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VL Ladumor
N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India

VB Parekh
Dept. of Basic Science and Humanities, ACHF, Navsari Agricultural University, Navsari, Gujarat, India

Stuti Krishna
N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India

HE Patil
Hill Millet Research Station, Navsari Agricultural University, Waghai (Dangs), Gujarat, India

Corresponding Author:
VL Ladumor
N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India

Genetic diversity in finger millet [*Eleusine coracana* (L.) Gaertn] Using ISSR markers

VL Ladumor, VB Parekh, Stuti Krishna and HE Patil

Abstract
Thirty five finger millet genotypes were evaluated using ISSR markers to study the genetic diversity in finger millet varieties. The study was conducted at the Forest Biotechnology laboratory, College of Forestry, Navsari Agricultural University, Navsari, Gujarat, India. The genetic variability among the 35 finger millet genotypes were studied in an initial screening using 60 ISSR primers. Fifteen of the 60 primers were produced informative, polymorphic products. Polymorphic information content (PIC) value ranged from 0.13 to 0.57. The highest PIC value observed for primer UBC-811 (0.57). The Jaccard's similarity coefficient obtained in this study ranged from 0.47 to 0.90. The maximum genetic similarity was found between the genotypes, VL-314 and PR-202 (0.90). The minimum genetic similarity was found between the genotypes, KOPN-235 and GN-5 (0.47). The information obtained in this study is useful in choice of related or diverse parents and genetic constitution of genotypes without any environmental influence.

Keywords: Genetic diversity, finger millet, ISSR primer

Introduction
Finger millet [*Eleusine coracana* (L.) Gaertn], is the most widely planted species amongst millets and the most important in East Asia. It is also known as African millet, Ragi and Coracan millet. Kezhvaragu, Aariyam, Panjapule, Maduva and Nachni are the common names of finger millet in different parts of India. Finger millet [*Eleusine coracana* (L.) Gaertn.] subspecies *coracana* belongs to family Poaceae. The cultivated *E. coracana* is a tetraploid (2n = 4X = 36); has morphological similarities to both *E. indica* (L.) Gaertn. (2n = 18) and *E. africana* (O.) Byrne (2n = 36). It is cultivated mostly as a rainfed crop in India for its valued food grains. Finger millet is more versatile due to its adaptability to wide range of geographical areas and agro-ecological diversity.

The crop originated in Africa and has been cultivated for thousands of year in the highlands of Uganda and Ethiopia. It was introduced to India at a very early date, probably over 3000 years ago. Through finger millet is reported to have reached Europe at about the commencement of the Christian era, however its utilization is restricted mostly to eastern Africa and India (Hittalmani *et al*., 2004) [1].

Finger millet is a tufted annual crop, growing to a height of 30-150 cm and maturing in 75-160 days. Leaves are narrow, grass-like and capable of producing many tillers and nodal branches. The panicle consists of a group of digitally arranged spikes often referred to as fingers. The spikelets are made up of 4–10 florets arranged serially on the finger. All florets are perfect flowers with the exception of the terminal ones which may sometimes be infertile. The grain is oblong to round and oval, reddish brown in colour with the grains surface finely corrugated. Typically, a tropical, rainfed crop, it is one of the best suited for dry farming. Finger millet is very adaptable and thrives at higher elevations than most other tropical cereals. (Vilas *et al.*, 2015) [2]

Complete emergence of inflorescence in finger millet required about 10 days and flowering remains continues for 7-8 days. The flower opens between 1 to 5 AM and progress from top to bottom in a finger. However, in a spikelet the order is reversed and proceeds from bottom to top and bigger to smaller flower. The stigma is receptive for a very short period after its emergence from the glumes. The period of anthesis being very short, is conducive for highly self-pollination.

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 requires several replications to establish the genotypic contributions.

Hence, there is a need to go for a highly reliable and precise method for assessment of genetic variability with no environmental effects. Assessment of genetic variability with molecular markers such as Restricted Fragment Length Polymorphism (RFLP), Random amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Interspersed Simple Sequence Repeats (ISSR) and Simple Sequence Repeats (SSR) or microsatellites over the various microcenters could enable to overcomes this problem. Lack of high yielding varieties adapted to diverse agro-ecological conditions is the major reason of low productivity. Evaluation of interaction of genotypes with environment and other agro-management conditions would help in getting information on adaptability and stability of performance of genotypes.

Materials and Methods

Plant material and DNA extraction

The material used for this study included 35 finger millet genotypes cultivated in different states of India (Table 1). DNA was extracted from young leaves, at the Forest Biotechnology laboratory, College of Forestry, NAU, Navsari. The genomic DNA was extracted by Cetyl tri-methy ammonium bromide (CTAB) method described by Doyle and Doyel (1990) [3] with some modifications. In present investigation, ISSR marker technique was used for the study of genetic divergence and establishment of distinctiveness and similarity.

Purity test and quantification of DNA

In order to perform PCR based analysis, the DNA has to be quantified. Spectrophotometry was performed to determine DNA concentration by using Nanodrop N.D.1000 (Software V.3.3.2, Thermo Scientific, U.S.A.). Dilutions of 50 ng/ µl working solutions prepared from stock solutions.

PCR amplification

The amplification cycles were undergone initial denaturation at 95^0C for 1 min, followed by 35 cycles of denaturation at 95^0C for 30 sec., annealing at 53^0C for 45 sec. synthesis at 72^0C for 1 min and finally extension step of 10 min at 72^0C. The PCR products were electrophoresed on 2% agarose gel. All amplified products of ISSR were run on 2% Agarose gel containing 4 µl of ethidium bromide (1 mg/ml). 10 µl of PCR product was mixed with 2 µl of 6X gel loading dye and loaded into the well. The gel was run at 80 V current (constant) to separate the amplified bands. The 100 bp standard DNA ladder was also run along with samples. The separated bands were visualized in U.V. transilluminator and stored using gel documentation system.

Table 1: List of Finger millet genotypes used in the experiment

No.	Genotype	Centre	State
1.	VL-352	ICAR-VPKAS, Almora	Uttarakhand
2.	VL-315	ICAR-VPKAS, Almora	Uttarakhand
3.	VL-149	ICAR-VPKAS, Almora	Uttarakhand
4.	VL-324	ICAR-VPKAS, Almora	Uttarakhand
5.	VL-376	ICAR-VPKAS, Almora	Uttarakhand
6.	VL-314	ICAR-VPKAS, Almora	Uttarakhand
7.	Dapoli-1	Dr. BSKKV, Dapoli	Maharashtra
8.	Dapoli-2	Dr. BSKKV, Dapoli	Maharashtra
9.	KOPN-235	MPKV, Rahuri	Maharashtra
10.	KOPN-942	MPKV, Rahuri	Maharashtra
11.	Phule Nachani	MPKV, Rahuri	Maharashtra
12.	VR-708	ANGRAUVizianagaram	Telangana
13.	VR-847	ANGRAU,Vizianagaram	Telangana
14.	VR-936	ANGRAU,Vizianagaram	Telangana
15.	PR-202	ANGRU, Perumallapalle	AndhraPradesh
16.	GPU-66	GKVK, Bangalore	Karnataka
17.	GPU-28	GKVK, Bangalore	Karnataka
18.	GPU-45	GKVK, Bangalore	Karnataka
19.	GPU-67	GKVK, Bangalore	Karnataka
20.	MR-6	GKVK, Bangalore	Karnataka
21.	KMR-340	GKVK, Mandya	Karnataka
22.	KMR-204	GKVK, Mandya	Karnataka
23.	KMR-630	GKVK, Mandya,	Karnataka
24.	OEB 532	OAUT, Bhubaneswar	Odisa
25.	Indira Ragi-1	IGKVV, Jagdalpur	Chhattisgarh
26.	Chhattisgarh Ragi-2	IGKVV, Jagdalpur	Chhattisgarh
27.	RAU-8	ARS, BAU, Dholi	Bihar
28.	GN-1	NAU, Waghai, Dangs	Gujarat
29.	GN-2	NAU, Waghai, Dangs	Gujarat
30.	GN-3	NAU, Waghai, Dangs	Gujarat
31.	GN-4	NAU, Waghai, Dangs	Gujarat
32.	GN-5	NAU, Waghai, Dangs	Gujarat
33.	GNN-6	NAU, Waghai, Dangs	Gujarat
34.	GNN-7	NAU, Waghai, Dangs	Gujarat
35.	GN-8	NAU, Waghai, Dangs	Gujarat

Results and Discussions

The genetic variability among the 35 finger millet genotypes were studied in an initial screening using 60 ISSR primers. Fifteen of the 60 primers were produced informative,

polymorphic products *viz.*, UBC-807, UBC-808, UBC-809, UBC-810, UBC-811, UBC-814, UBC-815, UBC-817, UBC-823, UBC-826, UBC-834, UBC-843, UBC-847, UBC-856 and UBC.

Table 2: Sequence, total number of bands, total numbers of alleles, number of monomorphic alleles, number of polymorphic alleles and percentage of polymorphisms using 15 ISSR primers in 35 finger millet genotypes

Primer	Sequence	Total number of bands	Total numbers of alleles (a)	Number of monomorphic alleles	Number of polymorphic alleles (b)	Percentage of polymorphisms (b/a x100)	PIC
UBC-807	AGAGAGAGAGAGAGAGT	173	06	02	04	66.66	0.25
UBC-808	AGAGAGAGAGAGAGAGC	200	07	01	06	85.71	0.26
UBC-809	AGAGAGAGAGAGAGAGG	353	12	04	08	66.66	0.13
UBC-810	GAGAGAGAGAGAGAGAT	210	09	00	09	100	0.49
UBC-811	GAGAGAGAGAGAGAGAC	149	07	00	07	100	0.57
UBC-814	CTCTCTCTCTCTCTCTA	142	06	00	06	100	0.50
UBC-815	CTCTCTCTCTCTCTCTG	256	09	05	04	44.44	0.26
UBC-817	CACACACACACACACAA	300	10	02	08	80.0	0.25
UBC-823	TCTCTCTCTCTCTCTCC	179	08	02	06	75.0	0.46
UBC-826	ACACACACACACACACC	342	12	03	09	75.0	0.30
UBC-834	AGAGAGAGAGAGAGAGYT	207	08	00	08	100	0.41
UBC-843	CTCTCTCTCTCTCTCTRA	115	05	01	04	80.0	0.53
UBC-847	CACACACACACACACARC	184	07	01	06	85.71	0.34
UBC-856	ACACACACACACACACYA	279	11	02	09	81.81	0.41
UBC-857	ACACACACACACACACYG	279	12	04	08	66.66	0.41
Total		3229	129	27	102	1343.28	5.57
Average		215.27	8.6	1.8	6.8	89.55	0.37

857 resolvable by agarose gel electrophoresis. Plates 1 to 15 shows the PCR products of these ISSR reactions.

The total number of amplification products found was 3229 with a maximum of 353 with the primer UBC-809 and minimum of 115 with the UBC-843. The range of polymorphism varied between 44.44% to 100%. Whereas 100% polymorphism of primer UBC-810, UBC-811, UBC-814 and UBC-834. The average number of total alleles per primer was 8.6. The highest numbers of total alleles were produced by primer UBC-809, UBC-826 and UBC-857 (12) followed by UBC-856 (11), UBC-817 (10), UBC-810 and 815 (09). UBC-843 (05) and UBC-807 (06) produces lowest total alleles among all.

The diversity among the 35 finger millet genotypes studied using the inter simple sequence repeat technique (ISSR). The present study exhibited the polymorphism with ISSR primers ranged from 44.44% to 100% among the 35 finger millet genotypes (Table 2). Animasaun *et al*. (2015) [4] reported 56.25% level of polymorphism. Kelkar *et al.* (2017) [5] reported 83.32% polymorphism. Shingane *et al*. (2018) [6] found 66.66% to 100% level of polymorphism. Zuge *et al.* (2018) [7] reported 72.79% polymorphism. Rajput *et al*. (2019) [8] reported highest frequency of polymorphism was found in the Dindori region (69.84%).

Polymorphic information content (PIC) value ranged from 0.13 to 0.57. The highest PIC value observed for primer UBC-811 (0.57) followed by primer UBC-843 (0.53) whereas lowest PIC value was reported with primer UBC-809 (0.13). While PIC value observed for other primers UBC-807 and UBC-817 (0.25), UBC-808 and UBC-815 (0.26), UBC-810 (0.49), UBC-814 (0.50), UBC-823 (0.46), UBC-826 (0.30), UBC-847 (0.34), UBC-834, UBC-856 and UBC-857 (0.41). Prabhu and Ganesan (2013) [9] reported PIC value was highest for the primer RM 440 (0.729) followed by RM 492 (0.726) while, the lowest PIC value recorded by the primer RM 244 (0.32). Babu *et al.* (2016) [10] reported PIC values of all the polymorphic loci across the 149 finger millet genotypes varied from 0.292 to 0.703 at an average of 0.442. Kelkar *et al.* (2017) [5] reported the average PIC value was 0.70 among the all 40 germplasms. Shingane *et al*. (2018) [6] reported the average PIC value for RAPD and ISSR was 0.74 and 0.73, respectively. Rajput *et al*. (2019) [8] reported the highest PIC (Polymorphism Information Content) value of 0.58 was observed by primer UBC-884 revealing 07 alleles among 42 accessions. Joshi *et al*. (2020) [11] reported polymorphism information content was of 0.314 for RAPD and 0.37 for SSR.

The similarity values produced from the ISSR was constructed by Jaccard's coefficient matrix for 35 genotypes of finger millet (table 4.5.2). The maximum similarity was found between the genotypes, VL-314 and PR-202 (0.90) followed by VL-315 and VL-314, Phule Nachni (0.89), VL-376 and VR-708 (0.89) and VL-376 and VL-314. So, these types of genotypes were closely related to each other. The minimum similarity was found between the genotypes, KOPN-235 and GN-5 (0.47) followed by Dapoli-2 and GN-5 (0.48), KOPN-235 and GN-4, Dapoli-2 and GN-3, GN-4 (0.50) and KOPN-235 and GN-3, GNN-7 (0.51). So, these genotypes were most diverse and these diverse genotypes can be used in breeding programme for hybridization and heterosis.

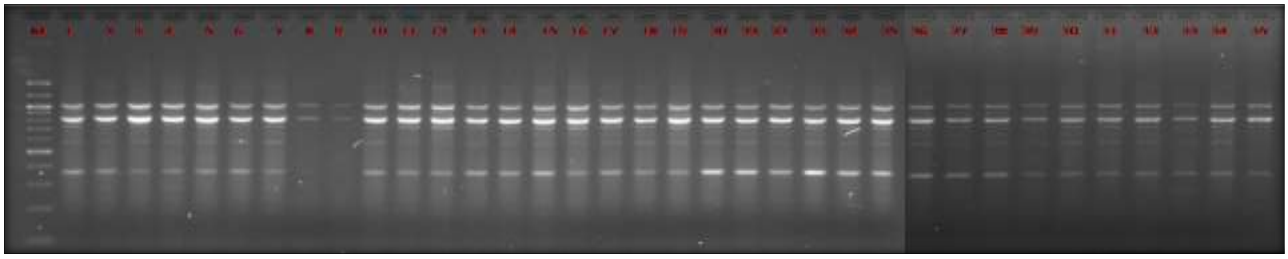


Plate 1: ISSR gel profiling of finger millet genotypes for UBC-807 primer

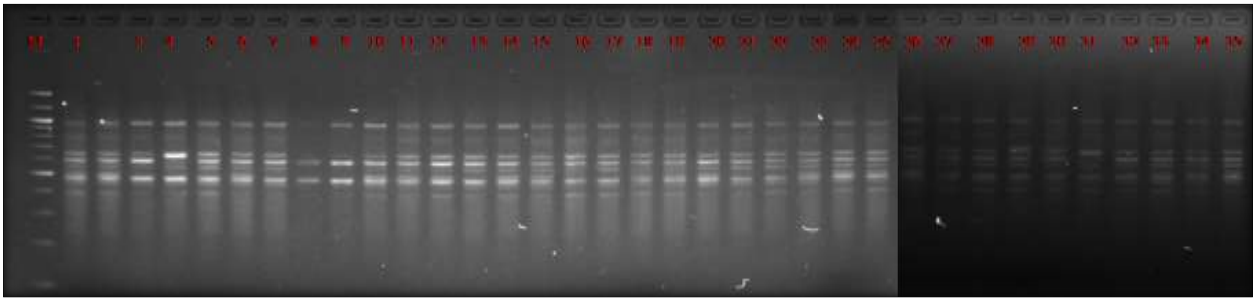


Plate 2: ISSR gel profiling of finger millet genotypes for UBC-808 primer

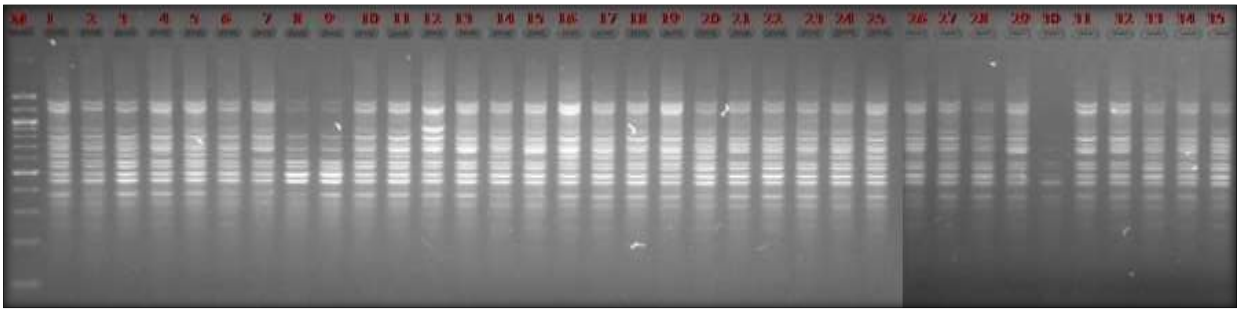


Plate 3: ISSR gel profiling of finger millet genotypes for UBC-809 primer

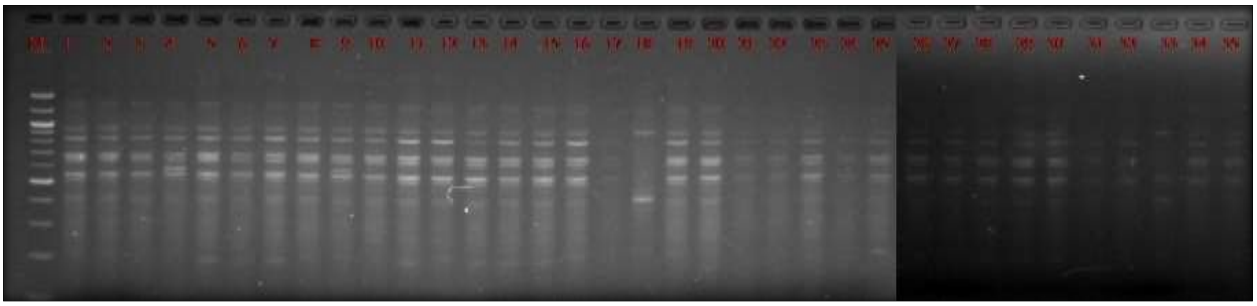


Plate 4: ISSR gel profiling of finger millet genotypes for UBC-810 primer

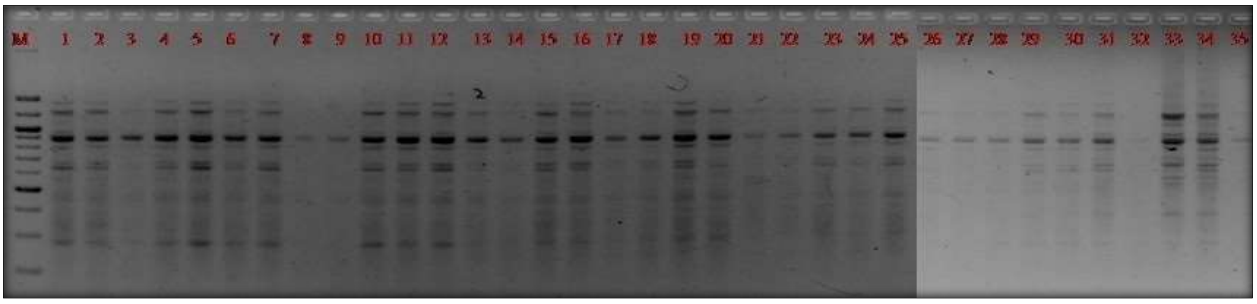
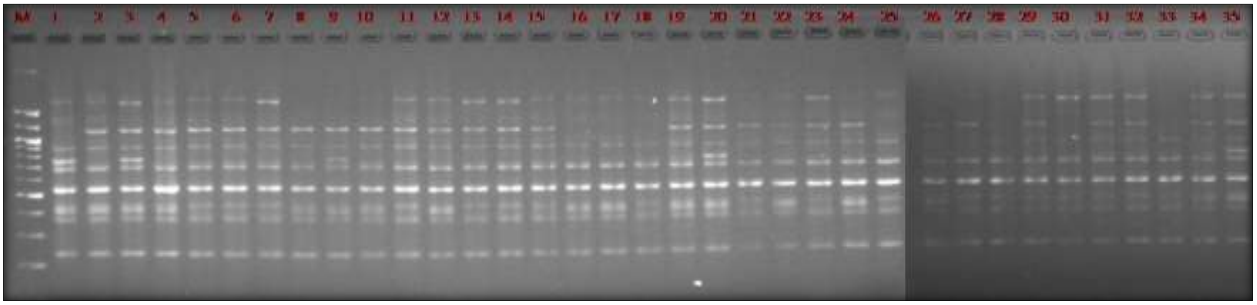


Plate 5: ISSR gel profiling of finger millet genotypes for UBC-811 primer

Plate 6: ISSR gel profiling of finger millet genotypes for UBC-814 primer



M = 100 bp marker and 1 to 35 = Finger millet genotypes

Plate 7: ISSR gel profiling of finger millet genotypes for UBC-815 primer

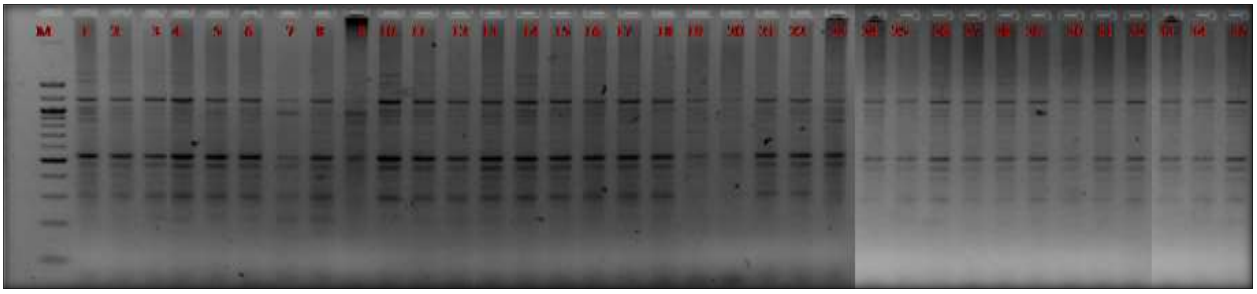


Plate 8: ISSR gel profiling of finger millet genotypes for UBC-817 primer

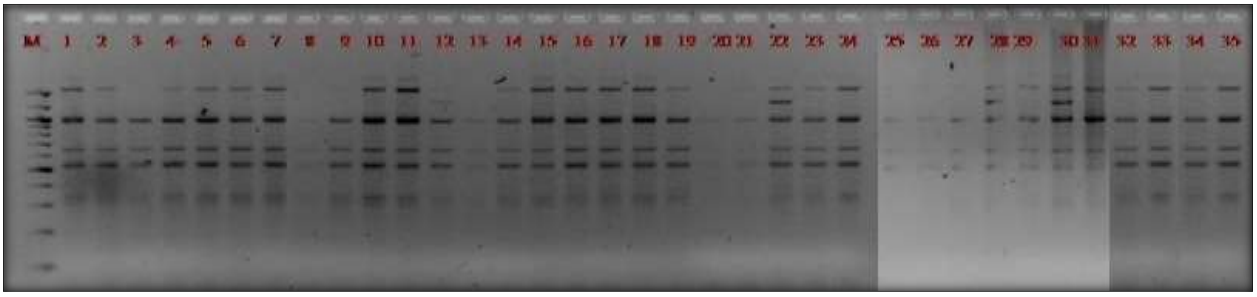


Plate 9: ISSR gel profiling of finger millet genotypes for UBC-823 primer

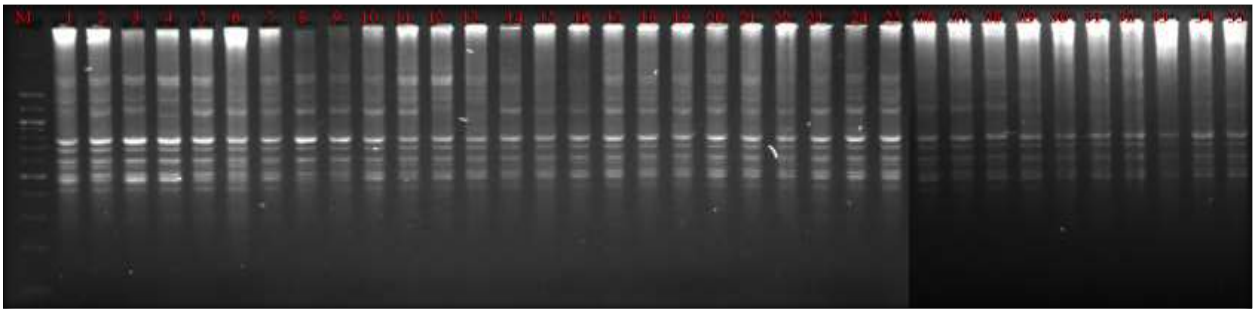
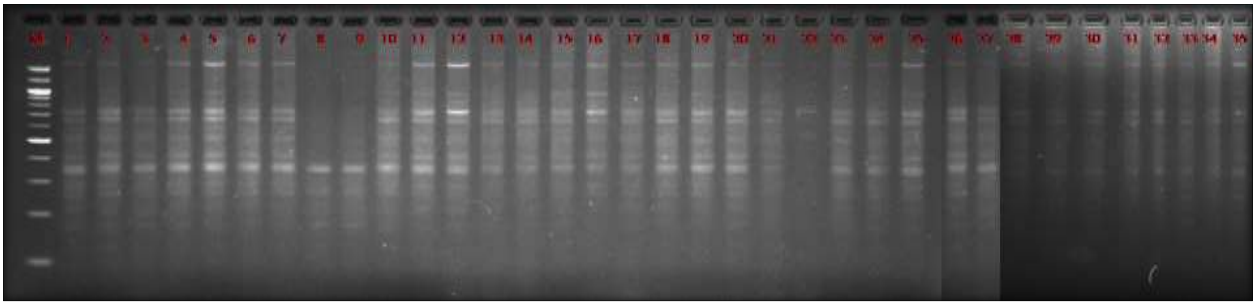


Plate 10: ISSR gel profiling of finger millet genotypes for UBC-826 primer



M = 100 bp marker and 1 to 35 = Finger millet genotype

Plate 11: ISSR gel profiling of finger millet genotypes for UBC-834 primer

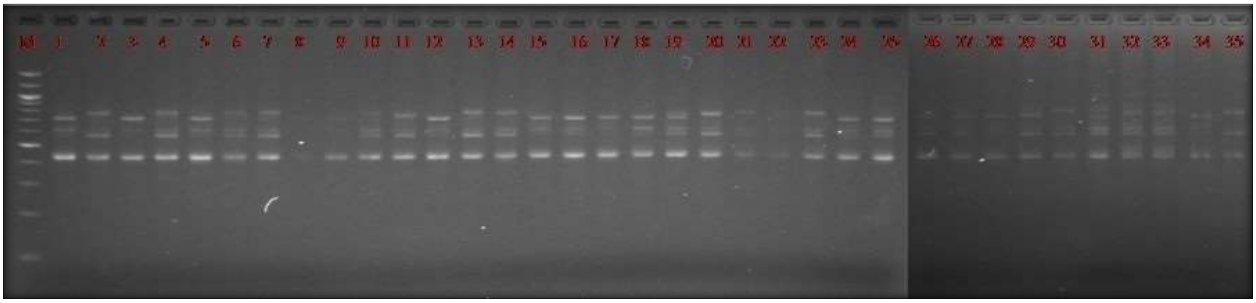


Plate 12: ISSR gel profiling of finger millet genotypes for UBC-843 primer

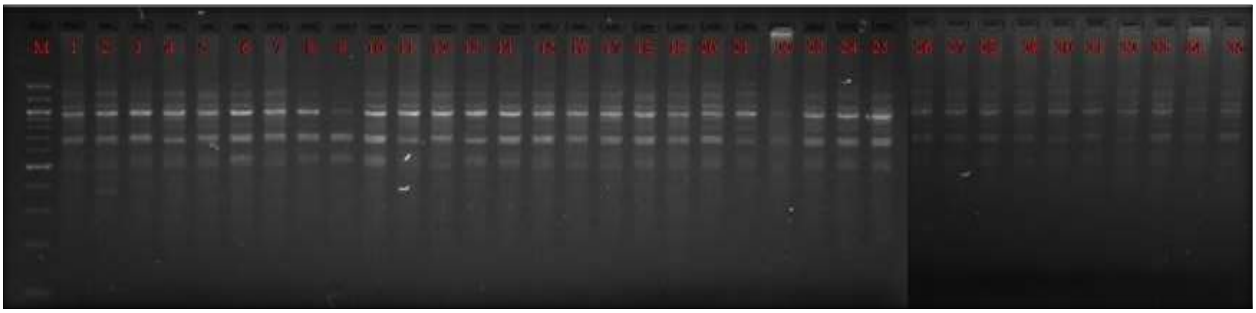


Plate 13: ISSR gel profiling of finger millet genotypes for UBC-847 primer

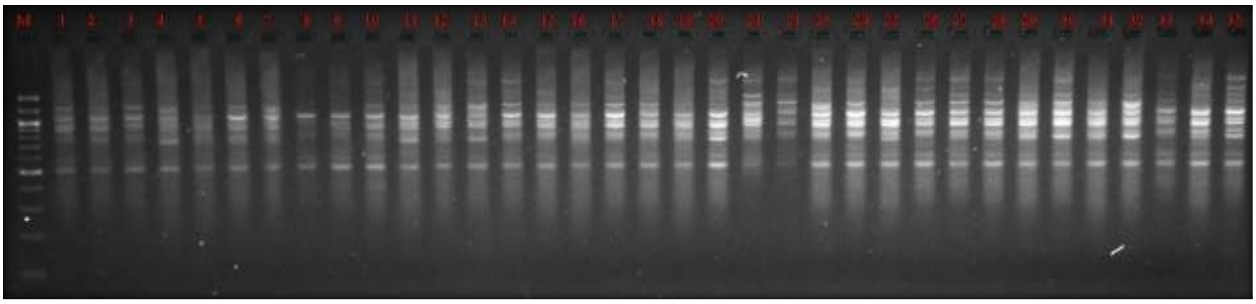
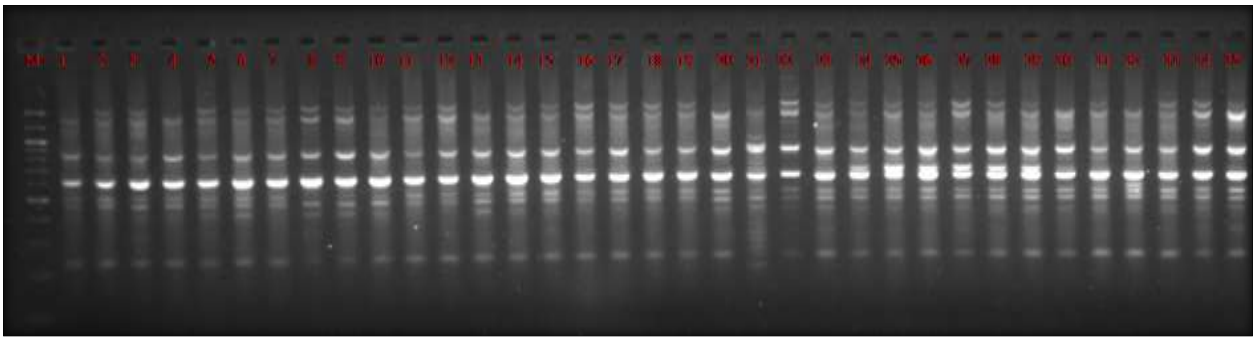


Plate 14: ISSR gel profiling of finger millet genotypes for UBC-856 primer



M = 100 bp marker and 1 to 35 = Finger millet genotypes

Plate 15: ISSR gel profiling of finger millet genotypes for UBC-857 primer

Table 3: Jaccard's similarity coefficient for 35 genotypes of finger millet based on ISSR marker

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	1.00																																		
2	0.86	1.00																																	
3	0.82	0.80	1.00																																
4	0.76	0.80	0.79	1.00																															
5	0.85	0.88	0.82	0.79	1.00																														
6	0.79	0.89	0.77	0.80	0.88	1.00																													
7	0.83	0.87	0.79	0.87	0.87	0.85	1.00																												
8	0.57	0.59	0.64	0.59	0.63	0.61	0.63	1.00																											
9	0.56	0.57	0.64	0.57	0.60	0.57	0.59	0.75	1.00																										
10	0.76	0.85	0.75	0.76	0.83	0.84	0.80	0.62	0.66	1.00																									
11	0.85	0.89	0.76	0.76	0.86	0.85	0.85	0.57	0.55	0.83	1.00																								
12	0.87	0.85	0.77	0.78	0.89	0.84	0.86	0.56	0.54	0.76	0.89	1.00																							
13	0.79	0.82	0.78	0.84	0.84	0.82	0.88	0.63	0.60	0.78	0.79	0.80	1.00																						
14	0.77	0.83	0.77	0.76	0.84	0.85	0.81	0.66	0.63	0.80	0.82	0.81	0.86	1.00																					
15	0.83	0.83	0.79	0.79	0.87	0.90	0.87	0.57	0.57	0.78	0.84	0.88	0.78	0.79	1.00																				
16	0.82	0.79	0.76	0.74	0.85	0.79	0.82	0.56	0.57	0.74	0.83	0.83	0.77	0.75	0.84	1.00																			
17	0.81	0.81	0.82	0.72	0.83	0.75	0.77	0.54	0.54	0.73	0.78	0.79	0.76	0.75	0.77	0.82	1.00																		
18	0.80	0.82	0.77	0.71	0.82	0.82	0.78	0.55	0.58	0.78	0.79	0.77	0.78	0.77	0.81	0.77	0.85	1.00																	
19	0.86	0.87	0.79	0.79	0.87	0.87	0.86	0.62	0.58	0.80	0.87	0.85	0.83	0.81	0.82	0.81	0.77	0.79	1.00																
20	0.84	0.86	0.77	0.78	0.85	0.86	0.81	0.57	0.56	0.82	0.87	0.85	0.82	0.83	0.83	0.76	0.76	0.78	0.83	1.00															
21	0.65	0.68	0.69	0.71	0.72	0.67	0.73	0.55	0.59	0.70	0.67	0.68	0.74	0.71	0.67	0.65	0.72	0.65	0.66	0.70	1.00														
22	0.58	0.60	0.65	0.56	0.64	0.60	0.58	0.53	0.60	0.65	0.59	0.57	0.57	0.62	0.60	0.63	0.65	0.57	0.59	0.57	0.72	1.00													
23	0.76	0.82	0.76	0.77	0.84	0.87	0.86	0.63	0.61	0.80	0.80	0.81	0.83	0.79	0.82	0.73	0.71	0.79	0.83	0.80	0.69	0.58	1.00												
24	0.82	0.81	0.78	0.69	0.84	0.81	0.77	0.59	0.58	0.77	0.78	0.80	0.77	0.80	0.83	0.74	0.81	0.81	0.79	0.81	0.66	0.62	0.80	1.00											
25	0.85	0.85	0.74	0.74	0.86	0.85	0.82	0.56	0.54	0.79	0.88	0.86	0.78	0.82	0.85	0.82	0.81	0.82	0.87	0.84	0.66	0.58	0.81	0.84	1.00										
26	0.64	0.66	0.68	0.64	0.66	0.70	0.66	0.57	0.58	0.68	0.65	0.66	0.66	0.74	0.74	0.66	0.65	0.63	0.65	0.67	0.65	0.71	0.69	0.73	0.69	1.00									
27	0.69	0.73	0.73	0.63	0.75	0.75	0.68	0.58	0.61	0.69	0.69	0.70	0.69	0.77	0.75	0.69	0.69	0.72	0.69	0.69	0.65	0.68	0.74	0.81	0.74	0.80	1.00								
28	0.64	0.68	0.68	0.62	0.70	0.69	0.63	0.59	0.63	0.70	0.67	0.65	0.67	0.76	0.68	0.67	0.69	0.70	0.66	0.71	0.66	0.67	0.67	0.72	0.70	0.76	0.83	1.00							
29	0.72	0.76	0.74	0.74	0.79	0.79	0.78	0.62	0.59	0.73	0.73	0.77	0.79	0.79	0.77	0.76	0.69	0.68	0.76	0.72	0.65	0.62	0.79	0.76	0.76	0.77	0.80	0.71	1.00						
30	0.67	0.64	0.61	0.62	0.66	0.67	0.66	0.50	0.51	0.64	0.68	0.67	0.72	0.65	0.66	0.69	0.60	0.63	0.68	0.64	0.61	0.52	0.68	0.63	0.66	0.63	0.64	0.60	0.76	1.00					
31	0.68	0.73	0.66	0.71	0.69	0.73	0.73	0.50	0.50	0.66	0.71	0.73	0.74	0.70	0.72	0.73	0.72	0.67	0.74	0.66	0.62	0.59	0.71	0.71	0.71	0.67	0.71	0.65	0.80	0.70	1.00				
32	0.63	0.66	0.64	0.69	0.64	0.69	0.70	0.48	0.47	0.59	0.64	0.66	0.74	0.69	0.69	0.66	0.68	0.67	0.65	0.63	0.67	0.54	0.68	0.65	0.65	0.64	0.71	0.61	0.72	0.66	0.80	100			
33	0.66	0.70	0.65	0.63	0.69	0.74	0.65	0.50	0.54	0.73	0.67	0.63	0.73	0.71	0.68	0.68	0.70	0.72	0.70	0.69	0.61	0.60	0.64	0.70	0.68	0.65	0.69	0.72	0.71	0.64	0.73	0.66	1.00		
34	0.74	0.75	0.71	0.69	0.80	0.78	0.73	0.54	0.51	0.70	0.77	0.76	0.71	0.74	0.78	0.79	0.76	0.68	0.79	0.76	0.63	0.61	0.73	0.76	0.83	0.70	0.72	0.66	0.75	0.64	0.73	0.67	0.66	1.00	
35	0.71	0.72	0.74	0.66	0.74	0.78	0.68	0.56	0.53	0.70	0.73	0.72	0.70	0.77	0.76	0.71	0.75	0.69	0.74	0.76	0.68	0.63	0.71	0.75	0.76	0.73	0.72	0.68	0.74	0.64	0.71	0.72	0.67	0.85	1.00

The Jaccard's similarity coefficient obtained in this study ranged from 0.47 to 0.90. This result is in agreement with other results reported by Ramakrishnan *et al.* (2016) [12] reported Jaccard's similarity coefficient ranging from 0.011 to 0.836. Kelkar *et al.* (2017) [5] reported Jaccard's similarity coefficient ranging from 0.197 to 0.679. Shingane *et al.* (2018) [6] reported Jaccard's similarity coefficient ranging from 0.35 to 0.98.

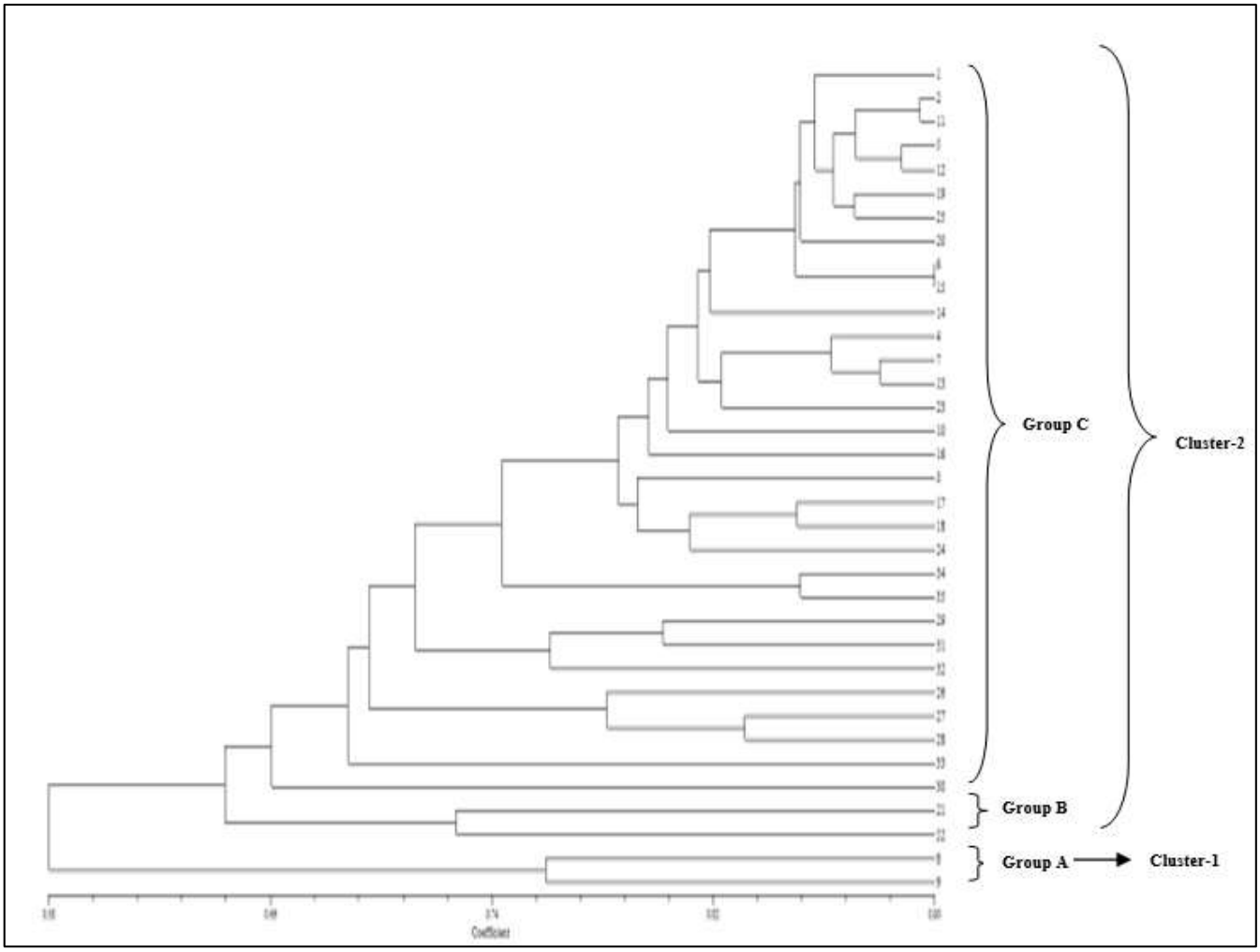


Fig 1: Dendrogram generated for 35 finger millet genotypes using UPGMA cluster analysis based on Jaccard's similarity coefficient using ISSR marker.

A dendrogram was constructed by using the UPGMA cluster in Fig. 1 showed two main distinct clusters of finger millet genotypes, which were also genetically diverse amongst themselves. The genetic coefficient of similarity among the genotypes ranged from 0.58 to 0.90. Cluster-1 comprised of only two genotype, KOPN-235 and Dapoli-2. While, Cluster-2 was the largest and it included 33 genotypes of finger millet with similarity coefficients ranging approx. 0.66 to 0.90. Further zooming in for simplify the comparative study, and found that the Cluster-2 divided into 2 groups. Group-B includes 2 genotypes of finger millet *viz*., KMR-204 and KMR-340. Group-C includes 31 genotypes *viz*., GN-3, GNN-6, GN-1, RAU-8, Chhattisgarh Ragi-2, GN-5, GN-4, GN-2, GN-8, GNN-7, OEB-532, GPU-45, GPU-28, VL-149, GPU-66, KOPN-942, KMR-630, VR-847, Dapoli-1, VL-324, VR-936, PR-202, VL-314, MR-6, Indira Ragi-1, GPU-67, VR-708, VL-376, Phule Nachni, VL-315 and VL-352 among PR-202 and VL-314 are more related kind of genotypes.

The association amongst different genotype was presented in the form of dendrogram prepared using rescaled distances. The resemblances coefficient between the two genotypes is the value at which their branches join. The dendrogram also showed the relative magnitude of resemblance among different clusters.

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