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In-vitro Propagation of *Elephantopus scaber* Using Seeds as Explants in Various Culture Growth Media

Yuliani
Department of Biology, Faculty of Mathematics and Natural Sciences
Universitas Negeri Surabaya
Surabaya, Indonesia
yuliani@unesa.ac.id

Fida Rachmadiarti
Department of Biology, Faculty of Mathematics and Natural Sciences
Universitas Negeri Surabaya
Surabaya, Indonesia
fidarachmadiarti@unesa.ac.id

Sari Kusuma Dewi
Department of Biology, Faculty of Mathematics and Natural Sciences
Universitas Negeri Surabaya
Surabaya, Indonesia
saridewi@unesa.ac.id

Mahanani Tri Asri
Department of Biology, Faculty of Mathematics and Natural Sciences
Universitas Negeri Surabaya
Surabaya, Indonesia
mahananiasri@unesa.ac.id

***Abstract*—*Elephantopus scaber* plant contains various secondary metabolites, such as flavonoids, phenolics, saponins, terpenes, triterpenoids, sesquiterpenes lactones, elephantopyns, and deoxyelephantopin, which can act as antimicrobial, antifungal, and bioinsecticides. *Elephantopus scaber* can be potentially grown by tissue culture method and isolated its secondary compounds. But, the presence of tricomes at stem, leaf midrib, or leaves of *E. scaber* hinder its in-vitro propagation, because of it can facilitate contamination agents, such as fungi and bacteria. To avoid contamination, seeds were used as source of explants. The aim of this study was to describe the use of seeds as an alternative explant source of *E. scaber* in various culture growth medium and to determine the right medium for *E. scaber* callus induction. This research used four growth mediums: 1) MSO medium (with no additional Plant Growth Factor), 2) MSC medium (MS medium with additional charcoal), 3) MS + 0.5 mg/L BAP and 1 mg/L 2.4 D, and 4) MS + 1.5 mg/L Kinetin and 1.5 mg/L 2.4 D. There were 20 bottles media for experiment with five seed explants put into each bottle of medium, thus total of 100 seeds were planted. The propagation steps were sterilization of mature seeds, preparation and sterilization of growth medium, and inoculation of seeds. The observation of callus induction was performed for four weeks. The parameters observed in the research was the formation of the callus induced from seeds of *E. scaber*. The results showed that explants in 5 bottles had grown, 7 seeds were planted as seedlings, while seeds in 15 bottles showed no signs of growth. All seeds planted in MSC medium (20 bottles) produced seedlings and explants. About 82 calli from 20 bottles were induced on MS + 0.5 mg/L BAP and 1 mg/L 2.4 D, while 17 bottles of MS + 1.5 mg/L Kinetin and 1.5 mg/L 2.4 D produced 48 calli and 3 bottles produced seedlings. The results showed that seed explants of *E. scaber* could reduce microbe contamination because there was no tricomes in seeds, as opposite on the leaves during callus induction. Seeds of *E. scaber* could be effectively used as an explant for callus induction through in-vitro propagation. The most optimal medium for callus induction of *E. scaber* seed was MS + 0.5 mg/L BAP and 1 mg/L 2.4 D. These findings support recommendation for callus induction using the seeds for plants with numerous tricomes on their leaves, such as *Elephantopus scaber*.**

***Keywords*—seeds, source of explant, *E. scaber*, growth medium, in-vitro**

I. INTRODUCTION

The level of secondary metabolite production in plant tissue is relatively low (less than 1% of the dry weight of plants) and highly dependent of physiological conditions and stages of plant development [1]. One strategy to increase its content is through tissue culture techniques, which can induce production of bioactive compounds under controlled conditions in a relatively short time [2]. In addition to being used for seed production, propagation via tissue culture techniques through callus formation can also be used to produce chemical compounds from secondary metabolism. The formation of these compounds can occur based on biochemical totipotency, where a plant cell has genetic potential to produce the same compound in large numbers if grown in in vitro conditions. The secondary metabolite compounds are obtained by extracting callus obtained from explants.

Various studies related to the multiplication of secondary metabolites through tissue culture have been carried out where the level of secondary metabolite production in callus culture was found to be higher than the level of production in the original plant [3]. The production of flavonoid compounds through tissue culture was also reported to be more effective using callus culture, because glycosides and aglycones produced was more likely to be obtained using this method [4]. Stem culture of *Pueraria*

candollei plant on MS media with the addition of growth regulators 4.5 M 2.4-D and 0.46 M kinetin produced daidzein at 5.12 mg / g dry weight and genistein 2.77 mg / g dry weight [2]. Callus formation is strongly influenced by the supply of exogenous hormones. Other factors that affect callus formation are genotype, explant physiological conditions, nutrient composition in growth media, and endogenous hormone content [4].

Elephantopus scaber L, a family of Asteraceae, is used by the community for traditional medicines, such as to treat bronchitis, liver problems, dysentery and so on. *Elephantopus scaber* was found to contain sesquiterpenes lacton, deoxyelephantopin, isodeoxyelephantopin, flavonoids, phenols, saponins, steroids, tannins, scabertropin, scabertopinol, terpenes, triterpenoids, elephantopines, besides also containing epifriedelinol, lupeol, steroids, tannins, scabertropin, scabertopinol, terpenes, triterpenoids, elephantopines, in addition to also containing epifriedelinol, lupeol, steroids, tannins, scabertropin, scabertopinol, terpenes, triterpenoids, elephantopin, besides also containing epifriedelinol, lupeol, stan-triac -1-ol, lupeolacetate, and luteolin-7-glucosida [5]. Alcohol and chloroform extracts from *E. scaber* found toxic germacranolide (sesquiterpene lacton), while its water extracts were found to have analgesic, diuretic and anti-inflammatory effect [6]. Ethanol extract of *E. scaber* leaves and roots could function as anti-bacterial agent [3]. Methanol extract of leaves of liman tread plants at 4 and 6% were able to effectively kill *S. litura* by 90% and *P. xylostella* by 83.3-100% [7]. This shows that *E. Scaber* is a plant that has the potential to be developed in tissue culture for isolating its secondary metabolites. [7].

However, in terms of explants source, *E. scaber* proved difficult to culture, because of trichomes found in the stems, leaf midribs, and even *E. Scaber* leaves [7]. The presence of trichomes causes explants from leaves and stems are often contaminated with bacteria and fungi, and experienced browning. Various sources of explants such as leaf bones, leaves and stems had been tried to produce callus, and the best results were obtained from leaf bones explants, which produced eleven calli (personal documentation). Thus, we needed another method to produce callus from *E. scaber* to overcome the presence of trichomes found in various plant organs, which was using seed as source of explants.

Explants using seeds in tissue culture have the advantage of seeds are part of plants still juvenile, its genetic material is still stable to be propagated, and free of microbes. However, it is also necessary to take into account the viability of seeds and environmental factors. Based on the background, this study was aimed to describe the use of seeds as an alternative source of *E. scaber* explants using various culture growth medium and determine optimum medium for callus induction *E. scaber*.

II. METHODS

This study used descriptive, by observing the growth of callus and using seeds of *E. scaber* as explant source and Murashige and Skoog (MS) basic medium. Plant hormones used were Benzyl Amino Purine (BAP), 2.4-D, and kinetin. This research used four growth mediums; 1) MSO medium (with no additional Plant Growth Factor, 2) MSC medium (MS medium with additional charcoal), 3) MS + 0.5 mg/L BAP and 1 mg/L 2.4-D and 4) MS + 1.5 mg/L Kinetin and 1.5 mg/L 2.4-D. A total of 20 bottles medium were used for study with five seed explants were planted in each bottle, hence total number of seeds used during study was 100 seeds. The propagation stages consisted of sterilization of mature seeds, preparation and sterilization of growth medium, and inoculation of seeds. The observation of callus induction was performed for four weeks. The parameter of this research was the emergence of induced callus from *E. scaber* seed explants. Data were analyzed descriptively.

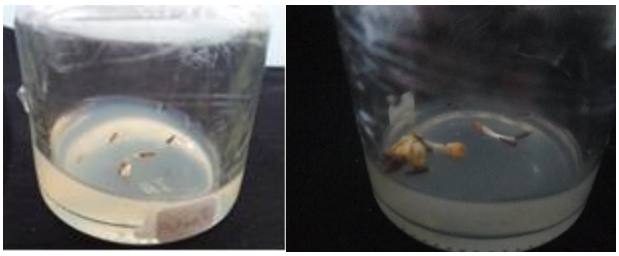
III. RESULTS

Results showed that seed explants planted in five bottles of medium had grown, seven seeds were able to be planted as seedlings, and 15 bottles of media showed no signs of growth. Seed explants planted in 20 bottles filled with MSC medium produced seedlings and explant. A total of 82 calli were found on MS + 0.5 mg/L BAP and 1 mg/L 2.4 D medium, and 17 bottles of MS + 1.5 mg/L Kinetin and 1.5 mg/L 2.4 D produced 48 calli, while 3 bottles produced seedlings. The results showed that using seeds of *E. scaber* as explant could reduce microbial contamination due to presence of tricomes on leaves during callus induction. Callus induction was initiated on the 7th day after inoculation on MS + 0.5 mg/L BAP and 1 mg/L 2.4 D medium, while sprouting growth began on 5th on MSC medium (MS0 medium with activated charcoal).
The number of seedlings and callus induced during experiment is presented on the Table 1.

TABLE I. THE AMOUNT OF CALLUS INDUCTION FROM SEEDS ON 4 WEEKS.

Medium	Number of callus	Number of seedlings	Additional information
MSO medium (MS without PGR)	0	7 seedlings	The others seeds did not show progress
MSC medium (MS medium + charcoal)	0	100 seedlings/explants	The others seeds did not become callus
MS + BAP 0,5 mg/L + 2,4 D 1 mg/L	82 calli	0	The others seeds did not show progress and contaminated
MS + Kinetin 1,5 mg/L + 2,4 D 1,5 mg/L	48 calli	10 seedlings/explant	The others seeds did not show progress

**PGR : plant growth regulator*



(a) (b)

Fig 1. The formation of Seedlings (a) and callus induction (b)

IV. DISCUSSIONS

Propagation of *E. scaber* through seeds is still limited, due to the low viability of seeds. Sometimes *E. scaber* seeds are damaged before germination due to environmental influences [8]. Propagation using tissue culture is an alternative method for propagating this species. Propagation of *E. scaber* using leaf explants had been carried out [9], but there were still few studies which used seed explants from *E. scaber* for callus induction. Callus induction from *E. scaber* leaves as explants is not optimal, due to trichomes in *E. scaber* leaves inhibit tissue initiation to become callus, mostly cause of microbial contamination [10]. Thus, seed explants were used in this study to induce *E. scaber* callus. Seeds are juvenile part of plants with genetic material stable to be propagated.

Elephantopus scaber seed explants were inoculated on MSO medium grew sprouts but could not produce callus, as well as seed explants inoculated on MSC medium. Medium without growth regulators was unable to induce callus growth [11], as found in this study, medium without 2.4-D or with additional of low concentration of BAP could not induce callus, even though cell proliferation had occurred.

The size (age) of the embryo, genotype, type of medium, concentration of growth regulators, and the conditions under which explants are grown are factors affecting callus induction [12]. From results, MS medium with additional combination of 2.4-D and BAP or kinetin could induce callus. Low concentration of BAP and high concentration of 2.4-D could induce as much as 82 clumps. Medium added the same concentration of kinetin and 2.4-D could induce 48 clumps. Administration of auxin in the form of 2.4-D either singularly or in combination with cytokines could grow and multiply callus [13]. In addition, many researchers concluded that 2.4-D auxin was the best auxin type for callus induction in both monocotyledonous and dicotyledonous plants [14].

Callus culture is the initial stage of propagation highly important for regeneration of all parts of the plant. Callus is a group of unorganized cell mass, generally not formed during the life cycle of plants. However, as a way to propagate plants, callus culture can be carried out using organized plant parts as explants. Growth regulators are needed for callus induction and can be modified with other nutrient content in the medium [15]. The need for 2.4-D auxin in culture medium for inducing callus growth in various plants had often been reported, such as in *Cassia obtusifolia* [16] and *Abutilon indicum* [17]. In addition, the combination of auxin and cytokinin is generally effective for callus formation. There had been many studies that proved the use of 2.4-D and kinetin could accelerate callus emergence. 2.4-D is a growth regulator essential for callus induction and kinetin can be used to accelerate the emergence of callus in MS medium [17].

V. CONCLUSION

Seeds of *Elephantopus scaber* seed could be effectively used as explant to induce callus via in-vitro propagation. The appropriate medium for callus induction from seeds of *Elephantopus scaber* was MS + 0.5 mg/L BAP and 1 mg/L 2.4-D. These findings support recommendation for callus induction using seeds from plants with numerous trichomes on their leaves, such as *Elephantopus scaber*.

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