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Genotype identification and assessment of genetic relationships in pearl millet [*Pennisetum glaucum* (L.) R. Br] using microsatellites and RAPDs

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Abstract The potential of DNA markers such as microsatellites, minisatellites and RAPDs was investigated in pearl millet [*Pennisetum glaucum* (L.) R. Br] with respect to their abundance and variability. Southern analysis, using 22 different di-, tri-, tetra- and penta-oligonucleotide probes and five minisatellite probes, identified $(GATA)_4$ as the most useful probe for the detection of multiple polymorphic fragments among pearl millet cultivars and landraces from India. The clustering patterns of pearl millet cultivars and landraces based on $(GATA)_4$ and RAPD (randomly amplified polymorphic DNA) markers differed. The landraces, representing eight states in India, could not be grouped based on their geographical distribution with the DNA markers. RAPD analysis revealed a high degree of genetic diversity among the cultivars and landraces employed in this study. The probability of an identical match by chance for any two genotypes using $(GATA)_4$ and RAPDs was 3.02×10^{-20} for cultivars and 5.2×10^{-9} for landraces. The microsatellite $(GATA)_4$ and RAPDs provide useful tools for genotype identification and for the assessment of genetic relationships in pearl millet.

Key words *Pennisetum glaucum* · Landraces · Genetic diversity · Microsatellites · RAPDs

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Introduction

Pearl millet is the fourth most important world food crop next to rice, wheat and sorghum. It is the most drought-tolerant of all the domesticated cereals and is grown in regions with 200–600 mm of annual rainfall where no other cereal crop can be grown. The nutritive value/content of pearl millet is higher than rice and wheat (Uprety and Austin 1972). Soon after its domestication, pearl millet became widely distributed across the arid, semi-arid, and tropical regions of Africa and Asia under scanty water conditions. Although pearl millet is grown in drought-prone semi-arid and arid areas of the world, it is also ideally adapted to irrigated farming. Pearl millet is an excellent forage crop because of its low hydrocyanic acid content. The green fodder which is rich in protein, calcium, phosphorous and other minerals contains oxalic acid within safe limits (Athwal and Gupta 1966; Gupta 1975). The major factors that restrict the production potential of pearl millet are low hybrid coverage, slow varietal spread and diseases (Gill 1991). These problems, however, can be overcome to some extent by the diversification of male-sterile lines, the improvement of restorers, and breeding heterogeneous and heterozygous single, as well as multicross, hybrids to combat the twin problems of low yield and disease susceptibility.

The germplasm of pearl millet landraces shows a wide range of variation for several characters. Landraces from West Africa are in general very late maturing, tall, thick stalked with short spikes and bold grains. In Western India, the landraces are generally earlymaturing, thin-stemmed and profusely branched, leading to asynchronous flowering, probably as an adaptive mechanism for low-rainfall conditions. Photoperiod-sensitive types are present in hilly areas of Tamil Nadu and Andhra Pradesh. The lines collected from Tamil Nadu contain more soluble sugars in their stalks during drought (Appa Rao et al. 1982).

Pearl millet cultivars are generated from a narrow gene pool and current breeding programs do not make use of wild pearl millets. Genetic diversity studies in *Pennisetum* germplasm offer possibilities for their use in improving pearl millet varieties. The genetic differences have been studied by morphological and isozyme analysis (Tostain et al. 1987; Tostain and Marchais 1989; Tostain 1992). A disadvantage of isozyme markers is that they are affected by environmental conditions and different stages of development (Falkenhagen 1985). In addition, the number of isozyme loci that can be analyzed is limited and discrimination between different genotypes is not always possible (Tobolski and Kempery 1992). Hypervariable repetitive DNA sequences such as micro- and mini-satellites can be of great value in deciphering a high level of polymorphism as they recognise multiple loci and are distributed throughout the genome (Jeffreys et al. 1985; Tautz et al. 1986). The utility of these genetic markers in plants has been demonstrated in a number of crops such as wheat, rice, barley (Wu and Tanksley 1993; Becker and Heun 1995; Roder et al. 1995) and many other plant species (Morgante and Oliveri 1993). Microsatellites, in particular, have been shown to be ten-times more polymorphic than RFLPs in plants (Akkaya et al. 1992; Senior and Heun 1993; Bell and Ecker 1994). Microsatellites such as $(GATA)_n$, $(TG)_n$ and $(CAC)_n$ are found to be highly informative in animal and human systems and have been used for constructing saturated genetic linkage maps, sex determination, paternity testing, and individual identification. Similar attempts in plant systems are being increasingly explored for their application in plant breeding (Weising et al. 1995) but the full potential of these markers remains largely unexplored. The minisatellite M13 has been shown to be polymorphic in plants such as apples and rice (Nybom and Hall 1991; Ramakrishna et al. 1995). The human minisatellite pV47 has been shown to be polymorphic in both rice and its pathogen *Xanthomonas* (Ramakrishna et al. 1995; Rajebhosale et al. 1997).

The development of randomly amplified polymorphic DNA (RAPD) markers by Williams et al. (1990), based on the use of short primers of arbitrary nucleotide sequence in PCR, has revolutionized the area of plant molecular genetics. RAPDs have a number of advantages over other DNA-based marker systems (Rafalski and Tingey 1993). RAPD analysis is an effective, cost-efficient method for genotype identification, pedigree analysis (Welsh et al. 1991) and genome mapping (Reiter et al. 1992).

DNA-based markers have been applied successfully to discriminate between individual genotypes in a wide range of plant and animal species (Epplen et al. 1991). In pearl millet, limited efforts have been made to study genetic diversity. Chloroplast DNA polymorphism has been found to be very much less among several wild and cultivated millet samples (Clegg et al. 1984). Using ribosomal DNA sequences, greater diversity is observed in wild millets than in cultivated millets (Gepts and Clegg 1989). The mitochondrial DNA sequences have been shown to be polymorphic in different male-sterile and -fertile cytoplasms of pearl millet (Smith and Chowdhury 1989). Restriction fragment length polymorphisms (RFLPs) have also been found to be highly polymorphic in pearl millet species (Liu et al. 1994).

In the present study, we demonstrate for the first time the usefulness of micro- and mini-satellites, as well as RAPD markers, to assess the genetic variation in Indian pearl millet cultivars and landraces.

Materials and methods

Plant material

Twenty two pearl millet cultivars and 36 landraces (Tables 1 and 2) were collected from IARI, New Delhi and ICRISAT, Hyderabad, India.

DNA analysis

Genomic DNA was extracted from young leaves of various genotypes as described by Sharp et al. (1988). DNA was digested with an excess of an appropriate restriction enzyme (6–8 units/μg) under the conditions recommended by the suppliers, separated on 1% or 1.4% agarose gels in 1 × TPE buffer (0.09 M Tris phosphate, 0.002 M EDTA, pH 8.0) and transferred onto nylon membranes.

Table 1 Pearl millet cultivars used in the present study

Name	Characteristics
Tift23A	First line introduced in India from USA, downy mildew susceptible
5141A	Best general combiner, tillering type, medium size, compact earheads
5054A	Early, tillering type, narrow leaves
L-111-A	Late, long earheads, broad leaves
81A	Dwarf, tillering type
843A	Very early, dwarf
863A	Non-tillering, purple glumes
841A	Derivative of MS-5141, medium size, compact earheads
3383A	Tillering type, dwarf
5122A	Dwarf, long earheads
5644A	Highly tillering, very thin stem, small earheads
189A	Tall, compact bold earheads
393A	Loose earhead
267A	Derivation of MS5141 through backcrossing with DM donors
ICMB88004	High tillering, yellowish green leaves
J-104	Best general combiner, drought tolerant, downy mildew susceptible
K-560-230	Tall, fast growing, broad leaves
D-23	DMR derivative of K-560-230
PPMI-301	Bold earheads
PPMI-493	Compact bold earheads
J-254	DMR derivative of J-104
ICM-451	Tall, bold, semi-compact bristled earheads

Table 2 Pearl millet landraces used in the present study

Sample no.	Genotype	State	Sample no.	Genotype	State
1	IP6901	Uttar Pradesh	19	IP3126	Rajasthan
2	IP7251	Utter Pradesh	20	IP3114	Rajasthan
3	IP7093	Uttar Pradesh	21	IP3143	Rajasthan
4	IP7346	Uttar Pradesh	22	IP3348	Rajasthan
5	IP7133	Uttar Pradesh	23	IP4138	Maharashtra
6	IP4593	Madhya Pradesh	24	IP17876	Maharashtra
7	IP4668	Madhya Pradesh	25	IP4195	Maharashtra
8	IP13535	Madhya Pradesh	26	IP4147	Maharashtra
9	IP4708	Madhya Pradesh	27	IP4197	Maharashtra
10	IP3516	Tamil Nadu	28	IP15098	Karnataka
11	IP3522	Tamil Nadu	29	IP15065	Karnataka
12	IP13601	Tamil Nadu	30	IP15075	Karnataka
13	IP3605	Tamil Nadu	31	IP15056	Karnataka
14	IP3652	Tamil Nadu	32	IP11796	Andhra Pradesh
15	IP3762	Gujarat	33	IP8521	Andhra Pradesh
16	IP4021	Gujarat	34	IP16282	Andhra Pradesh
17	IP3807	Gujarat	35	IP3018	Andhra Pradesh
18	IP3738	Gujarat	36	IP8532	Andhra Pradesh

Alternatively, gels were dried on a vacuum gel dryer. pV47, a human minisatellite sequence (Longmire et al. 1990), and a 282-bp fragment containing nine tandem repeats of a 15-bp core sequence from M13mp10RF (Vassart et al. 1987), were used in this study and were α^{32}P-labelled as described by Sambrook et al. (1989). The hybridizations were carried out for 16 h in 40% formamide, 5 × SSPE, 0.5%SDS, 5 × Denhardt's 0.1 × BLOTTO at 42°C. The filters were washed twice in 1 × SSPE, 0.5%SDS for 20 min at room temperature, followed by a hot wash at 55°C for 15 min. Oligonucleotides were synthesized on a gene assembler plus (Pharmacia), de-salted on NAP-5 columns and purified on a 20% denaturing polyacrylamide gel. Oligonucleotide probes, such as the *chi* sequence of *Escherichia coli* (Ehtesham et al. 1992), repeats of the *Per* gene of *Drosophila* (Ben-Shlomo et al. 1993), the 3′ hypervariable region of apolipoprotein B (Huang and Breslow 1987) and various di-, tri- and tetranucleotides, were end-labelled as described by Sambrook et al (1989). Hybridizations were performed at Tm − 5°C and were first washed with 5 × SSPE, 0.1%SDS twice for 15 min at room temperature and then at the hybridization temperature for 2 min and were exposed to X-ray films at − 70°C with intensifying screens.

PCR amplification

Operon series OPA, OPB and OPK (Operon Technologies Inc. Alameda, USA) were used and PCR was performed in 25-µl volumes consisting of 100 µM each of dATP, dTTP, dCTP and dGTP, 5 pmoles of primer, 20 ng of genomic DNA, 1 × reaction buffer and 0.5 u of *Taq* polymerase. Samples were amplified in a Perkin Elmer Cetus 48-well DNA thermal cycler programmed with a 3-min step at 94°C for initial denaturation. This was followed by 45 cycles of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C. The final cycle was followed by a 5-min final extension step at 72°C. Amplified products were separated on 1.4% agarose gels in 1 × TAE buffer.

Statistical analysis

Evaluation of fragment patterns and statistical calculations were performed according to Wetton et al. (1987). A similarity coefficient (F) between two DNA fingerprints was calculated as

$$F = 2N_{AB}/(N_A + N_B),$$

where N_{AB} was the number of bands shared by fingerprints A and B, and N_A and N_B the total number of bands present in fingerprints A and B, respectively. The chance occurrence that two different genotypes would exhibit identical fragment profiles was then calculated as the average F raised to the mean number of fragments per genotype. The dendrogram was constructed using UPGMA (unweighed pair-group method with arithmetic averages) with the computer program Taxan 4.0.

Results and discussion

Occurrence and abundance of hypervariable DNA sequences in the pearl millet genome

Table 3 depicts the occurrence and polymorphism of di-, tri-, tetra-nucleotide and minisatellite repeats in the pearl millet genome. Among the tested micro- and mini-satellites, $(GATA)_4$ and pV47 generated the most polymorphic patterns, both with cultivars and landraces (Table 3). Signals were obtained with all the probes except for $(AT)_{10}$, $(GC)_{10}$, $(ACG)_5$, $(ACC)_5$, 3′ hypervariable region in Apolipoprotein, telomere and *Per* DNA sequences. Although other DNA markers produced many bands, the number of polymorphic bands was either less or else they were monomorphic or very faint. A few microsatellites such as $(GAA)_6$ and $(TG)_{10}$ produced a very large number of bands, some of which were also polymorphic (Table 3); however, heavy background smears were observed in both cases in spite of stringent washing. Interestingly, microsatellites such as $(GATA)_4$, $(CAC)_5$ and $(GAA)_6$ produced a high number of bands with all the three restriction enzymes *Dra*I, *Hae*III and *Hin*fI (Table 3), indicating that most of the bands detected by hybridization are derived from separate loci in the genome, rather than from internal cuts within a cluster of repeats (Figs. 1, 2 and 3). Earlier, in chickpea, Sharma

Table 3 Occurrence and polymorphism of di-, tri-, tetra-nucleotides and minisatellite repeats in the pearl millet genome: a with cultivars. b with landraces

a With cultivars

Probe	Total no. of bands			Total no. of polymorphic bands		
	*Dra*I	*Hin*fI	*Hae*III	*Dra*I	*Hin*fI	*Hae*III
1 $(GATA)_4$	21	19	18	20	19	18
2 $(GGAT)_4$	–	14	16	–	0	0
3 $(GACA)_4$	–	–	13	–	–	4
4 $(CAC)_5$	19	12	20	5	0	3
5 $(GAA)_6$	33	23	20	8	3	0
6 $(TG)_{10}$	30	–	–	8	–	–
7 pV47	21	27	–	6	15	–
8 M13	–	18	–	–	12	–

b With landraces
Probes which generated polymorphic bands in landraces: $(GATA)_4$ and pV47
Probes which generated a high no. of bands which were mostly monomorphic: $(AG)_{10}$, $(AAG)_5$, $(AAC)_5$, $(ATG)_5$, $(ATC)_5$, $(AGA)_5$, $(AGT)_5$, $(AGG)_5$, $(AGC)_5$, $(ACA)_5$, $(ACT)_5$, *chi* and M13 oligonucleotides
Probes which generated very faint bands or no signal: $(AT)_{max}$, $(GC)_{max}$, $(ACG)_5$, $(ACC)_5$, apolipoprotein, telomere and *per* DNA sequences

et al. (1995) have shown that $(GATA)_4$ hybridization bands were derived from different loci in the genome. Several initial investigations of plant microsatellites in barley, soybean, wheat, rice and tropical trees have demonstrated their informativeness and random distribution in plant species (Wang et al. 1994).

Diffuse background regions were observed previously in rice with a microsatellite $(TG)_{10}$, indicating the presence of large number of loci containing TG repeats (Ramakrishna et al. 1994). This was also reported earlier when DNA fingerprinting in plants was compared to that in humans (Rogstad et al. 1988). Background cross-hybridizing sequences indicate that either they are divergent from mini- or micro-satellite repeats or else fewer sub-repeats are present. Compared to microsatellites, the bands obtained with pV47 were clear and polymorphic between the 3–6 kb region and diffused below 3 kb (data not shown) thus indicating that minisatellites are dispersed and clustered at certain loci in the genome.

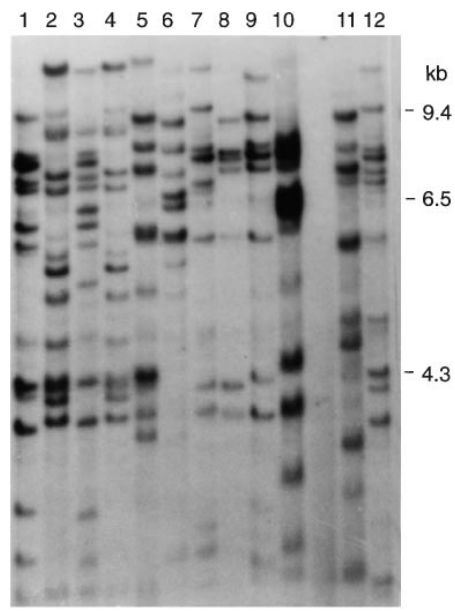

Fig. 1 Hybridization patterns of *Dra*I digests of pearl millet DNA detected by probe $(GATA)_4$. *Lanes 1–12* are Tift23 A(*1*), 5141 A(*2*), 5054 A(*3*), L-III-A(*4*), 81 A(*5*), 863 A(*6*), 841 A(*7*), 3383 A(*8*), 5122 A(*9*), 5644 A(*10*), 393 A(*11*) and 267 A(*12*)

DNA fingerprinting using the $(GATA)_4$ microsatellite

Southern analysis using 22 oligonucleotide probes and five minisatellite probes identified $(GATA)_4$ and pV47 as useful probes for the detection of multiple polymorphic fragments among pearl millet cultivars and landraces. Thirteen cultivar DNAs digested with *Dra*I, *Hae*III and *Hin*fI and hybridized with a $(GATA)_4$ probe exhibited unique DNA fragment profiles (Figs. 1, 2 and 3). In all three figures 12–19 clear resolvable bands were observed per lane. *Dra*I showed the maximum number of bands per lane (Fig. 1) and the average number of polymorphic bands between pairs 16.4 ± 5.16. Figures 2 and 3 show distinct bands between 4 and 6 kb and more background smear with bands below 4 kb. The smear may be a result of the presence of a large number of different loci with short repeats. The cultivars 841A (lane 7), 3383A (lane 8) and 5122A (lane 9) showed a smaller number of bands compared to the other genotypes in Figs. 1 and 2, whereas with *Hin*fI they showed almost an equal number of bands as the other cultivars (Fig. 3).

Similarity index values for $(GATA)_4$-derived fingerprint patterns were calculated for all possible pairwise comparisons which ranged from 0.33 (Tift 23A compared with 5141A) to 0.65 (5644A compared with 5054A). The average similarity index values were lower for *Hin*fI (0.33) compared to *Dra*I (0.47) in the case of cultivars. In landraces the similarity coefficient values ranged from 0.64 [IP6901(U.P) compared with

IP7251(U.P)] to 0.90 [IP7093(U.P) compared with IP3762(Gujarat)]. The average similarity index values were much higher for landraces compared to cultivars indicating more variability at $(GATA)_n$-containing loci in cultivars as compared to landraces of pearl millet. This may be due to the stable nature of landraces, on one hand, and to recombinations created while developing cultivars in order to pyramid desired genes, on the other hand. We have estimated the probability of identical fingerprints by chance to be 7.78×10^{-6}, 5.87×10^{-5} and 1.41×10^{-6} for cultivars with $(GATA)_4$ in combination with *Dra*I, *Hae*III and *Hin*fI, respectively (Table 4). Thus the mean probability that two pearl millet cultivars have identical fingerprints with $(GATA)_4$ alone is 6.4×10^{-16}. However, the mean probability that two pearl millet landraces have identical fingerprints is very high and the landraces cannot be distinguished completely with the $(GATA)_n$ probe alone, since the collection of pearl millet landraces at ICRISAT, Hyderabad, is itself in excess of 40 000.

Thus our results indicate that $(GATA)_4$ has good potential as a fingerprinting probe in pearl millet cultivars. Similar results have been obtained in other crops such as rice (Ramakrishna et al. 1994), chickpea (Weising et al. 1992) and tomato (Vosman et al. 1992) where $(GATA)_n$ in combination with various restriction enzymes produces informative fingerprint patterns. Use of this microsatellite as a PCR primer can also be attempted, since tetranucleotide repeat primers have been shown to be most effective in amplifying polymorphic patterns (Gupta et al. 1994).

DNA fingerprinting using RAPD markers

RAPD analysis was performed with 20 primers on 21 cultivars and with 60 primers on 36 landraces. The

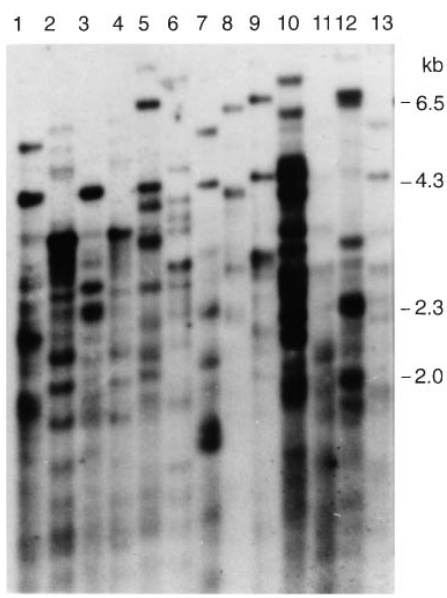

Fig. 2 Hybridization patterns of *Hin*fI digests of pearl millet DNA detected by probe $(GATA)_4$. *Lanes 1–10* as in Fig. 1, 189 A(*11*), 393 A(*12*) and 267 A(*13*)

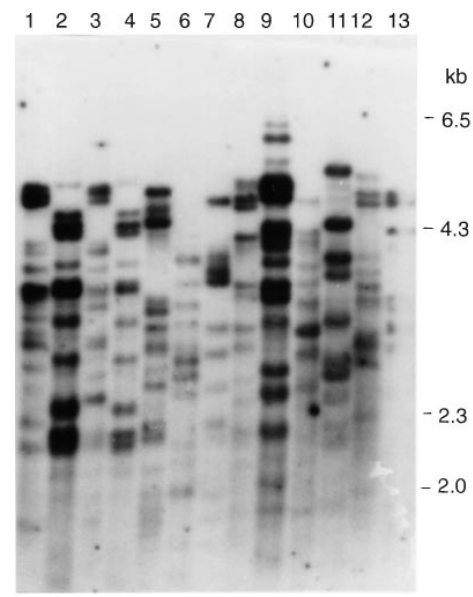

Fig. 3 Hybridization patterns of *Hae*III digests of pearl millet DNA detected by probe $(GATA)_4$. *Lanes 1–8* as in Fig. 1, 5644 A(*9*), 189 A(*10*), 393 A(*11*), 267 A(*12*) and 5122 A(*13*)

Table 4 Analysis of DNA fingerprints in cultivars[a] and landraces[b] of pearl millet. The values indicated for RAPDs were obtained with five and nine primers in cultivars and landraces, respectively

Item	*Dra*I/GATA	*Hae*III/GATA	*Hin*fI/GATA	RAPDs
Average no. of bands (n) $\pm$ SD	15.58 ± 3.5^a	11.23 ± 1.78^a	12.15 ± 3.1^a	21.42 ± 5.62^a
	11.00 ± 1.7^b	–	5.61 ± 1.33^b	30.66 ± 3.94^b
No. of comparisons	66^a	78^a	78^a	209^a
	153^b	–	153^b	153^b
Average no. of polymorphic bands between pairs $\pm$ SD	16.4 ± 5.16^a	12.9 ± 3.35^a	15.9 ± 4.0^a	16.31 ± 3.95^a
	4.88 ± 2.9^b	–	4.72 ± 2.26^b	18.80 ± 3.72^b
Average similarity index (X_D) $\pm$ SD	0.47 ± 0.14^a	0.42 ± 0.14^a	0.33 ± 0.15^a	0.628 ± 0.08^a
	0.78 ± 0.12^b	–	0.53 ± 0.11^b	0.66 ± 0.068^b
Probability of identical match by chance $(X_D)^n$	7.78×10^{-6a}	5.87×10^{-5a}	1.41×10^{-6a}	4.7×10^{-5a}
	6.5×10^{-2b}	–	2.8×10^{-2b}	2.9×10^{-6b}

primers giving inconsistent polymorphic patterns in various runs were eliminated from our analysis. All the RAPD primers used resulted in the amplification of 1–10 bands with an average of eight bands per primer in cultivars and five in the case of landraces. OPA10, OPA12, OPA16, OPA19, OPA20 and OPB12 generated polymorphic banding patterns in cultivars. In landraces, four different region-specific genotypes were chosen for initial screening to identify informative primers. OPA4, OPA12, OPA14, OPA16, OPB5, OPB8, OPB12 and OPB20 were found to be polymorphic. The polymorphism detected in cultivars and in landraces is represented in Figs. 4 and 5 using primers OPA20 and OPB20, respectively. In general, the extent of polymorphism generated by each primer was different in cultivars and in landraces (Table 5). This might be because of out-crossing in the present cultivars and more inbreeding in the landraces. Based on the polymorphic patterns obtained, similarity index values were calculated, which ranged from 0.54 to 0.86 for cultivars and 0.64 to 0.85 for landraces. The average similarity index values were almost similar for both (Table 4) and the probability of an identical match by chance was 4.7×10^{-5} for cultivars and 2.9×10^{-6} for landraces. Although, the values are higher compared to $(GATA)_n$, RAPDs can identify a greater number of genotypes by including more primers.

Problems with the reproducibility of RAPD banding patterns have been reported by Penner et al. (1993). In our laboratory they were solved by optimizing the conditions, running replications for screening primers, and discarding primers which generated inconsistent polymorphic banding patterns. In addition only strong, reproducible bands were used in the analysis while variable, faint, bands and those above 2 kb which were not repeatable were excluded. The RAPD analysis revealed a high degree of genetic diversity among the cultivars as well as between the landraces used in this study.

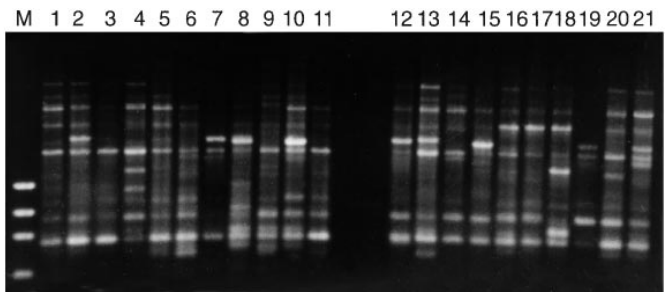

Fig. 4 RAPD fingerprinting of pearl millet genotypes with primer OPA 20. *Lanes 1–21* are as shown in Table 1

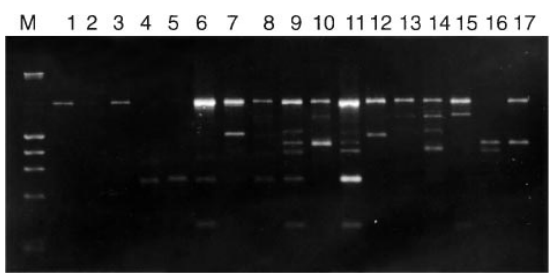

Fig. 5 RAPD fingerprinting of pearl millet landraces with primer OPB. 20. *Lanes 1–17* are as shown in Table 2

Table 5 Amplification patterns of pearl millet DNA with operon primers

Primer	Total no. of bands		Total no of polymorphic bands	
	Cultivars	Landraces	Cultivars	landraces
OPA4	–	8	–	7
OPA10	9	–	3	–
OPA12	10	9	10	2
OPA14	–	6	–	2
OPA16	12	12	7	8
OPA19	10	–	2	–
OPA20	15	–	15	–
OPB5	–	9	–	9
OPB12	11	10	11	4
OPB20	–	10	–	10

Genetic relationships

The grouping of 15 cultivars ($L \times T$ mating design) based on D^2 statistics is as shown in Table 6. Although 841 A, 267 A and 5141A were grouped under the same cluster, J104 and its derivative J254 represent two different clusters on the basis of D^2 statistics. Clusters generated using $(GATA)_n$ and RAPD data were also not completely in agreement with the available pedigree data. All the three genotypes 841A, 5141A and 267A were grouped in different clusters based on $(GATA)_4$ as well as RAPD data (Fig. 6a, b). Genotypes J254 and J104 were present in two different clusters, while K-560-230 and D-23 formed one cluster based on RAPD data (Fig. 6 b). The two cultivars 5141A and L-III-A having 97% similarity with $(GATA)_n$ hybridization (Fig. 6 a) showed no striking similarity with RAPDs (Fig. 6 b). In general, the groups established by D^2 analysis and the clusters developed by $(GATA)_n$ and RAPD analysis did not show much correlation.

Table 6 Grouping of 14 parents ($L \times T$ mating design) into clusters based on D^2 statistics

Cluster	No. of inbreds	Name of inbreds
I	6	267 A, 5141 A, 81 A, 393 A, Tift23 A, 841 A
II	2	J104, 843 A
III	4	PPMI493, 189 A, ICMP451, PPMI-301
IV	1	J-254
V	1	863 A
VI	1	D-23

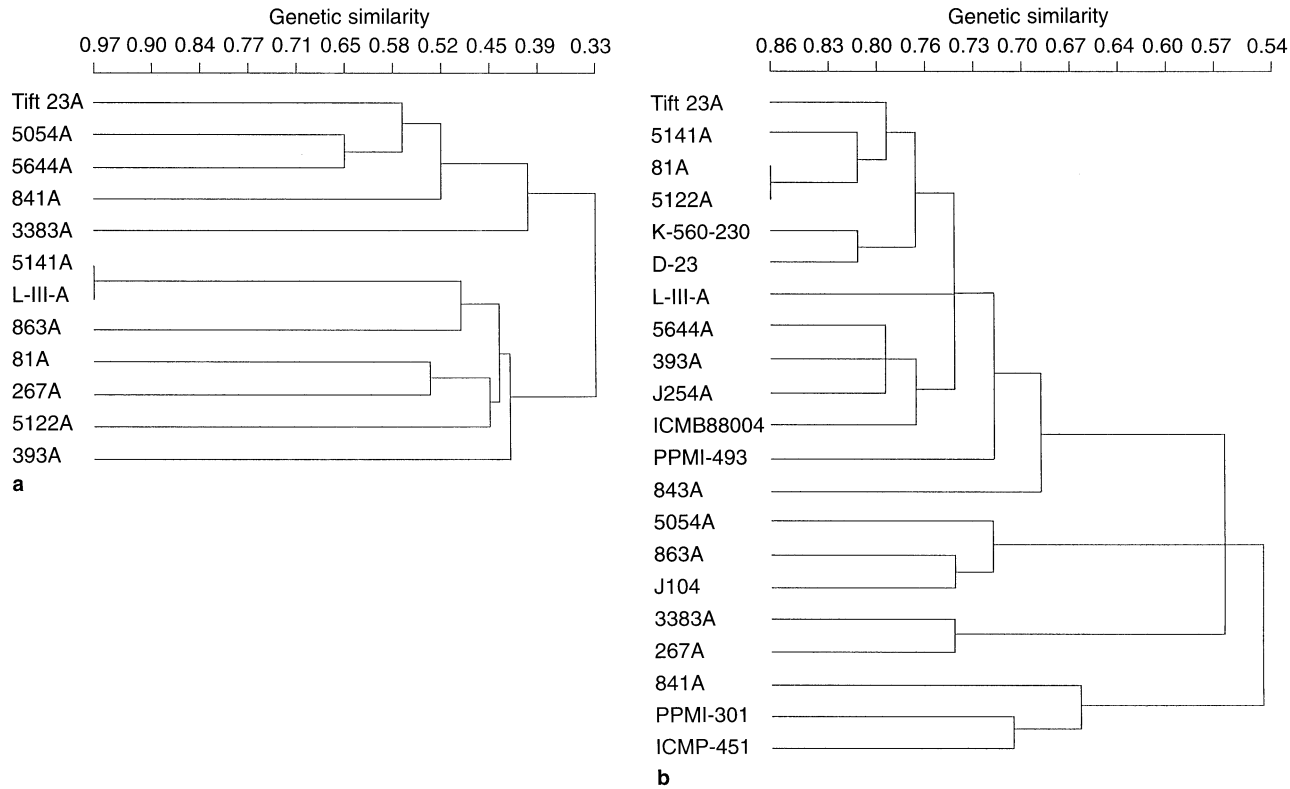

Fig. 6a Dendrogram of genetic relationships among 12 cultivars of India based on $(GATA)_4$ hybridization. **b** Dendrogram of genetic relationships among 21 cultivars of India based on RAPDs

Interestingly, the landraces representing eight states of India also could not be grouped based on their geographical distribution using DNA marker data (Fig. 7).

In the present study, pedigree data was not available for all cultivars. Tift23 A, the first line inroduced in India, shares a common genetic background with most of the present CMS lines. MS-5141 was derived from Tift23 by a backcrossing program (Pokhriyal et al. 1976). MS-841 was derived from a cross between MS-5141 and an unknown downy mildew resistant source (Singh et al. 1990). MS-267 was developed from 5141 through a backcross breeding program utilizing the 700651 downy mildew resistant donor from West Africa. MS-81 was a simple mutation of Tift23 A (Kumar et al. 1984). D-23 was a re-selection from K-560-230, and J-254 was an end product of a backcross program from J-104 utilizing a very diverse downy mildew resistant source from West Africa. The three groups, MS-5141 A, 841 A, 267 A and 81 A; K-560-230 and D-23; J-104 and J-254, share a common genetic background but in view of their pedigrees it cannot be assumed that they are genetically similar. In fact they differ in their combining ability (unpublished data). Out-crossing might be a reason for the large number of morphological changes in these cultivars (Rai and Hanna 1990).

Several studies have clearly demonstrated the utility of RAPDs and microsatellites for identifying plant varieties (Akkaya et al. 1992; Rongwen 1994; Morrel et al. 1995). Earlier results indicated that the exact number of primers and the number of bands required to distinguish varieties would be difficult to predict as it would vary from group to group and would have to be determined empirically. Akkaya et al. (1992) reported that the microsatellites in soybean could detect heterozygosity per locus, which varied from 52 to 88%. Our results have shown that all the cultivars and landraces can be identified using RAPDs and GATA hybridization patterns, which will prove useful for varietal identification in pearl millet. The probability of identical match by chance for any two genotypes using $(GATA)_n$ and RAPDs is 3.02×10^{-20} for cultivars and 0.52×10^{-10} for landraces. For highly inbreeding plants, or crops with a narrow genetic base, it is more difficult to find probes or primers that will discriminate between genotypes. In the out-crossed species *Plantago lanceolata*, a M13 probe could distinguish all 32 individuals whereas in the case of *Plantago major*, which is an inbred species, only a few distinguishing banding patterns were identified among 40 individuals (Wolff et al. 1994). Based on our data, a large collection of pearl millet landraces in India can be identified and exploited in the breeding program, including the identification of duplicates in germplasm collections. As shown by our studies, the loci containing many types of SSRs can be effectively used by converting them into sequence-tagged microsatellite sites (STMSs).

The success of a crop-improvement program largely rests on the availability and knowledge of genetic

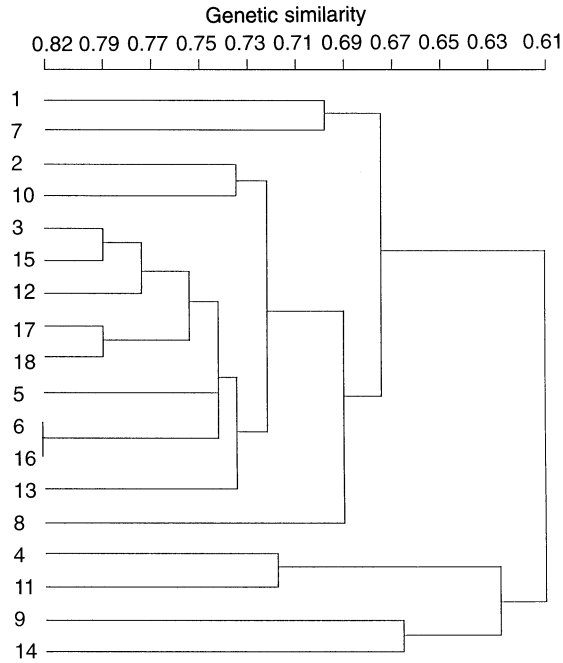

Fig. 7 Dendrogram of genetic relationships among pearl millet landraces from India based on RAPDs. *Numbers 1–18* as in Table 2

resources in germplasm collections. In case of pearl millet, the cultivars were developed from a narrow gene pool. Wild *Pennisetum* species offer diverse germplasms that can be used to improve pearl millet, and useful characteristics for this purpose are disease and insect resistance, genes for fertility restoration of A1 cytoplasm, cytoplasmic diversity, QTLs for yield, apomixis, maturity and many inflorescence and plant-morphological characteristics. At present, no serious attempt has been made to study the primary, secondary and tertiary gene pools in *Pennisetum* using DNA markers. $(GATA)_4$ and RAPDs could be of great value both for exploiting the wild germplasm during introgression breeding programs and in the identification of varieties having good combining abilities, without the evaluation of F_1 hybrids in the field, in order to predict heterotic combinations.

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