ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Biotechnology

ISSN 1682-296X
DOI: 10.3923/biotech.2019.9.14

Research Article
Effect of Somaclonal Variation in *Musa acuminata* cv. Berangan Through Micropropagation Using RAPD

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Abstract

Background and Objectives: Banana cv. Berangan is among the most important fruit plants in Malaysia. The study aimed to use benzylaminopurine (BAP) in the laboratory using a long cycle of time to detect somaclonal variation on morphological and genetic changes of banana cv. Berangan. **Materials and Methods:** Scalps culture from 15 mg L^{-1} of BAP at 20th subculture were cultured into MS medium with different concentrations of BAP (0, 5, 10 and 15 mg L^{-1}) until the 5th cycle to analysis the morphologies and number of shoots. Fourteen RAPD primers were used to detect between different BAP concentrations. **Results:** The result showed that the scalp culture was able to revive to normal shoot and had the highest number of shoots in control treatment after 5th cycles. Similarly, RAPD analysis suggested that there were genetic variations exist after the scalp developed into normal shoot in comparison to the other treatments. **Conclusion:** This study showed that BAP can produce morphological different such as rosette-like structure and scalp morphology after 20th subculture. Four of the 14 RAPD primers showed polymorphism between control and BAP treatment that BAP can lead to genetic variation in banana cultivar Berangan.

Key words: Somaclonal variation, banana cultivar, benzylaminopurine, berangan, genetic variations, scalp, fruit plants

Citation: Muslim Razani, Fatimah Kayat, Raimi Mohamed Redwan and Dwi Susanto , 2019. Effect of somaclonal variation in *Musa acuminata* cv. Berangan through micropropagation using RAPD. Biotechnology, 18: 9-14.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Banana is a first fruit crop that uses tissue culture for mass propagation and it is still multiplied until now as compared to other fruit crops with an annual production of more than two million[1,2]. In Malaysia, the banana is a first major fruit crop in term of area cultivated which comprises about 34,894.06 h and the total production[3] of 350,492.6 metric t in 2017. Plant growth regulators (PGR) are generally active at very low concentrations and naturally present inside the plant for growth and development[4]. Micropropagation is generally involved two important plant growth regulators. Firstly is cytokinin for inducing shoots and auxin for root differentiation. The PGR is important in plant tissue culture and a specific mode of growth can be developed by changing the amount and types of PGR in the medium to promote shoots and roots[5]. The rate of shoot multiplication is important in banana micropropagation for mass production especially in the commercial laboratory[6,7]. Shoot multiplication of plantlets in tissue culture depends on the presence or absence of PGR individually or in combination with the culture medium[5]. The higher the chances of abnormalities and not true-to-type plants can occur in the presence of high levels of cytokinins[8]. This condition can generate abnormalities which may lead to somaclonal variation[9]. The increase of cytokinin concentration in the culture medium did not increase the shoot length[10]. The higher concentrations of BAP can inhibit the banana plantlets growth which resulting in high abnormality index[7]. A high concentration of cytokinin can cause somaclonal variation in banana cv. Cavendish[11]. A high level of cytokinin did not cause somaclonal variation in the tissue culture for banana cv. Nanjanagudu[12]. Random Amplified Polymorphic DNA (RAPD) analysis was applied to detect DNA polymorphisms in tissue culture plantlets. It also to identify somaclonal variation arise from the tissue cultured banana varieties that were regenerated from the shoot tip culture[9]. In the present study, RAPD was used to identify the genomic differences associated with abnormalities that can lead to somaclonal variation in banana tissue culture plantlets. The first objective of this study was to study the morphological differences in banana tissue culture at a different BAP concentration and a number of subcultures. The second objective was to investigate the possible genetic changes appeared in abnormal banana tissue culture at the 25th subculture by using RAPD.

MATERIALS AND METHODS

Plant material: The banana micropropagated cultures of cultivar at 7th subculture (November 2014) were collected from the tissue culture laboratory at Kelantan Biotech Corporation, located in Machang, Kelantan, Malaysia and continue subculturing at Plant Tissue Culture Laboratory, Faculty of Agro Based Industry, Universiti Malaysia Kelantan, Jeli Campus, Kelantan, Malaysia.

Tissue culture: The experiment was starting in January 2016 until May 2016. The medium for the maintenance of culture for 1-10th subculture was followed the common practice by commercial laboratories using MS medium supplemented with 30 g L^{-1} of sucrose, 2.75 g L^{-1} of Gelrite and 5 mg L^{-1} of BAP[13]. The pH of all media was adjusted to 5.8 with NaOH. Autoclaved was performed at 121°C for 15 min at 15 psi. All cultures were maintained at 25±2°C with a photoperiod of 16 h day^{-1}. In order to produce scalps morphology in this study, the clump with multiple shoots was cultured into 15 mg L^{-1} of BAP from 11-20th subcultures. Clumps with scalp morphology (Fig. 1) in the cultures with a diameter ranging from 2.0-2.5 cm were divided into four (1.0-1.5 cm), at 20th subculture was used as starting material for subsequent treatment. Clumps with scalp morphology from 15 mg L^{-1} of BAP at 20th subculture with a diameter ranging from 1.0-1.5 cm were subjected to different BAP concentration (0, 5, 10 and 15 mg L^{-1}) in at least three replications with MS medium. The cultures produced were maintained at their respective treatments until 25th subculture and the morphological data were recorded for every 25 days. The leaves from each treatment were isolated for RAPD analysis after the 25th subculture.

Fig. 1: Clump with multiple shoots used as plant material at 20th subculture. (Bar = 1 cm)

Data and statistical analysis: All of the experiments were conducted in at least triplicates and set up in a completely randomized design. The statistical analyses were carried out using the IBM SPSS statistics. The means values and standard deviation (SD) were expressed based on the average of the 10 replicates. The Duncan's range test was used for dissociation of means.

DNA extraction: Genomic DNA was isolated using the CTAB method protocol[14]. The DNA qualification and quantification were checked and the samples were diluted with at 25 ng μL^{-1} concentration.

Random amplified polymorphism DNA (RAPD): The RAPD analysis and PCR methods were used according to the protocols[15,16]. The DNA amplification reactions were prepared in 25 µL reactions containing 100 ng μL^{-1} of banana genomic DNA, 1×PCR buffer, 200 µM of dNTPs, 2.5 mM $MgCl_2$, 10 pmoles of 14 random decamer oligonucleotide primers (OPA01, OPA02, OPA06, OPA15, OPA19, OPA21, OPA24, OPA25, OPC03, OPH09, OPJ04, OPJ10, OPJ13 and OPU06) and 1.25 U *Taq* DNA polymerase. The selection of RAPD primers was based on previous studies that can detect somaclonal variation in banana tissue culture[17-20].

RESULTS

Effect of different BAP and number of subculturing: This study showed the effect of different concentrations of BAP and number of subculture (21-25th) on the morphology of tissue culture plantlets (Fig. 2a-t). The scalp's production had reduced in treatment without BAP from the 23rd until 24th subculture at 29 and 14%, respectively (Table 1).

In 25th subculture, there was no scalp produced in the control as clump had transformed into normal individual shoots with the absence of BAP in the culture. This result indicated that the scalp culture can be reverted to normal shoot when BAP was absence. Furthermore, at 5 and 10 mg L^{-1} BAP concentration, the scalp formation had reduced from 57-50 and 50-49% in the 24th and 25th subculture, respectively. However, BAP only showed changes when the subculturing was began after three cycles (71%) and produced all normal shoots after five cycles in the treatment without BAP. In the scalp conditions, there was no shoots formation due to the high concentration of BAP that causes the structure changes to scalps morphology.

The number of the shoot was recorded at every cycle (25 days) until the five cycles. The highest number of shoots was in the control with 26.44±2.30 at 25th subculture

Table 1: Effects of different concentrations of BAP number of subcultures on morphologies

	Banana tissue culture morphology/concentration of BAP (mg L^{-1})											
	Normal shoot (%)				Rosette-like structure (%)				Scalp (%)			
No. of subcultures	0	5	10	15	0	5	10	15	0	5	10	15
21	0	0	0	0	0	0	0	0	100	100	100	100
22	0	0	0	0	0	0	0	0	100	100	100	100
23	71	0	0	0	0	0	0	0	29	100	100	100
24	86	0	0	0	0	43	50	0	14	57	50	100
25	100	0	0	0	0	50	51	0	0	50	49	100

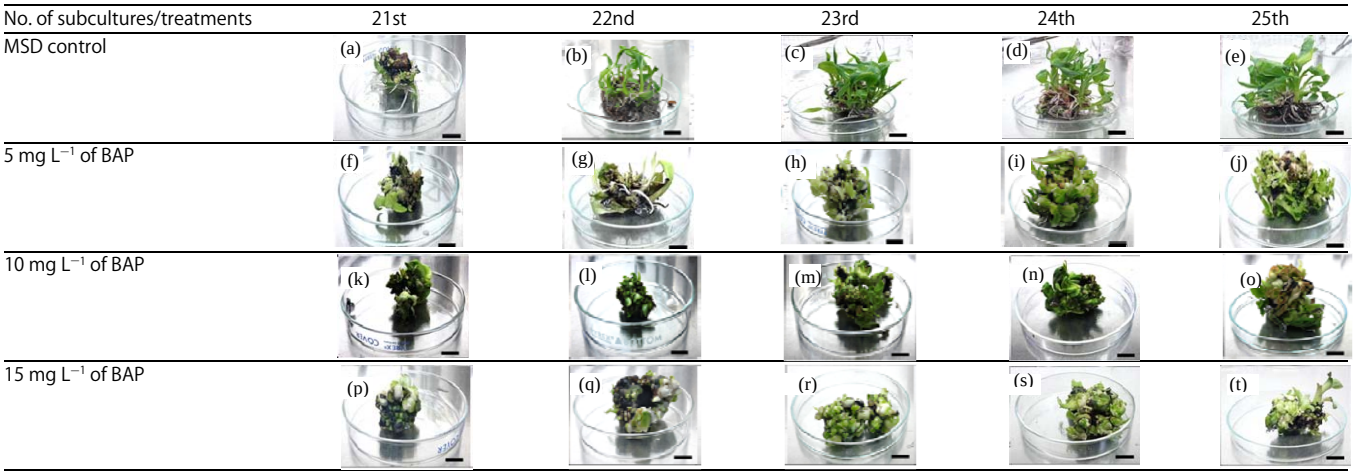

Fig. 2 (a-t): Morphology of shoot multiplication per clump (bar = 1 cm)

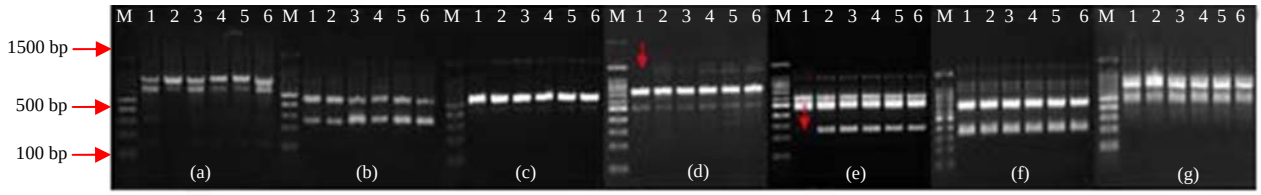

Fig. 3(a-g): RAPD profile primers (a) OPA01, (b) OPA02, (c) OPA06, (d) OPA15, (e) OPA19, (f) OPA21 and (g) OPA24, respectively at 25th subculture. The lanes are labelled as M: 100 bp ladder marker. Lane 1 -6 following Table 3

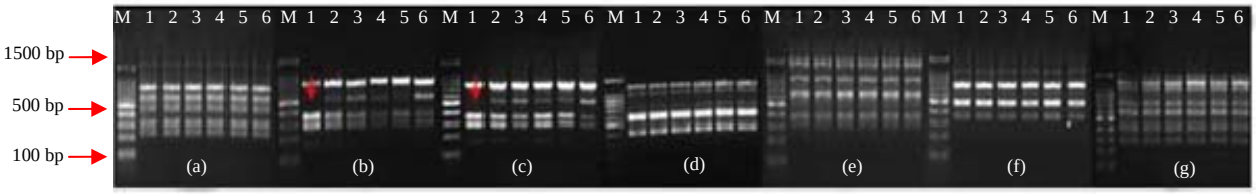

Fig. 4(a-g): RAPD profile primers (a) OPA25, (b) OPC03, (c) OPH09, (d) OPJ04, (e) OPJ10, (f) OPJ13 and (g) OPU06, respectively at 25th subculture. The lanes are labelled as M: 100 bp ladder marker. Lane 1 -6 following Table 3

Table 2: Mean number of shoots at different concentrations of BAP and number of subcultures

	Mean number of shoot per clump BAP (mg L^{-1})			
No. of subcultures	0	5	10	15
21	23.11 ± 3.22^{bc}	21.20 ± 3.36^{cd}	17.40 ± 2.91^{e}	6.90 ± 2.23^{f}
22	23.11 ± 3.22^{bc}	23.13 ± 3.56^{bc}	20.20 ± 1.93^{d}	7.89 ± 1.45^{f}
23	25.71 ± 3.10^{ab}	24.00 ± 2.77^{ab}	20.63 ± 3.46^{cd}	8.09 ± 1.87^{f}
24	26.43 ± 2.38^{a}	24.00 ± 2.77^{ab}	20.88 ± 3.91^{cd}	8.09 ± 1.87^{f}
25	26.44 ± 2.30^{a}	25.33 ± 2.24^{ab}	21.13 ± 3.04^{cd}	8.95 ± 1.91^{f}

Mean with different letters indicate values are significantly different ($p \leq 0.05$) by Duncan's Multiple range test, Values are Mean±standard deviations based on at least seven replicates

Table 3: Name produced by different morphologies and concentrations of BAP

No.	BAP concentration (mg L^{-1})	Morphology
1	0	Normal shoot
2	5	Rosette-like structure
3	5	Scalp
4	10	Rosette-like structure
5	10	Scalp
6	15	Scalp

(Table 2). When clumps with scalp morphology were cultured in 5, 10 and 15 mg L^{-1} of BAP, the number of shoots increased from the 21st until 25th cycles. There was an increased trend in the shoot multiplication in the clump culture for 5 mg L^{-1} of BAP treatment. The number of shoots in 10 mg L^{-1} of BAP treatment showed increased in number and at the 21st subculture with 17.40±2.91 up to 21.13±3.04 at the 25th subculture. There was a significantly lowered number of shoots in the 15 mg L^{-1} of BAP as compared to other treatments.

RAPD analysis: In the second experiment that used scalp morphology from 15 mg L^{-1} of BAP at 20th subculture, four (OPA15, OPA19, OPC03 and OPH09) out of 14 primers that were used in the previous experiment showed a unique banding pattern that differentiates between the control and the BAP treated scalp after five subcultures with the arrows represent as a missing band as shown in Fig. 3a-g and 4a-g. The lanes for the RAPD results as listed in Table 3. Primer OPA15 showed a missing band of 1,500 bp in the control but the presence in all the BAP treatments (Fig. 3d). In addition, one band at 300 bp was disappeared for the primers OPA19 (Fig. 3e). In addition, one band at 550 and 500 bp were disappeared for the primers OPC03 (Fig. 4b) and OPH09 (Fig. 4c), respectively. These unique banding patterns suggested that there were some genetic changes occurred in the culture.

DISCUSSION

The use of plant growth regulator such as BAP at a certain concentration can induce somaclonal variation[9]. High level of BAP can cause abnormalities which resulted in the production of non-true-to-type plants[21]. In the present finding, the

observed abnormalities were rosette-like and scalp which produced by 10 and 15 mg L^{-1} BAP treatments at the 21-25th subcultures (Fig. 2). Jafari *et al.*[22] reported that the 33 μM of BAP produced the highest number of abnormalities and also produced rosette-like and scalp morphologies in banana cv. Berangan after 7 months of culture. The scalp cultures still in its form when maintained in the same concentration of BAP (15 mg L^{-1}) and it can be used to produce scalp formation as long as it remains in the cultures for 15 cycles (Fig. 2p-t). According to the present finding, BAP was the main influence that changes the number of shoots for each treatment. A higher concentration of BAP (15 mg L^{-1}) had produced the least number of shoots as compared to the other treatments. However, further treatment without BAP leads to the highest number of shoots after 23rd subculture. This may due to the decreased of BAP concentration effect in the banana clump cultures. Only 15 mg L^{-1} of BAP treatment showed the lowest number of shoots in the cultures and this result may be related to the morphological changes in the formed of the cultures due to the effects of BAP. At 100 μM of BAP, the banana cv. Tanduk produced scalp morphology after 4th subculture[23]. However, banana cv. Berangan only showed the production of scalp morphology after 4 months of culture using 11.1 μM of BAP[24]. In this study, the banana tissue culture from scalp cultures required the time of subculturing to change the morphology to the rosette-like structure.

In order to confirm the genetic stability of different morphology and number of subculturing, RAPD analysis was used to detect any genetic changes. However, there were no markers associated with different morphology displayed between different BAP treatments. This outcome can be detected between BAP treatments and control. The genetic changes from scalp morphology to the normal shoots showed the different band in the RAPD analysis at a 25th subculture. However, the genetic changes between scalp morphology and the rosette-like structure in the BAP treatments at 25th still were still unknown. There were many successful reports had been recorded for the use of the RAPD technique for detection of somaclonal variants in banana tissue culture[25-27]. However, there was no somaclonal variation at high levels of cytokinin in tissue culture for banana cultivar Nanjanagudu[21]. Many successful reports on the use of RAPD technique in the detection of presence or absence of somaclonal variants of banana tissue culture were reported[21,26,27]. High levels of BAP concentration (58.5 μM) in banana cv. Nanjanagudu indicated that there was no somaclonal variation observed[25]. No genetic variation using RAPD for banana cv. Williams comparing with their mother plant[28]. However, several RAPD markers able to differentiate between the control and BAP treatments in this study. Polymorphism among banana tissue culture in this study might be associated with pre-existing recessive traits or transposable elements. This study discovered the scalp morphology at 20th subculture can revert back to the normal plantlets after the fifth cycle in the MS medium without BAP and RAPD analysis only detected between BAP and without BAP treatments.

CONCLUSION

The scalp culture from 20th in 15 mg L^{-1} of BAP also had produced three morphological differences such as normal shoot, rosette-like structure and scalp subculture after five cycles of subcultures. The molecular marker using RAPD results obtained in this study confirmed the difference between BAP treatments and without BAP treatment at 25th subculture.

SIGNIFICANCE STATEMENT

There are many studies on this banana tissue culture but there is limited study after 10th subculture. In this experiment, the subculture was continued until the 25th subculture to identify the morphological and genetic changes that occur among the banana cultures. Abnormal morphology such as scalp can return back to their normal plantlet after cultured in treatment without BAP in fifth cycles and showed genetic different using RAPD analysis. This study discovered the morphology and genetic changes for banana culture at 25th subculture that can be beneficial for researchers and commercial plant tissue culture laboratories. Thus, the outcome of this study also can be used for crop improvement in the future.

ACKNOWLEDGMENTS

The authors would like to express sincere gratitude to RAGS-UMK (R/RAGS/A07.00/00626A/002/2013/000124) and Skim Zamalah Khas - MyRA, Universiti Malaysia Kelantan for fully funded this project.

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