UNIVERSITI PUTRA MALAYSIA

GROWTH RESPONSE OF BANANA IN CONTROL ENVIRONMENT

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By

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ABSTRACT

Banana is one of the most famous edible fruit in the world which belong to the genus of *Musa*. The banana plantation have several problems that affect the growth performance and yield production such as infestation by panama disease and nematode problem. This research was conducted to identify the growth performance of banana species and varieties using macro-propagation method to increase the rate of plantlets production in control environment. The samples of banana from two main group of banana which is cultivar and wild banana collected from Belaga and Bintulu area. About 10 samples were analyzed in this study namely Awak, Nipah, Berangan Raja Udang, Kelat, Merah, Blood, Acuminata, Campestris and Tebem. The number of shoot, diameter, height, length and width of leaves were done and recorded in tabulated table. Result of the study shows that Merah, Campestris and Tebem have the highest number of shoots produced compare others. In fact, based on data the growth response in term of diameter of stems, height of stems, length of leaves and width of leaves represent by Nipah and Awak species compared to others species which shows varied growth response based on the morphology of species. Based on this study, can be concluded that number of shoots produced, growth response in term of diameter, height, length and width of leaves varies based on the species.

ABSTRAK

Pisang merupakan tanaman buah boleh dimakan yang paling popular dunia yang tergolong dalam genus *Musa*. Tanaman pisang mengalami beberapa masalah yang mempengaruhi kadar pertumbuhan dan juga hasil tanaman seperti dijangkiti oleh penyakit panama dan juga masalah nematod. Kajian telah dijalankan untuk mengenalpasti kadar pertumbuhan pelbagai variati spesis pisang dengan menggunakan kaedah makro propagasi untuk menambahkan kadar bilangan pertumbuhan tanaman dalam keadaan yang terkawal. Sampel pisang dari jenis kultivar dan juga liar telah dikumpulkan daripada kawasan Belaga dan Bintulu. Sepanjang kajian sebanyak 10 spesis telah dianalisis termasuk Awak, Nipah, Berangan Raja Udang, Kelat, Merah, Blood, Acuminata, Campestris dan Tebem. Bilangan jumlah pucuk yang tumbuh, diameter batang, ketinggian pokok, panjang serta lebar daun diukur dan direkodkan di dalam jadual. Hasil kajian mendapati Merah, Campestris dan Tebem menghasilkan bilangan pucuk yang paling banyak berbanding jenis pisang yang lain. Tindak balas pertumbuhan dari segi semua parameter pertumbuhan tumbuhan termasuk diameter batang, ketinggian, panjang daun dan lebar daun diwakili oleh spesis kultivar iaitu Nipah dan Awak berbanding spesis pisang lain yang menunjukkan pertumbuhan berbeza berdasarkan morphologi spesis tersebut. Berdasarkan kajian, dapat disimpulkan bahawa bilangan pucuk yang dihasilkan, pertumbuhan dari segi diameter dan ketinggian batang serta panjang dan lebar daun berbeza berdasarkan spesis.

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APPROVAL SHEET

I certify that this research project entitled "Growth response of banana in control environment" has been examined and approved as a partial fulfilment of the requirement for the degree Bachelor of Bioindustrial Science in the Faculty of Science Agriculture and Food Sciences, University Putra Malaysia Bintulu Sarawak Campus.

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HDPE	High density polyethylene
WAAP	West Africa Agricultural Productivity Program
AB	Abscisic acid
IAA	Indole-3-acetic acid
PIF	Plant Issus des Fragment de Tige
LDK2	Ladang Kongsi 2

CHAPTER 1

INTRODUCTION

1.1 Research Background

Banana is one of the most famous staple food in tropical region. The sweet and cream taste with high nutritional value make it high value for domesticated purposes. Banana being planted in Malaysia based on the population size, geographical location and the races in the area. . These the factor that determine the type of banana species were planted whether for personal use or business purposes. Banana is giant monocotyledonous perennial herbs, with scientific name *Musa* belonging to the order *Zingiberales* and family *Musacae*. The Malesia which include Malaysia and Indonesia is the center of the banana diversity with *M. acuminata* which known as the origin of the cultivar banana species. The modern day, cultivar banana are a mix of wild species and cultivated species (associated with *M. acuminata* and *M. balbisiana)* (Daniels *et al.* 2001). Banana are an important food crop which known as the most famous staple food in the subtropics and tropics area in the world.

Borneo were considered to be the center of biodiversity in tropical Asia and being part of banana origin with a large number of wild banana. In Borneo, banana plants prefer an open exposure, their growth is usually limited to slightly small and isolated populations therefore it show much genetic variation (Beccari 1902). Wild banana can be group into 4 different group based on the diploid number which is Australimusa (2n=20), Callimusa (2n=20), Musa (2n=22) and Rhodochlamys (2n=22).

The banana fruit can be eaten raw or cooked with sweet and creamy taste, it also can be processed into flour, fermented for the production of beverages such as juice, beer (Pillay *et al.* 2002). Even the pulp of banana being eaten raw before match or tournament for the athlete due to high contain of potassium mainly function to avoid muscle cramp especially for the field track athlete. Thus, banana mostly known as the staple food most tropical country due to pleasant taste, high nutritional value with affordable price. The banana by-product also have numerous function such as banana fiber as natural sorbent, even being used for the women pad production.

Malaysia diseases problem regarding banana plantation due to attack of Moko/Bugtok bacterial wilt *(Ralstonia solanacearum)*, blood bacterial wilt *(R. syzygii subsp. celebesensis)* that be the limiting factor in the banana production. The monoculture of the same banana species or monoculture method enhance the spread of the diseases which can cause 100% total loss to the farmer especially when panama disease attack. (Teng *et al.* 2016) has reported that the banana blood disease is currently spreading in peninsular Malaysia where it coexists with the Moko and Fusarium wilt diseases. The disease has been first detected in the province of Perak and Selangor. The Cavendish species were planted in large scale cultivation due to the nice texture, cream, sweet taste and have longer shelf life which is 2 month suitable for the export purposes.

The widespread threat to Musa cultivation by fungal disease especially the fusarium wilt virus (panama disease) in recent years has heightened interest in *Musa*. The most shocking crisis in the banana plantation was when the banana species of Gros Michel being planted as the banana for the commercial used and also for the export purpose

with United States as the main producer which cover 80% of banana production had being destroyed by panama disease which cause total loss of 100%. All banana export cultivars grown today are still selections from somatic mutants of the group Cavendish and have a very narrow genetic base (Perrier *et al.* 2011).

Thus, the programs for genetic improvement of banana were established among the banana exporter country with the main objective for the genetic improvement of banana is to breed sterile triploid hybrids through the recombination of fertile cultivars and species that meet farmers' needs and consumers' demands (Ortiz and Vuylsteke 1996). The genetic improvement can produce banana with the good taste for eating with high nutritional value and production. Hence, the seedless banana being produced as an alternative method to encounter panama disease as it is resistant to it. However, the cultivar species have disadvantages because it difficult for the breeding and produce only small amount of plantlet or sucker throughout life span.

In fact, *Musa* breeding programs started about one century ago in Trinidad, aiming to develop disease- and pest-resistant cultivars for the export trade. There are over a thousand Musa cultivars embracing a wide genetic diversity, which reveal their multiple origins through hybridization from these two ancestral diploid species, *Musa acuminata* and *Musa balbisiana* (Heslop Harrison and Schwarzacher 2007). Stimulation of lateral bud development and plantlet production is generally accomplished through decapitation methods which is one of the macro propagation method for the propagation banana. Thus, the macro propagation being used in poor

country as the application of micro propagation or tissue culture due to the financial issue which require high, best facilities.

The main focus of breeding programs is to improve the quality of plantain and banana for consumption. While the genetic diversity of cultivar banana is comparatively well studied and only a few studies have been conducted into wild population of *Musa acuminata* (Bhat *et al.* 1992; Loh *et al.* 2006). In this study, several wild banana species from central Sarawak were taken for the macro-propagation technique for the mass multiplication of new plantlet production and also the survival rate to adapt in new environment.

1.2 Objectives

The research objectives are:

1. To identify the diversity of banana species in Northern Sarawak region

The research mainly focus on the diversity and distribution of banana species including wild banana in central Sarawak region. The construction, land use due to the increase human population have led to the interruption of nature habit of banana.

2. To measure the growth performance of the banana in control environment.

The research also being done to indicate the difference in term of growth performance between species and also method of propagation.

1.3 Significant of Study

The simple, cheap and efficient propagation method of banana such as macro-propagation need to be practices by farmer in order to increase the production of healthy new plant as it is applicable for the farmer in all scale. The application of macro-propagation as the main method of banana propagation famous is in African country and shows significant advantage compare to the conventional method of propagation using tissue culture in term of cost and technique.

The main problem that limit the large scale of banana plantain is due to the slow natural regeneration in banana is comparatively slow due to hormone- mediated apical dominance exerted by main plant. Depending on the variety of the banana species, a plant produces 5-15 side sucker during its life span. Shy suckering is the major limitation in the production of sufficient planting material through conventional approach.

Micro-propagation or Tissue Culture (TC) ensure rapid production of healthy, vigorous and disease-free planting material. However, due to the large capital investment required for the facility, the plantlet produced are fairly expensive and beyond the reach of poor farmers make it not applicable especially for the small, poor farmer. Hence, through macro-propagation method which cheap, simple technique that increase the sucker multiplication at farm level and minimum technical skill need to practice by small-scale farmer.

Thus, macro-propagation being invented by the researcher and farmer in the African country as the limited financial sources restrict them from practicing conventional

breeding programs. Macro-propagation is one such cost effective technique where the removal of apical meristem will stimulate the regeneration of lateral meristem (Uma *et al.* 2013). Based on the previous study, after 90days, four or five healthy plants of banana can be used as healthy plants of banana which indicate this method as economically beneficial, cheap, simple and relatively rapid technique for vegetative multiplication of *Musa* sp. Thus, it suit for the resource poor, unskilled and small farmers.

CHAPTER 2

LITERATURE REVIEW

2.1 Diversity of Banana

Banana is a tropical lowland plant that widely used among citizen of tropical country especially Malaysia as the food sources. In fact, banana in the list of the most plant being planted for the commercial purposes. Banana species can be specifically determine based on the geographical location due to the nature habit which significantly different with each other. Banana consist of two main group which is cultivar and wild banana. Sandy soil, which rich of loam soil is the nature for the banana plant to growth. However due to the human intrusion the habitat of the wild banana slightly has changed and reduced in number (Scot *et al.* 2006).

The high nutritional value in every pulp of banana make the demand increasing trend every years. The pulp of banana with the sweet and creamy taste sugar rich and easily digested food. Based on research by (Arvanitoyannis *et al.* 2008) show that the composition each pulp of banana composed of 70% water, 27% carbohydrates, 1.2% protein and 0.3% fat. Besides each gram of banana composed of one calorie of energy. It also good sources of Vitamin A, B1, B2 and C.

The high nutritional value of banana and well-awareness on the health properties among the community especially in modern country ensure the market demand on banana in increasing demand trend from years to years. Thus the producer country require large quantities of planting material and uniform plantation for the

sustainable export of the crop as uniform ripening and systematic handling can delay the ripening period especially for the export to the other country (Roux *et al.* 2001; Ray *et al.* 2006)

2.2 Morphology of Banana Plant

The banana tree well-known as the biggest perennial herb in the world due to its physiology properties. It an herb because the aerial parts of the parent plant die down to the ground after the growing season. Whereas it is a perennial because the sucker growing out at the base of the plant. (Scot *et al.* 2006).

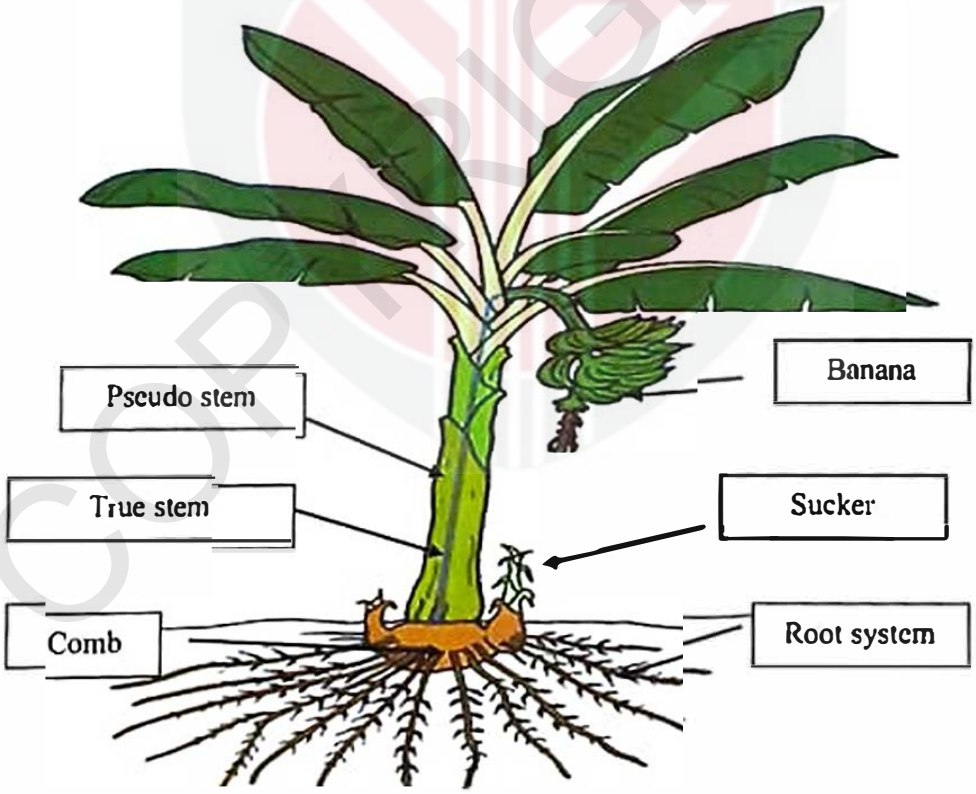

Figure 2.1 The morphology of banana plant

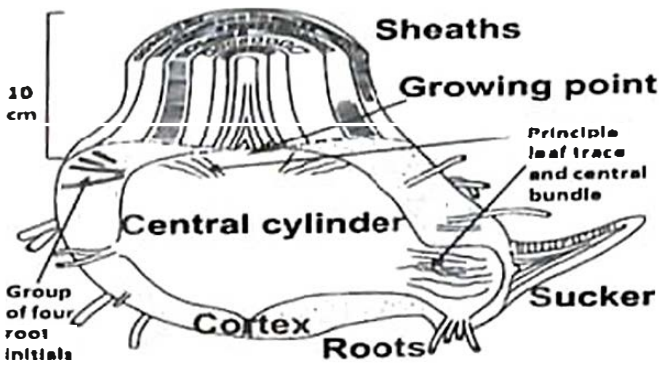

Figure 2.2 The genesis of sucker from mother corm by Siddhesh and Sajan (2015)

2.2.1 Root Anatomy

The root structure consist of three tissue systems, epidermis, ground parenchyma and vascular cylinder. The periderm, cortex of mature root had many layers of thick walled cells in outside surface (Tomlison 1969).

2.2.2 Stem morphology

The pseudo stem is made of large overlapping leaf sheath that are tightly rolled each other to form cylindrical structure. The aerial stem entirely depends on the leaf sheaths for mechanical support and it's essentially provides vascular connection (Purseglove 1972; Stover and Simmonds 1987).

2.2.3 Fruit Morphology

Fruit is elongated, curved and round in cross-section with the triangular form of the ovary still visible. Fruit length varies from 5cm to more than 30cm. The pulp of the

banana protected by an epidermis and underlying parenchyma layer. At the tip of the fruit, the perianth, androecium and the style become withered and separated from the fruit by a brown corky layer (Scot C *et al.* 2006).

2.2.4 Leaf Morphology

The leaf of banana belong to the complete type because they have midrib, stalk (petiole) and blade (lamina). The hollow U-shape section of the petiole and the longitudinal strengthening elements in its outer skin and give it adequate rigidity (Scot *et al.* 2006).

2.3 Why Macro-Propagation is the Best Among Others Method for Banana Propagation

According to Hartmann *et al.* (2010) stated that the concept of cloning mainly in perennial crops have been applied by the agriculturalists and horticulturalists for at least 800 years because the multiplication by vegetative means is a more applicable in term of productivity compare the multiplication by seed. Thus, it showed the application of propagation for the mass multiplication of cash crop had been applied by the agriculturalists and horticulturalists since 800 years ago.

The capacity to rapidly develop cultivars by propagation for cash crop using the cheap and simple techniques with high rates of multiplication especially crop with high economical and exports value (Leakey 2012). The sufficient and high quality of planting material would ensure the sufficient food supply whether for the local

consumption or export purposed. Thus, indirectly the nation food security issues can be solved as Malaysia highly depend others agricultural country for certain commodity.

In traditional method of banana planting, natural regeneration is very slow due to the apical dominance of mother plant. In a stalk's lifetime of 12-14 months, it will only produce 5-20 suckers (Singh *et al.* 2011). Plantation of banana are important staples and source of income for the smallholders that grow them in the humid forest and mid-altitude agro-ecologies of sub-Saharan Africa. However, the productivity and lifespan of banana and plantain fields have drastically reduced due to pest and disease problem. This situation occur due to the farmers totally reliance on natural regeneration of plants for the supply of planting materials, which are contaminated by pests and diseases. Major pests are the banana weevil (*Cosmopolites sordidus*) and a complex of plant parasitic nematodes (*Radopholus similis, Pratylenchus spp., Helicotylenchus multicinctus*, and *Meloidogyne spp.*). Major diseases are fusarium wilt, caused by *Fusarium oxysporum f.sp. cubense* and bacterial wilt, caused by *Xanthomonas campestris pv. musacearum.*

For growth and flower production, optimal temperature are between 22°C to 31 °C with rainfall of 2000-2500mm spread evenly throughout the year (Robinson and Sauco 2010). Macro-propagated plantlet are more adaptable to the field conditions because they are photosynthetically active as they are regenerated under in vivo conditions, while tissue cultured plants are partially photosynthetic and hence are very delicate and do not establish easily under field conditions.

Increased suckering rate can be achieved through complete or partial decapitation on a field grown plant or detached corm technique and sawdust is the best substrate over others like rice hull, sand etc. with higher water holding capacity. The principle of generating sucker for clean planting material is by removing the apical dominance and exposing lateral bud to allow the rise of many shoot (Baiyeri and Aba 2005). In the recent study, attempt have been made to enhance the rate of plantlet production through macro-propagation through macro-propagation by the addition of bio-fertilizer and phytohormones to the explant or substrate.

2.4 Current Issue on the Banana Plantation

Malaysia banana industry begin with the availability of diverse germplasm, varied production system and positive response to Good Agricultural Practices (GAP) contributed to high productivity of banana which directly transformed banana from a backyard crop to a high value crop. The spread of disease infected sucker as planting material lead to the growth of tissue culture industry in 1980's. Presently, tissue culture were the major biotechnology tool to supply disease free, mass production and season independent production of planting material which contributed to the growth and development of banana (Molina 2002).

New challenges include epidemics, drought and temperature extremes due to climate change, which will affect banana farming. For example the changes of climate due to the global warming will cause Mycosphaerella leaf spot incidence (Torrado-Jaime and Castaño Zapata 2008). The genetic knowledge and the genomic tools

accumulated in the last two decades will facilitate and speed breeding host-plant resistance to pests affecting the *Musa* crop.

As noted by the late Dirk R. Vuylsteke 1996, "A broad-based, improved *Musa* germplasm with pest/disease resistance will be a major component to achieve sustainable production of this vegetative propagated, perennial crop. Such germplasm can be produced through conventional cross-breeding, enhanced by the utilization of innovative methods for the introduction of additional genetic variation. Also, the increased use of molecular markers will accelerate the process of recurrent selection of improved *Musa* germplasm and, hence, facilitate the development of new hybrids. The prospects of banana and plantain breeding are unlimited and increased efforts will at once initiate a new phase of *Musa* evolution.

Currently, based on studies by Ortiz *et al.* (2011) stated that the focus of *Musa* breeding globally needs to gradually shift from addressing existing constraints to assessing the risk potential of emerging threats and preparing to respond to them. The global phenomenon such as climate change will enhance the problem for the banana plantation as environmental factor can worsen the threat of fusarium wilt disease, nematode toward the banana as the changes of climate greatly affect the plant physiology and immunity system and reduce the capability to resist these threat.

2.5 Micro-propagation

Tissue Culture (TC) is one of the available propagation methods that produce seedlings free from diseases and pests, with genetic purity and uniform growth (Sheela and Ramachandran 2001). However, adoption of this technology has been low due to high capital investments and subsequent high cost of seedlings. This has led to the plantlets being too expensive for majority of the small holders to acquire.

Based on (Gitonga *et al.* 2010) research stated on the benefit of tissue culture technique in the mass propagation of planting material. However it is not sustainable to the farmer in developing and poor country due to the cost of producing planting material. In vitro plantlet is an expensive, delicate method that can't be carried out easily by the farmers in their own because require high skill to develop new plantlet. Thus, macro-propagation technique being introduced for the propagation of banana plantlet. Macro-propagation can be classified into two separate section which is field condition refer as in-situ and nursery refer as ex-situ. Both section involves the decapitation and decortication technique also hardening of the plant.

Micro-propagation, *in-vitro* propagation or commonly known as tissue culture technique capable in producing plantlet production of healthy, vigorous and disease-free planting material. However due to the large capital investment required for the facility, the plantlet produced are fairly expensive and beyond the reach of poor farmers. Thus tissue culture as a method of propagation not suitable for the small-scale farmer. Hence, through macro-propagation method which cheap, simple technique that increase the sucker multiplication at farm level and minimum technical skill need to practice by small-scale farmer. Thus it showed that high initial

cost was the main reasons for their low adoption of tissue culture plants mostly among the small-holder farmer and poor country. The high technology facilities required to be built for the tissue culture technique as the aseptic condition is the main requirement for the successful of tissue culture. The method for the tissue culture difficult as requires officer with the high skill in tissue culture. Thus the different method of macro-propagation are being developed in order to break the apical dominance of the banana to promote axillary growth that can be used as starting growth materials (Arinaitwe *et al.* 1999). Macro-propagation method is capable to produce large number of plantlet within short time using the same concept of tissue culture.

In Cameroon, researcher use macro-propagation technique which known as Plant Issus des Fragment de Tige (PIF) which the horizontal cut were made through apical dominance to break the apical dominance, then place the corm in a moist bed of sawdust until plant emerge (Boss 2008). The technology transfer via West Africa Agricultural Productivity Program (WAAP) introduce this technique to the farmers in the Cameroon not only create job opportunity and also enhance the involvement of stakeholder in the banana plantain production. The established of WAAP ultimate goals to contribute to the economic and social development through enhanced adoption of agricultural research and innovative practices.

CHAPTER 3

MATERIALS AND METHODS

3.1 Banana Species and Cultivar Collection Site.

The location of sampling being done in Sungai Asap, Belaga, Sarawak due to the large distribution of wild banana according to Marku and Kalu (2005). The wild species commonly growth at the deep tropical forest, moist clay and shady area. Thus, during the sample collection we had to cross the river and enter the bushy area to reach the parent plant and collected the sample. For the cultivar species, most of the species collected in commodity area and easily recognized.

Figure 3.1 Banana Species and Cultivar Collection Site.

Figure 3.2 Study site at LDK2 UPM Bintulu Campus

3.2 Field Sampling Requirements

For the sampling there were several thing that need to bring along throughout sample collection which are the GPS 64s Garmin device to marking the exact location of sample. permanent marker and plastic marker for the labeling purpose. sharp knife and hoe for the removal of sucker from the parent plants.

3.2.1 Samples Collection Procedure

The samples taken were recognized the type of species based on the external observation and unique physical appearance that distinguish among the type of banana especially wild banana species. The sample from Belaga was taken based on the size of the sucker and also the physiological condition of the sucker 8 or 9

months old sucker. The sucker then slowly removed by using sharp hoe to avoid any injury to the sucker that will affect the plantlet production.

3.2.2 Sample Preparation for Macro-Propagation

The samples must be separated based on the type of species for the macro-propagation and also traditional method of planting as illustrated in Figure 3.3. The samples were tagging also with specific number for easy recognition for the data collection.

Figure 3.3 The sample with the tagging before proceed with the macro-propagation and traditional method

3.3 Removal of Sword Sucker from the Parent Plant

The healthy sword sucker with 15-30 cm diameter of girth varies based on the type o species will be taken as the sample for the project especially for the macro propagation method which the rate of successful of this method depend on the survival rate of the plant

3.3.1 Construction of Propagators

Propagators refer to the place for new plantlet production and hardening of the new plantlet. The sample were placed in it after macro-propagation technique were done. The bud proliferation and plantlet production was monitor to ensure the optimum growth performance of new plantlet and maximize banana plantlet production. The advantages of propagator as it can be constructed using cheap, recycle material. For my project. The thick (high density polyethylene) HDPE container was used and placed under the netting house and the wood as the main support to cover it up with transparent polyethylene sheet Figure 3.4 showed the fully covered propagator constructed in study site LDK2.

Figure 3.4 The construction of propagaton

3.3.2 Sterilization of Sawdust

Sterilization of sawdust for about 60 second an important step to control the plant pathogen and present of nematode from ruin the combs as initiation media essentially served for anchorage of the root act as mechanical support and provide moisture. In fact. the physical composition of the nursery plotting medium can have serious effect on the supply of water and air to the growing plants as well as affect anchorage

and nutrient and water holding capacity of the medium (Dayarani *et al.* 2013). Similarity of their effects on sucker plantlets initiation might be associated with their relative similarity in amounts of pore spaces and moisture retention capacity. Besides, saw dust have ability to heat build up to trigger the secretion of auxin hormone. The process of sterilize sawdust shown in Figure 3.5

Figure 3.5 The sterilization of sawdust method

3.3.3. Filling of Chambers

Propagator filled with steam sterilized fine sawdust which act to keep the heat buildup to trigger secretion of auxin hormone, retain the moisture for the root absorption and proper root aeration. The steamed saw dust can inhibit and destroyed the present of nematode, pathogen and microorganism that can affect the production of new plantlet production. The sample after being cleaned and apply decapitation method of macro-propagation were planted and covered by the sawdust to trap the heat buildup and trigger proliferation of bud. Then the daily monitoring and watering being done to observe the growth rate of new plantlet.

3.3.4 Selection of Sucker

There are three different type of sucker which is maiden, sword and watery sucker. However, sword sucker more prefer for the sampling as the maiden sucker the most mature sucker and give rise as the next parent plant whereas sword sucker refer to the young sucker whose leaves are pointed like a sword as the best condition to develop into new plant and watery sucker refer to the small sucker with perfect leaves. Thus, the selection of sucker must be done before sample were taken out as shown in Figure 3.6.

Figure 3.6 The selection on the healthy sucker.

3.3.5: Stabilize the Sucker

In Figure 3.7 shown the sample after 45 days of macro-propagation were kept in the polybag under netting house for a weeks to stabilize before the sample being transferred to the real field as the sudden environment changes can affect growth responses. Bareja (2011) stated that the temperature will affect the translocation rate of photosynthetic product to the plant part means the faster it being translocate the faster the plant mature as the enzyme activity and the rate of most chemical reaction in plant generally increase with the rise in temperature.

Figure 3.7 The sample being placed under the netting house for hardening before macro-propagation technique was conducted.

3.3.6 Macro-Propagation Technique

The comb were cleaned and trimmed by peeling the outer layer of the comb include the root, pseudo stem, stain which may host nematodes and weevil. The apical dominance which located at the middle of the comb were totally removed to trigger the growth of lateral bud meristem at side of the comb for the bud proliferation to aid new plantlet production. The comb were immersed in the hot water at range 70-80 °C to kill the nematodes and bacteria for 60 seconds by using thermometer. The water temperature were constantly monitored to ensure the effectiveness to kill nematodes and bacteria because after the samples were immersed the water temperature were dropped. Macro-propagation technique were done shown in Figure 3.8.

Figure 3.8 Macro-propagation technique

3.4 Propagator Management

There are several important precaution for proper management of propagator. Firstly make sure the transparent plastic that cover the entire propagator fully airtight to ensure the temperature trapped in it. The present of water vapor on the transparent plastic means the air trapped in it and high temperature in the propagator compare to the external environment. Hence, monitor the moisture of the sawdust daily as high moisture and dry sawdust will affect the growth response of the banana. Thus watering only being done when necessary to avoid water stress on comb which will reduce the possibility of plantlet production. Figure 3.9 showed the presence of water vapor on the transparent polyethylene plastic

Figure 3.9 The transparent sheet fully cover the sample

The samples with individual polybag and fully covered netting house as illustrated in Figure 3.10 showed to increase the rate of survival and growth response of banana in control environment.

Figure 3.10 The 3rd phase of macro-propagation with advanced method.

3.4.1 Propagation Stage Time Period

Following table shows the growth response of banana via macro-propagation method (Tenkouano *et al.* 2006). However, it varies between each other based on species.

Table 3.1 The growth response of banana via macro-propagation method

Stage	Period
1. Primary bud sprouting	3-5 weeks (depending on the climatic condition and variety)
2. Secondary bud sprouting	2-3 weeks
3. Rooting of detached plantlets	2 weeks
4. Acclimatization	3-6 weeks

3.5 Experimental Design

The samples were collected and macro-propagation technique applied into three different phases in control environment. The phases were divided based on the different propagator management which phase 1 begin after removal of apical dominance on the comb, phase 2 begin after the primary stem of plants were cut and phase 3 begin when the comb were placed into individual polybag with fully netting cover and fertilizer were applied compared phase 1 and phase 2 which only partially netting cover and no fertilizer applied. Each of the phases were compared with each other's and also compared with the direct planting in term of growth responses. Figure 3.11 shown the experimental design and growth response after 45 days via macro-propagation technique.

Figure 3.11 The experimental design and growth responses after 45 days

3.6 Data collection and Analysis

The growth performance of 10 different species of banana in different propagation method were measured to identify the banana species with the greatest growth performance. Vegetative growth of banana would be measured weekly and tabulated in the form include; (i) The number of shoot, (ii) Plant height from soil level, (iii) Stem girth, (iv) Length of leaf , (v) Width of leaves.

CHAPTER 4

RESULTS

4.1 The Diversity of Banana Species in Central Sarawak Region

In Sarawak, the type of banana species that mostly can be found in central Sarawak region was pisang kuch due to the local uses and high survive rate as it can be seen throughout my journey to the sampling location Belaga, Sarawak and also being planted by local community in Kuala Tatau and Bintulu area.

Table 4.1 Shows the GPS coordinates of sample

Sample	Location
1. *Musa Campestris and M. borneensis*	N3.02725 E113.94085
2. Pisang Kelat	N3.08221 E114.04925
3. Pisang Buaya	N3.08263 E114.04430
4. Pisang Kelat	N3.08455 E114.03401
5. Sawan	N3.08108 E114.00097
6. Pisang berangan, Pisang Merah, Pisang Midan	N3.01887 E113.90304
7. Pisang Mas, Tebem	N3.01602 E113.90034

Table 4.2 The diversity of cultivar banana being collected as sample.

Local name	Scientific name Cultivar sp	Features
1. . Pisang berangan	*Musa acuminata Colla (AA Group)*	Sweet and creamy taste Panting for the commercial purposes
2. Pisang raja udang	*Musa acuminata Colla (AAA Group) cv. 'Red*	Red strip on the stem and also the leaves.
3. Pisang saba	*Musa acuminata* × *Musa balbisiana*	Popular among Malaysian community due to its multipurpose
4. Pisang mas	*Musa acuminate colla*	small fruit with 4cm diameter, and whitish yellow
5. Pisang serendah	*Dwarf Cavendish*	Dwarf around 1-3m height Long peduncle
6. Pisang kelat	*Musa acuminata x balbisiana Colla (ABB Group) cv. 'Bluggoe.*	Less sweet, creamy taste
7. Pisang tanduk	*Musa x Paradisia fa. corniculata*	Bigger size compare to the other banana. Only 2,3 bunch on peduncle
8. Pisang jari buaya	*Musa eumusa*	The sharp on tips of the pulp of banana just like the finger of crocodile.

Table 4.3 The diversity of wild banana being collected as sample

Local name	Scientific name Wild s	Features
1. Pisang merah	*Musa coccinea*	The reddish stem and leaves
2. Pisang tebem	*Musa borneensis var. borneensis*	Purple stem and petiole based winged Long leaves with small width
3. Pisang campestris	*Musa campestris var miriensis*	Small fruit, clump
4. Pisang acuminata	*Musa acuminata sub. Microcarpa*	Small size fruit,
5. Pisang darah	*Musa acuminata var.zebrine*	Purple reddish spot on the top of the leaves

4.2 Result on the Growth Response of Banana in Control Environment

Linear measurement of stem growth are ordinarily a good indicator of the growth of the entire plant since growth is highly coordinated. The growth of one part usually reflect the growth of all part of other parts due to the cell division and cell enlargement. The progress of growth responses of banana in control environment throughout 45 days as shown in Figure 4.1

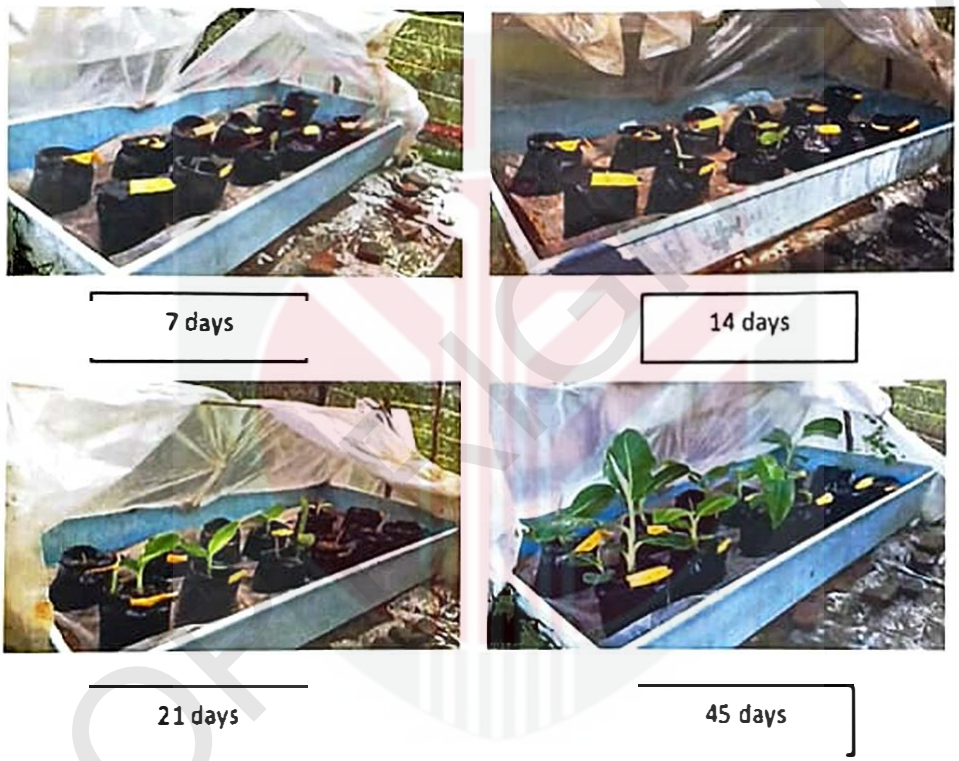

Figure 4.1 The progress of the samples during macro-propagation technique

The primary shoots and the apical dominance in the middle of the stem were removed again for the phase 2 for the next 45 days as shown in Figure 4.2. The growth of sample were recorded in the tabulated table to compare the growth performance of banana in control environment.

Figure 4.2 The primary shoot of the sample were cut off

Comb of samples that were infected with water stress showed several symptom such as poor emergence of primary root, stunted growth, failed bud and the comb decayed as illustrate in Figure 4.3 which affected the proliferation of the new bud to sprout

Figure 4.3 The comb of samples infected with water stress

4.2.1 Number of Shoots

After 45 days growth response in control environment by using macro-propagation technique the number of shoots were observed, measure and recorded in tabulated data as shown in Figure 4.4. Based on bar chart illustrate in Figure 4.5 showed that Merah, Tebem and Campestris produced highest average number of shoot for phase 1, 2 and 3 with 4.67, 2.67 and 2.67 per comb respectively. While others species showed average number of shoot 2 per comb.

Figure 4.4 The measurement of growth performance of sample for the 1st phase of macro-propagation after 45 days of macro-propagation

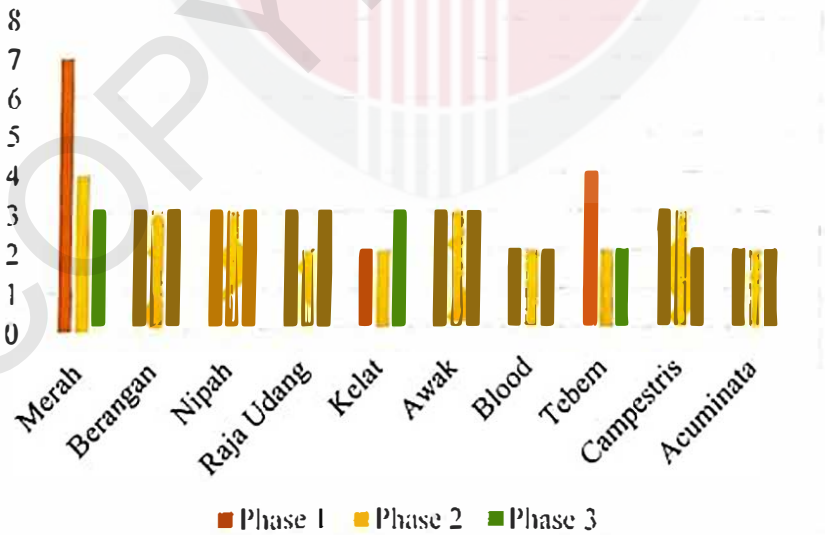

Figure 4.5 The graph showed the number of proliferation shoot produced after 45 days in control environment

4.2.2 Stem Diameter

Figure 4.6 showed how the diameter of stem were measured using rope. Based on the bar chart illustrated in Figure 4.7 shown that the growth response of banana based on the stem diameter illustrated the Traditional method have the highest values of means especially for wild species as Blood, Tebem, and Acuminata with 9.75 cm, 10 cm, 7.35 cm and respectively. However, Raja Udang species the exceptional from cultivar species with 11.8 cm. Phase 3 showed greater growth for cultivar species such as Merah, Nipah and Kelat with 6.75 cm, 11.25 cm and 10 cm respectively.

Figure 4.6 Showed how the diameter of stem were measured by using rope

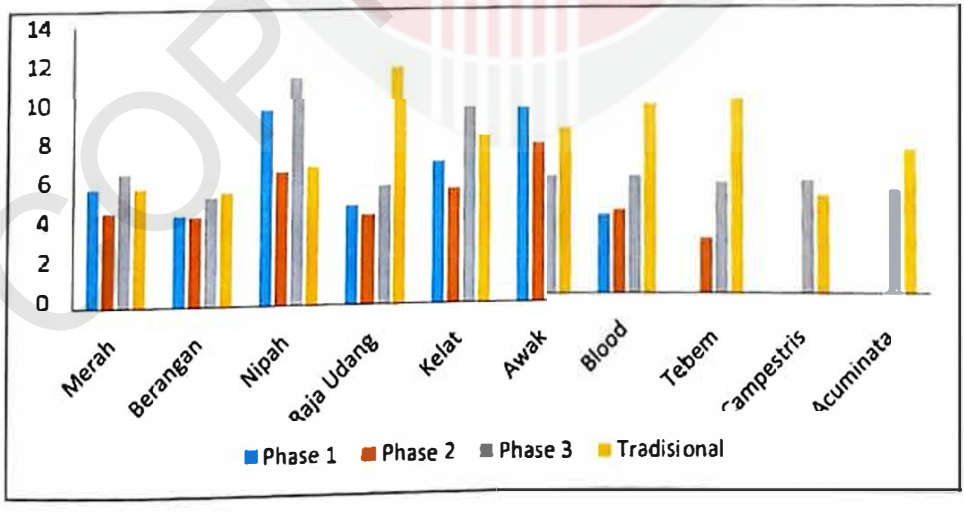

Figure 4.7 Shown the growth response of stem diameter (cm)

4.2.3 Height of Stem

From Figure 4.8 method of how the height of samples were measured. Based on Figure 4.9 showed that the comparison of height between wild and cultivar species. The height for cultivar species prefer phase 3 such as Merah, Berangan, Raja Udang Kelat and Awak with means values 14.15cm, 26 cm, 27 cm, 33.5 cm and 31 cm respectively compare others. While for the wild species 2 of the wild species which is Blood and Tebem with means values 21cm, 20.5cm respectively prefer in phase 3 However Acuminata and Campestris prefer traditional method with means values 20.75cm and 13.1cm

Figure 4.8 Shown method how the height of samples were measured

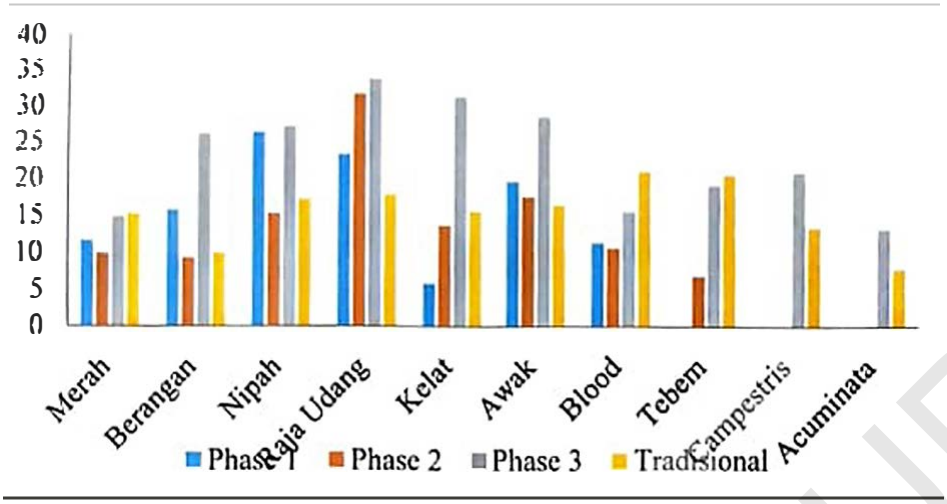

Figure 4.9 Shown the graph of mean height of banana growth responses. (cm)

4.2.4 Length of Leaves

Based on the graph shows in Figure 4.11 illustrated that the length of leaves for all species greater in Phase 3 and Traditional compare to Phase 1 and Phase 2. For Phase 3, Berangan, Kelat, Blood and Acuminata species and for the Traditional method, Awak, Tebem and Campestris species respectively showed highest means value for length of leaves compare others. However, the exception to Nipah which shows greater length in Phase 1 compare to others.

Figure 4.10 Shown the method of how the length of leaves were measured.

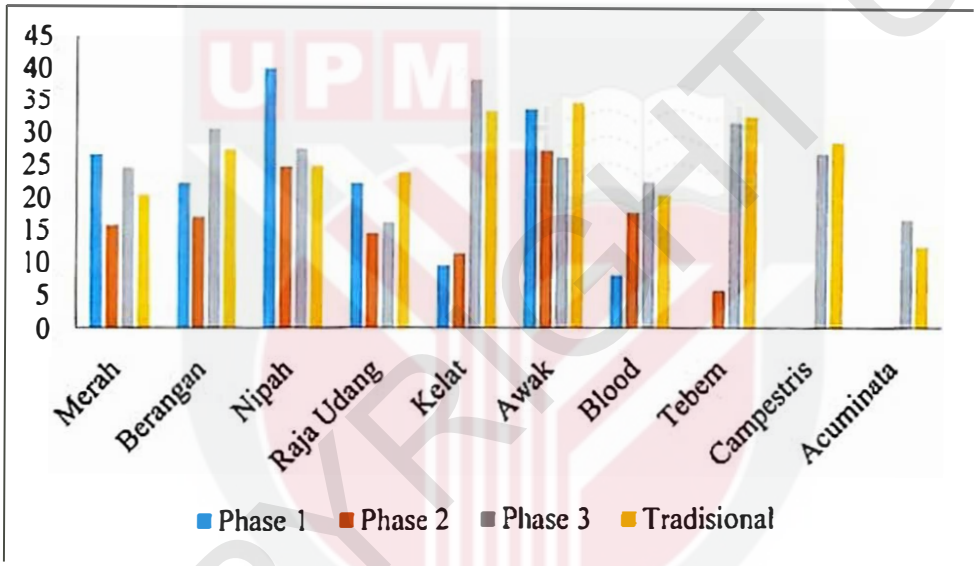

Figure 4.11 Shown the graph length of leaves (cm)

4.2.5 Width of Leaves

Figure 4.12 Shown the width of leaves were measured using rope. Based on the graph, in Figure 4.13 showed that the width of leaves in Phase 3 greater compared to Phase 1, Phase 2 and Traditional as most of the samples include Berangan, Nipah, Kelat, Awak, Tebem and Campestris. However, exceptional to Merah which shows greater width of leaves in Phase1 to compare Phase 3.

Figure 4.12 Shown the width of leaves were measured using rope

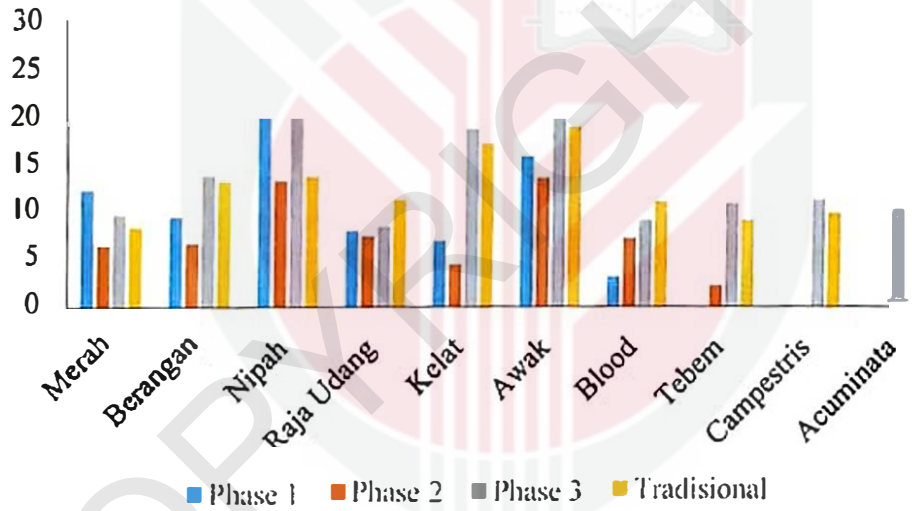

Figure 4.13 Shown the graph illustrates the width of leaves

CHAPTER 5

DISCUSSION

5.1 Number of Shoots

Based on the result of macro-propagation in phase 1, 2, 3 and also traditional method of planting shown the low new plantlet production with 4.67, 2.67, 2.67 and 2.0 per comb respectively. The situation occur due to the high endogenous auxin levels. All phase of macro-propagation shown low plantlet production which significantly different with the research was done by Singh et al. 2011 which recommended macro-propagation due to the ability to produce planting material about 50-60 shoots per sucker in 4-5 months varies depending on varies of species and 3-4 shoot for 30-35 days of first decortication and for the secondary phase of decortication done after the primary stem were cut down to encourage the multiplication of shoot and producing new plantlet span of time. In fact the failure for the total removal of apical meristem which recommended about 2 cm diameter and 4 cm depth to overcome apical dominance lead to low suckering production.

In banana low suckering ability associated with the strong apical dominance linked to high endogenous auxin levels (Arinaitwe *et al.* 2000). Thus, total removal of apical dominance very important during the macro-propagation method as it will affect proliferation of apical dominance. However in this research, the present of apical dominance affect the bud proliferation for the new plantlet thus show slow plantlet production. High endogenous auxin level play the main role for the new plantlet production as auxin homeostasis mediated by spatial and temporal. The

regulation of auxin biosynthesis plays a central role in determining root architecture. The root system very important for the plant growth responses as the nutrient and water absorption.

According to Osei (2006), cytokinin and auxin work antagonistically and thus, an application of cytokinin decreases the apical dominance, while an application of auxin increases the apical dominance. The balance between auxin and cytokinin hormone very important for the plant growth as auxin promoting the root and cytokinin stimulating shoot development. Thus, the strong link between auxin and cytokinin influences the growth responses and also the production of new plantlet during macro-propagation. Thus, the recent research found out that the shoot regeneration responses among banana influenced by the cytokinin type, concentration, species and their interaction. The recent study showed that treatment of decorticated suckers with Benzylaminopurine (BAP), synthethic cytokinin solution of 2.0mgL^{-1} enhanced the proliferation of plants when compared to the control, (Dayani *et al.* 2013).

Auxin have a role in root formation, from the initiation of a root meristem to the generation of a functional root system with a primary root, secondary lateral root branches and adventitious root (Damilola *et al.* 2017). Auxin is involved basically in all cellular process including cell division, cell expansion with implication for all stages of plant development process. The appropriate auxin distribution across the tissue is highly important to coordinate growth and tissue development. Auxin also act as an omnipotent regulator of root development which control the development and architecture of the root system via biosynthesis, metabolism, catabolism and

conjugation process. The high accumulation of auxin at the tips of the roots attributed to directional and transport of shoot-derived auxin moving down to the root tips. The roots development also influences by external factors such as light, temperature and nutrient availability which generate endogenous hormone signaling events.

5.2 Diameter of Stems

The average means value diameter of stems for each species in phases 1, 2, 3 and traditional method for Merah, Berangan, Nipah, Raja Udang, Kelat, Awak, Blood, Tebem, Campestris and Acuminata were 5.875 cm, 5.0875 cm, 8.738 cm, 6.825 cm, 7.875 cm, 8.13 cm, 6 cm, 6.17 cm, 5.375 cm and 6.3 cm respectively. From the analysis, shown that Nipah and Awak species have highest average of mean which is 8.738 and 8.13 cm respectively. The size of the comb for both species large to support the weight of stem and flowering stage. Oluwafemi (2013) found that the taller plants with thinner girth at higher planting densities. Wild species which is Blood, Tebem, Campestris and Acuminate have low rate of growth response in term of stem diameter due to the small comb size and growth in clump.

As noted by Marku and Meekiong (2005) stated that the wild species has thin pseudostem due to the high number of sucker produce and densely clustery with thin pseudostem. The physiological of the root system of the banana species which have different root architecture system. In fact, wild banana which have polyarchic architecture that capable to produce a numerous number of new plantlet from the

comb in favorable condition. However, for the cultivar the hierarchical pattern due to the strong dominance by middle part of main plant called apical dominance lead to the slow bud proliferation and low new plantlet production. For example, Blood and Campestris species capable to produce 13 sucker and 7 sucker respectively. The competition for the water, nutrient absorption by root and also the sun light sources for the photosynthesis process between suckers in a clump led to the thin pseudo stem.

In fact, the low new plantlet production and the low survival rate of wild banana in control environment occur due to the water stress. Water stress known as one of the main environmental factor that limit the plant production because it would produce reactive oxygen species (ROS) during photosynthesis, photorespiration caused damaged to cell as it toxic to plant cell and combined with vital component such as fats, protein, nucleic acid finally lead to the root death due to protein denaturation, DNA mutation and others. Water stress also would affected the plant physiology including growth, signaling pathway, gene expression and leaf photosynthesis (Flexas *et al,* 2004). Thus, in Figure 4.3 showed the effect of water stress on the comb of samples as it would affected the growth of plantlet caused stunted growth, poor root system and also reduced the number of bud proliferation due to interruption of plant physiology. Water stress occur due to the excessive and lack of water which would affect the plant physiology include the growth responses. For this research water stress occur due to the excessive water which cause an aerobic respiration. This situation occur due to the compactness of bedding media or sawdust which blocked the water channel at the bottom of propagator. Respiration consists of a series of reaction that occur primarily of plant cell. Respiration converts oxygen and

the sugars generated during photosynthesis into carbon dioxide, water and energy. It would occur under circumstances where no oxygen is present will cause the infected and also surrounding cells damaged. The infected and damaged roots will changes the root metabolism and produce physiological changes such as stunted growth

5.3 Height of Stems

The average means values height of Merah, Berangan, Nipah, Raja Udang, Kelat, Awak, Blood, Tebem, Campestris and Acuminata were 12.8 cm, 15.234 cm, 21.438 cm, 26.525 cm, 16.438 cm, 20.375 cm, 14.563 cm, 15.4 cm, 17.05 cm and 10.425 cm respectively. The cultivar species have greater height compared wild species as Nipah, Raja Udang and Awak species have highest average means value 21.438 cm, 26.525 cm and 20.375 cm. The cultivar species have low capability to produce sucker compare to wild species which can produce large amount of sucker. Acuminata have lowest with 10.425 cm due to the capability to produce 13 sucker and densely clustery in clump which highly competitive for the nutrient to growth (Marku and Kalu 2005).

Reddish and Singh (1993) reported that plant girth and height inversely proportional which primarily apply for the planting density not obey in this research which focus on the growth performance of wild and cultivar banana species in Central Sarawak because Awak and Nipah species highest growth response in both diameter and height. This research found that the size of the comb as the primary factor that influence the growth response of banana and the size of the comb varies to each other especially for the wild species.

5.4 Length of Leaves

The average means value length of leaves each species in all stages for Merah, Berangan, Nipah, Raja Udang, Kelat, Awak, Blood, Tebem, Campestris and Acuminata were 21.81 cm, 24.3 cm, 29.3 cm, 19.225 cm, 23.263cm, 30.493cm, 17.375cm, 23.25 cm, 27.575 cm and 14.4 cm. In term of length (cm) of leaves, Nipah and Awak species shown the highest length compared to others with 27.575 cm and 30.493 cm. Wild species which were Tebem and Campestris species have high growth for the length of leaves with 23.25 cm and 27.575 cm respectively. The average means value for Tebem affected by the means leaf length in phase 2 due to the water stress which caused stunted growth. In fact, Tebem species leaves can reach 3.5 m length and 0.8 m width which larger than mostly cultivar species. (Marku 2004). The number of functional leaves, leaves area include length of leaves and width of leaves depend on the competition for moisture and sunlight as wild species especially which generally growth in shady, deep forest area. The lack of sunlight as the main sources of photosynthesis process and produce photosynthetic for the plant growth. Light availability strongly affects whole plant growth and development especially leaf structure. Genetic adaptation to the light environment prevailing in the native habitat results shade tolerant species by modifying the girth stem, leaves length and width to optimize the growth rate (Larcher 1995). This situation occur in wild species banana to adapt with the native habitat.

5.5 Width of Leaves

The average means value width of leaves each species in all stages for Merah, Berangan, Nipah, Raja Udang, Kelat, Awak, Blood, Tebem, Campestris and Acuminata were 9.3 cm, 10.94 cm, 18.825 cm, 8.875 cm, 12 cm, 18.683 cm, 7.725 cm, 7.5 cm, 10.75 cm and 11.125 cm. Awak and Nipah species have the highest growth in term of leaves width with 18.825 cm and 18.683 respectively. In fact, Nipah and Awak species have overall great growth response among the banana species as this species dominate the growth in term of diameter of stems, height of stems, length of leaves and width of leaves due to the large comb size which facilitate and enhance growth rate.

CHAPTER 6

CONCLUSION

Based on the result of the research, I can conclude that the macro-propagation technique can boost and enhance the banana plantation in Malaysia. However due to the lack of experience in manipulating and totally removed of apical dominance caused the low plantlet production throughout the research. However, in this study I found that the size of the comb greatly affect the growth response of banana. Wild banana species with the small comb and growth in clump showed slow growth response and low survival rate compared to cultivar species. In fact, in Phase 1 and Phase 2 out of 4 species of wild banana Tebem, Blood, Acuminata, and Campestris only 2 species survive which is which represent Tebem *(Musa borneensis var. borneensis)* and Blood (*Musa* acuminata var. zebrine) species. The high sensitivity of the wild species toward the bud manipulation technique and also the water stress issue make it low production of new plantlet. The growth response of banana slightly difference to each other even belong to the same group. The species that show the highest growth in term of stem diameter, height of stem, length of leaves and width of leaves represent by cultivar species which is Nipah and Awak.

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PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Growth response of banana in control environment" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, University Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the faculty.

Mohamad Amirul Azlan Bin Abd Rahim
Date: