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RAPD Analysis of Somaclonal Variation on Different Abnormal Morphological Characteristics at Post Planting Stage in *Musa Accuminata* cv. Berangan.

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A thesis submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Agrotechnology) with Honour

Faculty of Agro-Based Industry

University Malaysia Kelantan

2018

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been seen submitted for a higher degree to any universities or institutions.

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RAPD Analysis of Somaclonal Variation on Different Abnormal Morphological Characteristics at Post Planting Stage in *Musa Accuminata* cv. Berangan.

ABSTRACT

Musa Accuminata cv. Berangan have high demand for local consumption and exportation. However, somaclonal variation can becomes a problem in micropropagation where it has been known to cause mutation, phenotypic variation or genetic variation. Somaclonal variation resulted during tissue culture stages and the explant were treated with different concentration of 6-Benzylaminopurine (BAP) treatments to induced somaclonal variation. From the treatments, the morphological changes of the explant were in parallel with the increased BAP concentration used during growth in tissue culture stages. Besides, genetic polymorphism of bananas with varied morphologies during tissue culture stages could also be detected by using the molecular marker RAPD. But, the plantlet could revert to normal when the BAP treatment was removed, but at post-planting the individuals further showed significant phenotypic changes especially their plant height. RAPD analysis were used in this study to assess the genetic diversity of somaclonal variation and sensitivity to detect genetic polymorphisms in banana clones. Six arbitrary RAPD primers were used to analyse the genetic polymorphisms within the treatments and the morphologies of the banana clones at post-planting stages. A total 44 bands were amplified using RAPD primers, OPJ-13, OPB-10, OPU-06, OPA-19, OPA-15 and OPA-06. Primer, OPU-06 produced the highest number of bands (12) while primer OPJ-13 produced the lowest which is 3 total bands. Primer OPA-06 produced the highest number of polymorphic bands which was 5 total bands followed by OPJ-13 and OPA-19.From all the primers used, three RAPD primers were identified (OPJ-13, OPA-19, OPA-06) as good primers to detect the polymorphisms between the individuals of the banana clones. This results shown, all the selected primers were effective to amplify higher number of bands within the individual but could not differentiated the genetics polymorphisms through the treatments and the morphologies of *Musa accuminata* cv. Berangan at post-planting stages.

Keywords: *Musa Accuminata* cv. Berangan, somaclonal variation, molecular marker, RAPD.

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Analisis RAPD terhadap variasi somaklonal terhadap ciri-ciri morfologi yang tidak normal dan berbeza pada peringkat pasca penanaman terhadap *Musa accuminata* cv. Berangan

ABSTRAK

Musa accuminata cv. Berangan mempunyai permintaan tinggi untuk penggunaan dan eksport tempatan. Walau bagaimanapun, variasi somaklonal telah menjadi salah satu masalah dalam mikropropagasi di mana ia boleh menyebabkan mutasi, variasi fenotip atau variasi genetik. Pada peringkat kultur tisu, anak pokok kultur tisu telah dirawat dengan rawatan yang berbeza mengikut perbezaan kepekatan Benzylaminopurine (BAP) menyebabkan variasi somaklonal telah berlaku. Perubahan morfologi anak pokok kultur tisu telah berlaku setelah dirawat selari dengan peningkatan kepekatan BAP yang digunakan untuk mempercepatkan pertumbuhan pada peringkat kultur tisu. Selain itu, polimorfisme genetik pisang dengan perbezaan morfologi semasa peringkat kultur tisu juga dapat dikesan menggunakan penanda molekul RAPD. Namun, anak pokok kultur tisu boleh kembali normal apabila rawatan BAP dibuang, dan apabila tanaman telah dipindah ke ladang, pertumbuhan anak pokok telah menunjukan perubahan dari segi fenotipik yang ketara terutama ketinggian pokok. Analisis RAPD telah digunakan dalam kajian ini bagi mengkaji kepelbagaian variasi genetik, variasi somaklonal dan sensitif untuk mengesan polimorfisme genetik dalam klon pisang. Enam primer RAPD yang rawak telah digunakan untuk menganalisis polimorfisme genetik klon pisang yang telah dirawat di peringkat penanaman. Sebanyak 44 jalur telah terhasil mengunakan primer OPJ-13, OPB-10, OPU-06, OPA-19, OPA-15 dan OPA-06. Primer OPU-06 telah menghasilkan bilangan jalur yang tertinggi (12) manakala primer OPJ-13 menghasilkan jalur yang terendah iaitu tiga. Primer OPA-06 menghasilkan jumlah jalur polimorfik yang tertinggi iaitu lima diikuti oleh OPJ-13 dan OPA-19. Berdasarkan semua primer yang digunakan, tiga primer RAPD telah dikenalpasti (OPJ-13, OPA-19, OPA-06) sebagai primer yang terbaik untuk mengesan polimorfisme antara individu klon pisang. Keputusan daripada eksperimen ini menunjukan, semua primer yang dipilih boleh menghasilkan jalur untuk setiap individu tetapi tidak dapat membezakan polimorfisme genetik melalui rawatan dan mengikut morfologi *Musa accuminata* cv. Berangan pada peringkat pasca penanaman.

Kata kunci: *Musa accuminata* cv.Berangan, variasi somaklonal, penanda molekul, RAPD.

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LIST OF ABBREVIATIONS AND SYMBOLS

DNA	Deoxyribonucleic acid
BAP	6-Benzylaminopurine
RAPD	Random Amplified Polymorphic DNA
kb	kilobase
bp	Base pair
cm	Centimeter
PCR	Polymerase Chain Reaction
cv.	Cultivar
2,4-D	2,4-Dichlorophenoxyacetic acid
2iP	N^6-(2-isopentyl)adenine
mg	miligram
psi	Pounds per square inch
CTAB	Cetyl trimethylammonium bromide
NaCl	Sodium chloride
Tris	Tris(hydroxymethyl)aminomethane
EDTA	Ethylenediaminetetra acetic acid
PVP	Polyvinylpyrrolidone
rpm	Rotations per minute
mM	Milimolar
M	Molar
TE	Tris-EDTA
HCl	Hydrochloric acid

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nm	nanometer
OD260/280	Optical density ratio of UV light absorbed by nucleic acids at a wavelength of 260 nm vs. 280nm
MgCl2	Magnesium chloride
KCl	Potassium chloride
dNTP	deoxynucleotriphosphate
TBE	Tris/Borate/EDTA
NCBI	National Center for Biotechnology Information

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Banana cultivated in other countries have many different cultivars and varieties including cooking and desert types. Most of the banana usually found cultivated in most of the country that located in subtropical and tropical area (Escalant and Paris, 2002). "Berangan" and 'Cavendish" have high demand for local consumption and exportation to the world market (Nik Mohd et al., 2002).

Somaclonal variation is a phenotypic variation, either genetic or epigenetic in origin, displayed among somaclones. Somaclonal variation could be generated from tissue culture. There are different types of variation which has been reported in banana. Most of the somaclonal variation occurred from tissue culture were reported with regard to plant morphology. Molecular marker and visual screening could detect the somaclonal variation in banana by using random amplified polymorphic DNA (RAPD) (L. Sahijram, J.R. Soneji, and K.T. Bollamma, 2003). According to Shirani et al. (2009), the somaclonal variation increases in multiplication phase when the BAP concentration and multiplication cycle increase, that was observed in micropropagation banana of cultivars "Berangan" (AAA). Increased culture duration, primary explant origin, and certain

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banana genotypes are factors that contributes to variations (Bairu et al., 2006). Benzylaminopurine (BAP) is the most commonly used in the micropropagation of banana (Shirani et al., 2009). Based on Sheidai et al., (2008), the different treatment of BAP concentration toward the banana plantlets have caused changes in length of shoots and the weight of the banana plantlet was significantly increased and this result was compared with others treatments such cytokinin which N- phenyl – N´- 1,2,3 – thiadiazol 5-yl urea (TDZ) and kinetin (KIN). So, when the BAP concentration were increased, the fresh weight and the length of the banana plantlet shoot were significantly increased.

In RAPD pattern observation, the primer annealing and the loss and gain can be one of the reason for such variation occurred. The deletion and insertion of sequence or transposable elements also the reason for the variation occurred and this due to certain mutation (Hernandez et al., 2007). RAPD analysis is widely used among molecular markers in various plant species and in studying genetic diversity of somaclonal variations. Damasco et al. (1996) reported that to detect the variation in banana such as "Cavendish" which is dwarf, the arbitrary RAPD markers have been used. Based on these studies, genetic polymorphism was revealed by using more than 60 markers among the normal and the dwarf plant (Supasanna et al., 2008).

In 2006, Asnita *et al.* found that the result scored as pattern of bands obtained from in-vitro micropropagated from male inflorescence of the same mother plant of *Musa accuminata* cv. Berangan have no polymorphism or genetic changes in the amplified DNA. The result were detected after amplification of PCR within micropropagated plants. In vitro-propagation from tissue culture laboratory provided control environment condition which is different than the natural condition of banana cultivated at post-planting stages.

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1.2 Problem Statement

Tissue culture techniques of banana propagation produces variants. This variation could be due deletion or insertion of sequence or transposable elements known as somaclonal variation. In separate study, during the tissue cultures stage, various concentration of benzylaminopurine (BAP) was used to induce somaclonal variation. The purpose of the study was to understand the genetic basis behind the somaclonal variation in tissue culture explant of banana. From the treatment, morphological changes of the explant were observed as a result to the increased BAP concentration used during growth in tissue culture stages. Plantlets that were exposed to high BAP developed into scalp morphology, however when the BAP treatment were removed, the plantlet revert back to normal with formation of normal roots and shoots. In addition, genetic polymorphism of bananas with varied morphologies during tissue culture stages could also be detected by using the molecular marker RAPD, which implies the genetic changes that occurred in parallel with the various morphologies observed. Early investigation of the polymorphism observed between the plantlets with different morphologies showed that the genetic changes observed were related to the repetitive element of the genome. In term of morphology, the plantlet can revert to normal when the BAP treatment was removed, however at post-planting the individuals further showed significant phenotypic changes especially their plant height. The genetic testing done during tissue culture was by bulking several samples in same treatment and morphology. Thus the result observed were not of the individual clones, which may explain variability between tissue culture and post planting stages. This study focuses on the assessment of the genetic polymorphism between the individuals that were treated with different BAP concentration at tissue

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culture stages and showing significant phenotypic changes at post-planting stages by using RAPD marker.

1.3 Objectives

I. To identify DNA polymorphism of *Musa accuminata* cv. Berangan between tissue culture clone of post-planting stage using RAPD analysis.

II. To correlate the identified DNA polymorphisms at post-planting stage to the different morphologies of the clones.

1.4 Hypothesis

H_0: There is no polymorphism identified using RAPD analysis at post-planting stage among the banana plantlet that were treated with different BAP concentration during its tissue culture growth.

H_A: There is polymorphism identified using RAPD analysis at post-planting stage among the banana plantlet that were treated with different BAP concentration during its tissue culture growth.

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1.5 Scope of the Study

In this study, the samples was taken from *Musa accuminata* cv. Berangan which have been planted at the field in Agro-Techno Park, University Malaysia Kelantan. All the samples were originated from tissues culture before transferred to the field and have been treated with different concentration of benzylaminopurine (BAP) in order to understand the somaclonal variation. The banana plantlet were treated with four different treatments (0.0 mg/l, 5.0 mg/l, 10.0 mg/l, 15.0 mg/l and 20 mg/l) to induce the somaclonal variation and caused the morphological changes during growth in tissue culture stages. The individual of banana clones were selected based on four different treatments and also from the morphology of the plants. The morphology were screened based on the plant growth (plant height) of the banana plantlets after 6 months transplanted at the filed. In this study, ten individuals were chosen per treatment and were assessed through RAPD analysis to identify the genetic polymorphism between the treatments and the morphology of the banana plantlets at the field. RAPD analysis were tested on selected primers which are OPJ-13, OPB-10, OPU-06, OPA-19, OPA-06 and OPA-15.

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1.6 Significant of Study

Banana is the most popular energy rich source of carbohydrates and a fair source of minerals and vitamins. Most of the small farmers prefer to plant the banana traditionally or by using the suckers but, there are also farmer that used the recent advances in tissue culture technology to provide plantlet and have the great impact on banana cultivation. DNA based marker system such as RAPD analysis can be used to detect the problem from somaclonal variation in tissue culture which can be caused by activation or mobile elements and mutations.

This study will help the researcher to collect more useful data about the genetic variability of banana and this data will be used to improve the tissue culture technique in banana to study the occurrence of variation among clones. Genetic variation provides the ability of species that can adapt to new climatic condition, changing environments, including problems from new pests and diseases.

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CHAPTER 2

LITERITURE REVIEW

2.1 Banana

Banana of genus *Musa* is one of the most important fruits and second mostly cultivated fruits in Malaysia. The production of banana in Malaysia covered about ten percent or 33 584 ha of total of total production fruits area which 297 860 ha. Besides, banana is the second most widely cultivated fruit plants with the total production of over 535 thousand tonnes annually. Fifty percent from all types of banana that cultivated in Malaysia are Berangan and Cavendish types. The development of banana becomes one of the 15 types of fruits cultivated in Malaysia which have given prioritised under the National Agriculture Policy.

The exportation of banana were increased from 1989 until 2009 which ranging from 20, 000 to 35, 000 tonnes production per years. The total consumption of banana in Malaysia were about 22 kg and this indicates that the production of banana was contributed in increase the higher income, population and greater healthy towards the local people. The banana plantation are important for the growth of economic in Malaysia because, banana fruits used for domestic and export and becomes one of others popular fruits to be importance for other usability. (Roff, Malik, & H, 2012)

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Based on Food Agriculture Organization (FOASTAT, 2016), banana cultivation produced 106 Million metric tons worldwide in that years under approximately five hectares of land areas. Most of the unripe bananas have high amount of starch and the lease levels of sugar with high amount of white latex. Besides, the quantity of starch in the bananas are high when the banana have ripen and if the starch is converted to sugar it containing decomposed latex and total sugar will be up to 25 percent (Arvanitoyannis, 2008).

Bananas that have the inflorescence such as male flowers and female flowers are monoecious plants which have the female parts are at the back, then at the top part is male part. Containing too much collenchyma are characterized as beery with a leathery outer peel and has parthenocarpy are characteristic to produce banana's fruit (Daniells et al., 2001). The samples that were used in this study are *Musa accuminata* cv. Berangan. The general characteristic of Berangan are, the fruit bunches have weight about up to 12 kilograms. Wood and bamboo have made as a support brackets to banana crop. (Perak Agricultural Department [PAD], 2010).

2.1.1 Banana Classification

Banana is classified based on "ploidy," number of chromosome sets and grouped under *Musa.* Cultivated banana derived from *Musa acuminata* (A) and *Musa balbisiana* (B). The banana classification can be triploid which is seedless banana such as diploid. Banana also can be classified to be seedless from cultivated varieties of banana which are triploid hybrids such as AAA, AAB, and ABB. While, other varieties are diploid such as

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AA, AB and BB and also tetraploids such as AAAA, AAAB, AABB, and ABBB (Profiles & Agroforestry, 2006).

The cross between inter- and intraspecific of two diploid in the banana (2n=2x=22) were wild species which *Musa acuminata* and *Musa balbisiana* (Pollefeys et al., 2004). The *Eumusa* is the most widely spread in the world and is found throughout South East Asia. Simmonds and Shepherd in 1955 have proposed genomic groups to classify the edible clones were AA, BB, AAA, AAB, ABB, AAAA, and ABBB. Majority of bananas are comes from *Musa acuminata* (AA) and *Musa balbisiana* (BB) and *Musa accuminata* were most selected species cultivated among the *Eumusa* (Arvanitoyannis et al., 2008).

2.1.2 Banana Micropropagation and the effect of BAP

The propagation of banana is usually done through vegetative ways based on its reputation become the most clearly healthy crop of the world. The most popular methods used to plant the banana is conventional propagation but its needed more worker, time consuming and low multiplication rate per plants. So, the more advance methods were introduced for rapid propagation (Khatri et al., 1997). Based on Darvari et al., 2010 micropropagation is an alternative ways to produce plantlets in a short times, more tolerance to disease and can produces higher yield per cycles compare to the conventional propagation (Darvari et al., 2010).

Tissue culture techniques is originated from recombinant DNA methods which the genome was characterized. Besides through this methods, it include gene transfer techniques, the cells, tissue and organs of the banana plantlet are grow in control aseptic condition and in vitro regeneration of plants (Brown and Thorpe, 1995). In tissue culture

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technique, the plant hormones or growth regulators plays the importance role for plant growth and development aspect (Hay et al., 2004).

There are five types of plant growth regulators which are auxins, cytokinins, gibberellins, abscisic acid, and ethylene. Auxins can encourage the cell growth for example indole-3-acetic acid (IAA) and 2,4-Dichlorophenoxyacetic acid (2,4-D). Second plant growth regulator are cytokinins which can generates cell division in plants such as zeatin, N6-(2-isopentyl) adenine (2iP), and 6-benzylaminopurine (BAP). Then, gibberellin which involved in regulating cell elongation while abscisic acid (ABA) inhibits cell division and is usually used to promote somatic embryogenesis. Other than that is ethylene which can release the gas that can fastest the fruit ripening and not specifically used as a hormone in tissue culture (Slater et al., 2008).

Usually, the common plant growth regulator used for banana micropropagation is cytokinin. Cytokinin can generates the shoot germination rates followed its concentration and type of banana cultivars and Benzylaminopurine (BAP) is commonly used in micropropagation of banana (Shirani et al., 2009). Based on Sheidai et.al (2008), different concentration of BAP treatment can increase the length of shoots and fresh weight of plantlets compared to two others cytokinin which N- phenyl – N´- 1,2,3 – thiadiazol 5-yl urea (TDZ) and kinetin (KIN). The increase length of shoots and the weight of plantlet is depend on the higher concentration of BAP used in the media.

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2.2 Somaclonal Variation in Banana

Tissue culture technology have been used to increase the production and mostly used by highly important crop. For in vitro culture the shoot tips of bananas can be used. Then, the micropropagation of shoot tips in tissue culture sometimes result in change of the genetic background. Besides, to detect the variation in micropropagation, it will take more time, laborious and expensive to be done visually and the application to detect the variability can only be done after 3-4 months at field establishment. The banana plants should also be evaluated at early stage to detect uniformity of growth for all plants and thus required individual inspection before any variability of banana can be detected (Bairu et al., 2006).

Banana cultivation can produce somaclonal variantion (Krikorian et al., 1993). Common somaclonal variants that have been reported occurred are have different types in plant morphology. Appearance of off-types during the in vitro multiplication process is an important drawback for mass propagation of bananas. In any micropropagation program, 3–5% somaclonal variation is allow (Hwang and Tang, 1996), but in banana, up to 10% variation is permitted (as practised by commercial micropropagation outfits) owing to the flexible genetic make-up of the crop (Smith, 1988; Cote et al., 1993).

In the genus Musa, somaclonal variation at the phenotypic level has been observed with 6% frequency on average. Hwang and Tang (2002) reported that the off-types banana from tissue-cultured plantlets ranged from 6 to 38% in Cavendish cultivars. They also reported, somaclonal variation detected in different types of bananas and plantains, which ranging from 0 to 69.1% (L. Sahijram, J.R. Soneji, and K.T. Bollamma, 2003).

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The somaclonal variation occurred in tissue culture becomes a manifestation of epigenetic influence or a change in the genome that differentiate the vegetative cells induced by tissue culture and its can generate stable plants that carrying interesting heritable traits (Soniya et al. 2001). The banana regenerated from in vitro culture that treated with higher percentage of BAP showed many abnormalities during vegetative propagated (Shepherd and Dos Santos, 1996).

2.3 Moleculer Marker

The molecular markers have been extensively used in germplasm characterization, finger printing, genetic analysis, linkage mapping, and molecular breeding. These markers are also been used in identification of possible somaclonal variants at an early stage of development which is considered to be very useful for quality control in plant tissue culture, transgenic plant production and in the introduction of variants (Soniya et al. 2001).

The use of molecular marker to track loci and genome regions in crop plants is now routinely applied in many breeding programs. Improvements in marker screening techniques have also been important in facilitating the tracking genes. As for the markers to be effective, they must be closely linked to the target locus and be able to detect polymorphisms in material likely to be used in a breeding program. Markers have also improved the strategies for gene deployment and enhanced the understanding of the genetic control of complex traits such as components of quality and broad adaptation (Agarwal et al., 2008).

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Morphological markers can be used to visualize traits with the naked eye including fruits or seeds, disease response, plant height, and so forth. Although the effect of linked minor genes are easily distinguished by using morphological markers (Bhat et al., 2010). Environmental situations influences many morphological characteristic and before accurately identify their discriminating traits, the plants need to fully grown and matured (Jonah et al., 2011).

Within the last two decades, important tool in genetic crop improvement have introduced these new genetic molecular techniques (Bhat et al., 2010). However, regardless of growth in tissue culture stages, differentiation, development, or defense status of the cell the marker are stable and detectable and not affected by the environment, pleiotropic and epistatic effects (Agarwal et al., 2008). Besides, others characteristic as a good molecular markers are easily accessible, and selectively neutral. Then, genetic trait that linked in molecular marker, and not fully affected by epistatic interactions and also pleiotropism (Jonah et al., 2011).

2.4 RAPD Analysis

RAPD was developed by William et al. in 1990 (Bhal et al., 2010). A single arbitrary nucleotide primer sequence of mostly ten bases long, called a decamer, used for amplification. These amplified DNA fragments are randomly selected thus providing random samples of DNA markers (Jonah et al., 2011; Bhat et al., 2010). The analysis of genome using this techniques does not needs any important knowledge and can be used as universal primers across the species. However, the reaction condition may differ within different laboratories since several distinct loci will be amplified by each primer, and

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heterozygous and the homozygous individuals are indistinguishable (Agarwal et al., 2008). Since the primers are short, a single nucleotide become a mismatch and can cause missing band and may prevent annealing process of the primer. Additionally, RAPD analysis process is efficient and fast (Jonah et al., 2011).

The technique of random amplified polymorphic DNA (RAPD) profiling using single primers of arbitrary nucleotide sequence allows random amplification of DNA sequences throughout the entire genome. The efficacy of RAPD as a tool to confirm the presence or absence of variation has been well established in banana (Gubbuk et al., 2004). RAPD (Random Amplified Polymorphic DNA) analysis using PCR in association with short primers of arbitrary sequence has been demonstrated to be sensitive in detecting variation among individuals. The advantages of this technique are the large number of samples can be quickly and economically analysed using only micro-quantities of material. Then, the DNA amplicons are independent from the ontogenetic expression and many genomic regions can be sampled with a potentially unlimited number of markers (Soniya et al. 2001)

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CHAPTER 3

METHODOLOGY

3.1 Plant Materials and Morphological Traits

The samples of young leaves from *Musa accuminata* cv. Berangan (AAA) were used as plant material. The samples were originated from tissue culture and then transplanted to post planting field at Agro-Techno Park, University Malaysia Kelantan. All these samples were chosen to represent the wide range of genomic groups. All the samples were collected 6 months after transplanting from tissue culture stages to the field. The banana plantlets have been treated with four different concentration of Benzylaminopurine (BAP) from 0 mg/l until 15 mg/l of BAP during tissue culture stages. The treatments have affected the morphologies of the plants in term of plants height at the fields. In this study, 50 total samples of banana plantlets were selected based on the morphological characteristics at the field and 10 samples of banana plantlets were selected per treatments (Table 3.1).

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Table 3.1: The plant's treatments and the plants height of the banana plantlet at the fields.

BAP Treatments	Total samples	Mean of plants Heights (cm)
T0 (0 mg/l of BAP)	10	94.88
T1 (5 mg/l of BAP)	10	196.60
T2 (10 mg/l of BAP)	10	118.22
T3 (15 mg/l of BAP)	10	73.68
T4 (Commercial Seedling)	10	296.85

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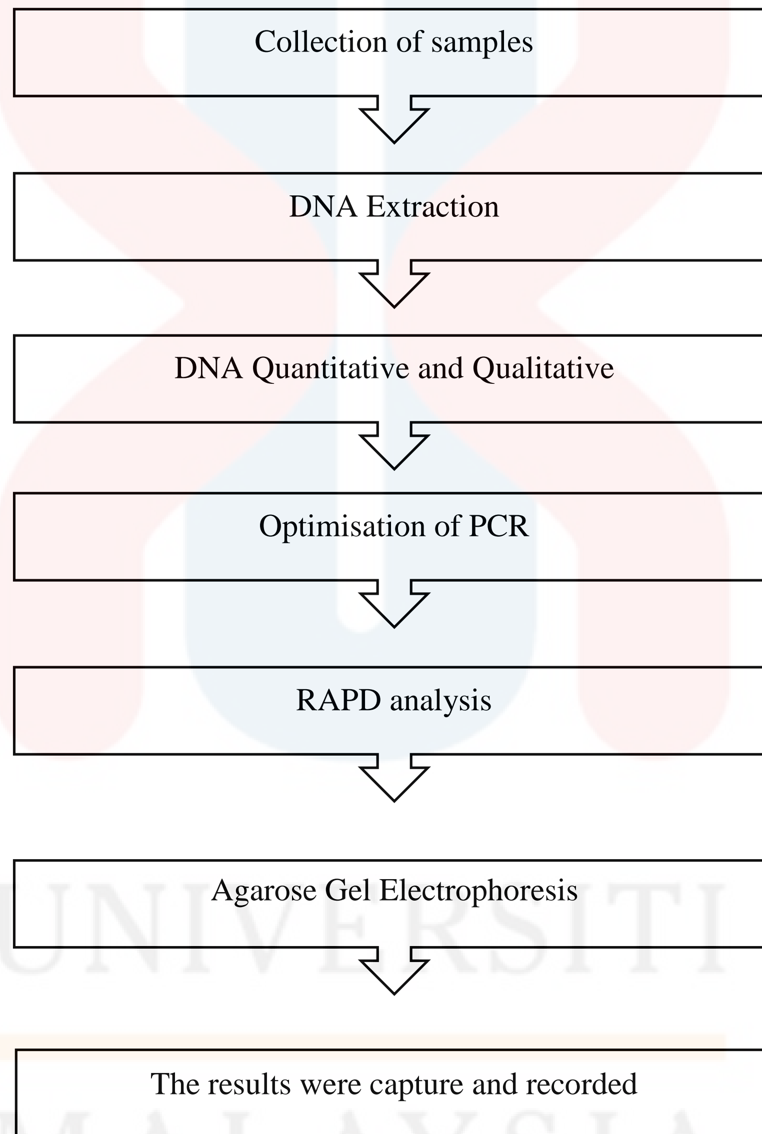
3.2 Experimental Design

Figure 3.2 show the summary of whole experiment procedures.



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3.3 DNA extraction

The DNA from *Musa accuminata* cv. Berangan was extracted using a modified CTAB method followed the protocol design by (Doyle, 1987). The young leaves sample of banana were weight around 3-5 g and were grinded into powder with liquid nitrogen by using mortar and pestle. Then, the grinded of banana powder were immediately transferred into 20 ml of pre-warmed (65 °C) of 2 X CTAB extraction buffer containing 2% CTAB (cetyltrimethylammonium bromide), 1.4M NaCl, 20Mm EDTA, 100 mM Tris-HCl, with the pH 8.0 in the 50 ml polypropylene tube to prevent degradation of DNA by cellular enzymes. The mixture of the grinder powder and the CTAB extraction buffer were incubated in the water bath at 65 °C for 30 minutes and mixed the content by inverting the tubes.

After that, an equal volume of chloroform-isoamyl alcohol (24:1) was added and mixed gently by using the shaker for 15 minutes to prevent degradation of DNA. The contents were centrifuged at ~ 2,700 rpm for 10 min and the aqueous (top) layer was carefully pipette out without disturbing the bottom into a new sterile 50 ml of polypropylene tube that already contains 2/3rds of a volume of ice-cold isopropanol. The contents were gently mixed by a few inversions. Then, the white threads of DNA were appeared and the samples were placed in a -20 °C freezer for 30 min or longer for incubation.

At this stage, the extractions were stored in the -20 °C freezer overnight. The extraction were centrifuged for 5 min, at ~ 10,000 rpm to pellet the DNA. The supernatant were poured away gently and be cautious not to discard the pellet. Then, 500 µl of 70% ethanol was added to wash the DNA. The tube was tap gently to allow it stand for a few

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minutes. The contents were centrifuged at 10,000 rpm for 5 min to re-pellet the DNA and the supernatant was discarded gently and then, by using the edge of a clean paper towel, the remaining excess liquid was drained away from the lip of the inverted tube. Tubes were opened upright for remaining liquid to evaporate that allowed it to stand for 30 min.

Then 100 µl of 1 X TE buffer was added and DNA were allowed to re-suspend in buffer before using it. The tubes were left open for several hours and occasionally tapping them to aid for re-suspension. All the DNA were stored at 4 °C. Using the same technique as for PCR product visualisation, the DNA quality was checked on 1% agarose gels. The DNA concentration was compared based on the DNA standards against of 10 ng/µl, 20 ng/µl and 100 ng/µl lambda DNA.

3.4 Determination of Quality and Quantity of DNA

3.4.1 Quantification of DNA

The solution of DNA was measured the concentration by using the nanodrop meter. The TE buffer was used for the Blank Measurement because have the same pH and contain similar ionic strength as the sample solution. 1 µl of DNA solution was used to measure the DNA quality by using nano drop meter (ultraviolet spectrophotometer). The 280 nm was indicated the protein contamination while 260 nm was indicated the DNA concentration. The DNA purity ratio A_{260}/A_{280} reading should showed the reading approximately from 1.8 but any samples reading showed in the range of 1.75 to 2.00 are considered to be of sufficient purity. The samples reading lower than 1.75, the DNA solution was treated with proteinase-K and the samples reading higher than 2.0, the DNA

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solution was treated with RNase. The amount of DNA obtained should be high concentration ranging from 400ng/ul to 800ng/ul.

3.4.2 Qualification of DNA

The quality of DNA obtained from the extraction were determined by electrophoresis in 1% of agarose gel. Then, a sharp band from large molecular weight DNA were appeared as while as DNA forms partially degraded a long smear consisting the large to small fragments. The samples were treated with RNase if the RNA contamination of the samples appeared near the end of the gel as fast running band. Then, the gel was loaded and run at 80 V for an hour and viewed under ultraviolet.

3.5 PCR Amplification

The Polymerase Change Reaction (PCR) was performed in reaction mixtures (1st Base, Malaysia) with total volume of 25 µl containing of 100 ng/ul DNA template, 1.5 Mm/ml $MgCl_2$, 10 mm dNTP, 100 Mm PCR buffer, 1.25 U *Taq* Polymerase,1 mm primer, and mixed with sterile distilled water (dH_2o) .The DNA solution of 50 individuals from *Musa accuminata* cv. Berangan were prepared. From total volume of 25 µl of PCR mixtures, 21 µl was standardised PCR mastermix and 4 µl was DNA template. The PCR was run in the Mastercycler Gradient (Eppendorf, USA) by using an initial denaturation at 94 °C for 4 minutes, then continued with 45 cycled of denaturation followed at 54 °C for 15 seconds, gradient annealing temperature ranging from 34 °C to 40 °C for 40

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seconds for all primer combinations. Then, the extension process was run at 72°C for 90 seconds, and then terminated by final extension at 72 °C and lastly incubated at 4 °C. After PCR was performed, stored the PCR products in the -20 °C for long term storage. All the samples were tested on 6 selected primers which are OPJ-13, OPB-10, OPU-06, OPA-06, OPA-19, and OPA-15 (Table 3.5).

Table 3.5: Nucleotide sequences of 6 arbitrary 10-mers primers used for screening.

No	Primers	'5- Sequence-3'
1	OPJ-13	CCACACTACC
2	OPB-10	CTGCTGGGAC
3	OPU-06	ACCTTTGCGG
4	OPA-19	CAAACGTCGG
5	OPA-06	GGTCCCTGAC
6	OPA-15	TTCCGAACCC

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3.6 RAPD Analysis

PCR was performed in Mastercycler Gradient (Eppendorf, USA) according to Williams et al. (1990) with same modifications as described in PCR amplification methods in 3.5. Final concentration of the reaction solution was 1.5 mM $MgCl_2$, 100 µM dNTPs, 100 Mm PCR buffer, 0.5 µM primer and 0.2U/10 1 of *Taq* Polymerase (1st base). A final volume of 25 µl, with a DNA concentration of 100ng were used for each reaction. The selected primers, OPJ-13, OPB-10, OPU-06, OPA-19, OPA-06 and OPA-15 were used to screen the banana clones samples. In 1.5% agarose gel, PCR products were run with 1:1 ratio of loading buffer. A 100bp ladder and 1kbp ladder were used as molecular marker and the gel electrophoresis was run at 80V for half an hour.

3.7 Agarose Gel Electrophoresis

Gel electrophoresis was used on the PCR products in order to analyse the DNA during the process. The 1.5% of agarose gel was used, so 1.75 g of agarose powder was weighed and put into a conical flask of suitable size and concentration of the gel. The agarose powder was mixed with 1 x TBE buffer in weight or volume ratio depends on the desired concentration and the contents were swirled. The gels were casted in different size of wells depending on the volume of DNA loaded into the gel and the number of samples in one run. Before the gels were casted, the mixture of agarose and 1 x TBE buffer was heated in the microwave until the solution become clear, and then, the flask was swirled. Then, 1 µl of FloroSafe DNA stain was put into the agrarose solution and the flask was swirled. FloroSafe DNA stain was the safer alternative to used compare to

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Ethidium Bromides because it low toxicity and non-carcinogenic alternative compare to Ethidium Bromide.

At the point at which the flask can be held (~ 55 °C, takes a few minutes) the agarose was cooled and poured into a previously prepared gel tray, to a depth of around 5 mm. Any obvious bubbles in the gel were pushed to the side of the tray by using a disposable pipette tip. The gel combs were placed into their allocated positions and the number of teeth used in the gel combs depends on how many samples need to be run on the gel, and the volume of material that needs to be loaded in each well. The gel was left to cool and harden for one hour and the gel combs were removed. After the gel harden, placed the gel below liquid level in an electrophoresis tank contains 1 x TBE buffer. The tank was top up with more 1 x TBE as the gel must be completely covered. Then, loaded the prepared samples carefully into a well and including markers at the start and end of each series of wells by using a pipette. Next, 5 µl of the prepared samples were mixed with 2 µl of 6 x agarose gel loading dye prior to loading into gel. The gels electrophoresis tank were run at 80 V for 90 minutes. Then, the result was screened by using gel documentation system, the gel was viewed were captured and recorded.

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CHAPTER 4

RESULTS AND DISCUSSION

4.1. DNA Extraction

In this study, the modified extraction method from (Doyle, 1987) was used to extract 50 samples of *Musa accuminata* cv. Berangan from the field and all the samples were successfully extracted with high quality of genomic DNA. The samples were collected at the Agro-Techno Park and the experiment was conducted in Post Graduate Laboratory, University Malaysia Kelantan, Jeli Campus. The method used had slightly different from the previous study in (Doyle, 1987) which the samples were weighted around 3 g until 5 g in order to produce high number of genomic DNA from *Musa accuminata* cv. Berangan. The fresh young leaves were chosen, for easy grind with liquid nitrogen and also to minimize the effect of phenolic and terpenoid compound. Based on previous study, the isolation of genomic DNA have its limitation because have high content of polysaccharides and polyphenols.(Deshmukh, Thakare, Chaudhari, & Gawande, 2007).

After grinded the young leaves in the liquid nitrogen, it is recommended to remove the polyphenols by using polyvinylpyrrolidone (PVP) and β-mercaptoethanol that contained in the CTAB extraction buffer. Then, the polysaccharides can also be removed

in the extraction buffer containing high NaCl concentration. The CTAB extraction buffer were preheat until 65 °C in the water bath before soak the grinded young leaves powder to break the plant cell walls. The quality of the extracted genomic DNA of *Musa accuminata* cv. Berangan were assessed using quantification and qualification of DNA.

4.2 DNA Purification

4.2.1 Quantification of DNA

The extracted of genomic DNA from *Musa accuminata* cv. Berangan were measured by using spectrophotometer (Nanodrop meter). The concentration of genomic DNA ranged from 500 ng/µl to 2400 ng/µl and this showed that the amount of DNA obtained from *Musa accuminata* cv. Berangan were high (Table 4.2). The good purity of the DNA were successfully extracted as shown in A_{260}/A_{280} reading that ranging from 1.8 to 2.00. If the purity of the genomic DNA was less than 1.8, the DNA probably contaminated with phenolic compound or protein and were treated with RNAse while, if the A_{260}/A_{280} reading were more than 2.00, the extracted DNA were treated with Proteinase-K (Table 4.2).

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Table 4.2: The DNA Purification Value

Sample Numbers	Sample Names	260/280	Concentration of DNA
1	T4R2	1.85	1412.939
2	T4R4	1.83	1776.141
3	T4R14	1.80	1519.728
4	T4R7	1.80	1663.56
5	T4R18	1.89	1156.288
6	T4R1	1.87	895.64
7	T4R12	1.99	594.275
8	T4R24	2.00	1447.684
9	T4R30	1.81	1181.426
10	T4R26	1.84	2439.039
11	T0R8	1.85	1626.159
12	T0R30	1.89	2440.938
13	T0R3	1.80	2037.21
14	T0R8	1.85	1038.973
15	T0R30	1.80	1971.743
16	T0R3	1.90	666.167
17	T0R5	2.00	1809.84
18	T0R6	1.99	550.046
19	T0R1	1.89	1852.491
20	T0R22	1.91	1313.047
21	T0R17	1.88	1814.703
22	T0R121	1.99	1880.098

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23	T0R2	2.00	1505.587
24	T1R7	1.98	634.571
25	T1R11	1.95	2144.156
26	T1R16	1.83	762.208
27	T1R121	1.87	1227.486
28	T1R6	1.85	2092.237
29	T1R3	1.81	1747.459
30	T1R28	1.83	1707.029
31	T2R17	1.95	2037.616
32	T2R9	2.00	914.616
33	T2R5	1.97	2168.814
34	T2R16	1.85	1279.01
35	T2R21	1.89	1797.277
36	T2R24	1.87	2130.525
37	T2R1	2.00	1483.117
38	T2R18	1.82	252.387
39	T2R20	2.00	787.571
40	T2R8	1.95	2049.868
41	T3R22	1.99	1003.854
42	T3R23	1.96	875.511
43	T3R6	1.93	2308.808
44	T3R21	1.93	2210.795
45	T3R1	1.98	2246.973
46	T3R18	1.86	1867.003

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47	T3R16	1.91	1301.883
48	T3R5	1.82	1477.739
49	T3R10	1.90	1293.265
50	T3R25	1.80	659.19

4.2.2 Qualification of DNA

The quality of extracted DNA solution were identified by electrophoresis in 1.5% agarose gel and the gel were run at 80V for 60 minutes. If the DNA were in the good quality, the sharps band were appeared while the partially degraded DNA formed a long smear because consisting large to small fragments (Figured 4.2.1). The extracted DNA that contaminated with others substances may cause inaccuracy in DNA qualification through spectrophotometer. Based on figure 4.2.2, those DNA extracted solution were treated with RNA-ase if the RNA contamination appeared as fast running at the end of the gel.

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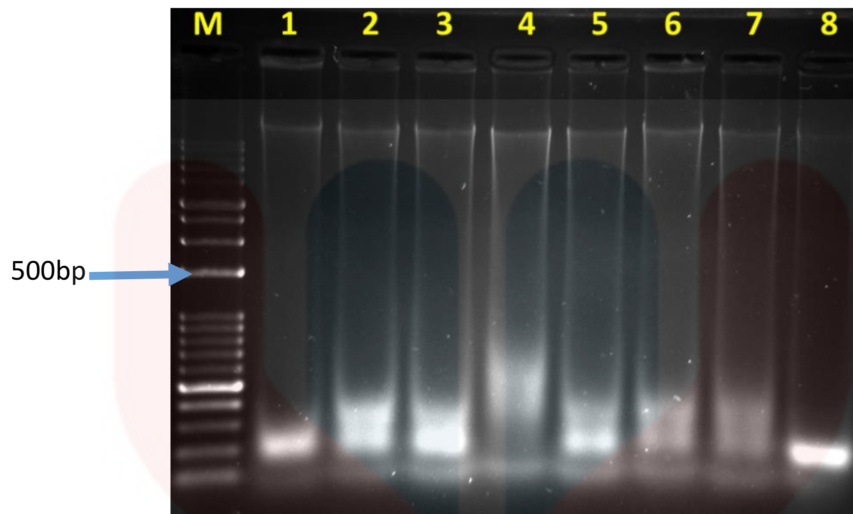

Figure 4.2.1: DNA qualification of *Musa accuminata* cv. Berangan before treated with RNA-ase, M= 100bp DNA Ladder, Lane 1-8 = DNA extracted solution.

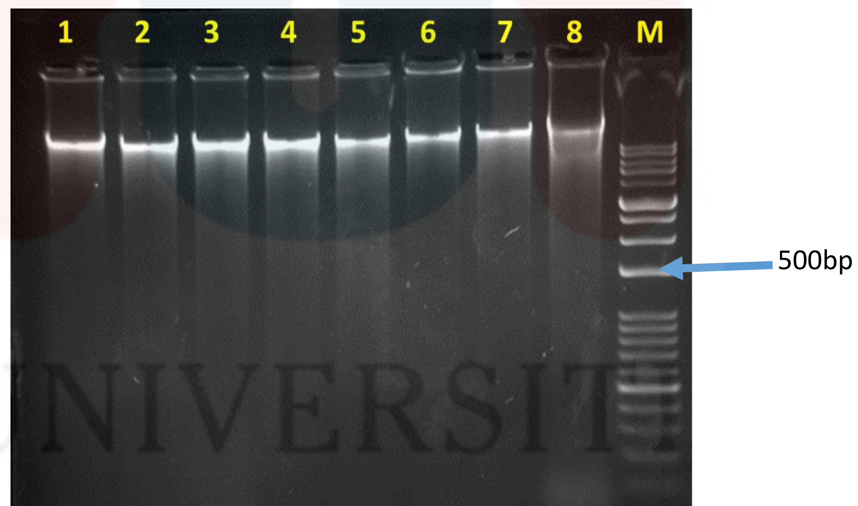

Figure 4.2.2: DNA qualification of *Musa accuminata* cv. Berangan after treated with RNA-ase, M= 100bp DNA Ladder, Lane 1-8 = DNA extracted solution.

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4.3 The Band Pattern of RAPD Analysis by using selected primers.

Based on the experiment that have been conducted, six arbitrary single primers were used to amplify segments of genomic DNA from *Musa accuminata* cv. Berangan that have been treated with different concentration of BAP. The banana clones that had been treated with BAP have slight different within the individual based on their morphologies. Some of the morphologies of banana clones can be classified as normal, stunted and giants in term of their growth which have different plant height at the field.

4.3.1 Evaluation of RAPD primers OPJ-13 on *Musa accuminata* cv. Berangan clones

Based on figure 4.3.1, the generated amplified bands count using selected primer OPJ-13 was three ranging from 450bp to 800bp. Polymorphisms detected was at 800bp, which bands were missing at lane 18 until 21. Due to the different treatments of BAP concentration, the plant height for sample 18 until 20 and sample 21 were different. Sample 18 until 20 were not treated with BAP treatment while, sample 21 have been treated with 5 mg/I of BAP. Due to that, the plant height for sample 21 were higher than sample 18 until 20. This shown that, the genetic differences identified by primer OPJ-13 could not differentiate among the treatments and the morphologies of the banana clones at the field. This is because, the polymorphism that detected using this primers was only specific to several individual within the same treatment but not consistent among the BAP treatments nor its morphology. So, there was no correlation between the genetic polymorphisms of banana clones at post-planting stage with the morphologies or by treatment by using this primers. These results showed that the primers OPJ-13 of arbitrary

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sequence can be used to amplify genomic DNA segments and that polymorphisms can be detected between the amplification products of different individuals of *Musa accuminata* cv. Berangan at post-planting stages. Based on Venkatachalam et al. (2007) analysed dessert banana cv. 'Nanjanagudu Rasabale' to study genetic stability of regenerated and micropropagated plantlets by using RAPDs did not observe genetically unstable plants grown in vitro.

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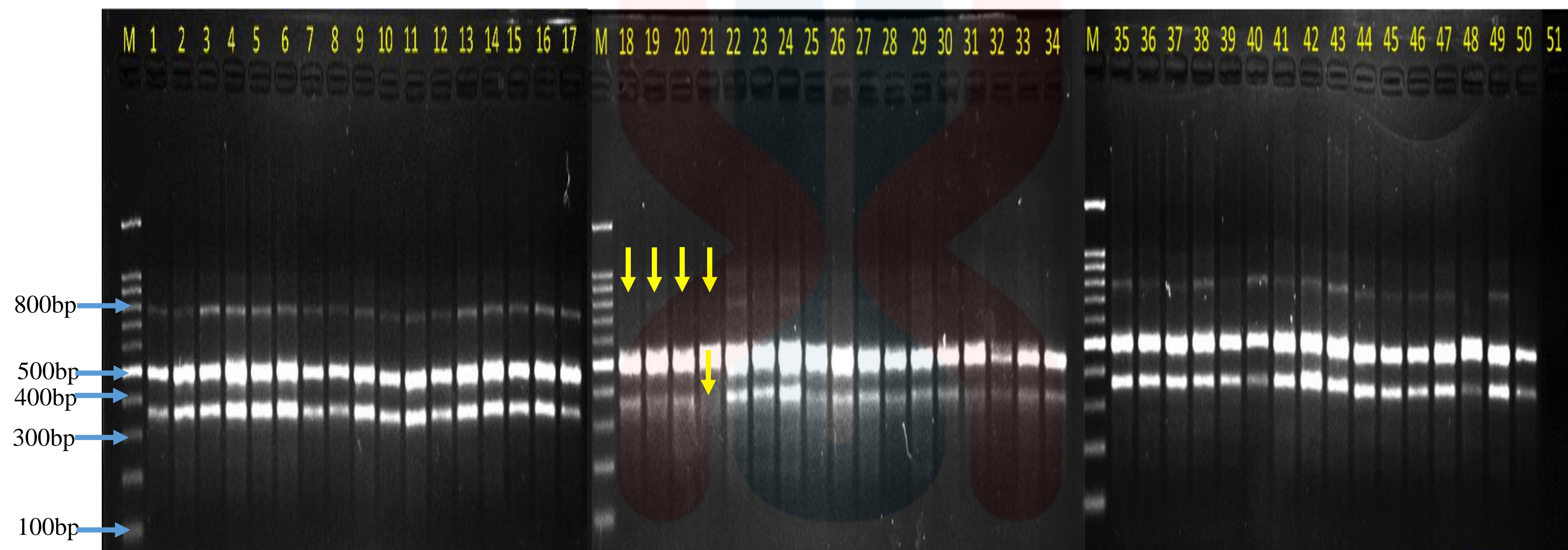

Figure 4.3.1: Random amplified polymorphic DNA of *Musa accuminata* cv. Berangan using primer OPJ-13. Lane M= 100bp of DNA ladder, Lane 1-10= Treatment 4 of commercial seedlings, Lane 11-20= Treatment 0 with 0 mg/I BAP, Lane 21-30= Treatment 1 with 5 mg/I BAP, Lane 31-40= Treatment 2 with 10 mg/I BAP, Lane 41-50= Treatment 3 with 15 mg/I BAP, Lane 51= Negative control. Arrow indicates polymorphic bands detected by using primer OPJ-13.

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4.3.2 Evaluation of RAPD primers OPB-10 on *Musa accuminata* cv. Berangan clones.

Based on figure 4.3.2 shown, nine amplified bands were detected from *Musa accuminata* cv. Berangan clones by using arbitrary primer OPB-10 ranging at 250bp to 1500bp. From all the amplified bands, there was no polymorphism and the genetic changes was detected within the individual and also not through the banana clones that have been treated with BAP treatment nor their morphologies. All banana clone showed 100% of monomorphic bands that ranging at 250bp until 1500bp. This showed that, there was no correlation between the genetic polymorphisms of banana clones at post-planting stage with the morphologies by using primers OPB-10. Therefore, it seems that very high percentages of monomorphic were generated from the RAPD primers used in this study. This result, could not support their used in characterization studies to differentiate the variants of *Musa accuminata* cv. Berangan clones that have been planted at post planting stage. The primers OPB-10 of arbitrary sequence thereby, can be used to amplify genomic DNA segments of *Musa accuminata* cv. Berangan, but the polymorphisms cannot be detected between the amplification products between the individuals nor between the BAP treatments.

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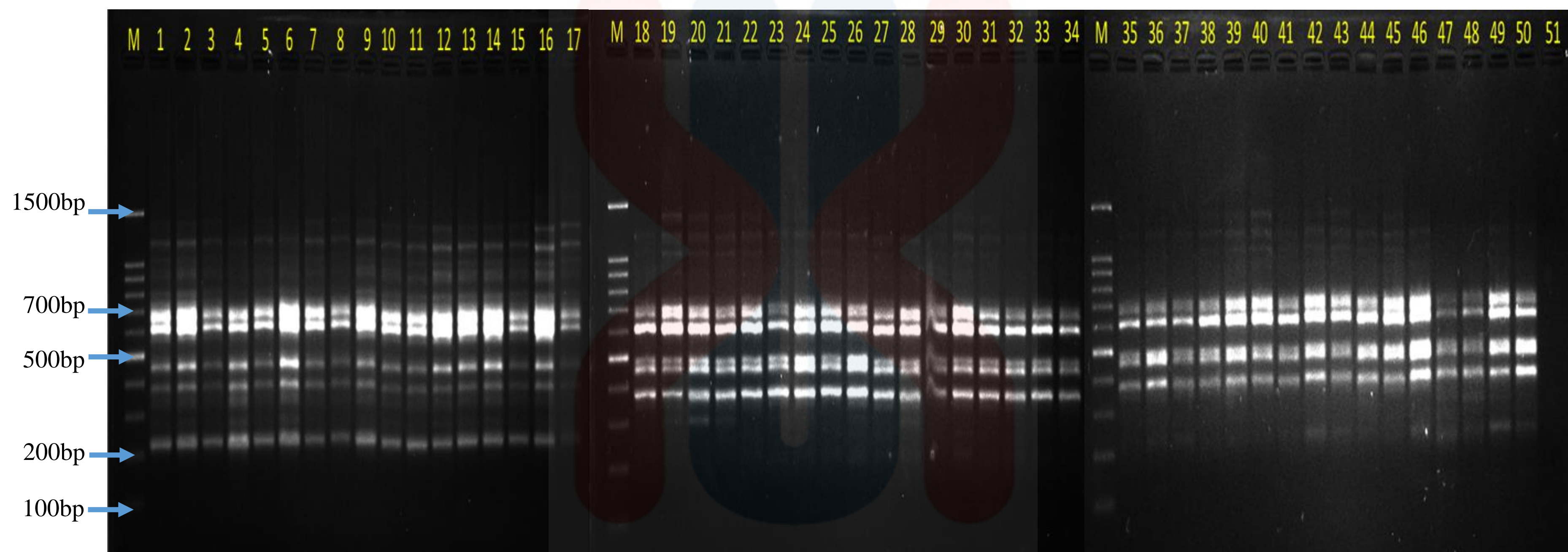

Figure 4.3.2: Random amplified polymorphic DNA of *Musa accuminata* cv. Berangan using primer OPB-10. Lane M= 100bp of DNA ladder, Lane 1-10= Treatment 4 of commercial seedlings, Lane 11-20= Treatment 0 with 0 mg/I BAP, Lane 21-30= Treatment 1 with 5 mg/I BAP, Lane 31-40= Treatment 2 with 10 mg/I BAP, Lane 41-50= Treatment 3 with 15 mg/I BAP, Lane 51= Negative control.

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4.3.3 Evaluation of RAPD primers OPU-06 on *Musa accuminata* cv. Berangan clones.

Based on the result shown, twelve total amplified bands were produced from *Musa accuminata* cv. Berangan by using RAPD primer OPU-06 ranging at 300bp to 1500bp. Based on figured 4.3.3, the result shown that there was no polymorphism and the genetic changes detected within the individual and also not through the banana clones that have been treated with BAP treatment and their morphologies. Thus, all banana clone showed 100% of monomorphic bands that ranging at 300bp until 1500bp. This showed that, there was no correlation between the genetic polymorphisms of banana clones at post-planting stage within the treatments nor its morphologies by using primers OPU-06 and this could not support this study to differentiate the banana clones based on the morphologies of the plants. Therefore, primers OPU-06 of arbitrary sequence can be used to amplify genomic DNA segments of *Musa accuminata* cv. Berangan, but the polymorphisms cannot be detected between the amplification products of different individuals nor between the BAP treatments.

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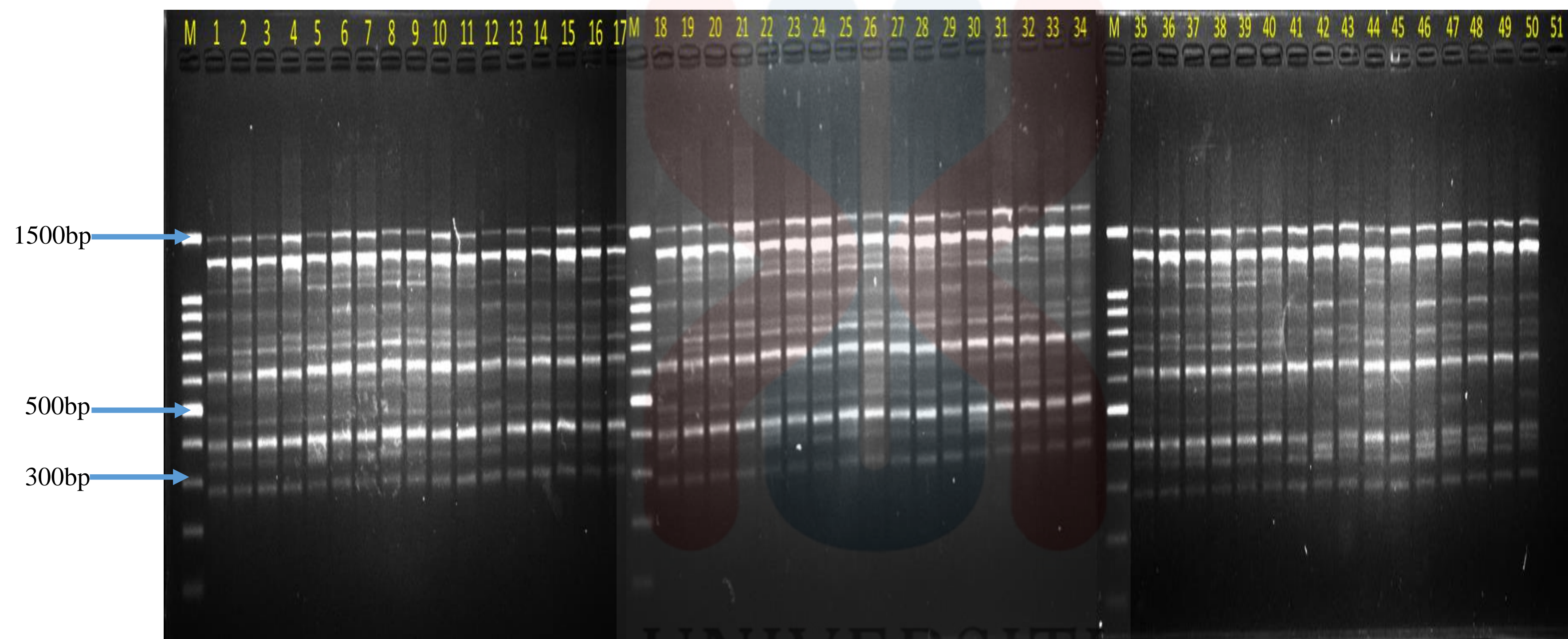

Figure 4.3.3: Random amplified polymorphic DNA of *Musa accuminata* cv. Berangan using primer OPU-06. Lane M= 100bp of DNA ladder, Lane 1-10= Treatment 4 of commercial seedlings, Lane 11-20= Treatment 0 with 0 mg/I BAP, Lane 21-30= Treatment 1 with 5 mg/I BAP, Lane 31-40= Treatment 2 with 10 mg/I BAP, Lane 41-50= Treatment 3 with 15 mg/I BAP, Lane 51= Negative control.

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4.3.4 Evaluation of RAPD primers OPA-19 on *Musa accuminata* cv. Berangan clones.

Based on figure 4.3.4 shown, the total numbers of amplified bands produced were eight and there was one polymorphism detected at approximately 1300bp which the band was missing at lane 18 for sample that was not treated BAP. According to Sheidai *et al*. (2008), the increased of BAP treatment can induces the somaclonal variation, which the plant growth of the banana clones were different. In this study, the sample with no different concentration of BAP treatment showed the genetic changes among the individuals of the banana clones but not within the treatments. Based on the figure 4.3.4, there were no genetic polymorphism were detected at the sample 21 until 50, which originally have been treated with BAP and this showed that, there was no correlation between the genetic polymorphisms of banana clones at post-planting stage with the morphologies by using this primers. This showed that, the polymorphism that detected by primer OPA-19, was only specific to several individual within the same treatment but the genetic was not consistent among the treatment nor its morphology. Based on previous study, primer OPA-19 were selectively used to estimate the genetic diversity among the banana clones because this primer produced strong, intense and unambiguous bands by detecting high yields of the reasonable number of polymorphic fragments for all samples examined. (Jain, Saini, Pathak, & Gupta, 2007). Thus, these shown that primers OPA-19 of arbitrary sequence can be used to amplify genomic DNA segments between individuals of *Musa accuminata* cv. Berangan at post-planting stage.

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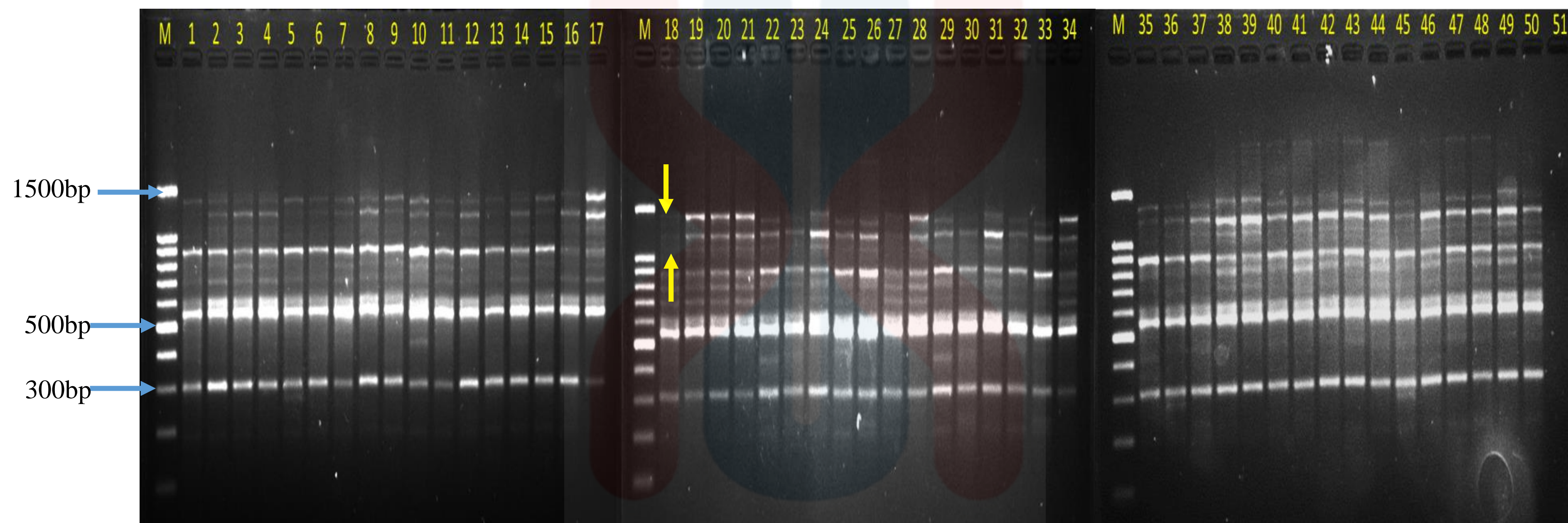

Figure 4.3.4: Random amplified polymorphic DNA of *Musa accuminata* cv. Berangan using primer OPA-19. Lane M= 100bp of DNA ladder, Lane 1-10= Treatment 4 of commercial seedlings, Lane 11-20= Treatment 0 with 0 mg/I BAP, Lane 21-30= Treatment 1 with 5 mg/I BAP, Lane 31-40= Treatment 2 with 10 mg/I BAP, Lane 41-50= Treatment 3 with 15 mg/I BAP, Lane 51= Negative control. Arrow indicates polymorphic bands detected by using primer OPA-19.

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4.3.5 Evaluation of RAPD primers OPA-15 on *Musa accuminata* cv. Berangan clones.

Based on figure 4.3.5, the generated amplified bands counts were six using the primers OPA-15 ranging at 250bp until 800bp. There was no polymorphism and the genetic changes was detected between the individual nor through the banana clones that have been treated with BAP treatment and their morphologies. Thus, all banana clone showed 100% of monomorphic bands that ranging at 250bp until 800bp. This result could not support this study and showed that the genetic polymorphism of the banana clones could not be correlated at post-planting stage with the morphologies by using primers OPA-15. Therefore, the result suggested that primers OPA-15 of arbitrary sequence can be used to amplify genomic DNA segments of *Musa accuminata* cv. Berangan, but the polymorphisms cannot be detected between the amplification products of different individuals nor between the BAP treatments. This result is consistent with previous study from Asnita et al., (2006) that showed 100 % similarity and could not differentiated the genetic changes of *Musa accuminata* cv. Berangan within the micropropagated plants.

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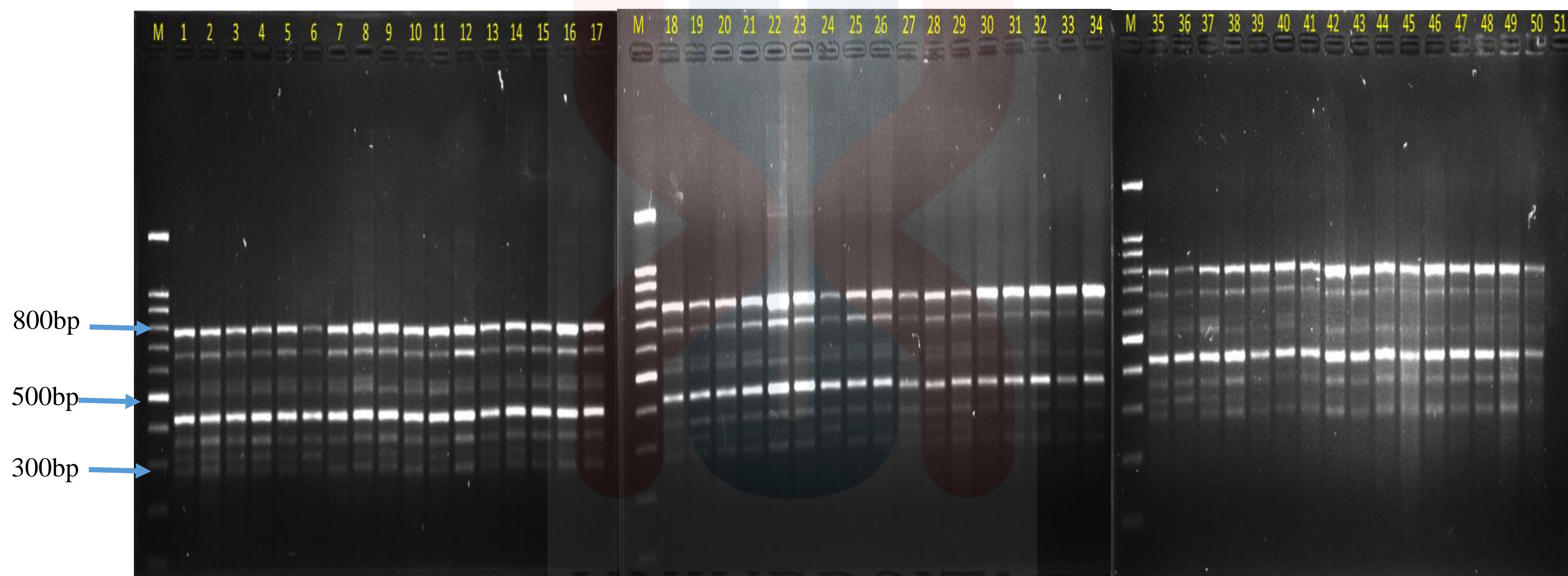

Figure 4.3.5: Random amplified polymorphic DNA of *Musa accuminata* cv. Berangan using primer OPA-15. Lane M= 100bp of DNA ladder, Lane 1-10= Treatment 4 of commercial seedlings, Lane 11-20= Treatment 0 with 0 mg/I BAP, Lane 21-30= Treatment 1 with 5 mg/I BAP, Lane 31-40= Treatment 2 with 10 mg/I BAP, Lane 41-50= Treatment 3 with 15 mg/I BAP, Lane 51= Negative control.

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4.3.6 Evaluation of RAPD primers OPA-06 on *Musa accuminata* cv. Berangan clones.

Based on figure 4.3.6 shown, six amplified bands were detected from *Musa accuminata* cv. Berangan clones by using arbitrary primer OPA-06 ranging at 300bp to 1500bp. From all the amplified bands, there was five total of polymorphic bands ranging from 300bp until 1500bp detected within the individual but not through the banana clones that have been treated with BAP treatment and their morphologies. Thus, this result shown only one monomorphic band that was detected at 550bp. Due to the different treatments of BAP concentration, the plant height for all samples were different. Nevertheless, the polymorphism identified by primer OPA-06 was specific to several individual within the same treatment but not consistent among the treatment nor its morphology. So, there was no correlation between the genetic polymorphisms of banana clones at post-planting stage with the morphologies by using this primers. The result suggested that the primers OPA-06 of arbitrary sequence can be used to amplify genomic DNA segments of *Musa accuminata* cv. Berangan, and that polymorphisms can be detected only to specific individual.

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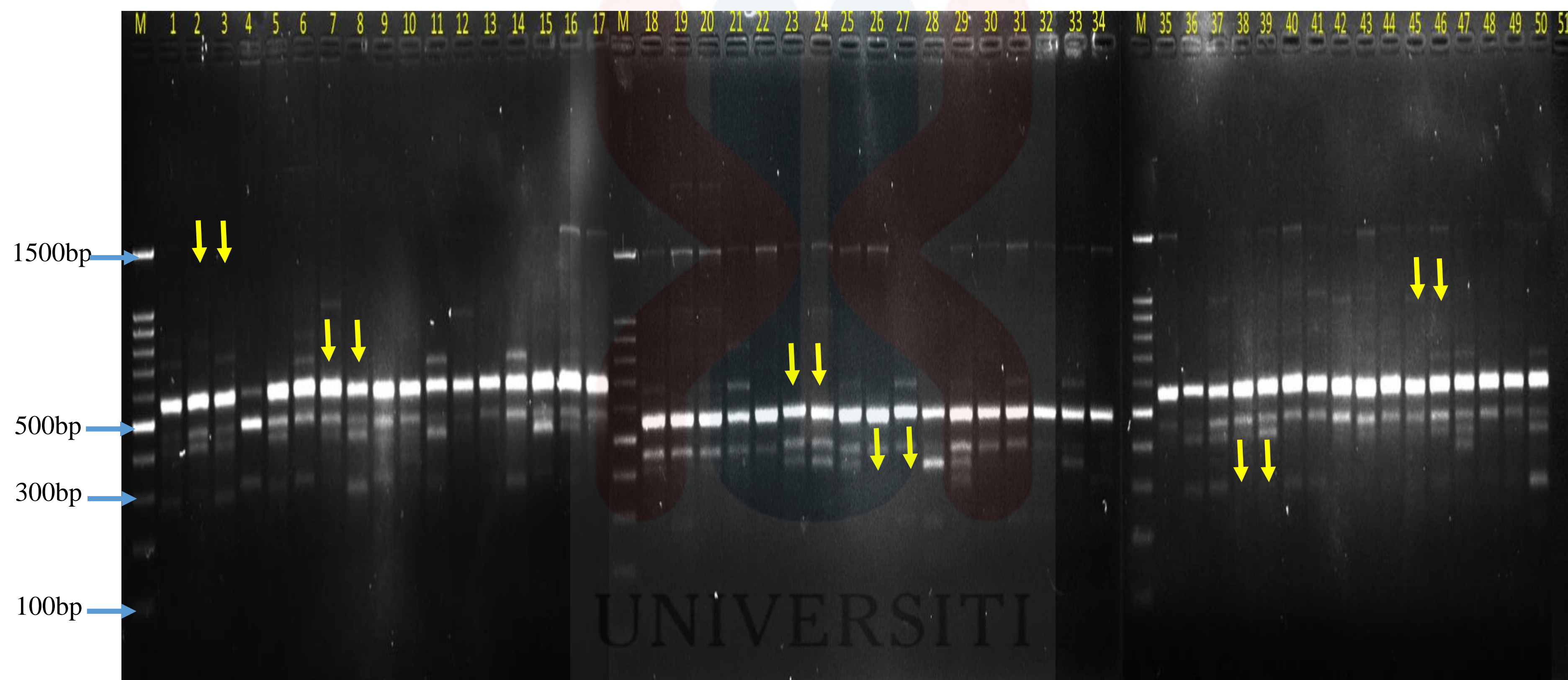

Figure 4.3.6: Random amplified polymorphic DNA of *Musa accuminata* cv. Berangan using primer OPA-06. Lane M= 100bp of DNA ladder, Lane 1-10= Treatment 4 of commercial seedlings, Lane 11-20= Treatment 0 with 0 mg/I BAP, Lane 21-30= Treatment 1 with 5 mg/I BAP, Lane 31-40= Treatment 2 with 10 mg/I BAP, Lane 41-50= Treatment 3 with 15 mg/I BAP, Lane 51= Negative control. Arrow indicates polymorphic bands detected by using primer OPA-06.

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4.4 Band statistic of RAPD markers by using selected primers.

There are six arbitrary primers that were selected to assess the genetic polymorphism of *Musa accuminata* cv. Berangan which were OPJ-13, OPB-10, OPU-06, OPA-19, OPA-15 and OPA-06 (Table 4.4). All these primers were used to evaluate the genetic polymorphism between the bananas clones that were treated with different BAP concentration at post-planting stages. All the primers successfully amplified a total of 44 reproducible bands ranging in size between 1500bp to100bp. The number of bands produced were varied with each primer and individual of the banana clones with a total 44 bands produced, 37 bands (84.1%) were monomorphic and 7 bands (15.9%) that were found to be polymorphic. Based on the level of percent polymorphism detected, three primers were identified (OPJ-13. OPA-19, OPA-06) as good primers to detect the genetic polymorphism between the individual bananas clones. The primers however could only differentiate between individuals within the same treatment and morphology and were not consistently unique to any specific treatment or morphology. Among the primers used, OPU-06 produced the highest number of bands (12) while primer OPJ-13 produced the lowest which was three total bands. The highest number of polymorphic bands (5) was observed in primers OPA-06 (83.3%) while the lowest numbers (1 bands) was observed in both primers which are OPJ-13 (33.3%) and OPA-19 (12.5%), respectively. Preferably, an ideal RAPD primer would be a primer that can produce specific banding pattern for all sample within a treatment or all sample that have same morphology. In this study, the primer was polymorphic only to certain individual within the same treatment and the same morphology. This indicates that the primer is not specific to the treatment nor to the morphology of plant height. However, the primer can be used for fingerprinting for that individual plantlet. The result also induced that changes occurred in somaclonal variation

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may be random, as in to say that the changes induced by exposure to high BAP occurred at certain individual and not to all of the explant. The selected primers used in this study was not specific to the polymorphism for the morphology of the plant height but may be specific to others morphology such as the characteristics of inflorescence and fruits that derived from banana clones. According to Harirah & Khalid (2006), RAPD analysis was carried out to assess the micropropagated banana from male inflorescence derived from *Musa accuminata* cv. Berangan and eighteen arbitrary primers was selected. Thus, no somaclonal variation and this showed that primer used in this study should be selected based on the morphology of the banana clones to induce the somaclonal variation that related to morphology of plant height in *Musa accuminata* cv. Berangan at post-planting stage.

Table 4.4: Amplification pattern of RAPD markers with polymorphic and monomorphic percentage.

Primers	Total number of bands	Number of monomorphic bands	Percentage of monomorphic band (%)	Number of polymorphic bands	Percentage of polymorphic band (%)
OPJ-13	3	2	66.7	1	33.3
OPB-10	9	9	100	0	0
OPU-06	12	12	100	0	0
OPA-19	8	7	87.5	1	12.5
OPA-06	6	1	16.7	5	83.3
OPA-15	6	6	100	0	0
TOTAL	44	37	-	7	-

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CHAPTER 5

CONCLUSION AND RECOMMENDATION

In this study, Random Amplified Polymorphic DNA (RAPD) genetic marker was used to analyse the genetic diversity and relationship among the banana clones with different morphological characteristic in *Musa accuminata* cv. Berangan at post planting stages. This research focussed on testing 50 individuals of the banana clones which have been treated with different concentration of BAP. This study found that, from all the selected primers used OPA-06, OPJ-13 and OPA-19 were shown to be good primers to assess the polymorphisms among the individuals of the banana clones but could not identify the differences between the treatments and the morphologies of the banana clones at post-planting stages. The number of bands produced varied with each primer and individual of the banana clones with out of total 44 bands produced, 37 bands (84.1%) were monomorphic and 7 bands (15.9%) were found polymorphic. The highest number of polymorphic bands produced were (5) observed in primers OPA-06 (83.3%), followed by OPJ-13 (33.3%) and OPA-19 (12.5%). From the result, it can be concluded that, six selected primers used in this study were useful to amplify higher number of bands but not to differentiate the banana clones based on their treatments and morphologies at post-planting stages. Based on the result obtained, it is recommended to increase arbitrary RAPD primers used and to select the primers based on the morphological characteristics shown on the banana clones at post-planting stage for better result. Besides, the genetic

assessment can be used by using other DNA base marker which more accurate and reproducible compared to RAPD analysis and lastly, RAPD primer can be developed to SCAR marker for more consistent result.

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APPENDICES

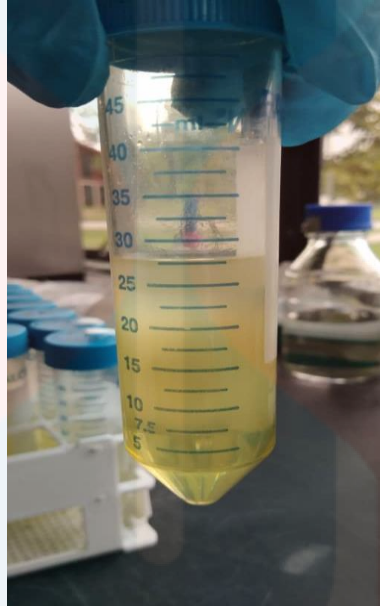

Figure 1: The white thread of DNA.

Figure 2: DNA pellet of *Musa accuminata* cv. Berangan.

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Figure 3: Grinded young leaves of *Musa accuminata* cv. Berangan were incubated in water bath at 65 °C.

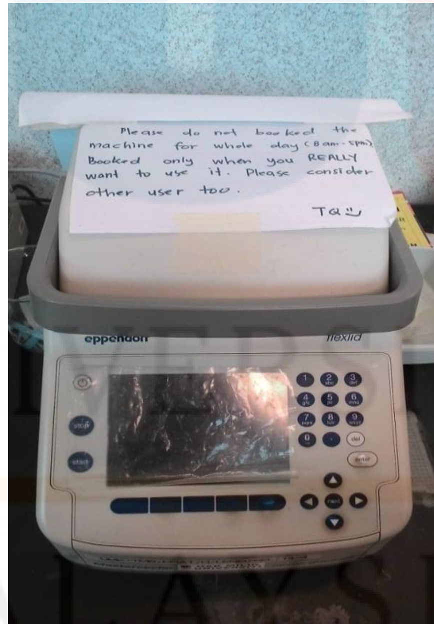

Figure 4: PCR machined.

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