Establishment of *In Vitro* of VitAto with Treatment of KIN and GA_3 Hormones

Anisah Fasiha Bt. Mustaffa
F16A0023

A Thesis Submitted In Fulfilment Of The Requirements For Degree of Bachelor of Applied Science (Agrotechnology) with Honours

Faculty of Agro Based Industry
Universiti Malaysia Kelantan

2020

DECLARATION

I hereby declare that the work embodied in here is the result of my own research except for the excerpt as cited in the references.

_____

Signature

Student's Name : ANISAH FASIHA BT. MUSTAFFA

Matric No : F16A0023

Date :

Verified by :

_____

Supervisor Signature

Supervisor's Name : DR. SUHANA BT. ZAKARIA

Stamp :

Date :

ACKNOWLEDGEMENT

First thing first, I would like to thank God for giving me this opportunity to study and gain knowledge as much as I can in Universiti Malaysia Kelantan (UMK).

Next, I would like to express my gratitude to my parents that have been supporting and understanding me along my journey towards a scroll for my future. Not to be forgotten, my supportive supervisor Dr. Suhana BT. Zakaria that has been very patient and helpful throughout the whole process in finishing this thesis. A big thank you for the advices, solutions, motivations and encouragement that had been given to me. This paper would never have been completed without their assistance and dedicated involvement in every step throughout the process. I am very grateful for the support and understanding over the past four years. I also want to thank to all the lecturers of UMK that helped me out directly or indirectly completing this thesis.

Lastly, I would like to state my deepest gratitude to my friends and family for helping and motivationing me physically and especially mentally.

Establishment of *In-Vitro* Culture of VitAto with Treatment of KIN and GA_3 Hormones

ABSTRACT

Micropropagation is the technique that involve the rapid vegetative propagation of plants under in vitro conditions of high light intensity, controlled temperature and a defined nutrient medium. Through micropropagation, not only huge amount of plants can be produced in short time, the specified or desired features can be added until it reaches the desired result of the plants. In this study, sweet potato(Ipomoea Batatas) is used.Sweet potato *(Ipomoea Batatas)* is one of the biggest consumed food in the world along with rice, maize, barley, wheat, cassava and potato.VitAto is another one of the new variety of sweet potato available that is known to have good potential in nutritional and functional values. At Malaysian Agriculture Research and Development Institute (MARDI), the researches have shown that this variety has higher content of Vitamin A (β-carotene). The problem statement of this study is a large number of loss will happen if we use conventional methods to propagate VitAto. For this study, the explant nodes will be used to be micropropagated. It is easier to be manage and surface sterilize the nodes. The explant node will be surface-sterilized before used. In this study, 10 different surface sterilizing method were used. Method D seem to be the best method for the explant survival with 37.33% rate of survival. This method use the combination of 70% ethanol, 5% hydrogen peroxide, 0.5% fungicide (Mancozeb) and 2 drops of Tween 20. The hormones used will be mixed into the media during media preparation. This study is aimed to increase the production of VitAto using micropropagation technique in optimum environment with the best ratio of hormones as supplement for the optimum growth of plant tissue. For this study, kinetin (KIN) and gibberellins (GA_3) were used and the ratio of 0.01mg/l of KIN and 0.1mg/l GA_3 is the best combination ratio for the root and shoot growth.

Keywords: VitAto, kinetin (KIN), gibberellins (GA_3), surface sterilization

Penumbuhan VitAto secara *In-Vitro* dengan Rawatan Hormon KIN dan GA_3

ABSTRAK

Mikropropagasi adalah teknik yang melibatkan penyebaran tumbuhan vegetatif yang cepat di bawah keadaan vitro dengan intensiti cahaya tinggi, suhu terkawal dan medium nutrien yang ditetapkan. Melalui mikropropagasi, bukan sahaja jumlah tumbuhan yang besar dapat dihasilkan dalam masa yang singkat, ciri-ciri tertentu atau yang dikehendaki dapat ditambah sehingga mencapai hasil yang diinginkan tanaman. Dalam kajian ini, ubi keledek (*Ipomoea Batatas*) digunakan. Ubi keledek (*Ipomoea Batatas*) adalah salah satu makanan yang paling banyak dimakan di dunia bersama dengan beras, jagung, barli, gandum, ubi kayu dan kentang. VitAto adalah satu lagi jenis ubi keledek yang sedia ada yang diketahui mempunyai potensi yang baik dalam nilai pemakanan dan fungsi. Di Institut Penyelidikan dan Pembangunan Pertanian Malaysia (MARDI), penyelidikan telah menunjukkan bahawa varieti ini mempunyai kandungan Vitamin A (β-karoten) yang lebih tinggi. Penyataan masalah kajian ini adalah sejumlah besar kerugian yang akan berlaku jika kita menggunakan kaedah konvensional untuk pembiakan VitAto. Untuk kajian ini, nod eksplan akan digunakan sebagai bahan mikropropagasi. Ia lebih mudah untuk diuruskan dan disterilkan. Nod eksplan akan disterilkan permukaannya sebelum digunakan. Dalam kajian ini, 10 kaedah sterilisasi permukaan yang berbeza telah digunakan. Kaedah D menjadi kaedah terbaik untuk meneruskan survival dengan kadar survival sebanyak 37.33%. Kaedah ini menggunakan gabungan 70% etanol, 5% hidrogen peroksida, 0.5% fungisida (Mancozeb) dan 2 titisan Tween 20. Hormon yang digunakan akan dicampur ke media semasa penyediaan media. Kajian ini bertujuan untuk meningkatkan pengeluaran VitAto menggunakan teknik micropropagation dalam persekitaran optimum dengan nisbah hormon terbaik sebagai tambahan untuk pertumbuhan optimum tisu tumbuhan. Untuk kajian ini, kinetin (KIN) dan gibberellin (GA3) telah digunakan dan nisbah 0.01mg / l KIN dan 0.1mg / l GA3 adalah nisbah kombinasi terbaik untuk pertumbuhan akar dan pucuk.

Kata kunci: VitAto, kinetin (KIN), gibberellins (GA_3), kaedah sterilisasi.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

KIN	-	Kinetin
GA_3	-	Gibberellins
MS	-	Murashige and Skoog Medium
mg/L	-	milligram per litre
Cm	-	centimetre
G	-	gram
^oC	-	degree Celsius

CHAPTER 1

INTRODUCTION

1.1 Research Background

Micropropagation is the technique involving rapid vegetative propagation of plants under controlled temperature, high light intensity and a defined nutrient medium under *in vitro* conditions. Only small amount of plant tissue is adequate to produce thousands of clones in only a year using this technique. It would take a longer time to produce same number of plants using traditional and regular methods to propagate the plants. For those plant species that shows negative response to practices of conventional propagation, the micropropagation method serves good alternative to ensure the plant species can be propagated effectively. Through micropropagation, not only huge amount of plants can be

produced in short time, the specified or desired features can be added until it reaches the desired result of the plants (Tan, 2015). In present days, micropropagation technique is used as a process for a rapid *in vitro* propagation of shoots selected as the best strain from the field, before getting them back to grow in the field. For this study, the explant nodes will be used to be micropropagated. It is easier to be managed and surface sterilized (Tan, 2015).

In this study, sweet potato (*Ipomoea Batatas*) was used. Sweet potato *(Ipomoea Batatas*) is one of the biggest consumed food in the world along with rice, maize, barley, wheat, cassava and potato. It is believed by the researches and scientists that sweet potato is originated from thousands years ago in Central America. A person known as Christopher Columbus took sweet potatoes to his home in Europe after his first voyage to America in 1492. China was one of the earliest country that received sweet potato as food supply in the late 16th. Then, it spreaded through Africa, Latin America, and Asia during the 17th and 18th centuries (Tan, 2015). Sweet potato is now cultivated in more developing countries compared to other root crops. Until 2015, the largest country in the world that cultivate sweet potato is China with more than 3.5 million hectares of cultivation area. This covers about 43% of the world's sweet potato supply. While in Malaysia the cultivation only about 3,041 hectares or area (Tan, 2015).

There are few varieties that are available in Malaysia. For example, Banting, Jalormas, Gendut and Biru Jepun. VitAto is another one of the new variety of sweet potato available that is known to have good potential in nutritional and functional values. At Malaysian Agriculture Research and Development Institute (MARDI), the researches have

shown that this variety has higher content of Vitamin A (β-carotene) (Sabeetha, 2017). In East Coast part of Malaysia, the cultivation of this variety is about 200 hectares in Terengganu with the production of 20 tonnes for 1 hectares. The production of VitAto is also said to reach until 25 to 30 tonnes in good planting season (MARDI, 2013).

1.2 Problem statement

VitAto is a new hybrid here in Malaysia, increasing the number of VitAto is the aim. The problem statement of this study is a large number of loss will happen if we use conventional methods to propagate VitAto. It is due to the outside environment such as humidity and temperature might be the reason for the loss of VitAto propagation at early stages. It includes the presence of pest and disease, and the insufficient nutrient supply for the growth. But, by using micropropagation method,the environment condition can be controlled to the optimum condition for better growth of plant tissue. Different ratio of hormones are also added to the media to identify the optimum ratio for the growth. The hormones act as the growth regulators to enhance the growth of the for the plant tissue. Conventional methods also consume more time than micropropagation technique. Micropropagation can produce large number of plants clones in short time.

1.3 Hypothesis

H_o =Addition of hormones has no significant on growth effects of the VitAto explants during the micropropagation.

H_1 =Addition of hormones is significant on growth effects of the VitAto explants during the micropropagation.

1.4 Scope of Study

This study is directed on increasing the number of VitAto using micropropagation technique with different methods of surface sterilization and addition of Kinetin (KIN) and Gibberellin (GA_3) hormones as plant growth regulators.

1.5 Significance of the Study.

This study is expected to increase the production of VitAto plant using micropropagation technique with the best ratio of hormones which are kinetin (KIN) and gibberellin (GA_3) as regulators for the optimum growth of plant tissue. Thus, help the sweet potato growers to increase their production with best quality of products in shorter period of time.

1.6 Objectives

The objectives of this study are:

a) To establish in vitro culture of VitAto using different sterilization techniques.
b) To investigate the effects of hormones kinetin (KIN) and gibberellin (GA_3) to the growth of the explants.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy of Sweet Potato (*Ipomoea Batatas*)

Sweet potato originally came from the western coast of South America and the Central America. But now, China is the largest cultivated area for sweet potato in the world, with more than 3.5 million hectares, making up almost 43% of the world source. The area under this crop in Malaysia is miniscule at 3,041 hectares. Root tubers, leaves and young shoots are the edible parts of the plant. Its beneficial protein content and high content of mineral,vitamins and antioxidants are the reasons that sweet potato is labeled as good for human health. There are some research that suggest to increase consumption of plant foods,

like sweet potatoes to reduce the risk of diabetes, obesity, heart disease, and overall mortality. A medium sweet potato can provide the daily requirements for vitamin A, vitamin C, vitamin B6 and potassium (MARDI, 2013).

Sweet potato is a family member of Convolvulaceae, and it is related to *Kangkung* (*Ipomoea aquatica*) and the ornamental morning glory (*I. indica* and other *Ipomoea* species). Indeed, these relatives can harbour the pests of sweet potato. Sweet potato is a spreading or trailing vine which can be harvested as early as after 5-6 months in the temperate region during the summer or 3½-4 months after planting in the tropics. With this short crop cycle, this crop can be grown not only in the tropics, but also in the temperate zone during the summer months. Sweet potato can also grow in a very wide range, from sea level to 2,500 meters in the tropics. While sweet potato produces sexual seed, using stem cuttings, the crop is propagated vegetatively. Shoot cuttings are the preferred planting materials. As in other root crops, the economic part is the storage roots, formed by bulking of and starch deposition in the adventitious roots which form wherever the stem nodes make contact with the soil. But, rather than using conventional methods propagating the sweet potato, micropropagation is used as an altenative technique to propagate the plants with higher number in short time (Olek, 2013). The variety of VitAto came from the mutation breeding of sweet potatoes. The hybrid of sweet potatoes from different variaties is hard to be done as it is complicated. The uncomplimentary of genetic is high. In Malaysia, MARDI and Malaysia Agency of Nuclear work together to do the mutation breeding to produce this variety. The sweet potatoes was infused with gamma ray enhance mutation in genetic information of the sweet potatoes. The produced VitAto has higher

content of anthocyanin (MARDI, 2013). Anthocyanins have been reported to be able of lowering blood pressure, enhancing visual acuity, decreasing the proliferation of cancer cells, inhibiting tumor growth, preventing diabetes, reducing the risk of cognitive and motor function modulating cardiovascular disease (CVD). Anti-inflammatory and anti-bacterial activity are also recorded (Guha, 2015). This new variety also has higher disease resistance. Because most cultivars are self-compatible, sweet potato plants generally do not produce seeds. The seed-producing plants are highly heterozygous and are genetically different from the mother plant (Olek, 2013). That is why, in this studies, internodes of sweet potato are used.

Figure 2.1 : VitAto

Source : MARDI, 2013

2.2 Tissue Culture

Tissue culture is the hygienic culture of *in vitro* cells, tissues, organs or entire plants, often under controlled nutritional and environmental conditions to create clones of plants. The resulting clones are true to the genotype chosen. Growing and multiplying environment for the culture is provided by the controlled conditions in tissue culture. These conditions include adequate nutrient amount, medium pH, adequate temperature, and adequate gaseous and liquid environment (Hussain, 2012).

Technology of plant tissue culture is commonly used for large scale multiplication of plants. In addition to their use as research tool, plant tissue cultivation techniques have became one of the major industrial significance in the field of plant propagation, disease elimination, crop enhancement and as secondary metabolite processing in the recent years. Small pieces of tissue can be used in a continuous process to produce hundreds of thousands of plants. Under controlled conditions, a single explant can be multiplied into several thousand plants in a relatively short time and space regardless of the season and weather, on a year round basis. Micropropagation has successfully grown and protected endangered, threatened and rare species due to high multiplication coefficient and low demands on the number of initial plants and storage. Micropropagation has successfully grown and protected endangered, threatened and rare species due to high multiplication coefficient and the low demands on the number of initial plants and storage. In addition, the

processing of somaclonal and gametoclonal variants is considered to be the most effective plant improvement technology. Micropropagation technology has extensive potential to produce high-quality crops, isolating useful variants in well-adapted high-yielding genotypes with enhanced disease resistance and stress tolerance capability. Other types of callus cultures result in clones with inheritable characteristics different from those of parent plants due to the possibility of somaclonal variation, which contributes to the production of improved varieties of commercial significance. Commercial plant growth through micropropagation techniques has several advantages over conventional methods of propagation in planting, slicing, grafting, and air-layering. It is rapid processes of propagation that can lead to virus-free plant growth. A major plant that is used in medicine field, *Coryodalisyanhusuo* was propagated by somatic embryogenesis from tuber-derived callus to produce tuber-free disease. Banana plants without banana bunchy top virus (BBTV) and brome mosaic virus (BMV) have formed the Meristem tip population. Higher yields were achieved through the cultivation of aseptic in vitro procedures. Increase in yield was achieved in controlled conditions up to 150 percent of virus-free potatoes. The main purpose of this chapter is to define tissue culture techniques, various developments, present and future developments and their application in different fields (Hussain, 2012).

2.3 Micropropagation Technique

Micropropagation is an alternative method that is ran in lab to produce genetically identical or the clones of plantlets by practicing *in vitro* procedures. Micropropagation is a technique that allow the propagation to be done from particular genotype using in vitro culture techniques. Micropropagation is related with mass production at a competitive price. Micropropagation only can be done when sufficient starting materials are available and used. The choice of stock material must be done discriminately. Usually, for fruit or ornamentals production, the starting material is chosen from an elite plant, it is selected according to some particular demanded or desired phenotypic characteristics. In forestry and vegetable production, the elite starting material will either be a selected phenotype (specific plant) or this plant will be the bearer of elite seed, commonly used as preliminary material for a more or less comprehensive micropropagation system(Hua, 2010) .

There are four stages in the whole micropropagation process. First, stage 0. Basically stage 0 was presented to be a solution for contamination problems. One of the steps toward avoiding contamination is raising mother plants under more hygienic conditions. It can reduce some of the contamination possibility, especially those related to fungi. Somehow, it is quite difficult to early detect the contamination especially when it is related to bacteria. Next stage is stage 1. The aim of stage I is to begin the axenic culture process. Most of the micropropagation work that has been done is by using apical or

axillary bud as the explant of choice (Hua, 2010). In the next stage, the culture can supply shoots better propagation as well as the material that is required to maintain the stock. There is possibility that the plant would not differentiate and produce any shoots that can be directly rooted if the stock is not increased or maintained. The plants could stay in stage 2 for quite a few generations. The physiology of the plants which are number of shoots, shoot length, number of rooted shoots, number of roots, and length of roots are by themselves insufficient parameters to judge the effectiveness of the treatments in stage 3. In stage 4, conditions of the plants are monitored to be transferred to the greenhouse. Efficiency is very important for this stage (Hua, 2010).

Micropropagation may produce several duplicates from a single seed or explants. It takes shorter time, no need to wait for seed production throughout the life cycle. For organisms with a long life span, rapid proliferation is possible along with low seed production levels, or seeds that do not germinate readily. It equates seasonal seed germination restrictions. But, in this study, internodes are used (Jehle, 2005). Micropropagation is the first major and widely accepted practice of plant biotechnology, popularly known for clonal propagation on a large scale. It is described as *in vitro* plant cultivation initiation, propagation and rooting for ex vitro establishment in the soil under controlled environmental conditions. Recent approaches to *in vitro* plant propagation techniques over the past decade have improved the science of micropropagation. It covers a big range of variety of plants, including economic, agronomic, and forest trees (Aladele, 2012)

Mass production of uninfected and even plants has been made possible by tissue culture. Therefore, the techniques bring the great benefit of high-quality planting materials to farmers. Production of planting materials is vital to the stem multipliers and producers in the overall research structure for preserving variety pureness and providing high yielding cassava cultivars as example (Aladele, 2012).

It was investigated the possibility of using screen house to sustain crops *in vitro* and to propagate essential vegetative crops with less pollution at a reduced cost. *In vitro* propagation was used to improve, develop and sustain economic trees and forest organisms through organogenesis and somatic embryogenesis. *Khayagrandifoliola*, for example, is important species to West Africa. Khaya wood, commonly known as African mahoganyas, is a high-priced wood that is often used for construction and furniture purposes. Micropropagation, with its endangered conservation status, was a useful tool for the mass reproduction of superior stock plants as well as genetic development and preservation. A robust plant regeneration protocol from *Khayagrandifoliola*'s matured seed embryo was established for conservation and multiplication purposes. *Plukenetiaconophora*, formerly known as *Tetracarpidium conophorum* and popularly known as African walnut, is another species of economic importance.

This tree species is an economically important annual climber, a nutritious plant that can be used in medicine field. A prolific shooting program (protocol) for *Plukenetia conophora* has been recorded. The need to preserve and regenerate recalcitrant species, however, has led to the development of in vitro protocols for many recalcitrant species,

including vegetables. Telfaria Occidentalis is a tropical vegetable grown in West African and widely consumed primarily for its protein wealth in tropical regions. Due to reproductive problems associated with the plant's sex, the female plant is preferred for the production of leaves and fruits. *T.occidentalis* in vitro culture was studied under various combinations of cytokinins and auxins.

The commercial use of micropropagation is mainly limited to high-value crops and due to the large annual numbers needed to start new farms, in addition to high production costs, the commercial use of this technique for the most essential plant species is inadequate. Protocols using bioreactors and liquid media are suggested to overcome the problems of traditional micropropagation. Bioreactor system incorporating a number of structures in its design was used to make the operation simpler and reduce the cost of production. Using a bioreactor, mechanization is one of the most efficient ways of reducing micropropagation costs (Aladele, 2012).

2.4 Surface Sterilization Agents

Earle H. Spaulding has been creating a coherent approach to disinfecting and sterilizing patient care products or supplies over 45 years ago. The classification system is so clear and logical that, when preparing procedures of disinfection or sterilization, it has been maintained, improved and widely used by practitioners in infection control and others. Spaulding claimed that the purpose of disinfection could be more easily understood if patient care devices and products were separated into 3 groups based on the level of risk of contamination involved in the use of the items. The three classes he identified are critical (enters sterile tissue and sterilization is a must), semi-critical (associates mucous membranes and requires high-level disinfection), and non-critical (enters intact skin contact and requires low-level disinfection). Such classes and procedures are intended to achieve high-level disinfection and sterilization (Rutala, 2013).

Explant surface sterilization is a procedure involving the soaking of explants in a suitable chemical sterilants or disinfectants concentration for a specified time resulting in a culture that is free from any contaminations to be produced. The disinfectants that are commonly used are ethanol (or isopropyl alcohol), sodium or calcium hypochlorite (NaOCl) or ($Ca(ClO)_2$), hydrogen peroxide (H_2O_2), mercury chloride ($HgCl_2$), silver nitrate ($AgNO_3$) and bromine liquid (Bello, 2018). The type, concentration and exposure time of the disinfectants required, however, differ for different parts of the plant and crop. Ethanol

is such an effective disinfectant, but also phytotoxic, sterilizing agent (Srivastava, 2008). It is typically used for only a few seconds or minutes at a concentration of 70%, followed by the applications or treatment with other disinfectants. Meanwhile, hypochlorite is a very active microorganisms killer that significantly reduces bacterial populations, even with micromolar concentrations. Nonetheless, for explant disinfection, sodium hypochlorite is quite aggressive, making use of calcium hypochlorite preferred as a minor sterilant (Abbasi, 2017). In a study by Bello (2018), it shows that among the treatments for *Ziziphus spina-christi* (L.) sterilization with three disinfecting agents (Ca(ClO)2, NaOCl and HgCl2), Ca(ClO)2 were most satisfactory.

Asepsis is the first requirement for a culture's survival. Maintaining uninfected (free from all microorganisms) or germ-free conditions is necessary for effective cultivation of tissue procedures. All culture vessels, materials and equipment used in tissue handling, as well as explant itself, must be sterilized to ensure an aseptic environment. The important thing is to keep dust away from the air, ground and floor. The killing or elimination of all types of microbial life in a material or object (including endospore) is known as the sterilization process (Badoni, 2010). Sterilization also is the process of removing contaminants from explants before cultures are formed. Similar sterilizing agents are used for tissue decontamination. These sterilants are also harmful to plant tissues, hence proper sterilant concentration, duration of exposure to the different sterilants, sequences of use of these sterilants need to be consistent to reduce the risk of explant injury and achieve better survival rate (Badoni, 2010). All operations in laminar airflow sterile cabinets should be conducted. The present study has used two different chemicals, mercuric chloride (0.1%)

and sodium hypochlorite (1%), to standardize the best sterilization protocol for potato in vitro cultivation (Badoni, 2010).

2.5 Kinetin (KIN) and Gibberellin (GA_3) As Plant Growth Regulators in Tissue Culture

Physiological procedure at low fixations is affected by a gathering of natural substances called plant hormones. The procedures affected are primarily in growth, separation and advancement. Different procedure, for example, stomatal development likewise could be affected. Plant hormones could likewise been referred to as phytohormones however this term is once in a while utilized (Akter, 2007). Plant hormones are an extraordinary compound set with special properties and digestion. The main all inclusive qualities of plant hormones are that they are regular aggravates that present in plants with a capacity to influence physiological procedures at focuses far underneath those where either supplements or nutrients would influence these procedures (Clark, 2000). The gibberellins (GAs) are a group of mixes dependent on the entgibberellane structure, more than 125 individuals exist and their structures can be found on the web. While the best broadly accessible compound is GA_3 or gibberellic corrosive, which is a contagious item,

the most imperative GA in plants is GA_1, which is the GA fundamentally in charge of stem extension. A significant number of different GA_3 are antecedents of the development dynamic GA1 (Phinney, 1955).

Hormones of plant are essential biotic variables to upgrade root development. There are seven sorts of plant hormones. In any case, auxin and gibberellin (GA) are solid controllers of shoot development, yet these are not generally quickening agents for root development. GA likewise assumes an irreplaceable job in the typical advancement of roots, since counterfeit GA exhaustion causes strange extension and concealment of root lengthening. The GA necessity for ordinary root development was divulged by the utilization of concoction inhibitors and freaks of GA biosynthesis. GA work that keeps root morphology long and slim is attributed to the game plan of cortical microtubules, cellulose microfibrils (Yamaguchi, 2008). Cytokinins are a very much concentrated group of plant hormones. During the 1950s, Skoog and Miller previously decided the basic recipe of a cytokinin, 6-furfurylaminopurine, a substance secluded from a herring sperm DNA arrangement that advances plant cell division in vitro. It was named kinetin. In any case, 6-furfurylaminopurine is falsely framed under explicit conditions, for example, when DNA arrangements disturb or are autoclaved, and were not known to happen normally (Miller, 1961). Initially, cytokinins were characterized as synthetic substances that actuate cell spread and improve callus duplication to shoot when connected with auxins, however at this point cytokinin is known as a distributer to the development and advancement of plants. It incorporates embryogenesis, support of root and shoot meristems, and vascular advancement.

They likewise balance root extension, sidelong root number, knob arrangement, and apical strength because of natural boosts. Cytokinins are therefore important signals for growth and development throughout the plant's life (Sakakibara, 2015).

Neither GA_3 nor kinetin could increase the amylase activities of roots while IAA reduced root amylase action. Action of corrosive and antacid invertases was the highest in shoots and low in cotyledons. Contrasted and antacid invertase, corrosive invertase movement was higher in every one of the tissues. The decreased corrosive and antacid invertase exercises in shoots of the seedlings were improved by GA_3 and kinetin. Roots of the stressed seedlings had higher basic invertase action and GA3 and IAA helped bring the level closer to the controls. GA3 and kinetin increase the activity or action of saccharose synthase (SS) and saccharose phosphate synthase (SPS) in seedlings-focused cotyledons, thus taking the increased level of root SPS to a near norm. The more elevated amount of lessening sugars in the shoots of GA_3 and kinetin treated focused on seedlings could be because of the high corrosive invertase action occurred in the shoots, and the abnormal state of bound fructose in the cotyledons of stressed seedlings could be because of the high action of SPS in this plant tissue (Kaur, 2000).

CHAPTER 3

MATERIAL AND METHOD

3.1 Plant Material and Apparatus

The variety of sweet potato which is VitAto will be obtained from Malaysian Agricultural Research and Development Institute (MARDI) center that is located in Bachok, Kelantan. The micropropagation cycles will be carried out with two different growth regulators which are kinetin (KIN) and gibberellin (GA_3) . The basic media that will be used is MS media (Murashige and Skoog 1962). There will be diverse amount of growth regulators in each cycle. The apparatus used in this study were pH meter, magnetic stirrer, measuring cylinder, schott bottles, digital weighing scale, beakers, microwave, test tubes,

forceps, scalpel, surgical blades, masks, gloves, laminar hood, autoclave machine, water distiller, spatula, aluminium foil, vegetables wrapper, parafilm, sonicator or ultrasonic bath, petri dish and cutting aluminium.

3.2 Preparation of stock solution

Four sets of stock solutions were made based on group of nutrient which is required for plant tissue culture. The required amounts of chemical were weight based on Table 1. The chemicals were then dissolved in sterilized distilled water. The solutions were then filled up to final volume once solubilisation is completed. After the stock solution is prepared, it was labeled to avoid error and stored at $4^{o}C$. Extra stock is prepared as back up for any possibilities such as contamination.

Table 3.1: Murashige and Skoog Stock solution

A. Macronutrient	**1X (g/L)**	**20X (g/500mL)**
NH_4NO_3	1.65	16.5g
KNO_3	1.90	19.0g
$CaCl_2.2H_2O$	0.44	4.4g
$MgSO_4.7H_2O$	0.37	3.7g
KH_2PO_4	0.17	1.7g
B. Micronutrient	**1X (g/L)**	**200X (g/500ml)**
$MnSO_4.4H_2O$	0.02230	2.23
$ZnSO_4.7H_2O$	0.00860	0.86
H_3BO_3	0.00620	0.62
KI	0.00083	0.083
$NaMoO_4.2H_2O$	0.00025	0.025
$CuSO_4.5H_2O$	0.000025	0.0025
$CoCl_2.6H_2O$	0.000025	0.0025
C. Vitamin	**1X (g/L)**	**(500X/125ml)**
Myo–inositol	0.1	6.25
Glycine	0.002	12.5
Thiamine-HCL	0.001	6.3
Nicotinic acid	0.0005	31.3
Pyridoxine-HCL	0.0005	31.3
D. Ferum Source	**1X (g/L)**	**(200X/500ml)**
$FeSO_4.7H_2O$	0.0278	2.78
$Na_2EDTA.2H_2O$	0.0373	3.73

3.3 Preparation of Explant Nodes and Surface Sterilization

The explants were extracted from the sweet potato plant for about 1-1.5cm long that consist of stem node with the axillary bud, fragments of internodes and fragments of petiole (Olek, 2013). The explants were sterilized by using 10 different methods for surface sterilization. These methods were used to identify the best method for longer survival period of the explants.

Table 3.2 : Procedures of surface sterilization techniques.

Method	Procedure
A	The explants were put under running tap water for 5 minutes and rubbed smoothly at the same time. They were immersed in 0.5% of sodium hypochlorite for 7 minutes with continuous shaking. Explants were washed with sterilized distilled water (Olek, 2013).
B	The explants were put under running tap water for 10 minutes and rubbed smoothly at the same time. The explants were shook continuously with 70% of ethanol, and immersed in 0.5% of fungicide (Mancozeb) for 5 minutes. The explants were then immersed in 5% hydrogen peroxide for 5 minutes before they were rinsed with sterilized distilled water for 3 times (Bakhsh, 2017).

C The explants were washed under running tap water for 10 minutes. Then immersed in 70% of ethanol for 5 minutes. 0.5% of fungicide (Mancozeb) was used to immerse the explants or 5 minutes. After that, the explants were put in 0.1% of sodium hypochloride for also 5 minutes. Lastly, the explants were rinsed 3 times using sterilized distilled water.

D The explants were put under running tap water for 10 minutes. They were immersed in 70% of ethanol for 5 minutes before they were immersed and shaken in 0.5% of fungicide for 5 minutes. Then, the explants were immersed 5% hydrogen peroxide with 2 drops of Tween 20 for 5 minutes. Explants were rinsed with sterilized distilled water 3 times.

E The explants were put under running tap water for 10 minutes and rubbed smoothly at the same time. They were immersed in 70% of ethanol for 5 minutes before they were immersed and shaken in 1% of fungicide (Mancozeb) for 5 minutes. Then, the explants were immersed 5% hydrogen peroxide with 2 drops of Tween 20 for 5 minutes. Explants were rinsed with sterilized distilled water 3 times. This time, the media was mixed with 0.2ml plant preservative mixture (PPM).

F The explants were put under running tap water for 10 minutes and rubbed smoothly at the same time. They were immersed in 70% of ethanol for 5 minutes before they were immersed and shaken in 1.5% of fungicide (Mancozeb) for 5 minutes. Then, the explants were immersed 5% hydrogen peroxide with 3 drops of Tween 20 for 5 minutes. Explants were rinsed with sterilized distilled water 3 times.

G The explants were put under running tap water for 10 minutes and rubbed smoothly at the same time. They were immersed in 70% of ethanol for 5 minutes before they were immersed and shaken in 2.0% of fungicide (Mancozeb) for 5 minutes. Then, the explants were immersed 5% hydrogen peroxide with 3 drops of Tween 20 for 5 minutes. Explants were rinsed with sterilized distilled water 3 times.

H The explants were put under running tap water for 10 minutes and rubbed smoothly at the same time. They were immersed in 70% of ethanol for 5 minutes before they were immersed and shaken in 3.0% of fungicide (Mancozeb) for 5 minutes. Then, the explants were immersed 10% hydrogen peroxide with 3 drops of Tween 20 for 5 minutes. Explants were rinsed with sterilized distilled water 3 times.

I The explants were put under running tap water for 10 minutes and rubbed smoothly at the same time. They were immersed in 70% of ethanol for 5 minutes before they were immersed and shaken in 1% of fungicide (Mancozeb) overnight. On the next day, the explants were immersed 5% hydrogen peroxide with 2 drops of Tween 20 for 5 minutes. Explants were rinsed with sterilized distilled water 3 times.

J The explants were put under running tap water for 10 minutes and rubbed smoothly at the same time. Explants were put in sonicator bath for about 5 minutes with the lowest wavelength. They were immersed in 70% of ethanol for 5 minutes before they were immersed and shaken in 5% hydrogen peroxide with 2 drops of Tween 20 for 5 minutes. Then, the explants were immersed in 1% of fungicide (Mancozeb) for 5 minutes. Explants were rinsed with sterilized distilled water 3 times.

3.4 Culture medium

The explants nodes were cultured on Murashige and Skoog (MS) media, supplemented with varying variable; hormone and control. The hormone MS media was fortified with of gibberellin (GA_3) and Kinetin (KIN) hormone. Meanwhile, control MS media that was used was the one without the gibberellin (GA_3) and Kinetin (KIN) hormone. Culture medium was prepared by using fifteen different concentrations of plant regulators shown in Table 2. First, 200mL of distilled water was added into a 500mL beaker. Next, prepared macronutrient stock, micronutrient stock and vitamin stock were added according to the required amount. A magnetic stirrer was put in the beaker and continuously stirred while adding 15g of sucrose on a hot plate. Distilled water was added to adjust the volume to 400mL. Plant growth regulators was added and the pH is adjusted to 5.8. The volume is then adjusted to 500mL. Solidifying agent, gelrite in the amount of 3g was added and let melt in a microwave. Pour the culture medium into glass jars and autoclave it. After autoclave, the medium were sealed, and stored in the storage room until they are ready to be used.

Table 3.3: The Content Ratio Of kinetin(KIN) and gibberellin(GA_3)

		GA_3 (mg/l)			
		0	**0.1**	**0.2**	**0.3**
KIN (mg/l)	**0**	CONTROL	J1	J2	J3
	0.01	J4	J5	J6	J7
	0.02	J8	J9	J10	J11
	0.03	J12	J13	J14	J15

The concentration for 1 GA_3 will be 1 mg/l and 1 KIN will also be 1 mg/l. So, the concentration of each jar is as follows:

Control= 0 mg/l kinetin, 0mg/l gibberellin

J1 = 0 mg/l kinetin, 0.1 mg/l gibberellin

J2 = 0 mg/l kinetin, 0.2mg/l gibberellin

J3 = 0 mg/l kinetin, 0.3 mg/l gibberellin

J4 = 0.01 mg/l kinetin, 0 mg/l gibberellin

J5 = 0.01 mg/l kinetin, 0.1 mg/l gibberellin

J6 = 0.01 mg/l kinetin, 0.2 mg/l gibberellin

J7 = 0.01 mg/l kinetin, 0.3 mg/l gibberellin

J8= 0.02 mg/l kinetin, 0 mg/l gibberellin

J9 =0.02 mg/l kinetin, 0.1 mg/l gibberellin

J10=0.02 mg/l kinetin, 0.2 mg/l gibberellin

J11=0.02 mg/l kinetin, 0.3 mg/l gibberellin

J12=0.03 mg/l kinetin, 0 mg/l gibberellin

J13=0.03 mg/l kinetin, 0.1 mg/l gibberellin

J14=0.03 mg/l kinetin, 0.2 mg/l gibberellin

J15=0.03 mg/l kinetin, 0.3 mg/l gibberellin

3.5 In Vitro Culture

For *in vitro* initiation of cultures, the explants were dissected into nodal sections and placed straight up on solidified regeneration medium in the test tubes. The test tubes were placed in growth chamber with temperature of 25^{o}C, photopriod 16/8 h, and white light with intensity 25-30 µmol m^{-2} s^{-1}.

3.6 Data collection and analysis

After about 7 days, each test tube of survived explants was calculated. Each treatment has five replicates. They were examined according to these features which are number of shoots and roots. Other than that, the rate of contamination and the growth were observed and recorded. The data analysis was conducted using ANOVA and Duncan's multiple range test (DMRT) in SPSS software. The best surface sterilization method and the best combination of hormone were identified.

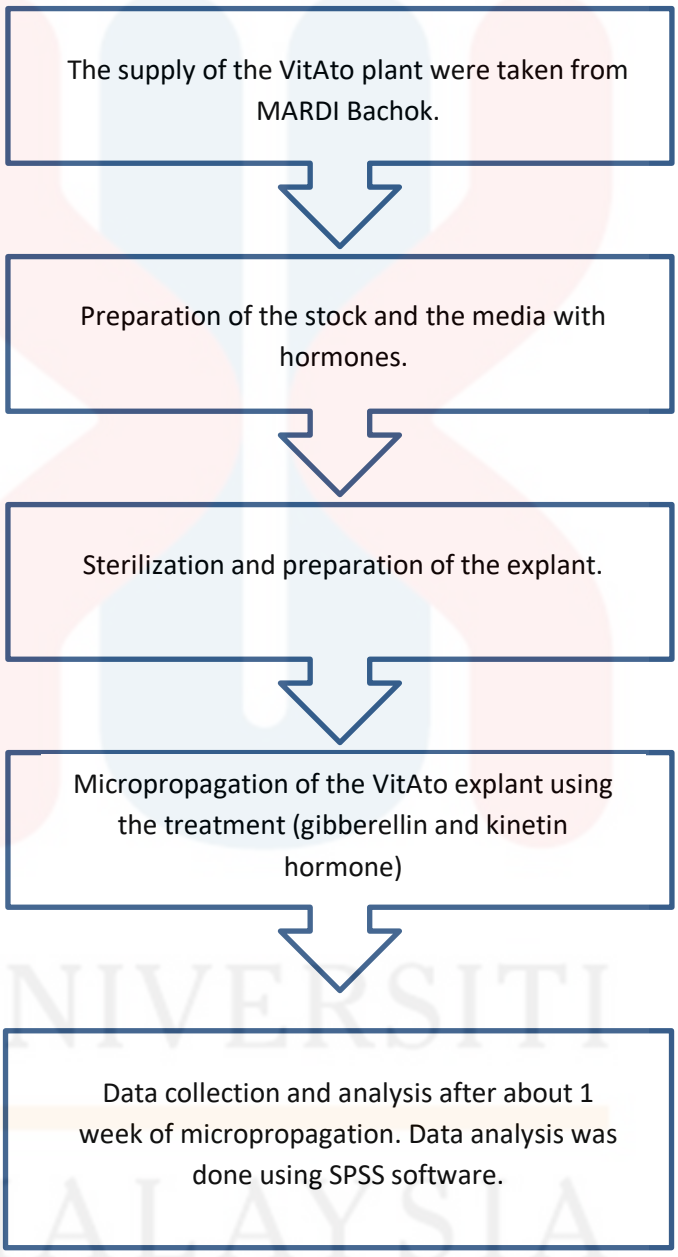

Figure 3.1: Flow chart of the research activities.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Surface Sterilization and the Percentage Of Survived Plant

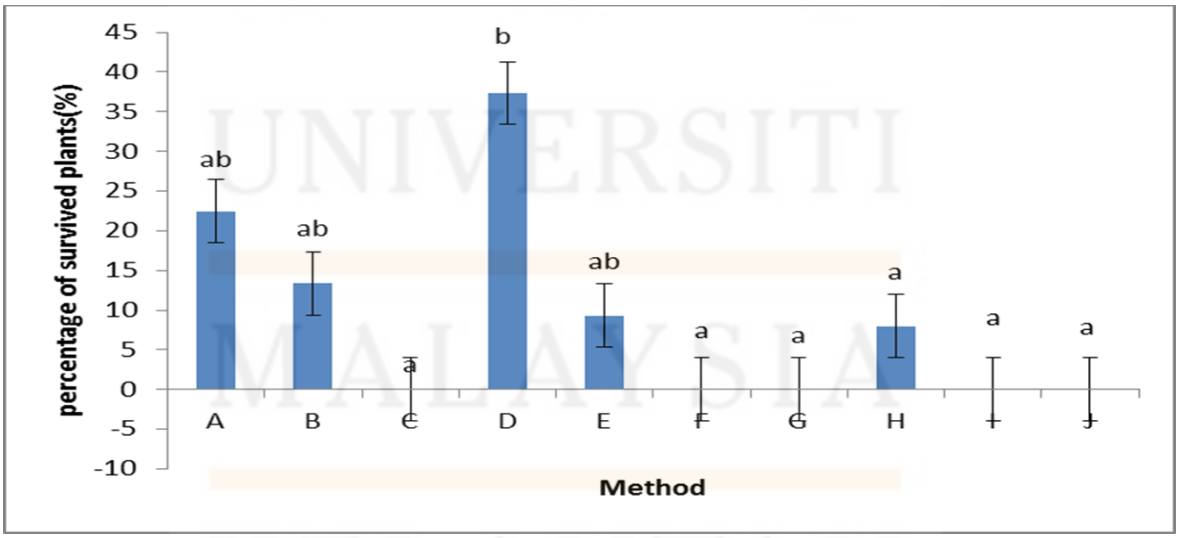

Figure 4.1 : Survival percentage of explants after different sterilization techniques.

Surface sterilizations were done with 10 different methods as in Table 3.2 to find the best method for the longest survival rate for the explants. The data of survived explants were taken until day 7 after every culture. The graph above shows the percentage of survived explants in different method of surface sterilization. Plant tissue culture is a biotechnological method and depends on different factors such as explant type, surface sterilizing agents, explant age, plant hormone concentration and combination to make this in vitro procedure a success (Murthy, 2019). For method A, 22.5% of the explants were survived. For method B, 13.33% of explants were survived, method D with 37.33% of survival, method E 9.33% and method H with 8 % of survival. For method C, F, G, I and J, there are no survived explants at all within 7 days. For method A, only 0.5% of sodium hypochlorite is used as the sterilizing agent. As the explants are from the soil, by using only sodium hypochloride may not be adequate to remove all the contaminants present on the explants. Commonly used surface sterilizing agents are ethanol, sodium hypochlorite, calcium hypochlorite, chlorine gas and mercury chloride used to sterilize the surface of different species of plant and seed material. Unfortunately, these agents frequently fail to remove all of the contaminants efficiently, especially when seeds are collected from the open field and stored in inadequate conditions (Barampuram, 2014).

The most widely used agents for the sterilization of plant material including seeds are ethanol, sodium hypochlorite, and calcium hypochlorite. Less commonly used agents like chlorine gas and hydrogen peroxide were successfully used to sterilize other seeds and plant material from Arabidopsis and soybean seed (Çavusoglu, 2010). In a study by Cavusoglu (2010), it showed that the use of a common surface sterilizer, such as bleach

(sodium hypochlorite), is not successful and leads to poor germination. Of the seeds available in their collection of cotton seeds, there were found to be the most contaminated with fungal and spores and had lower germination rates compared to other consents of seed. For the methods used afterwards, ethanol, fungicide (Mancozeb) and hydrogen peroxide were used. Previously, in the same study, hydrogen peroxide solution (3%) was used to sterilize seeds and improve Douglas-fir, wax currant, and barley germination (Çavusoglu, 2010). Higher hydrogen peroxide concentration (5 %) has been reported to have a negative effect on sunflower and rape seed germination (Modarressanavy, 2008).

That was what happened in method H, 10% of hydrogen peroxide was used and caused no growth of root and shoot at all and contamination occurred after days. But, in this study, explant nodes were used and a study from Baksh (2017) shows that In vitro crops with a combination of ethanol, hydrogen peroxide and Mancozeb, has less contamination and total regeneration response were found. This combination was used in method D along with Twen 20 which resulting to the highest percentage of survived explants. The percentage of crop contamination was low, showing this combination to be the most effective. The regenerated plants were healthy, fresh and medium-sized with robust growth. Mancozeb exhibits defensive action on plant surface contact it is a mixture of zineb and maneb, two dithiocarbamates (Bakhsh, 2017). Tween 20 is a widely applied detergent (surfactant) for immunohistochemistry buffers and reagents. The aim is to reduce background staining and improve the distribution of reagents in automated and manual procedures. On an automated staining method, it can also be used. Other than sterilizing methods, age of the explants also plays a big role in ensuring success of *in vitro* culture

(Prakash, 2010). According to the results above, the longer explants were stored, the higher chance of contamination occurred.

Based on the morphological characteristics observed, the fungus detected was *Aspergillus niger*. *Aspergillus* is a fungus genus consisting of approximately 300 species of mold (mold) recorded. *Aspergillus niger* is often one of the most common species found in fungal populations, suggesting that this species can easily spread in a wide range of environments. *Aspergillus niger* is mainly grown on dead plant material, consisting mainly of cell walls (Meijer, 2011). Their growth also depends largely on the availability of water. They reproduce asexually by developing conidium (fungi spore) spores. Once the spore land in a favorable environment (with humidity, heat and nutrients) they start to germinate where multiple hyphae will form the mycelium. The hyphae allows them to grow, spread and reproduce across the substrate surface (Ashour, 2014).

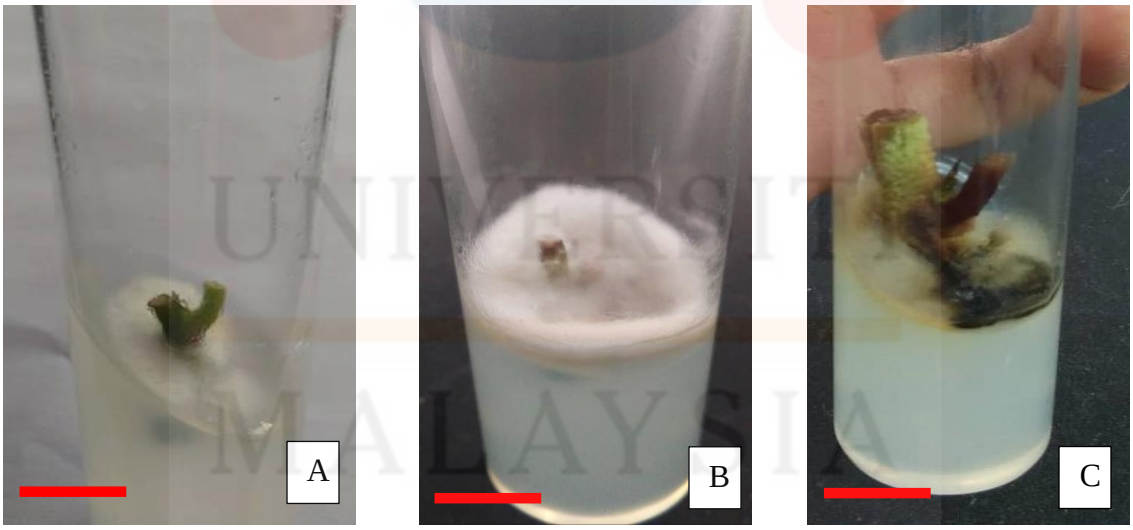

Figure 4.2 : The contamination occurred after 7 days of culture; (A) Contamination for day 8 to 10. (B) Contamination after 2 weeks. (C) Black mold appeared after more than 2 weeks. Bar = 1 cm

4.2 Effect of Combinations of Hormones (KIN and GA_3) on Number of Shoots

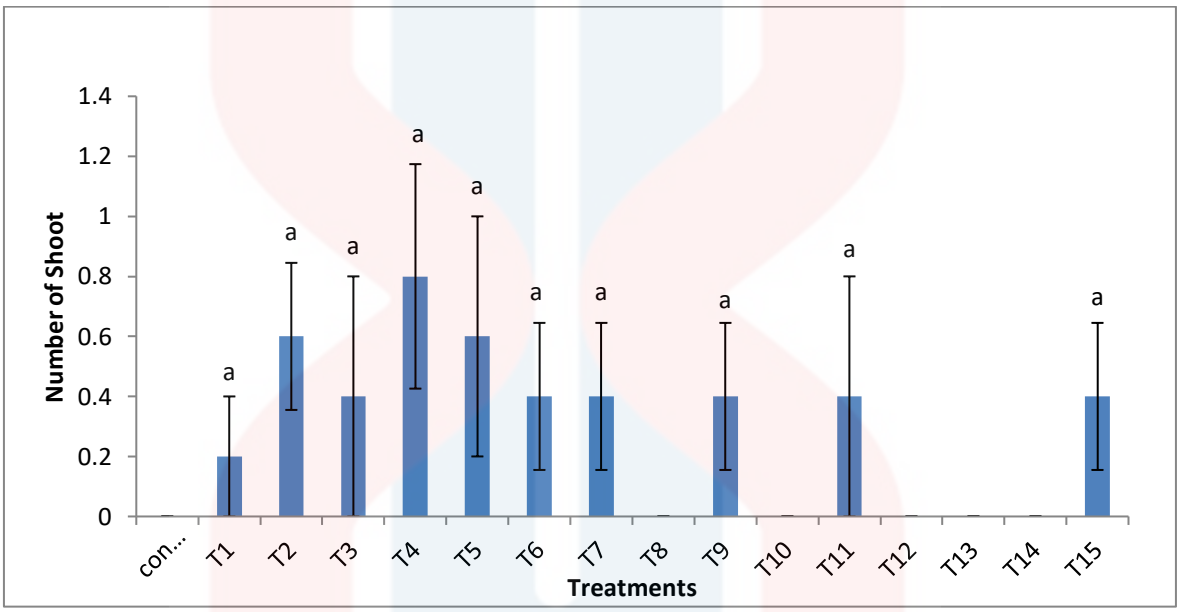

Figure 4.3 : Number of shoot in different treatments.

Figure 4.3 shows the data for number of shoot growth for every treatment and the mean value of 5 replicates per treatment. There are 5 replicates for each treatment. Control treatment has the mean value of 0, treatment 1 with $0.2^a \pm 0.2$, treatment 2 with $0.6^a \pm 0.245$, treatment 3 with $0.4^a \pm 0.4$, treatment 4 with $0.8^a \pm 0.374$, treatment 5 with $0.6^a \pm 0.4$, treatment 6 with $0.4^a \pm 0.245$, treatment 7 with $0.4^a \pm 0.245$, treatment 9 with $0.4^a \pm 0.245$, treatment 11 with $0.4^a \pm 0.4$ and lastly treatment 15 with $0.4^a \pm 0.245$. Treatment 8, 10, 12, 13 and 14 has no shoot growth at all. The graph above shows that treatment 4 has the highest rate of shoot growth in 7 days with mean of $0.8^a \pm 0.374$. Based on this study, the amount of KIN used in treatment 4 which was 0.01mg/l with no presence of GA_3 is the most ideal amount for shoot growth. But, there is no significant different between the treatments and number of shoot.

This is because the mean value in each column of the graph shows the same letter. There is no significant different between shoot and the treatment because the kinetin (KIN) hormone used in this study was in small amount compared to amount of gibberellin (GA_3).

Kinetin is widely used for shoot induction and rooting in tissue culture studies. The results show that there are differences in the effect of the different concentrations of KIN (Yarnia1, 2012). The best number of shoot is from treatment 4 as shown above by using 0.01 mg/l of KIN and 0 mg/l of GA_3. This is comparatively higher than the control rate. Data analysis revealed that KIN's effect on the number of shooting was important, but the combinations of KIN and GA_3 did not have any impact for the number of shooting since most of the treatment did not actively develop within 7 days. Cytokinins are generally known to facilitate bud formation in many organs grown in vitro. Like the results, other studies have shown that cytokinins have resulted in multiple shooting (Hesar, 2011). Ideal cytokinin concentrations differ from species to species and need to be accurately measured to achieve successful multiplication rates of shoot (Gomes, 2010). Some studies show that the addition of GA_3 can cause the reduction in number of shoot. But, using cytokinins alone like BAP and KIN only induced small amount of shoot growth (Pathak, 2009). A study has shown that MS media with combination of BAP (2.0mg/L), KIN (1.0 mg/L) and GA_3 (0.5 mg/L) was given best response of multiple shoots induction (Murthy, 2019). There were actually more shoots growth for the treatments. But, the data was disqualified as contamination occurred at the range of day 8 to 10.

4.3 Effect Combinations of Hormones (KIN and GA_3) on Number of Roots

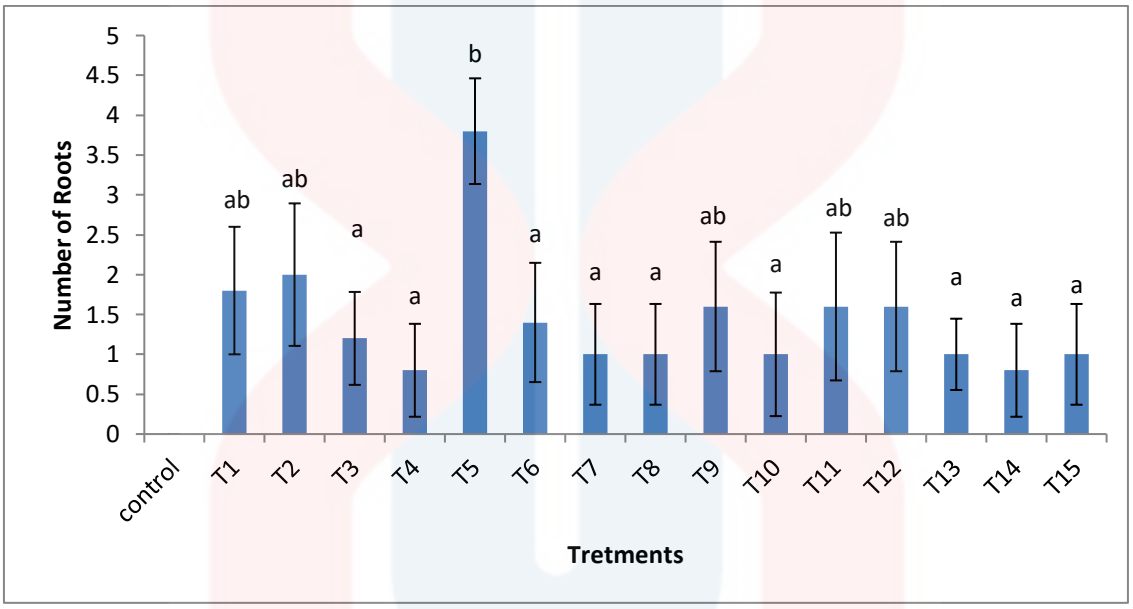

Figure 4.4 : Number of roots in different treatments.

For the growth of root in this study, Figure 4.4 shows the mean value for growth of root in 1 control and all 15 treatments. Control with 0 mean value, it means no growth of root at all in 7 days. Treatment 1 with $1.8^{ab}\pm0.8$, treatment 2 with $2^{ab}\pm0.894$, treatment 3 with $1.2^{a}\pm0.583$, treatment 4 with $0.8^{a}\pm0.583$, treatment 5 with $3.8^{b}\pm0.663$, treatment 6 with $1.4^{a}\pm0.748$, treatment 7 and 8 with $1^{a}\pm0.632$, treatment 9 with $1.6^{ab}\pm0.812$, treatment 10 with $1^{a}\pm0.775$, treatment 11 with $1.6^{ab}\pm0.927$, treatment 12 with $1.6^{ab}\pm0.812$, treatment 13 with $1^{a}\pm0.447$, treatment 14 with $0.8^{a}\pm0.583$, and lastly treatment 15 with $1^{a}\pm0.632$.The data shows that treatment 5 has the highest rate of root growth in 7 days with the mean of $3.8^{b}\pm0.633$. The lowest root growth rate is in treatment number 4 with mean value of

0.8^{a}±0.583. The graph shows there is significant different between the treatment and the growth of root.

There is no root growth for control and treatment 4 might because there is no application of GA_3 that enhance the rooting of the explant. There are studies that state that Kinetin caused decreasing root length (Yarnia1, 2012). But, in this study, the data shows that there was still growth of root even with presence of KIN. As the proof, there still were growth of root even in the presence of KIN in treatment 4 until 15.

The regeneration of roots on the medium containing 0.01mg/l KIN and 0.1mg/l GA_3 was larger than that of these regenerated on the medium with other amount of hormones (Olek, 2013).The plants grown on the cytokinin and gibberellin medium had more roots and longer stems than those regenerated on the medium that contain auxin alone (Olek, 2013). The application effect of gibberellic acid (GA_3) at very low concentrations could be beneficially utilized as its normal occurrence is known to regulate its production in plants in minute quantities. It is an established phytohormone used commercially to improve a number of crop plants ' productivity and quality (Qotob, 2019). A study shows that the plant hormone GA_3 is a major regulator of root growth in Arabidopsis. GA_3 has recently been reported to control root cell elongation. However, whether GA_3 also controls root cell production is currently unclear (Tomás, 2009). In the same study, they demonstrated how GA_3 is required after germination during root development to achieve and maintain root meristem size by regulating cell proliferation through degradation of DELLA proteins In addition, GA_3 was also proven to encourage root growth by increasing the expansion of endodermal cells in both meristematic and elongation areas, indirectly regulating the rates of division and expansion of other root tissues and root meristem length (Tomás, 2009).

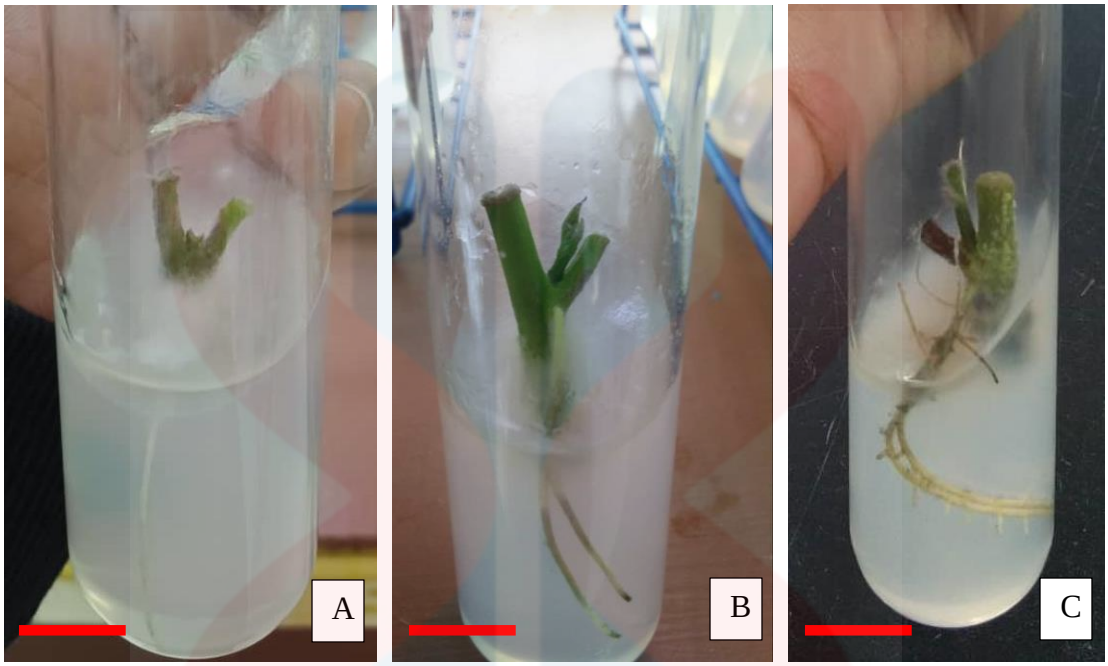

Figure 4.5 : Root and shoot growth of the explant; (A) Root growth in first 3 days. (B) Root and shoot growth in day 7. (C) Root and shoot growth after 7 days. Bar = 1 cm

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, the establishment in vitro culture of VitAto was done using the internodes. The study showed that *in vitro* cultivation can relatively easily propagate sweet potato from internodes. The best surface sterilization method for this study is method D that combines ethanol, hydrogen peroxide and fungicide The growth of root and shoot started at day 3 after cultured. The effect of combination of KIN and GA_3 hormones to the growth of the plants was investigated and treatment 5 with 0.01 mg/l KIN and 0.1 mg/l GA_3 was the best treatment for this study as it has the growth of shoots and roots. (Mancozeb) with

37.33% rate of survival. Contamination occurred at the range of 8 to 10 days and caused the data for the remaining days is disqualified. The stunting factor for this study is the rate of explants contamination.

5.2 Recommendation

The recommendation for better output in this study, first put the sample plants that will be used in a proper and conducive environment. Avoid the plants from mixing with the other sample plants. Next, spray suitable fungicide every week to the plants to reduce the chance of contamination. As for the media preparation, put some of the fungicide in the media (for this study, use Mancozeb) to reduce percentage of contamination and make sure to remove the excessive water in the media before culturing to avoid the growth of fungus. Benzyl Amino purine (BAP) can be added into the combination of hormones to induce more shoot growth. Then, always make sure that the lab is always in asthetic condition.

REFERENCES

Abbasi, Z. (2017). A Novel Aseptic Technique for Micropropagation of Aloe Vera mil. *Advanced Herbal Medicine*, 3(3), 47-60.

Akter, A., Ali, E., Islam, M. M. Z., Karim, R., & Razzaque, A. H. M. (2007). *Effect of GA3 on Growth and Yield of Mustard.* Mymensingh: Int. J. Sustain. Crop Prod.

Altaf Hussain & I. A. (2012, October 17). *Plant Tissue Culture: Current Status and Opportunities*. Retrieved November 20, 2019, from Intechopen: https://www.intechopen.com/books/recent-advances-in-plant-in-vitro-culture/plant-tissue-culture-current-status-and-opportunities

Ashour,A. (2014). *Control of Sexual Reproduction in Aspergillus species* . United Kingdom: University of Nottingham.

Badoni, A. C. (2010). In vitro Sterilization Protocol for Micropropagation of Solanum Tuberosum cv.'Kufri Himalini. *Academia Arena*, 2(4), 24-27.

Bakhsh, A., Hussain, T., & Caliskan, M. E (2017). A Promising and Cost Effective Surface Sterilizing Method for Sweet Potato (Ipomoea Batatas L.). *Fresenius Environmental Bulletin*, 3062-3067.

Barampuram, S., Allen, G., & Krasnyanski, S. (2014). Effect of Various Sterilization Procedures on the in Vitro Germination of Cotton Seeds. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 118(1), 179-185.

Bello, O. A., Esan, E. B., & Obembe, O. O (2018). Establishing Surface Sterilization Protocol for Nodal Culture of Solanecio biafrae. *Earth and Environmental Science*, 210(1),12007.

Çavusoglu, K., & Kabar, K .(2010). Effects of Hydrogen Peroxide on the Germination and Early Seedling Growth of Barley Under NaCl and High Temperature Stresses. . *EurAsian Journal of BioScience*, 4, 70-79.

Barciszewski, J., Siboska, G., Rattan, S. I., & Clark, B. F. (2000). Occurrence, biosynthesis and properties of kinetin (N6-furfuryladenine). *Plant Growth Regulation*, 257-259.

Gomes, F., Simões, M., Lopes, M. L., & Canhoto, J. M. (2010). Effect of Plant Growth Regulators and Genotype on the Micropropagation of Adult Trees of Arbutus unedo L.(strawberry tree). *New Biotechnology, 27*(6), 882-892.

Guha (2015, October 8). *Journal of Food Processing & Technology*. Retrieved November 10, 2019, from Health Benefits of Anthocyanins: https://www.longdom.org/proceedings/health-benefits-of-anthocyanins-30841.html

Hesar, A. A., Kaviani, B., Tarang, A., & Zanjani, S. B. (2011). Effect of Different Concentrations of Kinetin on Regeneration of ('Matthiola incana'). *Plant Omics*, 236.

Hua, Y. W., Huang, T. A., & Huang, H. S. (2010). Micropropagation of Self-rooting Juvenile Clones by Secondary Somatic Embryogenesis in Hevea Brasiliensis. . *Plant Breeding*, 202-207.

Matt, A., & Jehle, J. A. (2005). In vitro Plant Regeneration from Leaves and Internode Sections. *Cell Biology and Morphogenesis*, 468-475.

Kaur, S., Gupta, A. K., & Kaur, N. (2000). Effect of GA3, Kinetin and Indole Acetic Acid on Carbohydrate Metabolism. *Plant Growth Regulation*, 61-63.

Krikorian & A. D. (1995). Hormones in Tissue Culture and Micropropagation. In P. J. Davies, *Plant Hormones* (pp. 744-745). Ithaca,USA: Springer, Dordrecht.

Yarnia, M., & Tabrizi, E. F. M. (2012). Effect of Seed Priming with Different Concentration of GA3, IAA and Kinetin on Seedlin Growth. *Basic and Applied Scientific Research*, 2657-2660.

MARDI. (2013, April 8). *MARDI*. Retrieved March 12, 2019, from VitAto as The Substitute of Wheat Flour: https://blogmardi.wordpress.com/2013/04/08/vitato-ganti-gandum/#more-923

Meijer, M. H. (2011). Growth and Hydrolase Profiles Can be Used as Characteristics to Distinguish Aspergillus niger and other Black Aspergilli. *Studies in Mycology*, 69, 19-30.

Miller & C. O. (1961). Kinetin And Related Compounds. *Indiana University, Bloomington, Indiana*, 3-4.

Modarressanavy, Dolatabadian, A., & SANAVY, S. A. M. M& D. A. (2008). Effect of Ascorbic Acid, Pyridoxine and Hydrogen Peroxide Treatments on Germination, Catalase Activity, Protein and Malondialdehyde Content of Three Oil Seeds. *Notulae Botanicae Horti Agrobotanici ClujNapoca*, 36:61–66.

Murthy, M. K., Khandayataray, P., Samal, D., & Laha, R. (2019). Effects of Surface Sterilizing Agents, Sucrose and Plant Growth Regulatory Hormone Concentration Levels on Micropropagation of Bacopa monnieri L. *Asian Journal of Research in Botany*, 1-12.

Olek & Doliński, R (2013). Micropropagation of Sweet Potato(Ipomoea Batatas) from Nodes Plants. *Acta Scientiarum Polonorum*, 117-118.

Pathak, S., Lal, M., Tiwan, A. K., & Sharma, M. L. (2009). Effect of Growth Regulators on in vitro Multiplication and Rooting of Shoot Cultures in Sugarcane. . *Sugar Tech*, 86-88.

Phinney & West, C. A., B. O. (1955). Gibberellins As Native Plant Regulators. *University of California, Los Angeles*, 2-4.

Prakash, M. G., & Gurumurthi, K. (2010). Effects of type of Explant and Age, Plant Growth Regulators and Medium Strength on Somatic Embryogenesis and Plant Regeneration in Eucalyptus Camaldulensis. . *Plant Cell, Tissue and Organ Culture (PCTOC)*, 100(1), 13.

Qotob, M. A. (2019). Integrated Effect of Plant Growth Regulators with Boron Sources on Some Biological Parameters of Sugar beet. . *bioRxiv*, 1-12.

Rutala, W. A. (2013). Disinfection and Sterilization : overview. *American Journal of Infection Control*, 41(5), 2-5.

S.E. Aladele & A. O. (2012, October 17). *Intechopen*. Retrieved November 20, 2019, from The Science of Plant Tissue Culture as a Catalyst for Agricultural and Industrial Development in an Emerging Economy: https://www.intechopen.com/books/recent-advances-in-plant-in-vitro-culture/the-science-of-plant-tissue-culture-as-a-catalyst-for-agricultural-and-industrial-development-in-an-

Sabeetha, A. M. (2017). Physico-chemical characteristics of VitAto™. *Food Science Technology Research Centre, MARDI Headquarters*, 199-200.

,Sakakibara, H &. Osugi, A. (2015). How do plants respond to cytokinins. *BMC Biology*, 2.

Srivastava, N., Sharma, V., Dobriyal, A. K., Kamal, B., Gupta, S., Jadon, V. S., ... & Ameri, A. (2008). Standardization of Sterilization Protocol for Micropropagation of Aconitum heterophyllum-An Endangered Medicinal Herb . *Biotechnology*, 10(2), 1018-1022.

Tan, S.L. (2015). Sweetpotato(Ipomoea batatas)a great healthy food. *UTAR Agricultural Science*, 15-16.

Ubeda-Tomás, S., Federici, F., Casimiro, I., Beemster, G. T., Bhalerao, R., Swarup, R., ... & Bennett, M. J. (2009). Gibberellin Signaling in the Endodermis Controls Arabidopsis Root Meristem Size. *Current Biology*, 19(14), 1194-1199.

Yamaguchi, S. (2008). Gibberellin Metabolism and Its Regulation. *RIKEN Plant Science Center*, 225-227.

APPENDICES

Dilution equation

$$M_1V_1 = M_2V_2$$

M_1 = Concentration before dilution

M_2= Concentration after dilution

V_1= Volume before dilution

V_2= Volume after dilution

Table A1 : ANOVA Test

ANOVA

Shoots

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.188	15	.346	1.153	.331
Within Groups	19.200	64	.300		
Total	24.387	79			

ANOVA

Roots

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	39.680	14	2.834	1.113	.366
Within Groups	152.800	60	2.547		
Total	192.480	74			

Table A2 : Post Hoc Test

Shoots

Duncan

Treatment	N	Subset for alpha = 0.05
		1
0	5	.00
8	5	.00
10	5	.00
12	5	.00
13	5	.00
14	5	.00
1	5	.20
3	5	.40
6	5	.40
7	5	.40
9	5	.40
11	5	.40
15	5	.40
2	5	.60
5	5	.60
4	5	.80
Sig.		.062

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Roots

Duncan

Treatment	N	Subset for alpha = 0.05	
		1	2
4	5	.80	
14	5	.80	
7	5	1.00	
8	5	1.00	
10	5	1.00	
13	5	1.00	
15	5	1.00	
3	5	1.20	
6	5	1.40	
9	5	1.60	1.60
11	5	1.60	1.60
12	5	1.60	1.60
1	5	1.80	1.80
2	5	2.00	2.00
5	5		3.80
Sig.		.334	.059

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.