Micropropagation of purple yam (*Dioscorea alata L.*) from nodal and petiole explants using different types of plant growth regulators

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DECLARATION

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

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I certify that the report for final year project entitled "**Micropropagation of purple yam (*Dioscorea alata L.*) from nodal and petiole explants using different types of plant growth regulators** by **Nur Najihah Najwa Binti Mamat**, matric number **F15A0292** has been examined and corrected according to examiners for the degree of Applied Science (Agriculture Technology) With Honours, Faculty of Agro-Based Industry, University Malaysia Kelantan.

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Micropropagation of purple yam (*Dioscorea alata L.*) from nodal and petiole explants using different types of plant growth regulators

ABSTRACT

Yam is one of the tuber root-types that contain multifunctions and nutrients. Previous research quoted that purple yam has been commercially used as food colorant, but there is not much to extract that purple colour since we are lack of the purple yam production. Hence, tissue culture technology was manipulated to produce more purple yam in shortest time through multiple induction of shoots and roots. This was expected to be as the alternative source in gaining natural food colorant. Nodal and petiole were used as the source of explants to establish plant cell culture. Those explants were cultured on medium contained single cytokinin (BAP, TDZ, Kinetin) and combination of both Plant Growth Regulators (PGR) auxin and cytokinin (NAA+ BAP, NAA+ Kinetin, NAA+ TDZ, Kinetin+ IAA, NAA+IP, IAA+ IP). In this study, sterilization of purple yam explants using combination of Tween 20, 95% of ethanol and 10% chlorox were the most suitable surfactants. Moreover, the use of Thidiazuron (TDZ) and BAP hormones were functioned in inducing shoots meanwhile auxin hormone such as NAA actively promote roots rather than shoots. According to this research, the most suitable combination of auxin and cytokinin for inducing multiple shoots were MS + 0.5 mg/l KIN + 1.5 mg/l IAA meanwhile for in vitro rooting, the most suitable medium for cultured was on medium MS + 1.0 mg/l NAA + 0.5 mg/l TDZ. Therefore, these results proved that larger production of purple yam plantlet can be achieved by tissue culture technology.

Keywords: Medium, auxin, cytokinin, purple yam, nodal

Mikropropagasi ubi ungu (Dioscorea alata L.) daripada nodal dan petiole menggunakan berbagai jenis regulator pertumbuhan tumbuhan

ABSTRAK

Yam adalah salah satu jenis akar umbi yang mengandungi malfungsi dan nutrien. Kajian terdahulu mengatakan bahawa ubi ungu yang digunakan secara komersil sebagai pewarna makanan, tetapi tidak banyak untuk mengekstrak warna ungu itu kerana kita kekurangan pengeluaran yam ungu. Oleh itu, teknologi kultur tisu dimanipulasi untuk menghasilkan lebih banyak yam ungu dalam masa yang singkat melalui pelbagai induksi pucuk dan akar. Ini dijangka menjadi sumber alternatif dalam mendapatkan pewarna makanan semulajadi. Nodal dan petiole digunakan sebagai sumber penemuan untuk menubuhkan budaya sel tumbuhan. Penemuan ini dibiakkan pada sitokinin tunggal tunggal (BAP, TDZ, Kinetin) dan gabungan kedua-dua Pengawal Pertumbuhan tumbuhan (PGR) auxin dan sitokinin (NAA + BAP, NAA + Kinetin, NAA + TDZ, Kinetin + IAA, NAA + IP, IAA + IP). Dalam kajian ini, pensterilan eksplorasi yam ungu menggunakan kombinasi Tween 20, 95% etanol dan 10% chlorox adalah surfaktan yang paling sesuai. Selain itu, penggunaan hormon Thidiazuron (TDZ) dan BAP berfungsi lebih untuk merangsang pucuk manakala hormon auxin seperti NAA secara aktif mempromosikan akar dan bukan pucuk. Menurut kajian ini, gabungan yang paling sesuai untuk auksin dan sitokinin untuk menggerakkan pelbagai pucuk adalah MS + 0.5 mg / l KIN + 1.5 mg / l IAA manakala in vitro rooting yang paling sesuai untuk kultur pada MS + 1.0 mg / l NAA + 0.5 mg / l TDZ. Oleh itu, keputusan ini membuktikan bahawa pengeluaran tanaman ungu nip yang lebih besar dapat dicapai dengan teknologi kultur tisu.

Kata kunci: Medium, auxin, sitokinin, yam ungu, nodal

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LIST OF ABBREVIATIONS & SYMBOLS

IAA	Indole 3 acetic acid
NAA	Naphteleneacetic acid
TDZ	Thidiazuron
BAP	6-Benzylaminopurine
IP	Inositol trisphosphate
Mg/l	milligram per litre
PGR	Plant Growth Regulator
G	gram
Mm	millimetre

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Figure 1.1.1: Purple yam (*Dioscorea alata L.*) sp

Source: https://www.amazon.com/Filipino-Purple-Dioscorea-Potato-Rhizoma/dp/B06ZYLTSZJ

Yam are well known among Philippine cuisine because of their captivating colour. They have been used a lot in dessert because of their slightly sweet flavour and rich in texture. Besides, yam also been used for savoury dishes. Yam which high in carbohydrate content, starchy are being food base for over 300 million people around the world (Acevedo-Rodriguez & Strong, 2012).

The demand for yam has always exceeded its supply and expected to continue as world's population reaches 8 billion by the year 2025 (Osunde, 2008). Thus, yam become important in global food security strategy. Yam as the valuable source of carbohydrate, fibres, low level of fat and resembles as good dietary nutrient. But there are difficulties in propagation and to achieve high production of purple yam plant since purple yam is

limited by high cost of both labour and planting materials. More manpower required to make stakes as purple yam is the type of plant with climbing or trailing vines. Also seed of yam plants are tricky to store for reasonable time and bulky to transport (Andres, AdeOluwa, & Bhullar, 2017). Their export potential is realized to increase by years but since yam is the seasonal plants and only can be produced once a year, the production could not meet the demand of the export. Purple yam known to be tuber dormancy plant where as its tuber cannot be germinated immediately after harvest due to their dormancy. Its takes for about 3 to 4 months after harvesting period (Dhaliwal, 2017).

Although yam used to be staple food in some country all around the world, the demand for yam are constrained by inadequate production and losses in storage period. Yam are highly affected by disease such as nematodes, viruses and anthracnose besides environmentally affected by poor soil fertility. Hence in order to deal with those constrains, genetically improved by in vitro propagation are the most suggested since they are in controlled condition, resist to disease, and multiple production per culture. Micropropagation is the rapid vegetative propagation of plants under in vitro conditions in presence of light intensity, controlled temperature and a defined nutrient medium. In vitro technique most preferable because of their precise process in tissue culture where as in vitro meet demand by providing optimal level of mineral nutrients, environmental factors, vitamins and carbohydrate to achieve high regeneration rate. In vitro help most breeders by preserving high quality of planting material. Tissue culture techniques provide way to increase rapid production of virus free plant material.

Yam also known for their source of natural colorant that have based dyes which can be labelled as non-threatening vegetable juice. Anthocyanin, the pigment in yam that responsible giving those purple colour are excellent light and heat stability besides suitable for use in beverages, fruit bases, baking and confectionaries. Purple sweet potato

pigment are unique because they have tremendous colour stability with more intense colour and wider colour range from raspberry, red to grapelike purple (Barclay, 2013). They even contribute to health benefit since they are mildly anti-inflammatory and anti-carcinogenic. Apart from using artificial or synthetic colorants, natural colorants are the most health concern since they do not have harmful content and very reliable, excellent functionality, also biologically potential (Sezgin, 2018).

1.2 Problem Statement

Purple yam does have significant purposes all around the world. But there is less production of yam as they could not meet higher demand, thus this chance to cultivate purple yam by producing numerous qualitative plantlets using suitable explants and different concentration of plant growth regulators.

1.3 Objectives

1. To induce multiple shoots from nodal and petiole explants using different types of plant growth regulators.
2. To induce in vitro rooting of purple yam using different types of plant growth regulator.
3. To acclimatize purple yam plantlet to the tissue culture nursery.

1.4 Hypothesis

Different types of plant growth regulators have different effect towards the induction of shooting, rooting and acclimatization from nodal and petiole explants of purple yam.

1.5 Scope of Study

Shoot tips and petiole explants was used in micropropagation of purple yam as where meristematic cells are actively dividing hence leading rapid multiplication and differentiation. Those explant then cultured on Murashige and Skoog (MS) medium supplemented with different types and concentration of plant growth regulators. Then, cultured explants were kept in the growth room of tissue culture laboratory which supplied with constant light intensity and desired low temperature for explants to grow. Well-developed plantlets then transferred into tissue culture nursery for acclimatization.

1.6 Significance of Study

The present study enables the production of numerous yam platelets within short times by using shoot tips and petiole as explants. Establishment of tissue culture is important for the production of natural colorant from yam. Different type of plant regulator has different roles and may effect productivity of tissue culture for yam. Thus it is important that the different concentration and combination of plant regulator is studied to optimize the tissue culture of yam.

CHAPTER 2

LITERATURE REVIEW

2.1 Purple Yam

2.1.1 Taxonomy of purple yam

Domain:Eukaryote

Kingdom:Plantae

Phylum:Spermatophyta

Class:Monocotyledonae

Order:Dloscorealas

Family:Dioscoreaceae

Genus:Dioscorea

Species:Dioscorea alata

Figure 2.1.1: shows the taxonomy of purple yam plant.

Source: (Atherton & Rees, 2008)

2.2 Importance of yam

Yam was first cultivated earlier in Southeast Asia. The flesh colour of this yam usually bright lavender in colour. Yam was considered as economic importance as their use was worldwide. First thing first, purple yam has more benefits in medicinal use. Yam is kind of tuber crop which able to produce secondary metabolites of pharmaceutical

importance such as steroidal sapogenin, diterpenes and alkaloids. Studied before by Kansas State University showed that tubers contain lots of antioxidants that are not found in regular yams (Haider, 2015). A lot of antioxidants will benefit people from getting cardiovascular disease, strokes, even help prevent cancer and have the capacity to prevent DNA damage (Sezgin, 2018).

Yam have been used for centuries as alternative medicine. (Marengo, Katherin LDN, 2018) stated that yam able to help in treatment of menopause symptoms, rheumatoid arthritis, diabetes, and muscular cramps.

Also stated in (Haider, 2015), ube or purple yam can be boiled, fried, mashed, used in desserts such as cakes, cookies, flan, purple sticky rice, used in smoothies, used in crackers, made into ice cream and cakes, jams and spreads, used to make candy, and used in many culinary dishes to replace regular yams. Purple yam has been used in wide range of food such as in (Sutherlin, 2017) saying that from soft serve to pastries and cocktails, foods have been looking very, very violet showing that . The taste of purple yam with slightly sweet flavour and rich in texture making yam to well known among chefs and sweet tooth person. Their full of flavour also suitable to be consumed by the person who for one single dessert with varieties of taste. Purple yam also known for their benefits of colorant such as their stability, have wide range of colour as stated in (He, Li, Lv, & He, 2015) they displayed better stability as compared to the pigments of strawberries, red cabbage, perilla and other plants.

Cosmetic cream with anthocyanin extracts to provide additional protection against UV radiation, studied been done in (Chan, Lien, Lai, Huang, & Liao, n.d.) that the cosmetic cream with 0.61 mg of total anthocyanins (per 100 g cream) absorbed approximately 46% of the incident UV radiation and although the anthocyanins absorbed

both UV-A and UV-B radiation, they were particularly effective against UV-B rays. In addition, yam been used in the making of anti-aging skincare products as high content of hyaluronic acid will help to prevent wrinkles, keep the skin looking youth while the ability to keep skin and tissue moist and very effective treating dry skin and fine wrinkles. Hyaluronic acid could maintain the skin's elasticity and some studies have highlighted how it can even speed up healing times for wounds and scar tissue.

2.3 Anthocyanin

The word 'anthocyanin' is derived from two Greek words – anthos (flower) and kyanos (blue) (Kendrick, 2012). Anthocyanins are the pigments responsible for the red, purple, violet and blue colours seen in nature and are localised in the plant cell vacuole. Anthocyanin are the pigment that really pH dependent as their system will function according to pH surround. This pigments are most stable at low pH or in other words below pH 3.5 specifically. Typical applications for anthocyanins are soft drinks, confectionary and fruit preparations where an acidic pH exists as if they are into higher pH solution, they will form precipitate because the pigment only stable and favour at low pH. On top of that, recent studies have shown that purple yams contain a variety of acylated anthocyanins that exhibit higher levels of antioxidant activity than the corresponding non acylated compounds (Moriya et al., 2015). Anthocyanin will benefit consumers because of their abundant functions from solely pigment.

2.4 In vitro propagation

2.4.1 Growth media

Medium contains inorganic salt and organic compound such as plant growth regulator, vitamins, carbohydrate, hexitols and gelling agent. Amino acids, antibiotics may also include as the nutrient content of the medium. Vitamins may enhance cellular response like nicotine acid and pyrodixine. Vitamin stock are best stored in fridge and practically add vitamin stock solution to the medium before autoclaving. Sucrose or glucose was used in protoplast culture because green cell in culture are not photosynthetically active and require this carbohydrate source. Hexitol myo-inositol also vital for tissue culture in seed germination, sugar transport, mineral nutrition and more. Myo-inositol act as growth enhancer in vitro and provide carbohydrate source. Type of gelling agent will affect the response of explants towards media. Agar is the most medium used in tissue culture technology and they vary in brand like agar, agarose, phytagel, and gelrite. The use of agar is because of their stability characteristics, high clarity, non-toxic nature and resistance to its metabolism. Firm surface formation of medium will prevent the explant from sinking and may promote the morphogenic response in agar. Agar should always clean from impurities as contaminated medium will distract the growth of cultured explant.

Moreover, plant growth regulators (PGR) are vital in order to encourage plant cell development in tissue culture technology. Selection of suitable PGR in the tissue culture medium would help the explants to grow according to our experiment purposes. They will function differently depending to their types and concentration of plant growth regulator may vary according to cell culture purposes. An auxin is most required for plant cell to divide and root initiation. At high concentration, auxin can suppress

morphogenesis (Thimann, 1939). IAA, IBA and NAA are used for root induction while cytokinin will help promote cell division, shoot proliferation and shoot morphogenesis. It is observed that cytokinin required in optimal quantity for shoot proliferation in many genotypes but addition of low concentration of auxins along with cytokinins triggered the shoot proliferation. These both two PGR are the most used in tissue culture technology because of their availability and reliability.

2.5 Explant selection

Explants are small pieces of plant parts or tissues that are aseptically cut and used to initiate a culture in a nutrient medium. Explants can be taken from different parts of a plant such as shoots, leaves, stems, flowers, roots, and from many types of mature cells provided they are able to de-differentiate into totipotent cells (Thakur, 2018). Explant usually taken at the most dividing meristematic cell on part of the plant for instance shoot tips and leaf petiole. Part of the plant that contain actively dividing cell could work efficient through uptake of the nutrient from the media and elongate faster in growth room. Choosing explants most depend on the kind of culture to be done as if it is to observe clonal propagation, the explant will be lateral or terminal shoot or bud, and if for callus induction, pieces of cotyledons, hypocotyl, stem, leaf, or embryo are preferred. While if it is for protoplast isolation, leaf tissue from aseptically germinated seed is recommended (Tayeb, 2013). Besides, explant size also will influence the culture growth like if it is too small, the harder for the explants to be put on the medium. Thus, chosen suitable explant will enhance the performance of tissue culture growth and development.

Figure 2.4.1: Purple yam explant taken from the nodal

Source: https://picclick.com/UBI-PURPLE-YAM-PLANT-Edible-TUBER-Dioscorea-Alata-361313710666.html

2.6 Sterilization of purple yam

Plant that grow outside exposed to the environment are prompt to easily affected by diseases and pests. These contaminants mainly remain at the outer surface of the plant, then those surface microbes will easily remove from the explant by rinsing, but remaining contaminants will be killed by surface sterilization. The use of 95% alcohol in surface sterilization prior to treatment with combination of another disinfectant such as Tween 20 will assure that all the germs are killed. Since concentration of the alcohol used are higher, the time taken to immerse all those explants should less as if not, the plants cell will be destroyed before they be cultured on medium. Purple yam explants also washed with commercial bleach for about 10 minutes and rinsed thoroughly with distilled water before cultured on medium.

2.7 Micropropagation of Balbacious plant species

Recent studies on Potatoes (*Solanum tuberosum*) was reported in (Liljana, Mitrev, Fidanka, & Mite, 2012), and was conducted to study the effect of cytokinin and combination of cytokinin and auxinusing in vitro microtuber formation and the growth of two different potato cultivars, *Solanum tuberosum L*. In this study, sprout and nodal explants of potato cultivars Agrija and Andrea, were cultured on MS medium contained with different hormonal combinations. Rapid sprouting clean potato tubers were treated with 2 ppm GA_3. Between these two explants, sprout and nodal, nodal showed better microtuber formation. The Agrija cultivar showed greater ability for in vitro propagation, with 2.14 tubers per shoot and 13.33% microtuber formation.

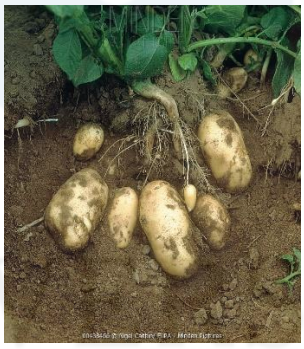


Figure 2.6.1: *Solanum tuberosum L sp.*

Source: https://www.mindenpictures.com/search/preview/potato-solanum-tuberosum-group-mature-variety-called-charlotte-england/0_00438466.html

While for sweet potatoes *(Ipomoea batatas* L.*)*, the studies been carried out to estimate the potential for larger production of well-rooted sweet potato, uniformity or in other words in vitro culturing. This study using two different cultivars which is 'Carmen Rubin' and 'White Triumph'. The selection of explants was the node of both cultivars

and were placed on growth media containing basic components of MS medium with 1 mg dm-3 gibberellin and 0.1 mg dm-3 kinetin, while the second one with 0.5 mg dm-3 IAA. After 9 weeks, 4 plants were produced from each primary explants and the properties planted depend on the cultivar itself where their weight of the explant and composition of the medium will influence those plants produced. The results recorded where the average weight of 'Carmen Rubin' plant much higher compared to the 'White Triumph' cultivar. Plus, 'Carmen Rubin' showed to produce longer shoots and induce more roots systems. The sweet potato able to acclimatize quickly based on the good appearance they showed.

Figure 2.6.2: *Ipomoea batatas* L. *sp.*

Source: https://vtechworks.lib.vt.edu/bitstream/handle/10919/74367/Sweet%20Potato%20Poster.pdf?sequence=1

Reported in previous studies (Abd-Alla, Ragab, El-Miniawy, & Taha, 2013) that cassava or (*Manihot esculenta*) also been cultured using stem nodes and treated with different concentrations of chlorox at different times (10, 20 and 30 % chlorox for 5, 10 and 15 min). 20% Clorox for 5 minutes show the lowest contamination occurred and the highest significant survival percentage. Nodal explants were cultured on MS medium supplemented with different concentrations of BA and Kin 0.1, 0.3, 0.5 and 1.0 mg L-1

from each one respectively incombination with 0.05 mg L-1 of NAA. 5.67 shoots performed on MS + 1.0 mg L-1 BA + 0.05 mg L-1 NAA. While roots formation experimented on different concentrations of NAA and IBA with 0, 0.5, 1.0, 2.0 and 4.0 mg L-1 respectively. MS medium supplemented with 2.0 mg L-1 IBA achieved highest number of roots with 10.2 formedand the root length with 14.4 cm. In vitro derived plantlets were successfully acclimatized on a mixture of peat moss and sand (1: 1) which gave the highest percentage of survival transplanting (100%).

Figure 26.3: Cassava or (*Manihot esculenta*) sp.

Source: http://ethnoherbalcure.blogspot.com/2016/08/medicinal-benefits-of-cassava-manihot.html

Arrowroot *(Maranta arundinacea L.; Marantaceae)* is another tuber roots plants that recently studied and cultured from rhizomes. The induction of shoot cultures from rhizome buds on semi solid medium supplemented with 3 mg.l-1 6-benzylaminopurine in the dark, which was also the best medium for shoot proliferation (Teixeira, Daquinta, Brown, Teixeira, & Sagarra, 2015). Also in this study managed to acclimatize shoots in zeolite and sugarcane filter substrate (1:1) with a 90% survival percentage.

Figure 2.6.4: Arrowroot *(Maranta arundinacea L.; Marantaceae) sp.*

Source: https://www.shutterstock.com/search/arrow+root

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and Reagents

Leaf petiole and shoot tips will be used as explant of purple yam. Murashige and Skoog (MS) medium chosen as the main medium for purple yam cultivation.10% of sodium hypochlorite, Tween20, sterile distilled water, and 70% and 95% of ethanol were used for explant sterilization.

3.1.2 Equipment

Autoclave machine, laminar flow, microwave oven, electronic balance, pH meter, glass petri dishes, Bunsen burner, pipette, beakers, containers, measuring cylinder, scalpel, forceps, blade, conical flask, centrifuge tube,15ml tube, hot plate stirrer and glassware used for preparing the media until culturing process take place.

3.2 Methods

3.2.1 Preparation of stock solutions

Table below shows the ingredients and the amount of substance to produce macronutrients, micronutrients, vitamin and ferum stocks and stored in refrigerator.

Table 3.2.1.1: Preparation of macronutrient

Macronutrient stock	g/L	20X(g/L)
NH_4NO_3	1.65	(1.65×20)=33g
KNO_3	1.9	(1.9×20)=38g
$CaCl_2.2H_2O$	0.44	(0.44×20)=7.4g
$MgSO_4.7H_2O$	0.37	(0.37×20)=7.4g
KH_2PO_4	0.17	(0.17×20)=3.4g

Table 3.2.1.2: Preparation of micronutrient

Micronutrients	g/L	200X(g/L)
$MnSO_4.4H_2O$	0.0223	(0.0223×200)=4.46g
$ZnSO_4.7H_2O$	0.0086	(0.0086×200)=17.2.g
H_3BO_3	0.0062	(0.0062×200)=1.4g
KI	0.00083	(0.00083×200)=0.166g
$NaMoO_4.2H_2O$	0.00025	(0.00025×200)=0.05g
$CuSO_4.5H_2O$	0.000025	(0.000025×200)=0.005g
$CoCL_2.6H_2O$	0.000025	(.000025×200)=0.005g

Table 3.2.1.3: Preparation of vitamins

Vitamin	g/L	100X(g/L)
Myo-inosol	0.1	(0.1×100)=10g
Glycine	0.002	(0.002×100)=0.2g
Thiamine-HCL	0.001	(0.001×100)=0.1g
Nicotine acid	0.0005	(0.0005×100)=0.05g
Pyridoxine-HCL	0.0005	(0.0005×100)=0.05g

Table 3.2.1.4: Preparation of ferums

Ferum	g/L	200X(g/L)
$FeSO_4.7H_2O$	0.0278	(0.0278×200)=5.56g
$NaEDTA.2H_2O$	0.0373	(0.0373×200)=7.46g

3.2.2 Preparation of PGR Treatments

Hormone stock will be prepared by dissolving 10mg of hormone into 100ml of distilled water and stored in fridge. Auxin hormone were prepared at concentration of 1.5mg/l. While mixture of auxin and cytokinin were prepared at concentration of 0.5mg/l of auxin and 0.1mg/l,0.3mg/l and0.5mg/l of cytokinin respectively.

Table 3.2.2.1 List combination of auxin and cytokinin

Treatments	Concentrations
NAA+BAP	1.0 mg/l+0.5 mg/l
NAA+TDZ	1.0 mg/l+ 0.5 mg/l
KIN+IAA	0.5 mg/l+ 1.5 mg/l
NAA+IP	1.0 mg/l+ 0.5mg/l
IAA+IP	1.5mg/l + 0.5mg/l

Table 3.2.2.2: List cytokinin hormones used

Treatments	Concentrations
BAP	0.5 mg/l
TDZ	0.5 mg/l
KIN	0.5 mg/l

3.2.3 Media Preparation

Firstly,200 ml distilled water will be added into 1L and 50 ml of macronutrient stock,5ml of micronutrient stock,5ml of ferum stock and 10 ml of vitamin stock will be added and stirred by using magnetic stirrer. Plant growth regulator were added into the stock solution with particular concentration and making sure to all dissolved.

Then, 30g of sucrose was dissolved in the beaker. The pH value of each beaker will be adjusted to 5.8 and NaOH will be added to increase the pH value and followed by agar to be put into each flask before distilled water was added. The solution then be placed in the oven until agar entire dissolved. The solution was poured into petri dish and

autoclaved for 15 minutes. The media preparation method is summarised as in Figure 3.2.3.1

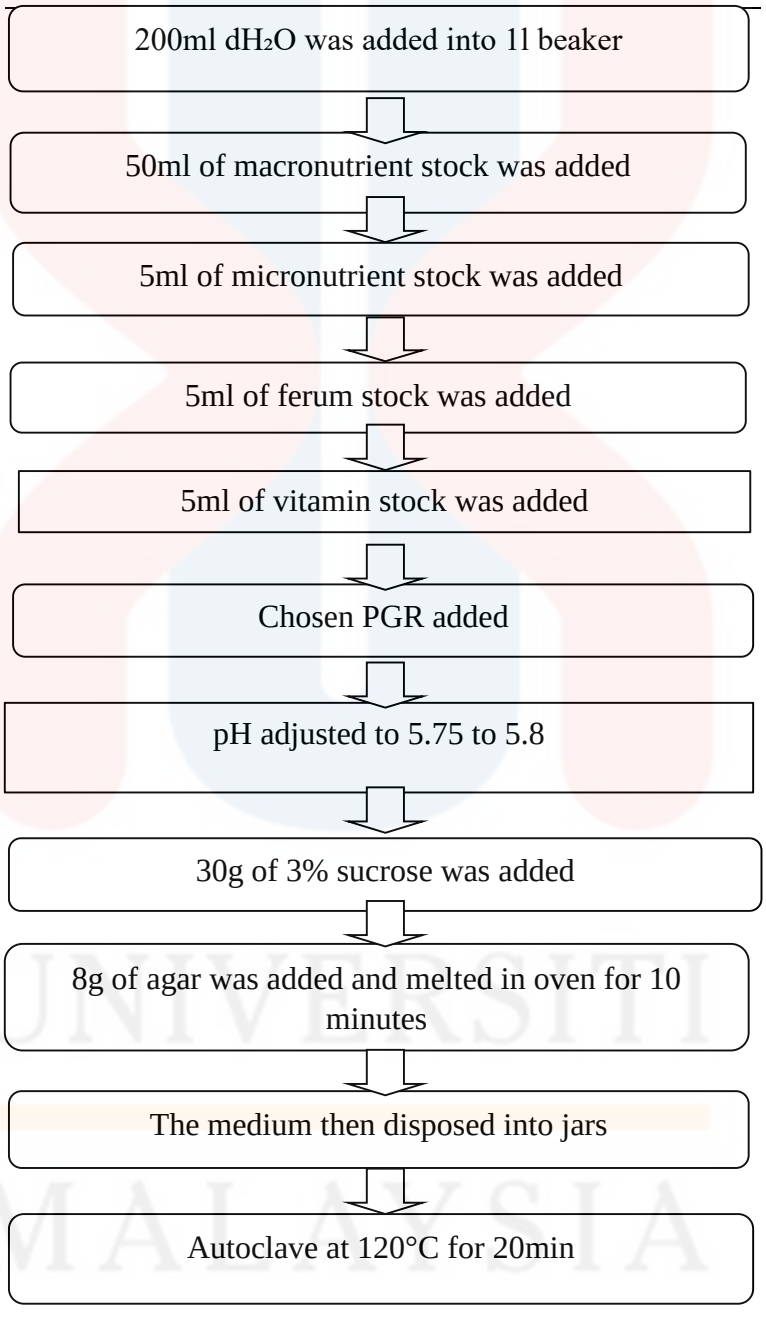


Figure 3.2.3.1.: Flow chart of media preparation

3.2.4 Collection of Explants

Nodal from purple yam was cut for about 2cm long and the young part of the plant was used as it has soft stem as if at this part cell may actively divide when put on growth media and induce shoots and roots in shorter time.

3.2.5 Explants Sterilization

All explants to be used are put in beakers with cotton gauze and placed under running tap water for 20 minutes before replace with detergent and later rinsed with distilled water. The explant will be sterilized with three drops of Tween-20 and left for 5 minutes. Then those explant were transferred into lamina flow chamber which already been set up and sterilized with 95% ethanol for 3 minutes. The explant will be sterilized with 5% of sodium hypochlorite for 5 minutes. Then the explants were rinsed off for 2 to 3 times with distilled water. The explant sterilization is summarised as in Figure 3.2.5.1.

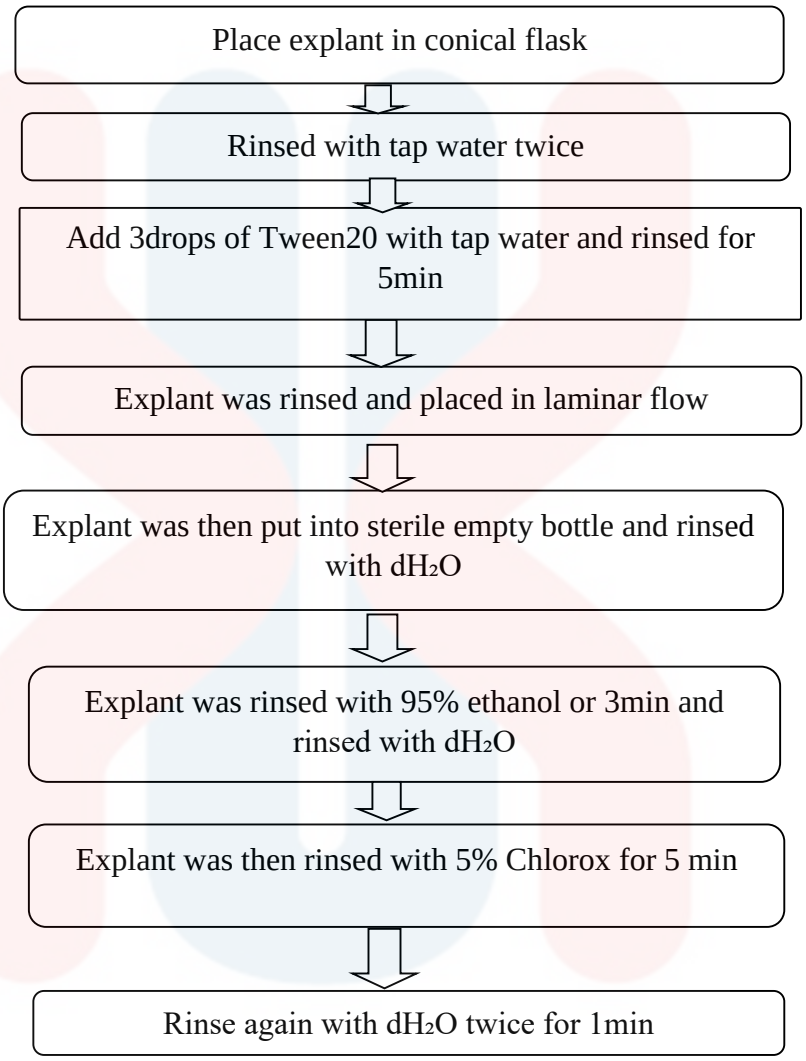


Figure 3.2.5.1 Flow chart of explants sterilization

3.2.6 Acclimatization

Plantlets with well-developed roots and shoots systems were washed with tap water to remove remaining media to avoid mould growth. They were then transplanted into mixture of soil and peat moss in the ratio 2:1 and dispensed in to polybags. Those polybags containing plantlets were kept in tissue culture nursery for a week. The number of surviving plantlets was recorded.

3.2.7 Experimental Design

Establishment of explants sterilization for purple yam was analysed using tables of collected data and formed into bar chart while the rate survival of purple yam plantlet during acclimatization after one week was analysed using pie chart in percentage readings.

3.2.8 Data analysis

A completely randomized design with eight treatments and four replications was used during initiation and multiplication experiments. Analysis of variance (ANOVA) with Dunken Multiple Range Test (DMRT) was carried to compare the number of hoots and roots growth formed in the MS medium.

CHAPTER 4

RESULTS AND DISCUSSION

Throughout this research, we are discussing on the production of edible colorant from nodal and petiole explants of purple yam using different types of plant growth regulators. But there are factors that will effect researcher to achieve their results such as parts of the plant taken as explant, method of sterilization, types and concentration of plant growth regulators used. The dependent variables in this study is the types of plant growth regulator used while the shoot and root growth act as independent variables. In order to obtain objectives of the study, surroundings should be in controlled condition for instance the presence of light should continuously provide for the culture to grow and temperature are set up that suit to the culture.

Nodal part of the purple yam act as explants and dissected from the mother plant. Each test tube contains single explants and half embedded into the media whereas each treatment were replicated four times. There were eight types of treatments including combination and single type of plant growth regulator as stated in the table below. There were four major parameters that were discussed in this chapter First is the rate survival of cultured explants while parameter number two is the number of shoots and roots produced

respectively. Lastly, the survival acclimatization of purple yam plantlets into tissue culture nursery after one week. Based on all these parameters, the best types of plant growth regulators were used for inducing multiple shoots and roots growth of purple yam were determined.

4.1: Rate survival of cultured purple yam explants.

Table 4.1.1 shows the data survival of cultured explants after one month treatments and transferred into bar chart as shows in the Figure 4.1.2 which indicates the percentage of surviving explants after 30 days of treatments. According to the figure, culture on treatments 3 and 4 with combination of 2 treatments are most achieved with 100% survivor especially culture on MS + 1.0 mg/l NAA + 0.5 mg/l TDZ and MS + 0.5 mg/l KIN + 1.5 mg/l IAA. While 88% survivor goes to culture on MS + 1.0 mg/l NAA + 0.5 mg/l BAP and the rest both on single hormone MS + 0.5 mg/l BAP and 0.5 mg/l TDZ.

Table 4.1.1: Data survival of cultured explants after one month treatments.

Treatments	Number of cultured tubes	Number of contaminated tubes	Percentage of survival (%)
MS + 1.0 mg/l NAA + 0.5 mg/l BAP	25	3	25-3=22 22/25×100=88
MS + 1.0 mg/l NAA + 0.5 mg/l TDZ	21	0	100

MS + 0.5 mg/l KIN + 1.5 mg/l IAA	6	0	100
MS + 1.0 mg/l NAA+ 0.5 mg/l IP	12	12	0
MS + 1.5 mg/l IAA + 0.5 mg/l IP	12	12	0
MS + 0.5 mg/l BAP	42	5	42-5=37 37/42×100=88
MS + 0.5 mg/l TDZ	18	16	18-16=2 2/18×100=11
MS+ 0.5 mg/l KIN	20	20	0

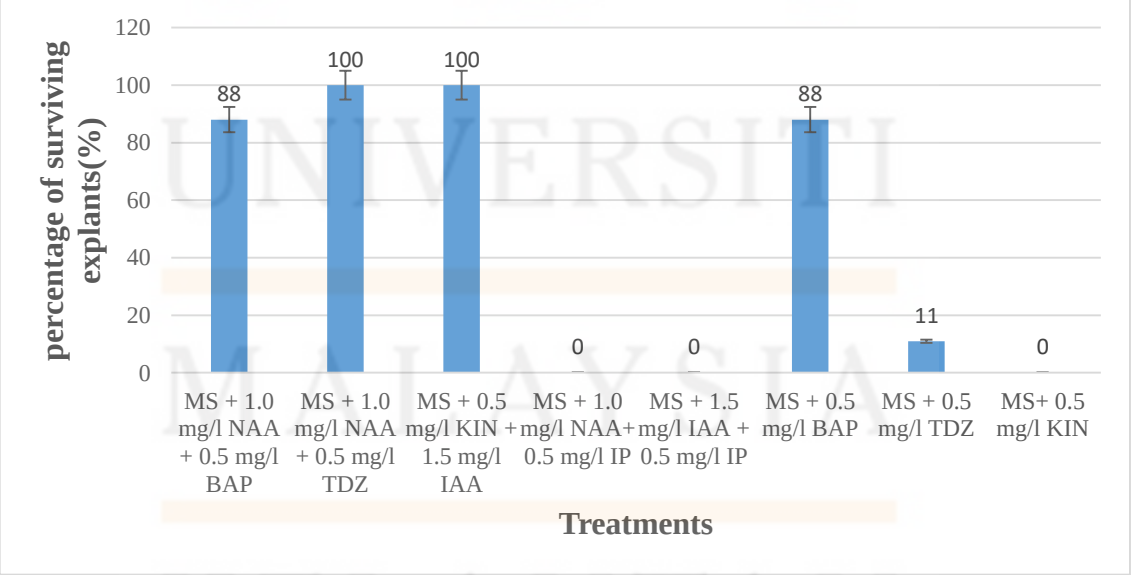


Figure 4.1.2: Percentage of explants survived after one month of treatments

Sterilization comes first when doing tissue culture to disinfect all contaminants like fungi, bacteria and viruses. Sterilization agents are varieties for example sodium hypochlorite, ethanol, calcium hypochlorite, hydrogen peroxide and Tween20. The time taken for those explants exposed to the surfactants and with higher concentration also matter like if immerse for too long, the explants will turn out brownish as the death of tissue cell in the explants occur during the sterilization process. In this research, surfactants used are sodium hypochlorite, Tween20 and ethanol. Commercial bleach with 5% concentration for five minutes, three drops of Tween20 also for five minutes, and 95% ethanol for three minutes.

Tween20 are widely used in tissue culture sterilization as it helps to wash collected explants and able to decrease the percentage of contamination caused by microbial besides ethanol are powerful surfactants depending to their concentration (Misra, 2012). Sodium hypochlorite commercially sold as bleach and also differ their effectiveness according to the concentration. Explants are soaked in surfactants for 3 to 5 minutes according to concentration and types of explant. Importantly, explants must be rinsed using sterile distilled water multiple times to ensure all surfactants washed off.

4.2 Number of shoots.

Figure 4.3 shows the effect of higher concentration in auxin hormones function inducing multiple shoots on the purple yam explants. Figure 4.3(a) shows the development of 1 shoot with branches on MS + 0.5 mg/l KIN + 1.5 mg/l IAA at 4th weeks from the starting culture. While figure 4.3(b) shows the induction of 2 shoots with 1 leaf each on MS + 1.0 mg/l NAA + 0.5 mg/l BAP. At the 2nd weeks of culture, 1 shoot with

wide leaf observed on MS + 1.0 mg/l NAA + 0.5 mg/l TDZ as shown in the figure 4.3(c) besides figure 4.3(d) proves the development of 1 shoot on MS + 0.5 mg/l IP + 1.5 mg/l IAA at 3rd weeks.

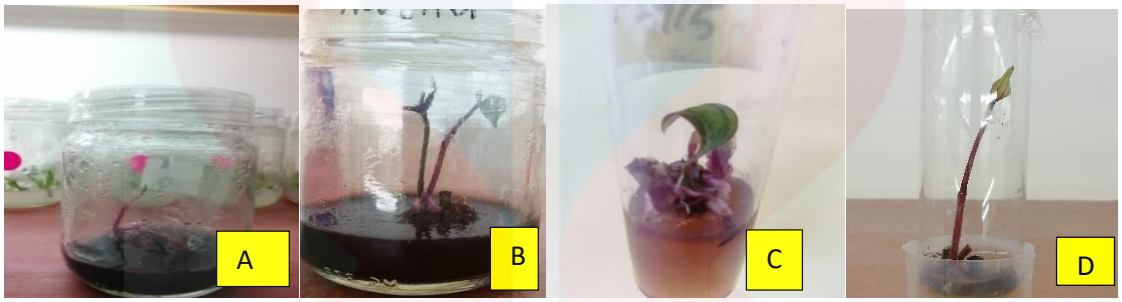


Figure 4.2.1: Effect of different types of PGR on shoots of yam culture (a) Purple yam shoot growth after 4th weeks of culture from nodal explants on MS + 0.5 mg/l KIN + 1.5 mg/l IAA (b) Purple yam shoots growth after 6th weeks of culture from nodal explants on MS + 1.0 mg/l NAA + 0.5 mg/l BAP (c) Purple yam shoot growth after 2nd weeks of culture from nodal explants on MS + 1.0 mg/l NAA + 0.5 mg/l TDZ (d) Purple yam shoot growth after 3rd weeks of culture from nodal explants on MS + 0.5 mg/l IP + 1.5 mg/l IAA.

4.3 Number of roots.

Figure 4.4 shows the effect of higher concentration in cytokinin hormones that promote multiple roots on the purple yam explants. Figure 4.4(a) shows the slightly development of 1 shoot and 4.4(b) are the closer look on the growth of 3 tiny roots on MS + 0.5 mg/l TDZ at 3rd weeks. While figure 4.4(c) and 4.4(d) shows the vigorous growth of purple yam roots on MS + 0.5 mg/l BAP at 5th weeks

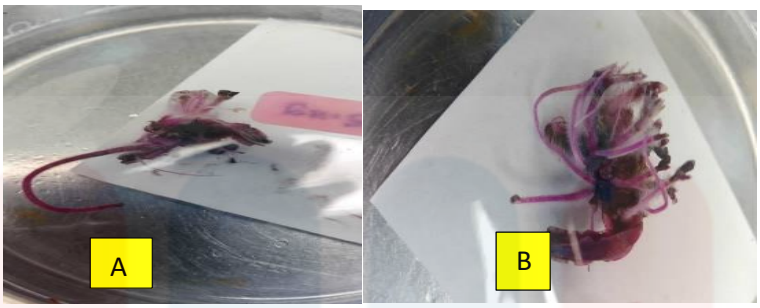


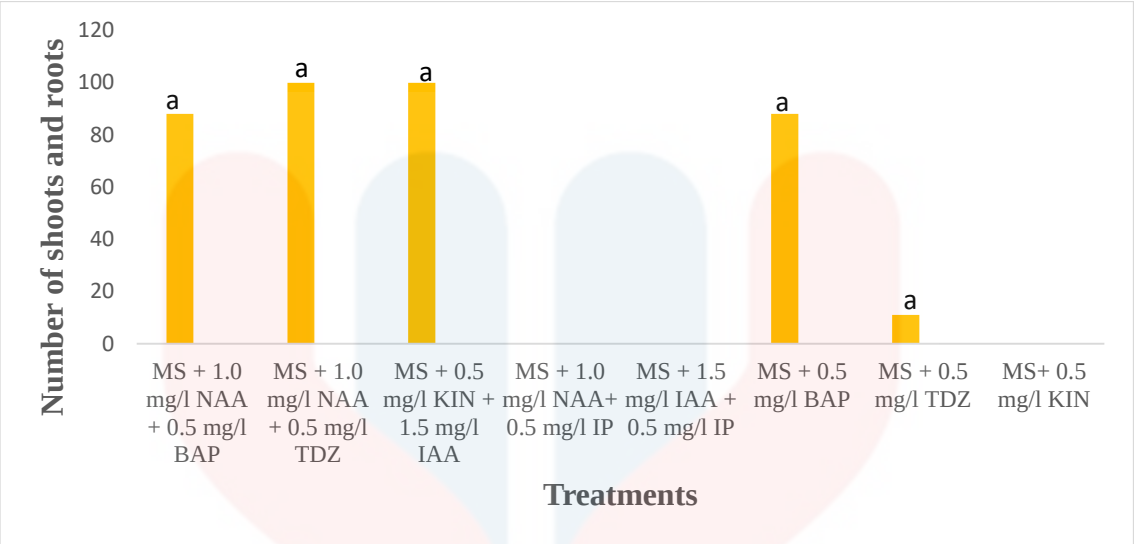
Figure 4.3.1: Effect of different types of PGR on roots of yam culture (a) Purple yam shoot and root growth on 3rd weeks of culture from nodal explants on MS + 0.5 mg/l TDZ (b) Purple yam shoot and root growth after 5th weeks of culture from nodal explants on MS + 0.5 mg/l BAP.

4.4 Effect of different types of PGR on the number of shoots and roots of yam culture

The data on the number of shoots and roots obtained were analysed as per Table 4.4.1 and the significant differences (≤0.05) were noticed between the eight media treatments in the number of shoots and roots induced of purple yam. The highest number of shoots produced was on MS + 1.0 mg/l NAA + 0.5 mg/l BAP medium followed by MS + 0.5 mg/l KIN + 1.5 mg/l IAA medium (Figure 4.3). MS + 1.5 mg/l IAA + 0.5 mg/l IP medium has the lowest number of shoots induced probably because of the lack of auxin in shoot. While in Figure 4.4.2 was the data shown in form of the bar chart according to previous table (Table 4.4.1).

Table 4.4.1: Effect of different types of PGR on the number of shoots and roots of yam culture

Treatments	Number of shoots	Number of roots
MS + 1.0 mg/l NAA + 0.5 mg/l BAP	0.75±0.47	1.00±1.00
MS + 1.0 mg/l NAA + 0.5 mg/l TDZ	0.25±0.25	0.75±0.75
MS + 0.5 mg/l KIN + 1.5 mg/l IAA	0.50±0.28	0.00±0.00
MS + 1.0 mg/l NAA+ 0.5 mg/l IP	0.25±0.25	0.00±0.00
MS + 1.5 mg/l IAA + 0.5 mg/l IP	0.00±0.00	0.00±0.00
MS + 0.5 mg/l BAP	0.00±0.00	5.00±2.50
MS + 0.5 mg/l TDZ	0.00±0.00	1.00±0.50
MS+ 0.5 mg/l KIN	0.00±0.00	0.00±0.00



***The simlar letter indicates that the results are not significantly different.

Figure 4.4.2: Effect of different types of PGR on the number of shoots and roots of yam culture.

4.5 Survival of acclimatized plantlets.

The data on the number of polybags containing purple yam explant survived throughout acclimatization stage for a week were observed and tabulated as in Table 4.5.1 according to their treatments in order to calculate their rate of explants survived (%).

Table 4.5.1: Data survival of plantlets after one week of acclimatization in tissue culture nursery.

Treatments	Acclimatized plantlet	Survived plantlet after 1 week	Rate of explants survived (%)
MS + 1.0 mg/l NAA + 0.5 mg/l BAP	1	0	0
MS + 1.0 mg/l NAA + 0.5 mg/l TDZ	1	0	0
MS + 0.5 mg/l KIN + 1.5 mg/l IAA	1	0	0
MS + 0.5 mg/l BAP	2	1	1/2×100=50
MS + 0.5 mg/l TDZ	1	0	0

The survival rates of the regenerated plants were found to be high if the root systems of the plantlets transplanted were stable and strong. As shown in the Figure 4.5.2, after a week about 50% of the regenerated plantlets survived and grew vigorously. For increasing the percentage of survival of these regenerated plants, they can be maintained longer in the polybags if the roots of the plantlets are stable and bring significant improvement in the hardening process and the survival rate was found to be less. The result of the plantlets on MS + 0.5 mg/l BAP showed high rate of survival (50%) of the regenerated plantlets which grew vigorously within a week.

It can be concluded that MS medium with 0.5 mg/l BAP was found to be the best for the growth of shoot elongation and rooting in purple yam.

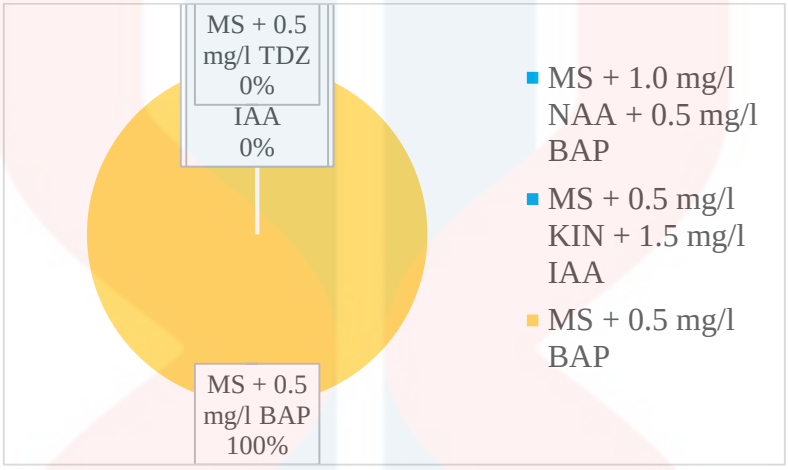


Figure 4.5.2: Rate of explants survived after one week of acclimatization in tissue culture nursery.

Figure 4.5.3 shows the effect of stable rooting of purple yam culture. Figure 4.7(a) shows the prior acclimatization of purple yam plantlet with 1 shoot emerged and 1 branch with 1 leaf each on MS + 0.5 mg/l BAP. While figure 4.7(b) shows the survived plantlet with greener leaf colour, strong stem on MS + 0.5 mg/l BAP at 6^{th} days of acclimatization in tissue culture nursery from nodal explant.

Figure 4.5.3: Effect of stable rooting of purple yam culture (a) Purple yam plantlet after 1 month of in vitro propagation from nodal explant on MS + 0.5 mg/l BAP (b) Purple yam plantlet after 6^{th} days of acclimatization in tissue culture nursery from nodal explant on MS + 0.5 mg/l BAP.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

From this study, proven that sterilization of purple yam explants using Tween20, ethanol and commercial bleach is the most preferable as surfactants to sterilize yam explants. Auxin hormone known to propagate shoots actively, while cytokinin will induce roots. Combination of auxin and cytokinin hormones rely to their concentration itself where as high concentration of auxin will promote shoots and high concentration cytokinin will trigger root elongation. Therefore, the use of combination hormones MS + 1.0 mg/l NAA + 0.5 mg/l BAP are suitable to induce multiple shoots while MS + 0.5 mg/l BAP are preferred for induction of roots in purple yam culture. After all, higher concentration of both auxin and cytokinin will induce shoots and root growth in tissue culture explants. Acclimatization of purple yam result best when the roots of the culture explants are strong enough and stable before planted in the tissue culture nursery. It is because external environment can be vulnerable to young plantlets as they are not used to the harsh environment.

5.2 Recommendations

Tissue culture workplace should be very sterile and always in controlled condition. Low hygiene of tissue culture laboratories will contribute to massive contaminations to all the cultures. Cultured explants will easily be infected to the microbial, viruses brought to the laboratories besides the surrounding of the explant growth area should always remain in controlled condition for instance light always turn on, suitable temperature.

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APPENDICES

Appendix 1: The ANOVA carried on the number of shoots and roots of purple yam.

		Sum of Squares	df	Mean Square	F	Sig.
No_Of_Shoot	Between Groups	2.219	7	.317	1.449	.232
	Within Groups	5.250	24	.219		
	Total	7.469	31			
No_Of_Root	Between Groups	20.969	7	2.996	.743	.638
	Within Groups	96.750	24	4.031		
	Total	117.719	31			

Appendix 2: The test of homogeneity of variances.

	Levene Statistic	df1	df2	Sig.
No_Of_Shoot	9.306	7	24	.000
No_Of_Root	6.688	7	24	.000

Appendix 3: The Tukey HSD[a] and Duncan[a] subset for alpha of number of shoots.

No_Of_Shoot			
	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	1.5 mg/L IAA + 0.5 mg/L IP	4	.0000
	0.5 mg/L BAP	4	.0000
	0.5 mg/L TDZ	4	.0000
	0.5 mg/L KN	4	.0000
	1.0 mg/L NAA + 0.5 mg/L TDZ	4	.2500
	1.0 mg/L NAA + 0.5 mg/L IP	4	.2500
	0.5 mg/L Kn + 1.5 mg/L IAA	4	.5000
	1.0 mg/L NAA + 0.5 mg/L BAP	4	.7500
	Sig.		.350
Duncan[a]	1.5 mg/L IAA + 0.5 mg/L IP	4	.0000
	0.5 mg/L BAP	4	.0000
	0.5 mg/L TDZ	4	.0000
	0.5 mg/L KN	4	.0000
	1.0 mg/L NAA + 0.5 mg/L TDZ	4	.2500
	1.0 mg/L NAA + 0.5 mg/L IP	4	.2500
	0.5 mg/L Kn + 1.5 mg/L IAA	4	.5000
	1.0 mg/L NAA + 0.5 mg/L BAP	4	.7500
	Sig.		.060

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Appendix 4: The Tukey HSD[a] and Duncan[a] subset for alpha of number of roots

No_Of_Root

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	0.5 mg/L Kn + 1.5 mg/L IAA	4	.0000
	1.0 mg/L NAA + 0.5 mg/L IP	4	.0000
	1.5 mg/L IAA + 0.5 mg/L IP	4	.0000
	0.5 mg/L KN	4	.0000
	0.5 mg/L TDZ	4	.5000
	1.0 mg/L NAA + 0.5 mg/L TDZ	4	.7500
	1.0 mg/L NAA + 0.5 mg/L BAP	4	1.0000
	0.5 mg/L BAP	4	2.5000
	Sig.		.650
Duncan[a]	0.5 mg/L Kn + 1.5 mg/L IAA	4	.0000
	1.0 mg/L NAA + 0.5 mg/L IP	4	.0000
	1.5 mg/L IAA + 0.5 mg/L IP	4	.0000
	0.5 mg/L KN	4	.0000
	0.5 mg/L TDZ	4	.5000
	1.0 mg/L NAA + 0.5 mg/L TDZ	4	.7500
	1.0 mg/L NAA + 0.5 mg/L BAP	4	1.0000
	0.5 mg/L BAP	4	2.5000
	Sig.		.139

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

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