The Influence of Various Growth Regulator of Growth Media on Biomass and Callus Induction in *Elephantopus scaber* Linn.

Yuliani*¹, F Rachmadiarti¹, S K Dewi¹ and M T Asri¹

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Indonesia. yuliani@unesa.ac.id fidarachmadiarti@unesa.ac.id saridewi@unesa.ac.id mahaniasi@unesa.ac.id

**Abstract.** *Elephantopus scaber* has the potential to be developed and isolated of secondary metabolites by tissue culture techniques. Secondary metabolites of *E. scaber* include scabertopin, scabertropinol, luteolin, flavonoids, deoxylephantopin, phenols, saponins, tannins, elephantopins which can be isolated from the callus, so that various growth media are used to get the largest *E. scaber* callus. The aim of this study is to describe the biomass and induction of *E. scaber* callus in various growth media in vitro (tissue culture techniques), and to determine the proper medium to produce *E. scaber* callus biomass optimally. *E. scaber* culture experiments were conducted descriptively, using 5 growth media, namely 1) MS + BAP media, 2) MS + Kinetin media, 3) MS + 2.4 D media, with concentrations of BAP, Kinetin and 2.4 D of 0.5; 1; 1.5; and 2 mg/L respectively, 4) MS + BAP + 2.4 D and 5) MS + Kinetin + 2.4 D, with the ratio of BAP + 2.4 D and Kin + 2.4 D are 0.5 + 0.5; 1 + 0.5; 1 + 1; 1.5 + 1.5 mg/L. Each medium contained 25 experimental bottles, each bottle are containing 5 *E. scaber* explants. Stages of research method are include explant sterilization, manufacture and sterilization of media, and planting explants into growth media. The research parameters are callus induction and callus biomass. The data were analyzed by quantitative descriptive. The results showed that the use of MS + 0.5 mg/L BAP growth media gave the fastest callus induction period, 14 days after planting, while MS + 1.5 mg/L BAP + 1.5 mg/L 2.4 D growth media gave the largest callus biomass that is 0.0797 grams with a friable callus texture.

1. **Introduction**

*Elephantopus scaber* (Family of Asteraceae) contains secondary metabolite compounds that can be used as traditional medicinal ingredients or for biopesticides. These compounds include scabertopin, scabertropinol, luteolin, flavonoids, deoxylephantopin, phenols, saponins, tannins, elephantopins, and *sesquiterpenes* [1], [2]. However, to get the secondary metabolite compounds needed a large supply of *E. scaber* plants. Therefore, in vitro culture is considered to be very efficient for producing secondary metabolites. Propagation by tissue culture techniques through callus formation can be used to produce secondary metabolic compounds. This can occur because a plant cell has genetic potential which is derived to produce the same compound in large numbers if it is grown *in vitro* conditions. Callus extraction was carried out to produce secondary metabolites [3]. Tissue culture can be used as an alternative method for obtaining secondary metabolites, because it can be done with media modification, growth regulators, carbon sources to produce the desired...
metabolites. In addition, tissue culture technique can form bioactive compounds under controlled conditions and in a relatively short time. To produce secondary metabolites usually used suspension culture and callus culture [4][5]. The need for growth regulators in most callus cultures is from the auxin and cytokinin groups. Giving auxin-cytokinin is very important in the regulation of cell division, cell lengthening, cell differentiation, and organ formation. Growth media using growth regulators can increase secondary metabolite compounds produced. The addition of 20M IBA and 18M BA results in a relatively high secondary metabolite content of grindelic acid in *Grindelia purchella* (1.85 mg/g dry weight) [6]. Stem organ culture studies of *Pueraria candollei* plants on MS media with the addition of 4.5M 2,4-D and 0.46M kinetin produced daidzein at 5.12 mg/g dry weight and genistein 2.77 mg/g dry weight [7]. *Elephantopus scaber* tissue culture research has been carried out to obtain the right explant sources in avoiding contamination and producing callus [8] using growth media for callus induction, namely MS media with the addition of auxin and cytokinins growth regulators. The auxin used is 2,4 D type, while for cytokinin it uses Benzyl Aminopurine (BAP) and Kinetin. Based on this, a study aimed to describing *E. scaber* biomass and callus induction on various growth media *in vitro* (tissue culture), and to determining the properly media to produce *E. scaber* callus biomass optimally.

2. **Methods**

*E. scaber* tissue culture research was conducted descriptively, using 5 growth media, namely 1) MS + BAP media, 2) MS + Kinetin media, 3) MS + 2.4 D media, with BAP, Kinetin and 2.4 D concentration each of 0.5; 1; 1.5 and 2 mg/L, 4) MS + BAP + 2.4 D and 5) MS + Kinetin + 2.4 D, with a ratio of BAP + 2.4 D concentration and Kinetin + 2.4 D are 0.5 + 0.5; 1 + 0.5; 1 + 1; 1.5 + 1.5 mg/L respectively (Mohr series). Each medium contained 25 experimental bottles, each bottle containing 5 pieces of *E. scaber* explants. Explants in the form of leaves obtained from germination of *E. scaber* seeds (germination age 14 days after planting). Stages of method include sterilization which includes sterilization of equipment, sterilization of media, sterilization of explants and sterilization of culture sites. Then making and sterilizing media, and planting explants into growth media. The research parameters are callus induction and callus biomass. The data were analyzed by quantitative descriptive.

3. **Results**

Calli are formed from *E. scaber* leaf explants starting at 3-4 weeks after culture. The response of callus formation begins with enlargement of explants and followed by the appearance of callus. (Picture 1). The colour of callus that is formed at the beginning of growth is white and has a friable texture.

![Figure 1. Elephantopus scaber Callus](image-url)
though cell proliferation has occurred. Media that can produce calli are B1 (MS + 0.5 mg/L BAP); D2 (MS + 1.0 mg/L 2.4-D); KD3 (MS + 1.5 mg/L Kinetin + 1.0 mg/L 2.4-D); KD4 (MS + 1.5 mg/L Kinetin + 1.5 mg/L 2.4-D), and BD4 (MS + 1.5 mg/L BAP + 1.5 mg/L 2.4-D) media.

Table 1. The callus induction data of *E. scaber* on various growth media

| The media treatments | Amount of calli | Callus Induction (Day after planting) | Callus texture |
|----------------------|-----------------|---------------------------------------|---------------|
| B1                   | 5               | 14                                    | Friabel       |
| D2                   | 5               | 19                                    | Friabel       |
| KD3                  | 5               | 19                                    | Friabel       |
| KD4                  | 5               | 19                                    | Friabel       |
| BD3                  | 5               | 19                                    | Friabel       |
| BD4                  | 5               | 19                                    | Friabel       |

*B1 = MS0 + BAP (MS + 0.5 mg/L BAP)*
*D2 = MS0 + 2.4D (MS + 1.0 mg/L 2.4D)*
*KD3 = MS0 + Kin + 2.4D (MS + 1.5 mg/L KIN + 1.0 mg/L 2.4D)*
*KD4 = MS0 + Kin + 2.4D (MS + 1.5 mg/L KIN + 1.5 mg/L 2.4D)*
*BD3 = MS0 + BAP + 2.4D (MS + 1.0 mg/L BAP + 1.0 mg/L 2.4D)*
*BD4 = MS0 + BAP + 2.4D (MS + 1.5 mg/L BAP + 1.5 mg/L 2.4D)*

Table 1. shows that the B1 treatment gave the fastest callus induction which was 14 days after planting. BAP growth regulators provide faster callus induction than others. Whereas the other treatment takes 19 days for callus induction.

Table 2. The biomass data of *Elephantopus scaber* calli

| Clump | Media               | The biomass of calli (g) |
|-------|---------------------|--------------------------|
|       | B1                  | D2                      | KD3 | KD4 | BD3 | BD4 |
| 1     | 0.0183              | 0.0099                  | 0.0311 | 0.0138 | 0.1092 | 0.0476 |
| 2     | 0.0172              | 0.0412                  | 0.1127 | 0.0238 | 0.0283 | 0.0277 |
| 3     | 0.0219              | 0.1207                  | 0.0000 | 0.0463 | 0.0251 | 0.1450 |
| 4     | 0.0420              | 0.0000                  | 0.0000 | 0.0358 | 0.0546 | 0.1129 |
| 5     | 0.0125              | 0.0000                  | 0.0000 | 0.0805 | 0.1137 | 0.0653 |
|       | Average             | 0.0224                  | 0.0344 | 0.0288 | 0.0400 | 0.0662 | 0.0797 |

The biomass data of Calli in table 2 shows that the largest is callus in BD4 media: MS0 + BAP + 2.4 D at 0.0797 grams followed by callus from growth media BD3 (MS0 + BAP + 2.4 D) and KD4 (MS0 + Kin + 2.4 D). The data also shows that addition of two growth regulators from auxin and cytokinin provides greater biomass compared to media with one growth regulator.

4. Discussion

The results showed that addition of auxin and cytokinin growth regulators with balanced concentrations (MS + 1.5 mg/L BAP + 1.5 mg/L 2.4 D) could provide the largest callus biomass (0.0797 grams). Auxin and cytokinin growth regulators for callus induction or for increased callus biomass are needed in tissue culture techniques given the function of the two Plant Growth Regulator. Auxin is a group of compounds that stimulate elongation of shoot cells, while Cytokinin is increase plant cell division in certain bioassay conditions, regulate plant growth and development as kinetin. Giving auxin-cytokinin are very important in the regulation of cell division, cell lengthening, cell differentiation, and organ formation [10]. Callus growth characteristics involve the relationship between plant material used for callus initiation,
medium composition, and environmental conditions during the incubation period. Callus formation from explants is divided into three development phases, namely induction, cell division, and differentiation. The duration of this phase is very dependent on the physiological conditions of the explant cells and the condition of the culture environment [11] [12].

The nutritional requirements for callus induction vary greatly for primary explants originating from different sources. However, most tissues require one or more growth factors to stimulate callus development [13]. An explant can require auxin, need cytokinin, need auxin and cytokinin, and require complex natural extracts.

The need for auxins and cytokinins for induction of callus and callus biomass are supported by research by [9] who reported, that the maximum callus frequency (71 g of callus fresh weight and 97% of callus induction of chick pea) were observed on 4 mg/L 2.4 D + 5 µM BAP in MS media, while the minimum callus percent (30 and 53%) was observed in 2 mg/L 2.4 D alone in MS Media after 2 and 4 weeks of culture. Lower concentration of NAA (0.50 mg/L NAA) and high concentration of 2.4 D (4 mg/L of 2.4 D) favored callus formation in both of MS and B5 media. Similar observation were recorded by [14] and [15] for cotton and pumpkin species, respectively. Similarly, the highest callus growth in term of fresh weight (0.411 and 0.787 g) was noticed in MS medium fortified with 4 mg/L 2.4 D + 5 µM BAP and (0.401 and 0.693 g) on 4 mg/L 2/4 D when used in B5 medium. BAP as cytokinin was more suitable in lower concentration (5 µM), whereas high concentration of this growth regulator is suboptimal in term of callus induction. Callus fresh weight increased significantly with lowering BAP and increasing 2.4 D concentration in both formulated media. This kind of auxin (2.4 D) alone or in combination with cytokinin (BAP) 100% callus induction on chick pea has been reported by [16].

5. Conclusion
The use of MS + 0.5 mg/L BAP growth media gave the fastest callus induction time which was 14 days after planting, while MS + 1.5 mg/L growth media BAP + 1.5 mg/L 2.4 D gave the largest callus biomass that was 0.0797 grams with a friable callus texture.

6. References
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