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Evaluation Of Rapd Analysis To Differentiate The Genotypes Of 11 Musa Cultivars

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Abstract: *Evaluation of RAPD technique in differentiating the genotypes of 11 Musa cultivars. RAPD and UPGMA analysis was carried out to differentiate the genotypes of 11 Musa cultivars. Among the 08 primers, OPC-06 demonstrated highest number of amplicons while OPA-11displayed the least amplicons. The dendrogram OPR-08 displayed three clusters and one outlier that differentiated 11 Musa cultivars into 4 genotypes (AAA, AAB, ABB and AB). Among the primers employed in the study, OPR-08 was found to be efficient in differentiating the genotypes of 11 Musa cultivars. The present study recommends further investigation for the validation of OPR-08 primer in the differentiation of the Musa cultivar genotypes.*

Keywords: *Musa cultivars, Banana, RAPD, Phylogenetic analysis.*

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I. Introduction

The genus *Musa* is of great significance globally with respect to its nutritive value and commercial varieties cultivated. The diversity of the *Musa* is well studied through morphological data, with precise characteristics correlating variety of indicators of the genome organization[1]. The genetic and diversity of banana is extensively analyzed by DNA marker technologies for taxonomy, cultivar true-to-type assessment and the development of genetic linkage map[1].

In tropical and subtropical countries, the bananas (*Musa* spp.) are the main staple food for rural and urban consumers[2]. Banana is the rich source of carbohydrates and vitamins, chiefly vitamin B; it is also an excellent source of potassium, phosphorus, calcium and magnesium[3].The present day cultivars have evolved, by the hybridization of the two main species of the banana that is *M. acuminate* and *M. balbisiana,* the main contributors of A and B genomes respectively[4]. Bananas cultivated for commercial purposes are seed sterile diploid, triploid or tetraploid clones which are categorized into various genome groups as AA, BB, AB, AAA, AAB, ABB, AAAA, AAAB, AABB and ABBB based on system formed by Simmonds and Shepherd, 1955[5]. This system allocates 1 to 5 for 15 chosen morphological features that distinguish *M. acuminate* and *M. balbisiana.*

Wide variety of B-rich genomes is prevailing in India (AB, AAB, ABB and ABBB) and huge diversity of the B genome (BB)[6]. Morphological and biochemical markers have demonstrated limitations; hence efforts are made to employ molecular markers for characterization of germplasm diversity. Molecular markers have clearly displayed a potential to identify the genetic diversity and also help in management of plant genetic resources[7, 8]. In comparison to morphological characters, molecular markers can divulge differences among genotypes at DNA level, giving a direct, reliable and efficient tool to characterize germplasm, conservation and management[9].

Currently there are many types of molecular markers available restriction fragment length polymorphism DNA (RFLP)[10,11] Radom amplified polymorphic DNA (RAPD)[12,13,14], amplified fragment length polymorphism (AFLP)[15,16], simple sequence repeats (SSRs)[17] and variable number tandem repeats (VNTRs)[18,19]. Among the molecular markers RAPD techniques has been employed extensively in characterization of various plant species[20]. RAPD has found to be advantageous as the technique is found to be simple, inexpensive, small quantity of sample DNA required and no prior knowledge of the genome and moderately reproducible. In this background, the study was designed with the following objectiveto evaluate the RAPD technique in differentiating the genotypes of the 11 *Musa* cultivars.

II. Materials And Methods

Sample collection: The 11 *Musa* cultivars used in this study were procured from different regions of Andhra Pradesh, representing *M. acuminate* and *M. acuminate* x *M. balbisiana.* All the collected plant materials were authenticated from the Department of Botany and Microbiology, Nagarjuna University, Guntur and

respective voucher numbers (ANUH2001 to ANUH2011) were obtained for the same. The list of *Musa* cultivars with their respective local name and ploidy is outlined in Table 1.

Table 1: Details of the 11 *Musa* cultivars employed in the study

Sl. No	Sample No.	Local Name	Ploidy
1	S1	Red Banana	AAA
2	S2	Grand Naine Banana	AAA
3	S3	Robusta Banana	AAA
4	S4	Tella Chakkarakeli Banana	AAA
5	S5	Nanjangud Rasbale Banana	AAB
6	S6	Cooking Banana	ABB
7	S7	Nendran Banana	AAB
8	S8	Udhayam Banana	ABB
9	S9	KarpuraValli Banana	ABB
10	S10	Monthan Banana	ABB
11	S11	Elakki Banana	AB

DNA extraction and purification: Fresh young leaves were harvested for DNA isolation, washed with water and ethanol to remove external contaminants. The DNA extraction was performed employing Aristogene kit (Catalogue number: ARK-11).

400mg of leaves were cut into small pieces and homogenized with dry ice using tissue homogenizer. Finely ground tissue was transferred into 30 ml centrifuge tube and 15ml of lysis buffer was added. The tubes were incubated at 65˚C for 1 h 30 min in water bath with intermittent mixing, followed by centrifugation at 10,000rpm for 10 min. The supernatant was carefully transferred into a fresh 30 ml centrifuge tube and equal volume of chloroform was added and mixed well. The tubes were centrifuged at 10,000 rpm for 15 min. Without disturbing the interface, aqueous layer was pipetted out into the fresh 30ml centrifuge tube. To the tube added equal volumes of isopropanol and $1/10^{th}$ volumes of 3M sodium acetate and mixed well. The tubes were incubated at room temperature for 5-10 min and later centrifuged at 10,000 rpm for 10-15 min. After discarding the supernatant, the pellet was washed with 1ml of 70% ethanol and air dried. Finally, the pellet was suspended in 400µl of 1X Tris- EDTA buffer. To remove inhibitors the DNA sample was further purified using column purification kit (Qiagen). The eluted DNA samples concentrations was determined by measuring A260nm using UV spectrophotometer (UV-1800 Shimadzu). The evaluation of purified DNA was done at A260/A280 ratio. 2µl of DNA sample with 10µl double distilled water and 2µl of DNA loading dye was electrophoretically checked on 0.8% agarose gel.

RAPD analysis: The RAPD analysis was carried out employing 9 primers as listed in Table 2. The RAPD amplification was performed using 40µl of PCR mixture which contained100ng of genomic DNA as template, 1 X PCR buffer, 100 µM each of dNTPs 1 U of *Taq* DNA polymerase and 10 p moles of RAPD primer. All the reagents used were from Aristogene Biosciences Pvt. Ltd. The PCR was performed at an initial denaturation at 94^{o}C for 5 min followed by 40 cycles of 30 s denaturation at 94^{o}C, 1 min annealing at 45^{o}C and 1.5 min extension at 72^{o}C with a final extension at 72^{o}C for 10 min using thermal cycler (PerkinElmer 2009).

Table 2: Primers employed for RAPD analysis

Primer	Sequence 5'-3'
OPA-02	TGCCGAGCTG
OPA-09	GGGTAACGCC
OPA-11	CAATCGCCGT
OPB-10	CTGCTGGGAC
OPC-06	GAACGGACTC
OPD-02	GGACCCAACC
OPF-02	GAGGATCCCT
OPH-05	AGTCGTCCCC
OPR-08	CCCGTTGCCT

Data analysis: The RAPD gel profiles for 8 primers which demonstrated amplification were analyzed using a bioinformatics tool PyElph 1.4 (Pavel and Vasile, 2012). Dendrograms were constructed for all the 8 RAPD profiles employingthe Unweighted Pair Group Method with Arithmetic mean (UPGMA) method for the differentiation of the 11 *Musa* cultivar genotypes.

III. Results

Among the 9 primers employed in the study, 8 primers responded to the DNA samples that demonstrated scorable banding patterns while 1 primer (OPB-10) did not amplify for all the 11 *Musa* cultivars. Fig. 1-8 represents the RAPD fingerprints of OPA-02, OPA-09, OPA-11, OPC-06, OPD-02, OPF-02, OPH-05 and OPR-08 respectively. The RAPD banding profile and percentage of polymorphism demonstrated by the primers involved in the study is represented in Table 3. Dendrogram constructed by UPGMA analysis (Fig. 9) for OPR-08 primer was found to be efficient in differentiating the genotypes of *Musa* cultivars when compared with other primers (data not shown).

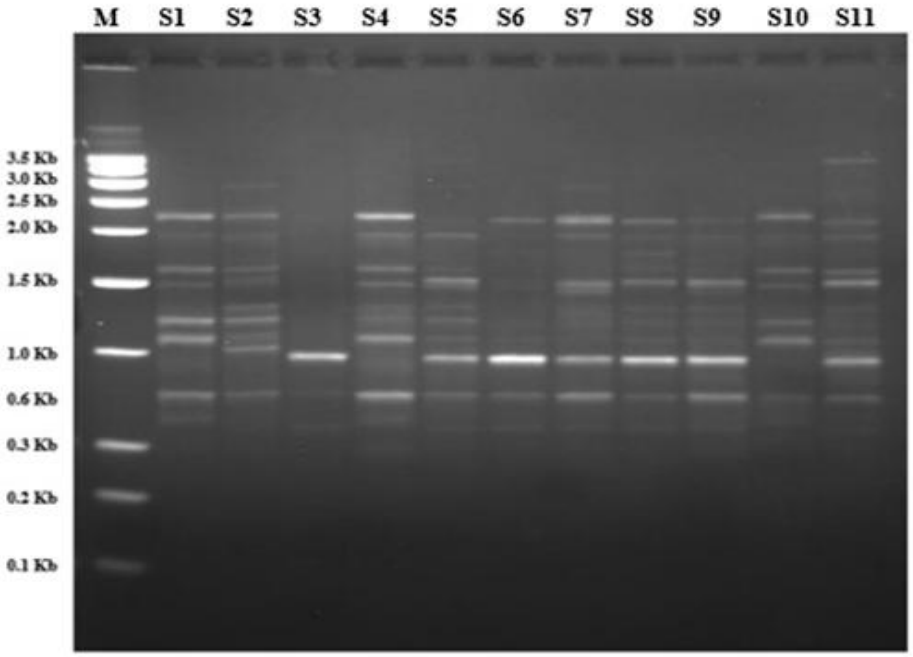

Figure 1: RAPD Fingerprints of 11 *Musa* varieties with OPA-02 primer

Figure 2: RAPD Fingerprints of 11 *Musa* varieties with OPA-09 primer

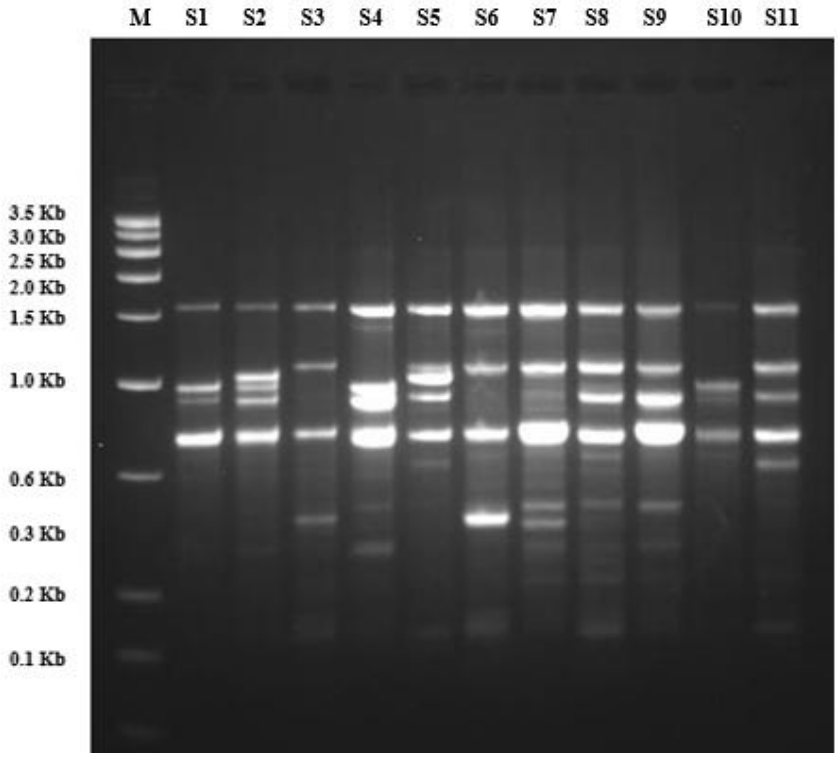

Figure 3: RAPD Fingerprints of 11 *Musa* varieties with OPA-11 primer

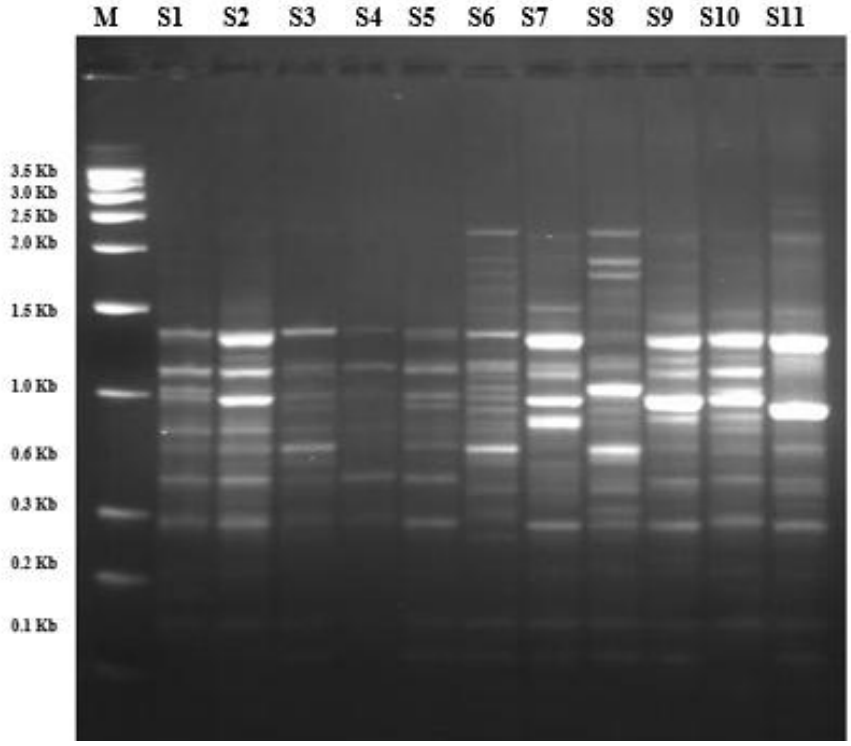

Figure 4: RAPD Fingerprints of 11 *Musa* varieties with OPC-06 primer

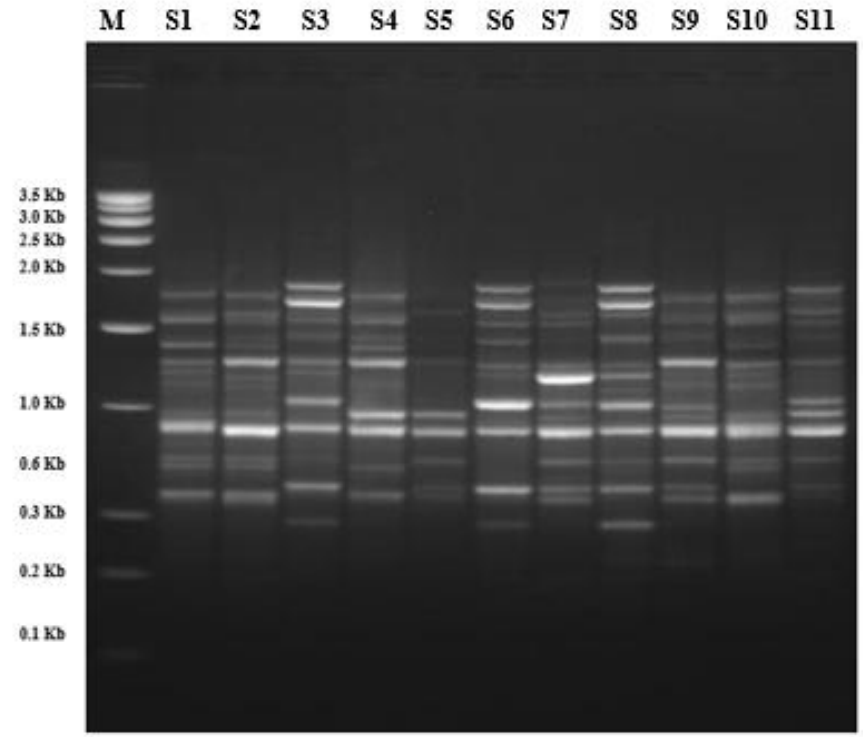

Figure 5: RAPD Fingerprints of 11 *Musa* varieties with OPD-02 primer

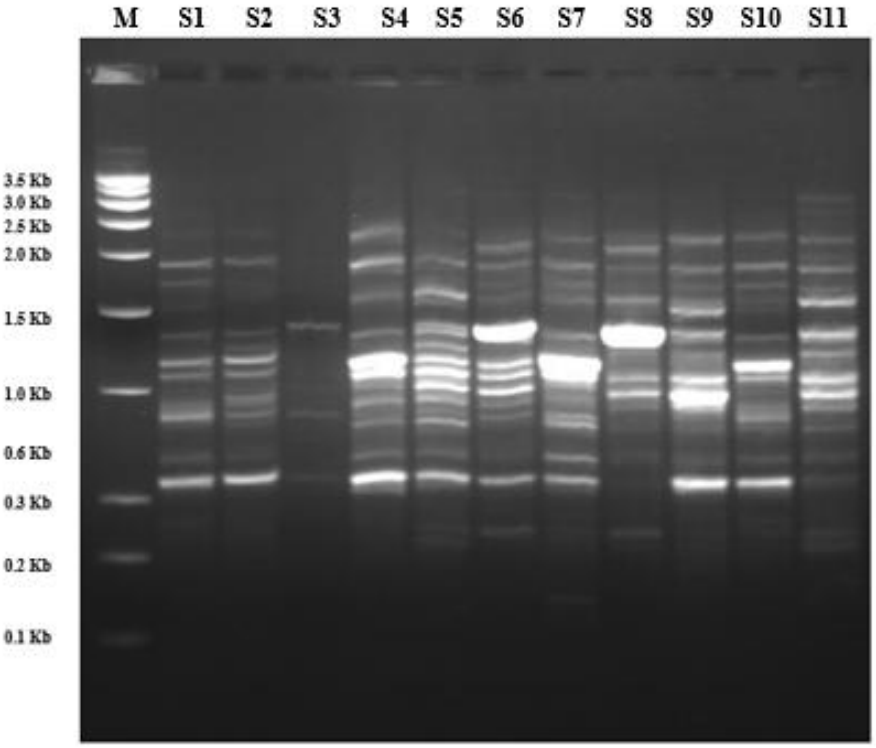

Figure 6: RAPD Fingerprints of 11 *Musa* varieties with OPF-02 primer

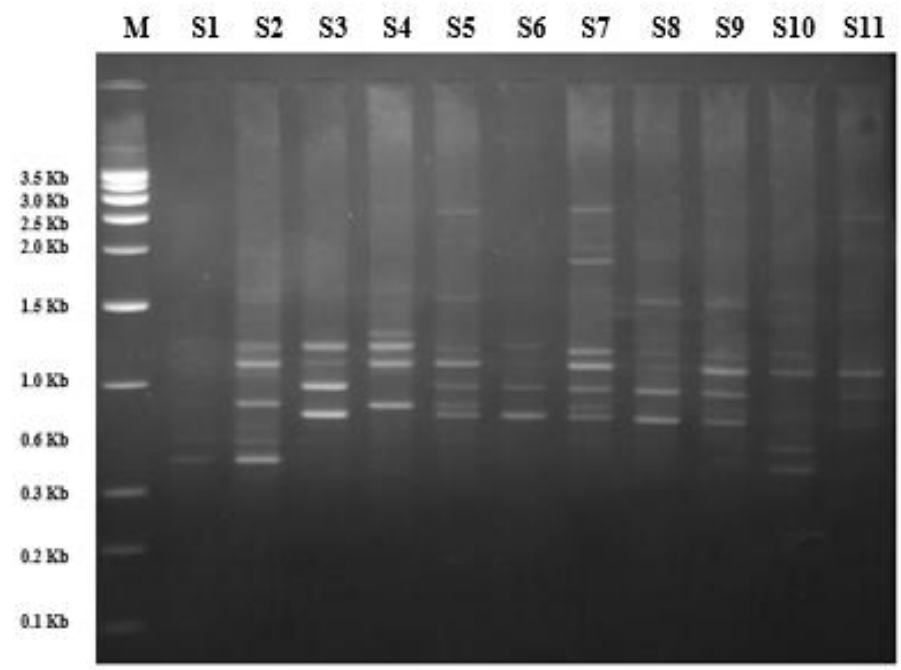

Figure 7: RAPD Fingerprints of 11 *Musa* varieties with OPH-05 primer

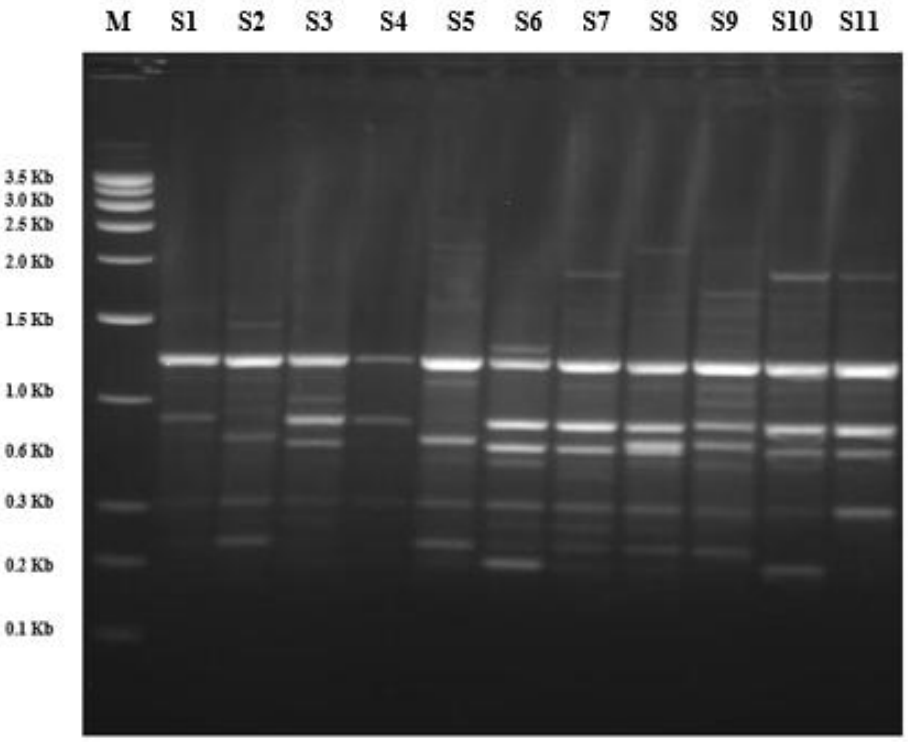

Figure 8: RAPD Fingerprints of 11 *Musa* varieties with OPR-08 primer

Table 3: RAPD banding profile and percentage of polymorphism displayed by the primers employed in the study

Primer	Total No. of bands	No. of polymorphic bands	No. of monomorphic bands	Polymorphic (%)
OPA-02	13	13	00	100
OPA-09	17	16	01	94.11
OPA-11	10	08	02	80
OPC-06	24	22	02	86.6
OPD-02	20	19	01	94.11
OPF-02	19	19	00	100
OPH-05	11	11	00	100
OPR-08	16	13	03	81.25

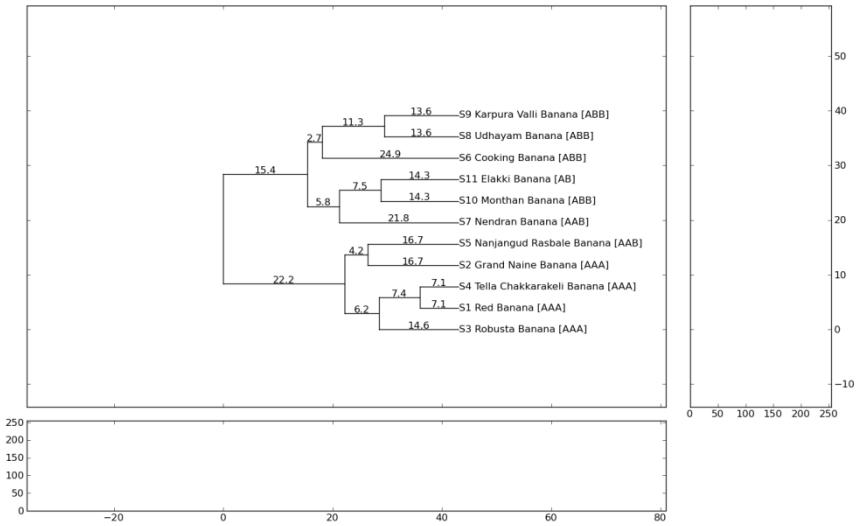

Figure 9: UPGMA derived dendrogram based on RAPD analysis by OPR-08 primer for 11 *Musa* cultivars

IV. Discussion

Previously, RAPD marker was investigated in *Musa* species for variety identification, genetic mapping among the clones, location of genes in conferring resistance to important diseases that affect the crop [21, 22.] The present study was designed to differentiate 11 *Musa* cultivar genotypes which are commonly cultivated in Andhra Pradesh employing RAPD marker. Among the 9 primers employed in the study amplification, 8 primers displayed scorable banding patterns except OPB-10 primer. The OPC-06 primer amplified highest number of amplicons (24) followed by OPD-02 (20) and OPF-02 (19) while OPA-11 produced least number of amplicons (10) [Table 3].The primers OPA-02, OPF-02 and OPH-05 displayed 100% polymorphism among the species while OPA-11 displayed least (80%). Among the 8 primers, OPR-08 displayed highest number of monomorphic bands (03).

Of the 8 UPGMA derived dendrograms employing different primers, OPR-08 dendrogram successfully differentiated the 11 *Musa* cultivars into 4 genotypes (AAA, AAB, ABB and AB). UPGMA analysis differentiated 11 *Musa* cultivars into two major clusters which were further sub-clustered. Similar results were also reported by Resmi *et al.*, revealing 4 major clusters which grouped cultivars of AB, AAB, AAA and ABB separately[23]. The phylogenetic analysis revealed the Red Banana (S1), Grand Naine Banana (S2), Robusta Banana (S3) and Tella Chakkarakeli Banana (S4) and Nanjangud Rasabale Banana(S5) are closely related varieties. Similarly, Cooking Banana (S6) and Nendran Banana (S7), Udhayam Banana(S8), Karpura Vali Banana (S9) and Monthan Banana (S10) were found to be closely related varieties.

V. Conclusion

In the present study OPR-08 primer was found to be efficient in differentiating the genotypes of 11 *Musa* cultivars that aids in understanding the gene flow among the banana cultivars. The study recommends further validation employing OPR-08 primer in differentiating the genotypes of *Musa* cultivars.

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