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In Vitro Mass Multiplication of *Artocarpus heterophyllus* Lam var. Tekam Yellow

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ABSTRACT

A protocol for rapid micropropagation of *Artocarpus heterophyllus* from seeds of a single fruit was established. The seeds were successfully sterilised using 40% Clorox (20 min) + 20% Clorox (15 min) and 50% Clorox (20 min) + 20% Clorox (15 min). The survivability percentage was 44.44%, while the contamination percentage was 14.81%. Experiments to assess the effect of shoot tip and different node positions on shoot induction, and to test the effect of decapitation on shoot proliferation were performed. The explants used for both experiments were derived from 8-week-old seedlings grown in half-strength MS basal media supplemented with 2.5 mg/L BAP. There was no significant difference in terms of percentage of explants regenerating shoots and mean shoot number produced per explants. However, node 2 significantly produced the highest mean shoot length (2.53 cm). In the decapitation experiment, there was no significant difference in terms of percentage of explants regenerating shoots and mean shoot length. Nevertheless, decapitated shoots significantly produced the highest mean shoot number per explant (18.33). 2.5 mg/L BAP was chosen as the best treatment for shoot induction from seed with a mean shoot number of 7.33 and mean shoot length of 2.95 cm. For shoot multiplication, 1.0 mg/L BAP significantly produced the highest mean shoot number (17.13), while 5.0 mg/L BAP significantly produced the

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highest shoot length (2.95 cm). For rooting, IBA at 2.5 mg/L and 5.0 mg/L produced the highest mean root number at 18.73 and 17.27 respectively. The highest mean root length (3.37 cm) was significantly obtained in the control treatment. The plantlets were successfully acclimatised in a potting mixture consisting of top soil and organic soil (1:1) with 88.89% survival rate.

Keywords: Acclimatisation, *Artocarpus heterophyllus* Lam, decapitation, micropropagation, shoot induction, shoot multiplication

INTRODUCTION

Artocarpus heterophyllus Lam. is a tropical tree that belongs to the mulberry family, Moraceae. Also known as jackfruit in English or *nangka* in Malay, it is considered a health food and a rich source of carbohydrate, protein, fat, minerals, vitamins, starch, calcium and thiamine (Baliga, Shivashankara, Haniadka, Dsouza, & Bhat, 2011; Burkill, 1997). In addition, all parts of the plant, in different forms, can be used for timber, fuel, fodder, and medicinal and industrial products, all of which have varying importance in economic values (Hasan, Ahmed, & Miah, 2008).

Jackfruit can be propagated through seeds and vegetative parts. There are, however, some problems still faced by growers. For propagation from seeds, the main problem is the recalcitrant nature of the seeds whereby the seeds cannot be stored outside for a long period of time. Moreover, seedlings from germinated seeds take longer time to reach fruit-bearing age than those trees propagated by vegetative methods.

Apart from that, seedling-propagated trees grow unnecessarily taller than those propagated by vegetative methods, which is a constraint in management and harvesting process. The trees, however, can also be dwarfs and tend to produce branches at low levels which in turn results in low quality timber with a short trunk (Hossain & Haq, 2006).

Therefore, culture technique of cells, tissues, organs or whole plant under controlled environment and nutrition is generally applied to produce clones or true-to type plants of a selected genotype (Hussain, Qarshi, Nazir, & Ullah, 2012; Thorpe, 2007). It is a very quick propagation process which can lead to the production of pathogen-free plants (García-Gonzáles, Quiroz, Carrasco, & Caligari, 2010).

However, non-woody species are usually studied for tissue culture. Woody species are often labelled difficult to propagate species. According to Mc Cown (1986), some woody plant species might take up to a year to complete their establishment and initiation stage as they contained less juvenile cells than herbs. Moreover, woody species contain high phenolic compound content which could be toxic for *in vitro* plant. This was confirmed by Dobránszki and Teixeira da Silva (2010) who reported oxidation of phenolic compounds mainly caused browning and necrosis in tissue culture explants of woody species. Additionally, obtaining tap root is also a problem for woody species due to recalcitrant rooting in woody species (Mohan Jain & Häggman, 2007), thus making the tissue

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culture technique for woody species more adventurous. Nunes et al. (2007) found the recalcitrance in a number of woody species to form their adventitious roots had restricted micropropagation.

Research in *in vitro* culture techniques for *A. heterophyllus* has been reported for potential mass propagation in order to produce planting materials on a large scale. There is, however, a dearth of studies on using seeds as the initial explants to produce aseptic culture for mass propagation of *A. heterophyllus* as well as to determine the best explant to provide high rejuvenation rate for *in vitro* mass propagation of *A. heterophyllus*. The position of nodes as well as the presence of shoot tips actually make a big difference in the rejuvenation rate of explants. Specifically, Shefakandeh and Khosh-Khui (2008) reported that shoot tip and the nodes from different parts of a plant shoot responded differently for growth and shoots proliferation. Usually, shoot tips are related to apical dominance. Apical dominance often plays a crucial role in shoot proliferation as it is termed as the control that the shoot tip has over axillary bud outgrowth (Cline, 1997). Apical dominance is best related to decapitation, which refers to the removal of the shoot tips (Dun, Ferguson, & Beveridge, 2006). Decapitation is normally executed as a way of testing the importance of the apical bud in controlling growth of lateral buds (Beveridge, Symons, & Turnbull, 2000).

This study investigated the possibility of micro propagating *A. heterophyllus* from seeds for mass production as they can be easily and abundantly obtained all year round which make them ideal for micropropagation. Specifically, the effects of shoot tips and different node positions on shoot induction were tested followed by the testing of the effects of decapitation on shoot proliferation in order to determine the best explants to be used for multiplication. Additionally, the effects of different plant growth regulators at varying concentrations on shoot multiplication and rooting in order to determine the optimum concentration for multiplication and rooting were tested, followed by assessment of different potting mixtures for successful acclimatisation of the plantlets.

MATERIALS AND METHODS

Six experiments were performed using seeds of *Atrocarpus heterophyllus*: (1) Effects of different concentrations of BAP and KIN on shoot proliferation from seeds; (2) Shoot induction and using shoot tips and different node positions; (3) Effects of decapitation on shoot proliferation; (4) Shoot multiplication; (5) Root induction; and (6) Acclimatisation.

Procurement of Plant Materials, Seed Surface Sterilisation and Seed Germination

Whole seeds were procured from 8-month fruits of *A. heterophyllus* trees grown at Malaysian Department of Agriculture plot in Seremban, Negeri Sembilan. Seed coats were removed, and seeds were surface-sterilised in 70% ethanol for 1 minute, followed by 20 minutes of sterilisation in

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three different concentrations (30%, 40% and 50%) of Clorox® (5.25% of sodium hypochlorite). Seeds were then rinsed once with sterile distilled water, followed by 15 minutes of sterilisation in three other concentrations (10%, 20% and 30%) of Clorox®, Tween 20 was added to Clorox® as surfactant. Seeds were later rinsed 5 times with sterile distilled water. Following this, seeds were germinated on a medium consisting of half-strength MS basal salts (Murashige & Skong, 1962), supplemented with 30 g/L sucrose, 3.0 g/L Gelrite (Duchefa, Haarlem, The Netherlands) without plant growth stimulators (half-strength MSO). The medium was adjusted to pH 5.7 prior to adding agar and autoclaved at 121°C at 103 kPa for 20 minutes. The cultures were incubated at 25 ± 2°C with a 16-h photoperiod of 35 - 40 μmol.m-2.s-1 provided by cool white fluorescent lights. The cultures were observed for 4 weeks. Two parameters were recorded: percentage of contamination and percentage of explant survival.

Effects of Different Concentrations of BAP and KIN on Shoot Proliferation from Seeds

Sterilised seeds were cultured in individual jars containing half-strength MS medium supplemented with 30.0 g/L sucrose, 3.0 g/L Gelrite™ and at different concentrations (0, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/L) of BAP and KIN separately. Seed response in term of shoot induction under two cytokinin types at different concentrations was observed. Following establishment of

surface sterilisation protocol obtained from this study, aseptic seeds were cultured in individual jars on half-strength MS medium supplemented with 30.0 g/L sucrose, 3.0 g/L Gelrite™ and different concentrations (0, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/L) of BAP and KIN separately. The cultures were maintained on the same media for 8 weeks with sub-culturing at 4-week intervals. Eleven treatments were done with 3 replications per treatment. Three parameters were recorded: percentage of explants regenerating shoots, mean number of shoots per seed and mean length of shoots (cm).

Shoot Induction Using Shoot Tip and Different Node Positions

Shoot tip, node 1 and node 2 excised from 8-week-old seedlings were cultured on half-strength MS medium supplemented with 30.0 g/L sucrose, 3.0 g/L Gelrite™ and 2.5 mg/L BAP (the best cytokinin concentration determined from previous experiment). This study was conducted to determine the part of the shoot that is most suitable for shoot induction. The cultures were observed for 4 weeks. Three treatments with 3 replications per treatment were done. The parameters recorded were the percentage of explants regenerating shoots, mean number of shoots per explant and mean length of shoots (cm).

Effects of Decapitation on Shoot Proliferation

Shoots were excised from 8-week old seedlings and cultured on half-strength MS medium supplemented with 30.0 g/L

sucrose, 3.0 g/L Gelrite™ and 2.5 mg/L BAP (the best cytokinin concentration determined from previous experiment). For decapitated explants, the tip of the shoot was removed while for the non-decapitated explants, the tip of the shoot was left alone. This was to determine whether the removal of the apical bud would have significant effect on the growth and proliferation of lateral buds. Two treatments were done with 3 replications per treatment. The parameters recorded were percentage of explants regenerating shoots, mean number of shoots per explant and mean length of shoots (cm).

Shoot Multiplication

Nodes of shoots derived from the seeds were cultured on half-strength MS medium supplemented with 3 different cytokinin, each cytokinin administered at 5 different concentrations, namely BAP (1.0, 2.5, 5.0, 7.5 and 10.0 mg/L), KIN (1.0, 2.5, 5.0, 7.5 and 10.0 mg/L) and TDZ (0.05, 0.1, 0.5, 1.0 and 2.0 mg/L). The cultures were maintained 12 weeks in the same media with sub culturing 4-week interval. Sixteen treatments were tested with 3 replications per treatment. The parameters recorded were percentage of shoot formation, mean number of shoots and mean length of shoot (cm).

Root Induction

Shoots derived from the multiplication step were cultured on medium without hormones for 2 weeks to eliminate the effects of hormones applied in the multiplication stage. The shoots which were 5 – 6 cm long were then separated individually and placed on half-strength MS rooting medium containing concentrations of IBA and NAA at 0, 1.0, 2.5 and 5.0 mg/L for 8 weeks. Seven treatments were tested with 3 replications per treatment. The parameters recorded were percentage of root formation, mean number of roots and mean length of roots (cm).

Acclimatisation

After rinsing the completely rooted plantlet under tap water to remove the agar, the plantlets were next rinsed with sterile distilled water and soaked for 5 minutes in 0.4% fungicide (Benocide 50WT®) (Hextar Chemicals Pte. Ltd., Klang, Malaysia). The plantlets were then transferred to medium sized pots (10 x 8 cm) (W x H) containing the following potting mixtures; organic soil + topsoil (1:1), perlite + sand (1:1), peat moss + sand (1:1) and organic matter + topsoil + sand (1:1:1). Each pot was maintained at 80% relative humidity by covering it with polythene bag. The pots were then incubated for 4 weeks in a misting chamber at 25°C to 30°C with 16/8-h photoperiod (light/dark) (21 µmol $m^{-2}s^{-1}$). Four treatments were tested with 3 replications per treatment. The parameter recorded was the percentage of survived plants.

Experimental Design and Statistical Analysis

Randomized Completely Block Design (RCBD) was used in all experiments except for "Shoot Induction Using Shoot Tip and

Different Node Positions" which employed the Completely Randomized Design (CRD). Data was analysed using ANOVA except for the experiment "Effect of Decapitation on Shoot Regeneration", [t-test analysis was used]. All data was subjected to normality test, and data transformation was done if data was abnormal or the coefficient of variation (CV) values was high. Also, for data in the form of percentages, Arcsin transformation was applied. Data was analysed using SAS program version 9.3.

RESULTS

Optimising Culture Sterilisation

In this experiment, contamination of seeds by bacteria was observed around five days after sterilisation while contamination by fungi was observed a little earlier on the third day and lasted until fifth day after the sterilisation process. Figure 1(a) shows the seedlings on the first day before contamination sets in. Figure 1(b) shows the survived seedlings.
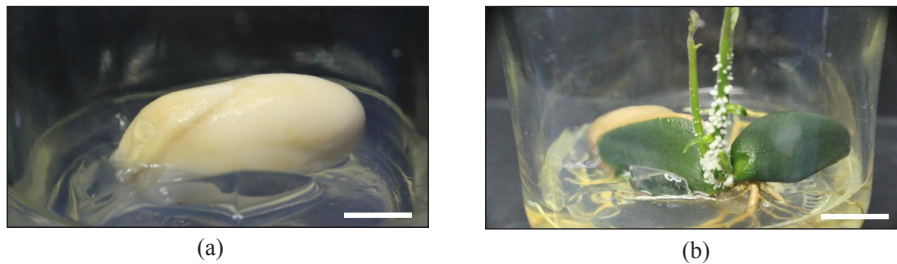

(a)   (b)

Figure 1. Seed culture of *A. heterophyllus*: (a) Seed at day 1 (Bar = 0.9 cm); (b) Survived and germinated seed explant (Bar = 1.0 cm)

Table 1

Mean contamination level (%) and mean survival rate (%) of A. heterophyllus *seeds 4 weeks after exposure to different sterilisation treatments*

Clorox (%)		Mean percentage of contamination (%)*	Mean percentage of survival (%)*
(20 min)	(15 min)		
30% Clorox +10% Clorox		$33.33 \pm 5.56abc$	$37.03 \pm 3.70abc$
30% Clorox + 20% Clorox		$40.74 \pm 4.90ab$	$33.33 \pm 5.56abc$
30% Clorox + 30% Clorox		$25.92 \pm 4.90abc$	$22.22 \pm 7.86c$
40% Clorox + 10% Clorox		$51.85 \pm 5.86a$	$51.85 \pm 9.80ab$
40% Clorox + 20% Clorox		$14.81 \pm 5.86cd$	$29.63 \pm 8.69abc$
40% Clorox + 30% Clorox		$3.70 \pm 3.70d$	$22.22 \pm 5.56bc$
50% Clorox + 10% Clorox		$22.22 \pm 9.62bcd$	$40.74 \pm 10.80abc$
50% Clorox + 20% Clorox		$14.81 \pm 5.86cd$	$44.44 \pm 7.86abc$
50% Clorox + 30% Clorox		$29.63 \pm 6.68abc$	$51.85 \pm 8.07a$

Note: *Means followed by the same letter(s) within each column are not significantly different ($p \leq 0.05$) using DMRT. (Data has been transformed using arcsine). In each treatment, seeds were subjected to 35 minutes of sterilisation with Clorox, split into two sterilisation stages, at 20-minute stage at a Clorox concentration followed by a 15-minute one at another Clorox concentration.

Empirical data for this part of the study are shown in Table 1 below. The table shows mean contamination level (%) and mean survival rate (%) of *A. heterophyllus* seeds 4 weeks after exposure to different sterilization treatments. The results indicated significant differences among some of the treatments.

The highest mean seed survival rates were from the sterilisation treatments consisting of 40% Clorox (20 min) + 10% Clorox (15 min) and 50% Clorox (20 min) + 30% Clorox (15 min), both giving a mean survival rate of 51.85% respectively at standard error of ±9.80% and ±8.07%. These two mean treatments did not differ significantly from each other, although each of them may or may not differ significantly from other treatments. The lowest mean survival rates were from sterilisation treatments consisting 30% Clorox (20 min) + 30% Clorox (15 min) and 40% Clorox (20 min) + 30% Clorox (15 min), both giving a mean survival rate of 22.22%, with respective standard error of ±7.86 and ±5.56. The means of the two treatments did not significantly differ from each other although each of them may or may not differ significantly from means of other treatments. Mean survival rates for other treatments range from 29.63% ±8.69 [for a sterilisation treatment consisting of 40% Clorox (20 mins) + 20% Clorox (15 mins)] to 44.44% ±7.86 [for a sterilisation treatment consisting of 50% Clorox (20 mins) + 20% Clorox (15 mins).

From Table 1, based on ANOVA and DMRT results, it can be seen that mean survival rates for some treatments are not significantly different from each other

(p>.05) [treatments: 30% Clorox (20 mins) +10% Clorox (15 mins) ; 30% Clorox (20 mins) + 20% Clorox (15 mins); 40% Clorox (20 mins) + 20% Clorox (15 mins); 50% Clorox (20 mins) + 10% Clorox (15 mins) and 50% Clorox (20 mins) + 20% Clorox (15 mins)] while mean survival rates for other treatments are significantly different from each other (p<.05) and from the above-mentioned means [treatments: 30% Clorox (20 mins) + 30% Clorox (15 mins); 40% Clorox (20 mins) + 10% Clorox (15 mins); 40% Clorox (20 mins) + 30% Clorox (15 mins); and 50% Clorox (20 mins) + 30% Clorox (15 mins)].

Effects of Different Concentrations of Cytokinin (BAP and KIN) on Shoot Proliferation from Seeds

In this experiment, seeds of *A. heterophyllus* were cultured on half-strength MS media supplemented with two types of cytokinin, BAP and KIN, at different concentrations. BAP was administered at the following concentrations: 1.0 mg/L, 2.5 mg/L, 5.0 mg/L and 7.5 mg/L. KIN was administered at 1.0 mg/L, 5.0 mg/L, and 10.0 mg/L. A control treatment was set up with neither BAP nor KIN (0 mg/L). The upper seed leaf started to bend backwards after four to five days of culture and the plumule could already be seen by this time. After around 4 weeks, the shoot length was about 1.0 cm. Multiple shoots were produced after 8 weeks of culture. Results from statistical analyses showed significant differences among the treatments on percentage of explants regenerating shoots (Table 2).

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Table 2

Effects of different concentrations of BAP and KIN on shoot proliferation from seeds of A. heterophyllus after 8 weeks of culture

Treatment (mg/L)	Quantity (%) of explants able to regenerate shoots*	Mean no of shoots per explant*	Mean length of shoots (cm)*
0 (control)	100.00 ± 0.00a	6.08 ± 2.12abc	2.60 ± 0.02b
1.0 BAP	100.00 ± 0.00a	3.75 ± 0.88abc	2.40 ± 0.02ed
2.5 BAP	100.00 ± 0.00a	7.33 ± 1.37abc	2.95 ± 0.03a
5.0 BAP	100.00 ± 0.00a	8.08 ± 1.90ab	2.58 ± 0.04bc
7.5 BAP	100.00 ± 0.00a	5.42 ± 1.44abc	2.45 ± 0.03cd
10.0 BAP	83.33 ± 3.55b	8.92 ± 2.56a	0.98 ± 0.04i
1.0 KIN	100.00 ± 0.00a	2.58 ± 1.04bc	2.30 ± 0.02ef
2.5 KIN	66.67 ± 3.55c	4.33 ± 0.78abc	2.23 ± 0.04gf
5.0 KIN	100.00 ± 0.00a	2.83 ± 0.58abc	2.09 ± 0.06gh
7.5 KIN	83.33 ± 3.55b	3.33 ± 1.73abc	2.09 ± 0.10gh
10.0 KIN	100.00 ± 0.00a	2.00 ± 0.51c	2.07 ± 0.14h

Note: *Means followed by the same letter(s) within each column are not significantly different (p≤0.05) using DMRT. (Data for percentage of explants regenerating shoots has been transformed using arcsine and data for mean number of shoots per explants has been transformed using square root). Shoot proliferation measured in terms of the quantity of shoots (%) regenerated by explants, mean number of shoots per explant and mean length of shoots.

The quantity (%) of explants that regenerate shoots was significantly higher for control (0 mg/L of either BAP or KIN), 1.0 mg/L BAP, 2.5 mg/L BAP, 5.0 mg/L BAP, 7.5 mg/L BAP, 1.0 mg/L KIN, 5.0 mg/L KIN and 10.0 mg/L KIN compared to the rest of the treatments. The results showed that under these treatments, all (100%) of the explants managed to regenerate shoots (100.00% ± 0.00%). At 100% rate of explants able to regenerate shoots, these treatments of course show no significant differences with each other with respect to the quantity of explants able to regenerate shoots (Table 2). In other treatments, the quantity (%) of explants regenerating shoots vary from 66.67% (2.5 mg/L KIN) to 83.33% (10.0 mg/L BAP and 7.5 mg/L KIN). Statistically, there were no

significant differences in % explants that regenerate shoots between 10.0 mg/L BAP and 7.5 mg/L KIN (both producing 83.33% explants regenerating shoots). However, 10.0 mg/L BAP and 7.5 mg/L KIN were significantly different from 2.5 mg/L KIN in producing higher quantity of explants (83.33%) that regenerate shoots compared with just 66.67% for 2.5 mg/L KIN. All treatments that produced less than 100% of explants that regenerate shoots were significantly different from those treatments that produce 100% of such explants (Table 2). For the BAP group, the results showed increasing BAP concentrations from 0 mg/L to 7.5 mg/L had no adverse effects on the quantity (%) of explants that regenerated shoots; all treatments within this range of BAP concentrations produced full quantity

(100%) of explants that regenerated shoots. At 10.0 mg/L BAP, however, the quantity of explants that regenerate shoots significantly dropped to 83.33% (Table 2). Further, there is a limit to increasing BAP concentration without negatively impacting the quantity of explants that can regenerate shoots: at 10.0 mg/L BAP, the shoot formation tends to be inhibited. As for the KIN group, the results showed that the quantity (%) of explants that can regenerate shoots fluctuates rhythmically with increasing concentrations of KIN. At 1.0 mg/L KIN, all the explants (100%) were able to regenerate shoots. But increasing the KIN concentration to 2.5 mg/L significantly reduced the quantity of explants able to regenerate shoots to 66.67% and yet, when KIN concentration was further increased to 5.0 mg/L, the quantity of explants able to regenerate shoots was restored to 100%. Astonishingly, this quantity fell significantly to 83.33% when the KIN concentration was further increased to 7.5 mg/L, yet achieving again a full 100% rate when the KIN concentration was increased to 10 mg/L, the final and the highest KIN concentration in this experiment. Results for the KIN group of treatments proved a rhythmic pattern of fluctuation in the quantity of explants able to regenerate shoots with increasing KIN concentrations. As for mean number of shoots per explant, results for the BAP group of treatments showed the mean number of shoots per explant varied from the lowest of 3.75 shoots per explant (in treatment consisting of 1.0 mg/L BAP) to the highest of 8.92 shoots per explant (in the treatment consisting of 10.0 mg/L BAP)

(Table 2). Results for the BAP group of treatments showed a fluctuating pattern in the mean number of shoots per explant with increasing BAP concentration. At 0 mg/L BAP (control treatment), mean number of shoots per explant was 6.08 (Table 2). But at 1.0 mg/L BAP, the mean number of shoots per explant fell to 3.75 and then increased to 7.33 at 2.5 mg/L BAP. And yet, results for these three BAP concentrations (0 mg/L, 1.0 mg/L and 2.5 mg/L) showed no statistically significant difference from each other (Table 2). Further, increasing BAP concentration to 5.0 mg/L further increased mean number of shoots per explant to 8.08, and yet when BAP concentration was increased to 7.5 mg/L, mean number of shoots per explant significantly fell to 5.42 before rising again, to 8.92 (the highest in the experiment), when BAP concentration was increased to 10.0 mg/L. The results for the BAP group of treatments showed a fluctuating trend in mean number of shoots per explant with increasing BAP concentration, and yet, all results for these treatments were not statistically different from each other as they all shared at least one superscript (Table 2). As for KIN group of treatments, the results also showed a fluctuating trend in the mean number of shoots per explant with increasing KIN concentration (Table 2). Results for KIN treatments showed mean number of shoots per explant varied between 2.00 (in treatment consisting of 10.0 mg/L KIN) to 4.33 (in treatment consisting of 2.5 mg/L KIN). Yet, statistically, KIN results were not significantly different from each other as they all shared at least

one superscript. In general, mean number of shoots per explant for KIN treatments is much lower than those obtained in BAP treatment, signifying that BAP is better as a plant growth inducer in terms of number of shoots per explant compared with KIN.

Shoot Induction Using Shoot Tip and Different Node Positions

Shoot tip, node 1 and node 2 explants excised from shoots of seed-derived plants of *A. heterophyllus* were cultured on half-strength MS media supplemented with 2.5 mg/L BAP for shoot induction. Figures 2(a), 2(c) and 2(e), respectively show the shoot tip, node 1 and node 2 during week 1 of culture. It was observed that bud break occurred within 7 to 8 days of culture. Figures 2(b), 2(d) and 2(f) respectively,

show multiple shoot formation from the shoot tip, node 1 and node 2 after four weeks of culture.

The quantity **(%)** of explants able to regenerate shoots from the shoot tip, node 1 and node 2, mean number of shoots per explant and mean length of shoots are shown in Table 3.

The mean number of shoots per explant ranged from 4.47 ± 0.96 (node 2) to 3.20 ± 0.40 (shoot tip). ANOVA results showed no significant difference among the treatments on mean number of shoots per explant. Nevertheless, numerically, the mean number of shoots per explant was the highest for node 2 at 4.47 ± 0.96. For the mean shoot length, the range was between 2.53 ± 0.04 cm (node 2) and 1.28 ± 0.05 cm (shoot tip) (Table 3). Significant differences were
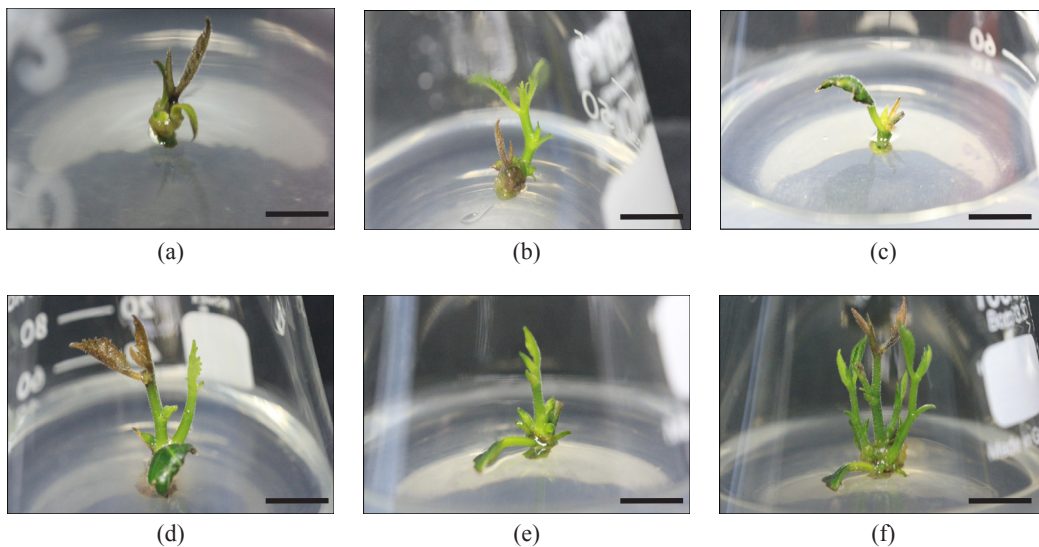

Figure 2. Shoot induction using shoot tip and different node positions of *A. heterophyllus*: (a) Week 1 shoot tip culture (Bar = 1.2 cm); (b) Week 4 shoot tip culture (Bar = 0.9 cm); (c) Week 1 node 1 culture (Bar = 0.7 cm); (d) Week 4 node 1 culture (Bar = 1.1 cm); (e) Week 1 node 2 culture (Bar = 0.8 cm); and (f) Week 4 node 2 culture (Bar = 1.1 cm)

Table 3
Effects of shoot tip and different node positions on shoot induction of A. heterophyllus *after 4 weeks of culture*

Explant	Quantity (%) of explants able to regenerate shoots	Mean no of shoots per explant*	Mean length of shoots (cm)*
Shoot Tip	100.00	$3.20 \pm 0.40a$	$1.28 \pm 0.05c$
Node 1	100.00	$4.07 \pm 0.42a$	$2.39 \pm 0.05b$
Node 2	100.00	$4.47 \pm 0.96a$	$2.53 \pm 0.04a$

Note: *Means followed by same letter within columns are not significantly different ($p > 0.05$). Data for mean number of shoots per explant has been transformed using square root.
These effects are measured in terms of the quantity (%) explants able to regenerate shoots, mean number of shoots per explant and mean length of shoots.

observed between the treatments on mean shoot length based on the ANOVA results. Results from DMRT revealed that the mean shoot length for node 2 (2.53 ± 0.04 cm) was significantly higher than node 1 and shoot tip. Shoots derived from shoot tip only reached 1.28 ± 0.05 cm in length.

Effects of Decapitation on Shoot Proliferation

The effects of decapitation on *A. heterophyllus* shoots was observed after six weeks of culture on half-strength MS media supplemented with 2.5 mg/L BAP. Figures 3(a) and 3(c) shows respectively, decapitated and non-decapitated shoot on day 1. Bud break from the axillary buds occurred
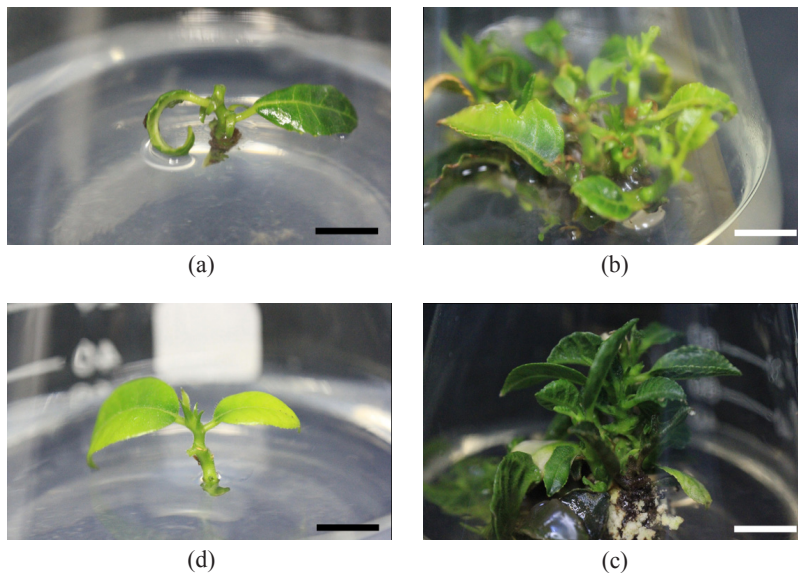

(a)　　　　　　　　　　　(b)

(d)　　　　　　　　　　　(c)

Figure 3. Effect of decapitation on shoot proliferation of *A. heterophyllus*: (a) Decapitated shoot at day 1 of culture (Bar = 1.1 cm); (b) Decapitated shoot after 6 weeks of culture (Bar = 0.9 cm); (c) Non-decapitated shoot at day 1 of culture (Bar = 1.2 cm); and (d) Non-decapitated shoot after 6 weeks of culture (Bar = 1.2 cm)

within 7 to 8 days of culture. Figures 3(b) and 3(d), respectively show the response of the decapitated and non-decapitated shoot after 6 weeks of culture. The quantity (%) of explants able to regenerate shoots from the shoot tip, node 1 and node 2, mean number of shoots per explant and mean length of shoots are shown in Table 4.

The result showed significant difference between the two treatments on mean number of shoots per explants. The mean number of shoots per explant for shoots without shoot tip (18.33 ± 2.12) was significantly higher than those attained by the non-decapitated shoots at 8.67 ± 2.31 (Table 4). As for the mean shoot length, there was no significant difference between both treatments. Nevertheless, shoots without shoot tip indicated the highest mean (2.31 ± 0.27 cm) compared with the shoots with shoot tip (1.89 ± 0.36 cm).

Table 4
Effects of decapitation on shoot proliferation from A. heterophyllus *shoot after 6 weeks of culture*

Explants	Quantity (%) of explants able to regenerate shoots	Mean no of shoots per explant*	Mean length of shoots (cm)*
With shoot tip	100.00	8.67 ± 2.31b	1.89 ± 0.36a
Without shoot tip	100.00	18.33 ± 2.12a	2.31 ± 0.27a

Shoot Multiplication

Nodal explants were cultured on half-strength MS media supplemented with different concentrations of BAP, KIN and TDZ for shoot multiplication. Bud break from the axillary buds began at day 7 to day 8 of culture. Shoot proliferation was observed after 2 weeks of culture. Table 5 shows the quantity (%) percentage of explants able to regenerate shoots, mean number of shoots produced per explant and mean shoot length attained after 12 weeks of culture.

The results show that quantity (%) of explants able to regenerate shoots varied from a low of 66.67% (in a treatment consisting 1.0 mg/L TDZ) to 100% (in treatments consisting of 0 mg/L (control treatment devoid of any cytokinin), 1.0 mg/L BAP, 2.5 mg/L BAP, 7.5 mg/L BAP, 10.0 mg/L BAP, 5.0 mg/ L KIN, 7.5 mg/L KIN, 10.0 mg/L KIN, 0.05 mg/L TDZ, 0.1 mg/L TDZ, 0.5 mg/L TDZ and 2.0 mg/L TDZ) (Table 5). All treatments, except for one that uses 1.0 mg/L TDZ, are not significantly different from one another in terms of the quantity (%) of explants able to regenerate shoots. For the treatment that uses 1.0 mg/L TDZ, the quantity (%) of explants able to regenerate shoots, at 66.67%, was significantly lower than values obtained in other treatments.

As for the mean number of shoots per explant, the results show that values ranged between 17.13 ± 3.54 (1.0 mg/L BAP) and 1.13 ± 0.09 (1.0 mg/L TDZ). Statistical analyses have bundled results into groups; within a group, results are not statistically

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Table 5

Effects of different types of cytokinin (BAP, KIN and TDZ) at different concentrations on shoot multiplication of A. heterophyllus *after 12 weeks of culture*

Treatment (mg/L)	Quantity (%) of explants able to regenerate shoots	Mean no of shoots per explant*	Mean shoot length (cm)*
0 (control)	100.00 ± 0.00a	5.80 ± 1.27b	2.30 ± 0.33a
1.0 BAP	100.00 ± 0.00a	17.13 ± 3.54a	1.09 ± 0.08c
2.5 BAP	100.00 ± 0.00a	4.00 ± 1.18bcd	0.92 ± 0.30c
5.0 BAP	86.67 ± 2.52ab	4.13 ± 0.66bc	2.95 ± 0.77a
7.5 BAP	100.00 ± 0.00a	2.67 ± 0.57cdef	1.12 ± 0.10c
10.0 BAP	100.00 ± 0.00a	3.87 ± 0.38bc	0.76 ± 0.04c
1.0 KIN	93.33 ± 2.521a	3.40 ± 0.48bcd	2.18 ± 0.36ab
2.5 KIN	80.00 ± 7.56ab	1.13 ± 0.13f	1.40 ± 0.14bc
5.0 KIN	100.00 ± 0.00a	3.47 ± 0.95bcde	1.14 ± 0.09c
7.5 KIN	100.00 ± 0.00a	2.67 ± 0.50cdef	1.01 ± 0.02c
10.0 KIN	100.00 ± 0.00a	1.67 ± 0.13def	0.99 ± 0.08c
0.05 TDZ	100.00 ± 0.00a	3.40 ± 0.62bcd	1.30 ± 0.10bc
0.1 TDZ	100.00 ± 0.00a	4.93 ± 1.28bc	0.72 ± 0.05c
0.5 TDZ	100.00 ± 0.00a	4.07 ± 0.78bc	0.85 ± 0.06c
1.0 TDZ	66.67 ± 6.67b	1.13 ± 0.09ef	0.66 ± 0.03c
2.0 TDZ	100.00 ± 0.00a	2.27 ± 0.49cdef	0.86 ± 0.05c

Note: *Means followed by the same letter(s) within each column are not significantly different ($p \leq 0.05$) using DMRT. (Data for percentage of explants regenerating shoots has been transformed using arcsine; data for mean number of shoots per explants has been transformed using square root and data for mean length of shoots has been transformed using square root).

Effects were measured in terms of quantity (%) of explants able to regenerate shoots, mean number of shoots produced per explant and mean shoot length after 12 weeks of culture.

different from each other. There is also overlapping between groups: some results in a group are not statistically significant from some results in other groups. For example, the result from the treatment that uses 1.0 mg/L TDZ is significantly different from the one that uses 0.5 mg/L TDZ, yet both are not significantly different from the treatment that uses 7.5 mg/L BAP (Table 5). A single treatment, one that uses 1.0 mg/L BAP, is in a group of its own. It produces the highest mean number of shoots per explant, at 17.13, significantly different and higher than values

from all the other treatments. The results thus show that at 1.0 mg/L, BAP induces the highest mean number of shoots per explant (17.13) compared with KIN (mean of 3.40 shoots per explant at 1.0 mg/L) and TDZ (mean of 1.13 shoots per explant at 1.0 mg/L). As concentration of each type of cytokine is increased from the initial 1.0 mg/L, the results showed that mean shoot number per explant for all types of cytokinin fluctuated and was at much lower value compared with 17.13 obtained from 1.0 mg/L BAP. Results showed that after

1.0 mg/L, mean shoot number per explant varied from a low of 1.13 (2.5 mg/L KIN and 1.0 mg/L TDZ) to 4.93 (0.1 mg/L TDZ). All values in this range are lower than 5.80 obtained for the control treatment (Table 5).

Results for mean shoot length meanwhile showed a range of values from a low of 0.72 cm (0.1 mg/L TDZ) to a high of 2.95 cm (5.0 mg/L BAP) (Table 5). The control treatment (0 mg/L and devoid of any growth regulator) yields a value of 2.30 cm, significantly high and not different from the numerically highest value of 2.95 cm obtained from concentration level of 5.0 mg/L BAP. Results from the control treatment and 5.0 mg/L BAP were significantly different and higher from those obtained in other treatments. Generally, shoot mean length values are statistically bundled in group based on results of statistical analyses. Within each group, mean shoot length values are not significantly different from

each, but there is some overlapping between groups whereby values within a group may be statistically similar to values in other groups. Most values are in the lowest range with letter 'c' attached to them as shown in Table 5, with values ranging from just 0.66 cm to just 1.40 cm (Table 5).

Rooting of Shoots

Shoots of *A. heterophyllus* were cultured on half-strength MS media supplemented with two types of auxins which (IBA and NAA) at different concentrations. The root initiation began as early as by the second week of culture. The results for this experiment are presented in Table 6. The results showed the quantity (%) of explants able to regenerate regenerating roots ranged from 100.00% (in treatment consisting of 2.5 mg/L IBA) to 66.67% (in treatment consisting of 5.0 mg/L IBA), after 8 weeks of culture (Table 6).

Table 6

Effects of two different types of auxins (IBA and NAA) at different concentrations on root induction of A. heterophyllus *after 8 weeks of culture*

Treatment (mg/L)	Quantity (%) of explants able to regenerate roots*	Mean no of roots per explant*	Mean root length (cm)*
0 (control)	80.00 ± 4.36ab	6.80 ± 0.35b	3.37 ± 0.18a
1.0 IBA	86.67 ± 5.04ab	9.13 ± 0.87b	1.62 ± 0.19c
2.5 IBA	100.00 ± 0.00a	18.73 ± 2.71a	1.74 ± 0.15c
5.0 IBA	66.67 ± 2.52b	17.27 ± 2.10a	1.50 ± 0.21c
1.0 NAA	80.00 ± 4.36ab	4.20 ± 1.24c	3.07 ± 0.27ab
2.5 NAA	93.33 ± 2.52ab	6.20 ± 0.75bc	1.85 ± 0.17bc
5.0 NAA	73.33 ± 2.52b	7.53 ± 0.57b	2.00 ± 0.19bc

Note: *Means followed by the same letter(s) within columns are not significantly different ($p \leq 0.05$) using DMRT. (Data for percentage of explants regenerating roots has been transformed using arcsine; data for mean number of roots per explant has been transformed using square root and data for mean length of roots has been transformed using square root).
Effects are measured in terms of quantity (%) of explants able to regenerate shoots, mean number of roots per explant and mean root length.

Most treatments showed no significant differences from each other in terms quantity (%) of explants able to regenerate, except for treatments 2.5 mg/L IBA which produces result (100% explants able to regenerate roots) significantly different from those of treatments 5.0 mg/L IBA (66.67% explants able to regenerate roots) and 5.0 mg/L NAA (73.33% explants able to regenerate roots) (Table 6). The latter two treatments (5.0 mg/L IBA and 5.0 mg/L NAA) do not significantly differ from each other in the quantity (%) explants able to regenerate roots. In general, most treatments produced high quantity (%) of explants able to regenerate roots except for treatments 5.0 mg/L IBA and 5.0 mg/L NAA which respectively yield relatively lower values of 66.67% and 73.33% (Table 6).

It can be clearly seen that both auxins showed relatively the same trend. The results show that as concentration increases from 1.0 mg/L to higher levels of 2.5 mg/L and 5.0 mg/L for both auxins, the quantity (%) of explants able to regenerate root increases (at 2.5 mg/L) followed by a decrease (at 5.0 mg/L). This shows that for both IBA and NAA, increasing the concentration to more than 2.5 mg/L tend to inhibit root formation in *A. heterophyllus*. Results for the mean number of roots per explant show that the values for mean number of roots per explants ranged from a high of 18.73 roots per explant (at 2.5 mg/L IBA) to 4.20 roots per explants (at 1.0 mg/L NAA). Statistical analyses using ANOVA (using square roots transformed data) and DMRT results have bundled the treatments into

groups. Within each group, mean number of roots per explant are not significantly different from one another (Table 6). Most groups are significantly different from each other in term of mean number of roots per explant, although there is a little overlapping between groups, whereby a mean within a group is statistically similar to a mean from another group. In general, IBA tends to produce significantly higher mean number of roots per explant (18.73 roots per explant to 9.13 roots per explant) compared with NAA (7.53 roots per explant to 4.20 roots per explant) (Table 6). The mean number of roots per explant for the control treatment which is devoid of any auxin (6.80 roots per explant) is generally significantly similar to values from NAA treatment but significantly lower than more values from IBA treatments.

As for the mean root length, overall results show a range of 3.07 cm (1.0 mg/L NAA) to 1.50 cm (5.0 mg/l IBA), not including the value from the control treatment (3.37 cm) (Table 6). The results for the control treatment (3.37 cm) is significantly higher than results from other treatment except 3.07 cm from treatment 1.0 mg/L NAA which is not significantly different. Most of the lower values are not significantly different from each other (Table 6). Results further indicate that mean root lengths for all NAA treatments are significantly higher than all values from IBA treatments, although IBA tend to result in significantly higher mean number of roots per explant (table 6). From this experiment it shows that IBA

is better than NAA for rooting of *A. heterophyllus* shoots as tap root (Figure 4(c)) was also obtained which is important to support the plant later on.

Acclimatisation

The survival rate (%) of *A. heterophyllus* plantlets was observed 8 weeks after transplanting them into different potting mixtures. The results for this experiment are show in Table 7.

The 0% survival rate for peat moss + sand at 1:1 (v/v) was due to the fact that all plantlets did not survive (Table 5). The results showed that survival rates range from 88.89% (organic soil + top soil at 1:1 ratio)

to 22.22% (Organic matter + topsoil + sand at 1:1:1 ratio). The survival rate of 88.99% in organic soil+topsoil (1:1) was significantly higher than survival the 44.44% survival rate in perlite+sand (1:1) and the 22.22% survival rate in organic matter+topsoil+soil (1:1:1). The survival rates in the latter two potting mixtures (perlite+sand at 1:1 ratio and organic matter + topsoil + sand at 1:1:1 ration) were not significantly different from each other. The results thus showed that organic soil + topsoil (1:1) is the best potting mixture for growth for *A heterophyllus*. Figure 4(d) shows a plantlet surviving in the best potting mixture of (organic soil + topsoil (1:1)).

Table 7

Survival (%) of A. heterophyllus *plantlets after 8 weeks of acclimatisation in different potting mixtures*

Potting mixture	Survived plantlets (%)
Organic soil + topsoil (1:1)	88.89 ± 5.56a
Perlite + sand (1:1)	44.44 ± 5.56b
Peat moss + sand (1:1)	0.00 ± 0.00c
Organic matter + topsoil + sand (1:1:1)	22.22 ± 5.56b

Note: *Means followed by the same letter(s) within columns are not significantly different (p≤0.05) using DMRT. (Data for percentage of explants regenerating roots has been transformed using arcsine)
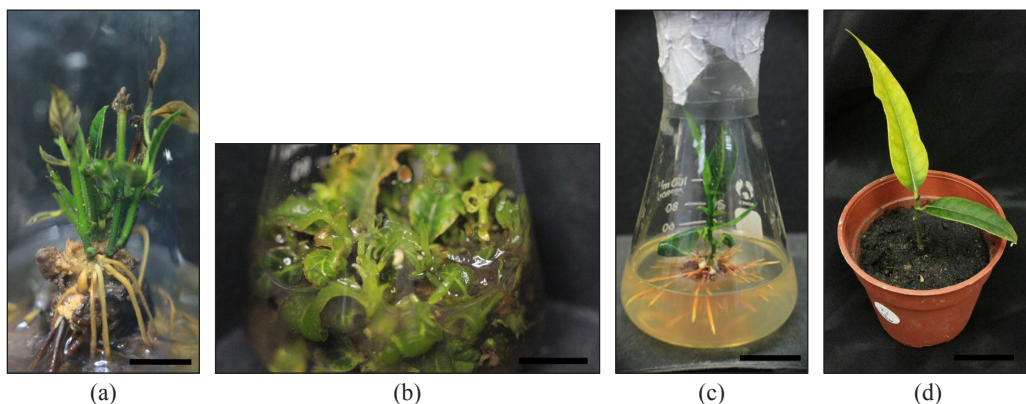

Figure 4. Micropropagation stages of *A. heterophyllus*: (a) Shoot induction after 8 weeks of culture on 2.5 mg/L BAP; (b) Shoot multiplication on 1.0 mg/L BAP after 12 weeks of culture; (c) Rooting in 2.5 mg/L IBA after 8 weeks of culture; (d) Plantlet surviving in a potting mixture consisting of organic soil + topsoil (1:1) after 8 weeks of acclimatisation

DISCUSSION

Plants growing in the external environment are invariably contaminated with micro-organisms and pests (George, Hall, & De Klerk, 2008). Anabestani, Behjatnia, Izadpanah, Tabein and Accotto (2017) reported pathogenic seed infection had a negative effect on the development of seed and plant. Thus, it is crucial to eliminate pathogens at the seed level through surface sterilisation for tissue culture. In this study, the experiment on optimisation of culture sterilisation showed the sterilization regimes of 40% Clorox for 20 min + 20% Clorox for 15 min and 50% Clorox for 20 min + 20% Clorox for 15 min can eliminate contamination. Both sterilisation treatments showed low rate (%) of contamination and high rate (%) of survival.

In comparison to previous works, Choy and Chan (2005) had used 20% Clorox for 10 min followed by 10% Clorox for 10 min for sterilising the apical shoots taken from a 12-year-old *A. heterophyllus* tree. Their sterilisation procedure was evidently different from the one used in this experiment. According to Abd El-Zaher (2008), different sterilisation procedures required by different types of explant could be maybe due to the various anatomical structures that these explants had. According to Lizarraga, Tovar, Jayasinghe and Dodds (1986) ethanol was used because it has a wetting property that can easily penetrate between leaf hairs and the wet surface of explants. Additionally, ethanol can partially remove hydrophobic waxes and resins which protect the microorganisms

from getting in contact with sterilant (Kunneman & Faaij-Groenen, 1988). On the other hand, sodium hypochlorite, also known commercially as Clorox, is mainly used as a surface sterilising agent in plant cell and tissue culture experiments (Çölgeçen, Koca, & Toker, 2011), and it is known to be an effective and powerful antimicrobial agent (Abd El-Zaher, 2008). The active components in Clorox bleach are water, sodium hypochlorite (it whitens and kills bacteria), sodium chloride, sodium carbonate (it removes alcohol and grease stains), sodium chlorate, sodium hydroxide (it removes soils that are fatty, oily, or acidic), and sodium polyacrylate (Anonymous, Wikipedia, 2018). Mng'omba, Sileshi, Toit and Akinnifesi (2012) reported that Clorox acts as an oxidising agent that kills a large range of pathogens. George (1993) reported that Clorox has the potential as a germicide which is related to its oxidation reaction properties.

In the experiment on shoot proliferation from seeds, results showed that the shoot-inducing regime of 10.0 mg/L BAP induced the highest number of shoots. Nonetheless, 2.5 mg/L BAP produced higher number of shoots of suitable length for multiplication. The discrepancy in 10.0 mg/L BAP regime is probably caused by the competition among the shoots in absorbing the hormones and nutrients from the agar which resulted in shorter shoots even though the number of shoots produced was higher. Ashraf, Aziz, Kemat and Ismail (2014) reported the same observation in *Chlorophytum borivilianum* when BAP concentration was increased.

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Thomas (2003), in contrast, obtained the best result of shoot induction using 1.2 mg/L BAP from cotyledon explants of *Morus alba*. Verma, Choudhary, Ashish, Kumar and Lal (2015) used seeds of ripened fruits of *A. lakoocha* for preparation of nodal segments as explants for synthetic seed production. Hassan and Khatun (2010) used germinated seeds of *Ficus glomerata* to produce shoot tips and node explants for adventitious shoot regeneration. The BAP requirement could be different for different species even though they are from the same family. Buah et al. (2010) mentioned that the variation in performance of *in vitro* cultures could be due to the differences in the genomic constitution or phenolic contents of a cultivar or species. Arinaitwe, Rubaihayo and Magambo (2000) studied proliferation rate effects of cytokinins on banana (Musa spp.) cultivars and reported that shoot proliferation is cultivar-dependent.

Cytokinin promote the growth of axillary buds by reducing the apical dominance of buds during the micropropagation phase (Van Staden, Zazimalova, & George, 2008). In addition, cytokinin are known to stimulate plant cell division as well as their involvement in releasing lateral bud dormancy, inducing adventitious bud formation, inducing growth of lateral buds, and their role in the cell cycle control (Melara & Arias, 2009). However, based on observation in this experiment, BAP, as a cytokinin, appears superior to kinetin as BAP produced higher mean number of shoots per explant as well as higher mean shoot length. Bogaert, Van Cauter,

Werbrouck and Dolezal (2006) stated that kinetin has a relatively low biological activity in certain bio-assays. Within the cytokinin group, BAP is more broadly used for in vitro shoot induction compared to other cytokinin as it exhibits higher shoot induction in many taxa (Isah & Mujib, 2013).

In the experiment on effects of shoot tip and different node positions on shoot regeneration of *A. heterophyllus,* the results obtained were similar to those of Rahman and Blake (1988). The researchers studied the effects of the same parameters on *A. heterophyllus* and found the number of shoots produced by nodal explants was not significantly different from the number of shoots produced from shoot tips, although numerically more shoots were produced by nodal explants than by shoot tips. However, the results on shoot length obtained in this study differed from those of Rahman and Blake (1988) who reported that shoot tip produced higher shoot length. The difference could be due to the fact that the cultivars used in this study are different from those used in the study by Rahman and Blake (1988). Apart from that, the varied response of different explant positions may be due to the variation in the endogenous auxin level of buds in different regions of the stem (Lane, 1978). Shirdel, Motallebi-Azar, Matloobi and Zaare-Nahandi (2013) also observed significant differences among node positions of Dog rose (*Rosa canina*), with the lower nodal position (node 2) having the longest shoot as well as having the highest number of shoots when cultured

in 6 mg/L BAP. Shekafandeh and Khosh-Khui (2008) also observed node 2 having the maximum number of shoots and shoot length in their study on effect of bud position in guava (*Psidium guajava* L.). Yadav, Lal and Jaiswal (1990) stated that shoot tips of *Syzigium cuminii* were less responsive than nodal explants. This is perhaps caused by the differences in the physiological state of the two explant types. Shoot induction from axillary or nodal bud is one of the most efficient methods of micropropagation in plants, considering the fact that buds emerging especially from meristematic tissues possess great potential for vigorous development due to their totipotency ability (Yadav, Malan, & Rajam, 1995). Taiz and Zeiger (2002) reported that carbohydrates played an important role in providing normal growth and development of shoots emerging from the nodes. Chern, Hosokawa, Cherubini, & Cline (1993) added that axillary bud growth was influenced by the node position in *Ipomoea nil*. They also observed that outgrowth of axillary buds at the lower part was reduced compared to that of the higher nodes. In addition, Punyarani and Sharma (2010) mentioned that cutting the stem of *Costus speciosus* into different node positions and then culturing them on suitable medium supplemented with suitable PGRs could break the dormancy of the bud, resulting in greater shoot proliferation. They also concluded that bud-break was affected by the position of the nodal buds on the stem.

Since there is no report on decapitation of *Artocarpus* species or any other species from the Moraceae family, the results obtained in this study were compared with those obtained from studies of plants of different families. For example, the findings from this study were similar to that reported by Singh and Tiwari (2012) who showed high frequency of shoot regeneration from decapitated embryonic axes of *Clitoria ternatea* in 2.0 mg/L BAP. Pumisutapon, Visser, & Klerk (2009) reported that decapitated rhizome of *Alstroemeria* showed the highest bud outgrowth whereby the axillary buds were released when rhizome tip and shoot tips were removed. Ngamau (2001) observed that seedling decapitation in *Zantedeschia aethiopica* 'Green Goddess' also resulted in an increased number of axillary shoot production. El Boullani, Elmoslih, El Finti, El Mousadik and Serghini (2012) reported that artichoke (*Cynara cardunculus* var. *scolymus* L.) seedlings produced greater shoot proliferation ratio (17 shoots per explant) when decapitated. Vieitez, Pintos, San-José and Ballester (1993) tested the effects of decapitation on a woody plant, *Quercus rubra* (Red Oak) and was able to attain vigorous axillary bud growth on woody plant medium with 0.2 mg/L BAP. Cline (1994) described apical dominance as the control exerted by the terminal bud (or shoot apex) over the outgrowth of lateral buds in order for plants to grow vertically. Apical dominance is said to significantly lessen shoot branching (Bressan, Kim, Hyndman, Hasegawa, & Bressan, 1982; Kucharska, Golis, Podwyszyńska, Wiśniewska-Grzeszkiewicz, & Orlikowska, 2000; Voyiatzi, Voyiatzis

and Tsiakmaki, 1995). Based on the auxin-inhibition hypothesis of apical dominance, Prasad *et al.* (1993) stated that auxin flowed down the stem and inhibits axillary bud outgrowth, either directly or indirectly. Dun *et al.* (2006) reported that decapitation had been widely used to study bud outgrowth which was best demonstrated via shoot tip removal. Tezuka *et al.* (2011) hypothesised that endogenous cytokinin synthesised after decapitation caused the promotive effect of CDM (complete decapitation method) on shoot regeneration. According to Punyarani, and Sharma (2010), it was possible to break bud dormancy in cultured nodal segments due to its separation from the shoot apex.

Punyarani, and Sharma (2010) in their experiment on shoot multiplication, observed that 1.0 mg/L BAP gave the highest mean number of shoots. The results were consistent with those of Amin and Jaiswal (1993) who also reported that 4.5 μM BAP (1.0 mg/L BAP) gave the highest multiplication of shoots of *A. heterophyllus*. However, Ashrafuzzaman, Kar and Prodhan (2012) and Khan, Rahman, Abbasi, Ibrahim and Abbas (2010) reported producing high shoot multiplication of *A. heterophyllus* on medium containing 2.0 mg/L BAP and 1.5 mg/L BAP respectively. Choy and Chan (2005) reported that 4.5 mg/L BAP was the best treatment in producing the most number of shoots per explant in *A. heterophyllus*. BAP is suitable for shoot regeneration and multiplication for most plants. Kumar, Krishna, Pradeepa, Kumar and Gnanesh (2012) reported that the

effects of BAP perhaps lied in its capability to trigger plant tissues to metabolise the natural endogenous hormones for shoot organogenesis induction. Taiz and Zeiger (1998) opined the tremendous activity of this plant growth regulator was probably related to its chemical structure, but high concentrations could induce reduction in shoot height and increase in hyperhydricity rates.

The highest mean shoot length obtained in this study was in 5.0 mg/L BAP. Choy and Chan (2005) showed the best mean shoot length in 4.0 mg/L BAP. Amin and Jaiswal (1993) and Ashrafuzzaman *et al.* (2012) obtained the best shoot length in 2.0 mg/L BAP. Meanwhile, Amany, Ali and Boshra (2007) achieved the best shoot length in 3.0 mg/L BAP. The difference may be due to different varieties of *A. heterophyllus* used in these studies which gave different responses. Lima da Silva, Rogalski and Guerra (2003) found the mean height of shoots of Prunus 'Capdeboscq' was reduced with increased concentration of the different cytokinin used and similar results were observed in this study. This is probably caused by competition among the proliferated shoots in absorbing hormones and nutrients from the agar (Ali, Mulwa, Norton, & Skirvin, 2003). Ledbetter and Preece (2004) in their work on *Hydrangea quercifolia* reported that BAP was more effective than TDZ as shoots in TDZ failed to elongate. Similar results were also obtained in this study for *A. heterophyllus* using BAP and TDZ.

In the experiment on rooting, the results of this study showed that 2.5 mg/L IBA was the best treatment as it produced the highest number of roots per explant with 100% of explants regenerating roots. Abd El-Zaher (2008) recommended half-strength MS + 3 mg/L IBA as the optimal treatment for producing good rooting in *A. heterophyllus*. On the other hand, Amany *et al*. (2007) reported that half-strength MS supplemented with 1.5 mg/L IBA produced the highest number of roots per explant in jackfruit while full MS medium supplemented with 1.5 mg/L NAA gave the greatest root length. Ashrafuzzaman *et al*. (2012) reported that half-strength MS supplemented with 2.0 mg/L IBA was the best in producing the highest number of roots for jackfruit.

Apart from playing a role in root formation, auxins also exert a strong influence on initiation of cell division, meristem organisation giving rise to unorganised tissue (callus) or defined organs (shoots), cell expansion, cell wall acidification, apical dominance and promotion of vascular differentiation (Gaspar et al., 1996, 2003). In this study, the highest mean root length was obtained in half-strength MS medium without hormone (control treatment). Ashrafuzzaman *et al*. (2012), however, obtained the best mean root length for jackfruit in half-strength MS medium supplemented with 2.0 mg/L IBA while Amin and Jaiswal (1993) obtained it in half-strength MS medium supplemented with 2.25 mg/L IBA. Earlier, Epstein and

Ludwig-Muller (1993) stated that the application of IBA to cuttings of many plant species resulted in the induction of adventitious roots, which in many cases was more effective than IAA.

According to Wareing and Phillips (1981), increasing the concentration of IBA from 10^{-7} to 10^{-5} M increased the number of roots, but the length was significantly decreased at the highest concentration. Such inhibition of root development by high concentrations of auxin may be due to the enhancement of ethylene biosynthesis in the root tissues. Sevik and Guney (2013) stated that auxins control the growth and development of roots in plants, including lateral root initiation and root gravity response. Generally, high level of auxin promoted the production of adventitious roots, despite the fact that it also inhibited root elongation (Blakesley, Weston, & Hall, 1991).

The results on acclimatisation of plantlets in this study were in accordance with that of Choy and Chan (2005) who also reported 70-80% survival of *A. heterophyllus* plantlets after 3 – 4 weeks maintenance in a mixture of organic soil + topsoil (1:1) in a chamber. On the other hand, Abd El-Zaher (2008) obtained the lowest survival percentage of *A. heterophyllus* plantlets in potting mixture consisting of peat moss + sand (1:1). In this study, the same potting mixture comprising peat moss + sand (1:1) resulted in no survival. Amin and Jaiswal (1993) reported that regenerated plantlets of jackfruit transferred to the soil resulted

in 50% survival rate. Roy, Rahmanand and Majumdar (1990) reported that they succeeded in the acclimatisation of jackfruit using potting mixture of sterile sand + soil + humus (1:2:1) where 75% of the plants survived.

CONCLUSION

This study showed the protocol for producing mass production of *A. heterophyllus* was successful in a short period of time. The technique can be used to produce clonal materials in place of the present method for propagating jackfruits using seeds. In this way, the seed recalcitrant problem and the lengthy grafting method often faced by farmers can be overcome. The established tissue culture protocol can be a platform for future transformation or future related studies on *A. heterophyllus*. As hormone combination was not tested during this study period, future study should consider that. Additionally, micrografting can be done using this protocol.

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