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Assessment of genetic relationship among varieties of finger millet using ISSR and SSR markers

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Abstract
The present study was carried out to asses the genetic charactorization among different varieties of finger millet by utilising 14 ISSR (dominant) and 6 SSR (co-dominant) markers. The DNA from leaf sample was isolated by standard protocol, while quite modifications in rapid protocol using 0.080 g ml^{-1} glucose, 0.020 g ml^{-1}polyvinylpyrrolidone and 0.0050 g ml^{-1} sodium dodecyl sulphate produced the best quantity DNA. The 14 ISSR and 6 SSR pattern of genomic DNA of 14 varieties were analyzed with respect to the fragments by standard PCR components. During ISSR assay, total of 795 scorable DNA fragments were produced and among them 235 DNA fragments were found to be polymorphic, i.e. 29.74 % of polymorphism. SSR assay yields total of 109 scorable DNA fragments were produced and among them 95 DNA fragments were found to be polymorphic i.e. 83.33% polymorphism. In ISSR assay, the primer UBC-812 (0.833) and SSR primer UGEP 12 (0.5) and UGEP 67 (0.5) produced maximum polymorphic information content. The similarity co-efficient ranged from 0.719 (between varieties D-2 and SCN-10) to 0.982 (between varieties SCN-14 and M-1) in ISSR and 0.444 (between genotype SCN-6 and M-3) to 0.889 (between genotype Dapoli-2, Dapoli-1, SCN-4, SCN-8, SCN-10, SCN-11, SCN-13, SCN-14, Dapoli-safed in SSR indicated the distinctness of these varieties. Hence, the study indicated the more efficiency of SSR than ISSR markers in investigating genetic variability at molecular level.

Keywords: Finger millet, genetic assessment, ISSR, SSR

Introduction
Finger millet (*Eleusine coracana* L.) commonly referred as Ragi, Nachani and Mandua ranks third in importance among millets in the country in area and production after sorghum and pearl millet [15]. It is also known as Dry land crop, cultivated in tropical and sub-tropical regions of south India [6]. Its cultivation is more widespread compared to other millets and accounts for 8% of the area and 11% production of all the millets cultivation in the world. Finger millet has wonderful health benefits also [18]. Genetic diversity is normally assessed by common morphological traits. However, such traits are affected by effects of environment, development stage of the plant and the type of plant material and also it require several replications to establish the genotypic contributions [4, 5]. Recently efforts have been made to develop genetic map of tetraploid finger millet genome (2n = 4x = 36) using RFLP, AFLP, EST and SSR markers, which might expedite the improvement of finger millet [1, 3, 8]. The use of molecular markers allows the direct assessment of genotypic variation at the DNA level. Marker analysis helps to understand the genetic makeup of the accessions and also make it possible to analyze the global organization of genetic diversity within a species [12, 13, 16]. Several statistical techniques are available for the analysis of genetic diversity using DNA fingerprinting data [19]. The present study, a set of 20 dominant and co-dominant markers were used asses the genetic diversity in ragi.

Materials and Methods
A) Plant material
For the experimental study, 14 different varieties of finger millet (Table 1) samples were collected from Plant Biotechnology Centre, Dapoli.

Table 1: List of varieties

Sr. No.	Name of variety	Sr. No.	Name of variety
01	Dapoli safed	08	SCN-10
02	D-1	09	SCN-11
03	D-2	10	SCN-13
04	SCN-4	11	SCN-14
05	SCN-5	12	M-1
06	SCN-6	13	M-2
07	SCN-8	14	M-3

B) DNA isolation

DNA was isolated from leaf as well as seed samples. Previously standardized protocol used for DNA isolation from leaf [11]. While slight modifications made to isolate DNA from seeds [7]. Three different solutions (T1, T2, and T3) were tested to extract DNA from seeds (Table 2).

Table 2: Quantity of components for seed

Components	T1	T2	T3
Glucose	0.700 g	0.800 g	0.900g
PVP	0.100 g	0.200 g	0.300g
Sodium Lauryl Sulphate	0.040 g	0.050 g	0.060g

Tender young leaf samples (0.1 g each) and newly germinating seeds (0.2 g each) were used to extract genomic DNA. RNA and protein contaminations were removed by treatment with 50 µg ml^{-1}RNase and 75 µg ml^{-1} Protease-K (HiMedia biosciences, Mumbai, India) respectively and incubated at 37 °C for 1 h. A total of 14 DNA samples were isolated from leaf and 14 DNA samples were isolated from seeds. The size range and concentration of genomic DNA from leaf samples as well as seed samples were determined after electrophoresis using a standard DNA ladder in 1% (w/v) agarose gels and by comparing the intensity of staining with 10 mg ml^{-1}ethidium bromide**.**

C) PCR Assay

A total set of 20 genetic primers (14 ISSR and 6 SSR primers; Bioresource Biotech Pvt. Ltd., Pune, India) for PCR amplification was used for amplification. A PCR assay was performed for all microsatellites as well as gene specific markers. Each 20 µl PCR contained 20-30 ng template DNA, 2.5 µl of 10× PCR buffer, 0.5 µl of 15 mM MgCl2, 1 µl of 10 mM dNTPs, 5 pmol of microsatellite and 10 pmol of gene specific primers and 3.0 units of *Taq* polymerase. According to the melting temperature of each primer, thermal profiles were standardised for each primer (i.e. marker) [14]. The standard annealing temperatures of all microsatellites and gene specific markers are given in Table 3 (A&B) [9, 17]. The PCR-amplified products were separated by electrophoresis in 2% (w/v) agarose gels at 80 V. The gels were stained with 10 mg ml^{-1}ethidium bromide and visualised under UV light using a Fire Reader gel documentation system (V10, Uvitec Ltd., Cambridge, UK) and the data were stored for further analysis.

Table 3 (A): List of ISSR markers with their sequence and annealing temp

Sr. No.	Primer	Primer Sequence (5' – 3')	GC Content (%)	Tm Value (ºC)	Standardized Annealing temperature (ºC)
1	UBC 807	AGAGAGAGAGAGAGAGT	47.05	42.4	45.5
2	UBC 808	AGAGAGAGAGAGAGAGC	52.94	46.4	52.0
3	UBC 810	GAGAGAGAGAGAGAGAT	47.05	48.1	50.3
4	UBC 812	GAGAGAGAGAGAGAGAA	52.94	44.3	54.0
5	UBC 816	CACACACACACACACACAT	47.05	51.1	55.0
6	UBC 817	CACACACACACACACAA	47.05	50.7	57.1
7	UBC 818	CACACACACACACACAAG	52.94	51.0	54.5
8	UBC 824	TCTCTCTCTCTCTCTCTG	52.94	48.5	46.0
9	UBC 825	ACACACACACACACACT	47.05	51.4	55.0
10	UBC 834	AGAGAGAGAGAGAGAGYT	47.05	51.4	55.0
11	UBC 841	GAGAGAGAGAGAGAGAYC	52.94	46.8	46.4
12	UBC 857	ACACACACACACACACCG	55.55	55.7	52.0
13	UBC 876	GATAGATAGACAGACA	37.5	39.6	46.0
14	UBC 891	AGATGTGTGTGTGTGTG	47.1	49.5	52.0

Single letter abbreviations for mixed base positions
B = (C, G, T) (i.e. not A) H = (A, C, T) (i.e. not G)
V = (A, C, G) (i.e. not T) D = (A, G, T) (i.e. not C)
Y = C OR T (i.e. not A,G) R = A OR G (i.e. not C,T)

Table 3(B): List of SSR primer with their sequence and annealing temp

Sr. No.	Code	Sequence (SSR Marker)	No. of Bases	Tm° value	Standardized Annealing temperature (ºC)
1	UGEP12	F: 5′ATCCCCACCTACGAGATGC3′ R: 3′TCAAAGTGATGCGTCAGGTC5′	19 20	56.5 ℃ 55.1 ℃	**52.3**
2	UGEP24	F: 5′GCCTTTTGATTGTTCAACTCG3′ R: 3′CGTGATCCCTCTCCTCTCTG5′	21 20	52.6 ℃ 56.3 ℃	49.8
3	UGEP53	F:5′TGCCACAACTGTCAACAAAAG3′ R: 3′CCTCGATGGCCATTATCAAG5′	21 20	54.2 ℃ 53.2 ℃	50.4
4	UGEP67	F: 5′CTCCTGATGCAAGCAAGGAC3′ R: 3′AGGTGCCGTAGTTTGTGCTC5′	20 20	56.1 ℃ 57.7 ℃	52.7
5	UGEP81	F: 5′AAGGGCCATACCAACACTCC3′ R: 3′CACTCGAGAACCGACCTTTG5′	20 20	57.3 ℃ 55.6 ℃	51.0
6	UGEP93	F: 5′TGGCCTCGTTAGGTGAAGTC3′ R: 3′AGCACCAAAACTCCCACAAC5′	20 20	56.7 ℃ 56.0 ℃	53.2

D) Statistical Analysis

ISSR and SSR markers across the 14 finger millet samples were scored for their presence (1) or absence (0) of bands for each primer. The generated data was used to calculate the level of polymorphism according to total number of bands scored. Similarity co-efficient matrix was generated using Jaccard's similarity co-efficients [10]. This was also leads to Unweighted Pair Group Method for Arithmetic Average analysis (UPGMA) to construct a dendrogram. The similarity co-efficient analysis and dendrogram construction were carried out by using MVSP-A Multivariate Statistical Package-5785 (Version 3.1).

Percent polymorphism computed by,

$$\text{Percent polymorphism} = \frac{\text{Total number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

Results and Discussion

A) DNA isolation: The DNA from leaf sample was isolated by standard protocol. While quite modifications in rapid protocol also gave good isolation of high-quality DNA from seeds (Plate 1). A protocol using 0.080 g ml^{-1} glucose, 0.020 g ml^{-1}polyvinylpyrrolidone and 0.0050 g ml^{-1} sodium dodecyl sulphate produced the highest yields and best quantity DNA. The quality and concentration of DNA isolated from seeds were good than leaf. Hence seed's DNA were utilises for further analysis.

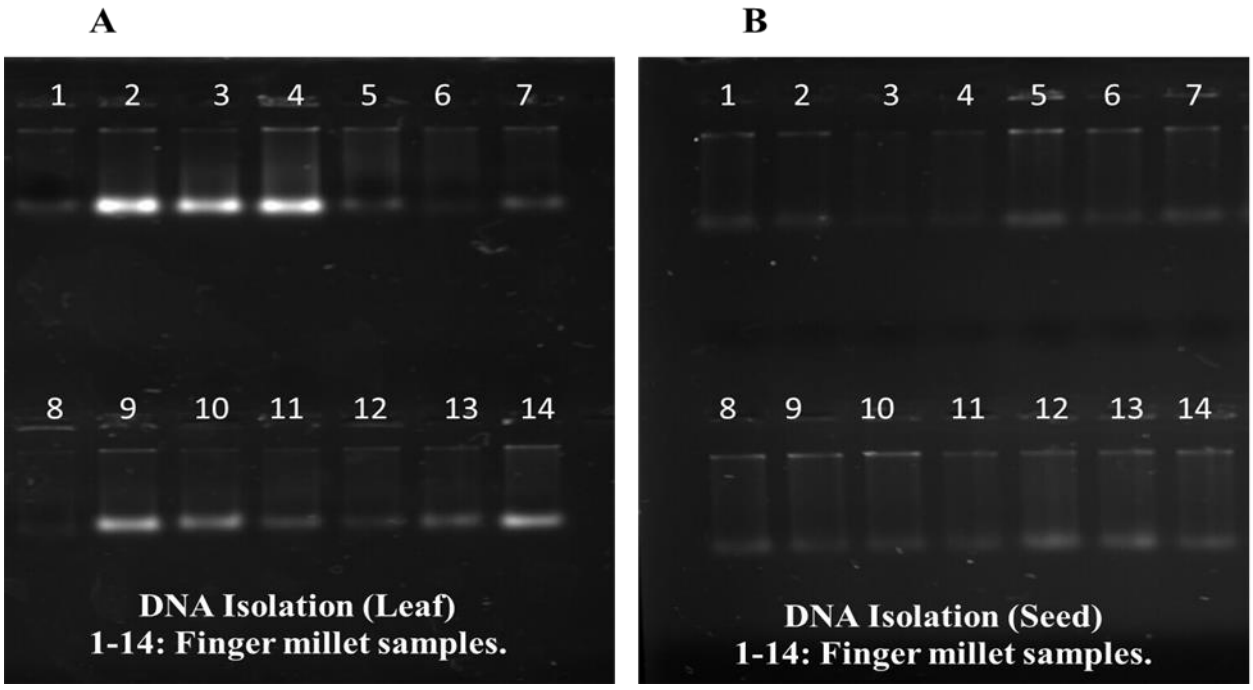

Plate 1: DNA bands of 14 varieties of finger millet; A) DNA bands of leaf samples, B) DNA bands of seed samples

B) Marker Analysis

For the present study, the 14 ISSR and 6 SSR pattern of genomic DNA of 14 varieties were analyzed with respect to the fragments, informative ness of the markers and polymorphism for the assessment of genetic diversity present among the genotype. The primer wise amplification detail of the genomic DNA of 14 finger millet varieties and percent polymorphism across the 14 ISSR and 6 SSR primers is presented in the Table 4 (A&B).

Table 4(A): ISSR primer wise amplification and percent polymorphism of Finger Millet varieties

Sr. No.	Primer Name	No. of Polymorphic Bands	No. of Monomorphic Bands	Total No. of Bands	Polymorphism %	Range of Amplification (bp)	PIC
1	UBC-807	17	28	45	37.77	342-1377	0.752
2	UBC-808	0	56	56	0	347-1428	0.750
3	UBC-810	25	42	67	37.31	293-1289	0.799
4	UBC-812	35	28	63	55.55	226-1450	0.833
5	UBC-816	20	28	48	41.66	420-1861	0.740
6	UBC-817	12	42	54	22.22	321-1580	0.748
7	UBC-818	22	28	50	44	409-1482	0.746
8	UBC-824	13	28	41	31.70	219-1777	0.680
9	UBC-825	22	28	50	44	304-1639	0.745
10	UBC-834	38	42	80	47.5	293-1858	0.832
11	UBC-841	8	56	64	12.5	446-1618	0.800
12	UBC-857	13	42	69	18.84	400-1582	0.822
13	UBC-876	7	42	49	14.28	385-1289	0.734
14	UBC-891	3	70	73	4.11	525-1978	0.814
Total		235	560	795	-	-	-
Average		16.78	40	56.78	29.74	219-1978	0.77

Table 4(B): SSR primer wise amplification and percent polymorphism of finger millet genotype

Sr. No.	Primer Name	No. of Polymorphic Bands	No. of Monomorphic Bands	Total No. of Bands	Polymorphism %	Range of Amplification (bp)	PIC
1	UGEP 12	26	0	26	100	50-300	0.5
2	UGEP 24	13	0	13	100	200-300	0
3	UGEP 53	16	0	16	100	50-300	0.21875
4	UGEP 67	22	0	22	100	100-300	0.5
5	UGEP 81	0	14	14	0	200-300	0
6	UGEP 93	18	0	18	100	100-250	0.40123
Total		95	0	109	-	-	-
Average		15.83	2.33	18.16	83.33	50-300	0.27

During ISSR assay, total of 795 scorable DNA fragments were produced and among them 235 DNA fragments were found to be polymorphic in the 14 finger millet varieties (Table 4A). The size of amplification ranged from 200 to 2000. The minimum numbers of polymorphic fragments produced by the primer UBC-891 (03) while the maximum numbers of polymorphic fragments were produced by the primer UBC- 834 (38). Primer UBC-808 produced monomorphic bands. Average number of polymorphic bands observed per primer was 16.78. The average percentage polymorphism across the 14 primers among the varieties found to be 29.74 %. While, SSR assay yields total of 109 scorable DNA fragments were produced and among them 95 DNA fragments were found to be polymorphic in the finger millet genotype (Table 4B). The size of amplification ranged from 50 to 300. Average number of polymorphic bands observed per primer were 15.83. The average percentage polymorphism across the 15 primers among the genotype found to 83.33%.

Polymorphic Information Content: In ISSR marker assay, the primer UBC-812 (0.833) produced maximum polymorphic information content. The minimum polymorphic information content was produced by the primer UBC-816 (0.740). Average polymorphic information content by ISSRs were 0.77 among the all 14 varieties of Finger Millet (Plate 2). In case of SSR assay, the maximum polymorphic information content produced by the primer UGEP 12 (0.5) and UGEP 67 (0.5). The minimum polymorphic information content was produced by the primer UGEP 24 and UGEP 81 (0). Average polymorphic information content was 0.2699 among the all 14 genotype of finger millet. It indicates that SSR markers have a great potential to execute the polymorphism among the finger millet genotype (Plate 3).

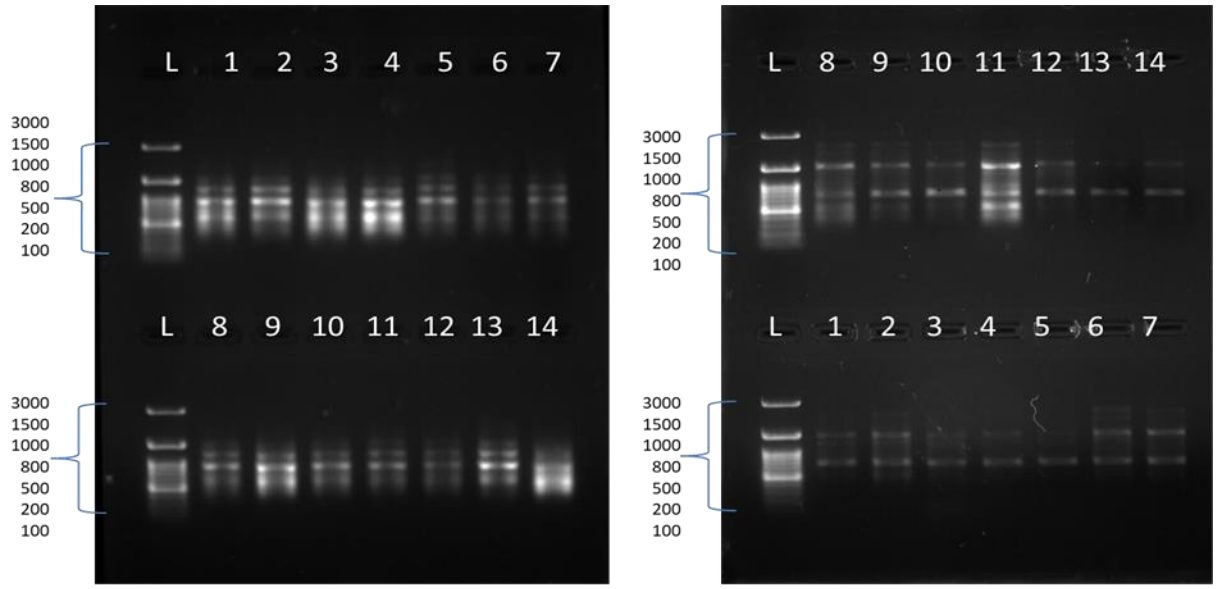

Plate 2: ISSR assay by using primer UBC-841 and UBC-857. Lanes M, 100 bp DNA ladder; lanes 1 - 14, amplicons of 14 varieties of finger millet

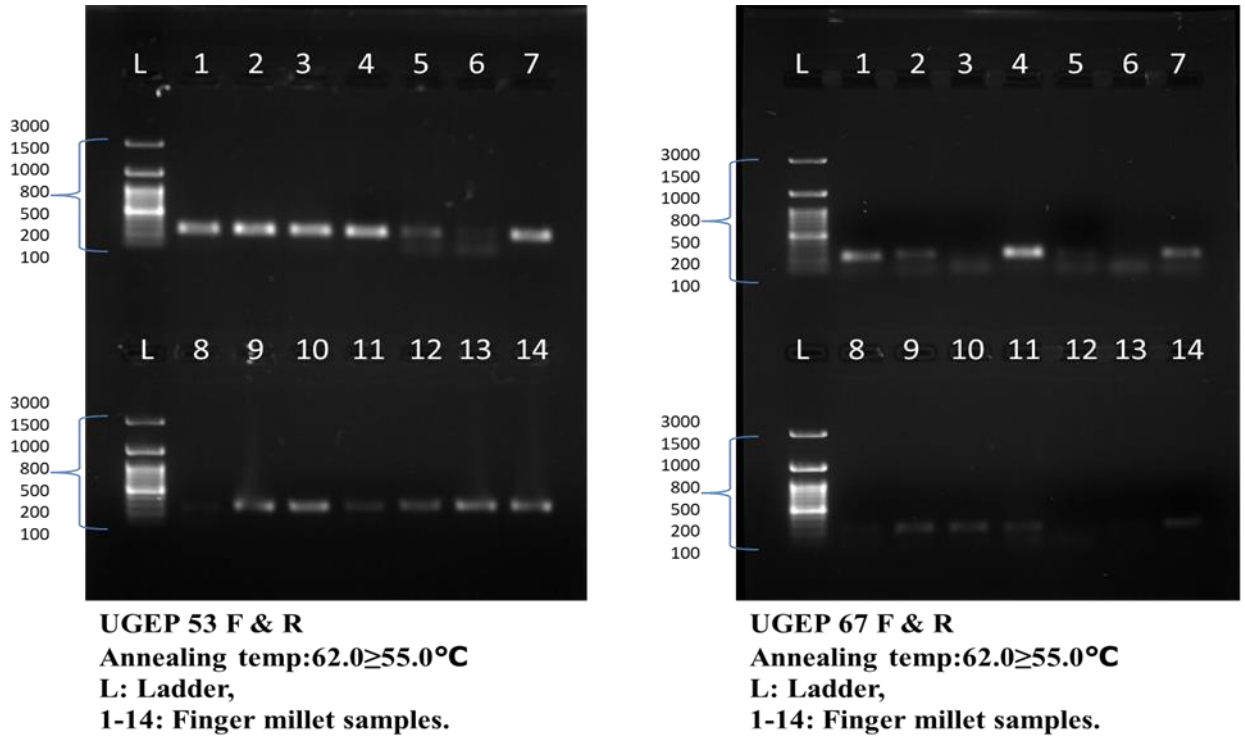

Plate 3: SSR assay by using primer UGEP 53 F&R and UGEP 67 F&R. Lanes M, 100 bp DNA ladder; lanes 1 - 14, amplicons of 14 varieties of finger millet

Genetic Relationship among Cultivars

Among ISSR primers: The genetic distance was computed considering all the genotypes from the pooled data and the dendrogram was constructed. The pair-wise Jaccard's similarity co-efficient for the genetic similarities among the 14 varieties are presented. The similarity co-efficient ranged from 0.719 (between varieties D-2 and SCN-10) to 0.982 (between varieties SCN-14 and M-1) indicated the distinctness of these varieties (Fig 1).

Among SSR primers: The genetic distance was computed considering all the genotypes from the pooled data and the dendrogram was constructed. The pair-wise Jaccards similarity co-efficients for the genetic similarities among the 14 genotype are presented. The similarity co-efficient ranged from 0.444 (between genotype SCN-6 and M-3) to 0.889 (between genotype Dapoli-2, Dapoli-1, SCN-4, SCN-8, SCN-10, SCN-11, SCN-13, SCN-14, Dapoli-safed (Fig 2).

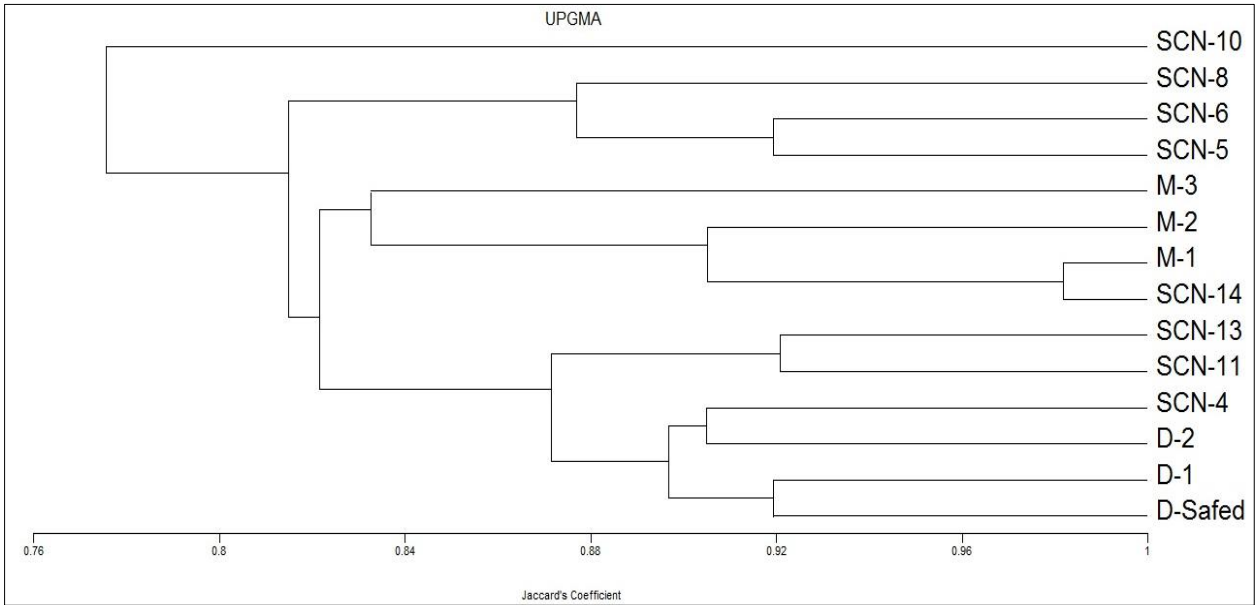

Fig 1: Dendrogram constructed using Jaccard's Similarity Coefficient by ISSR primers

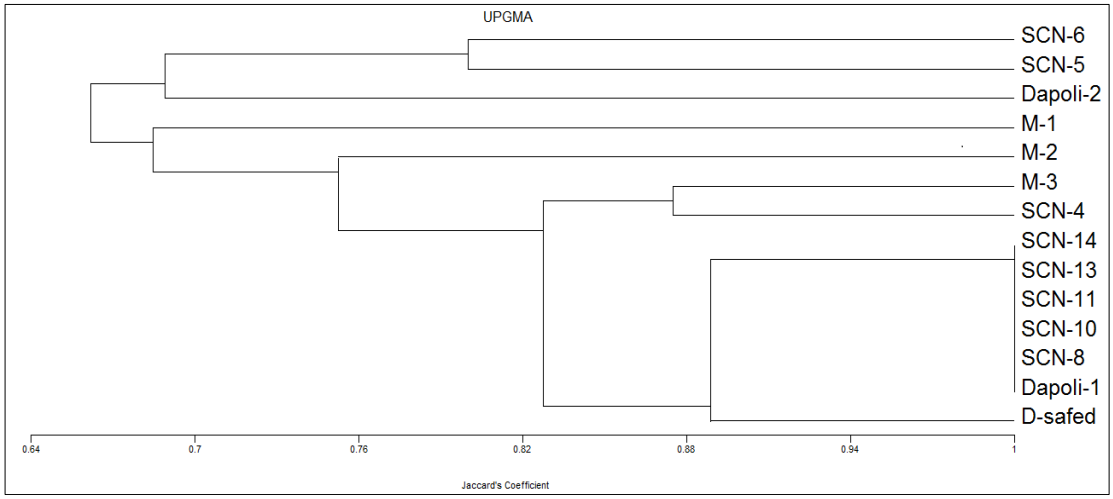

Fig 2: Dendrogram constructed using Jaccards Similarity Coefficient by SSR primers

Conclusion

The present study indicated that molecular markers are suitable for genetic assessment among different cultivars of finger millet. The ISSR and SSR analysis revealed substantial polymorphism in finger millet. The results of the present study indicated the more efficiency of SSR than ISSR markers in investigating genetic variability at molecular level, which is important for detecting distinctness of cultivars. Here we can conclude that, SSR markers as a co-dominant marker are more efficient and highly polymorphic than dominant ISSR markers. Such information is also helpful for characterization of genotypes as well as Marker Assisted Selection of desire trait.

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