HAYATI Journal of Biosciences December 2014
Vol. 21 No. 4, p 151-158
EISSN: 2086-4094

Available online at:
http://journal.ipb.ac.id/index.php/hayati
DOI: 10.4308/hjb.21.4.151

In Vitro Growth and Rooting of Mangosteen (*Garcinia mangostana* L.) on Medium with Different Concentrations of Plant Growth Regulator

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Received October 1, 2013/Accepted September 29, 2014

Propagation of mangosteen is challenging for many reasons, including limited seed set, slow rate of seedling growth, and difficulty with root formations. The objective of this research was to find the best combination of medium and plant growth regulator for *in vitro* growth and rooting of mangosteeen seed. Various types of explant (a whole seed; seed divided into 2, 3, and 4 cross sections; seed divided into 2, 3, and 4 longitudinal sections) were treated with five concentrations of benzyl amino purine (BAP; 0, 2.5, 5, 7.5, 10 mg/L) for shoot induction in ½ Nitrogen (N) Murashige and Skoog (MS) medium. The shoots were rooted on MS and woody plant medium (WPM) media with several combinations of indole butyric acid (IBA) and naphtalene acetic acid (NAA). Treatments for root induction were applied as follows: (i) low dose, given during induction of rooting, (ii) soaking the base of the shoots in medium treated with a high dose of auxin for 5 days, and then growing the shoots in MS ½ N with 1 mg/L NAA + 1 mg/L BAP medium. Our result show that BAP positively affected mangosteen bud growth. The best medium for mangosteen shoot regeneration was found to be MS ½ N + 5 mg/L BAP. This medium induced the highest number of shoots from the seed explant cut into four cross sections. We found the best medium to induce *in vitro* rooting of mangosteen shoot was MS ½ N + 3 mg/L indole butiric acid (IBA) + 4 mg/L NAA medium. Some treatment negatively affected growth. Soaking the mangosteen shoot base in a medium with an overly high dose of auxin seemed to disrupt and inhibit growth of the mangosteen shoot.

Key words: mangosteen, tissue culture, auxin, cytokinin

INTRODUCTION

Mangosteen, known locally as ”the Queen and the Finest of Tropical Fruits”, is native to Indonesia. Although the fruit has been recognized as an export commodity, production remains relatively low because mangosteen plantations are still traditionally managed (Dorly *et al*. 2008; Wulandari & Poerwanto 2010). In addition, many challenges have been encountered in attempts to increase economic development of the fruit (Sobir & Poerwanto 2007), such as selection of inappropriate cultivars (Liferdi *et al*. 2008), and low fruit production and quality (Efendi & Hermawati 2010). Some of these challenges can be traced to various physiological problems. Among these are low rate of photosynthesis, low rate of cell division in the shoot meristem, low seed production, long period of seed dormancy, slow seedling growth, problem with grafting compatibility, and poor root systems (Ray *et al*. 2006; Mansyah *et al*. 2010). Jawal and Syah (2008) used related species of *Garcinia* for double rootstock grafting to accelerate seedling growth and seedling availability. They experienced greater success with *Kandis pariaman, G. mangostana, G. dulcis*, compared with other mangosteen relatives. Other research has investigated possible methods to developed large quantities of mangosteen propagules as acclaimed by earlier researchers, but plant material (mangosteens) were not widely available (Sirchi *et al*. 2008b; Harahap *et al.* 2012).

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The poor root system found in mangosteen seedlings represents a significant problem in mangosteen propagation, becauce the roots growth was very slow. *In vitro* propagation through tissue culture techniques could be an alternative solution to solve the problem. The technique may also help production of more uniform and high quality seedlings. However, until present, rapid shoot proliferation and satisfactory rooting result have not been achieved (Harahap *et al.* 2012).

Some groups of plant growth regulator (PGR), such as auxins and cytokinins, are very effective for inducing organogenesis. Zuraida *et al.* (2011) claimed that 5 mg/L benzyl amino purine (BAP) is required to increase shoot development in pineapple plants during the *in vitro* stage. Rostika *et al.* (2008) reported that the highest number of mangosteen axillary buds were formed using a medium containing 3 mg/L benzyl adenine (BA). The objective of this research was to determine the best PGR combination for *in vitro* shoot and root inductions from several explant types of mangosteen seed.

MATERIALS AND METHODS

Shoot Induction. Mangosteen seeds from plantations on Pancur Batu City, North Sumatera were used as plant material for shoot induction. Experimental treatment consisted of five concentrations application of BAP (0, 2.5, 5, 7.5, 10 mg/L) to 7 types of explant [whole seeds ("group W"); seeds divided into 2, 3, and 4 cross sections (groups CS2-CS4); and seeds divided into 2, 3, and 4 longitudinal sections (groups LS2-LS4)] (Figure 1).

The mangosteen seeds were consecutively sterilized with 5% detergent, 0.008% mankozeb, 0.002% streptomycin sulphate, and 1.05% NaClO, and followed by rinsing with sterile distilled water, and then soaked in 0.5% antibiotic (amoxicillin) for 2 h. Seeds were cut into sections according to the treatment, then placed on shoot induction medium according to the treatment (MS ½ N with five concentrations of BAP (0, 2.5, 5, 7.5, 10 mg/L). These samples were incubated in the culture room and maintained at 24 °C by regulating the room air conditioner. Light level were maintained by application of flourescent light of 3000-3200 lux in a 16 h photoperiod. The number of regenerated explants and number of shoots, leaves and internodes were measured weekly from 1 until 12 weeks after planting.

Root Induction. 3 cm shoot explants were used for root induction. The explants were rooted on Murashige and Skoog (MS) and Woody Plant Medium (WPM) media with several combinations of Indole Butyric Acid (IBA) and Naphtalene Acetic Acid (NAA). The composition of the rooting medium combinations were (1) MS + 4 mg/L IBA + 3 mg/L NAA, (2) MS ½ N + 4 mg/L IBA + 3 mg/L NAA (Sinaga 2002), (3) WPM + 4 mg/L IBA + 3 mg/L NAA, (4) MS ½ N + 3 mg/L IBA + 4 mg/L NAA (Sinaga 2002), (5) WPM+ 3 mg/L IBA + 4 mg/L NAA, (6) MS ½ N + 500 mg/L IAA, (7) MS ½ N + 1000 mg/L IAA, (8) MS ½ N + 500 mg/L IBA, (9) MS ½ N + 1000 mg/L IBA, (10) WPM + 500 mg/L NAA (Tarwiyani 2002), and (11) WPM + 1000 mg/L NAA. Treatments for root induction were: (i) application of low doses of IBA and NAA, given to the groups receiving medium #1-5, during

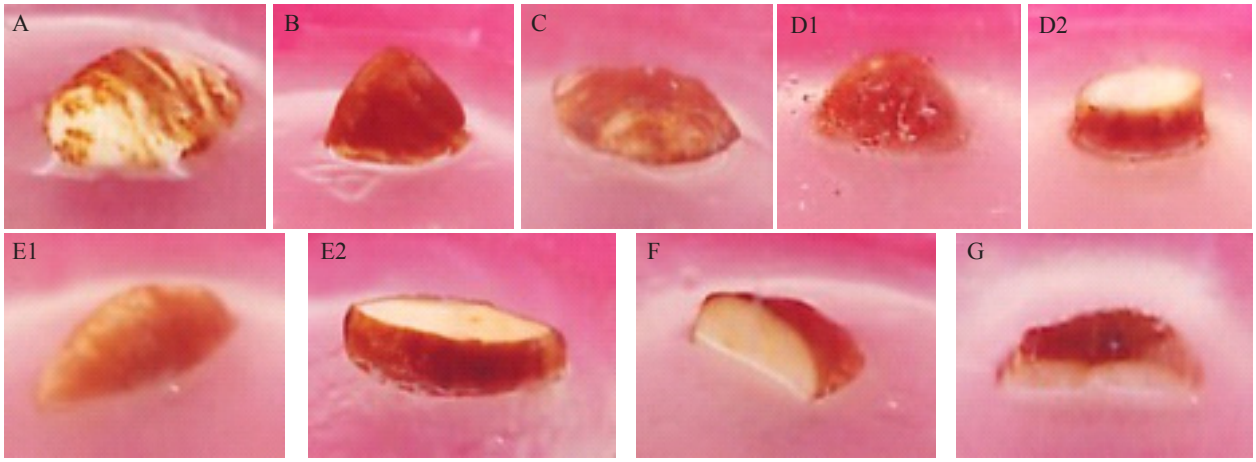

Figure 1. Pattern of cuts to mangosteen seeds; (A) whole (uncut seed), (B) divided into two cross sections, (C). divided into two longitudinal sections, (D1, D2) divided into three cross sections, (E1, E2) divided into three longitudinal sections, (F) divided into four cross sections, and (G) divided into four longitudinal sections.

the induction of roots; or (ii) soaking the base of the shoots in medium with a high dose of auxin for 5 days, and then transferring the shoots to MS ½ N + 1 mg/L BAP + 1 mg/L NAA medium for rooting until 12 weeks. This treatment was applied to groups receiving mediums #6-11. *In vitro* shoots were used as explants. The culture was maintained at 24 °C under flourescent light of 3000-3200 lux with a 16 h photoperiod as with the shoot induction method. Time of root appearance was observed weekly. The percentage of shoots that formed roots, and the number and length of roots, was measured at the end of 12 weeks after planting.

RESULTS

Shoot Induction. Shoots appeared earlier (2-3 weeks after planting) in all explant groups treated with BAP than in their untreated counter part (3-6 week after planting)**.** The smaller size of the explant, the later time of first shoot appearance. From visual observation, leaves appeared 1 to 2 weeks after bud appearance. Several buds produced normal roots from the base of the buds. Treatment groups receiving 5 mg/L BAP produced more roots compared with those receiving other concentrations of BAP. The highest concentration of BAP (10 mg/L) generally caused explant swelling with many initial buds visible, however those buds did not grow into shoots, or they produced stunted or dwarf shoots.

Bud Formation. Not all explants were able to form buds. Among explants planted on medium without BAP, only 41.43% among those formed buds, whereas 91.43% of explants planted on medium containing 5 mg/L BAP were able to form buds (Figure 2). Interestingly, all explants from the CS4 groups that were treated with 5 mg/L BAP were able to form a bud (Figure 3), but the same explants grown on medium with other BAP concentrations were not able to form a bud. This indicates that BAP treatment greatly improved bud formation in the seed explants

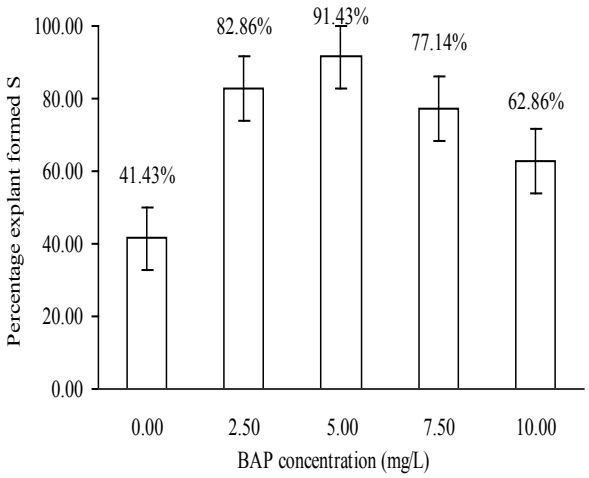

Figure 2. Effect of treatment with varied BAP concentrations on the percentage of explants forming shoots.

Number of Shoot, Leaves, and Internodes. The number of shoots was significantly affected by the interaction between BAP concentrations and types of explant. The highest number of shoots (11.80) was found in the CS4 group, cultured on medium containing 5 mg/L BAP (Figure 4). The number of leaves and internodes were significanly affected only by BAP concentration and not by explant group. Treatment with 5 mg/L BAP treatment produces the

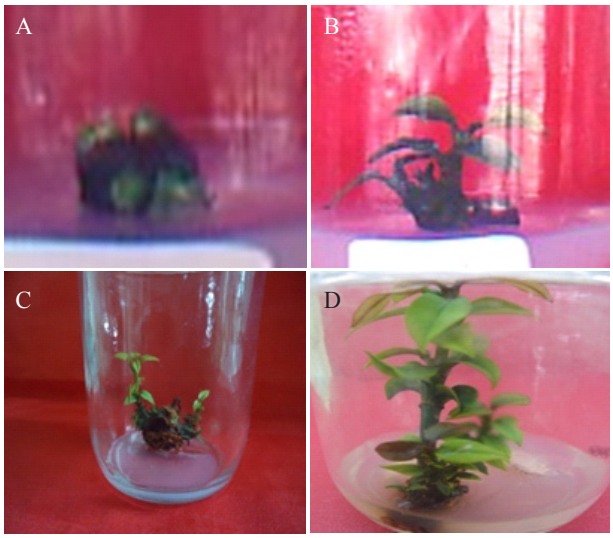

Figure 3. *In vitro* treatment of mangosteen explant with MS 1/2N + 5 mg/L BAP medium, seed divided into four cross sections: (A). Swelling of the explants with early bud (4 week after planting), (B). New shoot formation (6 weeks after planting), (C). Continued shoot growth (8 weeks after planting), (D). Shoot at final observation 12 week after planting.

Table 1. The effect of BAP concentration and explant types on the average number of leaves and internodes number (12 weeks after planting)

BAP (mg/L)	Leaf number	Internode number
0	1.91 ± 0.25c	1.94 ± 0.11c
2.5	2.94 ± 0.14b	2.46 ± 0.08b
5	3.51 ± 0.23a	2.78 ± 0.26a
7.5	2.83 ± 0.11b	2.49 ± 0.09b
10	2.00 ± 0.38c	2.02 ± 0.19c
Type of explants		
1	2.56 ± 0.42	2.27 ± 0.21
2	2.80 ± 0.28	2.44 ± 0.18
3	2.54 ± 0.42	2.30 ± 0.07
4	2.74 ± 0.00	2.37 ± 0.00
5	2.80 ± 0.28	2.43 ± 0.14
6	2.52 ± 0.00	2.31 ± 0.00
7	2.52 ± 0.14	2.25 ± 0.07

Type of explants. 1= whole, 2 = divided into two cross sections, 3 = divided into two longitudinal sections, 4 = divided into three cross sections, 5 = divided into three longitudinal sections, 6 = divided into four cross sections, 7 = divided into four longitudinal sections. Values followed by the same letter in the same column are not significantly different at 5% DMRT.

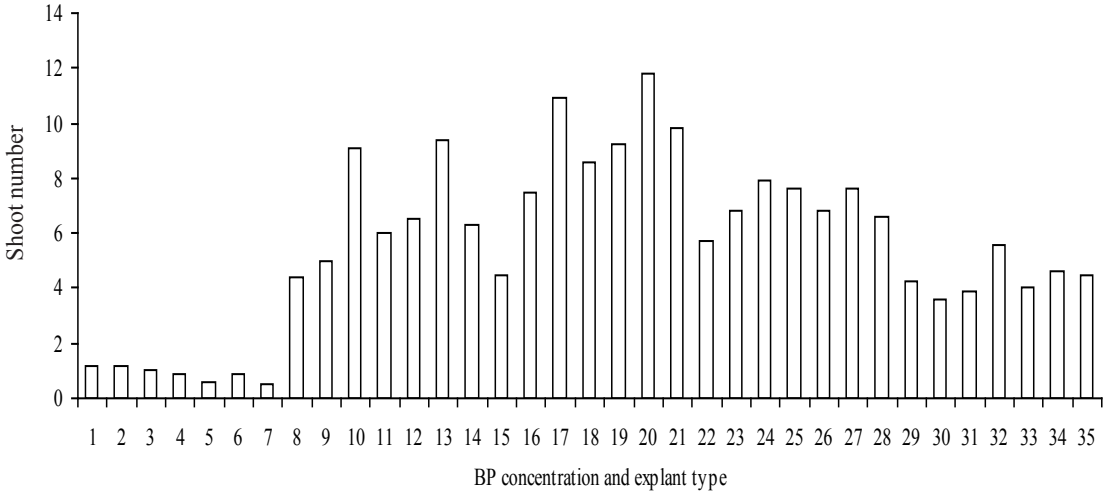

Figure 4. Effect of BAP and explant types to shoot number (12 weeks after planting). 1-7 = 0 ppm BAP with the whole seed, seed division type CS 2, 3, and 4, seed division type LS 2, 3, and 4; 8-14 = 2.5 ppm BAP with the whole seed, seed division type CS 2, 3, and 4, seed division type LS 2, 3, and 4; 15-21 = 5 ppm BAP with the whole seed, seed division type CS 2, 3, and 4, seed division type LS 2, 3, and 4; 22-28 = 7.5 ppm BAP with the whole seed; seed division type CS 2, 3, and 4, seed division type LS 2, 3, and 4; 29-35 = 10 ppm BAP with the whole seed, seed division type CS 2, 3, and 4, seed division type LS 2, 3, and 4.

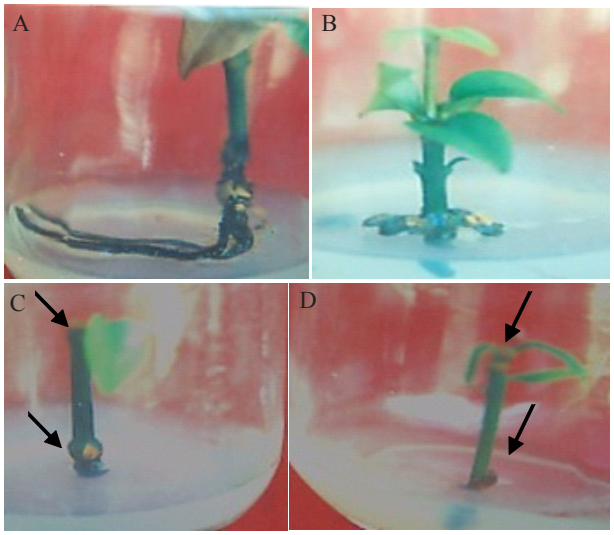

Figure 5. Shoots in treatment rooting medium. (A). The MS ½ N + IBA 3 mg/L + NAA 4 mg/L rooting medium, 12 weeks after treatment; (B). The MS + IBA 4 mg/L + NAA 3 mg/L rooting medium, 12 weeks after treatment; (C) The MS ½ N + IAA 1000 mg/L rooting medium, 4 weeks after treatment; (D) The MS ½ N + IBA 500 mg/L rooting medium, 12 weeks after treatment (A and B: roots grow from under base of the shoot, C and D: roots emerge above the base of the shoot, leaves fall.

highest average number of leaves and internodes: 3.51 leaves and 2.78 per shoot, respectively (Table 1). Different explant groups did not significantly differ with regard to number of leaves and internodes per shoot.

Root Induction. The specific combination of PGR significantly affected the time of root appearance, the percentage of root shoot, and the lenght of the roots. Based on visual observation, the roots that grew from the shoot explants that were cultured on MS ½ N + 3 mg/L IBA + 4 mg/L NAA medium looked better than the roots grown from the explants cultured on other media, and generally the roots from this medium, continued to grow until the last day of the experiment. This medium induced root formation within 3 weeks after planting, with the highest percentage of rooted shoot (85%) and the longest root length (1.49 cm) measured at the final observation.

Groups treated by soaking the base of the shoot with a high dose of auxin for 5 days, and then transferring the shoot to MS ½ N + 1 mg/L BAP + 1 mg/L NAA medium for rooting for 12 weeks (mediums #6 - 11), did not fare well. The bottom of the shoots became wrinkled and brown in colour. Roots did not appear near the bottom of the shoot, but at the top of shoot base. However, all the roots grew normally. We found that the leaves which formed on the plants in this group generally fell off several weeks later (between week 4 and 6). One to 2 new leaves appeared there after, but these leaves did not grow to maximum size (Figure 5C,D). Several shoots formed auxiliary buds but these also did not grow to maximum size (Figure 5D). The growth of mangosteen shoots in these groups was inhibited.

DISCUSSION

The result of our study showed that bud initiation from mangosteen seed was induced and enhanced by BAP. The seed explants treated with BAP formed buds sooner than those in control (untreated seed explant) groups. There seem to be limits to the benefit of BAP at high doses. High concentrations of BAP caused treated explants to swell and to produce many

initial buds, however those buds either did not grow into shoots, or the shoots appeared stunted. The size of the explant also correlated with the speed of shoot initiation. The smaller explant, the longer the delay before shoot initiation from the bud. BAP is a cytokinin, a type of phytohormone that induces cell division. It has been suggested that BAP may function by inducing cell division, manifested by initial bud formation and appearance. However, there may be obstacles to the further processes of differentiation, such that these buds do not develop into shoots. We found this phenomenon in our explant groups treated with high concentration of BAP. This study also indicates that BAP is a plant growth regulator for leaf induction and for internode formation from the shoots.

Past research indicates that there are optimum concentrations of BAP that work for specific plants, and specifically for mangosteen shoot induction. Explants treated in WPM medium with 0.5 mg/L BAP concentration produced the highest percentage of nodular calluses that form mangosteen shoots (Qasim *et al.* 2005; Qasim 2007). Zuraida *et al.* (2011) reported that a high concentration of BAP in medium induced more shoots in mangosteen explants, than did a low concentration of BAP. The study conducted by Harahap *et al.* (2012) found that 2 mg/L BAP stimulates the formation of mangosteen shoots from explants 2 cm in size. Usman *et al*. (2013) found that MS supplemented with BA (5 µM) produced the highest number of pineapple plantlets in his treatment groups. Zuraida *et al.* (2012) also reported that the proliferatoin of multiple shoots (*Pelargonium radula*) from nodal segments was the highest in MS medium supplemented with 0.5 mg/L BAP.

In the present study, explants treated with 5 mg/L BAP treatment were nearly all able to form shoots (91.43%), while explants treated at other BAP concentration (0, 2.5, 7.5, 10 mg/L) formed shoots at a lower rate (Figure 3). This suggests that the optimal BAP concentration for mangosteen shoot formation from seed explants is 5 mg/L BAP. Rostika *et al.* (2008) reported that the highest percentage of mangosteen seed growth and number of shoots formed per seed was obtained from seeds cultured on a basal medium containing 5 mg/L BA. However, Sirchi *et al.* (2008a) found that 2.0 mg/L BAP could induce shoot formation 60.2% from seed explants, it is most successfull groups of explans. With regard to other plants, Zuraida *et al.* (2012) reported that both liquid and solid media supplemented with 1 or 5 mg/L BAP, respectively, comprise the optimum conditions for pineapple shoot propagation.

Visual observation of the group treated with 7.5 mg/L BAP revealed many stunted/stubby buds and more calluses than in other groups. This condition occurs when the explant treated with high BAP concentration under goes continuous cell division, hindering any further differentiation process.

In the present study, we found that the CS4 group treated with 5 mg/L BAP produced the highest number of shoots (11.8 shoot; Table 1), while the LS4 group without BAP treatment produced the lowest average number of shoots (0.5 shoots). This maybe, because in the medium without BAP did not supply cytokinine that required for cell division.

Harahap *et al.* (2012) reported that the ability of seeds to form vegetative organs was influenced not only by the application of plant growth regulator, but also by other factors such as explant size. An explant 2 cm in size and treated with 4 ppm BA, produced shoots sooner than explants 1 cm in size. This can be explained as a function of the additional tissue in found in larger explants. Cultured mangosteen seeds basically already contain an embryo, that will developed into the buds. Two cm explants, which comprise almost 2/3 of each seed, certainly contained more ready tissue to form shoots compared to the explants of 1 cm in size.

The effect of BAP supplementation on the number of internodes was comparable with the effect of BAP on the increase of number of the leaves. This is because an increase in number of the internodes is always followed by an increase in the number of the leaves. Harahap (2011) notes that a very important role of cytokinin is to regulate cell division. In the present study, explants treated with 5 mg/L BAP produced more internodes and leaves than the groups receiving other BAP concentration treatment (0, 2.5, 7.5, 10 mg/L). We concluded that the optimum concentration of BAP to induce shoots, internodes and leaves from mangosteen seed explants is 5 mg/L, on medium MS with ½ N, and the best combination of medium and explant for *in vitro* mangosteen shoot induction was MS ½ N + 5 mg/L BAP with seed explants divided into four cross sections (the CS4 group).

This research indicates that, the types of medium generally has a very significant effect on the number of roots formed. Groups treated with MS ½ N + IBA 3 mg/L + NAA 4 mg/L medium, produced the highest number of roots per shoot (average = 1.05/shoot) and those treated with the MS ½ N + IBA 500 mg/L medium obtained the lowest (average = 0.3 roots/ shoot) (Table 1).

Table 3. The time of root appearance, percentage of rooted shoots, number of roots and root length (at 12 weeks after planting)

Medium	Medium number	Time of root appearance (weeks after planting)	% rooted shoots	Root number	Root length
MS + IBA 4 mg/L + NAA 3 mg/L	1	4	75	1.00 ± 0.29ab	1.16 ± 0.14a
MS ½ N + IBA 4 mg/L + NAA 3 mg/L	2	4	80	0.90 ± 0.36ab	1.10 ± 0.12a
WPM + IBA 4 mg/L +NAA 3 mg/L	3	4	85	0.90 ± 0.4ab	1.04 ± 0.19a
MS ½ N + IBA 3 mg/L + NAA 4 mg/L	4	3	85	1.05 ± 0.42a	1.49 ± 0.92a
WPM + IBA 3 mg/L + NAA 4 mg/L	5	3	80	0.90 ± 0.36ab	1.07 ± 0.21a
MS ½ N + IAA 500 mg/L	6	2	60	0.60 ± 0.49abc	0.41 ± 0.17b
MS ½ N + IAA 1000 mg/L	7	3	60	0.60 ± 0.49abc	0.34 ± 0.11b
MS ½ N + IBA 500 mg/L	8	2	30	0.30 ± 0.46c	0.36 ± 0.12b
MS ½ N + IBA 1000 mg/L	9	3	35	0.35 ± 0.48c	0.29 ± 0.10b
WPM + NAA 500 mg/L	10	2	55	0.45 ± 0.5bc	0.28 ± 0.11b
WPM + NAA 1000 mg/L	11	3	55	0.45 ± 0.5bc	0.48 ± 0.14b

In our study, not all mangosteen shoots could form roots, and only a small number of roots were formed (generally 1 or 2). The explants in our study rooted slowly, and with difficulty. This was due to the poor quality of the mangosteen root systems which developed very slowly and never developed healthy "hairy" roots. Our research showed that, despite the successful propagation of healthy shoots capable of forming roots, plant growth regulator is needed to induced the formation of roots from the shoots. Specifically, auxin has been shown to be effective in root induction. In the present research we used IBA and NAA. IBA and NAA are known as auxin that have a slow translocation, high persistent activities, and slow activity so that it required time for rooting mangosteen.

Our results showed that medium type had a significant effect on root length. The longest root (1.49 cm) grew from a shoot cultured on MS ½ N medium containing 3 mg/L IBA + 4 mg/L NAA (Table 3). According to Karjadi and Buchori (2007) root formation requires a high ratio of auxin to cytokinin. To maximize root length, auxin must be present as an important initiator of root growth, while cytokinin hormone should be reduced or eliminated. Sirchi *et al*. (2008a) achieved the best result for mangosteen root formation (90.4%) with explants cultured on one-quarter strength of MS salt medium supplemented with 0.1 mg/L NAA. Nisa and Rodinah (2005) found an interactive effect with NAA and Kinetin on *Musa paradisiaca* L. rooting.

IBA and NAA have been used to promote root growth in both of woody and herbaceus plants (Harahap 2011). Rostika *et al.* (2008) noted that auxin is required to induce rooting, and achieved 75% rooting in mangosteens using MS medium with 5 mg/L IBA. This medium was also very effective in increasing the absorption of nutrients from the mangosteen seeds.

In addition to IBA concentration, the period of pulse treatment has been shown to have significant effects on the average number of roots produced per shoot on *Catharanthus roseus* (Rupesh *et al.* 2013). Meera and Manjushri (2005) reported that when treating *Garcinia indica* Chois shoots with IBA, the longer the shoots were soaked in IBA, the greater the chance, the shoots would have two roots. However, in the present study, this did not occur. Commonly, explants treated with higher concentrations of IBA (containing auxin) and soaked for longer periods did not grow multiple roots. They did exhibit unconventional root growth, with roots forming above the base rather than from the bottom of the shoot base (Figure 5C,D).

Our result, indicated that mangosteen buds (*Garcinia mangostana* L.) require a long time to form roots. This parallels the finding of Pertamawati (2005) who reported new roots measuring only 4.7 mm long, maximum, from mangosteen shoots after 12 weeks of culturing. Another researcher suggests that the typically slow growth of mangosteen tissue might be caused by very low number of meristematic cells, or by the lack of an internal cofactor that acts as a precursor in the auxin biosynthesis. Auxin increases the rate of growth (Wulandari & Poerwanto 2010; Overvoorde *et al*. 2010).

Although auxin is clearly necessary for bud induction and root growth, at higher concentration auxin can actually disrupt and inhibit growth. Visual observation of explants pre-treated by soaking shoots in a liquid medium for 5 days, showed that the roots began to appear 2-3 weeks after transfer into MS ½ N + 1 mg/L BAP + 1 mg/L NAA medium for rooting. The base of the shoot would swell, eventually splitting to form roots. We found alteration in normal root appearance, with roots forming not out of the base of the shoot, but above the base, along the length of the shoot. In addition, the base of the shoot

changed appearance, becoming wrinkled and brown in color (Figure 5C,D). In groups treated with lower concentrations of auxin (< 5 mg/L), the shoots and roots kept a normal appearance, with roots emerging base of the shoot (Figure 5A,B). It is unclear why roots in the higher concentration groups emerged above the base of the shoot. It may have been due to high concentration of the IBA, NAA, and IAA, or because the auxin was absorbed and transported up the length of the shoots, inducing root growth above the base. Teale *et al.* (2005) reported that high concentration of auxin inhibits root elongation and stimulates cell differentiation.

Many prior studies have found that application of very high concentrations of auxin inhibits the growth of shoots directly, by retarding the rate of protoplasmic streaming. The highest concentrations tested in that experiment approached level that were definitely toxic to the plant material. Another effect seen in plant material treated with high concentrations of auxins, is the inhibition of the growth of parts morphologically above the point of the auxin application. Whether or not these phenomena related to high levels of auxin exposure have any bearing upon growth inhibitions is still not clear. They may play a part in pathological inhibition (Thimann 2008).

The shoots treated with high concentration auxin medium grew leaves that generally fell off the plant relatively soon. Some buds formed auxiliary shoots. Generally, in the plant treated with high level of auxin, apical buds fell out (Figure 5C,D) and were replaced by the appearance of auxiliary shoots. We have not yet as certained the precise mechanisms of this phenomenon, but possibly, it involves the plant nutrient transportation system. Disruption of auxin transport, caused too high auxin doses, may trigger metabolic dysfunction that causes the leaves fall. Overvoorde *et al.* (2010) noted that the combination of auxin transport, production, degradation, and conjugation in the cells of a plant will ultimately define, over time, the "auxin status" of a given cell, and the capacity of a given cell to generate a biochemical, physiological, or molecular response to the given level of auxin. These responses might be continuous, or defined by a threshold level of auxin, depending on the level and characteristics of the signaling components present in a given set of cells. In this research, treatment with overly high concentration of auxin caused leaves to fall. This result is unsurprising, given that we know high auxin concentration can disturb growth. (Gardner 1991) reported that treatment with high concentration of auxin can cause plant stress and inhibit growth. This condition illustrates that the metabolic disruption occurs at the shoot growth. The research by Teale (2005) with *Arabidopsis* mutants showed that, defects in either auxin transport or auxin responses also produced shoots with an altered morphology, and leaves with morphological defects.

ACKNOWLEDGEMENT

This study was supported by the Ministry of National Education of Indonesia, Directorate General of Higher Education, DP2M via Strategic Research Grant, contract number: 0187.0/023-04.2/II/2009 fiscal year 2009 and Fundamental Research Grant, contract number: 078/H33.8/SP2D/2010.

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