Analysis of molecular genetic diversity in a representative collection of foxtail millet [*Setaria italica* (L.) P. Beauv.] from different agro-ecological regions of India

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Abstract Foxtail millet [*Setaria italica* (L.) P. Beauv.], an important crop of East Asia is known for its drought tolerance and was once an indispensable crop of vast rainfed areas in semi-arid regions in India. In India it is cultivated in Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu, Rajasthan, Madhya Pradesh, Uttar Pradesh and north eastern states. The grain finds use in several local recipes such as *roti* (bread), *jaula*, *singal*, *sirol*. Foxtail millet grain contains 12.3 % protein, 4.7 % fat, 60.6 % carbohydrates, and 3.2 % ash. The present study was conducted to analyse the genetic diversity among foxtail accessions from different states of India and a few exotic accessions using RAPD and ISSR techniques and identify diverse accessions for use in variety improvement programmes. A set of 125 foxtail millet accessions selected from 11 different agro-ecological regions of India were analyzed using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) marker techniques. A total of 146 (115 RAPD and 31 ISSR) scoreable markers were generated with 16 RAPD and four ISSR primers. The dendrogram generated using Nei's genetic distances and principal component analyses revealed presence of two clusters and two subclusters in group I. The accessions from Andhra Pradesh, Karnataka, Maharashtra and Uttarakhand were more diverse since they were distributed in both the clusters. There was no clear geographical differentiation observable. The bootstrap support for the major groups identified was strong (above 80 %) indicating good

statistical support. The average value of Nei and Li's genetic distance was lowest (0.081) for accessions from West Bengal while the collections from Karnataka showed highest dissimilarity (average genetic distance = 0.239). The average genetic distance for all 125 accessions together was 0.177 indicating presence of only moderate genetic diversity in the collections. The analysis of molecular variance indicated that only 2.76 % variation was explained by variations among the groups and 11.55 % among populations within groups. However the percentage of variation observed within populations was high (85.68). The value of F_{st} was observed to be very low (0.028) indicating low differentiation of the accessions analysed. The population genetic analysis carried out indicates that highest number of alleles per locus (1.745 ± 0.438) was observed for Andhra Pradesh with 35 accessions. When four eco-geographic regions were considered, the southern region comprising AP, Karnataka and TN showed the highest number of alleles per locus (1.787 ± 0.411). The value of G_{st} was lowest for south (0.123) and highest for central west (0.455). This indicated that all the landraces from south share common alleles. The gene flow between the accessions from different regions was also observed to be high with the highest migration (3.557) recorded for south.

Keywords Foxtail · Genetic diversity · RAPD · ISSR markers · Spatial distribution

Introduction

Foxtail millet [*Setaria italica* (L.) P. Beauv.], is the second most widely planted species amongst millets and the most important in East Asia. It is also known as Italian millet, German millet and Hay millet. *Kangni*, *Navane*, *Tenai*,

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Table 1 Details of the foxtail germplasm accessions analyzed and their source

S. no.	Collection no.	Accession no.	State/origin
1.	BAR96	IC426717	Andhra Pradesh
2.	BAR252	IC426735	Andhra Pradesh
3.	BAS78	IC345014	Andhra Pradesh
4.	CMLS01_394	IC430606	Andhra Pradesh
5.	GS13	IC403438	Andhra Pradesh
6.	GS15	IC403476	Andhra Pradesh
7.	GS17	IC403480	Andhra Pradesh
8.	GS21	IC403579	Andhra Pradesh
9.	GS24	IC403814	Andhra Pradesh
10.	GS27	IC403846	Andhra Pradesh
11.	GS1384	IC479929	Andhra Pradesh
12.	GS1462	IC479267	Andhra Pradesh
13.	GS1464	IC479353	Andhra Pradesh
14.	GS1492	IC479708	Andhra Pradesh
15.	GS1494	IC479372	Andhra Pradesh
16.	GS1519	IC479479	Andhra Pradesh
17.	GS1566	IC479561	Andhra Pradesh
18.	KARS385	IC436863	Andhra Pradesh
19.	KARS408	IC436885	Andhra Pradesh
20.	KARS451A	IC436928	Andhra Pradesh
21.	KARS452	IC436929	Andhra Pradesh
22.	KDRS108	IC519642	Andhra Pradesh
23.	NDS96	IC328716	Andhra Pradesh
24.	NDS98	IC328718	Andhra Pradesh
25.	PSR10951	IC438725	Andhra Pradesh
26.	PSR10951A	IC4387251	Andhra Pradesh
27.	SIA326	SIA326	Andhra Pradesh
28.	SIA2829	SIA2829	Andhra Pradesh
29.	SIA2871	SIA2871	Andhra Pradesh
30.	SRAA380	IC309974	Andhra Pradesh
31.	Krishnadevaraya	Krishnadevaraya	Andhra Pradesh
32.	Lepakshi	Lepakshi	Andhra Pradesh
33.	Narasimharaya	Narasimharaya	Andhra Pradesh
34.	Prasad	Prasad	Andhra Pradesh
35.	SriLaxmi	SriLaxmi	Andhra Pradesh
36.	PS4	IC276598	Andhra Pradesh
37.	GS71	IC403989	Bihar
38.	GS77	IC403994	Bihar
39.	GS78	IC480474	Bihar
40.	GS102	IC404103	Bihar
41.	GS104	IC404112	Bihar
42.	GS105	IC404133	Bihar
43.	GS106	IC404144	Bihar
44.	GS111	IC404178	Bihar
45.	GS1377	IC480104	Bihar
46.	GS1380	IC479586	Bihar
47.	GS144	IC480227	Jammu & Kashmir
48.	GS146	IC403483	Jammu & Kashmir

Table 1 (continued)

S. no.	Collection no.	Accession no.	State/origin
49.	GS291	IC403871A	Karnataka
50.	GS308	IC403886	Karnataka
51.	GS326	IC403899	Karnataka
52.	GS337	IC480966A	Karnataka
53.	GS2076	GS2076	Karnataka
54.	GS2087	GS2087	Karnataka
55.	GS2092	GS2092	Karnataka
56.	GS161	IC403521	Madhya Pradesh
57.	GS162	IC403522	Madhya Pradesh
58.	GS163	IC403522A	Madhya Pradesh
59.	GS243	IC480833	Maharashtra
60.	GS245	IC480833B	Maharashtra
61.	GS253	IC403844	Maharashtra
62.	GS256	IC480554	Maharashtra
63.	GS260	IC480375	Maharashtra
64.	GS1430	IC479560	Maharashtra
65.	LS-131/2000	IC340216	Maharashtra
66.	LS-140/2000	IC340225	Maharashtra
67.	GS359	IC480415	Rajasthan
68.	GS200	IC403717	Tamil Nadu
69.	GS205	IC403739	Tamil Nadu
70.	GS219	IC403824	Tamil Nadu
71.	GS220	IC403825	Tamil Nadu
72.	GS227	IC403832	Tamil Nadu
73.	GS230	IC403834	Tamil Nadu
74.	GS231	IC403835	Tamil Nadu
75.	GS553	IC480440	Tamil Nadu
76.	GS563	IC479442	Tamil Nadu
77.	GS1917	IC479545	Tamil Nadu
78.	GS663	IC480185	Uttarakhand
79.	GS760	IC480045	Uttarakhand
80.	GS825	IC479797	Uttarakhand
81.	GS842	IC480109	Uttarakhand
82.	GS850	IC479615	Uttarakhand
83.	GS869	IC479746	Uttarakhand
84.	GS873	IC479780	Uttarakhand
85.	GS874	IC479578	Uttarakhand
86.	GS875	IC480201	Uttarakhand
87.	GS880	IC479481	Uttarakhand
88.	GS885	IC479991	Uttarakhand
89.	GS887	IC479674	Uttarakhand
90.	GS888	IC479419	Uttarakhand
91.	GS909	IC479883	Uttarakhand
92.	GS926	IC479731	Uttarakhand
93.	GS941	IC479985	Uttarakhand
94.	GS945	IC479684	Uttarakhand
95.	GS961	IC479762	Uttarakhand
96.	GS996	IC479890	Uttarakhand
97.	GS999	IC479753	Uttarakhand

Table 1 (continued)

S. no.	Collection no.	Accession no.	State/origin
98.	GS1001	IC479887	Uttarakhand
99.	GS1018	IC479989	Uttarakhand
100.	GS1036	IC479841	Uttarakhand
101.	GS1074	IC479702	Uttarakhand
102.	GS1097	IC479770	Uttarakhand
103.	GS1186	IC479544	Uttarakhand
104.	GS1196	IC480424	Uttarakhand
105.	GS1204	IC479582	Uttarakhand
106.	GS1211	IC480252	Uttarakhand
107.	GS1221	IC479738	Uttarakhand
108.	GS1242	IC479411	Uttarakhand
109.	GS1315	IC479661	Uttarakhand
110.	GS1339	IC479680	Uttarakhand
111.	GS401	IC480117	Uttar Pradesh
112.	GS1957	IC479243	Uttar Pradesh
113.	GS40	IC480268	West Bengal
114.	GS42	IC403922	West Bengal
115.	GS47	IC403929	West Bengal
116.	GS55	IC403962	West Bengal
117.	GS2095	GS2095	Bioversity International
118.	GS2097	GS2097	Bioversity International
119.	GS2116	GS2116	Bioversity International
120.	GS2145	IC97102	Bioversity International
121.	GS2152	IC 97176	Bioversity International
122.	GS2158	GS2158	Bioversity International
123.	GS2236	GS2236	Bioversity International
124.	GS1404	IC479384	China
125.	GS539	IC404017	Unknown

Korra, *Rala*, and *Kauni* are the common names of foxtail millet in different parts of India. Several hypotheses concerning the origin and domestication of foxtail millet have been proposed. Cytological studies indicated that the wild ancestor of foxtail millet is *Setaria viridis* (Kihara and Kishimoto 1942; Li et al. 1945). In China, foxtail millet is the most common millet and one of the main food crops, especially among the poor in dry northern parts of the country. Vavilov (1926) stated that the principal centre of diversity for foxtail millet is East Asia, including China and Japan. Recent molecular analyses based on isozyme (Jusuf and Pernes 1985), prolamine content (Nakayama et al. 1999); rDNA (Fukunaga et al. 1997; Schontz and Rether

1998); RAPD (Li et al. 1998; Schontz and Rether 1999); and AFLP (Le Thierry d'Ennequin et al. 2000) also supported the hypothesis of multiple origins, among which China is the centre of diversity of foxtail millet. In contrast to the hypothesis of multiple origin, Sakamoto (1987) based on extent of morphological variability of collections suggested that foxtail millet originated somewhere in Central Asia, Afghanistan, Pakistan and India. However genetic relationships among the *Setaria* species including crop evolution and species relationships are still poorly understood and no explicit genetic hypotheses have been proposed.

Foxtail millet is known for its drought tolerance and was once an indispensable crop of vast rainfed areas in semi-arid regions in India and other countries. At present it is cultivated on a limited scale in Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu, Rajasthan, Madhya Pradesh, Uttar Pradesh and north eastern states. Several local recipes such as *roti* (bread), *jaula*, *singal*, *sirol* are made from foxtail millet. Jaula made out of foxtail millet has special medicinal value for patients suffering from chicken pox. All the food products made from foxtail millet are observed to be effective in reducing blood glucose levels in normal as well as diabetic patients (Pathak et al. 2000). Foxtail millet grain contains 12.3 % protein, 4.7 % fat, 60.6 % carbohydrates and 3.2 % ash. The protein content of foxtail millet is greater than rice and is comparable to wheat. The fat content of foxtail millet is eight and three times more than rice and wheat respectively (Malleshi and Desikachar 1986).

Few molecular diversity analyses have been reported in foxtail millet. In a genetic diversity study using RFLPs, Fukunaga et al. (2002) found that foxtail millet landraces have differentiated genetically between different regions and that Chinese landraces were highly variable. This is in contrast to the results obtained by de Wet et al. (1979) and Jusuf and Pernes (1985), who reported that Chinese cultivars were uniform for storage protein and enzyme alleles.

The world foxtail millet germplasm collection of 1,535 accessions was analyzed extensively for morphological traits at ICRISAT, Hyderabad from 1977 to 2005. The analyses resulted in designation of 155 accessions as core collection representing most of the diversity observed. This core collection included about 93 accessions of Indian origin, which prompted us to undertake this study to analyze in detail the diversity of foxtail millet in India (Reddy et al. 2006). A few of the earlier workers had used RAPDs for the analysis of genetic diversity in foxtail millet (Li et al. 1998; Schontz and Rether 1999). Since codominant marker systems such as SSRs were not available in foxtail millet, this analysis was undertaken using the random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers techniques.

Materials and methods

Plant material

The material used for this study included 116 landraces cultivated in 11 states of India, 7 accessions from Bioversity International, Rome, one from China and one of unknown origin (Table 1). The Indian accessions were chosen from a collection of over 3,000 accessions from various states on the basis of analysis of morphological traits over 5 years at National Bureau of Plant Genetic Resources Station at Akola, Maharashtra (data being published separately). Seeds were sown in field and about 4–5 g of young, healthy leaves per accession were harvested from 15 to 20 days old seedlings for DNA isolation.

DNA extraction and purification

Total genomic DNA was isolated from a bulk of leaves from 20 to 25 plants per accession at 15–20 days after germination. The CTAB (cetyl trimethyl ammonium bromide) DNA extraction protocol of Saghai-Marooft et al. (1984) was followed. Approximately 4–5 g of leaf sample was ground in liquid nitrogen and extracted in 15–20 ml CTAB Buffer (100 mM Tris, pH-8.0, 1.4 M NaCl, 20 mM EDTA pH-8.0, 0.2 % β -mercaptoethanol, 2 % CTAB)

maintained at 60 °C. The extract was purified with 24:1 mixture of Chloroform-Isoamyl alcohol before precipitation of DNA with 0.7X volume of isopropanol. The precipitate was spooled out and washed with 70 % ethanol twice. After air drying, the precipitate was dissolved in 1 ml of TE (10:1). The DNA was then purified by RNase A treatment followed by Tris saturated phenol extraction. The purified DNA was finally precipitated by adding 1/10th volume of 3 M sodium acetate and 2.5X volume chilled ethanol. The precipitated DNA was spun down, washed with 70 % ethanol, air dried and dissolved in 500 μ l TE (10:1). The concentration of DNA in the samples was determined using a Hoefer DyNA Quant 200 fluorimeter using Hoechst 33258 dye. After quantification, a working DNA stock of concentration of 20 ng/ μ l was prepared and stored at 4 °C until use.

PCR amplification

One hundred and twenty RAPD primers from the six kits, OPA, OPC, OPD, OPG, OPM and OPO (Operon Technologies, USA) and 20 ISSR primers (University of British Columbia, UBC, Canada) were screened. Based on the survey, 16 RAPD and 4 ISSR primers producing polymorphic amplification products were selected for the final analysis (Table 2). PCR amplification was optimized and conducted in a reaction buffer of 25 μ l containing 1X PCR buffer; 1 Unit

Table 2 RAPD and ISSR primer sequences and the characteristics of amplification products from foxtail millets

S. no.	Primer	Sequence	Total no. of bands	No. of polymorphic bands	Percent polymorphism
1.	OPA-03	AGTCAGCCAC	08	06	75.0
2.	OPA-09	GGGTAACGCC	04	02	50.0
3.	OPC-05	GATGACCGCC	08	07	87.5
4.	OPC-06	GAACGGACTC	05	04	80.0
5.	OPC-08	TGGACCGGTG	06	05	83.3
6.	OPC-11	AAAGCTGCGG	04	04	100.0
7.	OPD-02	GGACCCAACC	12	10	83.3
8.	OPG-03	GAGCCCTCCA	08	06	75.0
9.	OPG-11	TGCCCCTCGT	07	06	85.7
10.	OPG-12	CAGCTCACGA	06	05	83.3
11.	OPG-16	AGCGTCCTCC	08	03	37.5
12.	OPG-19	GTCAGGGCAA	05	03	60.0
13.	OPM-05	GGGAACGTGT	06	05	83.3
14.	OPM-10	TCTGGCGCAC	07	07	100.0
15.	OPO-06	CCACGGGAAG	11	09	81.8
16.	OPO-12	CACAGACACC	09	08	88.9
17.	UBC885	BHBGAGAGAGAGAGAGA	03	02	66.7
18.	UBC888	BDBCACACACACACACA	10	10	100.0
19.	UBC889	DBDACACACACACACAC	09	08	88.9
20.	UBC891	HVHTGTGTGTGTGTGTG	09	08	88.9
	Total		146	119	

In primer sequence the notations indicate, B = (C, G or T); H = (A, C or T); D = (A, G or T); V = (A, C or G)

Fig. 1 RAPD profile obtained with primer, OPC-05 indicating the extent of genetic diversity observed in the foxtail millet collection. The lanes marked 1 to 125 correspond to the accessions as listed in

Table 1. The lane marked 'M' is DNA molecular weight standard, 1 kb ladder from MBI Fermentas, Germany

Taq DNA polymerase; 0.2 mM each of dATP, dGTP, dCTP and dTTP; 3 mM of $MgCl_2$ (all these reagents from Life Technologies, India), 0.1 μM of respective primer and 40 ng of genomic DNA. PCR amplification was carried out in BIOER XP Thermal Cycler (Hangzhou Bioer Technologies, Hangzhou, China). The thermal cycling conditions for RAPD were as follows: initial denaturation at 94 °C for 6 min, followed by 40 cycles of denaturation (94 °C) for 1 min, annealing (35 °C) for 1 min, primer extension (72 °C) for 1 min, followed by an extension at 72 °C for 10 min. For ISSR the cycling conditions were as follows: initial denaturation at 94 °C for 6 min, followed by 35 cycles of denaturation (94 °C) for 1 min, annealing (48–55 °C) for

1 min, primer extension (72 °C) for 1 min, followed by an extension at 72 °C for 10 min. The amplification products of both RAPD and ISSR were analyzed by electrophoresis on 1.8 % agarose gel, stained with ethidium bromide and photographed under short wave length UV light in a gel documentation system. A 1 kb DNA ladder (MBI Fermentas, Germany) was used as standard.

Data analysis

The amplification products were scored across the lanes comparing their respective molecular weights. Each band was scored for presence (1) or absence (0) in all samples.

Fig. 2 ISSR profile obtained with primer, UBC889 indicating the extent of genetic diversity observed in the foxtail millet collection. The lanes marked 1 to 125 correspond to the accessions as listed in

Table 1. The lane marked 'M' is DNA molecular weight standard, 1 kb ladder from MBI Fermentas, Germany

Fig. 3 Neighbor-Joining tree based on RAPD and ISSR profiles for 125 foxtail millet accessions. This tree is based on Nei and Li 1979 genetic distances generated after bootstrapping with 5,000 samples and rooted through the exotic, GS1404, an accession of Chinese

origin. Two major clusters were identified as indicated; the first cluster had two sub-groups as indicated. Only the bootstrap values of major clusters are mentioned at branch points for clarity. The bootstrap support for lower branches was higher

Fig. 4 Principal component analysis of 125 foxtail accessions indicating presence of 2 major groups, supporting the NJ tree. The first three principal components explained 25, 18 and 12 % of total variation

Only consistent amplification products were included for analysis and amplification failure was scored as '9', indicating missing data. RAPD and ISSR data were pooled for combined analysis.

Nei and Li's genetic distance (Nei and Li 1979) between pairs of accessions were calculated and the genetic distances were subjected to cluster analysis following Neighbor Joining procedure. The reliability of the nodes of the tree was tested by bootstrap analysis of 5,000 pseudo-samples. All the analyses were carried out using 'Free Tree' software (Pavlicek et al. 1999). The accession from China, GS1404 was used as an outgroup considering its diverse origin for rooting the tree. Principal Components analysis was also conducted using NTSYSpc in order to compare the grouping obtained with clustering procedures (Rohlf 1993). In order to test the degree of differentiation of the accessions from different eco-geographic regions, AMOVA was conducted. The accessions were further divided into four groups according to their eco-geographic regions of collection excluding the exotic introductions because of their doubtful origin. Analysis of molecular variance (AMOVA) was conducted using computer software

Arlequin 3.0 (<http://cmpg.unibe.ch/software/arlequin3>). This software was also used to compute F_{st} , the population subdivision measure. Further, population genetic diversity parameters were calculated using POPGEN32 software (Yeh et al. 2000); the statistics computed were: observed number of alleles per locus (n_a), effective number of alleles per locus (n_e), Nei's gene diversity (h), Shannon's information index (I), number of polymorphic loci (P), percent polymorphic loci, total heterozygosity (H_t), average heterozygosity (H_s), estimate of gene flow (N_m) and population substructure statistics G_{st} .

Results and discussion

Primer survey and selection

Out of 120 RAPD and 20 ISSR primers surveyed 16 RAPD and 4 ISSR primers were selected for the present study based on the extent of polymorphism observed in the amplicons. The amplification obtained with RAPD and ISSR primers was good and consistent as shown in Figs. 1

Table 3 Nei and Li's genetic distance, range and average between accessions from different geographic regions indicating the extent of diversity in the collections analyzed

State	No. of accessions	Range	Average
Andhra Pradesh	36	0.032–0.399	0.161
Karnataka	07	0.058–0.469	0.239
Tamil Nadu	10	0.046–0.247	0.144
Madhya Pradesh	03	0.065–0.258	0.192
Maharashtra	08	0.078–0.163	0.111
West Bengal	04	0.058–0.102	0.081
Bihar	10	0.056–0.269	0.154
Uttarakhand	33	0.018–0.286	0.133
Bioversity International	07	0.041–0.344	0.206

For Jammu & Kashmir, Rajasthan, China, and Uttar Pradesh only one or two accessions each were present, hence were not meaningful to include in this table

and 2 respectively. A total of 146 scoreable amplification products (115 RAPD and 31 ISSR) were generated. The number of amplicons generated by each primer varied from four (OPA-09) to twelve (OPD-02) for RAPDs and three (UBC885) to ten (UBC888) for ISSRs. The average number of amplicons detected were 7.3 per primer. Out of 146 bands generated 119 (81.5 %) were polymorphic and 27 (18.5 %) were monomorphic. The percent polymorphism for the primers varied from 37.5 % (OPG-16) to 100 % (OPC-11, OPM-10 and UBC888).

Genetic diversity for foxtail millet in India

Foxtail millet is cultivated in marginal lands and hilly tracts of India under diverse agro-ecological conditions. The cultivation regions extend from the foot hills of the Himalayas to coastal areas at sea level in the southern part of India. Such diverse climatic conditions have possibly contributed to the maintenance of genetic diversity in the cultivated germplasm of foxtail millet. This is evident from the results of the present analyses which indicate presence of substantial amount of variations among the 116 accessions originating from 11 states spanning more than five eco-geographical zones of the country. The RAPD and ISSR analyses grouped 116 Indian accessions and 9 exotics into two diverse clusters (Figs. 3 and 4). The cluster I was divided into two subclusters, namely, Ia and Ib. The cluster Ia included 62 accessions- 27 from Andhra Pradesh, 20 from Uttarakhand, 6 from Bihar, 2 each from West Bengal, Maharashtra and Karnataka and one each from Jammu and Kashmir and Rajasthan. The cluster Ib comprised 27 accessions- 7 from Uttarakhand, 5 each from Maharashtra and Tamil Nadu, 2 each from Andhra Pradesh, Madhya Pradesh, West Bengal, and Karnataka and one each from Jammu and Kashmir and Uttar Pradesh. The cluster II comprised 7 accessions each from Andhra Pradesh and Bioversity International, 5 each from Uttarakhand and Tamil Nadu, 4 from Bihar, 3 from Karnataka, and one each

Table 4 AMOVA, partitioning the molecular variance into groups divided region-wise, among accessions within a region and among germplasm accessions from different states, to access the differentiation of foxtail millet accessions from different regions

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation
Among regions	4	204.824	0.46761Va	2.76
Among accessions within regions	9	226.332	1.95463Vb	11.55
Among accessions within states	111	1609.132	14.49668Vc	85.68
Total	124	2040.288	16.91892	

Table 5 Fst values between 'States' based on expectation from the partitioning of molecular variation indicating the extent of differentiation of the germplasm accessions from various regions

	Andhra Pradesh	Bihar	China	Bioversity International	Jammu Kashmir	Karnataka	Maharashtra	Madhya Pradesh	Rajasthan	Tamil Nadu	Uttar Pradesh	Uttarakhand
Andhra Pradesh	0.000											
Bihar	0.040	0.000										
China	0.054	0.015	0.000									
Bioversity International	0.445	0.442	0.373	0.000								
Jammu Kashmir	-0.052	-0.065	0.034	0.499	0.000							
Karnataka	0.041	0.021	-0.169	0.258	-0.062	0.000						
Maharashtra	0.040	0.039	0.112	0.537	-0.121	0.062	0.000					
Madhya Pradesh	0.102	0.034	-0.200	0.299	-0.143	-0.088	0.060	0.000				
Rajasthan	-0.255	-0.152	1.000	0.550	-0.647	-0.279	-0.159	-0.244	0.000			
Tamil Nadu	0.208	0.139	-0.029	0.339	0.085	0.074	0.207	-0.028	0.175	0.000		
Uttar Pradesh	0.084	0.042	-0.037	0.316	0.051	-0.148	0.173	-0.157	-0.077	-0.016	0.000	
Uttarakhand	0.035	0.037	0.094	0.497	-0.065	0.071	0.034	0.103	-0.249	0.180	0.111	0.000
West Bengal	0.024	0.012	0.381	0.580	-0.026	0.064	0.020	0.106	0.063	0.253	0.292	0.036

from China, Maharashtra, UP and MP. Such similarity between the landraces of foxtail millet from India and China had also been reported earlier by Li et al. (1998) and Schontz and Rether (1999) which indicates exchange of germplasm between these two countries.

The accessions from Andhra Pradesh, Karnataka, Maharashtra and Uttarakhand were present in both the clusters indicating presence of higher diversity for foxtail millet in these states. However, presence of moderate diversity was indicated by the distribution of collections from Tamil Nadu in only cluster Ib and II. Similarly, the accessions from Bihar and West Bengal were present in clusters Ia & b and II. All the introduced accessions from Bioversity International (Rome) were grouped in cluster II indicating lower diversity in introduced accessions, but, these accessions were diverse from the other Indian accessions analyzed as they were placed in a separate subcluster. High bootstrap support was observed for the major clusters greater than 80 %. The results of both NJ based clustering and principal components analysis were in general agreement with each other (Figs. 3 and 4 respectively). The findings of this study are in agreement with the earlier conclusions that despite presence of high morphological variations, discrete grouping can be ascertained (Van Nguyen and Pernes 1985; Li et al. 1995, 1998; Schontz and Rether 1999). However, no distinct relationship between clustering of accessions and their geographic region of cultivation was evident, which may be due to frequent exchange of cultivars from one region to other on account of crop improvement and promotion programmes in the country. These findings are contrary to the geographic differentiation observed among the accessions of foxtail millet from five regions of the world (Fukunaga et al. 2002). However, in the above study it

may be noted that the accessions from India clustered together which is in agreement with the results of the present analysis as well as an earlier analysis based on RFLP patterns of mitochondrial DNA where all Indian accessions grouped together (Fukunaga and Kato 2002).

The average genetic distance (g.d.) was low (0.081) for accessions from West Bengal, while the collections from Karnataka showed high diversity (average g.d. = 0.239). The value of Nei and Li's genetic distances ranged from 0.058 to 0.102 for West Bengal and from 0.058 to 0.469 for Karnataka. The average genetic distances for accessions from Andhra Pradesh was 0.161 (range = 0.032–0.399). For collections from Maharashtra, the average genetic distances was 0.111; range = 0.078–0.163 (Table 3). These results indicate presence of greater diversity in the southern region of India encompassing the states of Andhra Pradesh, Karnataka and Tamil Nadu. In fact, the archeological evidences reported presence of foxtail millet in cultivation in the remains from Karnataka and Andhra Pradesh as early as 2800 cal B.C. (Fuller et al. 2004). These findings are in agreement with the reports correlating historic cultivations to diversity, based on study of morphological traits for Chinese foxtail millet landraces, which assigned the reason to very long history of cultivation of this millet in China (Li et al. 1996; Sakamoto 1987). Further, despite presence of large area of cultivation in Uttarakhand, Uttar Pradesh and Bihar region in the north, the diversity observed among the accessions was lower in comparison to the southern region. The analysis of storage protein polymorphism (de Wet et al. 1979) and isozyme alleles (Jusuf and Pernes 1985) in collections from different regions had similarly revealed little intra-regional variability. Such lack of variability may indicate common source populations for the landraces under cultivation in the Indian sub-continent.

Table 6 Genetic diversity parameters for different eco-geographic regions

Pop. ID	Population name	No. of accessions	No. of alleles/locus (na)	Effective no. of alleles (ne)	Nei's gene diversity (h)	Shannon's information index (I)	No. of polymorphic loci (P)	% polymorphic loci
1	AP	36	1.745±0.438	1.456±0.387	0.261±0.200	0.387±0.278	105	74.47
2	Bihar	10	1.567±0.497	1.387±0.403	0.218±0.214	0.319±0.304	80	56.74
3	JK	02	1.199±0.400	1.140±0.283	0.082±0.166	0.120±0.242	28	19.86
4	Karnataka	07	1.638±0.482	1.471±0.410	0.261±0.216	0.378±0.304	90	63.83
5	Maharashtra	08	1.390±0.489	1.271±0.394	0.150±0.207	0.220±0.293	55	39.01
6	MP	03	1.361±0.482	1.289±0.411	0.157±0.215	0.224±0.305	51	36.17
7	Rajasthan	01	1.000±0.000	1.000±0.000	1.000±0.000	1.000±0.000	0	0.00
8	TN	10	1.475±0.501	1.322±0.396	0.181±0.211	0.266±0.300	67	47.52
9	UP	02	1.199±0.400	1.140±0.283	0.082±0.166	0.120±0.242	28	19.86
10	Uttarakhand	33	1.610±0.490	1.384±0.389	0.221±0.207	0.327±0.293	86	60.99
11	WB	04	1.227±0.420	1.176±0.351	0.095±0.184	0.137±0.261	32	22.70

For Jammu and Kashmir, Rajasthan and UP, the sample size is too small, hence results not discussed

Differentiation and spatial distribution of diversity in India

The 125 accessions were classified based on geographic origin into 14 putative groups (since the cultivation conditions were similar within a region) and consequently into five adjoining groups. The reasoning behind the groupings was contiguity and possibility of germplasm sharing within a region due to similarities in cultivation conditions. Analysis of molecular variance indicated that variation among the regions was responsible for 2.76 % of total variation and 11.55 % was due to variations among accessions within regions. The percentage of variation among accessions within states was as high as 85.68 % (Table 4). The partitioning of variance are in general agreement with the trend observed in autogamous species; further, up to 2 % cross pollination between contrasting genotypes planted at 24 m distance have been recorded (Wang et al. 1997) which indicate the possibility of gene flow in farmers' fields.

The theoretical expectations from the AMOVA were used to calculate the F-statistics, namely, F_{st} , F_{it} and F_{is} . The value of F_{st} was observed to be 0.028, indicating very low differentiation of cultivars grouped state-wise, thereby indicating no significant population sub-division due to frequent gene flow as a consequence of exchange of cultivars between the regions as well as gene flow between the populations. The F_{it} value of 0.143 also indicates lack of substantial allelic differences in cultivars from different states. The moderate value of 0.119 for F_{is} further supports this conclusion as it indicates presence of very low differences for allelic frequencies between the regions. A comparison of pair-wise F_{st} between 14 geographical populations (Table 5) indicated that the pair-wise F_{st} were greater than 0.25 for Andhra Pradesh–Rajasthan, China–Rajasthan, China–West Bengal, Jammu & Kashmir–Rajasthan, Karnataka–Rajasthan, Rajasthan–Uttarakhand, Tamil Nadu–West Bengal, Uttar Pradesh–West Bengal and Bioversity International with all other populations. Such higher differentiation among disjoint populations is expected since the natural gene flow between these distant populations may be non-existent in the absence of human interventions. Further, role of climatic conditions on genotypic differences on population sub-division is also much likely since photoperiodism and temperature differences are higher for the above regions. Contrary to the expectations, low F_{st} values of less than 0.02 were observed between Bihar–China and UP–Tamil Nadu although these regions are geographically distant and isolated which could be attributed to the movement of genetic material between these regions as a result of crop improvement programmes at the national level. Similar results were reported earlier by Li et al. (1998) and Le Thierry d'Ennequin et al. (2000) where higher genetic affinities among the Asiatic collections of

Table 7 Estimates of genetic diversity parameters for foxtail millet accessions from different regions indicating the genetic diversity in different regions

Group	Region	No. of accessions	No. of alleles/ locus (na)	Effective no. of alleles (ne)	Nei's gene diversity (h)	Shannon's information index (I)	Ht	Hs	Gst	Nm	No. of polymorphic loci (P)	% polymorphic loci
South	AP, Karnataka, TN	53	1.787±0.411	1.473±0.388	0.270±0.198	0.402±0.272	0.267±0.038	0.234±0.032	0.123	3.557	111	78.72
Central-west	Maharashtra, MP, Rajasthan	12	1.553±0.499	1.342±0.387	0.197±0.204	0.294±0.290	0.188±0.039	0.102±0.013	0.455	0.599	78	55.32
North-hill	JK, Uttarakhand	36	1.617±0.488	1.384±0.389	0.220±0.206	0.327±0.293	0.188±0.036	0.151±0.025	0.193	2.090	87	61.70
North plain	Bihar, UP, WB	16	1.589±0.494	1.363±0.377	0.211±0.204	0.314±0.291	0.191±0.039	0.132±0.020	0.308	1.123	83	58.87

foxtail millet were observed. However, Fukunaga et al. (2002) had reported genetic differentiation of landraces from Europe and Asia; and even between Indian and Afghanistan accessions. Such ambivalent results are to be expected in such crops where modern breeding efforts are lacking since, majority of the crop improvement efforts are limited to introductions and population improvement procedures.

Allelic diversity and spatial distribution of diversity

The higher number of alleles per locus was observed for Andhra Pradesh (1.745 ± 0.438), Karnataka (1.638 ± 0.482) and Uttarakhand (1.610 ± 0.490); while for Bihar, Maharashtra and Tamil Nadu only 1.567 ± 0.497 , 1.390 ± 0.489 and 1.475 ± 0.501 alleles per locus respectively were recorded (Table 6); for Jammu and Kashmir, Rajasthan and Uttar Pradesh the sample size was too low, hence no significant conclusions can be drawn on existing diversity. Results observed were similar for the estimates of effective number of alleles per locus, Nei's gene diversity index, Shannon's information index and number of polymorphic loci. These statistics clearly indicate the presence of higher genetic diversity in Southern states of Andhra Pradesh and Karnataka and in Uttarakhand for northern states.

Analysis of the distribution of genetic diversity was done by grouping the cultivars from adjacent states and analysis of such four eco-geographic regions indicated similar trends which indicated the southern region comprising Andhra Pradesh, Karnataka and Tamil Nadu showed the highest number of alleles per locus (1.787 ± 0.411) followed by the northern hill region comprising Uttarakhand and Jammu and Kashmir (1.617 ± 0.488). The central-western region comprising Maharashtra, MP and Rajasthan showed the lowest number of alleles per locus (1.553 ± 0.499). The effective number of alleles was comparable, ranging from 1.342 for central-western region to 1.473 for south. The southern region had the maximum Nei's gene diversity (0.270 ± 0.198) while it was lowest for central-west (0.197 ± 0.204). The value of Shannon's information index was also maximum for south (0.402 ± 0.272) and minimum for central-western region (0.294 ± 0.290). The total heterozygosity was highest in southern region (0.234 ± 0.032). The value of average heterozygosity (0.234 ± 0.032) was high for south and moderate for central-western region (0.102 ± 0.013). The value of G_{st} was low for south (0.123) as well as for northern-hills (0.193); and high for central-western (0.455) and northern Plains region (0.308). This indicates that although southern and northern-hills region is rich in diversity, the landraces within the region share common alleles due to higher gene flow among the landraces within these regions (Table 7).

Results of this study indicate that the southern states of Andhra Pradesh, Karnataka and Tamil Nadu; and the northern hills inclusive of Uttarakhand and Jammu and Kashmir are diversity rich regions where greater landrace diversity exists and more intensive collections with a stress on sampling more number of landraces can be followed to capture the existing genetic diversity. However, in other foxtail millet growing regions sampling of larger regions which are well dispersed is essential to collect diverse genetic material.

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