POST-PLANTING EVALUATION OF BANANA cv BERANGAN TREATED WITH DIFFERENT CONCENTRATION OF BAP DURING MICROPROPAGATION

By

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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 submitted for higher degree to any universities or institutions.

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Student:

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I certify that the report of this final year project entitled “Post-Planting Evaluation of Banana (*Musa acuminata* cv Berangan) Treated with Different Concentration of BAP During Micropropagation” by Zahirah Haryati Binti Zamli, matric F14A0417 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Agrotechnology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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Penilaian Pasca Penanaman Di Ladang Pisang Cv Berangan Terhasil Melalui Kepekatan BAP Yang Berbeza Semasa Mikropropagasi

ABSTRAK

Musa acuminata kultivar Berangan ialah sejenis pisang jenis Cavendish yang ditanam di Malaysia. Kajian ini dijalankan adalah untuk menilai prestasi pokok *Musa acuminata* kultivar Berangan di peringkat ladang yang terinduksi dengan kepekatan BAP yang berbeza semasa di peringkat *in vitro***.** Matlamat kajian ini adalah untuk membandingkan prestasi tumbuhan tisu kultur yang dihasilkan dengan kepekatan BAP yang berbeza dan penyesuaian pokok setelah ditanam di peringkat ladang. Sekaligus dapat mengenalpasti variasi somaclonal pada pokok. Pokok yang terinduksi dengan 0mg/L, 5mg/L, 10mg/L dan 15 mg/L BAP telah ditanam di peringkat ladang selama 12 minggu. Parameter yang dikaji ialah panjang daun, lebar daun, jumlah daun semasa, ketinggian pokok dan ukur lilit batang pokok. Hasil dapatan kajian mendapati bahawa pokok yang terinduksi dengan 5mg/L BAP menunjukkan pertumbuhan yang cepat di peringkat ladang. Kajian selanjutnya perlu dijalankan sehingga ke peringkat pembungaan, pembuahan dan penuaian untuk membantu pemilihan pokok yang mempunyai ciri agronomi yang baik.

Post- Planting Evaluation of *Musa Acuminata* cv Berangan Treated With Different Concentration of BAP During Micropropagation

ABSTRACT

Musa acuminata cv Berangan is a Cavendish type banana that is popularly grown in Malaysia. This experiment was carried out to evaluate the field performance of *Musa acuminata* cv Berangan in the field treated with different BAP concentration during *in vitro.* The aim of this study is to compare the performances of the tissue culture seedlings produced from the different concentrations of BAP during *in vitro* and their adaptability in the farm. Furthermore, to identify any possible somaclonal variation showed in the plants. Plants treated with 0mg/L, 5mg/L, 10mg/L and 15mg/L of BAP were planted in the field for 12 weeks. Parameters measured were leaf length, leaf width, and numbers of functional leaves, plants height and the size of pseudo stem girth. The results show that the plants treated with 5mg/L of BAP shows vigorous growth than other treatment. Further studies need to be conducted until flowering, fruiting and harvesting stage to help selection of the plants with good agronomical characteristics.

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LIST OF ABBREVIATION AND SYMBOLS

ANOVA	Analysis of variance
BAP	6- Benzylaminopurine
Cv	Cultivar
mg/l	milligram per liter
sp	Species
SPSS	Statistical Package for the Social Sciences

CHAPTER 1

1.0 Introduction

1.1 Research background

Bananas and plantains are one of the major food crops for over 100 countries in tropical and sub-tropical regions and provide food for millions of people (Frison, 1998). Pisang Berangan is one of the popularly grown banana cultivars in Malaysia alongside with Pisang Raja and Pisang Rastali which are normally grown for home consumption or local market (Hui, 2012). *Musa acuminata* is diploid wild bananas that contribute to the A genome whereas the B genome was from *Musa balbisiana*. Most of commercial and domesticated banana are triploid.

Tissue culture is a fast method to meet the high demand of banana planting material. Tissue-culture is a cultivation techniques used widely in developed countries to achieve expeditious production, disease-free and invariant plants (Galo, 2009). Even though tissue culture is meant for the production of uniform plants, variation could appear through tissue culture techniques. This variant is called somaclonal variation (Moradi, 2015).

Through the existence and stability of DNA methylation in somaclonal variant plant, it can be used for crop improvement. Furthermore, the inferior types obtained from this study can be eliminated at early stage for the next culture and the superior off types obtained can be used to cultivate and improvise in the future. Somaclonal variation remarks a constructive in the production of useful new traits for both production and resistance.

1.1.1 Management of Banana plant on farm

A proper land preparation is vital and critical before planting in order to allow banana plantlet to grow to its full potential during the early stage as it is very tough to rectify the mistake after plant establishment (Robinson & Sauco, 2010). All aspects of farm management need to be taken into consideration such as the soil condition with good drainage system and adequate fertility. Next, weeding has to be done properly and regularly to reduce competition for nutrient and incidence of diseases. Proper sanitation of tools used is very vital as to avoid the spread of pathogen that could be infected to the plants.

1.2 Problem statement

Tissue culture plantlet are said to be succumb to common banana diseases like Panama, Black Sigatoka and Moko which spread rapidly causing the loss of yield in banana cultivation. Even though tissue culture plantlet are said to be disease-free and have uniform growth, the long exposure of PGR may cause off types which are abnormal size of leaf, abnormal size of girth, or abnormal plant height among the tissue

culture plants (Moradi, 2017). Verification at nursery stage and field condition is needed to identify this off types. Certain off types may display good agronomical characteristics and may also show inferior characteristic.

1.3 Hypothesis and objectives

If the treatment displays different morphological characteristics, there could be changes at their genetic level. Plants with superior characteristics can be selected for mass propagation. Otherwise, if the plants shows no different at their growth performance, high BAP concentration do not made any changes at the genetic level. Therefore, the objectives of this study are;

a) To compare the performance of tissue culture seedlings produced from different concentration of BAP *in vitro* and their adaptibility in the farm.
b) To identify any potential somaclonal variant showed by the plants.

1.4 Scope of the study

The experiment started with plantlets being acclimatized 8th week in nursery stage until 12th week grown in the farm *of Musa acuminata* cv *Berangan* plants. Acclimatized banana seedlings were planted in a 2 acre farm size, Kg Seberang Jeli (5°41'56.2"N 101°49'37.8"E).

1.5 Significance of study

This study is applicable to the banana industry in producing cheaper banana seedlings through application of optimum BAP concentration used during *in-vitro* with further verification during early stage for the selection of superior banana plant. Furthermore, once the off types with good characteristics such as rapid growth or good agronomical characteristics being identified, selected line can be chosen for further mass production. Meanwhile, off types with inferior characteristics can be eliminated at early stage to reduce cost of maintenance.

1.6 Limitation of study

This study was performed in one selected area which is Kg. Seberang Jeli, Kelantan that is known as flood affected area in 2014. Furthermore, the planting of tissue- culture plantlet will be during Mac – April (Transition Monsoon), Mei – July (Southwest monsoon) which in both season, the plot area received about 20% more rainfall than the usual time of the year.

CHAPTER 2

2.0 Literature review

2.1 Economic importance of banana

Banana has tremendous socio-economics significance. Banana produces many important products. In Australia, banana is used to produce paper and in Philippines the clothing industry made from banana fibre is one of the biggest industries. Other by-products using banana's fibre are natural sorbent, natural water purifier and the base material for paper and pulp industry. Malaysia produces 535,000 matric tones of banana every year. The commercial banana is called Cavendish banana that are the subgroup of the triploid "AAA". Cavendish banana has more sugar making in sweeter than plantains. The word "Banana" derived from an Arabic word *banan* which mean finger (Boning, 2006). *Musa* is a member of *Musacea* family and all *genera* are monocotyledons and that species able to grow up to 15m in height (OGTR, 2016). In 1955 a genome nomenclature was proposed and scientists assigned traits for selected morphological features that required chromosome counts to assign according to genome group (Pillay, 2007).

2.2 Banana origin

Bananas were widely spread in South East Asia while *Musa acuminata* was said to be originated from Malaysia (UNCST, 2007). Nowadays, tetraploids bananas are artificially bred for marketing purpose while most commercial banana varieties are triploids. The most popular dessert banana is from AAA genomes that are widely used in

cooking or juice making. Now the *Musa acuminata* is grown in many countries and became the important crop in Brazil, India and Equador (Matthew, 2016).

2.3 Tissue Culture

Tissue cultures provide disease-free planting material during the time of planting and it can be maintained that way with the implementation of good agricultural practice and proper crop management (Njukwe, 2013). It is also widely known that tissue culture plantlet performing better growth in the field thus resulting in less harvesting time and better yield compared to the conventional sucker (Njukwe, 2013). Other than that, tissue culture seedling enables uniform maturity during harvesting season enabling mechanization and facilitates marketing strategies. Next, the ability of tissue culture producing true to type which is similar to the mother plant enable expected yield return. Therefore, selection of the mother plant with superior characteristic is important in ensuring high productivity (Singh, Uma, & Karihaloo, 2011).

Although tissue culture can produce more plantlets than the conventional method over time, the cost of planting material is relatively high since the protocol requires aseptic condition with designated facility. Certain tissue culture procedure requires sophisticated equipment and expensive reagents thus incur high cost to cover the overhead cost of the machineries and specialties needed in this field. Some techniques in tissue culture require skilled breeders due to the complex procedure and the procedure varies according to the plants. Other than that, tissue culture plantlets required skilled personnel during the process which is not feasible to the smallholder in rural areas (Singh, 2011). This technique also requires special care attention in the field during their early stage of plant establishment.

However, the colossal benefit of tissue culture is it provides plant breeders with number of replicate to conduct experiment through regeneration and proliferation of plants through micropropagation techniques like synthetic seeds, conservation of germplasm, somaclonal variation and so forth (Pasquar, 2014). Plant tissue culture is one of the valuable tools to enhance studies in morphogenesis and molecular biology that could be exploited in crop improvement. Biotechnology improved crops can reduce the usage of pesticide and even fungicide of plants thus ensuring food security for the world population.

Tissue culture is a wide field involving may different scope of study. The popularly used tissue culture techniques are embryo culture, culture of meristem, *in vitro* selection, somaclonal variation, somatic embryogenesis, micropropagation, anther culture and many more. Tissue culture allows crop improvement to take place by allowing the breeders only cultivate the superior types of plants to be micropropagated after selection of somaclonal variant plants.

Plant Growth Regulator (PGR) is added in media of tissue culture to trigger various types of differentiation and growth during *in vitro.* There are 5 groups of PGR that are auxin, gibberellins, cytokinins, ethylene and abscisic acid. The usage of auxin at low volume is usually to induce root initiation meanwhile at higher concentration it can induce callus formation. 2,4-dichlorophenoxy acetic acid (2,4-D) is popular type of auxin. Whereas the usage of cytokinins is to trigger the shoot proliferation and 6-benzylaminopurine (BAP) are used widely in commercial plant micropropagation. The usage of Plant Growth Regulator (PGR) may induce the somaclonal variation (Pasquar, 2014).

2.4 Somaclonal variation

Somaclonal variation is commonly described as programmed losses of cellular control. The environment of tissue culture may portray a form of stress to the plant cell structure, which may cause the regeneration of off-type plants (Leva, 2017). The 2 types of factors are said contributed to these off-type plants which are endogenous factor and exogenous factor.

Endogenous factor is the genotype of mother plant used as starting material for culture. Culture seldom reveals any pre-existing variability and number of chromosomes of tissue may affect the variation. Different genomes react differently toward *in-vitro* stress (Leva, 2017). Meanwhile, exogenous plants are collected from original plant and cultured using nutritional media. Exogenous factors influence the chances of the tissue to adjust to the new conditions thus contributing to somaclonal variation (Leva, 2017).

In vitro propagation involves regeneration into plants through asexual reproduction which involves mitotic divisions and this resulted to the genetically uniform plant (Read, 2014). However, technical basis at genetic manipulation in plant *in vitro* resulted in the variation during culture process. The variation may involve superior growth and the inferior ones. Previous studies by Pasquar (2014) reported that during cell division, irregularities may occur, producing in numerical and structural abnormalities in regenerated plants.

Somaclonal variations were contributed by growth regulator, temperature, light, osmolarity and inadequate *in vitro* cell cycle control. Increase in plant growth regulator contributes to the instability of cell cycle thus resulting on somaclonal variation. Morphological variants occur according to the crop. However, it is reported by Sahijram *et al*, (2003) that off-types of banana from tissue culture ranged from 6% to 38%.The phenomenon of somaclonal variation in commercial tissue culture may be considered contemptible due to variation and loss of genetic fidelity.

2.5 Crop improvement

Induced mutation technique in order to produce the desired plant characteristics is a well-known method in crop improvement (Jain, 1998). Crop improvement is crucial in producing plants that can produce better yield and have all the good agronomical characteristics. Through the selection of superior off-types plants, studies and research in producing new cultivar can be done. One of the examples of new banana varieties made from the selection for crop improvement is variety of M19 released by National Banana Research Program in Uganda (NBRP, 2017)

2.6 Good Agricultural Practice (GAP)

Several benefits can be obtained from various aspects through the implementation of good agricultural practices (GAP). One of good practice before planting is by digging the planting hole about 30cm diameter and measure the distance of one plant to another is approximately 7 feet for good aeration and better farm management (Sailila, 2017).

GAP helps to optimize nutrient use and minimize nutrient losses. Optimum amount of fertilizer used according to the actual need could reduce the cost input at the same time icrease the farm production. Separation of fertilizers from other agro-chemical products during storage could prevent cross-contamination. Working area which is free from waste, spillage and leakage could create a pleasant environment for workers thus increase the productivity. Proper management of inorganic fertilizers by following the First-In First-Out (FIFO) practice to avoid wastage from expired chemical. A good record keeping of fertilizers with the specifics of product name, source, data and quantity obtained is also important to keep the information for further action and improvement. Proper application of fertilizers with good record keeping can help farmers tracking the expiry dates of the chemical used and estimates the amount of budget required for farm maintenance. Appropriate handling of equipment is important during the application or organic and inorganic fertilizers and most importantly is to recycle crop and organic residues to stabilize soil nutrients.

Weed control can be done manually or mechanically using barebone or any mechanical grasscutter. Manual method of weeding is needed to be done at one meter around the plant. Weeding is important and need to be done regularly to loosen the soil around the plant to allow water to infiltrate and reach the root zone of the plant. Besides,

it is vital as to reduce competition for soil nutrition with weeds and other unnecessary plant.

Other GAP that should be followed is leaf disease control. Farmers need to remove infected leaf by trimming and this should be done at least once a week. Infected disease may be left on the field as to mulch.

Selection of cultivars and varieties before planting is a critical phase to forecast the production, nutritional value and estimation their response towards fertilizers and pesticides. Contingency plans is an important thing as to improves preparedness and implement rapid response and containment procedures in case of pest and disease outbreaks and to promote integrated pest management (IPM) practices to overcome the problem.

Bananas are prone to several of diseases which some of them may be fatal to the banana industry. Thus, it is important to design a relevant biosecurity measures in growing banana to avoid the spread of disease to other banana plants. To control such disease from entering the farm, farmers need to conduct inspection for the presence of the disease and treatment area intervention. Inspection of the plant has to be done regularly trough visual of the leaves or the pseudo-stem of banana plant.

Contingency plans is an important thing as to improve preparedness and implement rapid response and containment procedures in case of pest and disease outbreaks and to promote integrated pest management (IPM) practices to overcome the problem. Banana can be infected with several of diseases and some of them may be fatal to the banana industry. Thus, it is important to design a very impeccable and relevant biosecurity restriction in growing banana to avoid the spread of disease to other banana plants (FAO, 2017).

CHAPTER 3

3.0 Materials & Methods

3.1 Location of the Study and planting materials

The experiment were carried out in Kg Seberang Jeli, Jeli which is flood affected area in 2014. Plantlets used for the experiment is coming from tissue culture treatment of different PGR concentration which are exposed to 0 mg/L, 5mg/L, 10mg/L and 15mg/L.

3.2 Materials used

The materials used for this study are tissue culture banana plantlets developed from the previous study using different BAP treatment (control, 5mg/L, 10mg/L, 15mg/L) during *in vitro* stage. Plantlets were acclimatized in nursery for about 3 months before being transferred to the field. Plantlets were sprayed with pesticide before planting and dead leaves were trimmed to remove any possible residue of pathogen.

3.3 Preparation of the field

The planting area was tillage after land clearing stage for about 25 cm with rotary cultivator once before planting. Holes digging were done after land tillage for about 15 cm deep.

3.4 Flow chart of the study

Below are the chronological steps involved in this study.

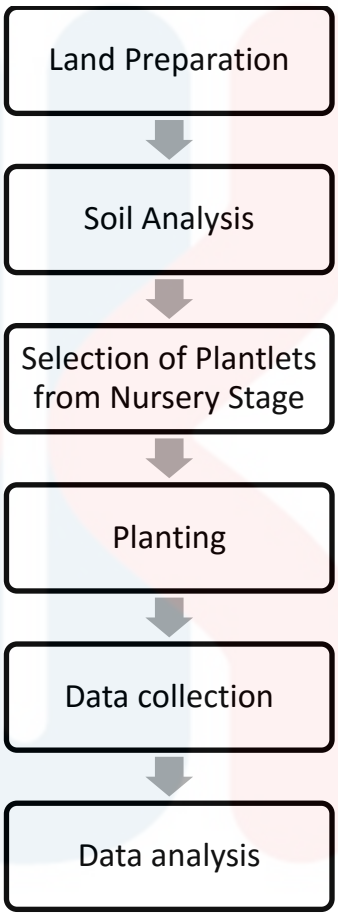

Figure 3.1 Flowchart of the study

3.5 Experimental design

Completely Randomized Block Design was used in this study. Treatments were assigned at random within blocks of subjects and any treatment can be adjacent to any other treatment but not to the same treatment within the block. Randomization is important in this study to ensure applicability of results to the entire population of inference and to reduce the chance of systematic bias affecting the accuracy of the

parameters of interest (Crossa, 1993). This experimental design is widely used especially in agricultural experiment.

3.5.1 Experimental design

Table 3.1: Plants assigned in one plot

T1	T0	T3	T2
T3	T2	T1	T0
T2	T3	T0	T1
T0	T1	T2	T3

T0 = Treatment 0

T1 = Treatment 1

T2 = Treatment 2

T3 = Treatment 3

3.6 Parameters measured

In this study, the parameters used are; girth size that were measured 2 cm from the soil ground (refer Figure 1). The heights of plants were measured from the bottom of pseudo stem above the soil until the bottom of new leaf opening (Figure 1). Other parameters observed were number of leaves, leaf length, leaf width, leaf area, time requires to flowering stage, disease incidence. Leaves length were measured from the tip of the lead until the bottom end of the leaf (Figure 1). Leaves width were taken from the end to end at middle of the leaf.

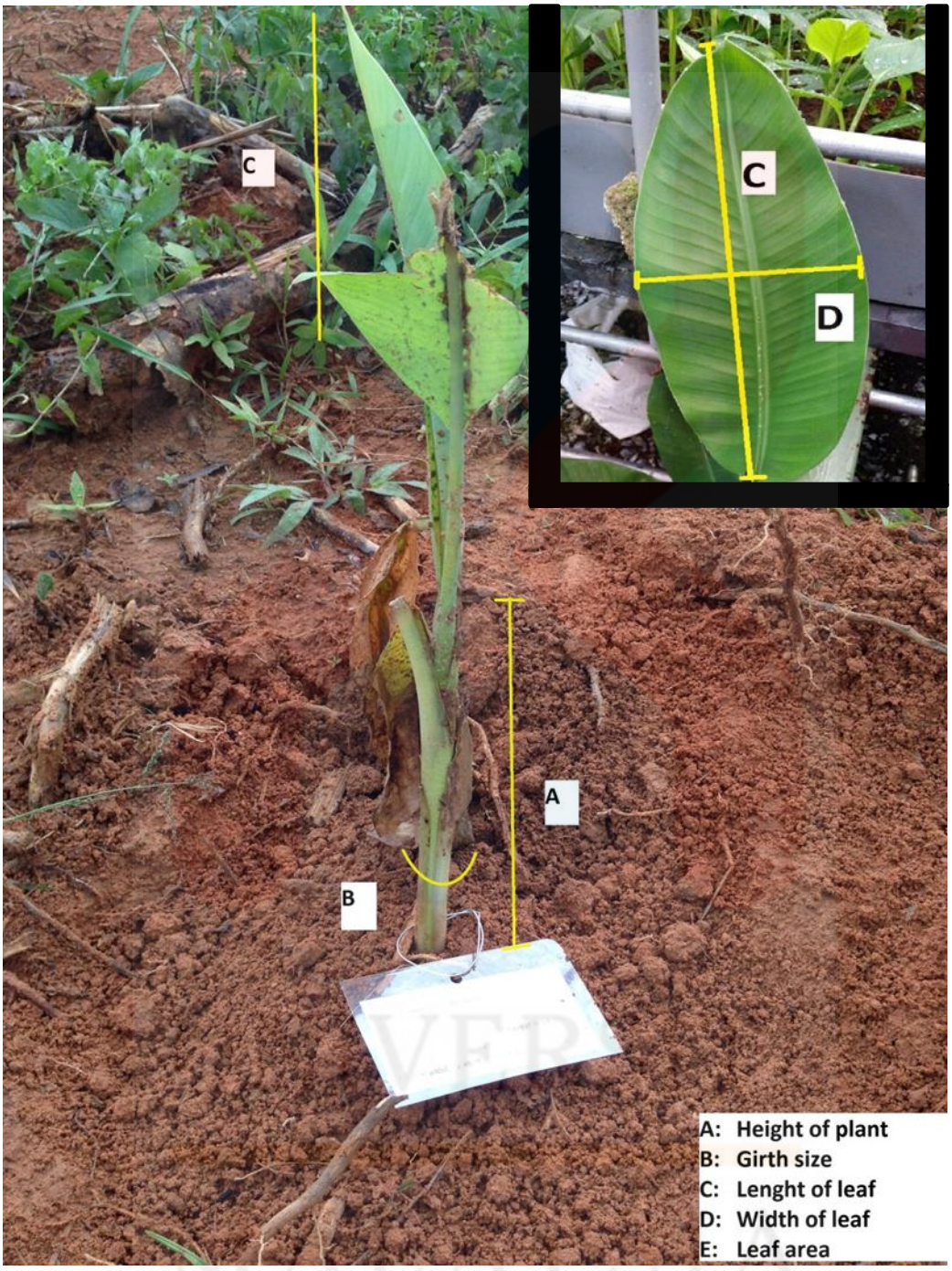

Figure 3.2: Parameters measured

3.7 Treatments

In this study, seedlings produced from four different treatments during *in vitro* evaluated 12 weeks being transplanted in the field. The four treatments used are; control (T0), 5 mg/l of BAP (T1), 10 mg/l of BAP (T2) and 15 mg/l of BAP (T3). The significance of establishing the replicates is to ensure scrutiny of all plantlets in each replicates exhibit similar responses during the field performance.

Table 3.2: Treatments induced

Name of Treatment	BAP induced	Number of planlets
T 0	0 mg/L	30
T 1	5 mg/L	30
T 2	10 mg/L	30
T 3	15 mg/L	30

3.8 Planting

3.8.1 Plantlets Screening

Banana can be affected to biotic and abiotic factors in the environment that may reduce the yield and make the plant stunted. Normally, 20-30 cm tall of banana seedlings are ready to be planted on the field with 4 to 5 broad leaves. The leaves must be healthy to run photosynthesis in the field because tissue culture plantlets are said to be sensitive to the environment (Singh, 2011). The seedlings that follow all the guidelines can be taken to field for planting. Before taking the seedlings to field, the plantlets were irrigated and sprayed an equal amount of pesticide.

3.8.2 Planting Distance

In this study, the planting distance used is 6 feet from one to another because tissue culture plantlet has high uniformity and high yield potential than conventional plants thus requires enough sunlight, optimum soil nutrient and to avoid shading from other plants thus making sure there is a good air circulation. This allows good aeration to plant canopy and allows wet condition to dry quickly. This is to reduce the chance for fungus attack.

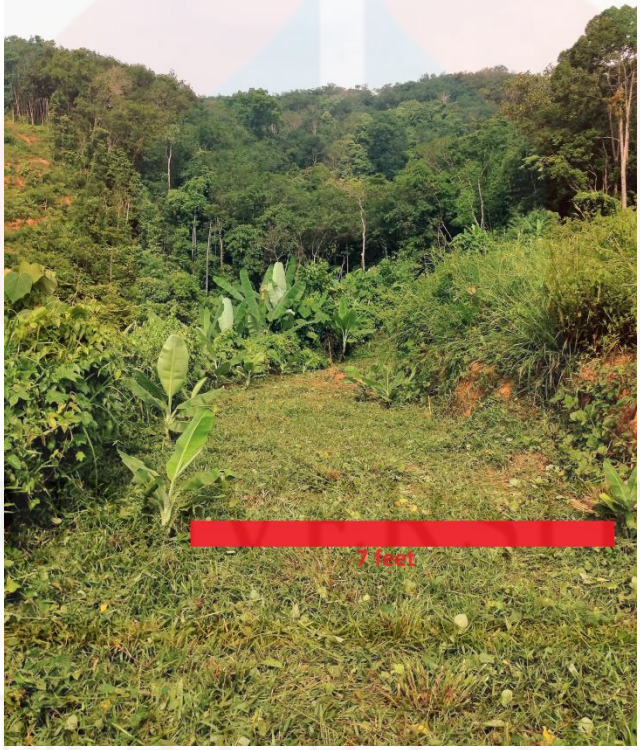

Figure 3.3: Planting distance

3.8.3 Planting Depth

In this study, the depths of the holes are 15 cm. Before planting, organic fertilizers were added inside the planting hole at equal amount and planting holes were slightly bigger than the polybag size. After planting, soils were pressed lightly to stabilize the plant and to avoid loose soil.

3.8.4 Plant management

The land need to be wet if the land do not receive rain at the day to avoid water stress. Banana has high water requirement. Banana plant may become stunted and resulting in small leaf area, weak pseudostem which may lead to plant fall, reduced yield, and discoloration of fruits and leaves which may be unfavorable to the market (OGTR, 2016). Plants were pruned to leave about 5 to 7 of leaves to allow optimum photosynthesis for the plant. Pruning of dead or infected leaves also is important to avoid the fungal spores to spread to other plants (UNCST, 2007).

3.4.5 Weed control

Weed that are typically grow at banana cultivation farm are ‘lalang’ (*Imperata cylindrica*), white blossom grass (*Asystasia intursa*), costus grass (*Borrenia latifolia*) and ‘kapal terbang’ grass (*Chromolaena odorale*). Weed can give competition for nutrient toward banana plants and should be removed immediately (Henriques, Jeffer, Lacher, & Kendall, 1996). In this study, weed control was done manually using hoe about 1 m around the plant. Herbicide should be sprayed during hot weather so that the herbicide will dry quickly and does not affect the banana plants.

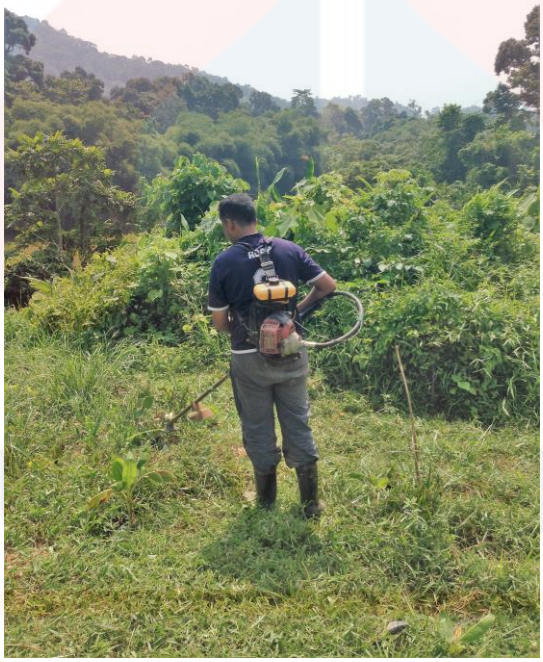

Figure 3.4: Weed control

3.4.5 Pruning

Pruning is a sanitary removal of leaves that will decrease the percentage of fungus attack and increase the efficiency of fungicide applied. The leaves pruned are left on the soil to mulch. Unremoved diseased leaves can be the sources for disease spread to the next coming seasons.

Figure 3.5: Pruning

CHAPTER 4

4.0 Results and discussions

The growth performance of *Musa acuminata* cv Berangan produce from different BAP concentration during *in-vitro* was evaluated at post-planting stage in the field. After 2 weeks being transplanted in the field, the growth performance was observed every 2 weeks until the 12th week. Evaluations were conducted by measuring the length of leaf, width of leaf, height of plants, number of functioning leaves and girth size of the pseudostem.

Table 4.1: Mean of leaf length (cm) of plants treated with different BAP concentration

Treatment	Control (cm)	5mg/L of BAP (cm)	10mg/L of BAP (cm)	15mg/L of BAP (cm)
Week 2	18.66 ± 4.87	23.68 ± 13.24	21.26 ± 11.00	23.64 ± 8.25
Week 4	29.56 ± 11.67	39.91 ± 20.48	27.77 ± 13.77	28.53 ± 8.86
Week 6	29.96 ± 9.14	40.09 ± 22.94	30.10 ± 14.14	31.75 ± 10.16
Week 8	34.47 ± 12.48	43.60 ± 25.13	31.19 ± 15.64	32.60 ± 11.90
Week 10	35.70 ± 12.02	47.40± 28.14	36.6± 18.10	35.83± 12.88
Week 12	40.17± 12.54	51.07± 30.11	40.07± 19.95	38.53 ± 13.23

Figure 4.1 depicts an increasing of length leaf in all treatment from the 2nd week until the 12th week in the field. At the 2nd week, the length of leaf for plants treated with 5mg/L was 23.68 ± 13.24 cm. The mean length of the leaf was increased to 51.07 ± 30.11 cm in the 12th week. Plants treated with 5mg/L of BAP during *in vitro* shows significant growth than plants treated with 0 mg/L, 10mg/L, and 15 mg/L of BAP.

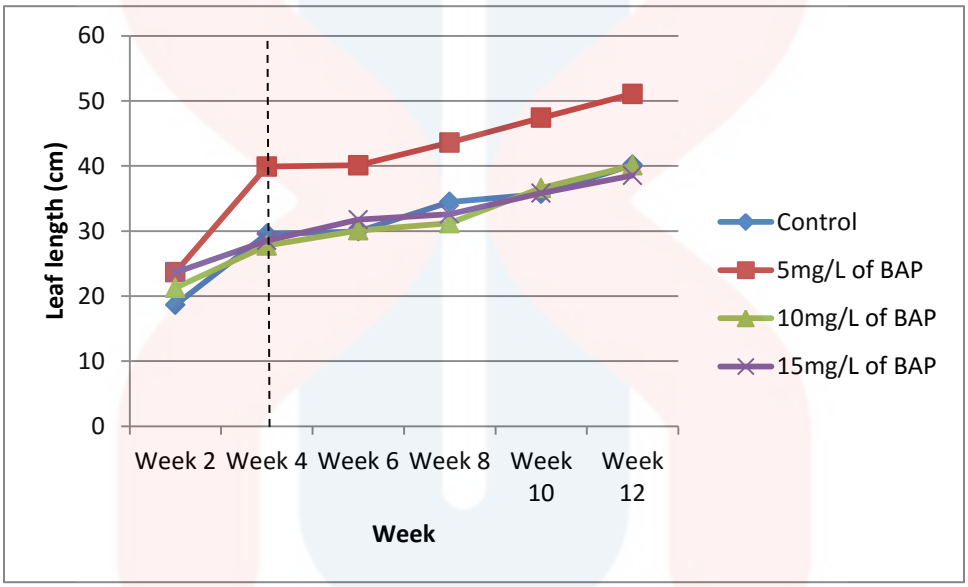

Figure 4.1: The leaf length of plants treated with different BAP concentration

The leaf length of plants treated with 5mg/L in week 4 shows the mean leaf length of 39.91 ± 20.48 cm. High standard deviation shown in Table 4.1 suggesting that the mean does not represent individual's performances of the same treatment due to high variance between the samples. However, plants with the longest leaf were found in individuals treated with 5mg/L of BAP. Since the photosynthesis takes place in the leaf, its length and width of leaf could influence the plant growth. Photosynthesis is vital in plant growth as it is the source of food for the plant and major growth of the plant as it contributed by this process.

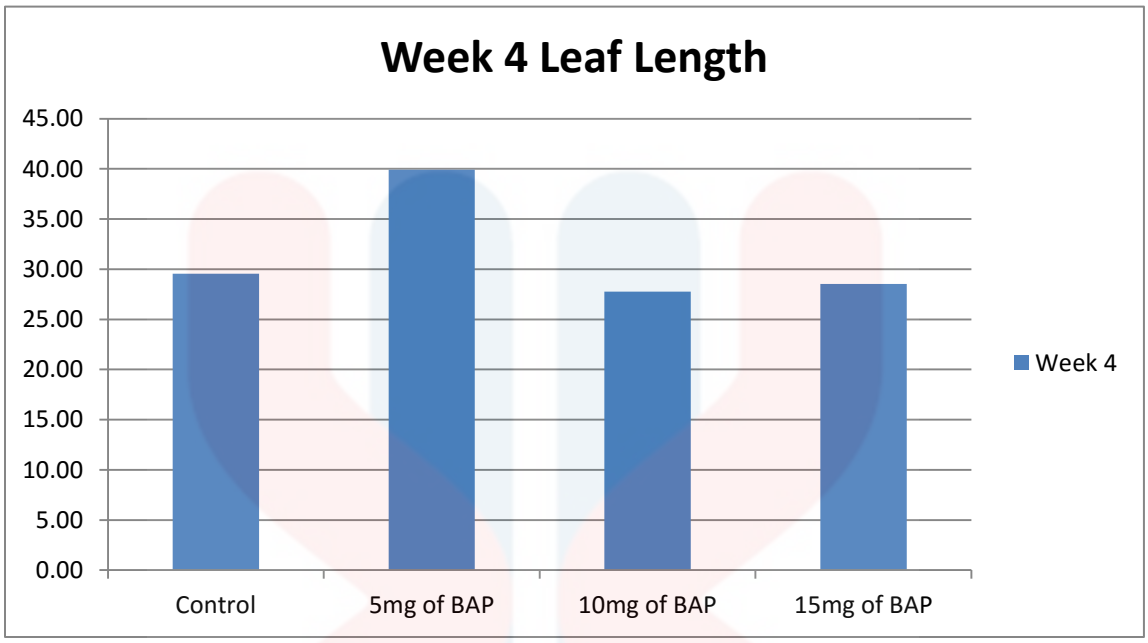

Figure 4.2: High variation different being observed in the leaf length of the treatment in the week 4

Figure 4.2 shows that plants treated with 5 mg/l of BAP shows the higher growth in week 4 with mean of 39.91 ± 20.48. Meanwhile, plants treated with 10 mg/l have the lowest mean of 27.77 ± 13.77 cm.

Table 4.2: Leaf width of plants treated with different BAP concentration

Treatment	Control	5mg of BAP	10mg of BAP	15mg of BAP
Week 2	8.35 ± 1.59	9.32 ± 4.00	9.63 ± 4.59	10.82 ± 2.95
Week 4	12.03 ± 5.46	16.55 ± 7.68	12.17 ± 5.25	14.95 ± 10.14
Week 6	10.93 ± 2.53	14.91 ± 8.63	11.83 ± 4.61	12.12 ± 4.40
Week 8	13.90 ± 5.95	16.53 ± 9.80	13.09 ± 5.33	11.53 ± 4.60
Week 10	14.46 ± 4.09	18.50 ± 10.51	14.7 ± 5.37	15.76 ± 4.20
Week 12	15.53 ± 4.14	20.63 ± 11.26	16.30 ± 5.38	18.23 ± 6.34

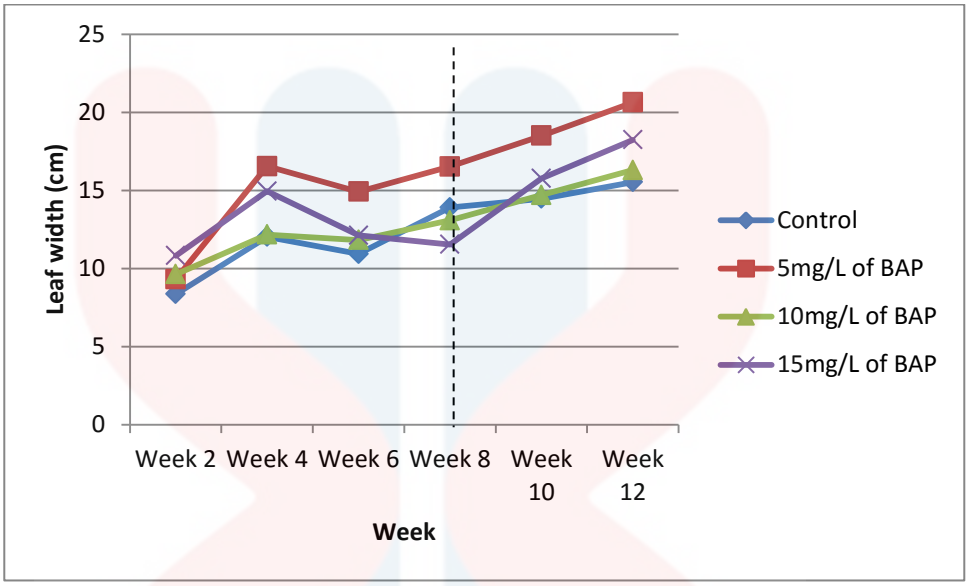

Figure 4.3: The leaf width of plants treated with different BAP concentration

Figure 4.3 show that the width of functional leaf grow rapidly from Week 2 until Week 4 but plants treated with 15mg/L of BAP decreasing in the measurements of leaf width at Week 6 and 8 (12.12 ± 4.40 cm and 11.53 ± 4.60 cm respectively). The size of length of leaf and width of leaf determine the surface area for the leaf. Larger surface area allows higher intake of sunlight and thus allowing more productive photosynthesis. The higher the photosynthesis rate, the bigger the plant can be. Weight of fruit bunch produced often determined by the size of the plant

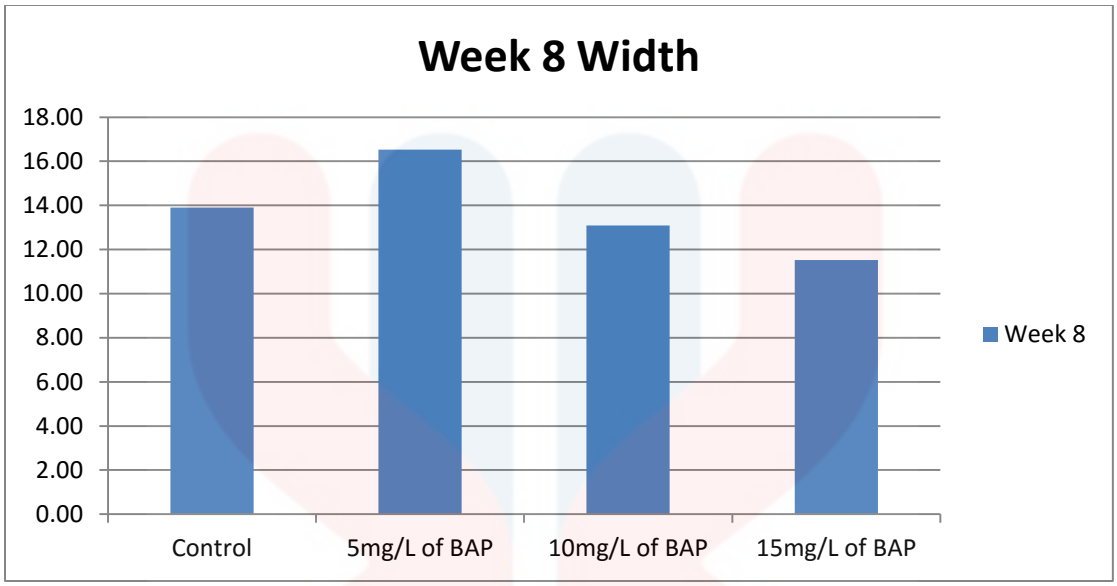

Figure 4.4: Week 8 of width measurement

Figure 4.4 shows that during week 8, Plants treated with 15 mg/l of BAP have the lowest mean of leaf width compared to other treatments (11.53 ± 4.60 cm). The highest mean value for leaf width in week 8 was recorded by plants treated with 5mg/l of BAP with mean of 16.53 ± 9.80 cm.

Table 4.3: Mean of number of functional leaves of plants (cm) treated with different BAP concentration

Treatment	Control (cm)	5mg/L of BAP (cm)	10mg/L of BAP (cm)	15mg/L of BAP (cm)
Week 2	5.96 ± 1.71	6.13 ± 1.71	6.73 ± 1.61	8.27 ± 2.08
Week 4	6.76 ± 1.67	6.16 ± 1.74	7.10 ± 2.7	6.90 ± 2.41
Week 6	6.33 ± 1.58	6.43 ± 1.69	6.80 ± 1.64	7.96 ± 2.57
Week 8	6.36 ± 1.47	6.36 ± 1.44	7.36 ± 1.88	7.36 ± 3.01
Week 10	5.83 ± 1.68	7.03 ± 1.37	7.83 ± 1.11	8.63 ± 2.56
Week 12	6.20 ± 1.34	7.56 ± 1.35	8.36 ± 1.12	8.23 ± 2.55

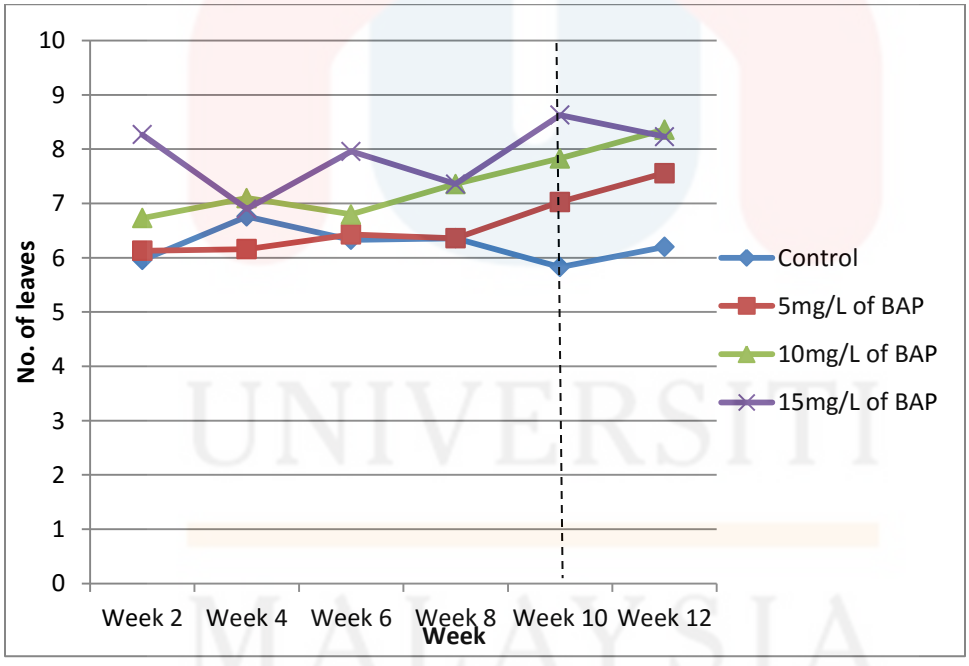

Figure 4.5: Number of functional leaves of plants treated with different BAP concentration

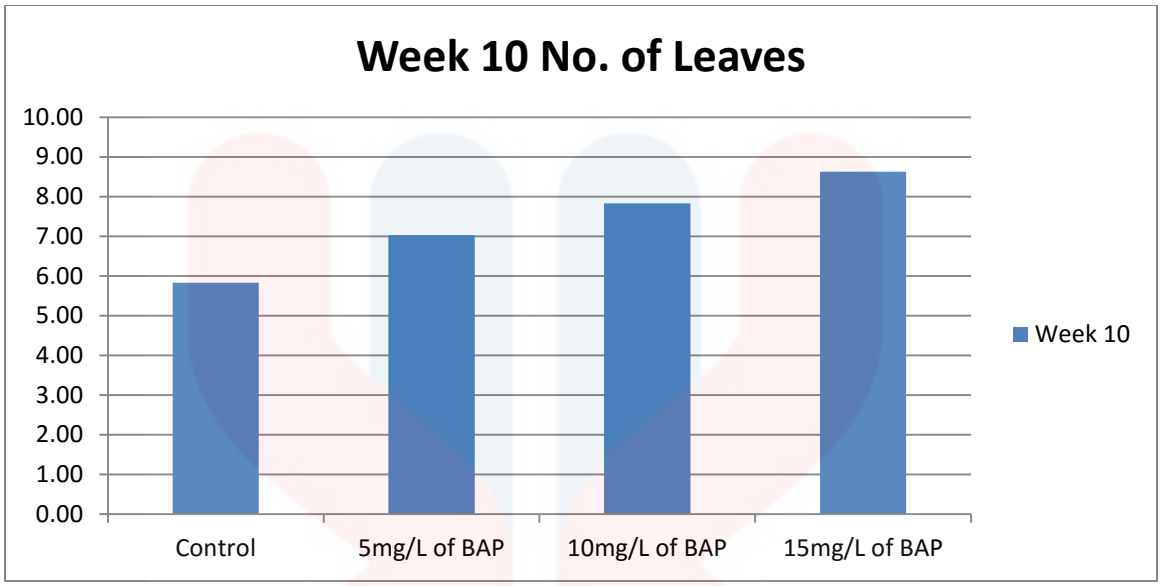

Figure 4.6: Critical point number of functional leaf of plants treated with different BAP concentration

Figure 4.5 showed that plants treated with 15mg/L of BAP depicts fluctuates number of functional leaves over the 12 weeks of study. However, plants from this treatment have higher number of functional leaves. Plants treated with 15mg/L of BAP are smaller size in height but higher number of functional leaves. At the 12th week, the number of functional leaves for plants treated with 15mg/L of BAP is 8.23 ± 2.55 cm. (Table 4.3)

The functional leaves for each treatment have unsteady growth. There are some reasons contributing to the high variance in the plants. Performance of growth of the plants shows high variety especially in plants treated with 5mg/L of BAP. Uneven nutrient content in the soil would contribute to high nutrient in the plant. Other contributing factor could be mutation caused by long exposure of PGR during *in vitro.* Growth regulator may induce the changes in cell cycle and thus producing somaclonal variation. Some plants shows minimal growth from week 2until week 12 suggesting the

plants are dwarf or stunted. This could be reaction from the environmental stress in the field.

In Figure 4.6, plants treated with 15 mg/l of BAP have the highest mean value of 8.63 ± 2.56 cm while the lowest mean was recorded by control treatment plants with mean of 5.83 ± 1.68 cm.

Table 4.4: Mean of height of plants (cm) treated with different BAP concentration

Treatment	Control (cm)	5mg/L of BAP (cm)	10mg/L of BAP (cm)	15mg/L of BAP (cm)
Week 2	12.25 ± 4.54	14.67 ± 7.25	10.84 ± 5.98	10.74 ± 4.39
Week 4	19.72 ± 7.77	26.08 ± 18.12	15.43 ± 7.20	20.63 ± 6.86
Week 6	21.88 ± 7.22	30.13 ± 20.88	18.26 ± 7.44	25.32 ± 7.57
Week 8	26.03 ± 7.64	34.36 ± 22.79	20.29 ± 7.89	25.97 ± 9.50
Week 10	30.25 ± 8.02	37.90 ± 23.7	23.81 ± 7.91	33.76 ± 12.3
Week 12	34.75 ± 8.69	41.30 ±24.7	26.50 ±8.23	40.73 ± 11.68

Table 4.4 showed that the plant height has gradual increase in all treatment. There were not much difference in the plant height at Week 2 probably plants were still adapting to the new environment in the field. The average plant height were 12.25 ± 4.54 cm, 14.67 ± 7.25 cm, 10.84 ± 5.98 cm, 10.74 ± 4.39 cm for plants treated with 0mg/L, 5mg/L,10mg/L and 15mg/L of BAP respectively. Plants gradually showing rapid growth starting from Week 3 onwards with plants treated with 5mg/L of BAP shows the vigorous growth compared to other treatments. However, plants treated with 15 mg/L managed to achieve the same height with plants treated with 5mg/L during the 12th week. Since the

plants managed to achieve 40.73 ±11.68 cm heights within 12 week, it can be suggested that plants possessing the characteristics of vigorous growth performance.

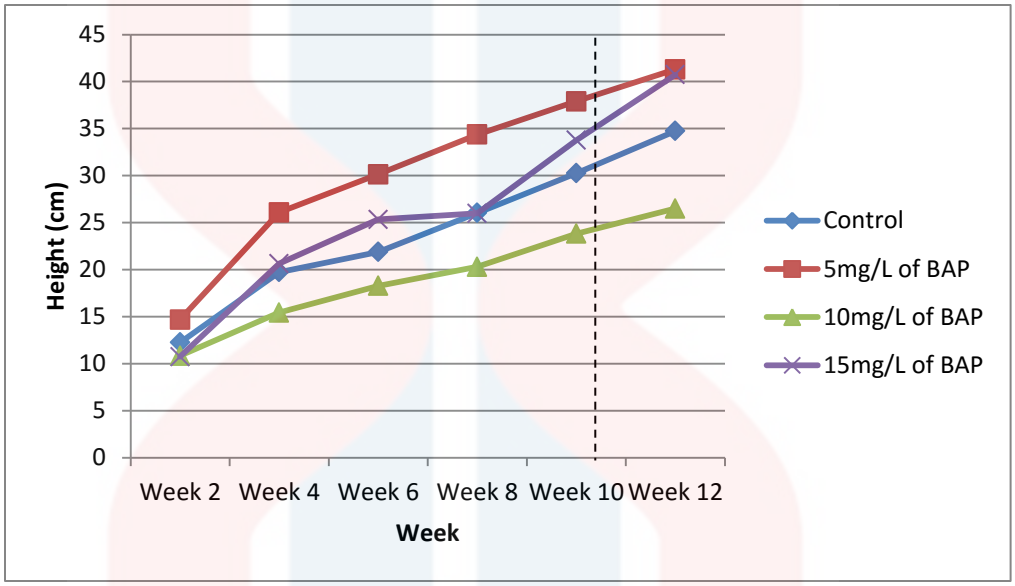

Figure 4.7: Height of plants treated with different BAP concentration

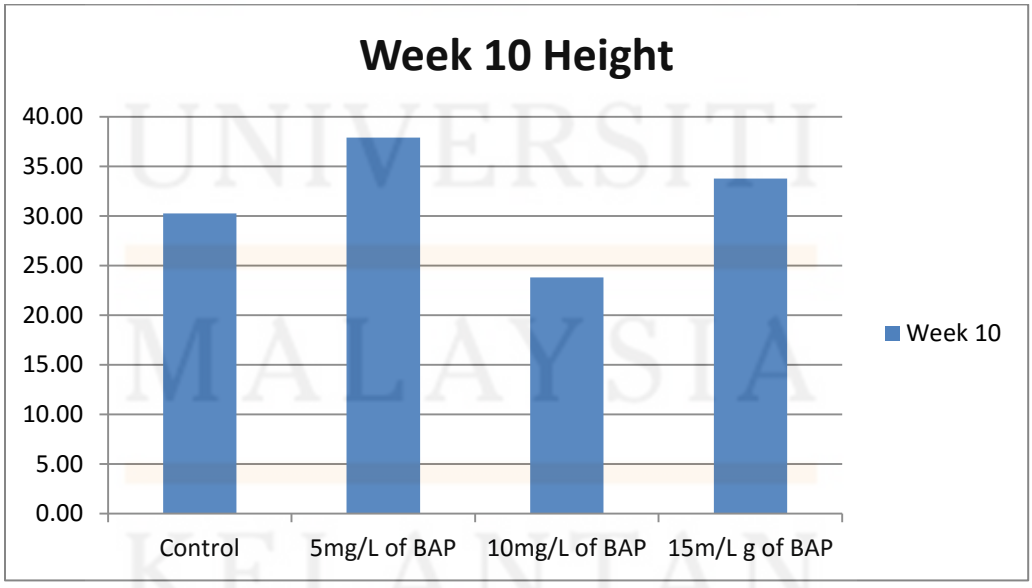

Figure 4.8: Week 10 of height measurement

Figure 4.8 shows that plants treated with 5 mg/l of BAP have the highest mean during week 10 with mean of 37.90 ± 23.7 cm. Meanwhile, plants treated with 15 mg/l of BAP have the second mean highest with mean of 33.76 ± 12.3 cm.

Figure 4.9: Plant treated with 5mg/L of BAP at week 6

Table 4.5: The mean girth size of the plants (cm) treated with different BAP concentration

Treatment	Control (cm)	5mg/L of BAP (cm)	10mg/L of BAP (cm)	15mg/L of BAP (cm)
Week 2	5.42 ± 1.57	7.64 ± 2.81	6.75 ± 2.21	9.36 ± 2.17
Week 4	8.42 ± 2.62	10.10 ± 3.44	8.17 ± 2.29	9.64 ± 1.98
Week 6	9.71 ± 2.94	12.98 ± 4.49	9.40 ± 2.50	12.2 ± 2.05
Week 8	11.63 ± 3.07	15.83 ± 5.88	10.26 ± 3.02	12.93± 4.50
Week 10	13.67 ± 3.55	18.10 ± 6.40	12.00 ± 2.84	15.00 ± 4.89
Week 12	15.7 ± 3.71	20.60 ± 6.60	13.76 ± 2.84	19.33 ± 4.42

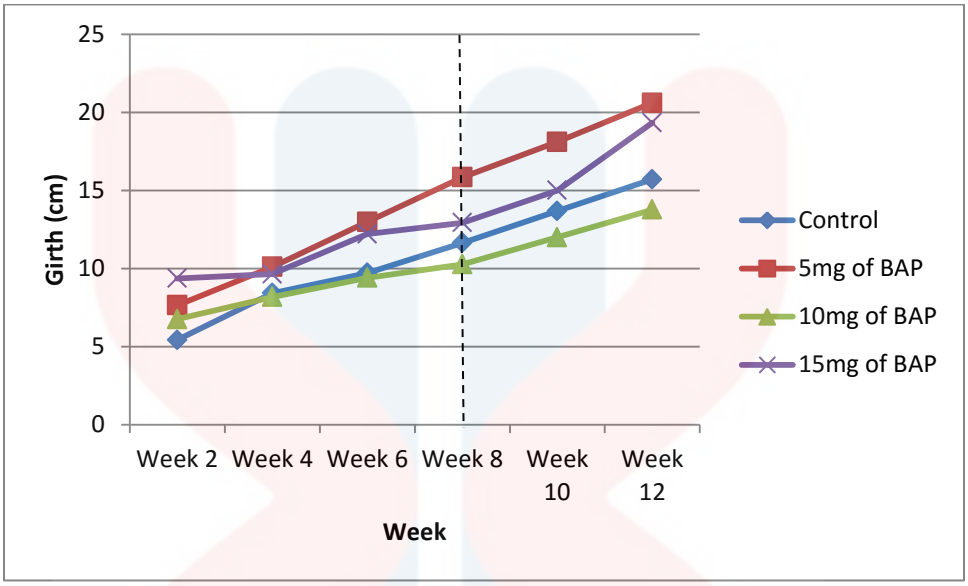

Figure 4.10: Girth size of plants treated with different BAP concentration

Figure 4.10 show the gradual increase of girth measurements in all treatments. Plants treated with 5mg/L of BAP have mean of 20.6 ± 6.6 cm at Week 12. There was no significant difference than the rest of the treatment because plants treated with 15mg/L of BAP has mean of 19.33 ± 4.42. The initial measurement at Week 2 also shows not significant difference.

Stem growth is a manifestation of vegetative growth. Table 4.5 shows the increasing behavior over the week without any fluctuation. Plants treated with 5mg/L of BAP recorded mean measurement of 5.42 ± 1.57 cm at 2nd week and 15.70 ± 3.71 cm at 12th week. The increase size of pseudostem girth size is directly proportional to the increasing of leaflength of leaf. The pseudostem size increased to support the plant height and future weight of the fruit bunch.

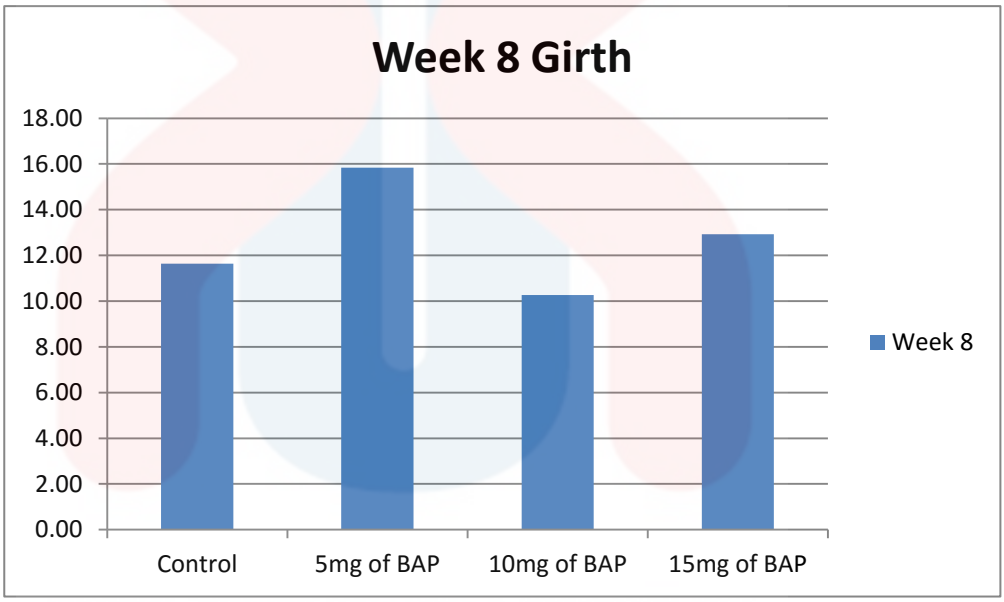

Figure 4.11: Week 8 of girth measurements

Figure 4.11 shows plants treated with 5mg/l of BAP has the highest mean of girth size compared to other treatments with mean of 15.83 ± 5.88 cm. the lowest mean recorded by plants treated with 10 mg/l of BAP with mean of 10.26 ± 3.02 cm.

5.0 Conclusion

As a conclusion, plants treated with 5mg/L of BAP during *in vitro* has higher performances of growth in the field as compared to plants that were treated with 0mg/L, 10mg/L and 15mg/L of BAP. Plants treated with 5mg/L of BAP showed superior growth in all parameters studied suggesting the superior off-types in banana plants could come from plants with this treatment. However, the evaluation to determine which treatment produced superior plant growth should be studied until flowering or post-harvest stage so the off - types plants can be detected and selected for future cultivation. Agronomic characteristics consider the growth performances and yield. Shorter plant height may facilitate the harvesting process which is beneficial for post-harvest handling. Thus the evaluation until flowering, fruiting and harvesting stage would help selection of the plants with good agronomic characteristic. Therefore, the study for growth of *Musa acuminata* cv Berangan treated with different concentration of BAP during *in-vitro* can bring significant impact to the commercial breeder regarding the optimum usage of BAP concentration during *in vitro.*

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APPENDIX

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
LL1	Based on Mean	9.134	3	116	0.000
	Based on Median	6.836	3	116	0.000
	Based on Median and with adjusted df	6.836	3	84.068	0.000
	Based on trimmed mean	8.767	3	116	0.000
LL2	Based on Mean	3.324	3	116	0.022
	Based on Median	3.239	3	116	0.025
	Based on Median and with adjusted df	3.239	3	70.415	0.027
	Based on trimmed mean	3.311	3	116	0.023
LL3	Based on Mean	4.794	3	116	0.003
	Based on Median	4.513	3	116	0.005
	Based on Median and with adjusted df	4.513	3	62.930	0.006
	Based on trimmed mean	4.635	3	116	0.004
LL4	Based on Mean	2.369	3	116	0.074
	Based on Median	2.111	3	116	0.103
	Based on Median and with adjusted df	2.111	3	60.471	0.108
	Based on trimmed mean	2.217	3	116	0.090
LL5	Based on Mean	2.739	3	116	0.047
	Based on Median	2.335	3	116	0.078
	Based on Median and with adjusted df	2.335	3	56.692	0.084
	Based on trimmed mean	2.540	3	116	0.060
LL6	Based on Mean	3.744	3	116	0.013
	Based on Median	2.916	3	116	0.037

	Based on Median and with adjusted df	2.916	3	55.690	0.042
	Based on trimmed mean	3.395	3	116	0.020
WL1	Based on Mean	9.933	3	116	0.000
	Based on Median	8.622	3	116	0.000
	Based on Median and with adjusted df	8.622	3	93.692	0.000
	Based on trimmed mean	9.924	3	116	0.000
WL2	Based on Mean	2.238	3	116	0.088
	Based on Median	1.089	3	116	0.357
	Based on Median and with adjusted df	1.089	3	71.071	0.359
	Based on trimmed mean	1.564	3	116	0.202
WL3	Based on Mean	7.306	3	116	0.000
	Based on Median	7.143	3	116	0.000
	Based on Median and with adjusted df	7.143	3	62.713	0.000
	Based on trimmed mean	7.199	3	116	0.000
WL4	Based on Mean	3.109	3	116	0.029
	Based on Median	3.064	3	116	0.031
	Based on Median and with adjusted df	3.064	3	72.136	0.033
	Based on trimmed mean	3.263	3	116	0.024
WL5	Based on Mean	5.163	3	116	0.002
	Based on Median	5.143	3	116	0.002
	Based on Median and with adjusted df	5.143	3	56.953	0.003
	Based on trimmed mean	5.226	3	116	0.002
WL6	Based on Mean	4.210	3	116	0.007
	Based on Median	4.009	3	116	0.009

	Based on Median and with adjusted df	4.009	3	66.778	0.011
	Based on trimmed mean	4.294	3	116	0.007
H1	Based on Mean	2.948	3	116	0.036
	Based on Median	2.156	3	116	0.097
	Based on Median and with adjusted df	2.156	3	94.247	0.098
	Based on trimmed mean	2.776	3	116	0.044
H2	Based on Mean	4.097	3	116	0.008
	Based on Median	3.654	3	116	0.015
	Based on Median and with adjusted df	3.654	3	47.515	0.019
	Based on trimmed mean	3.778	3	116	0.013
H3	Based on Mean	5.754	3	116	0.001
	Based on Median	5.683	3	116	0.001
	Based on Median and with adjusted df	5.683	3	45.150	0.002
	Based on trimmed mean	5.956	3	116	0.001
H4	Based on Mean	5.838	3	116	0.001
	Based on Median	5.772	3	116	0.001
	Based on Median and with adjusted df	5.772	3	46.509	0.002
	Based on trimmed mean	6.175	3	116	0.001
H5	Based on Mean	5.525	3	116	0.001
	Based on Median	5.573	3	116	0.001
	Based on Median and with adjusted df	5.573	3	48.342	0.002
	Based on trimmed mean	5.896	3	116	0.001
H6	Based on Mean	5.150	3	116	0.002
	Based on Median	5.031	3	116	0.003

	Based on Median and with adjusted df	5.031	3	46.228	0.004
	Based on trimmed mean	5.329	3	116	0.002
N1	Based on Mean	1.566	3	116	0.201
	Based on Median	1.148	3	116	0.333
	Based on Median and with adjusted df	1.148	3	112.786	0.333
	Based on trimmed mean	1.425	3	116	0.239
N2	Based on Mean	0.895	3	116	0.446
	Based on Median	0.763	3	116	0.517
	Based on Median and with adjusted df	0.763	3	87.539	0.518
	Based on trimmed mean	0.843	3	116	0.473
N3	Based on Mean	2.244	3	116	0.087
	Based on Median	2.132	3	116	0.100
	Based on Median and with adjusted df	2.132	3	99.904	0.101
	Based on trimmed mean	2.152	3	116	0.097
N4	Based on Mean	4.915	3	116	0.003
	Based on Median	4.112	3	116	0.008
	Based on Median and with adjusted df	4.112	3	86.163	0.009
	Based on trimmed mean	5.028	3	116	0.003
N5	Based on Mean	4.973	3	116	0.003
	Based on Median	3.809	3	116	0.012
	Based on Median and with adjusted df	3.809	3	74.557	0.013
	Based on trimmed mean	4.905	3	116	0.003
N6	Based on Mean	5.447	3	116	0.002
	Based on Median	4.402	3	116	0.006

	Based on Median and with adjusted df	4.402	3	80.829	0.006
	Based on trimmed mean	5.520	3	116	0.001
G1	Based on Mean	2.022	3	116	0.115
	Based on Median	1.878	3	116	0.137
	Based on Median and with adjusted df	1.878	3	85.175	0.139
	Based on trimmed mean	2.151	3	116	0.098
G2	Based on Mean	0.995	3	116	0.398
	Based on Median	0.919	3	116	0.434
	Based on Median and with adjusted df	0.919	3	84.050	0.436
	Based on trimmed mean	0.945	3	116	0.421
G3	Based on Mean	2.905	3	116	0.038
	Based on Median	2.616	3	116	0.054
	Based on Median and with adjusted df	2.616	3	77.182	0.057
	Based on trimmed mean	2.728	3	116	0.047
G4	Based on Mean	3.995	3	116	0.010
	Based on Median	3.406	3	116	0.020
	Based on Median and with adjusted df	3.406	3	87.909	0.021
	Based on trimmed mean	3.901	3	116	0.011
G5	Based on Mean	3.826	3	116	0.012
	Based on Median	2.592	3	116	0.056
	Based on Median and with adjusted df	2.592	3	82.801	0.058
	Based on trimmed mean	3.730	3	116	0.013
G6	Based on Mean	2.937	3	116	0.036
	Based on Median	2.616	3	116	0.054

	Based on Median and with adjusted df	2.616	3	72.796	0.057
	Based on trimmed mean	2.798	3	116	0.043