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Full Length Article

Detection of Abnormal Banana Plantlets Produced by High BAP Concentration and Number of Subcultures using Representational Difference Analysis

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Abstract

The disadvantage of somaclonal variation is mainly related to its limitation to provide disease-free and uniform plantlet in a large quantity and within a short duration. However, this technique faced a limited number of subculture and the use of the plant growth regulator to induce the shoot formation during propagation time. Hence, the objective of this study is to investigate the variation occur during prolong subculture with the increase of 6-benzylaminopurine (BAP) concentration, at morphological and molecular level. The results showed that the prolong subculture at 15 mg L^{-1} BAP had inhibited the shoot formation. The higher number of shoots per clump was observed at 5 mg L^{-1} at the 20th subculture. Genetic variation between clones was assessed by Representation Difference Analysis (RDA) technique. It was found that there was no genetic variation that can be amplified even though between the control and the BAP-treated explant. This implied a very small genetic variation had occurred among clones. The findings of this study provided a basis for further investigation to a genomic region in banana, that was susceptible to stress raised in tissue culture stage, especially when used higher concentrations of BAP (15 mg L^{-1}) and number of subcultures. © 2020 Friends Science Publishers

Keywords: Banana; BAP; Number of subcultures; RDA; Somaclonal variation

Introduction

Bananas are grown worldwide in more than 100 countries for their fruit, fibre and as ornamental plants (Nelson *et al.* 2006). It is widely consumed globally and also uses as an important staple food in many developing countries, especially Africa. Besides, banana provides an important source of basic nutrients for human nutrition. In 2016, it covers about 16.9% of the total acreage for fruit production with the estimation value of 309,507.6 metric tons of fruit in Malaysia (DOA 2017). However, a major concern arises which related to the existence of somaclonal variation in tissue culture plantlets. This problem can affect the use of tissue culture technique and become a limitation in the banana industry (Bairu *et al.* 2011).

The use of BAP as a plant growth regulator had provided various advantages in shoot tip cultures, particularly in promoting the growth of axillaries, adventitious buds and foliar development (Abeyaratne and Lathiff 2002; Buah *et al.* 2010). Besides, abnormalities and off-type plants might occur due to a high level of cytokinins, which can lead to somaclonal variation (Martin *et al.* 2006; Aremu *et al.* 2013). Previous research on banana cultivar Nanjanagudu Rasabale showed that the optimum concentration of BAP required for commercial production was 5 mg L^{-1} or 22.2 μ*M* (Bairu *et al.* 2008; Lalrinsanga and Vanlaldiki 2013). Bairu *et al.* (2008) also reported that the higher BAP concentration can inhibit the growth of banana plantlets which resulting in the high abnormality index. Similarly, application of 10 μ*M* BAP can cause somaclonal variation with the formation of the scalp in the plantain cultivar Bluggoe (Dhed'a *et al.* 1991). Furthermore, the scalp formation of *Musa* species was within 7–9 months (Strosse *et al.* 2003) and 8–12 months for plantain cultivar Spambia (Sholi *et al.* 2009). It has been known that the scalps morphology in banana tissue culture produced a somaclonal variation that is used for good or bad traits (Juli and Khalid 2002; Elhory *et al.* 2009; Shirani *et al.* 2010). In another finding, Sheidai *et al.* (2008) had reported that a number of subculturing had led to the production of somaclonal variation in banana tissue culture.

Molecular markers have been reported to use as tool for early detection of somaclonal variation at nursery stage to avoid commercial loss and save the time consumption during detection at field level (Bairu *et al.* 2011; Nwauzoma and Jaja 2013). In a banana tissue culture study in laboratory

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scale, somaclonal variation occurred after 7th subcultures which considered as the longest period for banana tissue culture (Jambhale *et al.* 2001; Borse *et al.* 2011; Devi *et al.* 2017). However, if the culture is kept for more than 7th subculture, it will reduce the cost and time consumption for a new batch in banana tissue culture. In the present study, the genetic of molecular change of explant to the 20th subculture was assessed in order to investigate the incidence of the somaclonal variant in banana tissue culture. For this reason, molecular marker is the best approach to detect somaclonal variation at the genetic level as this technique provides information on genetic variation among different individuals (Bairu *et al.* 2011). Representational Difference Analysis (RDA) is a subtractive methodology that combines the hybridization process and PCR. This method detects the differences between two complex genomes that associated with the genomic losses, rearrangement, mutation, amplification and pathogenic expression (Lisitsyn *et al.* 1993; Lisitsyn, 1995; Michiels *et al.* 1998). The potential of RDA as a molecular marker to detect early variation of Cavendish banana through tissue culture had been reported by many researchers (Cullis and Kunert 1999; Cullis and Kunert 2000; Oh *et al.* 2007). This method was also being done on other plant species such as date palm (Vorster *et al.* 2002; Johnson *et al.* 2009), oil palm (Cullis *et al.* 2007; Sarpan *et al.* 2013), rice (Panaud *et al.* 2002; Shim *et al.* 2010) and oak (Zoldos *et al.* 2011).

In the present study, RDA were used to identify the genomic differences associated with abnormalities that can lead to somaclonal variation in banana tissue culture plantlets. The objective of this study is to identify the morphological differences in banana tissue culture at a different BAP concentration and a number of subcultures. The second objective is to investigate the possible genetic changes appeared in abnormal banana tissue culture at the 20th subculture by using RDA.

Materials and Methods

Plant materials

Banana cv. Berangan tissue culture clumps with multiple shoots in 5 mg L^{-1} BAP media at the 7th subculture were collected from a commercial tissue culture laboratory of the Kelantan Biotech Corporation, Malaysia. After 10th subculture, clumps with a diameter range from 1 to 1.5 cm were used for the subsequent experiments with three replications for each treatment (Fig. 1). The cultures were maintained in the laboratory until another 10 subcultures. The basal medium contained the full strength of MS medium (Murashige and Skoog 1962) supplemented with 3% (w/v) sucrose and 0.275% (w/v) Gelrite® was prepared. The pH of the media was adjusted to 5.8 and autoclaved at 121°C for 15 min at 15 psi. All cultures were maintained at 25 ± 2°C with 16-h photoperiod per day. For every 25 days, multiple shoots were cultured in the fresh MS medium supplemented with 5 mg L^{-1} of BAP for multiplication.

Effect of different concentrations of BAP and number of subcultures

Banana clumps were cultured in a solid media consisted of the basal medium that supplemented with 5 mg L^{-1} of BAP for three cycles before subjected to 3 different concentrations of BAP (5, 10, and 15 mg L^{-1}), while MS without BAP was used as a control. The morphological parameters that were observed after 25 days of subculture comprised of the percentage of morphological difference (normal shoot, rosette-like structure and scalp), number of shoots per clump and number of scalps per clump. The clump culture with shoot, leaves and root were defined as a normal shoot. The rosette-like structure was defined as a circular arrangement of leaves or structures that resembled the leaves. The scalp contained many bulbous components, generating white tissues and appeared like cauliflower with the very high rate of proliferation derived from shoot tip culture (Dhed'a *et al.* 1991; Villalobos and Garcia 2008; Sholi *et al.* 2009). Cultures were maintained for another 10th cycle (11th to 20th subcultures) and morphological data were recorded for each subculture. Fresh leave samples of randomly selected shoots were collected at the 20th subculture and used as a source of genomic DNA to determine any possible genetic changes with control was collected from the cultures on BAP free medium.

DNA extraction

Genomic DNA was isolated using the CTAB method following the modified protocol of Doyle and Doyle (1987). The DNA qualification and quantification were checked and the samples were diluted with at 25 ng μL^{-1} concentration.

Representational different Analysis (RDA)

The RDA analysis was carried out following Lisitsyn *et al.* (1993) by DNA from normal plantlets was used as driver DNA while DNA from the off-type plant was used as tester DNA. The RDA experiment was designed with two treatments for driver and tester. Positive control in this experiment was normal shoot being used as a tester and driver (Table 1).

Statistical analysis

All experiments were conducted in at least triplicates and set up in a completely randomized design. The statistical analyses were carried out using the IBM S.P.S.S. Statistics. The means values and standard deviation (SD) were expressed based on the average of the three replicates. The Duncan's Range Test was used for dissociation of means.

Results

The effect of different BAP concentrations (0, 5, 10 and 15

mg L^{-1}) and a different number of subcultures from 11th until 20th subculture for normal shoot formation in MS media was evaluated. Results indicated that control group showed higher percentage of normal shoot formation as compared to in 5, 10 and 15 mg L^{-1} BAP treatments at 20th subculture (Table 2). The entire clump in control showed normal shoot at 15th subculture. Meanwhile, the normal shoots were not observed in BAP treated clumps until the 20th subculture. In this experiment, the rosette-like structure was observed in banana clumps cultured with 5 and 10 mg L^{-1} of BAP treatments after the 16th subculture onwards (Table 2). The percentage was increased continuously from 16th subculture onwards, which at 76.2% and 27% of the culture turn to rosette in the 5 and 10 mg L^{-1} BAP treatments, respectively. The highest rosette formed was recorded at 5 mg L^{-1} BAP treatment as compared to 10 mg L^{-1} BAP treatment. Besides, at the 20th subculture, the rosette in 5 mg L^{-1} and 10 mg L^{-1} of BAP were 95.9 and 57.6%, respectively.

For the scalp formation, it was observed in all treatments and control at 11th to 14th subculture. As the number of subcultures increased, the percentage of scalp structure was decreased due to the development of either normal shoot or rosette-like structure for 0, 5 and 10 mg L^{-1} BAP treatments. However, clumps were maintained in their scalp structure for 15 mg L^{-1} of BAP treatment until 20th subculture. The percentage of scalp structure was decreased from 41.7% at 11th subculture to 30.8% at the 14th subculture in the control treatment before it completely changed into normal shoots at 15th subculture onwards. The percentage of the scalp for 5 and 10 mg L^{-1} of BAP were optimum (100%) starting from the 11th until the 15th subculture. However, at 16th subculture, 5 mg L^{-1} of BAP treatment was decreased at about 3 quarters from 100% to 23.8%. A similar trend was also observed for 10 mg L^{-1} of BAP with a reduction at about one-quarter from 100% to 72.4%.

Results indicated that different BAP concentration leads to different morphology of banana clump (Fig. 2). Other than the production of normal shoots (Fig. 2–1a), the rosette-like structure (Fig. 2–2a) and scalp (Fig. 2–3a and Fig. 2–4a) also contained the small shoot. The shoot multiplication of banana cv. Berangan in different BAP concentration (0, 5, 10 and 15 mg L^{-1}) at 11th to 20th subcultures were recorded. It was observed that the highest number of shoots per clump was 8.75 ± 0.62 at 16th subculture, 22.1 ± 2.51 at 20th subculture, 11.3 ± 1.83 at 20th and 1.31 ± 0.95 at 14th subculture for 0, 5, 10 and 15 mg L^{-1} BAP respectively (Table 3). Based on the statistical data analysis, there was a significant difference between different BAP concentrations and a different number of subcultures on a number of shoots per clump. At 5 and 10 mg L^{-1} BAP treatments had shown similar increment in the number of shoots per clump from 11th subculture to 20th subculture. The number of shoots produced was significantly differed ($P < 0.05$) between control and 15 mg L^{-1} BAP treatment. Meanwhile, the number of shoots in medium supplemented with 15 mg L^{-1} of BAP started to reduce from 11th (1.31 ± 0.95) to 20th (0.31 ± 1.09) subculture. The highest mean number of shoots per clump was observed at 5 mg L^{-1} BAP treatment at 20th subculture with the mean value of 22.10 ± 2.51.

Table 1: Driver and tester used for RDA analysis

Treatment	Driver	Tester
1 (Control)	Normal shoot	Normal shoot
2	Normal shoot	Rosette-like structure
3	Normal shoot	Scalp
4	Scalp	Normal shoot
5	Rosette-like structure	Normal shoot
6	Banana cv. Tanduk	Normal shoot
7	Normal shoot	Banana cv. Tanduk

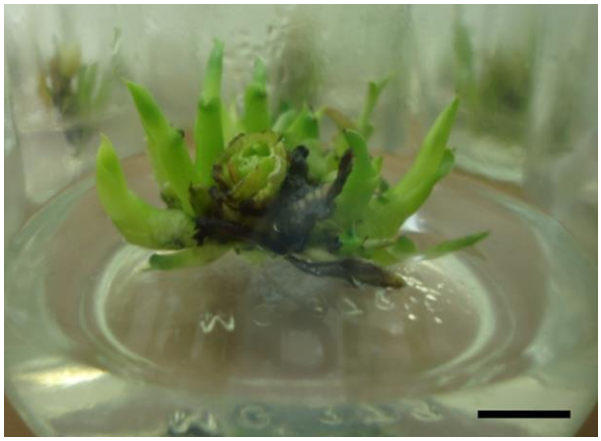

Fig. 1: Clump with multiple shoots used as plant material at 10th subculture. (Bar = 1 cm)

The RDA method in this experiment was developed as a result of unique genomic identification of different products from somaclonal variation that could be selected into DNA markers. This can be done by comparing of two representations (amplicon) for the identification of different abnormal morphologies in banana tissue culture. The RDA method was selected in order to differentiate between abnormal morphologies. The present study attempts to investigate that different product were isolated as the normal (control) used as a tester and the abnormal morphology used as a driver. The first step of RDA started with the preparation of amplicons or representation for driver and tester from two DNA samples by cutting using restriction enzyme following Lisitsyn (1995).

The result showed that there were no genetic differences between the clones despite the observed phenotypic variation at the tissue culture stages. In order to confirm the present finding, positive control was used in the next first round of subtraction, where the tester to driver ratio was 1 to 100. The use of different cultivars in this study act as a positive control. The results showed that only positive control from another cultivar had produced subtraction product which was proven successful in isolation of genome differences between bananas from

Table 2: Banana morphology; (Normal shoot, rosette-like structure and scalp) Produced at different concentrations of BAP and different numbers of subculturing

Number of subcultures	Normal shoot (%)				Rosette-like structure (%)				Scalp (%)			
	Concentration of BAP (mg L^{-1})				Concentration of BAP (mg L^{-1})				Concentration of BAP (mg L^{-1})			
	0	5	10	15	0	5	10	15	0	5	10	15
11	58.3	0	0	0	0	0	0	0	41.7	100	100	100
12	58.3	0	0	0	0	0	0	0	41.7	100	100	100
13	69.2	0	0	0	0	0	0	0	30.8	100	100	100
14	69.2	0	0	0	0	0	0	0	30.8	100	100	100
15	100	0	0	0	0	0	0	0	0	100	100	100
16	100	0	0	0	0	76.2	27.6	0	0	23.8	72.4	100
17	100	0	0	0	0	87.5	34.3	0	0	12.5	65.7	100
18	100	0	0	0	0	88.6	45.5	0	0	11.4	54.5	100
19	100	0	0	0	0	90.4	51.4	0	0	9.6	48.6	100
20	100	0	0	0	0	95.9	57.6	0	0	4.1	42.4	100

Fig. 2: Morphology of shoot multiplication per clump on control (MS0) treatments (1a) – (1j) = 11th – 20th subculture, 5 mg L-1 of BAP (2a) – (2j) = 11th – 20th subculture, 10 mg L-1 of BAP (3a) – (3j) = 11th – 20th subculture and 15 mg L-1 of BAP (4a) – (4j) = 11th – 20th subculture on MS solid media for banana cv. Berangan (bar = 1 cm)

different cultivar (Fig. 3). These results suggested that this technique was able to isolate genome differences, but not sufficiently sensitive to isolate the genomic difference between clones that derived from the same line.

Discussion

The effects of different concentration of BAP were investigated in the clumps culture of banana cultivar Berangan after 10th until 20th subculture. The morphological changes in this experiment were according to the morphological appearances. The abnormalities in banana tissue culture were a major concern associated with a somaclonal variation that can lead to non-uniform and off-type cultures (Bairu *et al.* 2011; Sahijram 2015; Krishna *et al.* 2016). In plant tissue culture industry, somaclonal variation can reduce the quality and commercial price in the market (Cullis 2005; Vroh-Bi *et al.* 2011; Abdellatif *et al.* 2012). Somaclonal variation of banana plantlet also can reduce the price value due to the lack of fruit quality and unhealthy plants (Bakry *et al.* 2009). In the present study, there were two types of morphological abnormalities have been observed which comprised of rosette-like and scalp at different concentration of BAP. These morphological abnormalities were reported in the previous study (Shirani *et al.* 2009; Sipen and Davey 2012). The use of plant growth regulator like BAP at a certain concentration in the medium can induce somaclonal variation (Martin *et al.* 2006). Additionally, a higher level of BAP can cause abnormalities which resulting in the production of not true-to-type plants (Venkatachalam *et al.* 2007). In present finding, abnormalities were observed at 5 and 10 mg L^{-1} BAP treatments, producing rosette-like and scalp at 11th and 20th subcultures respectively. Furthermore, at 15 mg L^{-1} of BAP treatment, abnormalities occurred and produced scalp morphology at 11th to 20th subculture. Jafari *et al.* (2011) stated that the use of 33 μM of BAP had produced the highest number of abnormalities which were the rosette-like

Table 3: The mean number of banana cv. Berangan shoots produced per clump at different concentrations of BAP

Number of subcultures	BAP Concentrations (mg L^{-1})			
	0	5	10	15
11	8.08 ± 0.76^{ij}	8.17 ± 0.72^{ij}	2.31 ± 0.48^{op}	1.31 ± 0.95^{pq}
12	8.08 ± 0.76^{ij}	8.50 ± 0.52^{ij}	2.69 ± 0.95^{no}	1.31 ± 0.95^{pq}
13	8.67 ± 0.79^{ij}	9.00 ± 1.13^{hi}	3.46 ± 0.66^{mn}	1.31 ± 0.95^{pq}
14	8.41 ± 0.79^{ij}	9.75 ± 1.42^{gh}	$4.15 \pm 0.9l^{m}$	1.31 ± 0.95^{pq}
15	8.57 ± 0.51^{hij}	11.15 ± 0.67^{f}	4.56 ± 1.16^{l}	1.13 ± 0.99^{q}
16	8.75 ± 0.62^{ij}	12.29 ± 1.15^{de}	5.83 ± 0.71^{k}	0.67 ± 0.90^{q}
17	8.45 ± 0.6^{ij}	12.73 ± 1.30^{d}	7.57 ± 0.65^{j}	0.68 ± 0.89^{q}
18	8.23 ± 0.69^{ij}	14.40 ± 1.65^{c}	8.54 ± 0.55^{ij}	0.48 ± 0.77^{q}
19	8.37 ± 0.49^{ij}	19.66 ± 2.43^{b}	10.51 ± 0.95^{fg}	0.36 ± 1.04^{q}
20	8.43 ± 0.5^{ij}	22.10 ± 2.51^{a}	11.32 ± 1.83^{ef}	0.31 ± 1.09^{q}

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 ten replicates

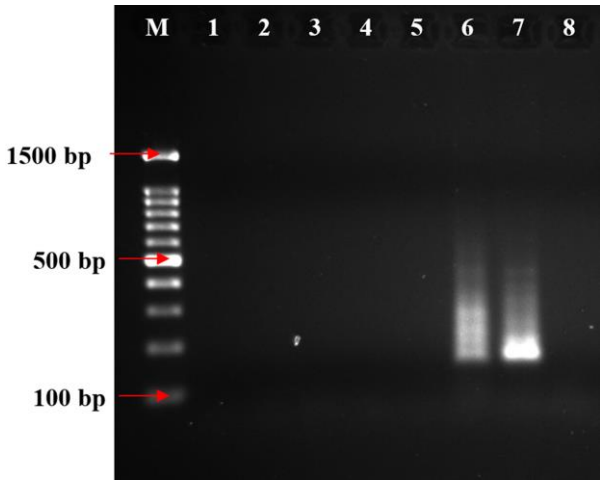

Fig. 3: Amplification of first hybridization [driver (100): tester (1)] after treated by mungbean nuclease using JBam24 primer for different morphologies as a driver and tester of banana cv. Berangan after separation on a 1.5 % agarose gel. [M = 100 bp ladder, lane 1 = driver (normal shoot) tester (normal shoot), lane 2 = driver (normal shoot) tester (rosette like structure), lane 3 = driver (normal shoot) tester (scalp), lane 4 = driver (rosette like structure) tester (normal shoot), lane 5 = driver (scalp) tester (normal shoot), lane 6 = driver (normal shoot) tester (Banana cv. Tanduk), lane 7 = driver (Banana cv. Tanduk) tester (normal shoot) and lane 8 = negative control (water)]

and scalp morphologies in banana cultivar Berangan after 7 months of culture. Moreover, Elhory *et al.* (2009) reported that banana cultivar Tanduk produced scalp morphology in 100 µ*M* of BAP after 4th subculture. However, in different finding by Shirani *et al.* (2010) the formation of scalp morphology in banana cultivar Berangan was recorded after 4 months of culture using 11.1 µ*M* of BAP.

The shoot multiplication of banana cultivar Berangan was counted per clump at different concentration of BAP (0, 5, 10 and 15 mg L^{-1}) after 10th until 20th subculture. These results showed that different concentration of BAP had produced a significantly different number of shoots. Treatment with 5 mg L^{-1} of BAP also produced a significantly higher number of shoots as compared to 10 and 15 mg L^{-1} of BAP. These results were similar to other findings that indicated that the optimal number in terms of shoot multiplication was observed in BAP concentration of 5 mg L^{-1} (McAlister *et al.* 2005; Bairu *et al.* 2008; Strosse *et al.* 2008). Previous reports highlighted that the different concentration of cytokinins leads to significant results on shoot multiplication rate (Arinaitwe *et al.* 2000; Gubbuk *et al.* 2004). Bhosale *et al.* (2011) had obtained 3 shoots per explant with 5 mg L^{-1} BAP for banana cultivar Ardhapuri, Basrai and Shrimanti on MS solid medium. However, a study by Shirani *et al.* (2010) had produced 8 and 10 shoots per explant for banana cultivar Berangan and Rastali after 60 days of culture. According to Jambhale *et al.* (2001), the rate of shoot multiplication on banana subculture was declined from 1st until 8th subculture and the plants became stunted after 14th subculture. However, in present finding, the shoot multiplication in 5 mg L^{-1} of BAP was increased from 11th to 20th of subculture.

In addition, treatment with 10 mg L^{-1} of BAP produced 19.9 shoots as compared to treatment with 3 mg L^{-1} of BAP after two subcultures for banana cultivar Grand Naine (Matsumoto *et al.* 2006). According to Bairu *et al.* (2006), high multiplication rate using plant growth regulators can lead to more variation. However, Bairu *et al.* (2008) stated that at 5 mg L^{-1} of BAP did not cause any effect of a somaclonal variation on the 5th subculture. The number of shoots multiplication increased with the number of subcultures using BAP as a plant growth regulator (Muhammad *et al.* 2004; Mukunthakumar *et al.* 2010). Sheidai *et al.* (2008) reported that a minimal number of subculturing processes may reduce the percentages of somaclonal variation in banana tissue culture. Mohamed (2007) suggested that somaclonal variation rate was higher after 6th clonal generation. In the present study, it was observed that 15 mg L^{-1} of BAP treatment showed inhibitory effects due to one or no shoot produced. Hence, it can be stated that the inhibitory effect was related to shoot multiplication of banana tissue culture. The study by many researches supported that the BAP concentration treatment causes the inhibitory effect on the shoot multiplication rate (Gubbuk *et al.* 2004; Bairu *et al.* 2008; Strosse *et al.* 2008).

The scalps were commonly used as a plant material for cell suspensions (Sholi *et al.* 2009), plant transformation (Acereto-Escoffié *et al.* 2005) and somatic embryogenesis culture (Strosse *et al.* 2006). According to Strosse *et al.* (2006), a high concentration of BAP can produce scalps after 9th subculture depending on the cultivar used. Besides, Sadik *et al.* (2007) reported that the used 100 µ*M* BAP can induce scalps for banana cultivar Musakala, Kibuzi, Mbwazirume and Lwandugu. These findings support the results of present study as the culture remained in scalp morphology when higher concentrations of BAP were used after 20th subculture. The frequent subculturing process to a new fresh medium can reduce phenolic compound excreted by banana plantlets by cutting off an unnecessary leaf, root or rhizome (Srangsam and Kanchanapoom 2003). It was

also shown that increased rate of shoots multiplication for every clonal generation in banana tissue culture (Bairu *et al.* 2006; Rout *et al.* 2009; Sadik *et al.* 2012). In addition, the profit in a banana tissue culture industry depends on the shoot multiplication rate for every number of subcultures (Chavan-Patil *et al.* 2010). The number of shoots multiplication rate increased with the increased number of subcultures using BAP as a plant growth regulator (Muhammad *et al.* 2004; Mukunthakumar *et al.* 2010). Sheidai *et al.* (2008) reported that the number of subculturing processes may produce a few percentages of somaclonal variation in banana tissue culture. However, Mohamed (2007) suggested that the somaclonal variation rate was only high after 6th clonal generation. According to Strosse *et al.* (2006), a high concentration of BAP can cause scalps formation after 9th subculture depending on the cultivar used.

The advantage of RDA was mainly in term of classification of specific polymorphisms in a specific individual by pooled DNA samples, rather than depending on the identification based on a particular pattern of polymorphic bands (Vorster *et al.* 2002). However, the disadvantage of RDA was related to a test on a small portion of the entire genome, which only revealed on some part of differences between the DNA tested (Cullis *et al.* 2002). The digested genomic DNA of three morphologies differences (normal shoot, rosette and scalp) with *Hpa*II showed incomplete digestion which was not appropriate for amplicon preparation. The *Bam*HI was tested and it showed a complete digestion after being viewed on agarose gel electrophoresis. The right selection of restriction enzymes to produce representational (amplicon) is a key success in RDA (Sarpan *et al.* 2013). According to Chang (2002), for optimal PCR amplification, the restriction fragments must between 200 to 1000 bp. Besides, Lisitsyn *et al.* (1993) suggested that each representation contained 2 to 15 % of the genome. This percentage was based on the restriction endonuclease site and efficiency of amplification using PCR to regenerate fragments. However, not all RDA will show a possible different product between two genomes (Chang 2002). The selection of restriction enzyme can be improved by sequencing of more RDA fragments from subtractions to develop a larger set of restriction enzymes in order to allow sufficient selection of informative differences (Sarpan *et al.* 2013). Another study showed that the RDA analysis can be distinguished by different interspecies such as rice (Panaud *et al.* 2002; Shim *et al.* 2010) and date palm (Vorster *et al.* 2002). In order to completely understand the changes in the somaclonal variation of abnormal banana tissue, particularly with different morphologies, more works need to be conducted in the future.

Conclusion

Based on the current finding, banana clumps from 11th – 20th subculture of banana cv. Berangan had been successfully carried out. Three morphologies different were derived from the different concentration of BAP and number of subcultures had produced the normal shoot, rosette-like structure and scalp in the culture at 20th subculture. RDA verification failed to indicate any changes in different morphologies of abnormal banana tissue culture at 20th subculture.

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References

Abdellatif KF, AE Hegazy, HM Aboshama, HA Emara, AA El-Shahed (2012). Morphological and molecular characterization of somaclonal variations in tissue culture-derived banana plants. *J Gen Eng Biotechnol* 10:47–53

Abeyaratne WM, MA Lathiff (2002). *In-vitro* propagation of 'Rathambala' (*Musa* AAA) and the occurrence of phenotypic variations in the pseudostem. *Ann Sri Lanka Dept Agric* 4:191–197

Acereto-Escoffié POM, BH Chi-Manzanero, S Echeverría-Echeverría, R Grijalva, AJ Kay, T González-Estrada, E Castaño, LC Rodríguez-Zapata (2005). *Agrobacterium*-mediated transformation of *Musa acuminata* cv. ''Grand Nain'' scalps by vacuum infiltration. *Sci Hortic* 105:359–371

Aremu AO, MW Bairu, L Szucova, K Dolezal, JF Finnie, JV Staden (2013). Genetic fidelity in tissue-cultured 'Williams' bananas – The effect of high concentration of topolins and benzyladenine. *Sci. Hortic*., 161: 324–327

Arinaitwe, G., P.R. Rubaihayo and M.J.S. Magambo, 2000. Proliferation rate effects of cytokinins on banana (*Musa* spp.) cultivars. *Sci Hortic* 86:13–21

Bairu MW, AO Aremu, JV Staden (2011). Somaclonal variation in plants: causes and detection methods. *Plant Growth Regul* 63:147–173

Bairu MW, CW Fennell, JV Staden (2006). The effect of plant growth regulators on somaclonal variation in Cavendish banana (*Musa* AAA cv. 'Zelig'). *Sci Hortic* 108:347–351

Bairu MW, WA Stirk, K Dolezal, JV Staden (2008). The role of topolins in micropropagation and somaclonal variation of banana cultivars 'Williams' and 'Grand Naine' (*Musa* spp. AAA). *Plant Cell Tiss Org Cult* 95:373–379

Bakry F, F Carreel, C Jenny, JP Horry (2009). Genetic improvement of banana. *In: Breeding Plantation Tree Crops: Tropical Species*, pp: 3–51. Jain, S.M. and P.M. Priyadarshan (eds.). Springer, New York, USA

Bhosale UP, SV Dubhashi, NS Mali, HP Rathod (2011). *In vitro* shoot multiplication in different species of banana. *Asian J Plant Sci Res* 1:23–27

Borse N, VP Chimote, AS Jadhav (2011). Stability of micropropagated *Musa acuminata* cv. Grand Naine over clonal generations: A molecular assessment. *Sci Hortic*, 129:390–395

Buah JN, E Danso, KJ Taah, EA Abole, EA Bediako, J Asiedu, R Baidoo (2010). The effects of different concentrations cytokinins on the *in vitro* multiplication of plantain (*Musa* spp.). *Biotechnology* 9:343–347

Chang Y (2002). Representational Difference Analysis. *In: Current Protocols in Molecular Biology*, pp: 12–27. Ausubel, F.M. (Ed.). John Wiley & Sons, Inc., New York, USA

Chavan-Patil VB, CD Arekar, DK Galkwad (2010). Field performance of *in vitro* propagated banana plants form 8th and 15th subculture. *Intl J Adv Biotechnol Res* 1:96–103

Cullis CA (2005). Mechanisms and control of rapid genomes changes in flax. *Ann Bot* 95:201–206

Cullis CA, KJ Kunert (2000). Isolation of tissue culture-induced polymorphisms in bananas by representational difference analysis. *Acta Hortic* 530:421–428

Cullis CA, KJ Kunert (1999). Isolation of tissue culture-induced polymorphisms in bananas by representational difference analysis. *In: Plant Biotechnology and In Vitro Biology in the 21st Century*, pp: 245–248. Altman, A., M. Ziv and S. Izhar (eds). Springer, Dordrecht, The Netherlands

Cullis CA, MA Cullis, AM Ong (2007). *Development of Markers for the Mantled Phenotype in Oil Palm. PIPOC 2007.* Proceedings agriculture, biotechnology and sustainability, Kuala Lumpur, Malaysia

Cullis CA, S Rademan, K Kunert (2002). *Method of monitoring genomic integrity and detecting genomic destabilization of plant cells in tissue culture*. United States Patent US006500616B1

Devi K, MB Gogoi, S Singh, BK Sarmah, MK Modi, P Sen (2017). *In vitro* regeneration of banana and assessment of genetic fidelity in the regenerated plantlets through RAPD. *Annu Res Rev Biol* 17:1–11

Dhed'a D, F Dumortier, B. Panis, D Vuylsteke, ED Langhe (1991). Plant regeneration in cell suspension cultures of cooking banana 'Bluggoe'cultivar (*Musa* spp. ABB group). *Fruits* 46:125–135

DOA (2017). *Fruit Crops Statistic Malaysia 2016.* Department of Agriculture, Putrajaya, Malaysia

Doyle JJ, JL Doyle (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19:11–15

Elhory SMA, MA Aziz, AA Rashid, AG Yunus (2009). Prolific plant regeneration through organogenesis from scalps of *Musa* spp. cv. Tanduk. *Afr J Biotechnol* 8:6208–6213

Gubbuk HD, M Pekmezci, AN Onus, M Erkan (2004). Identification and selection of superior banana phenotypes in the cultivar Dwarf Cavendish using agronomic characteristics and RAPD markers. *Pak J Bot* 36:331–342

Jafari N, RY Othman, N Khalid (2011). Effect of benzylaminopurine (BAP) pulsing on *in vitro* shoot multiplication of *Musa acuminata* (banana) cv. Berangan. *Afr J Biol* 10:2446–2450

Jambhale ND, SC Patil, AS Jadhav, SV Pawar, B Waghmode (2001). Effect of number of subcultures on *in vitro* multiplication of four banana clones. *InfoMusa* 10:38–39

Johnson C, TA Cullis, MA Cullis, CA Cullis (2009). DNA markers for variety identification in date palm (*Phoenix dactylifera* L.). *J Hortic Sci Biol* 84:591–594

Juli NA, N Khalid (2002). Comparative analysis of regenerants between single and naked meristem (scalps) of *Musa acumita* var. Berangan. *Asia Pacif J Mol Biol Biotechnol* 10:127–131

Krishna H, M Alizadeh, D Singh, U Singh, N Chauhan, M Eftekhari, RK Sadh (2016). Somaclonal variations and their applications in horticultural crops improvement. *3Biotech* 6:1–18

Lalrinsanga R, H Vanlaldiki (2013). *In vitro* shoot tip culture of banana cultivar *Meitei Hei Bioscan* 8:839–844

Lisitsyn N (1995). Representational difference analysis: finding the differences between genomes. *Trends Genet* 11:303–307

Lisitsyn N, N Lisitsyn, M Wigler (1993). Cloning the differences between two complex genomes. *Science* 259:946–951

Martin KP, S Pachathundikandi, CL Zhang, A Slater, J Madassery (2006). RAPD analysis of a variant of banana (*Musa* spp.) cv. grande naine and its propagation via shoot tip culture. *In Vitro Cell Dev Biol – Plant* 42:188–192

Matsumoto K, LS Caldas, Y Yamamoto (2006). Response of banana dwarf somaclonal variants to benzylaminopurine. *InfoMusa* 15:27–29

McAlister B, J Finnie, MP Watt, F Blakeway (2005). Use of the temporary immersion bioreactor system (RITA®) for production of commercial Eucalyptus clones in Mondi Forests (SA). *Plant Cell Tiss Org Cult* 81:347–358

Michiels L, FV Leuven, JJVD Oord, CD Wolf-Peeters, J Delabie (1998). Representational difference analysis using minute quantities of DNA. *Nucl Acid Res* 26:3608–3610

Mohamed AES (2007). Morphological and molecular characterization of some banana micro-propagated variants. *Intl J Agric Biol* 9:707–714

Muhammad A, I Hussain, SMS Naqvi, H Rashid (2004). Banana plantlet production through tissue culture. *Pak J Bot* 36:617–620

Mukunthakumar S, G Praveen, S Seeni (2010). Rapid propagation of two seedless diploid (AB) land races of *Musa* through *in vitro* culture of shoot and inflorescence apices. *Tree For Sci Biotechnol* 4:64–68

Murashige T, F Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plantarum* 15:473–497

Nelson SC, RC Ploetz, AK Kepler (2006). *Musa* species (Banana and Plantain). *Musaceae* (banana family). *In: Species Profiles for Pacific Island Agroforestry*, p: 15. Permanent Agriculture Resources (PAR), Hōlualoa, Hawaii, USA

Nwauzoma AB, ET Jaja (2013). A review of somaclonal variation in plantain (*Musa* spp.): mechanisms and applications. *J Appl Biol* 67:5252–5260

Oh TJ, MA Cullis, K Kunert, I Engelborghs, R Swennen, CA Cullis (2007). Genomic changes associated with somaclonal variation in banana (*Musa* spp.). *Physiol Plantarum* 129:766–774

Panaud O, C Vitte, J Hivert, S Muzlak, J Talag, D Brar, A Sarr (2002). Characterization of transposable elements in the genome of rice (*Oryza sativa* L.) using Representational Difference Analysis (RDA). *Mol Genet Genomics* 268:113–121

Rout GR, SK Senapati, S Aparajita, SK Palai (2009). Studies on genetic identification and genetic fidelity of cultivated banana using ISSR markers. *Plant Omic J* 2:250–258

Sadik K, G Arinaitwe, P Ssebuliba, P Gibson, C Lugolobi, SB Mukasa (2012). Proliferation and shoot recovery among the east african highland banana. *Afr Crop Sci J* 20:67–76

Sadik K, PR Rubaihayo, MJS Magambo, M Pillay (2007). Generation of cell suspensions of East African highland bananas through scalps. *Afr J Biol* 6:1352–1357

Sahijram L (2015). Somaclonal Variation in Micropropagated Plants. *In: Plant Biology and Biotechnology*, pp: 407–416. Bahadur B, MV Rajam, L Sahijram, KV Krishnamurthy (eds.). Springer, India

Sarpan N, SE Ooi, MO Abdullah, CL Ho, CF Chin, P Namasivayam (2013). Representational difference analysis (RDA) for the identification of DNA markers associated with tissue culture amenity in oil palm. *J Oil Palm Res* 25:305–313

Sheidai M, H Aminpoor, Z Noormohammadi, F Farahani (2008). RAPD analysis of somaclonal variation in banana (*Musa acuminata* L.) cultivar Valery. *Acta Biol Szeged* 52:307–311

Shim J, O Panaud, C Vitte, MS Mendioro, DS Brar (2010). RDA derived *Oryza minuta*-specific clones to probe genomic conservation across *Oryza* and introgression into rice (*O. sativa* L.). *Euphytica* 176:269–279

Shirani S, M Sariah, W Zakaria, M Maziah (2010). Scalp induction rate responses to cytokinins on proliferating shoot-tips of banana cultivars (*Musa* spp.). *Amer J Agric Biol Sci* 5:128–134

Shirani S, F Mahdavi, M Maziah (2009). Morphological abnormality among regenerated shoots of banana and plantain (*Musa* spp.) after *in vitro* multiplication with TDZ and BAP from excised shoot tips. *Afr J Biol* 8:5755–5761

Sholi NJY, A Chaurasia, A Agrawal, NB Sarin (2009). ABA enhances plant regeneration of somatic embryos derived from cell suspension cultures of plantain cv. Spambia (*Musa* spp.). *Plant Cell Tiss Org Cult* 99:133–140

Sipen P, MR Davey (2012). Effects of N6-benzylaminopurine and indole acetic acid on *in vitro* shoot multiplication, nodule-like meristem proliferation and plant regeneration of Malaysian bananas (*Musa* spp.). *Trop Life Sci Res* 23:67–80

Srangsam A, K Kanchanapoom (2003). Thidiazuron induced plant regeneration in callus culture of triploid banana (*Musa* spp.) "Gros Michel", AAA group. Songklanakarin *J Sci Technol* 25:689–696

Strosse H, E Andre, L Sagi, R Swennen, B Panis (2008). Adventitious shoot formation is not inherent to micro propagation of banana as it is in maize. *Plant Cell Tiss Org Cult* 95:321–332

Strosse H, H Schoofs, B Panis, E Endré, K Reyniers, R Swennen (2006). Development of embryogenic cell suspensions from shoot meristematic tissue in bananas and plantains (*Musa* spp.). *Plant Sci* 170:104–112

Strosse H, R Domergue, B Panis, JV Escalant, F Côte (2003). Banana and plantain embryogenic cell suspensions. *In: INIBAP Technical Guidelines 8*, pp: 1–31. Vézina, A. and C. Picq (eds.). Montpellier, France

Venkatachalam L, RV Sreedhar, N Bhagyalakshmi (2007). Genetic analyses of micropropagated and regenerated plantlets of banana as assessed by RAPD and ISSR markers. *In Vitro Cell Dev Biol – Plant* 43:267–274

Villalobos MR, ED Garcia (2008). Obtainment of embryogenic cell suspensions from scalps of the banana Cien-Bta-03 (*Musa* spp., AAAA) and regeneration of the plants. *Electr J Biol* 11:1–10

Vorster BJ, KJ Kunert, CA Cullis (2002). Use of representational difference analysis for the characterization of sequence differences between date palm varieties. *Plant Cell Rep* 21:271–275

Vroh-Bi I, A Anagbogu, S Nnadi, A Tenkouano (2011). Genomic characterization of natural and somaclonal variations in bananas (*Musa* spp.). *Plant Mol Biol Rep* 29:440–448

Zoldos V, S Siljak-Yakovlev, D Papes, A Sarr, O Panaud (2011). Representational Difference Analysis Reveals Genomic Differences between *Q. robur* and *Q. suber*: implications for the study of genome evolution in the genus *Quercus*. *Mol Genet Genomics* 265:234–241