Shoot initiation for *Macadamia integrifolia* explant with tissue culture technique

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Abstract. Macadamia nuts are grown in subtropical and tropical regions and endemic species in Greenland that can be commercially developed in Indonesia. Macadamia's generative propagation tends to have problems in its seed stock. It often experiences obstacles in field seed stock, and the production requires a long time because it has a thick shell (pericarp). Macadamia initiation needs technology to prevent extinction. One of the propagation is through the technique culture in vitro. This research was conducted to determine the initial response of basic media and to know the response of Macadamia growth in vitro. This research used five media which are Media 1 (DKW with BAP 0.1 ppm, kinetin 0.1ppm), Media 2 (WPM with BAP 1 ppm), media 3 (DKW), media 4 (MS), media 5 (MS with BAP 0.5 ppm). The results showed that media 1 and 3 (DKW media) had a good response for leaf and shoot growth in macadamia explants.

1. Introduction

Macadamia comes from the eastern part of the Australian continent, consisting of two types, namely *Macadamia integrifolia* and *Macadamia tetraphylla* [1]. Countries that cultivate macadamia for commercial purposes include Hawaii, Australia, South Africa, Malawi, Zimbabwe, Guatemala, Brazil, Costa Rica, and Fiji [2]. Macadamia is a plant species that have high economic value. However, Indonesian people do not know much about this plant. Macadamia plants can be found in Cibodas Botanical Gardens, Cianjur, West Java, and Horticulural Research Center in Cikole, Lembang, Bandung. It has been thoughtfully cultivated at the Kalisat Jampit Plantation and the Sempol Plantation (PTPN XII) in the Ijen Highlands, Bondowoso, East Java. The macadamia is already fruiting and processed in this plantation, although the volume is still minimal and only sold in the garden area to tourists [3].

Based on Heryana et al., (2008), macadamia products in the form of nuts have high economic value in local and international markets and become industrial raw materials that can be processed into various forms of food [4]. Macadamia oil contains palmitic acid and also has a lot of oleic acids, which are very good for softening the skin, regenerating skin cells, moisturizing the skin, and as a natural anti-inflammatory [5].

Commercial development of macadamia is faced with difficulties in obtaining good quality, producing uniform seeds in large quantities and in a short time. Currently, macadamia is propagated by seeds and grafting. Propagation by seed has several disadvantages, such as need a longer time, the number of seeds produced is limited, and the plants produced are very diverse. Grafting propagation also has several disadvantages, which are the procurement of rootstock depends on the season, while the procurement of
stem requires a lot of material and quality varieties, the number of seeds produced is limited (Ashari, 1995), and seed maintenance time takes around 18 to 24 months, require skilled labor, material inputs and wide space [6,7]. The price of seeds in Indonesia is still quite high, ranging from Rp. 50,000 – Rp. 85,000/stem. This provides an opportunity for macadamia development through tissue culture.

Efforts to overcome this problem can be made by using in-vitro culture techniques or often called micropropagation [8]. Shoot culture uses shoot tips or axillary shoots as explants and planted in a nutrient medium [9,10]. Thus, differentiation and organogenesis occur to form perfect plants. The shoots are the best explants used for plant propagation [11]. Tissue culture studies of macadamia tetraphylla have been conducted by Bhalla et al., (2001), Cha um et al., (2011) [12,13]. However, there has been no report on a stable tissue culture system for M. integrifolia [14]. Successful in vitro culture of any species requires basic empirical experiments to optimize nutrients, growth regulators, and culture growth conditions of the plant at each stage of the culture process. The purpose of this study was to determine the initial response of basic media on the regenerative potential of Macadamia integrifolia shoots in vitro.

2. Implementation method
2.1. Research material
2.1.1. Explant. The explant material used was young shoots of macadamia seedlings aged 8 months kept in the BPTH Region II Greenhouse. The macadamia seeds came from PTPN Bondowoso.

2.1.2. Medium. The medium used was MS (Murashige and Skoog), WPM (Woody Plant Medium), and DKW (Driver & Kuniyuki) basic media by adding growth regulators such as BAP and kinetin.

2.2. Method
2.2.1. Explant. Young shoots of macadamia seedlings with a length of 3 cm and aged two weeks were cut from macadamia seedlings and soaked in distilled water. After that, it was washed with running water and then soaked in a distilled water + tween solution for 20 minutes, then rinsed thoroughly. Then the explants were soaked with a 2% function alicide for 60 minutes and a bactericide for 60 minutes.

Sterilization was done in Laminar Air Flow. Explants were soaked with 70% alcohol for 1 minute, rinsed thoroughly, then soaked with commercial Clorox 20% plus tween for 10 minutes and 10% plus tween for 5 minutes, then rinsed again. The old leaves were cuts. The soft and light green leaves were left. The explants were then planted into the prepared sterile medium (Figure 1).

2.2.2. Medium. After weighing the macro and micro elements, it was diluted with distilled water mixed with 7 grams of agar and 30 grams of sugar. Growth regulators were added with concentrations of 0.1 ppm, 0.5 ppm, and 1 ppm. The available media were MS 0, MS BAP 0.5 ppm, DKW 0, DKW BAP 0.1 KIN 0.1, WPM BAP 1 ppm. The medium was not made by design because this study was to determine the initial response to the basic media. The medium used during the observation was adjusted to media stock in the BPTH Region II tissue culture laboratory.
3. Result and discussion

The basic media response test was carried out using the external and internal laminar sterilization method using a bayclin. The results of observations on the basic media responses are presented in Table 1 below:

| No | Medium          | Contamination | Number of Shoots | Number of Leaf | Shoot Height (cm) | Photo |
|----|----------------|---------------|------------------|----------------|-------------------|-------|
| 1  | MS 0           | -             | 1                | 4              | 2.5               | ![Image](image1.jpg) |
| 2  | MS BAP 0.5 ppm | -             | 2                | 4              | 2.4               | ![Image](image2.jpg) |
| 3  | DKW 0          | -             | 1                | 6              | 5.2               | ![Image](image3.jpg) |
| 4  | DKW BAP 0.1 ppm| -             | 1                | 4              | 3.5               | ![Image](image4.jpg) |
Based on Table 1, the explants planted on each basic media were not contaminated. It means that the sterilization process was appropriate. Sterilization is the most crucial step in producing sterile explants. Sterilization is done to reduce the occurrence of contamination. According to Moeso (1977), the failure of tissue culture is mainly because of failure to keep the explants sterile [15].

The sterilization method carried out was correct and showed the absence of contamination of the implanted explants (100% pure). According to Hussey (1978), the use of explants from young tissue will have a better success rate than old tissue, and the size of the cut shoot required for culture is small enough to obtain uncontaminated explants [16]. Larger explants will divide and enlarge more rapidly than small explants but may contain viruses [17].

Observation of the explant's growth was done by measuring the height and number of leaves. Based on these results, the explants planted on DKW 0 and DKW BAP 0.1 Kin 01 Ppm media gave a good response, seen with high explants and more leaves than MS and WPM media. According to Hamzah (2016), shoot height is directly proportional to the number of leaves because it can provide more for the leaves to grow [18]. This is also in line with Suyanti's (2013) statement that the higher the shoot, the more nodes as a place for leaf to grow [19]. When viewed from the nutrient content of the DKW medium, it contains more nutrients than MS and WPM media. Yuniastuti et al., (2010) stated that shoots are plant organs where growth and development are influenced by the presence of Nitrogen (N), Phosphorus (P), and Potassium (K) [20]. DKW media contains more phosphorus (P) elements than MS and WPM media. These results can be compared with research by Gitonga, et al. (2008), which stated that Macadamia integrifolia grew better on WPM media than MS media [7]. The results of this study showed that the three basic media, the MS, WPM, and DKW, gave a growth response, but the best results were shown on the DKW media; thus, it can be used as a reference for future research to obtain media stability and ZPT concentration. It is also more efficient in terms of the budget used in the production of macadamia seedlings through tissue culture.

4. Conclusion and suggestion
4.1. Conclusion
The sterilization method can be applied to initiate young macadamia shoot because it did not experience any contamination, and all basic media respond well. DKW media gave the best response from the height and number of leaves.

4.2. Suggestion
It is necessary to conduct further research using experimental designs with various concentrations of growth regulators such as BAP and Kinetin using DKW, MS, and WPM media to obtain the proper method for in vitro propagation of macadamia.

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