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ESTABLISHMENT OF SUGAR PALM (*Arenga pinnata)* SHOOT FROM ZYGOTIC EMBRYO IN MS MEDIUM SUPPLEMENTED WITH DIFFERENT CONCENTRATIONS OF BENZYLAMINOPURINE

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ABSTRACT

Arenga pinnata is one of the potential crops which provide a great number of products such as ethanol, starch and fiber. This plant usually propagated by seed but its seed growth is unpredictable and may take more than one year to germinate. The study was conducted with aim to obtain plantlets through initiation of half matured fruits of sugar palm. Zygotic embryo obtained from fresh half matured fruits were directly excised and cultured into MS free-hormone medium and MS medium treatments supplemented with 6-benzylaminopurene (BAP) at concentrations 0.1, 0.5, and 3.0 mg/L. Zygotic embryo cultured on MS0 served as control treatment. The results of the study indicated that MS medium supplemented with 0.5 mg/L BAP shows no regeneration of explant, shoot and radicle after four weeks culture. However, although after 8 weeks culture 59.5% explant was regenerated with increased radicle size, but the shoot emergence was only observed after 24 weeks culture. Meanwhile, regeneration of zygotic embryos in MS medium hormone-free resulted in highest percentage of plantlets produced (90%) with production of one shoot, longest radicle (4.5 cm) and longest plantlet length (6.0cm) as compared with other treatments. *In vitro* seedling was successfully developed after 32 weeks culture.

Key words: *Arenga pinnata*, sugar palm, *in vitro* culture, plant growth regulators

INTRODUCTION

Sugar palm is one of the multipurpose Arecaceae family grown in Malaysia (Sahari *et al.,* 2012) and nowadays widely grows in Southeast Asia and in other country such as Vietnam, Laos and Cambodia (Wolter and Leo, 2010; Endri *et al.,* 2011). All parts of sugar palm such as leaves, trunk, fruit and bark can be utilized (Wolter and Leo, 2010). Besides yielding neera sugar, sugar palm provides a great variety of products such as ropes, filters, brooms and roof materials (Sahari *et al.,* 2012). Fibers of sugar palm with its desirable properties, have great potential to be used as reinforcement in polymer composites. Besides that, the fiber is highly durable and resistant to sea water and it is readily available in the form of woven fibers, thus making it easy to process. Sugar palm tree also can produce juices from its fruits bunch which is white in colour and can be drunk (Helen and Priscilla, 2003). The juice of sugar palm can be used as a renewable source of biofuel energy like other bioethanol plant sources (Ishak *et al.,* 2010). Bioethanol is used as a raw material in production of varieties of chemical products, solvents, pharmaceutical, beverages and medicines (Ishak *et al.,* 2013). In Malaysia, especially in state of Negeri Sembilan, it was popular with activity of tapping palm sap as the material for making traditional sugar blocks locally known as *gula enau* or *kabung*. Sugar palm also can be as an alternative of commercialized sugarcane granular sugars which were processed to produce crystal and brown sugar (Ishak *et al.,* 2011). The fruit of the sugar palm can be processed for making pickles, juices, desserts, for canned foods, and also being cooked for making traditional sugar syrup (Wolter and Leo, 2010).

Sugar palm have a relatively long youth phase before they start producing flowers which can be tapped. Direct sowing is possible but the seed is short lived and takes a long time to establish well (van Dam, 2007). The seed are usually dormant and

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the germination takes more than one year to germinate (Wolter and Leo, 2010). Study conducted by Chairun (1994) shows that radicle and plumule emerged after five to six weeks germination. Due to the delayed germination problem, tissue culture was offered as the best methods to be practiced in order to produce high quality of sugar palm seedling. Tissue culture is one of the most effective cloning methods in existing palm (Endri *et al.,* 2011). There are many studies have been conducted using immature and mature sugar palm zygotic embryos toward different types of medium and hormones. Mirza (2013) indicated that BAP led to highest formation of root and shoot but did not affect growth of zygotic embryo. In contrast, auxin has been used to induce callus formation of zygotic embryo. The result shows that embryogenic calluses appear after 4 to 8 weeks. However, further treatment should be taken to develop or regenerate into a plantlet.

Although many studies have been conducted, little information and documentation on the *in vitro* culture of *Arenga pinnata* is available, thus was raised our interest to further investigate the mechanism. Considering that in practice the propagation of palm plants is accomplished mainly by seeds, therefore, the study was conducted to evaluate the growth performance of *Arenga pinnata* through *in vitro* culture of zygotic embryo. Besides, the current trend in Malaysian agriculture, toward fully utilized natural resources, less chemically intensive and less harmful to the environment, interest has increased to improve growth performances of sugar palm which can be utilized as sugar and natural resources of biofuel that beneficial for human health and environmental condition. Moreover, the research also can provide and publish significant information and data on sugar palm growth for future study.

MATERIALS AND METHODS

Plant material and sterilization procedure

Fresh mature green fruits of sugar palm (*Arenga pinnata*) were collected from local growers in Kampung Peruang, Benta, Pahang (latitude 3º 58' 12" North, longitude 101º 58' 5.52" East). The fruits were thoroughly washed under running tap water for few minutes, surface sterilized with 70% (v/v) alcohol for 10 minutes and immersed in 50% (v/v) sodium hypochlorite added with a few drops of Tween 20 for another 10 minutes. Sequential rinses with sterile distilled water were performed to remove the remaining chemical agents. Then, the fruits were dried on a petri dish containing sterile filter paper discs. Sterilization process was performed in a laminar air flow chamber. This research was carried out at Plant Tissue Culture Laboratory, Universiti Teknologi MARA Shah Alam.

Shoot establishment and regeneration from half mature *Arenga pinnata* zygotic embryos

The zygotic embryos were excised and cultured on medium containing approximately 20mL hormone-free Murashige and Skoog (MSO) medium (Murashige and Skoog, 1962). The MS medium was prepared by dissolved 4.41 mg/L commercial MS powder with vitamins (Duchefa, Holland), 8.0 g/L agar (Duchefa, Holland) and 30.0 g/L sucrose (Duchefa, Holland) in distilled water. The culture media were adjusted to pH 5.8 using 1M NaOH (Merck, Germany) and 1M HCl (Merck, Germany) and sterilized by autoclaving at 121ºC for 15 minutes. The cultures were maintained at 25±1ºC and a photoperiod of 16 hours.

Plant growth regulators consisting of benzylaminopurene (BAP) was used as treatment to obtain *in vitro* regeneration of sugar palm. The treatment media were as followed: MS0 (Control treatment), MS + 0.1 mg/L BAP, MS + 0.5 mg/L BAP, MS + 3.0 mg/L BAP. Excised zygotic embryos established from MSO medium were used as explant in *in vitro* plantlet regeneration protocol.

The experiment was arranged in a completely randomized design (CRD) with 10 replications. The percentage of germinated mature zygotic embryos of sugar palm was observed and evaluated after 4, 8, 16, 20, 24, 28, 32 weeks of culture on hormone-free MS medium and the explant length, radicle length, shoot length, root length and the color of the plantlets were recorded. *In vitro* regeneration of sugar palm from zygotic embryo was observed after four and eight weeks. The results were subjected to analysis of variance (ANOVA) and Duncan New Multiple Range Test (DNMRT) at a 5% probability level. Statistical parameters such as percentage, mean, and standard error were estimated using SPSS version 21.

RESULTS AND DISCUSSION

Shoot establishment and regeneration

Sugar palm shoot was successfully induced from fruits of *Arenga pinnata* by using hormone-free MS medium and regeneration protocol using MS medium supplemented with different concentrations of BAP. The results of the study indicate that, after four weeks culture, the color of the explant in MS free hormone medium turned from creamy (Fig. 1A) to green color (Fig. 1B). Explants start to produce yellowish brown root with white secondary roots after 8 weeks culture (Fig. 1C) and plumule sprout and then developed into shoot after 24 weeks

culture (Fig. 1D). Then, the green shoot with unopened leaflet (Fig. 1E) was fully developed into a leaf after 32 weeks culture (Fig. 1F). Hormone-free MS medium which performed as control treatment shows 90% of regenerated explants compared to other treatments. It also produced shoots and shows an increase in plantlet and radicle length. Previous study stated that growth of the embryos was observed through swelling or cracking on the explant surface. Likewise, changes of colour from cream to green also indicated the development of embryo (Najya *et al.,* 2013). Furthermore, embryos were considered germinated when the plumule developed and the radicle exhibited signs of improve-ment (Danson *et al.,* 2009).

The effects of MS medium supplemented with various concentrations of BAP after eight weeks culture are shown in Table 2. The result indicated that about 59.5% of the explants regenerated from zygotic embryos in medium containing 0.5 mg/L BAP after 8 weeks culture. This concentration also led to the increase in radicle size. But, no growth performances were observed in highest concentration of BAP (3.0 mg/L). The results indicated that a growth performance of sugar palm zygotic embryo was successfully established in hormone-free MS medium. Regeneration of the explant was found in MS medium containing 0.5 mg/L BAP. Previous study described that growth of explant observed in MS hormone-free medium indicated that the plants are embryonic. However, the use of hormones such as BAP in fostering the somatic embryo maturation and cotyledon development is very important. It has been reported that medium supplemented with BAP shows good performances of the plant development (Farzana *et al.,* 2008). This study represents an efficient protocol for improvement of *in vitro* regeneration via zygotic embryo of *Arenga pinnata* using 6-benzylaminopurene. It is expected that the developed protocols, which could help to satisfy the increased demand for plant material of *Arenga pinnata* as an alternative to sugarcane and biofuel in future.

Table 1. Observation on i*n vitro* germinated plantlet of sugar palm from zygotic embryos explants at 0, 4, 8, 16, 20, 24, 28, 32 weeks of culture on MS medium hormone-free

Weeks	Explant Length (cm)	Radicle Length (cm)	Shoot Length (cm)	Root Length (cm)	Observation
0	0.3	0	0	0	Creamy colour
4	0.5	0	0	0	Explant turned from creamy white to green colour
8	0.7	0.5	0	0	Greenish brown radicle
16	0.8	3.0	0	1.5	Yellowish brown root with white secondary roots
20	1.0	4.0	1.0	3.0	Yellowish brown root with white secondary roots
24	1.0	4.5	2.0	5.5	Yellowish green shoot
28	1.0	4.5	3.0	6.0	Green shoot with unopened leaflet
32	1.0	4.5	6.5	6.0	Green plantlet with opened leaf

Table 2. *In vitro* germinated of sugar palm from zygotic embryos explants after 8 weeks of culture on MS medium supplemented with different 6-BAP concentrations

No	Treatment	Explant regenerated (%)	Radicle size (cm)	Total shoot per culture	Observation
1.	MS0 (Control)	90.7 ± 0.94g	4.50 ± 0.05g	1.00 ± 0.05b	Green plantlet
2.	MS + 0.1 mg/L BAP	40.6 ± 0.52c	0.50 ± 0.03b	0.00 ± 0.00a	Green explant
3.	MS + 0.5 mg/L BAP	59.5 ± 0.52e	1.57 ± 0.06e	0.00 ± 0.00a	Green explant
4.	MS + 3.0 mg/L BAP	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	Green explant

** Each result was based on the mean of 10 replications. Means with same letter are not significantly different at P < 0.05)*

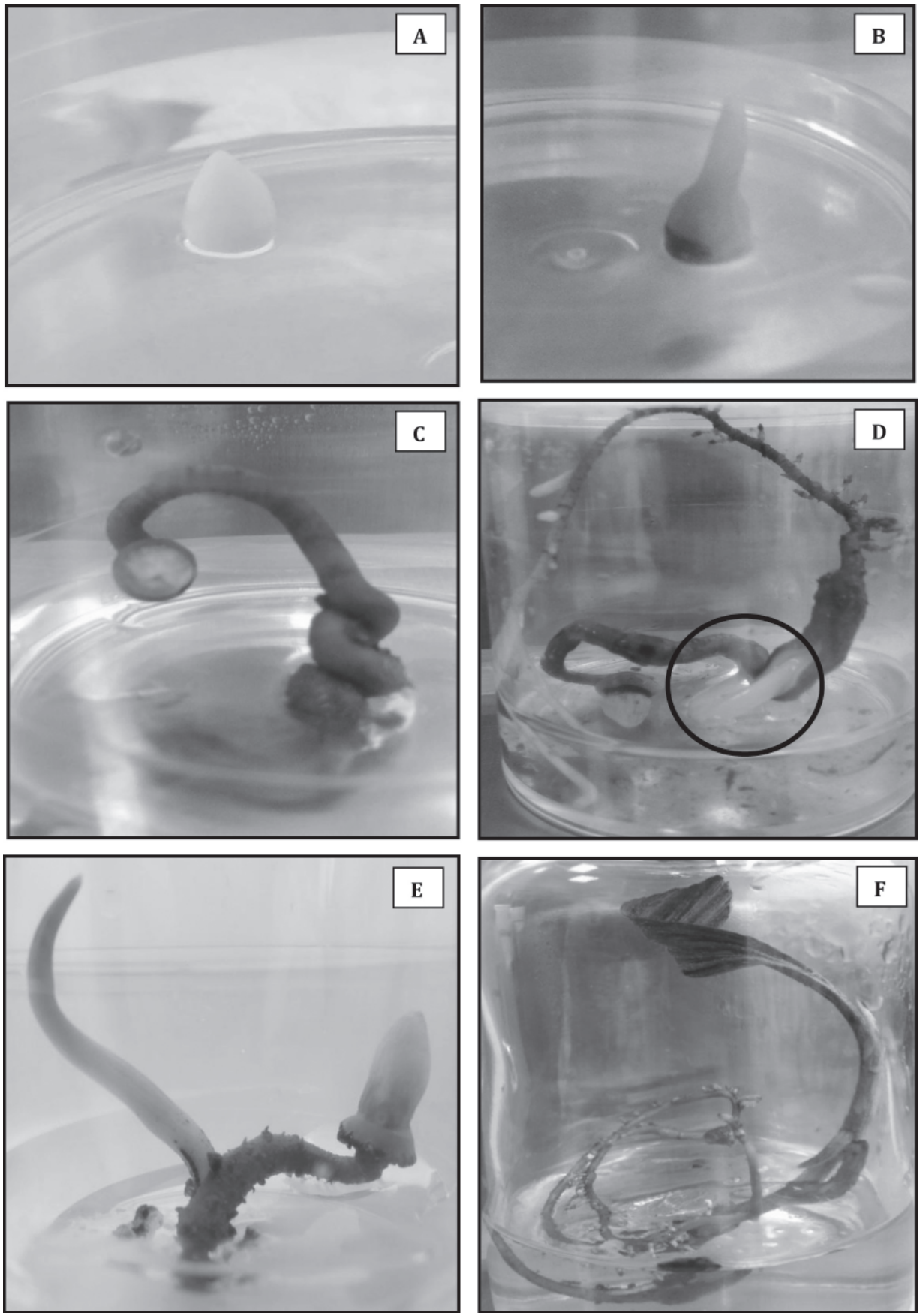

Fig. 1. (A) Zygotic embryo of *Arenga pinnata* excised from mature fruit; (B) Explant at four weeks old; (C) Radical emerged after 8 weeks culture; (D) Shoot emerged after 24 weeks culture; (E) Green shoot with unopened leaf at 28 weeks old and (F) *In vitro* seedling of *Arenga pinnata* after 32 weeks old culture.

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