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Morphological and Molecular Characterization of Banana in Kerala, India

Research Article

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Abstract

Banana is one of the most important food crops all over the world. There are around 365 varieties of bananas available throughout the world. Banana is a traditional medicine for diabetes, cancer, diarrhea and also highly nutritional food crop. In this study, commonly used varieties of banana are taken for characterization by morphology and genotype which is based on International Plant Genetic Resources Institute, 1984 (Descriptors for banana (*Musa* sp.,) and RAPD analysis. Five varieties were morphologically similar in parameters such as leaf habit, pseudo stem appearance and peel color. RAPD analysis proved that these varieties of banana are closely related which coincides with the morphological characterization.

Keywords: RAPD analysis; Morphological characters; Genotype

Introduction

Banana is naturally packed with nutrients, fibers, protein and other compounds. Various parts of plantain tree are used for medicinal purpose and as a food product. Banana is a monocotyledon plant in the genus *Musa* (Musaceae). They are giant herbs, commonly up to 3m in height and their stem is not as strong as other trees. Morphologically, each variety is different from others in parameters such as leaf habit, male bud size, color pedicle size, pedicle position, fruit numbers, pulp color, mature peel color and immature peel color. Due to this variation, Musa has been taxonomically classified to 50 species. Most of the edible bananas are from derived from two wild species like *M. acuminata* (having AA genome) and *M. balbisian* (having BB genome) through inter and intra specific hybridization, resulting in the generation of many genome groups such as AA,AB,AAA,AAB,ABB,AABB,AAAB and ABBB [1]. It is considered least because of the widespread vegetative reproduction and natural occurrence of many hybrids varieties [2]. Climate and soil fertility influences the growth of banana.

Differentiating varieties of banana based on their morphology has some limitations in the accurate identification because limited traits are available for characterization [3]. Molecular markers are used for studies such as AFLP, RFLP, RAPD, ISSR, SSR, and SNP for accurate analysis. Random Amplified Polymorphic DNA (RAPD) technique is widely used for population study and genetic linkage because of their high polymorphic nature [4], frequent occurrence in the genome, easy access, fast process and easy exchange of data between laboratories.

RAPD molecular markers are widely used for variety of species identification for apple (*Malus* species L), grapes (*Vitis*) and Rice (*Oryza sativa* L). It is an efficient and inexpensive technique without requiring prior knowledge of the genome [5]. RAPD analysis depends on the selection of primers; when five different primers are used for the selection process, it is helpful to identify which primers contain high polymorphic activity which is provides useful amplification products.

Materials and Methods

Five banana fruits samples and leaf samples were collected

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from Palakkad. They were identified as S1: *Musa acuminate Colla* (AAA) (Chenkadhali/Red banana), S2: *Musa x paradisiacal* L. (AB) (Njalipoovan), S3: *Musa Pisang lilin* (AA) (Mezhuthirikalan), S4: *Musa accuminata Colla* (AA) (Kadali) and S5: *Musa x paradisiaca* L. (AAB) (Nendran). The samples were authenticated by Botanical Survey of India and Banana Research Centre, Kannara, Kerala (Figure 1).

Morphological characterization

The collected five varieties of banana were morphologically analyzed for their features based on the IPGRI 1984, descriptors of banana. Leaf habit, leaf colour, pseudo stem colour, size, pedicle size, color, appearance, male bud color, shape, fruit color, shape, size were observed and tabulated.

Molecular characterization of Banana

RAPD is a PCR based method to detect variation between individuals of species by selective amplification of some polymorphic sequences in their genomes. RAPDs are of much use to construct genetic map [6].

Isolation of DNA from banana

DNA was isolated from the leaf samples using NucleoSpin ® Plant II kit ((Macherey-Nagel) as per manufacturer's instruction. The eluted DNA was stored at 4 °C.

Agarose gel electrophoresis

Agarose gel electrophoresis was performed according to standard protocol (Molecular Cloning -CSHL Press) to check quality of the isolated DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE buffer containing 0.5 µg/ml EtBr. After electrophoresis the gel was viewed on a gel docking station and photographed.

Figure 1: (a) *Musa acuminate Colla* (AAB) (Chenkadhali/Red banana), (b) *Musa x paradisiaca* L. (AAB) (Nendran), (c) *Musa x paradisiaca* L. (AB) (Njalipoovan), (d) *Musa Pisang lilin* (AA) (Mezhuthirikalan), (e) *Musa accuminata Colla* (AA) (Kadali).

RAPD analysis

Restriction enzymes and primers were purchased from Medox Biotech, India. PCR amplification was performed in two stages. The pre-selective amplification was performed with an amplification profile of 95 °C for 5 minute, annealing at 42 °C for 1min, extension at 72 °C for 1.3 min, repeated for 34 cycles, then extend at 72 °C for 10 min. Further amplification was performed with a cycling profile of 94 °C for 0.45 min, 42 °C for 1.00 min, 72 °C for 1.30 min cycles followed by extension at 72 °C for 10 min and a cooling of 4 °C for 30 min. Electrophoresis of the PCR products was carried out on agarose gel (1.2%), by loading 10 µl of each DNA samples .The electrophoresis was run at 75 V for 3 hrs and viewed on a gel docking station and photographed (Table 1).

Data scoring

The standard of scoring was generated for most dominant DNA markers and only clear distinctive bands were scored using NTSYS software. Bands were recorded into binary symbols "1" for band presence and "0" for band absence using and phylogenetic tree was constructed using NTSYS software.

Results and Discussion

The five banana samples collected from palakkad district were morphologically characterized based on several parameters are tabulated in (Table 2). From this table, it can be seen that the morphological parameters of all the samples are similar up to 75%. This is confirmed by the phylogenetic tree which forms the first out group of *Musa x paradisiaca L.(AB) (Njalipoovan)* and *Musa x paradisiaca L.(AAB) (Nendran)* second out group of (Chenkadhali/ Red banana) *Musa accuminata Colla (AA)* (Kadali/Kali). *Musa Pisang lilin (AA)* (Mezhuthirikalan/ sudhari) is found common between these two groups [7] (Figure 2).

Table 1: Primers used for RAPD.

S.No.	Primer Name	Primer Sequence (5'-3')
1.	OPA-01	CAGGCCCTTC
2.	OPA-03	AGTCAGCCAC
3.	OPB-05	TGCGCCCTTC
4.	OPC-05	GATGACCGCC
5.	OPZ-10	CCGACAAACC

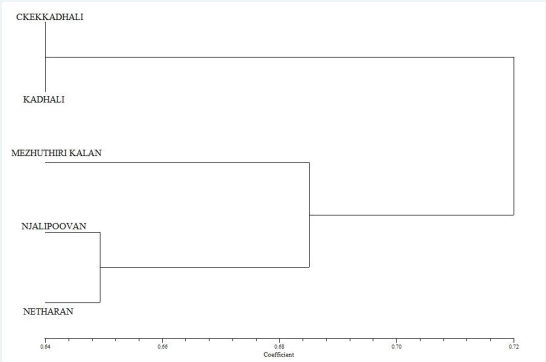

Figure 2: Phylogenetic relationship of morphological parameters of Musa samples.

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Table 2: Plant characteristics and properties of Musa varieties.

Samples	leaf habit	pseudo stem height	pseudo stem colour	petiole canal leaf	colour of leaf upper surface	peduncle length	peduncle colour	bunch shape	male bud type	male bud shape	male bud size	fruit shape	immature fruit peel colour	mature fruit peel colour	pulp colour before maturity	pulp colour at maturity	flesh texture	predo-minant taste
Musa acuminate Colla(AAB) (Chenkadhali/ Red banana)	Drooping	>3	Red	wide with Erect margins	Dark Green	>61 cm	Red	Asymmetric-bunch axis is nearly straight	Normal	Ovoid	21 to 30 cm	Straight in the distal part	Pink	Red-purple	Ivory	Yellow	Soft	Sweet and acidic
Musa accuminata Colla (AA) (Kadali).	Drooping	2.1 to 2.9	Dark Green	wide with Erect margins	Dark Green	31-60 cm	Green	Asymmetric-bunch axis is nearly straight	Normal	Like a top	>31 cm	Straight	Dark Green	grey spots	Ivory	Yellow	Firm	Sugary
Musa Pisang lilin (AA) (Mezhuthirikalan)	Erect	>3	Dark Green	wide with Erect margins	Green	>61 cm	Dark Green	Cylindrical	Normal	Lanceolate	<20 cm	Other (candle like shape)	Light Green	1	Ivory	Yellow	Firm	Slightly tasty
Musa x paradisiaca L.(AB) (Njalipoovan)	Erect	>3	Green	open with margins spreading	Green-yellow	<30 cm	Green	Cylindrical	Normal	Lanceolate	<20 cm	Straight	Green	Yellow	White	Yellow	Soft	Sweet
Musa x paradisiaca L.(AAB) (Nendran)	Inter mediate	>3	Green	wide with Erect margins	Dark Green	<30 cm	Dark Green	Csymmetric-bunch axis is nearly straight	normal	Inter mediate	<20 cm	Straight	Light Green	bright yellow	Orange	Orange	Firm	Slightly tasty

In order to further confirm the relationship between the samples, RAPD analysis was performed and the phylogenetic tree is shown in (Figure 3). This tree confirms the grouping of *Musa acuminate Colla(AAB) (Chenkadhali/Red banana) Musa accuminata Colla(AA) (Kadali)* and a close relationship between *Musa x paradisiaca L.(AB) (Njalipoovan)* and *Musa x paradisiaca L.(AAB)(Nendran)* (Figure 4 and 5).

From these results, it can be concluded that genotypic relationship between the samples also explicitly exhibited in their phenotype. It is well known that *Musa paradisiaca L.(Nendran)* is effective in curing breast cancer [8]. Since *Musa x paradisiaca L.(AB) (Njalipoovan)* shows close relation with *Musa x paradisiaca L.(AAB)(Nendran)*. Both morphologically and genotypically it is expected that this variety also has an anticancer potential (Figure 6). Further studies are needed to confirm the gene expression pattern in the collected varieties which can be used for pharmaceutical application [9].

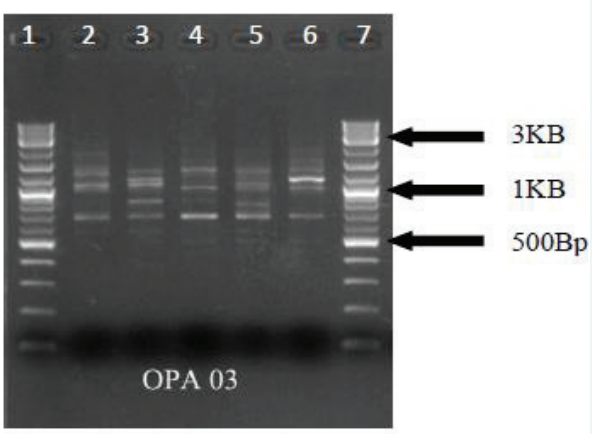

Figure 4: RAPD bands obtained using OPA 03 primer (Lane 1: MW ladder, Lane 2: S2, Lane 3:S5, Lane 4: S3, Lane 5: S4, Lane 6: S1, Lane 7: MW ladder).

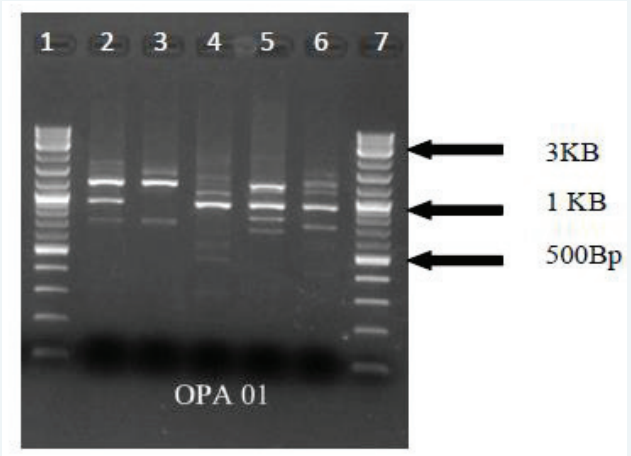

Figure 3: RAPD bands obtained using OPA 01 primer (Lane1: MW ladder, Lane 2: S2, Lane 3: S5, Lane 4: S3, Lane 5: S4, Lane 6: S1, 7: MW ladder).

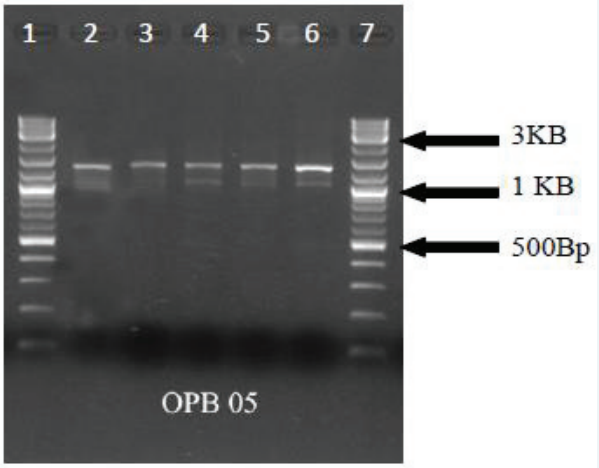

Figure 5: RAPD bands obtained using OPC 05 primer (Lane 1: MW ladder, Lane 2: S3, Lane 3: S5, Lane 4: S1, Lane 5: S2, Lane 6: S4, Lane 7: MW ladder).

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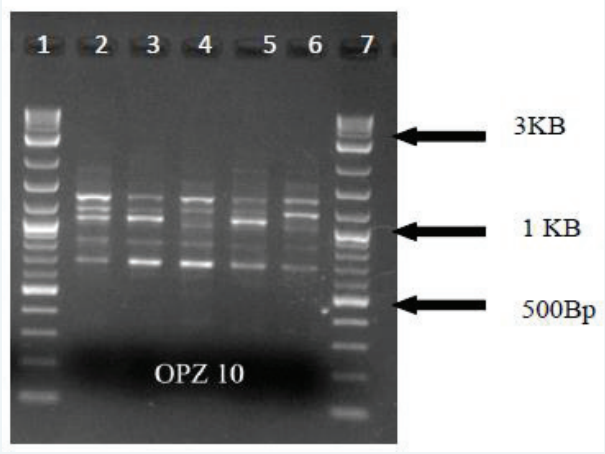

Figure 6: RAPD bands obtained using OPZ 10 primer (Lane 1: MW ladder, Lane 2: S2, Lane 3: S5, Lane 4: S3, Lane 5: S4, Lane 6: S1, Lane 7: MW ladder).

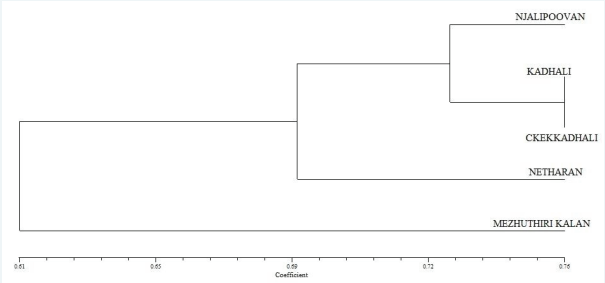

Figure 7: Phylogenetic relationship of the Musa samples based on RAPD analysis.

Acknowledgement

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Reference

1. Uma S, Siva SA, Saraswathi MS, Manickavasagam M, Durai P, et al. (2006) Variation and intraspecific relationships in Indian Wild Musa balbisiana (BB) population as evidenced by random amplified polymorphic DNA. Genet Res Crop Evol 53: 349-355.
2. Chandler S (1995) The Nutritional value of bananas. J Scrip 468-480.
3. Rao NK (2004) Plant genetic resources: advancing conservation and use through biotechnology. Afr J Biotechnol 3: 136-145.
4. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tinqey SV (1990) DNA polymorphisms amplified by arbitrary primer are useful as genetic markers. Nucleic Acids Res 18: 6531-6535.
5. Jain PK, Saini ML, Pathak H, Gupta VK (2007) Analysis of genetic variation in different banana (Musa species)Variety using Random Amplified Polymorphic DNAs (RAPDs). Afr J Biotechnol 6: 1987-1989.
6. Fasalu Rahiman, Balasubramanian T, Kumar P, Shejina M (2015) P-44 Random Amplified Polymorphic DNA (RAPD) - A tool for gene mapping. National conference on Advances in Laboratory Medicine 2: 163-166.
7. Vezina A, den Bergh IV, Rouard M (2016) Morphology of the banana plant. Promusa Musa community.
8. Kumar PS, Durgadevi S, Saravanan A, Uma S (2019) Antioxidant potential and antitumor activitites of Nendran Banana peels in Breast cancer cell line. IJPS 81: 464-473.
9. Sambrook JF, Russell DW (2001) Cold Spring Laboratory press 5: 735-738.

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