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In Vitro Callus Induction from (Antidesma bunius L.) Leaves

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Abstract: *Antidesma bunius L. is a tree species that has ecological and economic benefits, and has many bioactivities. Propagation of this tree can be done in several ways, one of which is callus induction. Callus is a collection of undifferentiated cells that can develop into new plants with the help of ZPT. The purpose of this study was to determine the effect and optimum combination of ZPT 2,4-D and BAP on the induction of leaf callus of A. bunius L. The research design was a non-factorial Completely Randomised Design (CRD) with 4 treatments namely P0: MS without the addition of zpt; P1: MS + 1 ppm BAP + 3 ppm 2,4-D; P2: MS + 2 ppm BAP + 2 ppm 2,4-D; P3: MS + 3 ppm BAP + 1 ppm 2,4-D. Parameters observed included callus emergence time, percentage of callus explants, and callus morphology. The results showed that P2 is the best concentration for callus induction, by looking at the observed effect on callus emergence time of 6 HST, percentage of callus explants 56% and callus morphology which is white with crumb texture, which can develop into embryogenic callus.*

Keywords: *Buni leaves; In vitro; callus; ZPT*

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1. INTRODUCTION

Antidesma bunius L. is native to Southeast Asia. This plant can be found in various countries, including in the regions of Indonesia, Malaysia (Lim, 2012), Thailand (Jorjonget al. 2015), and the Philippines (Lawaget al. 2012). In Indonesia, it has different names in each region. Sunda and Java (wuni), Bali (boni/buni) and Gorontalo is called Takuti/Malahengo. A. bunius L. is a multifunctional tree species, both ecologically and economically. It has quite large roots and often lives on riverbanks, making it a flood-fighting tree and excellent for crit ical land reclamation. The bark has practical uses as a rope material (Hazarika & Singh, 2017), while the young leaves can be enjoyed as a side dish to accompany rice or processed into spices (Lim, 2012). This tree has black fruits that contain antioxidant and anti-obesogenic compounds (Krongyut & Sutthanut, 2019). Which is utilized by the community as an ingredient for making salad and processed into herbal drinks (Sulfianiet al. 2022) jelly, jam and juice (Lim, 2012; Ong & Kim, 2017; Inonu, 2020).

According to IUCN (International Union For Conversation Of Nature) A.bunius L. is included in the IUCN Red List category LC (Least Concern) or low risk. However, the Gorontalo Watershed and Protected Forest Management Center report-

ed that the existence of A. bunius L. trees in Gorontalo is threatened with extinction. This tree is only found in two locations in the Gorontalo region, namely in Bone Bolango (Tilongkabila forest) and Gorontalo Regency (Bongohulawa forest). In Gorontalo City, North Gorontalo, Boalemo and Pohuwato, no parent trees were found (Lahay, 2022).

Efforts to prevent A. bunius L. plants from extinction can generally be done by propagating seeds vegetatively and generatively or through conventional methods. Both methods, however, require a long time to produce a large number of seeds. The utilization of biotechnology can be a solution to overcome this problem, including through tissue culture techniques. One of the techniques used is callus induction. Callus induction is the first step in in vitro culture that can utilize the potential of each cell to develop into new individuals. Research conducted by Sari & Sumadi (2014) showed that the percentage of callus into shoots was 78% in Carica pubescens leaf tissue explants. Thus, effective callus induction is a crucial step in obtaining quality seedlings and quickly in large quantities. Based on the description above, the study of the effect of the concentration of ZPT 2,4-D and BAP on callus induction of A. bunius L. leaveswas carried out. With the aim of knowing how the effect and optimum concentration of ZPT 2,4-D, and BAP are appropriate for callus induction of A. bunius L.

2. MATERIALS AND METHODS

The materials used were A. bunius L. leaves, MS base medium (Murashige & Skoog 1962), 2,4-D (2,4-Dichlorophenoxyacetic Acid), BAP (Benzyl Amino Purin), sucrose, agar, aluminum foil, plastic wrap, sodium hypochlorite, detergent, label paper, KOH 1N, NACL 1 N, Handscoon and mask. Tools used autoclave, laminar air flow, analytical scales, measuring cup, beaker glass, petridish, culture bottle, bunsen lamp, scissors, scalpel, tweezers, lighter, stirring rod, pH meter, micropipette, stationery, and documentation tools.

The research design is non-factorial Completely Randomized Design (CRD), namely, P0 = MS without the addition of zpt; P1 = MS + 1 ppm BAP + 3 ppm 2,4-D; P2 = MS + 2 ppm BAP + 2 ppm 2,4-D; P3 = MS + 3 ppm BAP + 1 ppm 2,4-D. Each treatment 3 replicates, in each replicate 3 bottles (4 x 3 x 3 = 36 bottles). Each bottle consists of 2 explants. Parameters observed were callus emergence time, determined based on the time interval (days) from planting until callus began to appear from each treatment. Percentage of callus was done in the last week. Calculated using the percentage formula referring to Prihastanti, et.al (2020) as follows:

$$Callus\ Percentage = \frac{\text{Number of explants producing callus}}{\text{Total number of explants per treatment}}\ x\ 100\%$$

Callus morphology was observed from the beginning to the end of the study by making visual observations of the color and texture characteristics of the callus formed. Determination of callus color is based on criteria conducted by Tarigan et al, (2023) with modifications (Table 1).

No	Color
1	Yellow
2	White-yellow
3	Brown
4	White
5	Brownish yellow
6	Brownish white

Determination of callus texture based on research by Sugiyarto & Kuswandi (2014) as follows:

1. Compact; tissue that is arranged tightly and firmly, with cells that are tightly bound together and not easily separated. Its dense and hard texture indicates the strength and durability of this structure.
2. Crumb structure; easily destroyed, cells that are not tightly bound and have large intercellular spaces.

Callus morphology was analyzed descriptively. The percentage of callus using the percentage formula and the time of callus appearance were analyzed using

Analysis of variance (ANOVA) and followed by Duncan's test at the 5% level. This test was conducted with the SPSS 21 application program.

3. RESULTS AND DISCUSSION

Callus emergence time

The formation of callus on explants is the main parameter in indicating growth and development in tissue culture. The results of observations obtained initially callus formed on the former part of the explant cut, in the form of a collection of plant cells that grow irregularly in the form of small spheres and white color. Tariganet al (2023) also stated that the initiation of callus tissue growth occurs on the former explant slices, round in shape and there are protrusions of callus aggregates. In line with the research of Waryastutiet al (2017), the sign of callus in the explant is swelling and the formation of white tissue in the former explant slice area. Analysis of variance results on the provision of ZPT 2,4-D and BAP showed a significant difference in the time of callus appearance (Table 2).

The P0 treatment which was used as a control had a significant difference in the Duncan test at the 5% level among the other treatments and there was no callus induction response. The reason is that the media used did not contain 2,4-D and BAP. In line with the research of Wulandari et al, (2022) that without the addition of ZPT has not been able to induce callus in Duku (Lansium domesticum Corr.) leaf explants. This indicates that the addition of ZPT is necessary because endogenous ZPT levels in plants are not sufficient to trigger callus formation. In addition, P1, P2 and P3 have no significant difference. Even so, P2 is the fastest in giving rise to callus, namely 6 DAP, compared to P3 callus grows at 6.53 DAP and P1 is the slowest concentration in callus emergence time which is 7.17 DAP. This is in line with the research of Waryastuti et al, (2017) where low concentrations of 2,4-D resulted in slower callus initiation towards callus induction. At high concentrations 2,4-D prevents callus growth and development (Konaet al. 2019). Similar research also revealed that the higher the concentration of growth regulators, the less the number of callusformed (Daret al. 2021).

Percentage of callus

Analysis of the percentage of callus formation is one indicator to assess the responsiveness of explants, to the effectiveness of the treatment combination applied in encouraging the growth of callus tissue. Based on the results of the research that has been done (Figure 1); P2 (2 ppm BAP + 2 ppm 2,4-D) is the highest concentration of callus explants, which is 56% compared to the percentage of P3 (3 ppm BAP + 1 ppm 2,4-D) and P1 (1 ppm BAP + 3 ppm 2,4-D) on explants that grow callus, which is 50% and 44%, respectively. This is due to the same ratio of auxin and cytokinin will cause callus induction (George, et al. 2008). If high cytokinin and low auxin will produce shoots. On the other hand, if the ratio of high auxin and low cytokinin will produce roots (Schalleret al. 2015).

Callus morphology

Analysis of callus color and texture is an important parameter to determine the physiological status, health, and potential for callus regeneration and differentiation. Based on the results of observations, the average callus formed in all treatments is white with a crumbly texture in Table 3 and Figure 2. Based on the research of Nurokhman et al.(2019) the addition of 2,4-D and BAP to leaf explants produces crumbly white callus, compared to the combination of NAA and BAP, the callus formed is compact in texture and green in color.

The advantage of crumb callus is that it has a faster cell division rate compared to compact callus (Prashariska et al.2021).

Callus with this texture is very useful for cell suspension culture because undifferentiated cell groups can grow quickly in liquid culture media (Ramulifhoet al. 2019). Other studies have also revealed that callus with a crumbly texture and white or yellow color is one of the characteristics of callus that can develop into embryogenic (Isda & Salsabilla, 2022). Based on research by Yelnititis (2012), crumbly callus changes color from white to yellowish or light yellow, then turns greenish yellow and then becomes green during the callus growth period.

Table 2: Effect of the combination of 2,4-D and BAP on callus emergence time

Treatment	Callus emergence time (Days)
P0 (0 ppm BAP +0 ppm 2,4-D)	*a
P1 (1 ppm BAP + 3 ppm 2,4-D)	7.17b
P2 (2 ppm BAP + 2 ppm 2,4-D)	6.00b
P3 (3 ppm BAP + 1 ppm 2,4-D)	6.53b

Notes: *= no callus; Numbers followed by the same letter have no significant difference in the Duncan test at 5% level.

Table 3: Color and texture of callus from the combination of 2.4-D and BAP

Treatment	Color	Texture
P1(1 ppm BAP + 3 ppm 2,4-D)	White	Friable
P2(2 ppm BAP + 2 ppm 2,4-D)	White	Friable
P3(3 ppm BAP + 1 ppm 2,4-D)	White	Friable

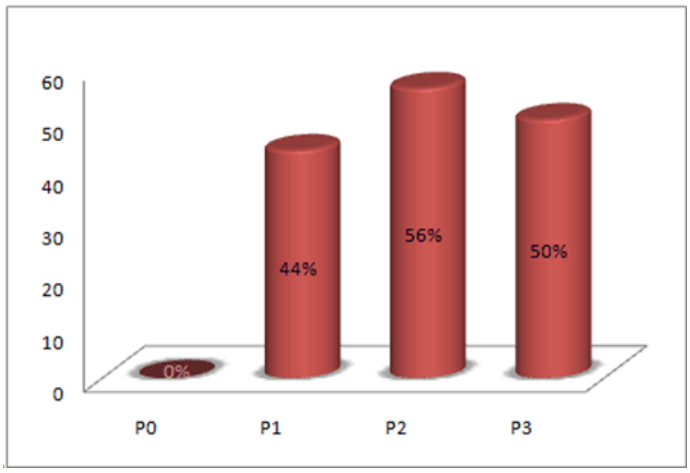
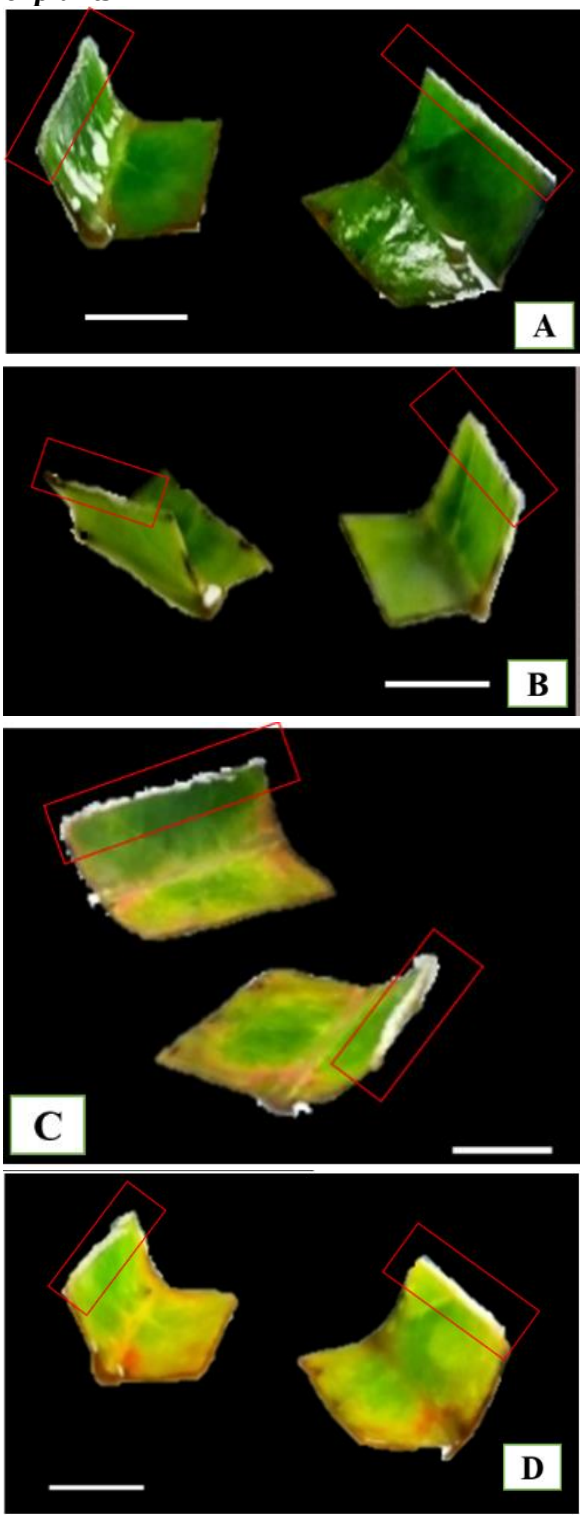


Figure 1: Graph of the percentage of callus explants



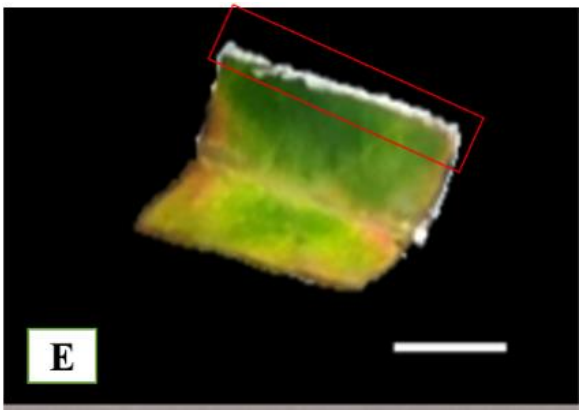


Figure 2: Morphology of callus. A) 1 MST explants; B) Explants 2 weeks after planting; C) Explants 3 weeks after planting; D) Explants 4 weeks after planting; E) White callus, friable; Red square line = observed callus; Bar 5mm

4. CONCLUSION

The use of ZPT 2,4-D and BAP in callus induction from the leaves of A. bunius L. on MS media gives an influence on the fastest callus emergence time of 6 DAP, the highest percentage of callus explants 56% and morphology of callus formed white color with crumb texture. The optimum concentration to induce leaf callus of A. bunius L. is a combination of ZPT 2 ppm 2,4-D and 2 ppm BAP. It is hoped that there will be further research to obtain a more optimal treatment concentration to induce leaf callus of A. bunius L. In addition, it is better not to plant 2 explants in one bottle because if one explant is contaminated, the other explants will also be contaminated automatically. This happens because the sterilization of explants is not optimal, and the explants used are not the result of in vitro sprouts, but come from open land.

5. CONFLICT OF INTEREST

The author declares that there is no conflict of interest concerning the content of this paper

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