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Molecular characterization of diploid and triploid banana germplasms through DNA fingerprinting using ISSR markers

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Abstract
Domesticated bananas and plantains are mainly triploids (AAA, AAB, ABB genomes) and develop fruit by vegetative parthenocarpy. Collection, conversation, characterization of banana germplasm is one of the most important breeding approach**.** Molecular characterization of germplasm using DNA marker is always reliable because it does not get influenced by external environmental factors. A total of 191 germplasm accessions were screened using 10 ISSR markers, a total of 37 polymorphic bands were obtained with an average of 3.7 amplified bands per primer. The average PIC of the germplasm accessions was 0.3751 and its ranges from 0.1403 (IS-13) to 0.4752 (UBC- 811). The percent polymorphism was observed the highest in IS-13 (83.33%) and the lowest was found in UBC 811 (40%). Dendrogram analysis grouped this 191 genotypes into three major clusters and several subclusters. The major cluster I consists of 5 genotypes, cluster II includes 118 genotypes and cluster III consists of 69 genotypes. The genotypes which presented higher similarity are allocated under same the cluster. Here in the present study the genotypes Dakshin sagar (ABB) and Bhimkol (BB), BCB-1(ABB), Bhimkol(BB), M.B. Sawai (BB), H 96/7 (AB) are genetically dissimilar and have wide genetic variation. Thus, they can be exploited for further banana improvement programme.

Keywords: banana, DNA finger printing, ISSR, diversity, genetic dissimilarity

1. Introduction
Banana belongs to the family Musaceace of order Zingeberales and is one of the most widely grown fruit crop in the world. India is one amongst top producers of banana in the world with the production of 30.80 Lakh MT from an area of 8.8 Lakh Ha (Horticultural statistics at a Glance, 2018). The vast majority of the cultivated bananas are diploids (2n=2x=22), triploids (2n=3x=33) or tetraploids (2n=2x=44) which evolved from intra and inter specific crosses between two diploids wild species (*Musa acuminata colla*. and *M. balbisiana colla.)* in the section *Eumusa* of the genus *Musa* (Simmonds, 1955) [24] which contributes the A and B genomes respectively. The taxonomic score card designed by Simmonds and Shepherd based on 15 diagonistic morphological characters was used to classify genomic groups of banana cultivars (Simmonds, 1955) [24]. However this genomic identification by morphological characters was subjective and generated different results between researchers (Gusmiati *et al.* 2018; Probojati *et al.* 2019) [8, 18]. Southeast Asia is the centre of origin of banana (Valmayor *et al.*, 2000) [26] but cultivation has spread throughout the tropics and sub tropics of the world. There are over 1000 cultivars or land races of banana known worldwide (Babu *et al*., 2018) [1]. As many synonyms exist for each clone but named differently in various regions. Therefore, the molecular characterisation is necessary to produce viable results and to identify the clone unambiguously.
Knowledge on genetic diversity is essential for efficient breeding programs (Venkatachalam *et al.*, 2008) [27]. Characterization of existing or available germplasm is important event for further development of new cultivar. Therefore, characterization of germplasm in phenotypic as well as molecular level is important. However phenotypic traits is influenced by genotype of the plant as well as environment. Molecular characterization of germplasm using DNA marker is always reliable because the external environmental factors does not have any influence on DNA. Therefore, the molecular approach is needed to create a valid result (Didik Wahyudi *et al*, 2020) [6]. Thus, molecular markers are therefore important tool to access this diversity (Chandra Das *et al.*, 2018) [4].
The inter simple sequence repeats (ISSR) is one of that DNA-based marker technique that has

been successfully used to determine the genetic variations in the germplasms of Musa L. (Chowdhury *et al*., 2014; Kumar *et al.*, 2017; Rahman *et al*., 2012) [5, 11, 19]. ISSR has an advantage over other molecular markers with its simplicity, fastness, reliability, low cost and most of all it does not require any prior sequence information (Huang and Sun, 2000; Sarma and Tanti, 2017) [9, 22]. Rout *et al.* (2009) [21] reported that inter simple sequence repeats (ISSR) markers were effective at demonstrating the genetic variability among banana cultivars. Mattos *et al.* (2010) [13], in a study with SSR markers in banana genotypes, identified four clusters of diploid and tetraploid banana trees. Lu Y *et al.* (2011) [12] found that it was possible to identify banana genotypes of clones from China using ISSR markers. Kiran *et al* (2015) [10] observed genetic variability among banana cultivars originating in Odisha using molecular markers. ISSR is proven to produce more polymorphism data than RAPD (Poerba *et al.* 2019) [15]. Furthermore, ISSR is widely used for assessing genetic diversity and genome clustering of bananas (Chandra Das *et al.* 2018; Babu *et al.* 2018) [4, 1]. ISSR technique is considered as a very simple, fast, cost-effective, highly discriminative and reliable technique for genetic diversity analysis (Pradeep *et al.*, 2002) [17]. Hence in the present study, ISSR marker technique is used to investigate genetic variability among diploid and triploid banana genotypes.

2. Materials and Method

2.1. Plant materials

The study was conducted to evaluate the genetic diversity of diploid and triploid banana germplasms/genotypes. The banana germplasm was maintained at college orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The diploid banana genotypes include 42 genotypes *viz.*, 18 genotypes of banana have AA genome, 17 genotypes have AB genome and 7 genotypes have BB genome. Triploid genotypes include 149 genotypes *viz.*, 21 genotypes have AAA genome, 61 genotypes have AAB genome and 67 genotypes have ABB genome. A total of 191 genotypes (Table.1) were screened for polymorphism through 10 ISSR markers (Table.2).

Table 1: List of germplasm accessions utilized for the study

S.no	Genotypes	Genome	S.no	Genotypes	Genome
1	Wather	AAA	61	Kerela	AAB
2	Gros Michel	AAA	62	Poovan	AAB
3	Williams	AAA	63	Njali poovan	AAB
4	Robusta	AAA	64	Mysore ethen	AAB
5	Red Banana	AAA	65	Sandhana vazhai	AAB
6	Green Red	AAA	66	Pisang Nangka	AAB
7	Agneeswar	AAA	67	Quintal Nendran	AAB
8	Chakrakleli	AAA	68	Rasthali	AAB
9	Naka Tembi	AAA	69	Jwaribale	AAB
10	YKM-5	AAA	70	Manik chmpa	AAB
11	Thella Chakrakleli	AAA	71	Malbhog	AAB
12	Dwarf cavendish	AAA	72	Kathubale	AAB
13	Manoranjitham(Flav)	AAA	73	BRS-2	AAB
14	Manoranjitham(Flav)	AAA	74	BRS-1	AAB
15	Grand Naine	AAA	75	Singan	AAB
16	There kanchi	AAA	76	Attu Nendran	AAB
17	Red Banana (mut)	AAA	77	Big-E-Banga	AAB
18	Raja vazhai	AAA	78	Manjeri Naveen	AAB
19	EC-3	AAA	79	Swarnamukhi	AAB
20	Lacatan	AAA	80	TMB	AAB
21	Gandevi sln	AAA	81	Rasthali sln	AAB
22	Nattu poovan	AAB	82	Martaman	AAB
23	Motta poovan	AAB	83	Senna chenkadali	AA
24	Rasthali	AAB	84	Anai komban	AA
25	Ayiranga Rasthali	AAB	85	Amala kadali	AA
26	Suganthi	AAB	86	Erachi vazhai	AA
27	Virupakshi	AAB	87	Pisang lilin	AA
28	Hill Banana	AAB	88	Pisang jaribuaya	AA
29	Krishna vazhai	AAB	89	Pisang mas	AA
30	Lady finger	AAB	90	Rose	AA
31	Sirumalai	AAB	91	Tongat	AA
32	Kullan	AAB	92	Nivedhya kadali	AA
33	Vannan	AAB	93	Sikuzani	AA
34	Ladan	AAB	94	Sannachen kadali (R)	AA
35	Kali poovan	AAB	95	Hatidat	AA
36	Kallar ladan	AAB	96	Matti	AA
37	Pacha nadan	AAB	97	Namarai	AA
38	Padathi	AAB	98	Chemmatti	AA
39	Malai Kali	AAB	99	Mayannan	AA
40	Kali Bow	AAB	100	Kariyannan	AA
41	Kali	AAB	101	Then kunnan	AB
42	Kali Bale	AAB	102	Thatilla kunnan	AB

43	Co 1	AAB	103	Veneetu kunnan	AB
44	Uthiran J	AAB	104	Adukka kunnan	AB
45	Uthiran E	AAB	105	Valliya Kunnan	AB
46	Cheena Bale	AAB	106	Kunnan	AB
47	Thiruvannathapuram	AAB	107	Kappu Kadali	AB
48	Chinali	AAB	108	Bile Kadali	AB
49	Padali moongil	AAB	109	Poom Kadali	AB
50	Kalibow (R)	AAB	110	Rasa Kadali	AB
51	Kali(R)	AAB	111	Pey Kadali	AB
52	Nendran	AAB	112	Ney Kadali	AB
53	Myndoli	AAB	113	Kadali	AB
54	Nendra kunnan	AAB	114	Ney poovan	AB
55	Nendran padathi	AAB	115	Putta bale	AB
56	Nedu nendran	AAB	116	Adukkan	AB
57	Zanziber	AAB	117	KNR Mutant	ABB
58	Mac vazhai	AAB	118	Bhoodi bale	ABB
59	Mysore ethen	AAB	119	Kanchi Kela	ABB
60	Malai ethen	AAB	120	Chakkiya	ABB
121	Ocataman	ABB	157	Poocha Kunnan	ABB
122	Bagner	ABB	158	Govakar	ABB
123	Bargrner	ABB	159	Gouria	ABB
124	Dakshin sagar	ABB	160	Jurmony	ABB
125	Burharia	ABB	161	Karpooravali	ABB
126	Monthan	ABB	162	Karpooravali (M)	ABB
127	Pacha Montha	ABB	163	H.201 x Pey kunnan	ABB
128	Pidi Monthan	ABB	164	Pey kunnan	ABB
129	Lambi	ABB	165	Boodi	ABB
130	Nalla Bonthan	ABB	166	Saba	ABB
131	Kari Bale	ABB	167	Singan	ABB
132	Nattu Vazhai	ABB	168	Peyan	ABB
133	Nattu Peyan	ABB	169	Mannan	ABB
134	Booditha Bontha Batheesa	ABB	170	Kombilla vazhai	ABB
135	Bankel	ABB	171	Ney vannan	ABB
136	EPX	ABB	172	Chakkia	ABB
137	Pacha Bonthan Batheesa	ABB	173	Udhyam	ABB
138	Ashy Batheesa	ABB	174	Mutheli	ABB
139	Alshy	ABB	175	Burre chemsa	ABB
140	Chengali Kondan	ABB	176	Seema	ABB
141	Chirapunji	ABB	177	BCB-1	ABB
142	Bangrier	ABB	178	BCB-2	ABB
143	Bibutia	ABB	179	BCB-3	ABB
144	Lakhandi	ABB	180	Kovvur Bontha Batheesa	ABB
145	Kanthali	ABB	181	NRCB-8	ABB
146	Onkamannan	ABB	182	Popuolu	ABB
147	Kothiah	ABB	183	Klu numkhom	ABB
149	Dudh munga	ABB	184	Athiakol	BB
150	Beula	ABB	185	Bhimkol	BB
151	Basia	ABB	186	M.B.Bhimkol	BB
152	Barsain	ABB	187	M.B.Sawai	BB
153	Bhurkel	ABB	188	Assam	BB
154	Singalal	ABB	189	H 96/7	AB
155	Sambarani	ABB	190	Elavazhai	BB
156	Buthibale	ABB	191	Culcatta-4	BB

Table 2: List of ISSR primers used for screening

S.no	Primer name	Sequence 5' to 3'
1	UBC 810	5' GAGAGAGAGAGAGAGAT3'
2	UBC 807	5' AGAGAGAGAGAGAGAGT3'
3	UBC 812	5' GAGAGAGAGAGAGAGAA3'
4	UBC 836	5'AGAGAGAGAGAGAGAGYA3'
5	UBC 811	5' GAGAGAGAGAGAGAGAC3'
6	UBC 850	5'GTGTGTGTGTGTGTGTYC3'
7	UBC 840	5' GAGAGAGAGAGAGAGAYT3'
8	UBC 842	5' GAGAGAGAGAGAGAGAYG3'
9	UBC 808	5' AGAGAGAGAGAGAGAGC3'
10	IS 13	5'ATCATCATCATCATCATCC3'

2.2. Genomic DNA isolation and quantification

Fresh leaf samples were collected from plants under ice cold condition and used for extraction of DNA. Isolation of DNA was done following modified cetyl trimethyl ammonium bromide CTAB method (Doyle, 1990) [7] with little modification by adding MCP (1%) and PVP (0.2%) to extraction buffer in order to remove phenolics. The DNA was quantified spectrophotometrically by taking the absorbance at 260 nm by using Nano Drop™ 2000/2000c spectrophotometer. To check the quality of the extracted genomic DNA, gel electrophoresis was carried out on 0.8 per cent agarose gel. For PCR reaction DNA concentration have been diluted with 50 ng per µl.

2.3. PCR amplification using ISSR primers

PCR was performed at initial denaturation at 94 ^{0}C for 5 min followed by 40 cycles of 1 min denaturation at 94 ^{0}C, 1 min at annealing temperature (varies with primers) and 1 min extension at 72 ^{0}C with a final extension of 72 ^{0}C for 15 min using a thermal cycler (Eppendorf Thermal cycler). The PCR reaction volume of 15 µl containing 2 µl working DNA sample, 2 µl primer, 8 µl master mix, 3 µl sterile water. The PCR products after amplification were electrophoresed through 1.5% weight/volume (w/v) agarose gel to achieve adequate separation of the DNA bands for easy scoring. The amplified bands are visualized under UV gel documentation unit. The 100 bp DNA ladder was used as a size marker.

2.4. Scoring and data analysis

ISSR have only two scoring state ('1', present and '0', absent) can be distinguished at each band position. Each band position corresponds to a locus with two alleles, presence and absence of the band, respectively (Chandra Das *et al*., 2018) [4]. Polymorphism was scored as presence or absence from distinct reproducible ISSR bands obtained from all 10 primers across all the 191 genotypes. The average polymorphism information content (PIC) were calculated by applying the formulas given by Powell *et al.*, (1996) [16] and Smith *et al.*, (1997) [25]. The binary data matrices were entered into the Darwin 6.0 software program. Dendrogram that summaries the clustering process. On the basis of this analysis, the populations were grouped into three clusters. Similarity matrices and Dendrogram were constructed based on diversity coefficients generated for individual primers and also for pooled data by using UPGMA methods of Darwin 6.0.

3. Results and Discussions

Compared with other molecular markers, ISSR can reveal high polymorphism, which is helpful in distinguishing individual at inter-and/or intra-species level. Moreover, ISSR has its specific advantage over other markers as follows: no prior sequence information, simple operation, high stability and low cost. Therefore, ISSR has been proposed as a more economical and reliable DNA marker system (Bornet *et al.*, 2001) [3]

3.1. ISSR profiles

In the present study out of ten primers, viz. UBC 810, UBC 807, UBC 812, UBC 836, UBC 811, UBC 850, UBC 840, UBC 842, UBC 808, IS-13 showed polymorphism for 191 Banana cultivars. In total, 37 scorable bands were produced in 191 cultivars with 10 ISSR primers. This data was utilized for further computations and analysis. ISSR is proven to produce more polymorphism data than RAPD (Poerba *et al.* 2019) [15]. Furthermore, ISSR is widely used for assessing genetic diversity and genome clustering of bananas (Chandra Das *et al.* 2018; Babu *et al.* 2018) [4, 1].

Table 3: Polymorphic banding pattern generated by 10 ISSR primers for 191 genotypes

S.no	Primer name	Band range (bp)	Total number of bands	Number of polymorphic bands	Percent polymorphism	PIC value
1	UBC 810	500-150	8	6	75.00%	0.2294
2	UBC 807	600-200	6	4	66.66%	0.2671
3	UBC 812	400-180	6	3	50.00%	0.3026
4	UBC 836	600-150	5	3	60.00%	0.2018
5	UBC 811	480-200	5	2	40.00%	0.4752
6	UBC 850	700-280	5	3	60.00%	0.3697
7	UBC 840	500-180	5	4	80.00%	0.3927
8	UBC 842	550-150	6	3	50.00%	0.4562
9	UBC 808	480-200	6	4	66.66%	0.3156
10	IS 13	1100-400	6	5	83.33%	0.1403
		Total	58	37	551.65	3.1506
		Mean	5.8	3.7	55.165	0.3151

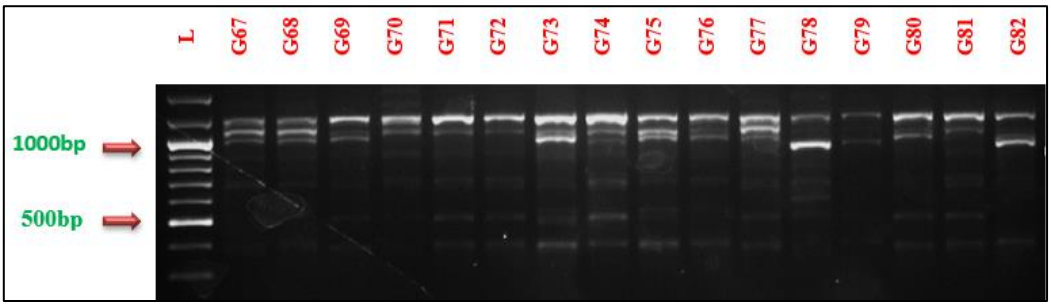

Fig 1: Banding pattern of IS13 ISSR Primer

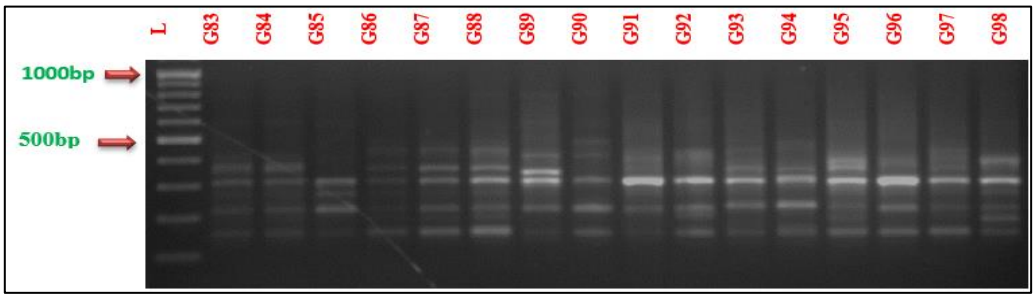

Fig 2: Banding pattern of UBC810 ISSR Primer

Among 10 primers screened the highest bands was obtained in IS-13 (1100 bp) (Fig.1) totally it produced 6 bands of which 5 were polymorphic (Table.3). The band size ranges from 400-1100 bp (Table.3). The lowest band size was observed in UBC 810 (fig.2) and UBC 842 (150 bp). The percent polymorphism value ranges from 40% (UBC 811) to 83.33% (IS-13). Lu Y *et al* (2011) [12] identified a high percentage of polymorphism 65.1% using ISSR markers. Silva *et al*., (2016) analysed the genetic diversity of 21 banana genotypes using 12 ISSR primers where they observed 97.5% polymorphism. All the 10 primers are effective in bringing out difference among 191 germplasm accessions by scoring the bands as presence '1' and absence '0'. The size of DNA bands ranges from 150-1100 bp. The average amplified band per primer was 3.7. This result almost similar to the results obtained by Babu *et al*. (2018) [1] where they evaluated genetic diversity of eight banana cultivars using 20 primers, the mean number of polymorphic ISSR bands was 3.5 per primer. PIC value for the marker ranges from 0.1403 in IS-13 to 0.4752 in UBC 811.The average PIC value of the 10 ISSR markers was 0.3151(Table.3).

3.2. Dendrogram analysis

Most of the genotypes were allocated in a main cluster with several subclusters. Genetic similarity and distances was computed considering all the genotypes from the pooled data set and the dendrogram was constructed. Ten primers were used to study the genetic relativity of 191 genotypes banana. Dendrogram was constructed using Darwin 6.0 version. On the basis of the analysis, the germplasm accessions were grouped into three major clusters figure.3.

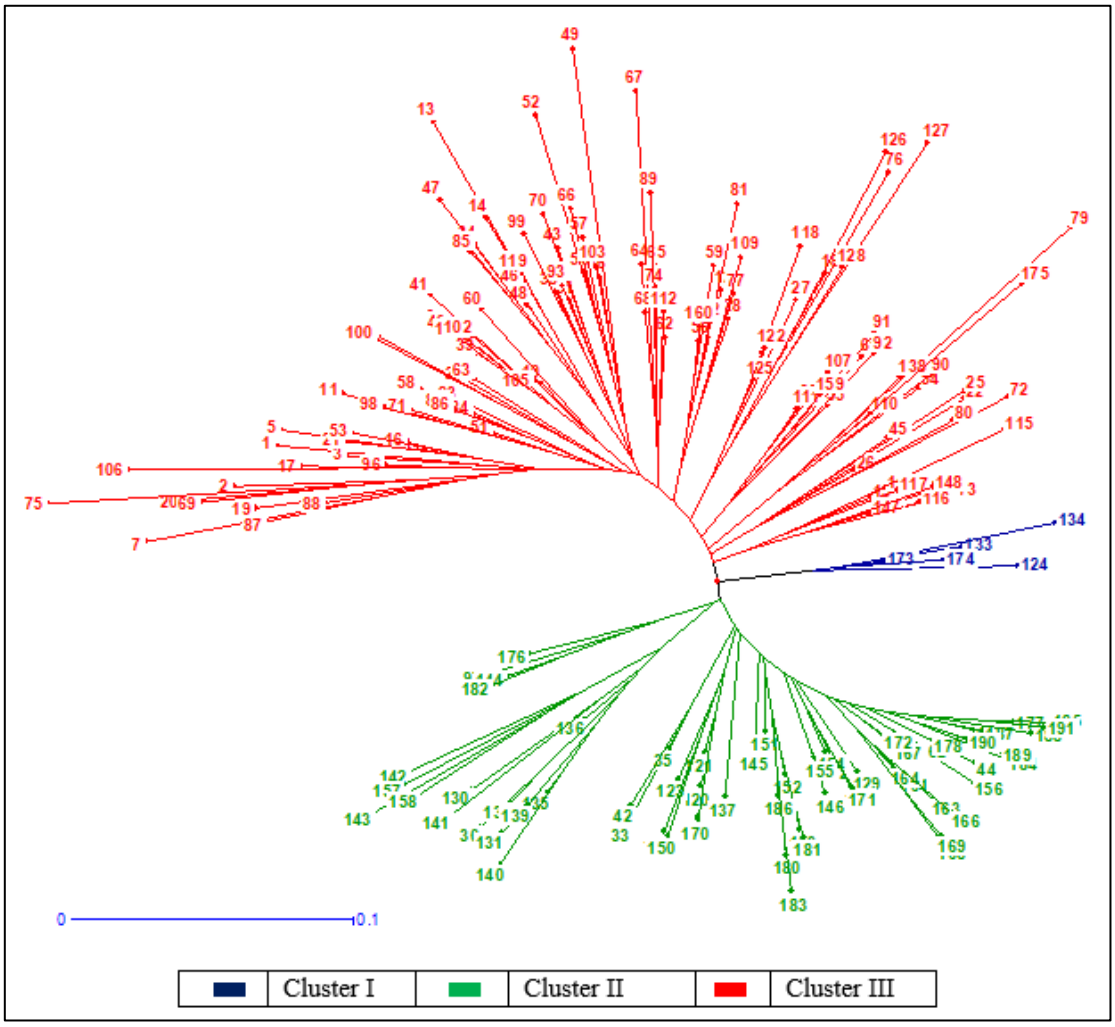

Fig 3: Cluster diagram generated by Darwin 6.0 for 191 genotypes

The first major clusters consists of the following 5 genotypes viz., Dakshin sagar (ABB), Nattu peyan (ABB), Booditha Bontha Batheesa (ABB), Udhayam (ABB) and Mutheli (ABB).

The second major clusters consists of 118 genotypes viz., Wather, Gros michel, Williams, Robusta, Red banana, Green Red, Agneeswar, chakrakkeli, Naka Tembi, YKM -5, Thella chakarakeili, Dwarf cavendish, Manoranjitham (Flav), Manoramjitham, Grand Naine, There kanchi, Red banana (Mut), Raja vazhai, EC-3, Lacatan, Gandevi selection, Nattu poovan, Motta poovan, Rasthali, Ayiranga Rasthali, suganthi, Virupakshi, Hill banana, Krishna vazhai, Sirumalai, Ladan, Kallar ladan, Pacha nadan, Padathi, Malai kali, Kali bale, Kali bow, Kali, CO1, Uthiran E, Cheena bale, Thiruvannathapuram, chinali, Padali moongil, Kalibow (R), Kali (R), Nendran, Myndoli, Nendra kunnan, Nendra padathi, Nedu nendran, Zanziber, Mac vazhai, Mysore ethen, Mysore ethen, Malai ethen, kerela, Poovan, Njali poovan, Mysore ethen, Santhana vazhai, Pisang nangha, Quintal Nendran, Rasthali, Jwaribale, Manick champa, Malbhog, Kathubale, BRS-2, BRS-1, Singan, Attu Nendran, Big-E-Banger, Manjeri Naveen, Swarnamukhi, TMB, Rasthali selection, Martaman, Sanna chen kadali(a), Anai komban, Ambala kadhali, Erachi vazhai, Pisang lilin, Pisang Jaribuaya, Pisang Mas, Rose, Tongat, Nivedhya kadali, Sikuzani, Sanna chen kadali (R), Hatitat, Matti, Chematti, Mayannan, Kariyannan, Then kunnan, Thattila kunnan, Vaneetu kunnan, Adukka kunnan, Valiya kunnan, Kunnan, Kappu Kadali, Poom kadali, Rasa kadali, Pey Kadali, Ney kadali, kadali, Ney poovan, Putta bale, Adukkan, KNR mutant, Bhoodi bale, Kanchi kela, Burharia, Monthan, Pacha Monthan, Pidi monthan, Alshy Batheesa, Kothiah, Muthiah, Gouria, Jurmony, Burre chemsa.

The third major clusters consists of 69 genotypes viz., Lady finger, Kullan, Vannan, kali poovan, Kali bale, Uthiran J, Namarai, Bile kadali, chakkiya, Octaman, Bagner, Bargner, Lambi, Nalla Bonthan, Bankel, EPX, Pacha Bontha Batheesa, Alsly, chengali kondan, Chirapunji, Bangrier, Bithutia, Lakhadi, Onkamannan, Urkhandi, Dudh munga, Beula, Basia, Bhurkel, Sambarani, Buthibale, Poocha kunnan, Govakar, Karpooravali, karpooravli (mut),H201 x Pey kunnan, Boodi, Saba, Singan, Peyan, Mannan, Kombila vazhai, Ney vannan, chakkia, Seena, BCB-1, BCB-2, BCB-3, Kovur Bontha Batheesa, NRCB-8, Populu, Klu Numkhom, Athiakol, Bhimkol, M,B.Bhimkol, M.B. Sawai,Assam, H96/7, Elavazhai,culcatta-4.

The present data supported with the result obtained by Babu *et al.,* (2018) [1] where they evaluated 8 banana cultivars using 20 ISSR primers and constructed a dendrogram using NTSY spc programme. Here the population were grouped into three major clusters which includes both diploid and triploids under similar group. Poerba *et al.* (2010) [14] assessed the molecular diversity of 36 accessions of plantain and cooking banana using both RAPD and ISSR. In the cluster analysis of the combined data of both RAPD and ISSR. The genotypes were separated into two distinct clusters. Ravishanker *et al.* (2017) [20] studied diversity among 22 AAB Indian banana cultivars along with AAB, AA, BB types using 16 ISSR markers. Based on the cluster analysis the genotypes were divided into two major cluster genotypes. Borborah *et al.* (2020) [2] used RADP marker to evaluate genetic diversity between 27 banana cultivars and the dendrogram separated the population into two main clusters and each main cluster have several subclusters.

The genotypes presented higher similarity are allocated under same cluster. Here in the present study the genotypes Dakshin sagar (ABB) and Bhimkol (BB), BCB-1, Bhimkol (BB), M.B. Sawai (BB) and H 96/7(AB) are genetically dissimilar and have wide genetic variation. Thus, they can be exploited for further banana improvement programme.

4. Conclusions

ISSR based DNA finger printing is a more reliable technique to determine the genetic diversity and relationship among the germplasm accessions. This study would facilitate the use of ISSR fingerprints for selecting valuable cultivar for effective utilization of banana genotypes among the accessions for further banana improvement programme. Among 10 ISSR primers representing tri, tetra, penta and other repeats. A total of 58 scorable bands were obtained out of which 37 bands shows polymorphism and giving an average of 3.7 bands amplified per primer. The primers used produces specific bands for each cultivar. By exploiting the most divergent genotypes can assist in developing a new cultivar.

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