Induction, Multiplication, and Acclimatization of Rodent Tuber (*Typhonium flagelliforme* Lodd.) Plant From Indonesia by In Vitro Organogenesis

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***Abstract*— Rodent tuber (*Typhonium flagelliforme* Lodd.) is a native Indonesian medicinal herb with high anticancer activity. Propagation of rodent tuber is hard to be achieved by using conventional methods. The purpose of this research is to optimize method and media for propagating rodent tuber through initiation, induction, multiplication, and acclimatization. Rodent tuber was obtained from Pekalongan, Indonesia. In vitro rodent tuber plantlets were induced through direct single node culture of tuber. Shoot induction was achieved on MS 1 mgL^{-1} 2,4-D combined with 0.3 mgL^{-1} BAP and 1 mgL^{-1} NAA combined with 0.5 mgL^{-1}. Induced shoots were initiated on MS media supplemented with (1, 0.5, 1.5 mgL^{-1}) NAA and (1, 0.5, 0.5 mgL^{-1}) BAP. Shoots were then multiplicated on MS media supplemented with (0.25, 0.5, 1, 1.5 mgL^{-1}) NAA and 0.5 mgL^{-1} BAP to investigate the effect of NAA concentrations on shoot proliferation. Plantlets were acclimatizated on husk and compost (1:1) media and post-acclimatizated on husk, soil, and compost (1:1:1) media. The highest percentage of viable induced explants (66.67%) was obtained on MS 1 mgL^{-1} 2,4-D and 0.3 mgL^{-1} BAP. The maximum number of shoots obtained in the initiation stage (14 shoots) was achieved on MS media supplemented with 0.5 mgL^{-1} NAA and 0.5 mgL^{-1} BAP. The maximum number of shoots obtained in the multiplication stage (8.2 ± 3.19) was achieved on MS media supplemented with 0.5 mgL^{-1} NAA and 0.5 mgL^{-1} BAP. The survival rate of rodent tuber during acclimatization period was 100% and during post-acclimatization period was 58%. Rodent tuber has been successfully multiplicated through direct organogenesis in vitro.**

Keywords—Typhonium flagelliforme* Lodd.*; Indonesian clone; in vitro organogenesis; acclimatization

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I. INTRODUCTION

Rodent tuber (*Typhonium flagelliforme* Lodd.) is a medicinal herb from the Araceae family [1]. It has white tuber, triangle-shaped leaves, and it could grow up to 30 cm height [2]. It is found between 1-300 m altitude above sea level [3]. Rodent tuber is effective to inhibit cancer cell growth in vitro and induce apoptosis [2].[4] showed that the plant's tuber extract possess antibacterial and antioxidant activity. According to [5], rodent tuber could be used as source for the treatment of human lung and breast cancer. [6] has showed the toxic activities of rodent tuber extract against *Artemia salina*. Phytochemical analysis shows that the plant contains alkaloid, steroid, flavonoid, and glycoside [1].

Rodent tuber has a slow propagation rate and is distributed only in damp and shady place in its natural habitat. On the other hand, the plant is highly demanded for cancer treatment. The problems of conventional propagation can be overcomed by tissue culture. Tissue culture is able to produce high-quality planting materials, uniform seeds, and disease-resistant plants rapidly [7]. Rodent tuber has low genetic diversity because it usually reproduces vegetatively through tuber separation [8]. The diversity and quantity of bioactive compounds will be enhanced in line with genetic diversity. Tissue culture is able to increase the genetic diversity of rodent tuber through somaclonal variation of propagated explants [9].

Direct organogenesis is one of the propagation methods involving the formation of buds or organs directly without passing through the callus. [10] have showed the micropropagation of Indonesian Rodent tuber through callus formation. Murashige Skoog (MS) medium is the most commonly used media

base in tissue culture method because it contains inorganic salt to support optimum plant growth. Composition of plant growth regulators in tissue culture media is an instrumental in morphogenesis [7].

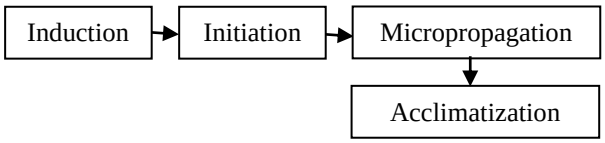
Plant growth regulators are chemicals which affect plant growth and physiological activity. Auxin and cytokinin have been known to play critical role in regulation and maintenance of shoot meristem, and in in vitro plant organogenesis [11]. The most common type of auxin is Naphthaleneacetic acid (NAA) and cytokinin is 6-Benzyl Amino Purine (BAP) [12]. Both NAA and BAP have been successfully used in combination for organogenesis of several different plants, such as soybean [13], bitter apple [14], *Albizia odoratissima* L.f. (Benth.) [15]. The success of plant propagation by in vitro method could be indicated by the survival rate of plants during acclimatization [7].

Research about in vitro culture of Indonesian rodent tuber plant had never been published before. The specific aim of this research is to obtain the optimal media for initiation and multiplication of in vitro rodent tuber plantlets.

II. Materials and Methods

A. Plant material

The plant materials were obtained from Pekalongan, Jawa Tengah, Indonesia. The flow of this research is:



B. Induction and Initiation of Explants

The plants were washed with detergent and rinsed in running water to remove soil particles. The rhizomes along with the buds of the plant were cut and sunk in 0.25 gram of both bactericide and fungicide in 200 ml of water for 30 minutes, 2.25 g/ml antibiotic rifampicin for four hours, 2% of Clorox® bleach for 15 minutes, 1.5% of Clorox® bleach for 15 minutes, 1% of Clorox® bleach for 10 minutes, and 0.1% of $HgCl_2$ for 10 minutes. The explants were then rinsed two times with sterile water, and planted into induction medium. Explants were cultured on MS media supplemented with sucrose, coconut water, and either 1 mgL^{-1} NAA and 0.5 mgL^{-1} BAP or 1 mgL^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.3 mgL^{-1} BAP. Viable induced explants were initiated on MS media supplemented with 1 mgL^{-1} NAA and 1 mgL^{-1} BAP, 0.5 mgL^{-1} NAA and 0.5 mgL^{-1} BAP, 1.5 mgL^{-1} NAA and 0.5 mgL^{-1} BAP. The parameter recorded in the experiment is the mean number of shoots produced per explant on the seventh week.

C. Multiplication of Plantlets

Explants were then multiplicated on MS media supplemented with different concentration of NAA (0.25, 0.5, 1, 1.5 mgL^{-1}) and 0.5 mgL^{-1} BAP in order to investigate the effect of NAA on shoot multiplication.

D. Acclimatization of Plantlets

In vitro propagated plantlets of rodent tuber were acclimatized after eight weeks. The plantlets were washed with flowing water and sunk into a mixture of 0.25 gram bactericide and 0.25 gram fungicide in 200 ml of water. The plantlets were then planted into acclimatization media containing husk and compost (1:1). The medias were always kept moisture in room temperature. Viable percentage of the plant was recorded for four weeks. Viable plants were then moved into post-acclimatization media containing husk, soil, and compost (1:1:1) in the green house. Viable percentage of plants was also recorded for four weeks of post-acclimatization.

E. Experimental design and statistical analysis

The experimental design on this experiment was completely random design with 10 replications per treatment. The mean shoot numbers of plantlet were compared statistically on the seventh week. The statistical analysis used in this research comprises the Kolmogorov-Smirnov normality distribution test, followed by Analysis of Variance (ANOVA) for parametric data because the normality test showed that the data obtained follow normal distribution pattern.

III. Results and Discussion

A. Induction and Initiation of Explants

Rodent tuber shoots were induced from single node of tuber explants. Single node culture is a plant micropropagation method involving the induction of axillary bud meristematic cells. Advantages of single node culture method are rapid propagation, simple method [16], and genetically stable [17]. [18] had induced rodent tuber in vitro explant from tuber. Single node culture also has been applied to micropropagation of *Solanum tuberosum* [19], *Dioscorea bulbifera*, *Dioscorea alata* [20], dan *Cucumis sativus* [21].

The highest viable induced explants percentage was obtained on MS media supplemented with 1 mgL^{-1} 2,4-D and 0.3 mgL^{-1} BAP (66,67%) as compared with 1 mgL^{-1} NAA and 0.5 mgL^{-1} BAP media (25%) (Table 1). MS media had been known as the most suitable media for micropropagation of rodent tuber compared to the other media such as Nitsch and Nitsch (NN), Gamborg B5 (GB5) and White (W) media [22]. 2,4D is a synthetic auxin which is able to induce cell division, cell elongation, and calli initiation [23]. 2,4D is able to induce somatic embryogenesis of celery (*Apium graveolens*) and lettuce (*Lactuca sativa*) [24].

TABLE I. Induction of Rodent Tuber Explants

Growth Regulator Composition (mgL^{-1})			**Viable Induced Explants Precentage**
NAA	***2,4-D***	***BAP***	
1	0	0.5	25%
0	1	0.3	66.67%

BAP is a cytokinin which is able to stimulate axillary bud proliferation [7, 25]. BAP affects the availability of nitrogen

and potassium in media which is important in protein synthesis and cell division [26]. BAP contained in rodent tuber induction medium is able to promote cell differentiation to become shoots and leaves primodia [27; 28]. 2,4D and BAP had also been used to induce the direct organogenesis of peanut (*Arachis hypogaea* L) [29] and *Mentha viridis* L. [30].

Multiple shoots were transferred to MS initiation media supplemented with different concentrations of NAA and BAP (Fig. 2A, Table 2). The highest mean number of shoots (14 shoots) was obtained on MS media supplemented with 0.5 mgL^{-1} NAA and 0.5 mgL^{-1} BAP. The lowest mean number of shoots (4 shoots) was obtained on MS media supplemented with 1.5 mgL^{-1} NAA and 0.5. BAP and NAA are effective in increasing the proliferation of shoots and explant fresh weight of rodent tuber [18]. NAA is an auxin which is able to promote cell elongation, apical dominance, and root formation [31]. NAA induce cell elongation through binding with Auxin Receptor Binding Protein 1 (ABP1) [32]. High concentration of BAP can inhibit the differentiation and multiplication of shoot due to the toxic effect of BAP in shoot tissue [33].

TABLE II. MEAN NUMBER OF SHOOTS ON INITIATION MEDIA

Growth Regulator Composition (mgL^{-1})		**Mean Number of Shoots**
NAA	***BAP***	
1	1	10.9
0.5	0.5	14
1.5	0.5	4

Fig. 1. Control plant grown in MS0 media (without growth regulators)

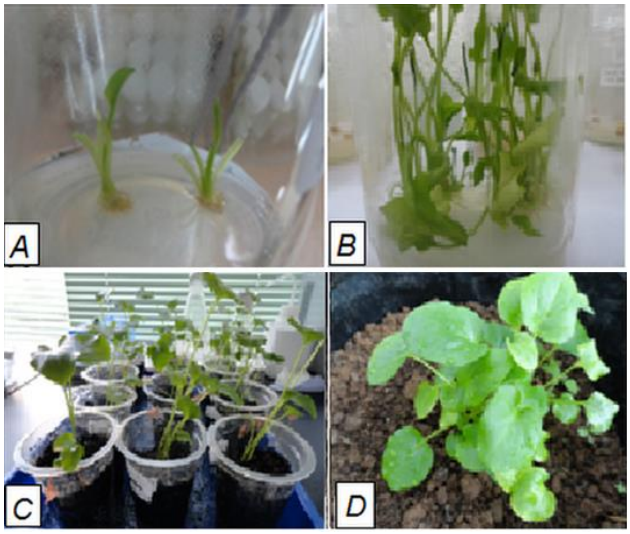


Fig. 2. Initiation, Micropropagation, and Acclimatization of Indonesian Rodent Tuber
(A) Initiation of explants, (B) micropropagation of plantlets through direct organogenesis, (C) acclimatization of plantlets, (D) post-acclimatization of plants

B. *Multiplication of Plantlets*

Rodent tuber plantlets were multiplicated through direct organogenesis on MS media supplemented with different concentration of NAA and BAP. Fig. 3 showed the mean number of shoots produced by the plantlets during seven weeks of culture. Propagation of plantlets was observable on second week.The highest increase of shoots was obtained on MS media supplemented with 0.5 mgL^{-1} NAA and 0.5 mgL^{-1} BAP.

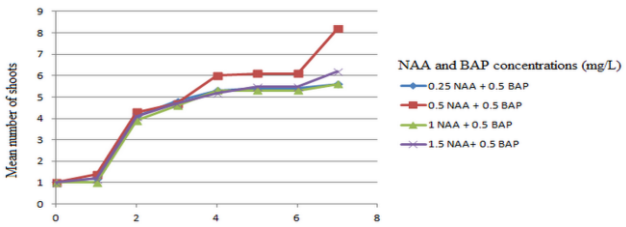


Fig. 3. Mean number of shoots of rodent tuber plantlets during seven weeks of in vitro culture on micropropagation media

Table 3 and Fig. 4 showed the mean number of shoots on seventh week. The highest mean number of shoots (8.2 ± 3.19) was obtained on MS media supplemented with 0.5 mgL^{-1} NAA and 0.5 mgL^{-1} BAP. Mean number of shoots produced on micropropagation media were not statistically different in inducing the adventitious shoots. NAA and BAP has been used to induce in vitro plant cell division [34], such as in *Dioscorea Rotundata* L. [35] and *Alstroemeria* [36].

TABLE III. MEAN NUMBER OF SHOOTS ON MICROPROPAGATION MEDIA

Growth Regulator Composition (mgL^{-1})		Mean Number of Shoots[a]
NAA	*BAP*	
0.25	0.5	5.6 ± 2.91
0.5	0.5	8.2 ± 3.19
1	0.5	5.6 ± 2.01
1.5	0.5	6.2 ± 1.69

[a] On seventh week, number of replication : 10

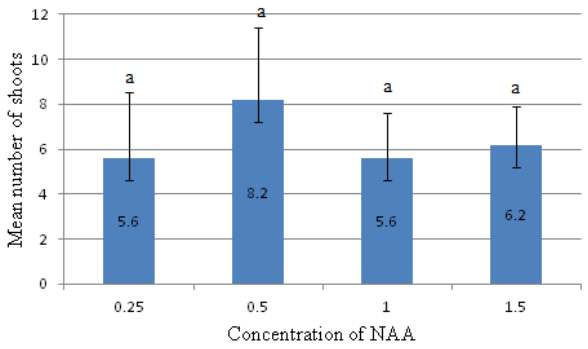


Fig. 4. Mean number of shoots on micropropagation media on seventh week

The highest number of shoots was obtained in MS media containing 0.5 mg/L NAA and 0.5 mg/L BAP. [37] had obtained the optimum media for propagation of in vitro rodent tuber plantlets (*Typhonium flagelliforme*), i.e. MS media supplemented with 0,5 mg/L BAP and 0,5 mg/L NAA. However, the shoots had abnormal morphology and spirally arranged leaves. Rodent tuber propagated in this research (Fig. 2B) had normal shoots, leaves, and roots morphology. Micropropagation of Goji (*Lycium barbarum* L.) [38] and Tukish woad (*Isatis aucheri*) [39] also produce the highest number of shoots on medium containing 0,5 mg/L NAA and 0,5 mg/L BAP. High NAA concentration is able to inhibit the formation of shoot [40].

Medium containing 0.25 mg/L NAA and 1 mg/L BAP produced only a few shoots (Table 3). G-proteins transduce the auxin-triggered signal from a receptor with low affinity for NAA toward the machinery responsible for the stimulation of cell division. Therefore, NAA can induce cell division only at very high concentration [32]. Low NAA concentration in multiplicaton medium (< 0,1 mg/L) is able to produce abnormal rodent tuber shoots [22]. Plant organogenesis responses to the composition of plant growth regulators in medium is affected by the concentration of endogenous plant hormones, such as auxin indoleacetic acid (IAA), and cytokinin 6(2-isopentenyl)adenine (iP), N6(2-isopentenyl)adenosine (iPR), zeatin (Z), zeatin riboside (ZR) and N6-benzyladenine (BA). NAA and BAP has been known to increase the concentration of endogenous iP cytokinin which is able to stimulate organogenesis [41].

C. *Acclimatization of Plantlets*

The *in vitro* propagated plantlets were acclimatized for four weeks (Fig. 2C), and then transferred to the greenhouse for post-acclimatization (Fig. 2D). Fig. 5 shows the viable percentage of plant through four weeks of acclimatization and four weeks of post-acclimatization period. The survival rate of rodent tuber during acclimatization period was 100% and it decrease over time during post-acclimatization period.

The survival rate of rodent tuber after four weeks of post-acclimatization period was 58%. Acclimatization of rodent tuber (*Typhonium flagelliforme*) by [18] obtained the viability precentage of 90% in peatmoss: perlite: vermiculite media = 3:1:1, while acclimatization of rodent tuber by [22] had obtained the viability precentage of 92% in sand : coconut husk = 1 : 1. Tissue cultured plants are susceptible to acclimatization shocks leading to high mortality in the final stage of micropropagation [42]. This is due to the growth conditions on in vitro media which is able to induce abnormal morphology and physiology of plants [43]. Therefore, pre-acclimatization should be done in lower air humidity than post-acclimatization [44] Viability of plants during post-acclimatization is low due to instable environment conditions [7], such as temperature, humidity, irradiance, CO_2 concentration, and air flow rate [45].

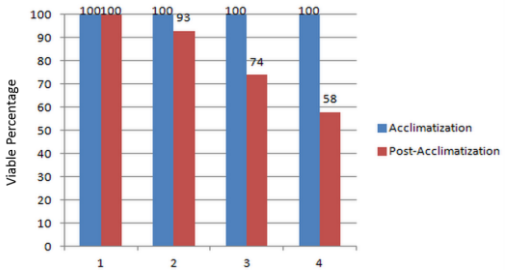


Fig. 5. Viable percentage of plants during acclimatization and post-acclimatization period

IV. CONCLUSION

Rodent tuber (*Typhonium flagelliforme* Lodd.) explants had been induced on MS 1 mgL^{-1} 2,4-D and 0.3 mgL^{-1} BAP. Optimum media for initiation was MS 0.5 mgL^{-1} NAA and 0.5 mgL^{-1} BAP. The maximum number of shoots obtained in the multiplication stage was achieved on MS 0.5 mgL^{-1} NAA and 0.5 mgL^{-1} BAP. The survival rate of rodent tuber during acclimatization period was 100% and during post-acclimatization period was 58%. Rodent tuber has been successfully multiplicated through direct organogenesis in vitro

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