EFFECT OF PLANT GROWTH REGULATORS ON MICROPROPAGATION OF SABA BANANA

(*Musa balbisiana* cv. Saba)

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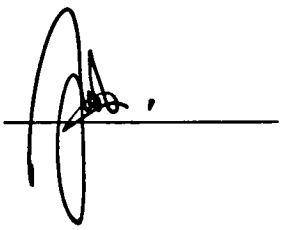

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ABSTRACT

A laboratory based experiment was conducted in Plant Tissue Culture laboratory at Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan Campus to study the effect of plant growth regulators on micropropagation of Saba banana (*Musa balbisiana* cv. Saba). The objective of this study was to determine the effect of different concentrations of plant growth regulators on micropropagation of Saba banana. The experimental design used was Completely Randomized Design using Murashige and skoog media, there were seven treatments which were MS 0 (control), MS media supplemented with different types and concentrations of plant growth regulators which were 2.5 mg/L BAP, 5.0 mg/L BAP, 7.5 mg/L BAP, 2.5 mg/L BAP with 0.5 mg/L NAA, 5.0 mg/L BAP with 0.5 mg/L NAA, 7.5 mg/L BAP with 0.5 mg/L NAA respectively, each treatment had seven replicates. Data were recorded twice a week for four months. Results were analysed using Statistical Analysis System (SAS) version 9.4. All the collected data were analyzed using the One Way Analysis of Variance (ANOVA) with the significant level at 0.05. Least Significant Difference was used to determine the significant difference between the treatments in this study. Results obtained from this study showed that MS media supplemented with 5.0 mg/L performed better than other treatments, however the growth response of Saba banana is rather slow, phenolization of explant caused necrosis on explant.

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ABSTRAK

Satu kajian makmal telah dijalankan di Makmal Tisu Kultur, Fakulti Pertanian Lestari, Universiti Malaysia Sabah, kampus Sandakan untuk mengkaji kesan pengawal atur pertumbuhan terhadap pisang Saba (*Musa balbisiana* cv. Saba) melalui mikropropagasi. Objektif kajian ini adalah untuk menentu kesan-kesan pengawal atur pertumbuhan terhadap pisang Saba melalui mikropropagasi. Rekabentuk eksperimen yang diguna ialah rekebentuk rawak lengkap dengan menggunakan Murashige and skoog media, tujuh jenis rawatan digunakan dalam kajian ini, iaitu MS 0 (terkawal), MS media yang ditambah 2.5 mg/L BAP, 5.0 mg/L BAP, 7.5 mg/L BAP, 2.5 mg/L BAP dan 0.5 mg/L NAA, 5.0 mg/L BAP dan 0.5 mg/L NAA, 7.5 mg/L BAP dan 0.5 mg/L NAA masing-masing. Setiap rawatan mempunyai tujuh replikasi. Data dicatat dua kali setiap minggu selama empat bulan. Data dianalisis dengan 'Statistical Analysis System' (SAS) versi 9.4. Data yang dikumpul dianalisis menggunakan ANOVA sehala dengan tahap signifikan pada 0.05. 'Least Significant Difference' digunakan untuk menentu perbezaan signifikan antara rawatan dalam kajian ini. Keputusan yang diperolehi daripada kajian ini menunjukkan bahawa MS media yang ditambah dengan 5.0 mg/L BAP mempunyai pertumbuhan yang lebih baik berbanding dengan rawatan yang lain, walau bagaimanapun pertumbuhan Pisang Saba mengambil masa yang lama, *phenolization* menyebabkan nekrosis pada eksplan.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percent
±	Plus/minus
°	Degree
ANOVA	Analysis of Variance
BA	Benzyladenine
BAP	6-benzylaminopurine
BBB	BBB genome
C	Celcius
cm	Centimetre
cv.	Cultivar
FAO	Food and Agriculture Organization
FNCA	Forum for Nuclear Coorporation in Asia
g	Gram
HCl	Hydrochloride
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
kg	kilogram
L	Litre
m	Metre
m^2s^{-1}	Metre Square per Second
mg	Miligram
mL	Mililitre
mm	Milimetre
MS	Murashige and skoog
NAA	1-Naphthaleneacetic acid
NaOH	Sodium hydroxide
SAS	Statistical Analysis System
T	Treatment
µmol	Micromole

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CHAPTER 1

INTRODUCTION

1.1 Background

Banana is an edible fruit crop which belongs to Musacea family. It is a great source of potassium and vitamins such as A, B6, C and D (Sampath Kumar *et al.*, 2012). Banana ranked fourth in global gross value of production, right behind rice, wheat and maize, it is considered as an important staple food crop in many countries (Singh *et al.*, 2011). It is grown in more than 130 tropical and subtropical countries. Global gross banana exports held a record of 16.5 million tonnes in 2012, it helps to improve income security for smallholder farmers through local and international trade (FAO, 2014A). Banana occupies an important position in the agricultural economics of Australia, Malaysia, Taiwan, Sri Lanka and South China (Al-Amin *et al.*, 2009). The fruit taste, seediness, color, size and other characteristics of banana depend on the species and variety (Sampath Kumar *et al.*, 2012). The cultivars of banana varies depending on the genome which can be diploids, triploids or tetraploids.

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Among the many cultivars grown, Saba banana is one of the leaders in terms of production and trade. Saba banana is of BBB genome, it is a pure *Musa balbisiana* clones which have been identified in Southeast Asia. Saba banana is also known as 'Pisang Kepok' in Indonesia, 'Pisang Nipah' in Malaysia and 'Kluai Hin' in Thailand. It is a cooking banana with medium to large fruits. The shape of Saba banana fruit is highly angular, the pulp is creamy white in colour, the flesh becomes sweet on ripening. (Robinson and Galan Sauco, 2010).

According to FAO (2014B), production of banana is attacked by serious disease known as Fusarium wilt or Panama disease in the past years. This disease is caused by *Fusarium oxysporum* f.sp *cubense.* The fungus grows in soil and invades banana

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through fine roots, spores are produced in the xylem and blocked the water flow which result in wilting of banana plants. This fungus can remain in soil for a long period of time. It has been stated that this disease originated from Southeast Asia but was first reported in Australia in 1876 and recent outbreak of this disease is reported in Jordan and Mozambique.

Micropropagation is a widely accepted application of plant tissue culture technique in which cell from a single plant is cloned under enviromentally controlled area. Plants are totipotent, which means its cells have the ability to undergo cell division and regenerate into a whole plant when culture into a right condition. Because of this characteristic of plants, it has great potential to be multiplied using plant tissue culture techniques. Cultivation of banana through micropropagation techniques has raised attention because it has the ability to produce genetically uniform planting stocks rapidly (Lohidas and Sujin, 2015).

Plant growth regulators are produced naturally in plants and greatly affect the growth development of plants. However, plant cultures normally do not manufacture enough amount of plant growth regulators to sustain its growth development. Therefore, plant growth regulators are normally added to media culture to promote the growth of cultures. Auxins, cytokinins, gibberellins and abscisic acids are the four classes of plant growth regulators most often used in plant tissue culture. Different type of plant growth regulators have its specific function. The type and concentration of plant growth regulators supply to media varies according to the need of cell culture (Kumar and Reddy, 2011).

1.2 Justification

This study was conducted to investigate the effect of plant growth regulators on micropropagation of Saba banana. Saba banana was selected for this study due to its increasing demand from Peninsula Malaysia, some of the main importers are Kuala Lumpur and Johor. Generally, banana cannot be propagated through seed as the seeds are sterile. Hence, propagation of banana has to be done in alternative methods. Sucker and rhizome propagation are most commonly practiced. However, the attack of Fusarium wilt or otherwise known as Panama disease has greatly reduced the

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availability of high quality, healthy disease free planting materials. These issues deserves to be given more attention from agriculturists.

It is important to come up with a solution to increase disease free planting materials of banana in order to meet the demand of consumer. Therefore, micropropagation plays an important role in this situation. Application of micropropagation techniques on banana has been a major focus, several studies are ongoing to use different type, concentration and combination of Plant growth regulators, mainly auxins and cytokinins, to promote the growth of banana through plant tissue culture in order to improve yield production of banana. However, there are limited research on the BBB genome banana. Through this study, more information related to micropropagation of Saba banana can be greatly increased.

1.3 Objective

The objective of this research was to determine the effect of plant growth regulators on micropropagation of Saba banana.

1.4 Hypothesis

Hypothesis tested in this research is as follow:

H_0 : There is no significant difference on the effect of plant growth regulators on micropropagation of Saba banana.

H_a : There is significant difference on the effect of plant growth regulators on micropropagation of Saba banana.

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CHAPTER 2

LITERATURE REVIEW

2.1 Classification of Banana

Banana belongs to the order of Zingiberales, the family of Musaceae (Madhava Rao, 2010) and to the *Musa* genus (Robinson and Galan Sauce, 2010). The numbers of species and cultivars of *Musa* comprises are constantly changing since many new species have been identified recently (Argent, 2010). Wild banana species are diploids, cultivated bananas are originally from two major species which are *Musa acuminata* and *Musa balbisiana*; *Musa acuminata* is A genome donor while *Musa balbisiana* is B genome donor (Pillay and Tenkouano, 2011). The fruits of these wild species banana are barely edible due to its small amount of edible pulp and contain numerous large seeds. The modern edible bananas grown today are hybridization of two *Musa acuminata* or two *Musa balbisiana* or between *Musa acuminata* and *Musa balbisiana* (Simmonds, 1966). Hybridization has resulted in different genome group and ploidy level in bananas. Therefore, the composition of genomes in *Musa* plays an important role in its classification and it has been internationally accepted that all banana cultivars should be referred by following a code denoting the genome group and ploidy level, followed by subgroup name and popular name of cultivars (Robinson and Sauce, 2010; Pillay *et al.*, 2012). Generally, banana is divided into two categories, dessert banana and cooking banana.

2.2 Botanical Description of Banana

Banana is monocotyledonous and herbaceous plant, it has no woody component. The life cycle is perennial and the height of banana plant varies on species, it ranges from 1.5 m to 9 m (Robinson and Galan Sauce, 2010).

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The root system of banana plant is fleshy and adventitious.They are responsible for the water and mineral uptake of plant, the potential water and mineral uptake of plant depends on the number of primary roots produced and the vigour of root extension through soil. Primary roots normally originate in groups of two to four from the surface of central cylinder within the rhizome. Primary roots are about 5-8 mm in diameter. The roots are white in colour when they are new and healthy and will turn into grey or brown in colour before it dies off (Robinson and Galan Sauce, 2010). A healthy rhizome has the ability to produce 200 to 500 primary roots (Draye *et al.*, 2005). Secondary and tertiary roots develop from primary roots, they are thinner and shorter compared to primary roots. Secondary roots originate from protoxylem near the root tip of primary root, they continue to produce as the primary roots extends through the soil. The same applies to tertiary roots emerging from secondary roots (Robinson and Galan Sauce, 2010).

Rhizome is an important storage organ that plays a key role in sustaining the growth of bunch and developing suckers, it has short internodes and covers by closely packed leaf scars externally. Inside rhizome, it is differentiated into the central cylinder and cortex. Mature rhizome is about 300 mm in diameter. Rhizome should remain underground completely. Exposed rhizome will affect the yield potential as the plant becomes unstable due to the drying of primary roots (Robinson and Galan Sauce, 2010).

Suckers are lateral shoots that emerge from buds. Morphologically, there are two types of sucker which are sword sucker and water sucker. Sword suckers have narrow leaves base and broad rhizome, they depend strongly on the mother plant for development. Water suckers have broad leaves and narrow rhizome, unlike sword suckers, they have weak connection with the mother plant and hence they produce broad leaves to compensate the lack of parental support (Robinson and Galan Sauce, 2010). Continous emergence of new suckers perpetuates the plant's life, once the mother plant dies down, sucker will regenerate the plant, this gives banana plant its perennial status. Growth of suckers is affected by the maturity stage of parent plant and the field operation such as pruning or manure application (Pillay and Tenkouano, 2011).

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Pseudostem is made of leaf sheaths overlapping and tightly rolled on one another, forming a firm cylindrical structure. Pseudostem contains 95% of water and it is very fleshy. It can support a bunch heavier than 50 kg and plays an important role in providing mechanical support and vascular connection between all parts of the plant. (Pillay and Tenkouano, 2011; Robinson and Galan Sauce, 2010).

Leaf disposition on plant enables them to receive maximum amount of light for photosynthesis. The lamina, which also known as leaf blade, develops as rolled cylinder during its passage through pseudostem and it unrolls itself as the leaf sheath grows, it has stomata on top and bottom surface. The formation of lamina is completed when the leaf sheath on both side narrow to form petiole. Lamina expands gradually with a pronounced supporting midrib to form canopy. Most banana varieties produce 30-40 leaves in a cycle. The light green banana leaves have smooth and glossy texture. The final leaf, which is known as shot blade, is smaller compare to the rest and it curves to protect the developing infloresence from rain and sun. The lamina turns yellow in colour, hangs down against the pseudostem and eventually dies (Madhava Rao, 2010; Pillay and Tenkouano, 2011; Robinson and Sauce, 2010).

Inflorescence is a complex spike with a stout peduncle which flowers are arranged in nodal cluster on transverse cushions, subtended by large spathe-like bracts that are almost ovate and normally in purple-red colour (purseglove, 1972). At a certain stage of plant development, the apical growing point in pseudostem stop producing leaves and start to produce inflorescence, there are no external sign can be observed when the transformation occur. The trigger for flower initiation could be hormonally induced. The peduncle continually extends until it pushes the inflorescence out through the top of plant. Each nodal cluster has two closely appressed rows of flowers, one above another and enclosed in large subtending bract. Female bracts appear first then followed by male bracts (Robinson and Sauce, 2010).

Fruit of banana plant is elongated, curved and round in cross-section. Its length varies depending on species, it ranges from 5 cm to more than 30 cm. Fruits vary in their arrangement, position, shape, apex and waxiness. The fruit is covered by epidermis and underlying parenchyma layer which consists of vascular bundles and latex tubes. Banana fruit contains edible pulp which is filled with starch and converted to sugars during the ripening process (Pillay and Tenkouano, 2011).

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2.3 Economic Importance of Banana

Banana consumption rates are high, ranging in between 200 to 250 kg per capita annually in New Guinea and the surrounding countries., it is a valuable source of income through local trade for millions of people in the world. Other than that, it is also an important source of employment and export earnings. The total world production of banana is about 98 million metric tonnes, of which around a third is produced in each of the African, Asia-Pacific, Latin American and Caribbean regions. In 2004, the world total exports of banana was about 15.9 millions and in 2012, the volume of world's gross banana exports has increased and reached 16.5 million tonnes. This shows that banana is becoming more and more important as a source of revenue, it is the main source of income for rural communities, especially for small-holder farmers. Hence, banana plays an important role in income security and poverty alleviation (Jain and Swennen, 2004; Madhava Rao, 2010; Pillay *et al.*, 2011).

2.4 Cultivation Problems of Banana

In Malaysia, banana production by smallholder farmers are not well-organized. Other than that, smallholder farmers often use poor quality planting materials with low input, this leads to lower yield and fruit quality. On the other hand, large scale farmers use better quality planting materials from tissue cultured plantlets and with higher inputs; they also adopt more modern technologies, good agricultural practices and better pest and disease management, therefore it results in higher yield and better quality fruits (FNCA, 2010).

However, the diseases caused by fungi and nematodes are the major limiting factors in a successful quality production of banana. Nearly all the commercial cultivars of banana are highly susceptible to certain deadly diseases. Fusarium wilt, also known as Panama disease caused by *Fusarium oxysporum* f. sp. *cubense*, a soil-borne disease, is the major constraint in banana production in Malaysia which affects many important cultivars of banana and plantain. The disease has caused serious crop losses and it has worldwide, recent outbreak is reported in Jordan and Mozambique (FAO, 2014B).

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Furthermore, nematodes are major causes of diseases of banana production in Malaysia. The root-knot nematodes like *Meloidogyne* spp. and the burrowing nematode *Radopholus similis* weakens the root systems, reduce yields, topple plants before harvest, make plants more prone to wind knockdowns, reduce fertilizer uptake and thereby reduce the banana-growing lifespan (FNCA,2010).

2.5 History Plant Tissue Culture in Banana

The science of plant tissue culture takes its roots from path breaking research in botany like discovery of cell followed by propounding of cell theory (Rai, 2007). It produces more planting material from one explant and only small space required. Many commercial companies produce over 30 minutes of banana plantlets by micropropagation. 2000 in vitro plantlets are produced using one original explant within a year (Robinson, 1995). Propagation of bananas through plant tissue culture techniques has been well-established for several years. Several parts of the banana can be used as explant because it possess totipotency, which is the ability to regenerate into a whole plant by one cell. Most common parts of banana used are the apical meristem and shoot-tips. Only healthy plants are chosen to be as explant because viruses are not eliminated by micropropagation techniques. Plant tissue culture has improved the germplasm handling for clonal propagation and also uniformity of propagules.

2.6 Factors Affecting Micropropagation of Banana

There are some factors that can affect the propagation of banana through plant tissue culture technique such as type of explant, basal media, plant growth regulators used, organic additives, carbon source, gelling agent, culture vessel, light source and light intensity.

2.6.1 Type of Explant

An explant is cell, tissue or organ of a plant that used to start in plan tissue culture. Past studies reported that explant characteristics such as the type, source, genotype and history affect the success and commercial viability of tissue culture systems (Bhau

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and Waklu, 2001; Chan and Chang, 2002; Hoy *et al.*, 2003). Appropriate tissue used in micropropagation will have better growth development.

Shoot tip cultures in micropropagation of banana have been studied in the past and it showed good result when it is cultured in the right condition (Muhammad *et al.*, 2004; Muhammad *et al.*, 2007). Other than that, meristem culture is also commonly practised, auxiliary meristems are the source of bud formation (Burrow, 1989; Al-Amin *et al.*, 2009). However, the development of auxiliary meristems is often inhibited by apical dominance (Razdhan, 2003). Some explants possess strong apical dominance, apical dominance is influenced by the growth substances released by terminal bud, which inhibit the growth of lateral bud (De Langhe *et al.*, 1983). In a study conducted by Mateille and Foncelle using *Musa* cv. poyo (1988), longitudinal cuts of buds induced a threefold increase in multiplication, the multiplication rate was also found to depend on the origin of sucker bud, whereby lateral buds doubled in size within three weeks while the apical buds reached three times their size. However, there are no recent study found using bud splitting technique as it is not commonly practised in micropropagation of banana.

2.6.2 Basal Media

A nutrient media used in plant tissue culture consist of inorganic salts, vitamins, growth regulators, gelling agent and carbon source. The decision on using type of media for metabolic needs of cultured plant is an important factor of success in plant regeneration process. There are various types of media that have been formulated such as Linsmaier and skoog (1965), Nitsch and Nitsch (1969). However, most of the past studies on micropropagation of banana preferred to use Murashige and skoog (1962) (Muhammad *et al*, 2004; Al-Amin, *et al.*, 2009; Lohidas and Sujin, 2015).

2.6.3 Plant Growth Regulators

Plant growth regulators play an important role in promoting the growth of plant cultures. There are four classes of plant growth regulator used in plant tissue culture, which are auxins, cytokinins, gibberellins and abscisic acids (Kumar and Reddy, 2011). In plant tissue culture of banana, cytokinin and auxin are most commonly used plant growth regulators to promote shooting and rooting.

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Cytokinin is responsible for shoot initiation and shoot proliferation, there are various types of cytokinin, which are BAP, kinetin, zeatin, adenine sulphate. Cytokinins such as BAP and kinetin are generally known to reduce the apical dominance and help to induce axillary and adventitious shoot formation from meristamatic explants in banana (Jafari *et al.*, 2011). Abeyaretne and Lathiff (2002) states in their study that BAP is widely used to increase the multiplication rate on micropropagation of banana. The study reported that 2-3 mg/L of BAP with basal media is an advisable concentration for banana shoot tip culture. Their statement is supported by Rahman *et al.* (2004) who also reported that BAP is effective in shoot proliferation compared to any other types of cytokinins. The most established banana shoot tip culture system was achieved by using BAP as a supplement to basal media, this statement was supported by another study conducted by Anbazhagan *et al.* (2014), the study states that BAP is better than kinetin in shoot initiation for micropropagation of banana. They reported that 3 mg/L BAP resulted in 7.25±0.28 number of shoot buds while the same amount of kinetin only induced 2.50±0.21 number of shoot buds. In the same study, MS medium supplemented with combination of two cytokinins, which are 2 mg/L BAP and 0.5 mg/L kinetin, showed 7.50±0.26 number of shoot initiation, it is relatively higher compare to other media supplemented with only one type of cytokinin. However, the best result of shoot initiation from this study is a combination of cytokinin and auxin with concentration of 2.0 mg/L BAP and 0.5 mg/L of IAA, which has resulted in 7.85±0.26 number of shoot buds. From this study by Anbazhagan *et al.* (2014), it can be concluded that basal media supplemented with two type of cytokinins is better than single cytokinin but to obtain even better result of shoot initiation, basal media supplemented with combination of cytokinin and auxin is preffered.

Other than the study done by Anbazhagan *et al.* (2014), there are many studies conducted in the past that proved combination of cytokinin and auxin enhances shoot initiation and shoot proliferation. A study on micropropagation of banana using combination of cytokinin and auxin by Ahmed *et al.* (2014) reported that MS medium with 4.0 mg/L BAP and 2.0 mg/L IAA showed maximum culture establishment in lesser time for *Musa* cv. Grand naine, it achieved 100% culture establishment in 14.33 days, the same medium also achieved maximum shoot multiplication with 10.66 shoots per culture and attained 18.30 cm of shoot length. Kalimuthu *et al.* (2006) also reported that combination of cytokinin and auxin in basal media promote higher shoot initiation, MS medium supplemented with 3 mg/L BAP and 0.2 mg/L NAA showed 95% of shoot

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formation, the same medium also showed the most vigorous shoot proliferation. They reported that MS medium with concentration of BAP lesser than 0.5 mg/L or 5.0 mg/L and higher showed poor response of shoot initiation for *Musa sapientum*. On the contrary, same species of banana was studied by Iqbal *et al.* (2013) who reported that MS medium supplemented with 5.0 mg/L BAP and addition of 1.0 mg/L IAA attained the stage of vigorous proliferation.

Auxin plays an important role in root initiation. There are various types of auxins such as IAA, NAA, IBA and many more. Various studies have proven that addition of auxins in media help explant to initiate roots. The root elongation phase on micropropagation of banana was found to be very sensitive to auxin concentration, medium concentration of IBA supports the initiation of root, it was reported that high concentration of auxin will inhibit the root elongation of explant (Ganapathy *et al.*, 2005). Rahman *et al.* (2013) stated that full strength MS medium with 1.0 mg/L IBA resulted in highest number of roots and longest root length which were 8.6±1.16 cm and 3.69±0.34 cm respectively. Based on the study done by Ahmed *et al.* (2014), half strength of MS medium with the addition of 1.0 mg/L IBA and 200 mg/L of activated charcoal gives maximum rooting for *Musa* cv. Grand naine with 98.66% rooting in 6.33 days and also showed the longest root length compared to other treatments. Anbazhagan *et al.* (2014) conducted a study using the same treatment but without the addition of activated charcoal, it has also resulted in highest number of roots per explant and longest root length. As for IAA, Iqbal *et al.* (2013) found that 2.0 mg/L in MS medium is the most efficient root inducing condition for micropropagation of banana. As for NAA, both studies done by Anbazhagan *et al.* (2014) and Iqbal *et al.* (2013) reported that MS media supplemented with concentration between 0.2 mg/L to 2.0 mg/L NAA resulted in poorer response of root initiation compared to other treatments such as IBA and IAA.

2.6.4 Organic Additives

It is common to add complex additives in basal media. Sugarcane juice was added in basal media as organic additive on micropropagation of banana, it was reported that plants that were cultured on 5% sugarcane juice had higher fresh weight, shoot height and number of shoots compared to other treatments (Buah *et al.*, 2011). Beside sugarcane juice, Ssamula *et al.* (2015) added banana juice in MS media on

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micropropagation of banana, the highest number of shoots and shoot height was observed when bananas were cultured on media supplemented with 50 mL/L Kayinja juice (A type of banana juice). Results also showed that banana juice not only enhanced micropropagation but also improved micropropagation plantlet vigour and reduced the cost of energy sources by 30%.

In micropropagation of other plants, organic additives such as coconut water, coconut milk, grind spinach leaves, grind potato tubers, grind carrot, rice flour, green gram, grind pumpkin, banana fruit and orange were used. For micropropagation of Radish (*Raphanus sativus* L.) Var. Beeralu reported that the highest mean number of shoots, which are 12 shoots per explant, was observed in MS medium with 2.5 mg/L BAP and 0.1 mg/L NAA with 10% orange juice, whereas the second highest shoots were obtained with 20% coconut water (Manawadu *et al.*, 2014). 70 mL/L young coconut water and 50 mL/L mature coconut water were tested on micropropagation of *Celosia* sp and it showed high shoot initiation, which were 14.21+±8.2 and 13.14±10.33 respectively (Daud *et al.*, 2011). From these previous studies, it can be concluded that organic additives are good sources of vitamins and inorganic ions required growth, they are also good carbon source.

2.6.5 Carbon Source

Carbon sources in plant tissue culture media are important for plants whose photosynthetic efficiency is insufficient under in vitro condition. The type and concentration of sugars are known to influence the success of any *in vitro* protocol (Waman, 2014). Sucrose is the preferred carbon source for most plant tissue culture protocol as it is an easily assimilated macronutrient that rapidly provides energy, However commercially available table sugar has been used to replace tissue culture grade sucrose, this reduced medium costs without compromising on the micropropagation rate or on the quality of the plants produced in micropropagation of *Centella asiatica* (Raghu *et al.*, 2007). The cost of table sugar commonly used in commercial tissue culture laboratories and a substitute for tissue culture grade sucrose is also relatively high given the volumes used. There are also other alternatives to choose from such as D-Mannitol, a sugar alcohol helps to plant protoplast formation and fusion. The cost of tissue culture grade carbon sources is high, thus making tissue culture derived plantlets expensive.

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