IN VITRO CULTURE OF MANGOSTEEN USING COMBINATION OF PLANT GROWTH REGULATOR AND ORGANIC MATTER

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ABSTRACT

The producing of high quality clones through tissue culture is expected to support the successful development and improvement in cultivation of mangosteen crop. This study was aimed to obtain the best combination of growth regulator and organic matter for induction of mangosteen shoots in vitro by using seed explants. The experiment was conducted in Laboratory of Tissue Culture, Faculty of Agriculture, Siliwangi University in February 2016 to August 2016. This experiment used a randomized completely block design with 9 treatments and 4 replications. The treatments consisted of a combination of growth regulators and organic matter : BAP (6-benzylaminopurine) (1 mg L^{-1}, 3 mg L^{-1} and 5 mg L^{-1}), each of which was combined with addition of coconut water (0%, 10% and 20%) and banana extract (0 g L^{-1}, 100 g L^{-1} and 200 g L^{-1}). Each combination of growth regulator and organic matter was incorporated to MS basal medium with a sugar of 30 g L^{-1} and phytagel 2.5 g L^{-1}. As explant planted was the mangosteen seed. The results showed the growth response of cut seed explant on media added by ZPT BAP, coconut water and banana extract, that was by the forming of callus, nodule and bud. MS medium added by BAP 3 mg L^{-1} + coconut water 10% + banana extract 100 g L^{-1} yielded 2 nodules per explant and no different from media control (without coconut water and banana extract) and higher than other media treatment with coconut water and banana extract. MS medium supplemented by BAP 3 mg L^{-1} + coconut water 20% + banana extract 200 g L^{-1} was capable of producing shoots although the number and percentage is still low (0.25 buds per explant) than on the media without the addition of coconut water and banana extract.

Keywords: banana extract, 6-benzyladenine, coconut water, in vitro culture, mangosteen

INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) is native to Indonesia and more popular as a horticultural crop to be consumed as fresh fruit, juice and a health drink supplement. Recently, mangosteen fruit is also used as raw material in the healthcare industry. Mangosteen shell produce secondary metabolites of the xanthone that are useful as antioxidants, antitumors, allergies, anti-inflammatories, antibacterials, and antivirals (Chomnawang *et al.*, 2007; Pedraza-Chaverri *et al.*, 2008).

The demand for mangosteen from year to year is increased both for domestic and export need. Data in 2014 (January-December 2014) showed that

mangosteen exports remained relatively high at 6.54 million US dollars with volume of 10.08 million tons (BPS, 2015). Thus, mangosteen has a high prospect to be developed and become a priority of Indonesian export fruits.

One of the problems faced in the development of mangosteen is the availability and quality of seeds. Mangosteen plants are usually propagated by seed (generative propagation) or grafting method (conventional vegetative propagation), but both still have limitations. Mangosteen planting with seed is often low-growing and there are constraints in the provision of seeds: the number of seeds per fruit a little (1-2 seeds per fruit) and even fruit is not seeds at all, and the mangosteen seeds are recalcitrant that are difficult to store for long. Planting with conventional seedlings often results in slow-growing, weak, not uniform, and slow-growing plants (Normah *et al.*, 1995; Cruz, 2001). Trees grown from new seeds bloom by 10-15 years, while those grown from seedlings can flower at age 5-7 years (Hernowo, 2011). In vitro culture is one alternative that can be used to overcome these problems, in addition to producing mangosteen clone with large quantities, relatively uniform and short life cycle.

In vitro propagation technique is a method of planting protoplasts, cells, tissues and organs on artificial media in aseptic conditions in order to regenerate a new plants. The success of in vitro cultures is strongly influenced by the media compounds generally comprising: inorganic salts, carbon and energy sources such as glucose and sucrose, growth regulators (PGRs), amino acids, and vitamins. PGRs or exogenous hormone is required to influence the growth and morphogenesis of cell cultures, organs, and tissues (Gunawan, 1988), but the use of PGRs can be combined with organic matter such as coconut water and banana extract containing hormones or nutrients needed for growth of explant. The use of organic matter can increase efficiency of culture because the extracts of natural substances can reduce PGRs or other media components that have the same function.

Study on in vitro culture of mangosteen has been done by Goh *et al.* (1990) that the WPM medium plus BA 5 mg L^{-1} gave the best multiplication of mangosteen shoots. The results by Roostika *et al.* (2008) showed the best shoot induction on seed explants cultured on MS medium plus BA 5 mg L^{-1}, while the best shoot multiplication was produced on MS medium plus BA 3 mg L^{-l}. However, in vitro mangosteen culture experiment using natural substances or its combination with PCRs has not been widely performed or is still limited in information. In other crops, the addition of autoclaved coconut water at 15% concentration as a synthetic benzyladenine substitution resulted best multiplication of temulawak shoots in vitro with an average of 3.4 buds within 2 months (Seswita, 2010). The interaction between organic media and NAA showed the addition of ambon banana extract 150 g L^{-1} and NAA 20 ppm could induce and produce the highest average number of black orchid shoots (Untari, 2006).

Therefore, in mangosteen plants, research on the use of natural substances (coconut water and banana extract) combined with PGRs in propagation in vitro is necessary to further stimulate the growth and development of explant to form the desired plant regeneration. The aim of this research was to obtain best combination of BAP growth regulator (6-benzylaminopurine) and organic matter of coconut water and banana extract to induce mangosteen shoots in vitro.

MATERIALS AND METHODS

The experiment was conducted in Laboratory of Tissue Culture Study Program of Agrotechnology Faculty of Agriculture, Siliwangi University, Tasikmalaya in February 2012 to March 2014. The in vitro experiment was set up in completely randomized design with 9 treatments of PGRs combined with organic matter that incorporated to MS basal medium (Murashige & Skoog, 1962), as follows:

(a) BAP 1.0 mg L^{-1} + coconut water 0% + banana extract + 0 g L^{-1}
(b) BAP 1.0 mg L^{-1} + coconut water 10% + banana extract + 100 L^{-1}
(c) BAP 1.0 mg L^{-1} + coconut water 20% + banana extract + 200 L^{-1}
(d) BAP 3.0 mg L^{-1} + coconut water 0% + banana extract + 0 g L^{-1}
(e) BAP 3.0 mg L^{-1} + coconut water 10% + banana extract + 100 g L^{-1}
(f) BAP 3.0 mg L^{-1} + coconut water 20% + banana extract + 200 g L^{-1}
(g) BAP 5.0 mg L^{-1} + coconut water 0% + banana extract + 0 g L^{-1}
(h) BAP 5.0 mg L^{-1} + coconut water 10% + banana extract + 100 g L^{-1}
(i) BAP 5.0 mg L^{-1} + coconut water 20% + banana extract + 200 g L^{-1}

Each combination of PGRs and organic matter was added to MS medium containing 30 g L^{-1} sugar, 2.5 g L^{-1} phytagel. Each treatment was repeated 4 times. As explant material was used mangosteen seeds of Puspahiang local variety. Mangosteen seeds were cleaned from the flesh, then consecutively sterilized by soaking in 70% alcohol for 10 minutes and rinsed with sterile distilled 3 times. Further, the seeds were soaked into 20% NaOCl solution for 15 minutes, and rinsed with sterile distilled water 3 times each for 5 minutes. Seed were cut into three sections, then placed on each flask of shoot induction medium according to the treatment (BAP combined with coconut water and banana extract). The pH of the media was adjusted to 5.6-5.8 and autoclaved at 121 ^{o}C for 15 min. The culture was incubated under white fluorescent light of 1.500 lux at 24 h photoperiod at 24±3 ^{o}C. The percentage of explant performing callus, the number of nodules per explant, and the number of shoots per explant at 10 weeks after culture (WAC) on the media were recorded. An analysis of variance was performed to all quantitative data obtained, and significant differences among treatment means were calculated by the Duncan's Multiple Range Test (DMRT) at a probability level of 0.05.

RESULTS AND DISCUSSION

In this experiment growth response of cut seed explants were obtained on media MS suplemented with growth regulator BAP and organic materials coconut water and banana extract. Explant growth was characterized by the formation of callus tissue, nodules and shoots (Tables 1-3, Figures 2, 4, 5 and 7) indicating that there was effect of the combination of BAP and coconut water and banana extract to growth of explant cultured.

Percentage of Explant Performing Callus

In addition to nodules and shoots, also found callus tissue in the explant which is generally white and yellowish colour (Fig. 2) and initially formed on 2 WAC. Addition of BAP combined with coconut water and banana extract on MS medium was able to produce explant performing callus, that were on medium supplemented by BAP 1 mg L^{-1} + coconut water 10% + banana extract 100 g L^{-1}

(B), BAP 1 mg L^{-1} + coconut water 20% + banana extract 200 g L^{-1} (C), BAP 3 mg L^{-1} + coconut water 10% + banana extract 100 g L^{-1} (E) and BAP 3 mg L^{-1} + coconut water 20% + banana extract 200 g L^{-1} (F) (Table 1, Fig. 1), and showed no significant difference with control except with control D (BAP 3 mg L^{-1} + coconut water 0% + banana extract 0 g L^{-1}) according to Duncan test at 5% probability level.

Table 1. Effect of combination of growth regulator and organic matter in medium to percent explant performing callus after 10 weeks of culture

Induction media	Explant performing callus (%)
A. BAP 1.0 mg L^{-1} + coconut water 0% + banana extract + 0 g L^{-1}	50.00 ab
B. BAP 1.0 mg L^{-1} + coconut water 10% + banana extract + 100 L^{-1}	8.33 b
C. BAP 1.0 mg L^{-1} + coconut water 20% + banana extract + 200 L^{-1}	25.00 b
D. BAP 3.0 mg L^{-1} + coconut water 0% + banana extract + 0 g L^{-1}	72.49 a
E. BAP 3.0 mg L^{-1} + coconut water 10% + banana extract + 100 g L^{-1}	8.33 b
F. BAP 3.0 mg L^{-1} + coconut water 20% + banana extract + 200 g L^{-1}	8.33 b
G. BAP 5.0 mg L^{-1} + coconut water 0% + banana extract + 0 g L^{-1}	50.00 ab
H. BAP 5.0 mg L^{-1} + coconut water 10% + banana extract + 100 g L^{-1}	0.00 b
I. BAP 5.0 mg L^{-1} + coconut water 20% + banana extract + 200 g L^{-1}	0.00 b

Values followed by the same letter at column were not significantly different according to DMRT (α= 0.05)

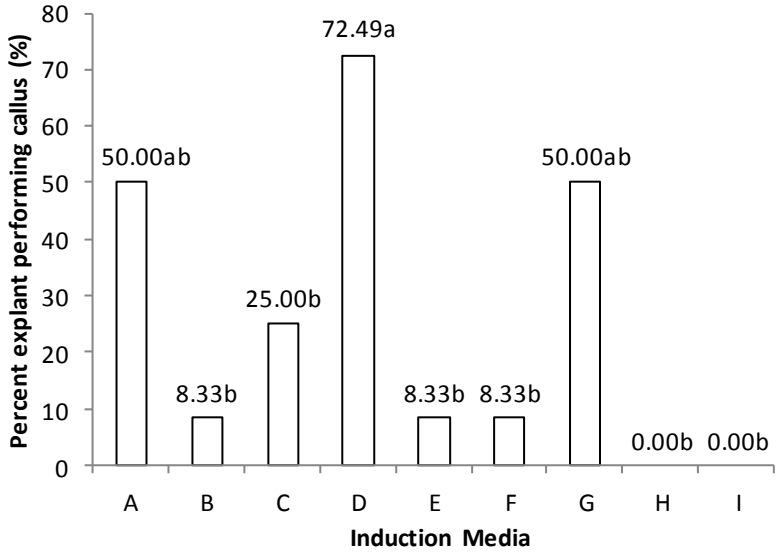

Figure 1. Percent explant performing callus on media MS added by various combination of PGRs (BAP) and organic matter (banana extract and coconut water)

The concentration of plant growth regulators (auxin, cytokinin, gibberellin, ethylene, etc.) is one of the major factors that control callus formation in culture media (Smith, 1992). The concentration of plant growth regulators may vary for

each plant species and may even depend on the source of the explants to be cultured. Explant on control media (A, D and G) were suspected to have adequate endogenous auxin and addition of BAP (exogenous cytokinin) increased stimulation of callus formation. It was parallel to Wetter and Constabel (1991) that auxin and its combination with cytokines such as kinetin and benziladenin have important effect on the formation of callus. Percent of explant performing callus on the medium with the addition of coconut water and banana extract was not significantly different with the control but tended to be lower, even in H medium (BAP 5 mg L^{-1} + coconut water 10% + banana extract 100 g L^{-1}) and medium I (BAP 5 mg L^{-1} + coconut water 20% + banana extract 200 mg L^{-1}) has not produced explant performing callus (0%), this was thought to be due to increased cytokinin content from coconut water and banana extract (especially on H and I) disrupting the balance of auxin and cytokinin concentrations required for callus formation.

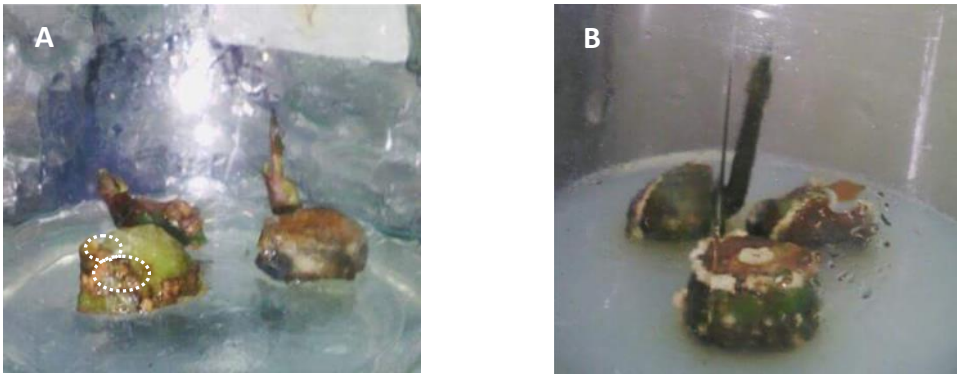

Figure 2. Growth of callus. nodule. and shoots produced. (A) callus tissue. nodule. and shoots produced in control media D (BA 1.0 mg L^{-1} + coconut water 20% + banana extract + 200 g L^{-1}). (B) callus growing located on explant cutting parts

Callus tissue grown both on the control and on the media with coconut water and banana extract were very small in volume and generally located on the explant cutting parts. Smith (1992) stated that callus is a cell mass that can be formed from a wound response (wound response). The resulting callus tissue has the potential to regenerate into shoots, except for a white callus resembling cotton. According to Qosim (2006) callus that like cotton can not regenerate, eventually the callus will dry, brown and die.

Number of Nodules per Explant

The combination of BAP with coconut water and banana extract gave effect to the number of nodules per explant, as shown in Table 2 and Figure 3. Duncan test result (α = 0.05) showed there was significant difference in number of nodules per explant.

MS medium added with BAP 3 mg L^{-1} + coconut water 10% + banana extract 100 g L^{-1} (E) yielded 2 nodules per explant and not significantly different with control media A (BAP 1 mg L^{-1} + coconut water 0% + banana extract 0 g L^{-1}), medium D (BAP 3 mg L^{-1} + coconut water 0% + banana extract 0 g L^{-1}) and G medium (BAP 5 mg L^{-1} + coconut water 0% + banana extract 0 g L^{-1}), but was higher than the five other treatment of coconut water and banana extract. The content of the auxin hormone in coconut water and banana extract in

combination with BAP could induce the formation of nodules. The auxin content in 10% coconut water and 100 g L^{-1} banana extract was presumed to be too low to combine with 1 mg L^{-1} of BAP, on the contrary, the auxin content of more 20% coconut water and 200 g L^{-1} banana extract was also supposed to be excessive to be combined with 5 mg L^{-1} of BAP, thus both could not initiate nodulation growth. Media containing low auxin concentrations compared to the concentration of cytokinin, the growth of explant will be induced into shoot organogenesis, whereas if the concentration of auxin is high compared to cytokinin concentration then growth is pushed toward root formation (Phillips *et al.*, 1995).

Table 2. Effect of combination of growth regulator and organic matter in MS medium to number of nodules after 10 weeks of culture

Induction media	Number of nodule per explant
A. BAP 1.0 mg L^{-1} + coconut water 0%+ banana extract + 0 g L^{-1}	2.75 a
B. BAP 1.0 mg L^{-1} + coconut water 10%+ banana extract + 100g L^{-1}	0.00 c
C. BAP 1.0 mg L^{-1} + coconut water 20%+ banana extract + 200g L^{-1}	0.50 bc
D. BAP 3.0 mg L^{-1} + coconut water 0% + banana extract + 0 g L^{-1}	2.75 a
E. BAP 3.0 mg L^{-1} + coconut water 10%+banana extract + 100g L^{-1}	2.00 a
F. BAP 3.0 mg L^{-1} + coconut water 20%+banana extract + 200g L^{-1}	1.50 ab
G. BAP 5.0 mg L^{-1} + coconut water 0%+ banana extract + 0 g L^{-1}	3.00 a
H. BAP 5.0 mg L^{-1} + coconut water 10%+banana extract + 100g L^{-1}	0.25 bc
I. BAP 5.0 mg L^{-1} + coconut water 20%+banana extract + 200g L^{-1}	0.00 c

Values followed by the same letter at column were not significantly different according to DMRT (α = 0.05)

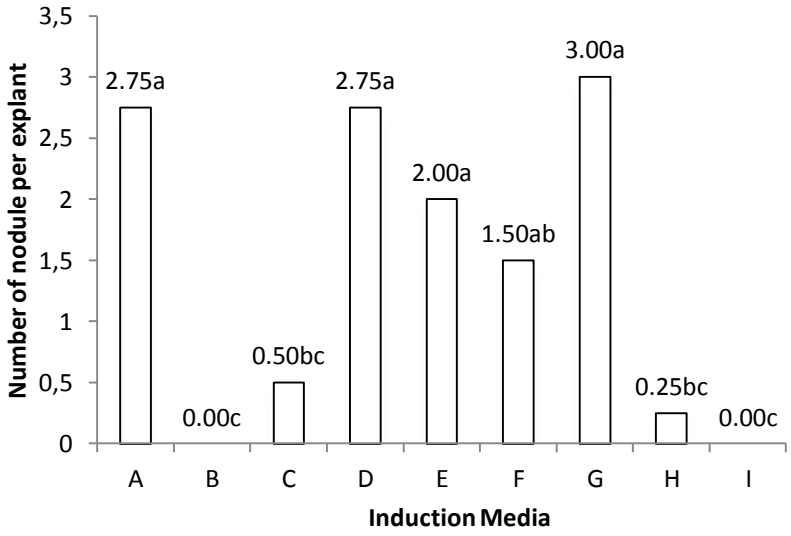

Figure 3. Number of nodule per explant on media MS added by various combination of PGRs (BAP) and organic matter (banana extract and coconut water)

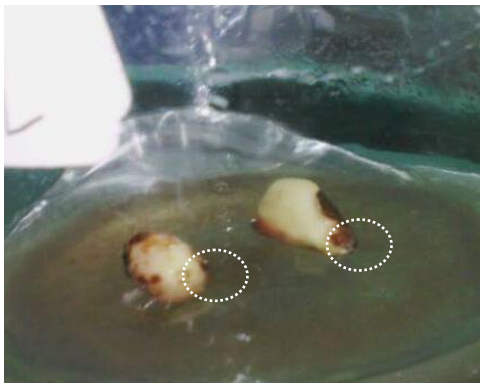

Figure 4. The nodules obtained on media F (MS media contaning BAP 3.0 mg L^{-1} + coconut water 20% + banana extract + 200 g L^{-1})

Based on the appearance of recorded nodules, the nodules consisted of: nodules that grew directly from the explant tissue (Figure 4), and some grew from callus tissue. Generally the nodules were yellow and bright green and brownish red. According to Joni et al. (2014) nodules appearing on mangosteen explants are capable for shoot morphogenesis, but do not regenerate further on the same medium, and presumably it require new formulation media and the addition of in vitro periods to regenerate them.

Percentage of Explant Performing Shoots and Number of Shoots per Explant

In this experiment, mangosteen shoots were obtained in vitro although the number of shoots per explant produced was relatively low (maximum of 2.25 shoots per explant). Control media (without addition of coconut water and banana extract) A, D and G (Figure 5) were able to induce shoots with higher percent of explant performing shoots and number of shoots per explant than those produced on media containing coconut water and banana extracts. The successful shoot induction on coconut water and banana extract media was on F medium (BAP 3 mg L^{-1} + coconut water 20% + banana extract 200 g L^{-1}) although was extremely less in number of shoots per explant (0.25 shoots) compared to control media.

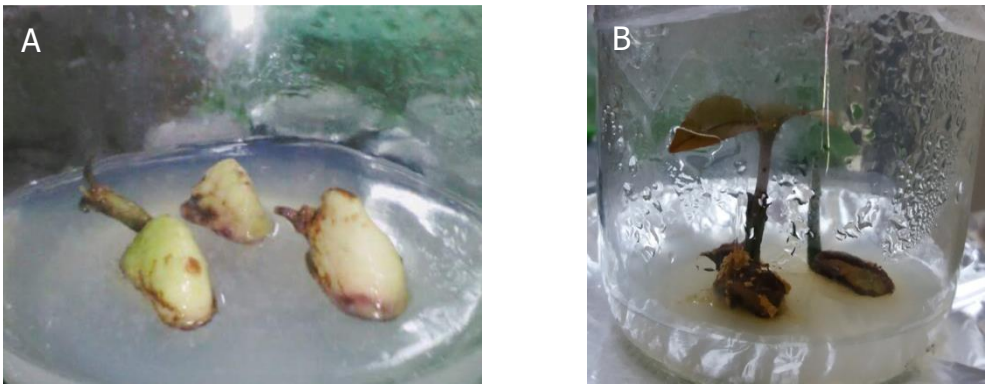

Figure 5. Shoots and callus produced on control media G (MS media contained BA 5.0 mg L^{-1} + coconut water 0% + banana extract 0 g L^{-1}) at 8 WAC (A) and 12 WAC (B)

As shown in Table 3 and Figure 6 the other media supplemented with coconut water and banana extract (B, C, E, H and I) did not produce shoots. Shoots produced from the media control and from media F were the shoots result of direct organogenesis or shoots directly grew on the tissue explant without growing of callus phase. The shoots begin to appear at 2-3 WAC. Direct organogenesis is the process of forming direct adventive shoots from explants (Joni *et al.*, 2014). The resulting adventive buds are unipolar structures with vascular tissue still connected to the parent tissue (Qosim, 2006).

Table 3. Effect of combination of growth regulator and organic matter in MS medium to percent of explant performing shoots and number of shoots per explant after 10 weeks of culture

Induction media	Percent of explant performing shoots	Number of shoots per explant
A. BAP1.0 mg L^{-1} + coconut water 0% + banana extract +0 g L^{-1}	49.99 a	1.00 b
B. BAP1.0 mg L^{-1} + coconut water 10% + banana extract+100g L^{-1}	0.00 b	0.00 c
C. BAP1.0 mg L^{-1} + coconut water 20% + banana extract+200g L^{-1}	0.00 b	0.00 c
D. BAP3.0 mg L^{-1} + coconut water 0% + banana extract +0 g L^{-1}	83.33 a	2.25 a
E. BAP3.0 mg L^{-1} + coconut water 10% + banana extract+100g L^{-1}	0.00 b	0.00 c
F. BAP3.0 mg L^{-1} + coconut water 20% + banana extract+200g L^{-1}	8.33 b	0.25 c
G. BAP5.0 mg L^{-1} + coconut water 0% + banana extract+ 0 g L^{-1}	58.33 a	1.00 b
H. BAP5.0 mg L^{-1} + coconut water 10% + banana extract+100g L^{-1}	0.00 b	0.00 c
I. BAP5.0 mg L^{-1} + coconut water 20% + banana extract+200g L^{-1}	0.00 b	0.00 c

Values followed by the same letter at columns were not significantly different according to DMRT (α = 0.05)

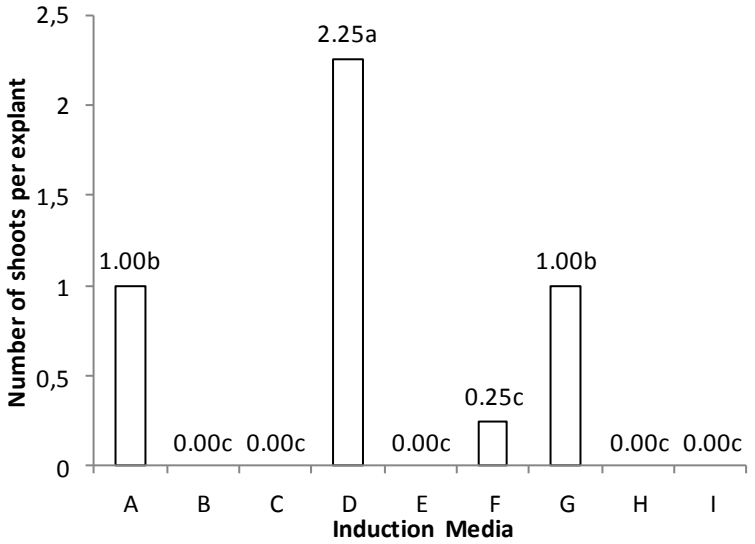

Figure 6. Number of shoots per explant on media MS added by various combination of PGRs (BAP) and organic matter (banana extract and coconut water)

The success of tissue culture is strongly influenced by the media comprising inorganic salts, amino acids, vitamins, growth regulators and other organic substances such as coconut water, yeast extract, bananas, bean sprouts,

oranges, papaya, etc. used as additives in tissue culture medium. According to Widiastoety and Bahar (1995) banana extract added to tissue culture medium can stimulate cell division and promote cell differentiation, and in turn the explants can grow and develop.

Ambon banana extract is known to contain elements of potassium (K), phosphorus (P) and iron (Fe) that can give positive effect on shoot growth. Tulecke *et al.* (1961) stated coconut water contains nutrients, vitamins, amino acids, nucleic acids and substances such as auxin and gibberellic acid that act as a stimulator of tissue proliferation, enhance metabolism and respiration. Besides containing natural auxin, in coconut water (Molnar, 2011) and banana extract (Van Staden *et al.*, 1975) also contain natural cytokinin which can reduce the need for supplemented (exogenous) cytokinin to induce bud growth. This was as shown in the results of this experiment, MS medium containing BAP 3 mg L^{-1} + coconut water 20% + banana extract 200 g L^{-1} (F) was able to produce shoots although was still in low capability of performing shoots compared to control media (Figure 7).

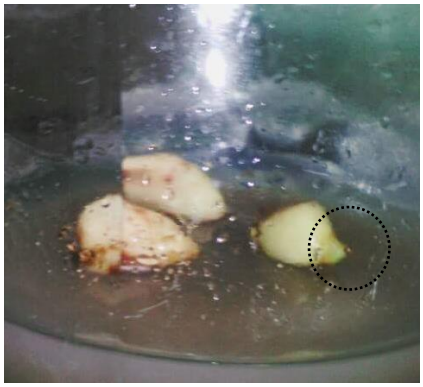

Figure 7. Shoot growing on MS media suplemented by BAP 3 mg L^{-1} + coconut water 20% + banana extract 200 g L^{-1} (F)

Based on percent of explant performing shoots and number of shoots per explant obtained, there was a tendency on the control medium BAP concentrations above 1 mg L^{-1} the number of shoots increased, and then decreased again at the concentration of BAP 5 mg L^{-1}. The determination of the concentration of growth regulators and organic matter in the media is crucial to the ability explant in inducing the formation of shoots. The unappropriate combination of PGRs and organic matter concentrations was apparently to be the cause of the treatment of medium B (BA 1.0 mg L^{-1} + coconut water 10% + banana extract + 100 g L^{-1}), C (BA 1.0 mg L^{-1} + coconut water 20% + banana extract + 200 g L^{-1}), E (BA 3.0 mg L^{-1} + coconut water 10% + banana extract + 100 g L^{-1}, H (BA 5.0 mg L^{-1} + coconut water 10% + banana extract + 100 g L^{-1}) and I (BA 5.0 mg L^{-1} + coconut water 20% + banana extract + 200 g L^{-1}) still have not produced shoots.

CONCLUSION

In this experiment there was the effect of the combination of BAP and coconut water and banana extract to growth of explant cultured which were indicated by the formation of callus, nodules and shoots. MS medium contained

BAP 3 mg L^{-1} + coconut water 10% + banana extract 100 g L^{-1} produced 2 nodules per explant and was not significantly different with control media (without coconut water and banana extract), but was higher than the five other media with coconut water and banana extract. MS medium with BAP 3 mg L^{-1} + coconut water 20% + banana extract 200 g L^{-1} was capable of producing shoots although was extremely lower in number of shoots (0.25 shoots per explant) than on the media without addition of coconut water and banana extract (highest 2.25 shoots per explant).

Based on the results of the study, further research is required to increase the percentage of in vitro mangosteen shoots produced, in addition to rooting and plantlet initiation.

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