*Available online at* www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5570

**ISSN: 2320 – 7051**

*Int. J. Pure App. Biosci.* **5 (6):** 172-176 (2017)

*Research Article*

International Journal of Pure & Applied Bioscience

OPEN ACCESS

# Molecular Characterization of Chemically Induced Banana Mutants

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Received: 1.09.2017 | Revised: 28.09.2017 | Accepted: 5.10.2017

## ABSTRACT

*Banana cultivar Nanjanagudu Rasabale is one of the important geographically tagged crop in Mysore region of Karantaka. Now facing the serious threat of extinction due to its severe susceptibility to Panama wilt disease. In order to improve its resistance, the present investigation on induction of variation through chemical mutagens and molecular characterization of putative mutants was carried out at Centre for Horticulture Biotechnology, Bagalkot. Different concentrations of EMS () and $NaN_3$ were utilized to induce in vitro mutagenesis. Among the morphological mutants obtained were subjected for Random Amplified Polymorphic DNA analysis. OPN, OPJ, OPS, OPT, OPA and OPR series primers were used to determine genetic variation between the various morphological mutants along with mother plant. A total of 631 amplification products were produced from the selected 09 primers and the number of bands varied from 1-11 with an average of 7.7 bands per primer. With OPR 07 primer presence of a major band at 800 bp and absence of band at 2000 bp in lane 2 and 3 showed the presence of polymorphism among the various morphological mutants. RAPD analysis is little cheaper method of analysing the genetic variability hence it can be utilized to identify the variation among the mutants.*

***Key words:*** *in vitro, mutagenesis, molecular*

## INTRODUCTION

Banana is vegetatively propagated crop which have India as one of its centre of origin. With existence of wide natural variability in these regions afford a rich pool of genotypes which are acclimatized to different agro climatic conditions. Nanjanagudu Rasabale is one such ecotype having geographical indication in Nanjanagudu area of Mysore region Karnataka now under threat of extinction due to its susceptibility to Fusarium wilt[10]. So crop improvement is must for enhancing variability and incorporate resistance in banana.

Conventional breeding methods have been of limited success in *Musa* species due to parthenocarpy, polyploidy in many cultivars, and limited available information on genetic and genomics[2,3].

**Cite this article:** Kishor, H., Prabhuling, G., Prakash, D. P., Babu, A. G., Manjunatha, N. and Abhijith, Y. C., Molecular Characterization of Chemically Induced Banana Mutants, *Int. J. Pure App. Biosci.* **5(6):** 172-176 (2017). doi: http://dx.doi.org/10.18782/2320-7051.5570

As an alternate method mutation breeding *in vitro* is a powerful tool for the induction and selection of desirable mutants which can be utilized in banana improvement either for higher yields, good quality and resistance to biotic and abiotic factors[13].

The purpose of induced mutations is to enhance the mutation frequency rate in order to select appropriate variants for plant breeding. The mutation frequency rate of spontaneous mutations is rather very low and difficult to exploit by the plant breeders. Mutations are induced by physical (e.g. gamma radiation) and chemical (e.g. ethylmethane sulfonate) mutagen treatment of both seed and vegetatively propagated crops. The mutagen treatment breaks the nuclear DNA and during the process of DNA repair mechanism, new mutations are induced randomly and heritable[9]. The mutants obtained after mutagenesis should be identified for its successful establishment in crop fields. In order to characterize the variants morphological and molecular markers are utilized as reported by several researchers.

Until recently, morphology-based methods had been used for the characterisation of *Musa* germplasm[11,12]. Morphological characteristics are influenced by the environment. Molecular markers have a number of perceived advantages over the morphological markers for the assessment of genetic diversity. Therefore, molecular methods including PCR-based analysis techniques such as Random Amplified Polymorphic DNA (RAPD)[15], Simple Sequence Repeats (SSRs) and Amplified Fragment Length Polymorphisms (AFLPs) have been used to elucidate genetic relationships among different *Musa* genotypes[14]. RAPD assays have been used to distinguish plantain landraces[8], for the identification of dwarf mutants within the Cavendish group[5], and for classification of *Musa* clones in India[1]. In addition, this method was proven to be efficient in efforts to determine genetic diversity among 76 plantain landraces[4] and for the evaluation of genetic relationships among 19 East African highland bananas (*Musa* spp.). Keeping these points in view the present was conducted to characterize the putative mutants through molecular markers.

## MATERIAL AND METHODS

The present investigation was carried out at Centre for Horticulture Biotechnology, Directorate of Research, Bagalkot. Banana cultivar Nanjanagudu Rasabale with two chemical mutagens *viz*., Ethyl Methane Sulphonate (EMS) and Sodium Azide ($NaN_3$) were selected for induction of *in vitro* mutagenesis. The explants were treated with various concentrations of EMS (0.30 %, 0.60 %, 0.90 % and 1.20 %) and $NaN_3$ (0.01 %, 0.02 % and 0.03 %) and rooted after four subsequent cultures. These plantlets were further hardened and utilized for molecular characterization.

**Isolation of DNA**

DNA was extracted from banana variants from each chemical concentration treatments. At 1.20 % of EMS concentration we observed no rooting so these plants couldn't be taken for molecular analysis. In hardened plants young cigar leaf were choosen for DNA extraction. CTAB method was used for the isolation and extraction of DNA as a Standard protocol.

**Polymerase chain reaction (PCR)**

The PCR reaction was performed in a 25µl reaction volume containing 10X incomplete buffer, 25 mM Mgcl2, 1 mM dNTP's, 0.30 µM primers, 0.50 U of Taq DNA polymerase (Genei, Bangalore) and 20 ng template DNA in Eppendorf master cycler. The RAPD primers were obtained from Junifer, Bengaluru.

**PCR reaction components and programs**

**For RAPD marker**

PCR reaction was carried out using RAPD markers in Master Cycler gradient 533 (Eppendorf, India). The cycles were programmed as mentioned below.

| Steps | Temperature ($^0$C) | Duration (Minutes) | Cycles |
|---|---|---|---|
| Initial denaturation | 95 | 4 | |
| Denaturation | 94 | 1 | |
| Annealing | 38 | 1.15 | 35 |
| Extension | 72 | 2 | |
| Final extension | 72 | 10 | |
| Hold | 4 | | |

The amplified products (after PCR) were stored at $4^0$C till GEL electrophoresis

**Testing of Polymorphism using RAPD markers**

The 10 RAPD (Random Amplified Polymorphic DNA) markers were chosen to assess polymorphism among induced banana variants. The details of primers used were as follows.

| Primers | Nucleotide sequence (3' to 5') |
|---|---|
| OPJ 10 | AAGCCCGAGG |
| OPJ 16 | CTGCTTAGGG |
| OPN 06 | GAGACGCACA |
| OPN 04 | AAGCGACCTG |
| OPN 16 | AAGCGACCTG |
| OPR 07 | ACTGGCCTGA |
| OPR 08 | CCCGTTGCCT |
| OPS 12 | CTGGGTGAGT |
| OPT 08 | AACGGCGACA |
| OPT 20 | GACCAATGCC |

**Resolutions of amplified product on agarose gel electrophoresis**

**Procedure**

1. The frame of the gel casting unit was cleaned, dried and sealed with a tape to form a mould. The frame was placed on a flat platform to ensure a flat and level base. The comb was then positioned parallel to the open edge of the frame about 2 mm above the surface.
2. Sufficient 1x electrode buffer was prepared from 50x stock.
3. 1.8 % agarose gel was prepared using 1 x TAE buffer and ethidium bromide (5 µl/100 ml) was added as a stain. Then agarose solution was poured after keeping the comb in position after solidification, the gel was transferred to the electrophoresis unit such that the wells were towards the negative pole. The gel tank was filled with TAE buffer (1x) just enough to cover the surface of the gel.
4. The PCR product (20 µl) was mixed with 4 ml of loading dye (50 % sucrose + 5 µl of bromophenol blue) and slowly loaded into the wells of the submerged gel using a disposable micropipette tip.
5. The electrodes were connected to the power supply and electrophoresis was carried out at 100 volts for 1 hr or till the dye migrated to the end of the gel.
6. After the completion of electrophoresis, the DNA was visualized and documented in a gel documentation system.

**Gel scoring**

It was done to identify resistant specific band (band which is present in resistant mutant and absent in susceptible individuals or vice-versa) and for testing variation among various morphological mutants, it was expected that the resistant specific band should be present in resistant mutants as RAPD is a dominant marker system.

## RESULTS AND DISCUSSION

Random amplified polymorphic DNA markers were used to detect the variation among the mutants. OPN, OPJ, OPS, OPT, OPA and OPR series primers were used to determine genetic variation between the various morphological mutants along with mother plant.

For molecular analysis 10 Operon primers were used. Among these 09 primers showed amplification and 03 primers amplified unambiguous, readable and showed polymorphic bands. A total of 631 amplification products were produced from the selected 09 primers and the number of bands varied from 1-11 with an average of 7.7 bands per primer. The bands which are more than 100 kb are selected for analysis. The details are presented in Table 1.

**Table 1: Analysis of genetic variation using random Operon primers**

| Primers | Nucleotide sequence (3' to 5') | Total bands | Polymorphic bands | Monomorphic bands |
|---|---|---|---|---|
| OPJ 10 | AAGCCCGAGG | 100 | 78 | 22 |
| OPJ 16 | CTGCTTAGGG | 67 | 38 | 29 |
| OPN 06 | GAGACGCACA | 121 | 96 | 25 |
| OPN 04 | AAGCGACCTG | 55 | 37 | 18 |
| OPN 16 | AAGCGACCTG | 88 | 60 | 28 |
| OPR 07 | ACTGGCCTGA | 88 | 70 | 18 |
| OPR 08 | CCCGTTGCCT | 101 | 90 | 11 |
| | TOTAL | 620 | 469 | 151 |

Each and every individual could be identified using gel profiles. A polymorphism was found among the various morphological mutants and mother plant indicating there was a high molecular variability among the mutants.

Putative morphological mutants amplified with OPN-16 showed the absence of major band at 800 bp shared by all morphological mutants was observed in lane 4. In lane 6, absence of the major band at 600 bp

shared by other morphological mutants were observed. When these mutants amplified with OPR 07 presence of a major band at 800 bp and absence of band at 2000 bp in lane 2 and 3 showed the presence of polymorphism among the various morphological mutants and 800 bp band was consistently observed in most of the other putative morphological mutants and mother plant (Plate 1 & 2).

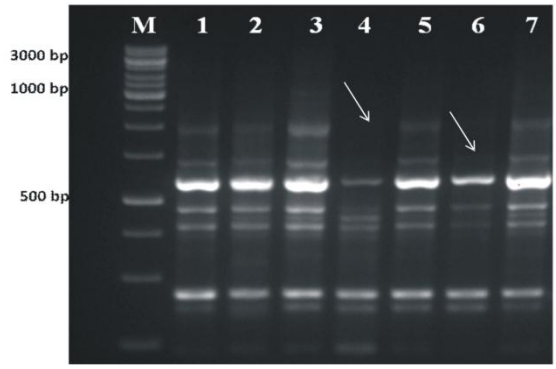


**Plate 1. RAPD profile of mother plant and mutants obtained with OPN 16**

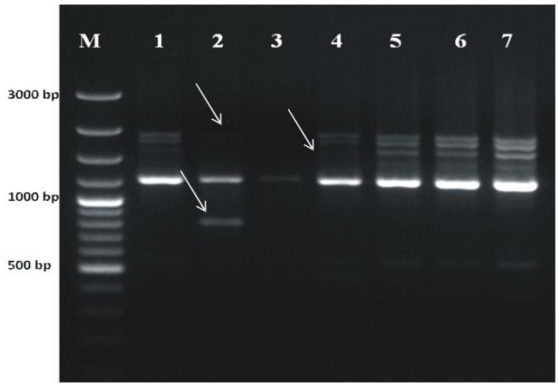


**Plate 2. RAPD profile of mother plant and mutants obtained with OPR 07**

**Legends; M-Ladder, 1-EMS at 0.30 % variant, 2-EMS at 0.60 % variant, 3-EMS at 0.90 % variant, 4-$NaN_3$ at 0.01 % variant, 5-$NaN_3$ at 0.02 % variant, 6- $NaN_3$ at 0.03 % variant, 7-Control mother plant**

Problems associated with clonal classification, and the various ways that molecular approaches can be utilized to overcome these difficulties, have been reported previously[1]. Our results confirmed that RAPD markers could be readily detected and analyzed for different banana putative mutants. Damasco *et al*[5]., successfully demonstrated the use of RAPD markers and detected a marker linked with dwarfness in Cavendish bananas. Crouch *et al*[4]., identified only a weak relationship between RAPD-based genetic and phenotypic similarities in study involving 76 plantain landraces. However, Engelborghs *et al*[6]., found a significant correlation between molecular diversity and morphotype grouping. Genetic similarities between the types ranged from 0.550 to 0.913 and genetic differences from 0.088 to 0.413, as determined by RAPD analysis[7]. The high levels of genetic polymorphism among banana types indicated that the RAPD technique can be useful in evaluating banana intra-varietal genetic variation types.

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