Research Article

Genetic Fidelity Studies of Tissue Culture Raised Plants of Banana (*Musa spp*.) Cultivar G-9

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Abstract

The present investigation was undertaken to develop *in vitro* protocol to assess the genetic fidelity of *in vitro* raised plants in banana cultivar Grand Naine. Cetyl Trimethyl Ammonium Bromide (CTAB) extraction method was used for genomic DNA isolation from young leaves taken from *in vitro* raised plants as well as mother plant of banana cultivar Grand Naine. *In vitro* raised plants were examined for genetic stability using fifty RAPD primers. Out of fifty primers screened, twenty eight primers produced amplification while twenty two primers did not show any amplification. The results showed that all RAPD profile for *in vitro* raised plants were monomorphic and similar to their mother plant, which showed that all the plants raised through micropropagation were true to type or identical to the mother plant.

Keywords: banana, genetic fidelity, RAPD primer, true to type

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Introduction

Banana is conventionally propagated by sword sucker. Although propagation by sucker retains all the characters of the parent but viral diseases are also transmitted through suckers especially when parent material is infected and also suckers are found in touch to soil therefore, they become infested with those diseases for which organisms are present in the soil around the banana plant. Besides, sufficient number of suckers is not available at a time for sowing on a large scale. Hence, non availability of disease free planting material is barrier in its cultivation. Regeneration through *in vitro* culture has now become a viable and alternate method to conventional one to produce disease free planting material within a short time of period.

In a micropropagation programme, clonal fidelity is one of main concerns as true to type propagules and genetic stability are primary objectives of clonal reproduction. The origin and loss of genetic fidelity in tissue culture regenerates is the result of alterations that can occur in nuclear DNA, cytoplasmic DNA or in all genomes present in a cell. The early detection of the presence of somaclonal variants remains critical as it saves valuable time and minimize the overall economic loss to the users of tissue cultured planting materials (Bairu *et al*., 2011). Somaclonal variation can be identified by morphological, biochemical, physiological and genetic characteristics. Clouteir and Landry (1994) reported that DNA marker are power tools for early detection of somaclonal variation and not influence by environmental factors like morphological marker. Somaclonal variation, a major problem of clonal propagation, has been reported in several cultivars of *Musa* spp. (Tang *et al*., 2000; Vidal and Garcia, 2000; Hwang, 2002; Vidhya and Nair, 2002; Martin *et al*., 2006). Hence, the need arises to study the genetic fidelity of tissue culture raised banana plants.

Materials and Methods

The present study on "Genetic fidelity studies of tissue culture raised plants of banana cultivar G-9" was conducted at Centre for Plant Biotechnology, Government of Haryana, CCS HAU Campus, Hisar during 2015-2016. The mother plants of banana cultivar Grand Naine growing in experimental polyhouse of Centre of Plant Biotechnology, Hisar and *in vitro* regenerated plantlets were used for the extraction of DNA and further characterization.

Genomic DNA isolation

Cetyl Trimethyl Ammonium Bromide (CTAB) extraction method of Murray and Thompson (1980), modified by Saghai-Maroof *et al.* (1984) and Xu *et al.* (1994) was used for genomic DNA isolation from young leaves (5gm) taken from *in vitro* raised plants as well as mother plant of banana cultivar Grand Naine.

Amplification of DNA by Polymerase Chain Reaction (PCR)

PCR reaction condition was standardized by using mother plant DNA with LD 3230 and LD 3231 primers. The variations were made in genomic DNA concentration (50ng, 100ng and 200ng), Taq. DNA polymerase concentration (1.5 units and 2.0 units), primer concentration (0.2µl and 1µl) and annealing temperature (36^0C, 37^0C and 40^0C). All possible combinations were tried in 20µl of reaction mixture for PCR amplification. Amplification was carried out in PTC-100 programmable thermal cycler (MJ research and Biometra personal).

PCR reaction was performed at an initial denaturation temperatue of 94^0C for 1minute (1 cycle) followed by 40 cycles of each,

Denaturation	94ºC	1minute
Annealing	36ºC, 37^0C and 40^0C	1minute
Extension	72ºC	2 minutes
Final extension was carried out at 72^0C for		10 minutes.

Agarose gel electrophoresis

Agrose gel (2.5 per cent; *w/v*) were used to resolved amplified DNA fragments. PCR products were submerged in horizontal agarose gel electrophoresis and visualized by staining with ethidium bromide.

Results and Discussion

PCR Amplification

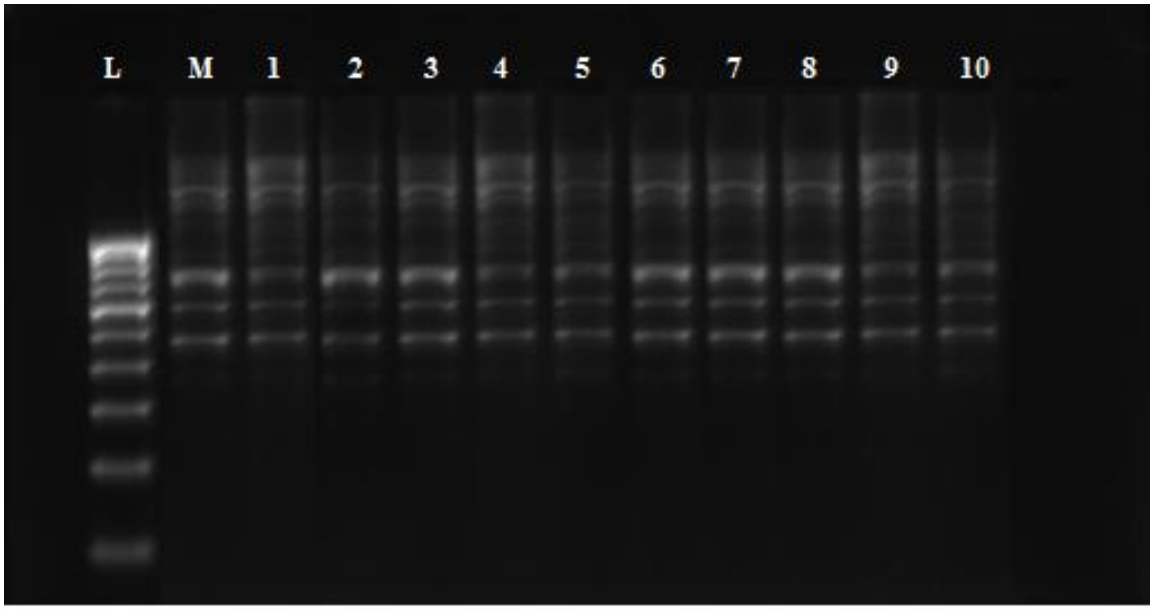
The PCR conditions need to be well defined to obtain reproducible patterns because DNA profiling and reproducibility of RAPD technique is influenced by any variation in template DNA, primer, Taq DNA polymerase concentration and annealing temperature (Bassam *et al.*, 1992 and Kernodle *et al*., 1983). Therefore, variable concentrations of reaction mixture's components were used to standardize the PCR reaction. Visual assessment of the amplified products on 2.5 percent agarose gel electrophoresis revealed that the banding pattern was influenced by the different combinations of reaction conditions. In the present investigation very little amplification was observed with 1.5 unit Taq. DNA polymerase, while with the increase in the concentration of Taq. DNA polymerase (2 unit), higher number of bands were observed. Annealing temperature of 36^0C was observed to be optimal for producing highest number of reproducible DNA bands using the RAPD primers. At higher concentration of primer, indiscriminate bands were generated. No amplification was observed at lower concentration of DNA (50 ng, 100 ng). Therefore, in the current work clear banding patterns were obtained in a reaction mixture of 20µl containing 200 ng template DNA, 2.5µl of dNTPs mix, 0.2µl of primer, 3µl of 10 X Taq DNA polymerase buffer, 2 units of Taq. DNA polymerase and 10.3 µl of sterile distilled water. PCR reaction was performed at an initial denaturation temperatue of 94^0C for 1minute (1 cycle) followed by 40 cycles of each denaturation at 94ºC for 1minute, annealing at 36ºC for 1minute and extension at 72ºC for 2 minutes. Final extension was carried out at 72^0C for 10 minutes. Similar PCR conditions have been reported by Martin *et al.* (2006).

Table 1 Random primers showing amplification

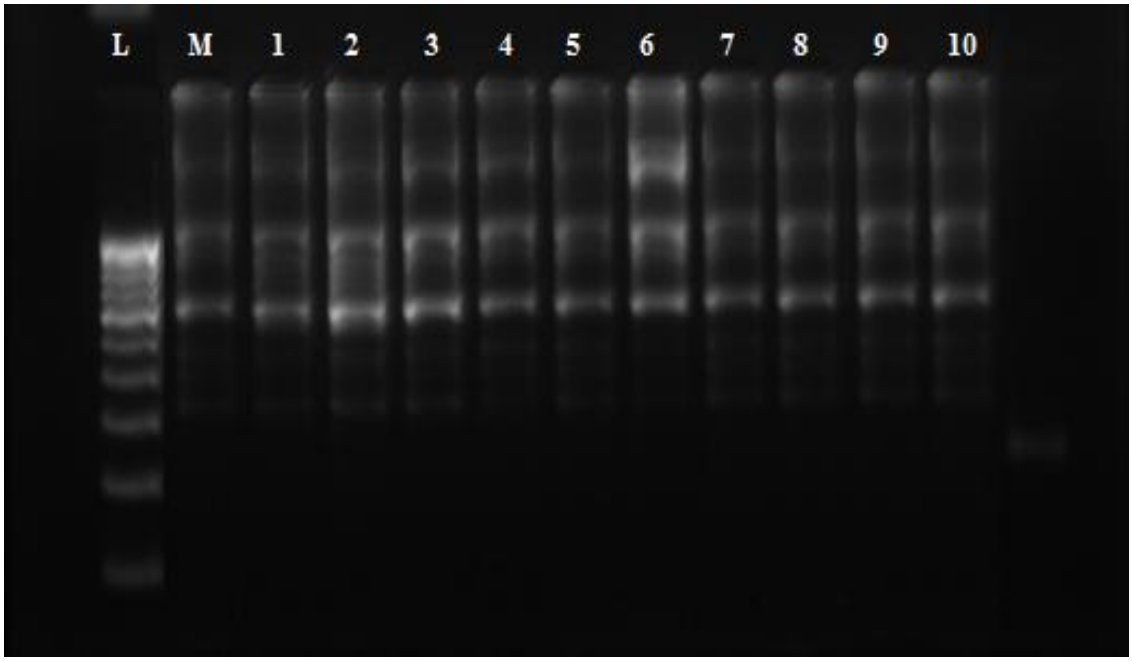
Sr. No.	Primer code	Sequence (5'-3')	Sr. No.	Primer code	Sequence (5'-3')
1	LD 3230	AATCGGGCTG	15	LD 3260	CCTTGACGCA
2	LD 3231	CAATCGCCGT	16	LD 3263	GGAGGGTGTT
3	LD 3233	GACCGCTTGT	17	LD 3265	AGGGAACGAG
4	LD 3245	CCGCATCTAC	18	LD 3266	CCACAGCAGT
5	LD 3246	GTCCCGACGA	19	LD 3268	GGACCCTTAC
6	LD 3249	TGAGCGGACA	20	LD 3269	TGCTCTGCCC
7	LD 3250	GGACCCAACC	21	LD 3272	GATGACCGCC
8	LD 3251	ACCGCGAAGG	22	LD 3273	GAACGGACTC
9	LD 3252	GTTGCCAGCC	23	LD 3274	TGGACCGGTG
10	LD 3253	TGAGTGGGTG	24	LD 3275	CTCACCGTCC
11	LD 3255	GGTGACGCAG	25	LD 3276	TGTCTGGGTG
12	LD 3256	GTCCACACGG	26	LD 3277	AAGCCTCGTC
13	LD 3258	CTGCTGGGAC	27	LD 3278	TGCGTGCTTG
14	LD 3259	GTAGACCCGT	28	LD 3279	CACACTCCAG

Table 2 Random primers not showing amplification

Sr. No.	Primer code	Sequence (5'-3')	Sr. No.	Primer code	Sequence (5'-3')
1	LD 3232	TCTGTGCTGG	12	LD 3244	GTGAGGCGTC
2	LD 3234	GTTGCGATCC	13	LD 3247	AAAGCTGCGG
3	LD 3235	TCGCCGCAAA	14	LD 3248	GACGGATCAG
4	LD 3236	AGCGTCACTC	15	LD 3254	TTCCCCCCAG
5	LD 3237	GTCCGTACTG	16	LD 3257	TGGGGGACTC
6	LD 3238	GGTGCTCCGT	17	LD 3261	TTCCCCCGCT
7	LD 3239	GACCGACCCA	18	LD 3262	TCCGCTCTGG
8	LD 3240	AGGGTCTGTG	19	LD 3264	TTTGCCCGGA
9	LD 3241	GGGTAAGGCC	20	LD 3267	ACCCCCGAAG
10	LD 3242	GTGATCGCAG	21	LD 3270	TTCGAGCCAG
11	LD 3243	GACCGCTTGT	22	LD 3271	GGGGGTCTTT

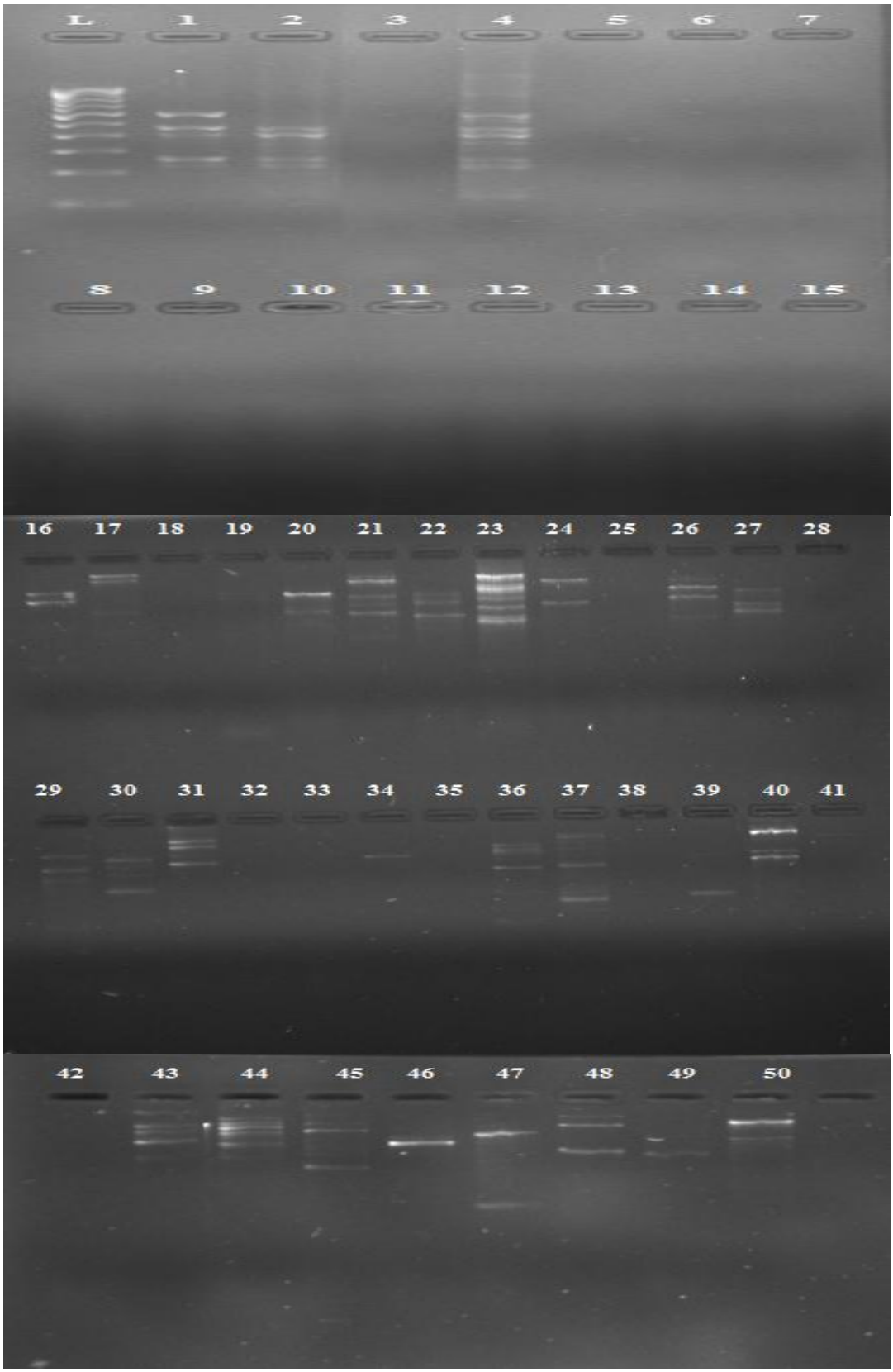


(a) RAPD profiles of mother plant and *in vitro* raised plants using primer LD 3272



(b) RAPD profiles of mother plant and *in vitro* raised plants using primer LD 3273

Figure 1 RAPD profiles of mother plant and *in vitro* raised plants (L = Ladder (100 bp), M = Mother plant, 1-10 = *in vitro* raised plants)



L = Ladder (100 bp), 1-50 = LD3230 - LD3279
Figure 2 Random primers showing amplification

RAPD Analysis

Mother plant and *in vitro* raised plants were screened to assess genetic fidelity by using fifty RAPD primers. Out of fifty primers used, twenty eight primers showed amplification and twenty two primers did not show any amplification. The DNA amplification products, could result from changes in either the sequence of the primer

binding sites or changes which alter the size and present the successful amplification of target DNA. Banding pattern of tissue culture raised plants was similar to mother plant and was monomorphic which showed that all the plant raised through micropropagation were true to type. Although, in some micropropagated plants, minor morphological variations were recorded. The developed RAPD profiles of tissue culture raised plants were typically identical, because there were no changes in the banding pattern observed in micropropagated plants and mother plant irrespective of phenotypic variation. The phenotypic variations in tissue cultured plants might be due to higher concentrations of growth regulators (cytokinins and auxins). Similar results were reported by Kajla *et al.* (2014) where they observed a homogenous amplification profile of all micropropagated plants of banana cultivar Grand Naine and their mother plant. Choudhary *et al.* (2015) found that all tissue cultured raised plants of banana cultivar Robusta were true to type. Gaffar and Sarkar (2006) reported epigenetic changes in tissue culture raised plants due to applications of plant growth regulators, but such trait is not passed to their offspring through sexual cycle or might entirely disappear during plant maturation. Ray *et al*. (2006) highlighted the genetic stability of *in vitro* raised plants of banana cultivars- Robusta, Gaint Governor and Mataman by using 21 RAPD and 12 ISSR primers, however they found 3 somaclonal variants in Robusta and 3 in Gaint Governor.

Conclusion

In the present investigation, attempts were made to develop an efficient protocol for to assess genetic fidelity in banana cultivar Grand Naine. Salient features of the results obtained in the present study are as given below.

- Clear, consistent and reproducible bands were obtained in a total of 20µl reaction mixture containing 200 ng genomic DNA, 3 µl of 10X buffer, 0.2 µl of each primer, 2.5 µl of dNTPs, 2 units Taq. DNA polymerase and 10.3µl of sterile distilled water.
- PCR reaction was performed at an initial denaturation temperatue of 94^0C for 1 minute (1 cycle) followed by 40 cycles of each denaturation at 94ºC for 1 minute, annealing at 36ºC for 1 minute and extension at 72ºC for 2 minutes. Final extension was carried out at 72^0C for 10 minutes.
- Mother plant and *in vitro* raised plants were screened to assess genetic fidelity by using fifty RAPD primers. Out of fifty primers screened, twenty eight primers produced amplification, while twenty two primers did not show any amplification.
- Banding pattern of tissue culture raised plants was similar to mother plant and was monomorphic which showed that plants raised through micropropagation were true to type.

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