GROWTH OF ARENGA PALM (*Arenga pinnata* (Wurmb) Merr.) EXPLANT WITH BENZYL AMINO PURINE (BAP) APPLICATION

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**ABSTRACT**

The research was aimed to obtain the optimum concentration of BAP to buds growth as in vitro. It was conducted in the Tissue Culture Laboratory at Forestry and gardening seedling Unit of Forestry and Gardening Office of Banten Province from April until December 2008. The research used Randomized Completely Design on MS base medium with different BAP concentration: (1) B1 = MS + Kinetin 0.1 ppm + BAP 1 ppm, (2), B2 = MS + Kinetin 0.1 ppm + BAP 2 ppm, (3) B3 = MS + Kinetin 0.1 ppm + BAP 3 ppm, (4) B4 = MS + Kinetin 0.1 ppm + BAP 4 ppm, (5) B5 = MS + Kinetin 0.1 ppm + BAP 5 ppm. Each treatment was replicated ten times. The data was analyzed and continued with Duncan Multiple Range Test (DMRT) 5 percent. The explant which grew in MS media with different BAP concentration showed radicle appearance. It tended to grow to be embryo, where it began with radicle length and was followed by bud formation. Higher BAP application for each treatment increased the germination. The best concentration of embryo germination which would be grown to be bud was 4 or 5 ppm BAP.

Keywords: tissue culture, Arenga palm, BAP

**INTRODUCTION**

There are problems in the conventional arenga palm cultivation because arenga palm seeds have a hard structure and a thick skin, causing a low permeability. Palm seed dormancy period varies between 1 to 12 months (Mujahidin *et al.*, 2003). According to Copeland and Mc.Donald (2001) dormancy can be broken by mechanical or chemical scarification.

The duration of seed germination is inhibiting factor in the provision of seeds for palm cultivation. Palm seeds need special treatment for good germination. According to the research by Mashud, Ilat and Allorerung (2001), stripping and storage of seeds affect the speed of germination but do not affect germination. Both kinds of seed that are peeled or unpeeled have a high germination at 71 % when stored for 2 to 4 weeks.

Naturally, arenga palm plants are developed by weasels (*Paradoxurus hermaproditua*). In addition, arenga palm seed procurement is quite difficult because it is an annual plant. Vegetative propagation is the solution that can be achieved by tissue culture technique in which the procurement of plant materials are cheap and uniform can be realized (Gaba, 2005; Gunawan, 1987).

In vitro propagation techniques for plantation material procurement have several advantages such as the quality improvement of gene, physiology, and purity. Good quality of seed and pest-free plants can be obtained through tissue culture techniques. Another advantage of tissue culture selection is that it can be done on the properties of the desired plant early (Haris and Mathius, 1995; Mariska and Purnamaningsih, 2001; Budiarto, 2010).

Substances of growth regulator such as auxin and cytokinin play a very prominent role in plant propagation in vitro. Auxin plays a role in encouraging cell renewal, cell division, differentiation of xylem and phloem tissue, adventitious root formation and apical dominance. Physiological role of cytokines is to promote cell division, morphogenesis,
performance, formation of chloroplasts, breaking dormancy, the formation of stomata, inhibiting senescence and absisi (Wattimena et al., 1991; Gunawan 1987; and Pierik 1987).

Balance of auxin and cytokinin in explant will determine the type of organs formed. When the ratio of auxin and cytokinin is high, then the organs form roots, whereas, in the contrary, the shoots will be formed. The role of auxin and cytokinin is important for the reprogramming of somatic cells that will determine the next dedifferentiation stage. The re-differentiation of meristem produces cells that will develop into embryogenic cells and somatic embryos (Gaba 2005). Formation of somatic embryogenesis is influenced by several factors including the formulation of different media in each somatic embryo development and the type of explant used.

The research is aimed to determine the concentration of growth regulators (BAP) as given in the explant culture medium for the growth of arenga palm explant as in vitro.

MATERIALS AND METHODS

The research was conducted on MS basal medium with Kinetin 0.1 ppm at Tissue Culture Laboratory at Forestry and gardening seedling Unit of Forestry and Gardening Office of Banten Province from April to December 2008.

The materials used were seeds (embryozygotic) arenga palm, MS medium, BAP, distilled water, alcohol 70% and 96%, and other materials.

The tools required consisted of autoclave, scalpel, tubes, culture bottles, scales, laminar flow cabinets, and various laboratory equipments.

The design used was completely Randomized Design on MS basal medium containing 0.1 ppm Kinetin with various concentrations of BAP treatment, each 1, 2, 3, 4, and 5 ppm. The treatment was repeated 10 times so that there were 50 combinations of the experiment. Data were analyzed with ANOVA followed by DMRT at 5% level.

Regeneration media preparation was done by diluting the stock solution in accordance with the provisions of MS medium for plant growth regulator added in accordance with the treatment. Total media solutions are made in accordance with the required number of culture bottles. The number of bottles was prepared and the manufacture of media was adjusted to the number of explants which would be planted.

The determination of MS medium pH in the range of pH 5.8 was conducted by adding 1 N NaOH solution when the pH is too low or adding 1 N HCl when the pH is too high, then stirring the solution until it became clear. To reach the boiling point, 7.5 g per liter of media was added. After the solution became clear, the heating was stopped and the media was immediately put into 10 ml culture bottles and covered with aluminum foil. Furthermore, the media were sterilized in an autoclave at a pressure of 15 psi with a temperature of 121°C for 20 minutes. Once sterilization was complete, the culture bottles were incubated for 2 weeks in the transfer chamber before planting explants. Contaminated media were removed from the culture room and were not used for the cultivation of explants.

Explants were taken from embryosmatic palm that has been copied into shoots in vitro were uniform in accordance with the treatment media. In order to obtain shoots in vitro initiation of palm plants, sub-cultures were performed every 8 weeks with the same media composition.

RESULTS AND DISCUSSION

The results reveal that the embryos which have been sterilized subsequently and grown on MS medium in accordance with the treatment show palm embryo culture without under-developed endosperm but still sterile.

The number of buds per explant cannot be displayed because the newly formed buds are only in the form of an elongated radicle. The number of radicle contained in each treatment can be seen in Table 1.
Table 1. The average number per explant radicle (fruit) on MS medium explants + kinetin 0.1 ppm + different concentrations of BAP 0.1 ppm + different BAP concentration

<table>
<thead>
<tr>
<th>BAP Concentration</th>
<th>Weeks 1</th>
<th>Weeks 2</th>
<th>Weeks 3</th>
<th>Weeks 4</th>
<th>Weeks 5</th>
<th>Weeks 6</th>
<th>Weeks 7</th>
<th>Weeks 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>2 ppm</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Percentage radical explant grown on MS medium + 0.1 ppm kinetin + different concentration of BAP

<table>
<thead>
<tr>
<th>BAP Concentration</th>
<th>Weeks (%) 1</th>
<th>Weeks (%) 2</th>
<th>Weeks (%) 3</th>
<th>Weeks (%) 4</th>
<th>Weeks (%) 5</th>
<th>Weeks (%) 6</th>
<th>Weeks (%) 7</th>
<th>Weeks (%) 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>90</td>
<td>100</td>
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<tr>
<td>2 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>40</td>
<td>90</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>80</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>4 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>40</td>
<td>90</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>50</td>
<td>90</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3. Average explant growth (cm) on MS medium + kinetin 0. ppm + different concentrations of BAP

<table>
<thead>
<tr>
<th>BAP Concentration</th>
<th>Weeks 1</th>
<th>Weeks 2</th>
<th>Weeks 3</th>
<th>Weeks 4</th>
<th>Weeks 5</th>
<th>Weeks 6</th>
<th>Weeks 7</th>
<th>Weeks 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>2 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>3 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>0.1</td>
<td>1.3</td>
<td>2.0</td>
</tr>
<tr>
<td>4 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>0.5</td>
<td>2.1</td>
<td>3.0</td>
</tr>
<tr>
<td>5 ppm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>0.5</td>
<td>2.2</td>
<td>3.1</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Remarks: Figures followed by small letters on the same column, each of which is not significantly different according to DMRT at 5% significance level. The values followed with small letters of each same column show significant notes according DMRT at 5% level.

The growth of explants on MS medium with different BAP concentrations shows the emergence of radicle and there is a tendency that the first embryo of arenga palm always starts with the elongation of the radicle, and then followed by bud formation (Table 2-3). The development of explants can be seen in Figure 1a, 1b, 1c, 1d, 1e. The embryo began to grow in the explant in week 4 after cultured. Concentrations of BAP used influence the growth rate of arenga palm embryos. Giving BAP in each treatment would further improve the seedling arenga palm. The concentration of 4 and 5 ppm BAP showed better germination of embryos developing into buds than others.

BAP is the most widely used cytokinin in tissue culture because it is more stable and more effective than other types of cytokinin, and it can easily be obtained. Morphogenesis of shoots and roots is influenced by the ratio of auxin and cytokinin. In this study, cytokines was the only substance applied in term of the formation of buds because it allegedly contains the endogenous auxin in the explant. This is in line with the notion of Wattimena (1992) which stated that the appropriate ratio of cytokinin and auxin would encourage bud formation.
Figure 1. Explants plant growth of plants in various combinations of treatments on arenga palm

Remarks=
1a. Explant age of 8 weeks on medium B1 = MS + kinetin + 0.1 ppm 1 ppm BAP
1b. Explant age of 8 weeks on medium B1 = MS + kinetin 0.1 ppm + 2 ppm BAP
1c. Explant age of 8 weeks on medium B1 = MS + kinetin 0.1 ppm + 3 ppm BAP
1d. Explant age of 8 weeks on medium B1 = MS + kinetin + 0.1 ppm + 4 ppm BAP
1e. Explant age of 8 weeks on medium B1 = MS + kinetin 0.1 ppm + 5 ppm BAP

CONCLUSIONS AND SUGGESTIONS

CONCLUSIONS
The concentration of BAP given to the culture medium provides a different effect on the average growth of explants. The concentration for bud formation of arenga palm explants is obtained on delivery of 4 mg l⁻¹ BAP + 0.1 mg l⁻¹ kinetin.
SUGGESTIONS
Based on the results obtained, it is advisable to conduct further research. It is recommended to use a combination of 4 mg l-1 BAP + 0.1 mg l-1 kinetin as the initial medium for the growth of arenga palm plant explant.

ACKNOWLEDGMENTS
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REFERENCES