 days), lower in grain yield but either higher or similar in grain Ca, Fe, Zn and protein as compared to the best controls. ISe 1419 was late in flowering but superior in all the four nutritional traits. The selected accessions on an average had 27.6% higher Ca, 16.1% higher Fe, 12.0% higher Zn and 5.1% higher protein; all flowered 5 days earlier with grain yield similar to the average of control cultivars (56 days flowering, 860 kg ha^{-1} grain yield, 125 mg kg^{-1} Ca, 45 mg kg^{-1} Fe, 45 mg kg^{-1} Zn and 13% protein).

The clustering based on the first five principal component scores of 16 agronomic and nutritional traits, captured 85.1% variation,

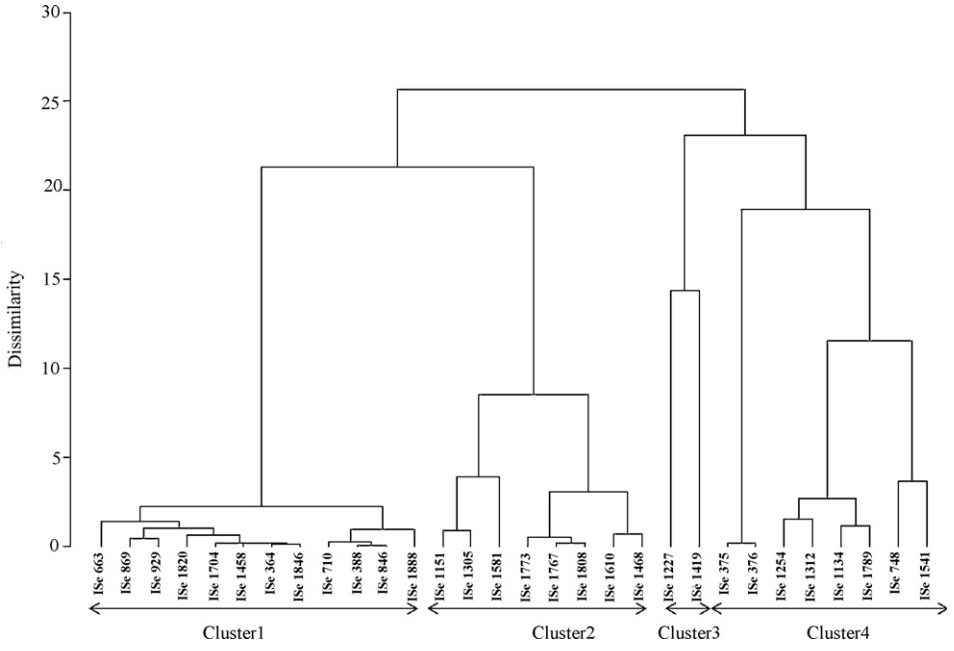

Fig. 1. Dendrogram of 26 selected accessions and four control cultivars based on scores of first five principal components (85.1% variation).

Table 1
Variance due to genotype (σ^2g), and genotype × environment interaction (σ^2ge) for 12 quantitative traits among 155 foxtail millet core collection accessions evaluated at five locations, 2008 rainy season, India.

Trait	Pooled		Environment		Nandyal (σ^2g)	Patancheru (σ^2g)	Dholi (σ^2g)	Mandya (σ^2g)	Vizianagaram (σ^2g)
	σ^2g	σ^2ge	Wald statistics	*F* prob					
Days to 50% flowering	44.6**	20.1**	503.7	<0.001	8.8	142.9**	61.8**	46.8**	45.4**
Plant height (cm)	315.6**	101.5**	1351.0	<0.001	58.4	687.7**	557.1**	357.1**	60.4
Basal tiller (no.)	0.1*	0.1*	172.7	<0.001	0.5**	0.2	1.1**	0.2**	1.6**
Flag leaf blade length (mm)	1683.0**	1806.7**	400.6	<0.001	671.0	8047.0**	6866.8**	2223.7**	2604.0**
Flag leaf blade width (mm)	4.8**	6.5**	186.4	<0.001	2.4	18.2**	16.2**	11.2**	12.2**
Flag leaf sheath length (mm)	122.8**	121.5**	151.9	<0.001	148.6	677.7**	807.3**	171.3**	5.6**
Peduncle length (mm)	253.0**	362.0**	3057.5	<0.001	1238.0	2247.0**	1452.0*	71.4**	15.4**
Panicle exertion (mm)	200.0**	355.0**	255.3	<0.001	729.8**	1049.1**	1560.9**	807.3**	13.3**
Inflorescence length (mm)	883.6**	750.8**	287.3	<0.001	256.1	3139.2**	2747.2**	746.4**	1611.2**
Inflorescence width (mm)	4.7	5.2**	929.4	<0.001	7.7**	17.4**	4.7**	7.4**	7.7
Panicle weight (g)	0.2**	0.7**	1132.8	<0.001	0.9**	0.8**	4.5**	<0.001	2.6**
Grain yield (kg ha^{-1})	33519.0	138128.0**	113.4	<0.001	<0.001	393988.0**	<0.001	35624.0**	<0.001

* Significant at $P = 0.05$.
** Significant at $P = 0.01$.

and grouped the accessions into four clusters (Fig. 1), indicating diversity among the identified 26 accessions and four control cultivars. The majority of the high yielding accessions were grouped in cluster 1, whereas the accessions with high yield and better nutritional traits (Ca, Fe and Zn) or with only high nutritional traits grouped in cluster 2 including the control cultivar ISe 1468. Cluster 3 included only ISe 1227 and ISe 1419 (landraces), both yielded low and were superior in nutritional quality. The accessions with high protein concentration in combination with either earliness or high Ca and Fe/Zn concentrations were grouped in cluster 4. A high protein control cultivar ISe 1541 was also grouped in cluster 4. These results indicate diversity among the identified accessions, which can be utilized to broaden the genetic base by bringing an additional source of variability for sustainable genetic improvement of foxtail millet for grain yield and nutritional traits.

3.2. Development of mini core collection

Data on 21 traits of 155 core collection accessions were subjected to estimate phenotypic distance matrix (Gower, 1985) that was used for hierarchical cluster analysis (Ward, 1963), which resulted in classifying these accessions into 25 clusters, with number of accessions in the individual cluster ranging from 2 to 11. A mini core collection of 35 accessions was formed using the sampling

Table 2
Top 21 accessions identified for two agronomic and four nutritional traits in core collection of foxtail millet.

Accession	Grain yield (kg ha^{-1})	Accession	Days to 50% flowering	Accession	Ca (mg kg^{-1})	Accession	Fe (mg kg^{-1})	Accession	Zn (mg kg^{-1})	Accession	Protein (%)
ISe 710	1118	ISe 1312	36	ISe 1227	288.7	ISe 1151	68.0	ISe 1286	74.2	ISe 1312	18.5
ISe 969	1113	ISe 1151	37	ISe 1181	271.6	ISe 1286	66.0	ISe 748	60.1	ISe 1227	17.8
ISe 1820	1093	ISe 1227	37	ISe 1059	248.4	ISe 1400	65.1	ISe 1387	59.3	ISe 1789	17.7
ISe 388	1092	ISe 1201	38	ISe 1419	241.0	ISe 1305	63.9	ISe 195	58.5	ISe 1254	17.2
ISe 842	1072	ISe 1234	38	ISe 827	236.4	ISe 1332	63.7	ISe 1134	58.3	ISe 1541	17.2
ISe 49	1065	ISe 1335	38	ISe 751	221.3	ISe 1059	63.3	ISe 1408	57.9	ISe 827	16.9
ISe 1888	1034	ISe 1286	39	ISe 1474	204.8	ISe 1581	62.4	ISe 1419	57.2	ISe 748	16.8
ISe 90	1030	ISe 1161	40	ISe 1685	200.3	ISe 1320	62.2	ISe 1161	56.4	ISe 1305	16.7
ISe 364	1011	ISe 1320	40	ISe 900	199.5	ISe 1312	61.9	ISe 900	56.2	ISe 1647	16.5
ISe 1767	1009	ISe 1647	40	ISe 840	192.5	ISe 144	61.7	ISe 1820	56.0	ISe 1335	16.2
ISe 362	1008	ISe 1638	41	ISe 1629	190.8	ISe 1163	61.1	ISe 1320	55.9	ISe 751	16.1
ISe 1808	1004	ISe 1037	41	ISe 1851	188.1	ISe 1460	60.0	ISe 1654	55.9	ISe 1118	16.0
ISe 846	996	ISe 1181	41	ISe 769	187.3	ISe 160	59.4	ISe 1704	55.5	ISe 1134	16.0
ISe 869	996	ISe 1563	41	ISe 1581	184.2	ISe 1037	59.3	ISe 1605	55.5	ISe 1151	15.9
ISe 1511	996	ISe 1254	41	ISe 1286	181.5	ISe 1597	59.1	ISe 403	55.3	ISe 195	15.8
ISe 909	992	ISe 1204	41	ISe 1136	180.5	ISe 1009	58.9	ISe 1808	55.2	ISe 1234	15.7
ISe 1846	986	ISe 1547	42	ISe 1161	179.7	ISe 1161	58.9	ISe 751	55.2	ISe 1067	15.7
ISe 1610	984	ISe 1187	42	ISe 1773	176.6	ISe 1704	58.8	ISe 1674	54.9	ISe 1419	15.7
ISe 795	983	ISe 403	42	ISe 931	174.8	ISe 1187	58.6	ISe 144	54.9	ISe 144	15.7
ISe 1458	981	ISe 1118	43	ISe 869	173.9	ISe 1745	58.4	ISe 1234	54.5	ISe 735	15.6
ISe1704	981	ISe 1163	43	ISe 663	171.2	ISe 838	58.2	ISe 985	54.5	ISe 1161	15.6
Mean	1025.8		40.1		204.4		61.4		57.2		16.4
Controls											
ISe 375	705		55		126.7		40.1		42.0		11.3
ISe 376	790		54		114.6		43.9		46.4		11.4
ISe 1468	979		51		152.8		48.6		41.3		13.4
ISe 1541	966		64		107.4		47.6		51.9		17.2
Mean	860.0		55.7		125.4		45.0		45.4		13.3
Trial mean	806.33		50.92		145.82		49.78		47.85		13.50
SEM±	13.3		3.766		18.15		5.92		4.88		0.81
LSD ($P = 0.05$)	36.9		10.45		50.79		16.57		13.65		2.26
CV (%)	21.9		5.29		17.76		16.89		14.42		8.55
Trial range											
Minimum	485		36		17.8		5.9		4.9		0.8
Maximum	1118		79		288.7		68.0		74.2		18.5

Table 3
Best 26 accessions identified with three or more agronomic and nutritional traits combinations in core collection of foxtail millet.

Accession	Days to 50% flowering	Grain yield (kg ha^{-1})	Ca (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Protein (%)
ISe 364	53.2	1010.8	123.9	42.8	53.1	12.4
ISe 388	52.2	1091.6	152.6	40.9	45.9	12.4
ISe 663	54.2	842.3	171.2	51.6	54.3	11.9
ISe 710	53.5	1118.0	156.3	45.4	44.2	11.4
ISe 748	70.5	671.4	154.2	43.1	60.1	16.8
ISe 846	51.4	995.7	147.3	51.1	47.9	11.9
ISe 869	53.9	995.7	173.9	46.5	46.2	13.0
ISe 969	51.5	1113.1	145.7	49.4	47.3	12.6
ISe 1134	44.0	625.6	153.7	55.8	58.3	16.0
ISe 1151	37.4	525.2	163.7	68.0	49.3	15.9
ISe 1227	37.4	560.1	288.7	48.7	51.7	17.8
ISe 1254	41.2	563.4	162.8	49.8	47.8	17.2
ISe 1305	43.2	583.6	153.9	63.9	51.0	16.7
ISe 1312	36.1	489.3	134.6	61.9	50.3	18.5
ISe 1419	78.4	765.4	241.0	52.2	57.2	15.7
ISe 1458	54.1	981.2	143.5	54.8	52.2	13.1
ISe 1581	44.9	512.6	184.2	62.4	46.8	12.5
ISe 1610	49.6	983.9	136.8	51.0	42.0	11.5
ISe 1704	51.8	981.2	148.6	58.8	55.5	12.6
ISe 1767	53.4	1008.9	145.6	57.3	53.9	12.8
ISe 1773	52.4	830.5	176.6	55.4	53.4	13.2
ISe 1789	43.0	733.1	140.9	49.8	47.8	17.7
ISe 1808	50.9	1003.8	152.7	55.3	55.2	13.3
ISe 1820	50.7	1092.5	145.5	50.1	56.0	13.1
ISe 1846	52.6	985.6	136.3	51.2	50.9	12.5
ISe 1888	50.2	1033.5	125.6	42.1	44.3	11.7
Mean	50.4	849.9	160.0	52.3	50.9	14.0
Control						
ISe 375	54.7	704.8	126.7	40.1	42.0	11.3
ISe 376	54.2	790.4	114.6	43.9	46.4	11.4
ISe 1468	50.5	978.9	152.8	48.6	41.3	13.4
ISe 1541	63.6	965.8	107.4	47.6	51.9	17.2
Mean	55.7	860.0	125.4	45.0	45.4	13.3
Trial mean	50.92	806.33	145.82	49.78	47.85	13.50
SEM±	3.766	153.349	18.15	5.92	4.88	0.81
LSD (P = 0.05)	10.45	425.69	50.79	16.57	13.65	2.26
CV (%)	5.29	51.99	17.76	16.89	14.42	8.55

strategy of 10% or a minimum of one accession from each cluster. It represented 22.6% of core collection accessions or 2.4% of the entire collection of foxtail millet in the ICRISAT genebank. Thus, adequate diversity from the core or entire collection has been preserved in the mini core. Other statistics revealed that this mini core represented 86–100% range variation for most of the traits, except for panicle weight (60%), from the core collection (Tables 4 and 5). The differences between means of the core and mini core were found to be non-significant for all the traits, while the variance of the core and mini core was homogeneous for all traits, except for flag leaf blade length and peduncle length ($P < 0.05$) (Table 4).

The Shannon–Weaver diversity index (H') is used in genetic studies as a convenient measure of both allelic richness and evenness. A low H′ indicates an extremely unbalanced frequency of classes for an individual trait and a lack of genetic diversity. The average H′ for 12 quantitative traits in the mini core (0.571 ± 0.0074) was comparable to the core collection (0.589 ± 0.0101), indicating that diversity of the core was well represented in the mini core. The variance and coefficient of variation in the selected subset should be higher than the initial collection (Hu et al., 2000). In the present study, high coincidence rate (86–100% for 11 of the 12 traits) and higher variable rate (100% to 129% for all the 12 traits) (Table 5) further confirmed that the mini core is representative of the core collection.

The χ^2 probabilities of the frequency distribution of foxtail millet core and mini core accessions were non-significant for

Table 4
Comparison of range, means and variances for 12 quantitative traits in foxtail millet core and mini core collections.

Trait	Range		Mean*		Variance			
	Core	Mini core	Core	Mini core	Core	Mini core	F value	Probability
Days to 50% flowering	36.09–78.54	38.34–78.54	50.8a	50.5a	47.76	69.63	0.89	0.35
Plant height (cm)	58.2–131.4	62.9–127.5	102.8a	101.5a	330.42	344.09	0.01	0.93
Basal tiller (no.)	2.264–3.135	2.355–3.102	2.7a	2.7a	0.03	0.04	<0.001	0.97
Flag leaf blade length (mm)	158.2–370.1	158.2–370.1	271.9a	265.4a	1900.15	3005.47	4.12	0.04
Flag leaf blade width (mm)	11.94–22.47	11.94–22.44	17.0a	17.1a	4.78	7.21	3.58	0.06
Flag leaf sheath length (mm)	110.5–157.2	110.5–152.7	136.3a	135.9a	100.28	139.13	2.17	0.14
Peduncle length (mm)	232–297.1	235.8–297.1	263.0a	263.4a	176.94	278.57	4.32	0.04
Panicle exertion (mm)	112.3–177.5	115.6–171.5	138.8a	140.7a	178.68	235.47	1.27	0.26
Inflorescence length (mm)	55–204.3	55–189.2	140.7a	136.3a	995.23	1323.32	1.51	0.22
Inflorescence width (mm)	15.41–26.65	16.41–26.65	19.3a	19.5a	3.51	5.48	1.65	0.20
Panicle weight (g)	2.12–5.985	2.12–4.436	2.9a	2.9a	0.18	0.18	<0.001	0.98
Grain yield (kg ha^{-1})	485.2–1118	485.2–1118	762.8a	733.2a	28018.13	31039.15	0.18	0.98

* Means followed by same letters were not significantly different at P = 0.05.

Table 5
Coincidence rate (CR%), variable rate (VR%) and Shannon diversity index (H') in foxtail millet core and mini core collections.

Traits	CR%	VR%	H' index	
			Core	Mini core
Days to 50% flowering	94.7	121.4	0.549	0.524
Plant height (cm)	88.3	103.3	0.584	0.583
Basal tillers (no.)	85.8	100.0	0.619	0.601
Flag leaf blade length (mm)	100.0	128.8	0.596	0.574
Flag leaf blade width mm)	99.7	122.2	0.610	0.599
Flag leaf sheath length (mm)	90.4	118.2	0.628	0.586
Peduncle length (mm)	94.2	125.2	0.609	0.592
Panicle exertion (mm)	85.7	113.2	0.598	0.550
Inflorescence length (mm)	89.9	119.1	0.618	0.574
Inflorescence width (mm)	91.1	123.3	0.584	0.572
Panicle weight (g)	59.9	100.7	0.504	0.526
Grain yield (kg ha^{-1})	100.0	109.5	0.571	0.577
Mean ± SEM	90.0 ± 3.11	115.4 ± 2.86	0.589 ± 0.0101	0.571 ± 0.0074

geographic regions as well as countries within region, indicating that accessions from the regions (except for Africa and Europe) or those from countries within a region were well represented in the mini core collection (Table 6). Biologically, the foxtail millet core collection accessions are represented by three races and 10 subraces (Upadhyaya et al., 2008). The χ^2 probabilities of the frequency distribution of races and subraces within race accessions of core and mini core revealed that core collection accessions from both races and subraces were well represented in the mini core (Table 7). Furthermore, the non-significant χ^2 probabilities of the frequency distribution of nine qualitative traits in core and mini core collections revealed that these traits were well represented in the mini core (Table 8).

Proper and adequate sampling of accessions in core from the entire collection helps to conserve phenotypic associations arising from co-adapted gene complexes (Ortiz et al., 1998). In the present study, there is a fair degree of similarity in phenotypic correlation coefficients among 12 quantitative traits (data not presented), suggesting that this mini core has preserved most of the co-adapted gene complexes controlling these associations. Further, the proportion of variance in one trait that can be attributed to its relationship with a second trait is indicated by the square of correlation coefficient (Snedecor and Cochran, 1980), and the estimate of this value greater than 0.71 or lower than −0.71 suggests meaningful correlations (Skinner et al., 1999). Few correlation coefficients in the present study (10 in core and 12 in mini core) were close to this value in both the core and mini core, with coefficient, in general, greater in the mini core than that in the core collection (Table 9).

The various statistical tests (Tables 4–9) indicated that for all the quantitative traits substantial diversity of the core collection (155 accessions) (Upadhyaya et al., 2008) was retained in the mini core collection (35 accessions) (Table 10). The average H′ of 12 quantitative traits in the mini core collection (0.571 ± 0.0101) was 97% of the core collection (0.589 ± 0.0074) (Table 5). Further, the means of core and mini core collections did not differ significantly for any of the 12 quantitative traits (Table 4). The CR% reflects the percentage of range captured in the mini core collection, which was high (86–100%) for 11 of 12 traits. The VR% reflects CV% in the mini core, and ranged from 100% to 129% (Table 5). The results in this study confirmed our results in formulating mini core collections

Table 6
Frequency distribution and χ^2 probability (P) of accessions when grouped based on geographical groupings (regions and countries within region) in core and mini core collection of foxtail millet.

Regions and countries within region	Core	Mini core	df	χ^2	P
Africa	4	4	1	7.466	0.006
Ethiopia	1	1	1	0.250	0.617
Kenya	1	1	1	0.250	0.617
Malawi	1	1	1	0.250	0.617
South Africa	1	1	1	0.250	0.617
Heterogeneity			3	6.466	0.091
Russia and CIS countries	7	2	1	0.111	0.739
Europe	4	4	1	7.466	0.006
Hungary	1	1	1	0.250	0.617
Spain	1	1	1	0.250	0.617
Switzerland	1	1	1	0.250	0.617
United Kingdom	1	1	1	0.250	0.617
Heterogeneity			3	6.466	0.091
East Asia	11	4	1	0.925	0.336
China	6	1	1	0.640	0.424
Republic of Korea	5	1	1	0.368	0.544
Heterogeneity			1	0.083	0.773
South and Southeast Asia	103	17	1	1.684	0.194
India	93	12	1	0.731	0.393
Myanmar	1	1	1	0.680	0.410
Nepal	2	1	1	0.087	0.767
Pakistan	3	1	1	0.000	0.995
Sri Lanka	1	1	1	0.680	0.410
Taiwan	3	1	1	0.000	0.995
Heterogeneity			5	0.494	0.992
West Asia	20	5	1	0.052	0.820
Afghanistan	2	1	1	0.000	1.000
Iran	1	1	1	0.250	0.617
Lebanon	3	1	1	0.083	0.773
Syria	12	1	1	1.333	0.248
Turkey	2	1	1	0.000	1.000
Heterogeneity			4	1.615	0.806
North America (USA)	5	1	1	0.015	0.903
Unknown	1	0	1	2.333	0.127

Table 7
Frequency distribution and χ^2 probability (P) of accessions when grouped based on races and subraces in core and mini core collection of foxtail millet.

Races and subraces within race	Core	Mini core	df	χ^2	P
indica	102	20	1	0.399	0.528
erecta	2	1	1	0.030	0.863
glabra	16	6	1	2.612	0.106
nana	81	12	1	0.949	0.330
profusa	3	1	1	0.222	0.638
Heterogeneity			3	3.414	0.332
maxima	24	6	1	0.062	0.803
assamense	1	1	1	1.960	0.162
compacta	20	4	1	0.200	0.655
spongiosa	3	1	1	0.053	0.818
Heterogeneity			2	2.151	0.341
moharia	29	9	1	0.918	0.338
aristata	11	3	1	0.050	0.823
fusiformis	1	1	1	1.318	0.251
glabra	17	5	1	0.014	0.906
Heterogeneity			2	0.465	0.793

Table 8
Frequency distribution and χ^2 probability (P) of qualitative traits representing in core and mini core collections in foxtail millet.

Descriptor	Core	Mini core	df	χ^2	P
Plant pigmentation			1	0.261	0.609
Green	129	28	1	0.044	0.834
Pigmented	26	7	1	0.217	0.641
Leaf color			1	0.726	0.394
Green	119	29	1	0.169	0.681
Yellow	36	6	1	0.558	0.455
Growth habit			3	0.689	0.876
Decumbent	4	1	1	0.180	0.671
Erect	118	26	1	0.016	0.899
Erect geneculate	24	5	1	0.032	0.858
Prostrate	9	3	1	0.461	0.497
Culm branching			2	0.894	0.640
High	69	17	1	0.129	0.720
Low	44	11	1	0.114	0.736
Medium	42	7	1	0.651	0.420
Bristle length			2	2.127	0.345
Long	89	16	1	0.835	0.361
Medium	39	12	1	1.158	0.282
Short	27	7	1	0.134	0.714
Panicle lobing			2	0.144	0.931
Dense lobed	35	7	1	0.103	0.748
Medium lobed	89	21	1	0.041	0.840
Non lobed	31	7	1	0.000	1.000
Inflorescense compactness			2	1.155	0.561
Compact	109	24	1	0.015	0.903
Loose	15	2	1	0.568	0.451
Medium	31	9	1	0.571	0.450
Lobe compactness			2	0.599	0.741
Compact	125	30	1	0.112	0.738
Loose	7	1	1	0.213	0.644
Medium	23	4	1	0.274	0.601
Grain color			3	4.038	0.257
Black	2	0	1	2.005	0.157
Black and white	2	0	1	2.005	0.157
Red	9	2	1	0.001	0.975
Yellow	142	33	1	0.027	0.870

in chickpea (*Cicer arietinum*) (Upadhyaya and Ortiz, 2001), groundnut (*Arachis hypogaea*) (Upadhyaya et al., 2002), pigeonpea (*Cajanus cajan*) (Upadhyaya et al., 2006), sorghum (Upadhyaya et al., 2009b), finger millet (*Eleucine coracana*) (Upadhyaya et al., 2010b) and pearl millet (Upadhyaya et al., 2011b), as the mini core collection of foxtail millet retained most of the diversity of the core collection.

The development of the mini core in foxtail millet has dramatically reduced the number of entries, and thus provides the pool of diversity that can be extensively evaluated for economically important traits through multilocation evaluation to identify new sources of variation for use in foxtail millet breeding. Research con-

Table 9
Correlation coefficient with values more than 0.649 among some agronomic traits in foxtail millet core and mini core collection.

Trait	Correlation coefficient (r)	
	Core collection	Mini core collection
Days to 50% flowering and plant height	0.758	0.721
Days to 50% flowering and flag leaf blade length	0.699	0.645
Days to 50% flowering and inflorescence length	0.694	0.674
Plant height and flag leaf blade length	0.817	0.834
Plant height and flag leaf sheath length	0.740	0.828
Plant height and inflorescence length	0.862	0.877
Flag leaf blade length and flag leaf sheath length	0.699	0.699
Flag leaf blade length and inflorescence length	0.861	0.876
Flag leaf sheath length and inflorescence length	0.733	0.759
Peduncle length and panicle exertion	0.759	0.762
Flag leaf width and inflorescence width	0.667	0.811
Flag leaf width and panicle weight	0.457	0.734
Flag leaf sheath length and peduncle length	0.602	0.708
Inflorescence width and panicle weight	0.452	0.725

Table 10
Country of origin, cluster no., races and subraces description of 35 accessions included in foxtail millet mini core collection.

Accession identity	Cluster no.	Country	Race	Subrace
ISe 2	1	India	*indica*	*nana*
ISe 132	20	India	*indica*	*nana*
ISe 375	7	India	*maxima*	*assamense*
ISe 507	12	Kenya	*indica*	*nana*
ISe 663	23	Switzerland	*indica*	*nana*
ISe 710	22	India	*indica*	*nana*
ISe 719	2	Pakistan	*moharia*	*aristata*
ISe 745	17	India	*indica*	*nana*
ISe 748	10	India	*indica*	*nana*
ISe 751	13	India	*indica*	*glabra*
ISe 783	16	India	*indica*	*nana*
ISe 907	15	India	*indica*	*nana*
ISe 963	14	India	*indica*	*nana*
ISe 1037	21	Lebanon	*moharia*	*aristata*
ISe 1161	4	Syria	*moharia*	*glabra*
ISe 1181	7	China	*maxima*	*compacta*
ISe 1209	25	Russia	*moharia*	*fusiformis*
ISe 1254	3	Russia	*moharia*	*glabra*
ISe 1269	24	South Africa	*indica*	*nana*
ISe 1299	25	Iran	*moharia*	*glabra*
ISe 1302	9	Afghanistan	*moharia*	*aristata*
ISe 1305	9	Spain	*moharia*	*glabra*
ISe 1335	19	Hungary	*moharia*	*glabra*
ISe 1338	8	Turkey	*maxima*	*compacta*
ISe 1387	17	Sri Lanka	*indica*	*glabra*
ISe 1474	6	United Kingdom	*indica*	*glabra*
ISe 1563	11	Republic of Korea	*maxima*	*compacta*
ISe 1610	20	Malawi	*indica*	*glabra*
ISe 1655	11	Taiwan	*indica*	*glabra*
ISe 1687	18	India	*maxima*	*spongiosa*
ISe 1736	10	Nepal	*maxima*	*compacta*
ISe 1745	17	Myanmar	*indica*	*glabra*
ISe 1820	9	India	*indica*	*erecta*
ISe 1888	5	Ethiopia	*indica*	*nana*
ISe 1892	7	USA	*indica*	*profusa*

ducted at ICRISAT and at other locations by our partners revealed that when chickpea, finger millet, groundnut, pigeonpea, pearl millet and sorghum mini core collections were evaluated, researchers were able to identify new sources of variation for various agronomic traits (Upadhyaya et al., 2009a, 2010c; Meena et al., 2010; Parameshwarappa et al., 2011), seed quality (Upadhyaya et al., 2009a, 2010c, in press, 2011a), resistance to biotic (Pande et al., 2006; Upadhyaya et al., 2009a, 2010c; Sharma et al., 2010) and abiotic (Serraj et al., 2004; Krishnamurthy et al., 2003, 2010, 2011a,b; Upadhyaya et al., 2009a, 2010a, 2010c; Upadhyaya, 2005; Kashiwagi et al., 2005, 2006, 2010; Srivastava et al., 2006; Vadez et al., 2007) stresses. Similarly, trait-specific germplasm were identified using groundnut mini core in China (Jiang et al., 2010), USA (Holbrook and Dong, 2005; Chamberlin et al., 2010) and India (Yugandhar, 2005).

The process and concept of mini core advocated by Upadhyaya and Ortiz (2001) has now been recognized as an "International Public Good (IPG)", with the result that many national programs have shown great interest in evaluating mini core subsets as reflected by the supply of 114 sets of mini core of chickpea, finger millet, foxtail millet, groundnut, pigeonpea, pearl millet and sorghum to researchers in 18 countries, and the researchers from these countries have reported new and diverse sources of variation for several agronomic and nutritional traits, and for resistance to biotic and abiotic stress in these crops (Upadhyaya et al., 2009a, 2010c). This mini core collection can also be used for molecular characterization to identify genetically diverse germplasm for use in breeding and genomics studies in foxtail millet. Researchers can avail limited seeds of foxtail millet mini core accessions following the Standard Material Transfer Agreement (SMTA), free of charge from the genebank at ICRISAT, Patancheru, India.

4. Conclusions

The multilocation evaluation of the foxtail millet core collection led to (i) the development of mini core and (ii) identification of trait-specific diverse germplasm superior to controls. A few germplasm with early flowering, high grain yield and with better nutritional profiles (greater seed protein, Ca, Fe and Zn) have been identified. The targeted evaluation of mini core developed in this study will facilitate identification of new sources of variation for other agronomically desirable traits and biotic and abiotic stresses, which will lead to better utilization of germplasm by the breeders.

Acknowledgements

The authors gratefully acknowledge the financial support of the BMZ/GTZ project on "Sustainable conservation and utilization of genetic resources of two underutilized crops-finger millet and foxtail millet- to enhance productivity, nutrition and income in Africa and Asia" funded by the Federal Ministry for Economic Cooperation and Development (BMZ), Germany to carry out this activity.

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