SHORT COMMUNICATION ARTICLE

Assessment of Genetic Fidelity in Micropropagated Banana Plantlets Using DNA Extraction and RAPD Analysis

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Abstract

This study examines the genetic diversity and fidelity of in-vitro propagated banana plantlets using Random Amplified Polymorphic DNA (RAPD) markers. As a vital fruit crop with substantial economic value, bananas are frequently propagated in-vitro to facilitate the large-scale production of uniform, disease-free plants. However, the tissue culture process can introduce somaclonal variations, potentially leading to genetic instability. This research focuses on evaluating the genetic uniformity of micropropagated banana plantlets in comparison to their parent plants. RAPD markers were selected for their effectiveness in detecting polymorphisms across multiple genomic loci. A total of 36 RAPD primers were analyzed, generating 256 scorable bands, with 2% showing polymorphism. The findings indicate a high level of genetic fidelity among the in-vitro propagated plantlets, with only minimal variation observed. This study highlights the utility of RAPD analysis as a fast and cost-efficient tool for assessing genetic stability in micropropagated banana plantlets, ensuring the preservation of desired traits in large-scale cultivation efforts.

Keywords: DNA extraction, RAPD analysis, Genetic Fidelity, Micropropagation, Plantlets.

Highlights:

- The study evaluates the genetic fidelity of micropropagated banana plantlets using RAPD markers.
- In-vitro propagation can cause somaclonal variations, affecting genetic stability.
- 36 RAPD primers produced 256 bands, with only 2% polymorphism detected.
- Findings show a high genetic uniformity in the plantlets with minimal variation from parent plants.
- RAPD analysis proves to be a fast, cost-effective tool for assessing genetic stability in banana propagation.

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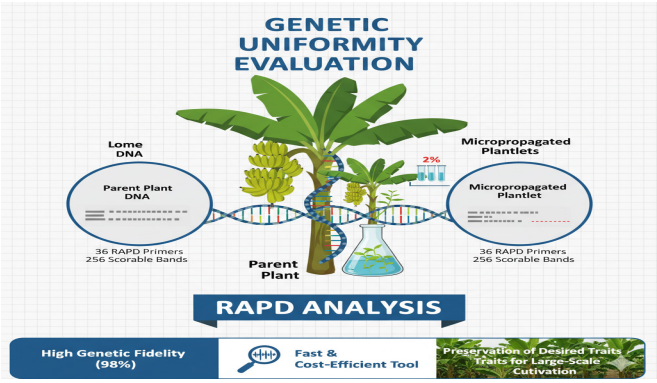

Graphical Abstract:

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Introduction

Banana is a key crop in tropical and temperate regions, with an annual global production reaching 40 million metric tons (Christelová *et al*., 2017). Most edible banana varieties are sterile triploids or tetraploids, primarily propagated through vegetative methods. Tissue culture has gained significant attention as a method for producing genetically uniform, pest-free, and disease-free banana planting materials. Various studies have reported the successful *in-vitro* propagation of bananas using different explants and regeneration pathways. The development of effective propagation techniques is crucial for the conservation of banana germplasm and the cultivation of genetically superior varieties through micropropagation (Subrahmanyeswari, T., & Gantait, S., 2022).

The banana is the fourth most important crop in the world. (Bebber *et al*., 2023) Many high-yielding, premium cultivars have been created to fit different agroclimatic situations. Northeast India (Bihar) is home to the G9 and Malbhog varieties, which are well-known for their fruits that are high in protein and vitamins (Vazhacharickal *et al*., 2022). Particularly prized for its great production potential and inherent tolerance to the majority of viral illnesses is Malbhog. But there hasn't been any coordinated or methodical attempt to develop these particular varieties. Because there is currently a dearth of a timely and sufficient supply of good planting materials, they are currently grown in a few regions in a disorganized manner. These cultivars have not yet been extensively used for commercial bulk multiplication. This problem might be resolved by mass-producing planting

materials using tissue cultures, which would increase the profitability and viability of cultivation. Banana *in-vitro* regeneration has a well-established track record of preserving true-to-type plants and facilitating the quick growth of cultivars free of disease (Syombua *et al*., 2021).

The G9 banana, also known as "Grand Nain", is a popular variety of the Cavendish banana group, widely cultivated for its high yield and resistance to diseases (Navik *et al*., 2023). It is characterized by its medium to large size, smooth yellow skin, and sweet, creamy flesh. The G9 variety is favoured in commercial banana production due to its ability to produce uniform fruit, making it a staple in global markets. Its plants are robust and well-suited to various climatic conditions, contributing to their widespread cultivation in tropical and subtropical regions.

Malbhog is a traditional banana variety native to the northeastern regions of India, particularly Assam and Bihar. Known for its rich flavor and aromatic scent, Malbhog bananas have a distinct golden-yellow skin when ripe and a slightly firm texture. The fruit is smaller compared to commercial varieties like Cavendish, but is highly prized for its sweetness and taste. Malbhog is often consumed fresh or used in local delicacies. Although it is less widely cultivated than other banana types, Malbhog holds significant cultural and economic importance in its native regions, where it is valued for both its taste and its contribution to the local agricultural economy.

Researchers and farmers need reliable, high-yielding cultivars to optimize production. However, traditional methods based on phenotypic selection are complex and often inconsistent for identifying true cultivars. This highlights the need to explore and develop novel molecular markers. These markers are invaluable for assessing genetic diversity and can effectively complement traditional breeding approaches. Molecular markers are increasingly used in plants to uncover the genetic basis of variation, determine genetic relationships within germplasm, identify duplicate accessions, and ensure genetic fidelity (Tyagi et al., 2022).

A popular molecular tool for evaluating genetic diversity and fidelity is random amplified polymorphic DNA (RAPD) analysis, especially in plant species that are cultivated in-vitro. Often used to generate huge numbers of homogeneous, disease-free plantlets, in-vitro propagation is used in the context of banana (Musa spp.), a crop of major worldwide economic importance (Kumar, N.S. et al., 2011). On the other hand, somaclonal differences can be induced during the tissue culture process, which can lead to genetic instability and compromise the uniformity of the plants that are propagated. Because RAPD markers can amplify random DNA segments and expose polymorphisms throughout the genome without the need for prior sequence information, they are an invaluable tool for identifying these differences. By assessing the genetic homogeneity of banana plantlets grown in-vitro, this technique offers a quick and affordable way to ensure the propagated plants retain the desirable characteristics of the parent stock. RAPD analysis supports the sustainable production of this vital crop by evaluating the genetic diversity among micropropagated populations, which helps with quality control of large-scale banana farming projects. (Ferreira et al., 2024).

This study focuses on developing an optimized regeneration protocol for the G9 and Malbhog banana Cultivars and validating its effectiveness in maintaining genetic fidelity using RAPD markers. Ensuring genetic stability in tissue-cultured plantlets is vital for quality control in large-scale banana propagation, as genetic uniformity directly influences the reliability and commercial success of micropropagated plants. The research emphasizes establishing a micropropagation system that consistently produces genetically identical and stable plants prior to large-scale release. The novelty of this work lies in providing clear evidence of the high genetic fidelity of *in-vitro* propagated banana plantlets through comprehensive RAPD marker analysis, demonstrating that large-scale micropropagation can effectively preserve genetic uniformity with minimal somaclonal variation. It further highlights the reliability of RAPD profiling as a rapid, cost-effective, and sensitive molecular tool for assessing clonal stability in commercially important banana cultivars.

Materials and methods

Plant materials

Twenty tissue-cultured banana plantlets of G9 and Malbhog were obtained from the Tissue Culture Laboratory of TPS College, Patliputra University, Patna.

DNA extraction

Genomic DNA was isolated from the young, fresh leaves of two *Musa spp*. Cultivars, G9 and Malbhog, using a modified version of the CTAB extraction method (Dhanapal *et al*., 2014). DNA was extracted from the tender leaves of ten randomly selected micropropagated plants from the same batch, as well as the mother plant. About 500 mg of fresh leaves was finely powdered using liquid nitrogen and a mortar and pestle. The fine powders were transferred to 50 ml centrifuge tubes containing 1000 µl of preheated CTAB buffer (65 °C) and 10 µl of beta-mercaptoethanol. The homogenates were centrifuged at 10,000 rpm for 10 minutes. The obtained supernatants were mixed with a volume ratio of 25:24:1 of phenol: chloroform: isoamyl alcohol and centrifuged again. DNA was precipitated from the aqueous phase by adding isopropanol and was stored at –20°C for 12 hours (Islas-Flores *et al*., 2006). The DNA was washed with 70% ethanol, dried under nitrogen, and stored in sterile distilled water. RNase treatment was performed using a 1 mg/ml concentration, with the solution incubated at 37°C for 20 minutes. The purity and concentration of the genomic DNA were subsequently determined by measuring absorbance at 260 and 280 nm (Turaki *et al*., 2017). The DNA was subsequently separated on a 1% agarose gel stained with ethidium bromide and photographed using a gel documentation system.

PCR amplification

RAPD analysis was conducted using a modified method with 40 arbitrarily designed decamer primers provided by Xploragen Discoveries Pvt. Ltd. These primers, with 60-70% G+C content and no internal complementarity, were used to evaluate the genetic fidelity of micropropagated plantlets during the secondary hardening stage. The plantlets were randomly selected from a single batch, alongside the mother

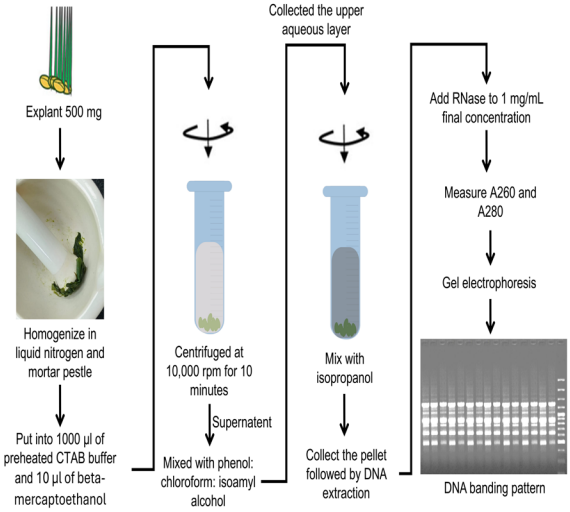

Fig 1: Schematic diagram showing the steps of genomic DNA extraction from Musa spp. leaves using the CTAB method.

plants, for comparison. Genomic DNA (10 µl) was amplified in a 25 µl reaction mixture that included 1 µl primer, 2.5 µl PCR buffer (G-Biosciences), 0.5 µl dNTP (G-Biosciences), 0.25 µl Taq polymerase (G-Biosciences), and 18.75 µl sterile distilled water. Amplification was performed in a thermocycler (Bio-Rad) with the following program: an initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of denaturation at 94 °C for 1 minute, annealing at 42 °C for 45 seconds, and extension at 72 °C for 1 minute (Hadidi, A., *et al*., 2017) The final synthesis step was extended by 7 minutes, after which the amplification products were cooled to 4 °C (Rodriguez-Garcia *et al*., 2016). The amplified products were separated on a 2% agarose gel stained with 0.5 µl ethidium bromide (Venkatachalam *et al*., 2007). To increase the molecular weight, a dye loading solution was added, and the gel was photographed under UV illumination using a gel documentation system (Selvakumar et al., 2020). **Fig. 1** shows the schematic representation of the CTAB-based genomic DNA extraction workflow from young leaves of Musa spp. (G9 and Malbhog), illustrating key steps including tissue grinding, cell lysis, organic extraction, DNA precipitation, purification, and quality assessment.

Result and Discussion

The augmentations of *Musa spp.* (cv. G9 and Malbhog) holds significant importance as a vital food source for various sectors of society. Therefore, it is essential to conserve the genetic resources of these varieties while ensuring they meet practical needs. A crucial step toward achieving this is understanding

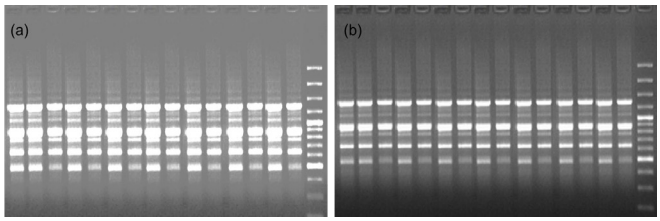

Fig. 2: DNA banding pattern of (a) G9 (b) Malbhog

Table 1: Total number of purity and genomic DNA concentrations of two varieties Musa spp

Sample	*Purity (A260/A280 ratio)*	*Concentration (mg/µl)*
G9	1.72	151.7
G9	1.79	113.5
G9	1.18	111.7
Malbhog	1.44	36.9
Malbhog	1.0	30.3
Malbhog	0.8	37.9

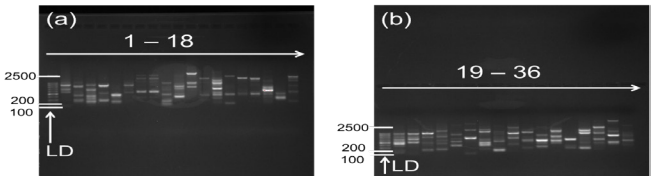

Fig. 3: RAPD analysis of micropropagated plants of Musa spp. G9. LD-ladder, (a) Primer 1-18 (b) 19-36- The primers are used sequentially in the order listed in Table 2.

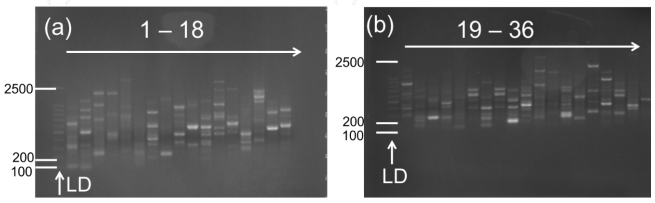

Fig. 4: RAPD analysis of micropropagated plants of Musa spp. Malbhog, LD-ladder, (a) Primers 1-18 (b) 19-36-The primers are used sequentially in the order listed in Table 2.

the genetic fidelity of *Musa* spp. Varieties propagated through micropropagation. In this study, RAPD analysis was employed to evaluate the genetic uniformity of micro-propagated *Musa spp.* Plantlets. The quality and quantity of the extracted DNA were assessed using spectrophotometry and agarose gel electrophoresis (Fig. 2). The genomic DNA of both cultivars showed satisfactory purity and concentration levels, as confirmed by the amplification results, which are presented in Table 1. These findings indicate that the DNA extracted from the micro-propagated plantlets was of suitable quality for further molecular analysis, providing a reliable basis for assessing clonal fidelity.

The RAPD analysis of genetic diversity in *in-vitro* raised banana plantlets revealed a high level of genetic uniformity among the samples (Fig. 3 and Fig. 4). A total of 36 RAPD primers were used, out of which 32 generated clear and reproducible banding patterns. These primers produced a total of 256 distinct bands ranging in size from 250 bp to 2500 bp, with each primer producing an average of 7.14 bands per plantlet (Table 2).

Comparative analysis of the banding patterns between the micropropagated plantlets and the mother plants demonstrated a high degree of genetic similarity. The similarity

Table 2: List of RAPD primers, their sequences and size of amplified fragments generated along with the number of bands, polymorphic bands for detecting the genetic fidelity in micropropagated plants of banana cultivar G9 and Malbhog

S. No.	*Primer Code*	*Primer Sequence*	*Number of bands*		*Number of polymorphic bands*		*Number of monomorphic bands*		*Size range (bp)*
			G9	*Malbhog*	*G9*	*Malbhog*	*G9*	*Malbhog*	
1.	*OPA-1*	*CAGGCCCTTC*	*5*	*9*	*0*	*1*	*3*	*8*	*300-1500*
2.	OPA-2	TGCCGAGCTG	5	6	0	0	2	6	300-1000
3.	OPA-3	AGTCAGCCAC	7	2	0	0	4	2	500-2500
4.	OPA-4	AATCGGGCTG	3	2	1	0	3	2	300-2000
5.	OPA-5	AGGGGTCTTG	2	3	0	0	5	3	500-1600
6.	OPA-6	TGGGCGTCAA	2	4	0	0	4	4	300-1000
7.	OPA-7	GGCATGACCT	4	6	1	0	2	6	300-1800
8.	OPA-8	TGGGCGTCAA	4	4	0	0	5	4	400-1900
9.	OPA-9	CCAGCAGCTT	5	4	0	0	1	4	400-1800
10.	OPA-10	GACTGCACAC	2	8	1	1	3	7	600-1200
11.	OPA-11	CAATCGCCGT	7	6	0	0	2	6	200-900
12.	OPA-12	TCGGCGATAG	3	1	0	0	4	1	150-1030
13.	OPA-13	CAGCACCCAC	2	4	0	0	6	4	150-650
14.	OPA-14	CTCGTGCTGG	2	2	0	0	2	2	200-2000
15.	OPB-5	TGCGCCCTTC	2	4	0	0	3	4	300-1100
16.	OPB-06	TGCTCTGCCC	2	5	0	0	4	5	220-1400
17.	OPB-07	GGTGACGCAG	5	3	0	0	2	3	270-750
18.	OPB-8	GTCCACACGG	0	2	0	0	1	2	350-1000
19.	OPC-01	TTCGAGCCAG	3	1	0	0	3	1	280-1800
20.	OPC-02	GTGAGGCGTC	4	4	0	0	5	4	300-2500
21.	OPC-04	CCGCATCTAC	4	7	0	0	4	7	220-2000
22.	OPC-07	GTCCCGACGA	5	4	0	0	2	4	300-2500
23.	OPC-08	TGGACCGGTG	3	3	0	0	4	3	290-2500
24.	OPD-07	TTGGCACGGG	4	3	0	0	3	3	380-2500
25.	OPD-16	AGGGCGTAAG	4	1	0	0	5	1	400-2000
26.	OPM-16	GTAACCAGCC	2	4	0	0	2	4	400-2000
27.	OPM-20	AGGTCTTGGG	3	1	0	0	4	1	320-2000
28.	OPN-03	GGTACTCCCC	3	5	0	0	2	5	3000-2500
29.	OPN-09	TGCCGGCTTG	3	2	0	0	3	2	240-2500
30.	OPN-10	ACAACTGGGG	2	7	0	0	3	7	240-2500
31.	OPA-16	AGCCAGCGAA	3	8	0	1	1	7	550-1400
32.	OPA-17	GACCGCTTGT	4	5	0	0	5	5	300-1100
33.	OPA-18	AGGTGACCGT	4	3	0	0	3	3	290-1200
34.	OPA-20	GTTGCGATCC	2	6	0	0	4	6	200-2500
35.	OPB-01	GTTTCGCTCC	0	2	0	0	2	2	480-1000
36.	OPB-04	CGACTGGAGT	0	2	0	0	3	2	200-900

indices ranged from 0.92 to 0.98, indicating that the in-vitro propagation process maintained the genetic integrity of the plantlets. However, minor variations were observed in a few plantlets, suggesting possible somaclonal variations or mutations introduced during the micropropagation process. No significant differences in the RAPD profiles were observed between plantlets at different stages of secondary hardening, further supporting the genetic stability of the propagation method. The observed polymorphism was minimal, with an average polymorphism rate of 2%, indicating a very low level of genetic diversity among the plantlets.

The results highlighted the effectiveness of RAPD markers in assessing genetic diversity and fidelity in in-vitro raised banana plantlets. The micropropagated plantlets and the mother plants showed a high genetic similarity which suggested that the tissue culture techniques used in this study were effective in preserving the genetic integrity of the banana plants. The minimal polymorphism observed among the plantlets indicates that the in-vitro propagation method used is reliable for producing genetically uniform plants, which is crucial for maintaining the desired traits in commercial banana production (Subrahmanyeswari, T., & Gantait, S., 2022). The low level of genetic variation detected may be attributed to the stringent selection of explants and the controlled micropropagation environment, which minimizes the chances of genetic alterations (MS, S, & P, D., 2023)

However, the minor genetic differences observed in a few plantlets warrant further investigation. These variations could be the result of somaclonal variation, which, while often considered a disadvantage in clonal propagation, can sometimes lead to the development of new traits that may be beneficial. Understanding the causes and implications of these variations could be valuable for optimizing micropropagation protocols and improving the overall quality of the propagated plants.

The RAPD analysis confirms that the micropropagation protocol employed in this study is effective for generating genetically uniform banana plantlets with minimal genetic diversity. This finding is significant for the large-scale production of banana plants, ensuring consistency in crop quality and yield. Further studies could focus on long-term field evaluations of these plantlets to monitor any potential genetic drift or variations that may arise over successive generations

Conclusion

In conclusion, ensuring clonal fidelity remains a critical aspect of successful micropropagation for any crop species. One of the main challenges in this process is the occurrence of somaclonal variation, which can arise during the *in-vitro* culture of plant cells, tissues, or organs. The RAPD analysis conducted on randomly selected banana plantlets (cv. G9 and Malbhog) and their mother plants revealed a high degree of genetic uniformity, with most of the samples showing monomorphic patterns. However, the presence of some polymorphic bands indicates a degree of genetic variation, highlighting the importance of continuous monitoring for somaclonal variation in micropropagation efforts. The RAPD primers generated 256 amplified fragments ranging in size from 250 to 2500 bp, with 2% of the amplification bands being polymorphic. This study demonstrated that RAPD markers are a valuable tool for detecting DNA polymorphisms and assessing genetic relationships in bananas. The results of this investigation achieved the primary goal of banana micropropagation producing genetically uniform plantlets. Additionally, it was observed that RAPD-PCR analysis is an effective method for testing the genetic fidelity and uniformity of tissue-cultured plants. Overall, this study underscores the importance of making such tests mandatory in all tissue culture laboratories to ensure the production of genetically pure clones or plantlets.

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Author Contribution

AK: Data collection, analysis of the data, and conceived of the idea, GA: Edited and Revised the manuscript, VBK: Developed the methods, and edited the manuscript.

Conflict of interest

We declare that there is no conflict of interest.

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