The 2nd International Conference on Natural Resources and Life Sciences (NRLS-2018)

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PREFACE: the 2nd International Conference on Natural Resources and Life Sciences (NRLS) 2018

The 2nd International Conference on Natural Resources and Life Sciences (NRLS) 2018 has been organized by the Faculty of Biotechnology of University of Surabaya, Indonesia. The theme of this conference is *"Managing Natural Resources for Better Health"*. Focusing on biological aspects, the conference has facilitated chances of collaborations in research and development – as well as enlarged joint activities regarding natural resource management – among academics and professionals in their attempts to contribute further to the community through their respective fields.

The 2nd NRLS 2018 was held on August 23–24, 2018. This conference presented eight international speakers from five countries: Indonesia, Malaysia, the Netherlands, Singapore, and Thailand. Over 200 representatives of 48 institutions participated in this event, involving more than 74 abstracts submitted in the form of oral and poster presentations. After a rigorous selection process, the Scientific & Editorial Board have decided to publish 43 manuscripts in IOP Conference Series: Earth and Environmental Science (EES), an international proceedings indexed in Scopus, Scimago, Conference Proceedings Citation Index-Science (CPCI-S) of Clarivate Analytics's Web of Science, etc. From 43 selected ones above, 21 manuscripts were results of joint researches between Indonesia and various countries, e.g. Australia, England, Georgia, Germany, India, Japan, Latvia, Lithuania, Malaysia, the Netherlands, the Republic of Korea, Spain, and Sweden. Those manuscripts cover various biological themes, i.e. Food Biotechnology, Agricultural Biotechnology, Medical Biotechnology & Forensics, and Environmental Biotechnology & Renewable Energy. Each of the 43 manuscripts in IOP Conference Series–EES have been reviewed by at least two experts using double-blind system. The published manuscripts have passed all necessary improvement requirements (according to the IOP Proceedings standard), reviewer's comments, SI (*Système International d'Unités*), and similarity tests (with the highest threshold of 25 %) as well as editing procedure by professional editors from seven countries (Georgia, India, Indonesia, Latvia, Lithuania, Malaysia, and Sweden).

Our appreciation goes to the reviewers, editors and members of the Scientific & Editorial Board for their big efforts in reviewing and improving the manuscripts. For the generous supports in succeeding the NRLS-2018, we extend our gratitute toward the University of Surabaya's management and supporting units, our co-hosts the Faculty of Pharmacy and the Faculty of Medicine, and our sponsors VISION TEKNIK, SCIENCEWERKE, INDOLAB UTAMA, and MEGAH SEJAHTERA SCIENTIFIC.

Last but not least, we thank you all presenters and attendees for the active contribution to share scientific ideas, inspire new researches, and exchange new contacts for closer co-operations. We hope you have had enjoyable time with us and are currently encouraged to collaborate further in order to explore natural resources and life sciences in various aspects of living. We look forward to welcoming you and your team in the 3rd NRLS-2020!

Surabaya May 02, 2019
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Johan Sukweenadhi, Ph.D.
Executive Chief of the 2nd NRLS-2018

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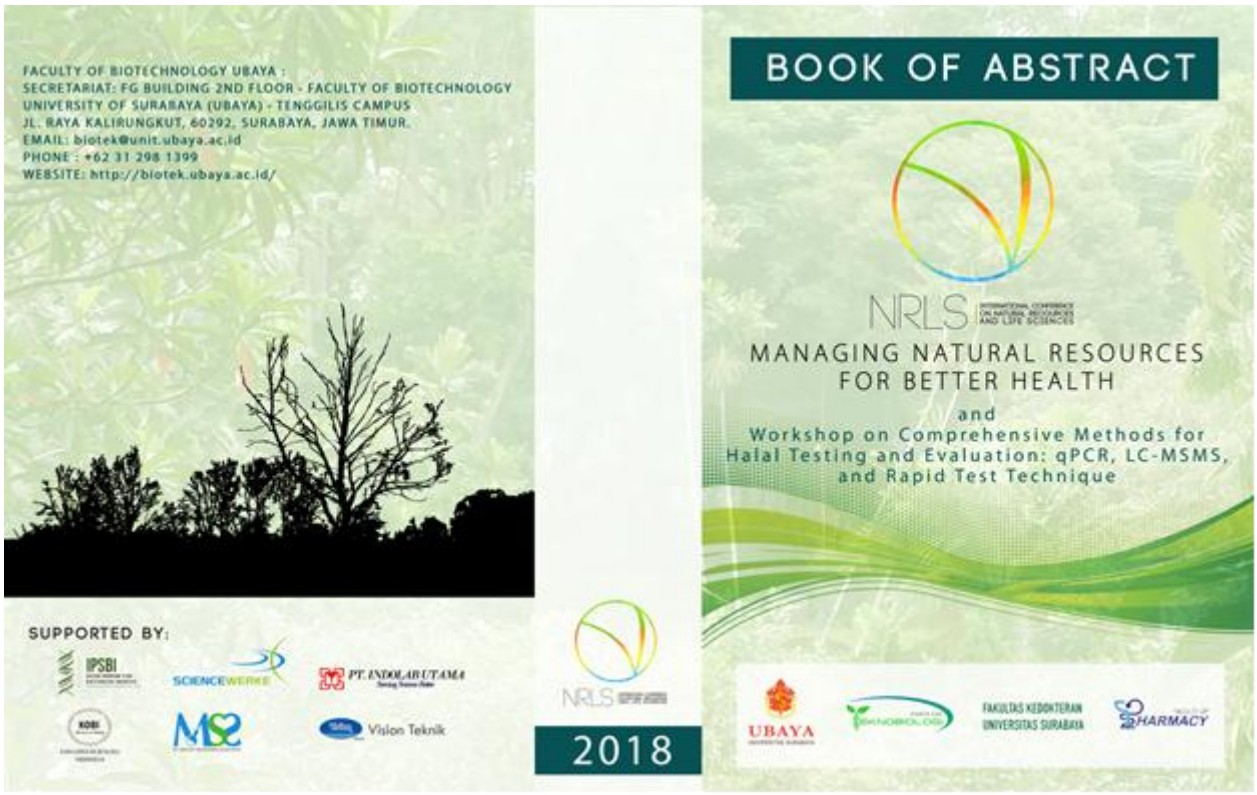


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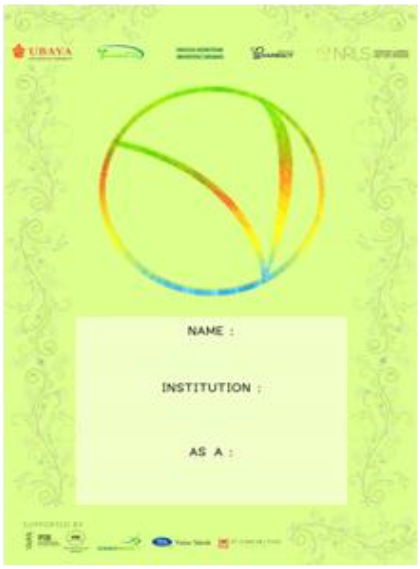
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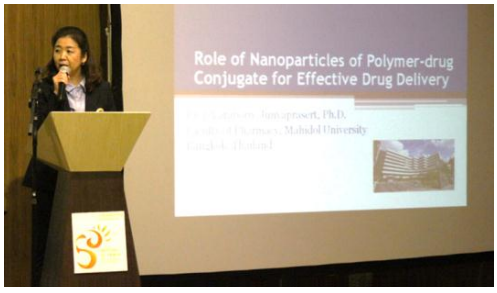

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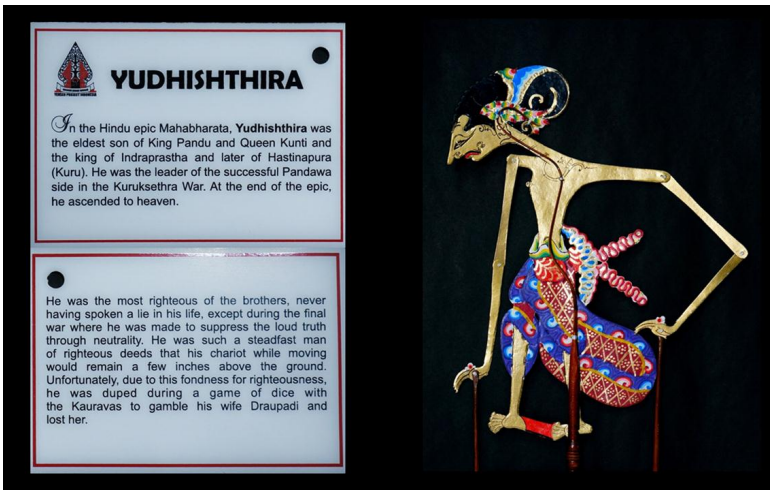

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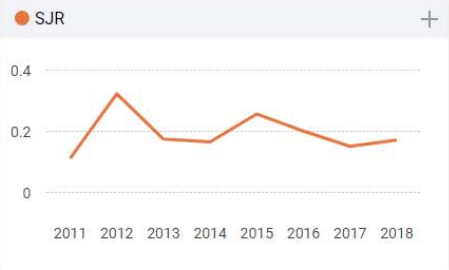

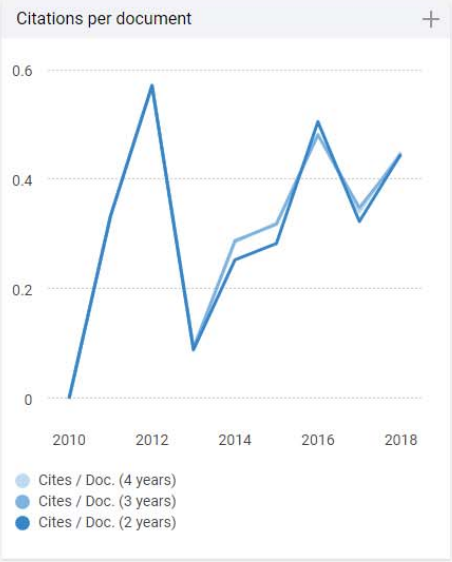

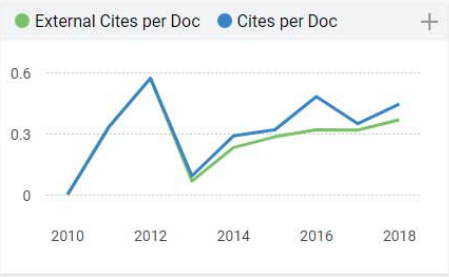

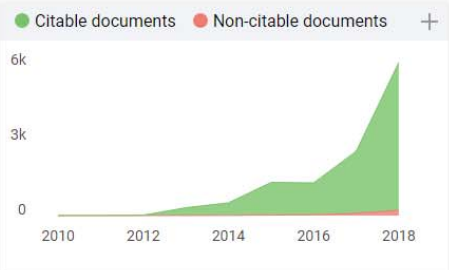

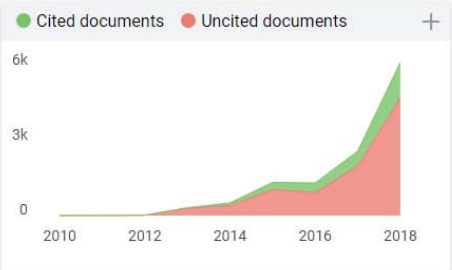

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Callus induction and *in vitro* mass culture of adventitious roots from leaf segment explants of *Dendropanax morbifera* Lev.

Callus induction and *in vitro* mass culture of adventitious roots from leaf segment explants of *Dendropanax morbifera* Lev.

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Abstract. *Dendropanax morbifera* Lev. is a unique species and natively found in Korea and distributed in the South regions, such as Jeju, Goheung, and Wando. In this study, tissue culture system for native *D. morbifera* was developed. The callus from native *D. morbifera* leaves was cultured on Woody Plant Media (WPM) supplemented with 30 g L^{-1} of sucrose, with addition of 2.4-D and BA [(0.5, 1.0, 2.0) mg L^{-1}], separately and mixed. After 5 wk of culture, the highest induction of callus was obtained from 0.5 mg L^{-1} of 2.4-D mixed with 2.0 mg L^{-1} of BA. Adventitious root formation on different media (MS, WPM, B5) with various auxins (IBA, IAA, and NAA) and different concentration [(0, 1, 3, 5) mg L^{-1}] were tested. After 8 wk of culture, WPM showed better induction of adventitious root. The highest induction of adventitious root was obtained on 3.0 mg L^{-1} IBA. Root growth was best in WPM liquid medium with 3.0 mg L^{-1} of IBA and 30 g L^{-1} of sucrose. The same formulation with modified ½ WPM was successfully established *in vitro* adventitious roots culture in 18-L bioreactor system. This study also proposed as the mass production technique of adventitious roots from native *D. morbifera*.
Keywords: Adventitious roots, bioreactor system, gingseng tree, *in vitro* culture, tissue culture

1. Introduction

The *Dendropanax morbifera* Lev. is a perennial woody plant belonging to *Araliaceae* and it is a native species of Korea. It is mainly distributed in Jeollanam-do and sporadically abundant in the islands such as Jeju Island, Goheung and Jangheung [1]. This plant is also known as “Trees that heal the world” or “Ginseng tree,” and its economic and pharmacological value has been reevaluated since 2010 [2]. Some research reported the usage of this plant to improve blood circulation and prevent thrombosis [3], thereby preventing heart disease [4] and improve insulin sensitivity [5, 6]. In addition, it is believed that *Dendropanax* extract has a unique directional component and contains benzoic acid which calms or strengthens the nervous system [7–9]. Moreover, it also has been reported that the

compound is fractionated and tested in cells for 10 yr or more to inhibit the production of melanin, thereby excelling hair whitening problem [7]. From this point of view, studies on *Dendropanax* are continuously carried out, and it is suitable for use as a natural material for new drug development. Thus, continuous cultivation and production are necessary needed. However, the seeds of *Dendropanax* has weak tolerance against cold stress. In order to obtain the seeds, it takes more than 6 yr after a long process of flowering and fruit production [10]. Furthermore, the germination rate of seeds obtained through a long process has been reported to be very low [1]. In this way, it is many difficulties to grow it in populations because of the geographical limitation of Hwigae-gil, which requires a longer time for cultivation and seed breeding in other regions [11].

In 2007, 479 yr *Dendropanax* tree was designated as a natural monument; located in Wando Island, Jeollanam-do. Wild *Dendropanax* tree is grown in some areas of Jeollanam-do, but its production is not very high [12]. It is necessary to seek a method for solving the problems that occur in such a way as well as a method for supplying and receiving raw materials smoothly. Studies on the growth and propagation of one species of *Dendropanax* have been carried out by Choi et al. using *in vitro* propagation method, and the characteristics of germination of its seed were done according to the climate change environment [13]. This study aims to stabilize the supply and demand of raw materials used industrially by mass production of adventitious roots through *in vitro* culture of *Dendropanax morbifera* Lev.

2. Materials and methods

2.1. Plant material

On the July 2016, a 100 yr of leaf *Dendropanax morbifera* Lev. (figure 1) were harvested from the area of Wando Island, Jeolla-nam Province, and the experiment was carried out at Kyung Hee University tissue culture laboratory. For leaf disinfection, 2 to 3 drops of tween 20 solution were added to 70 % ethanol for 1 min and 2 % sodium hypochlorite solution for 15 min and then washed five times with sterilized water. The pH of the medium used was adjusted to 5.7, sterilized at 121 °C for 30 min using a high-pressure sterilizer, and dispensed into a petri dish (90 mm × 20 mm). The culture conditions were maintained at room temperature (23 ± 1) °C, indoor humidity 40 %, and cultured in the dark room where light was completely blocked.

Figure 1. Leaves of 100 yr. *D. morbifera*. White bar indicated 5 cm length.

2.2. Induction of callus by cytokinin hormone treatment

In order to select the hormone treatment suitable for callus induction of *D. morbifera*, the auxin-type hormone 2.4-D and the cytokinin-type hormone BA were administered at various concentration [(0, 0.5, 1, and 2) mg L^{-1}] with additional of sucrose 30 g L^{-1} as a nutrient source. The leaves of the plants

were cut into 0.5 cm × 0.5 cm sized of pieces, and 10 pieces were plated on a petri-dish medium and cultured at (22 ± 1) °C for 5 wk. The callus formation rate was observed weekly.

2.3. Induction of adventitious roots by auxin hormone treatment

IAA, IBA and NAA with various concentration [(1, 3, and 5) mg L^{-1}] were added to MS medium, WPM medium and B5 medium to select the medium suitable for inducing roots from leaves. Additional sucrose (30 g L^{-1}) was added as a nutrient source to all kind of media. The pH of the medium was adjusted to 5.7, and then 30 mL of each was dispensed into a petri dish. Seven individuals were placed in the culture medium and cultured for 8 wk. IBA [(1, 2, and 3) mg L^{-1}] was then administered to the WPM medium to select secondary adventitious roots for the induced adventitious roots, with addition of sucrose (30 g L^{-1}) as a nutrient source. After adjusting the pH to 5.7, the medium was divided into 30 mL of petri-dish and 10 individuals were placed in the culture medium. After 4 wk of incubation, growth was examined.

2.4. IBA concentration and sucrose content condition in liquid culture medium

In order to investigate the effect of IBA concentration on the roots growth, various concentration of IBA [(1, 2, and 3) mg L^{-1}] was added to 30 g L^{-1} sucrose-treated WPM medium. As many as 0.1 g of adventitious roots was added to 100 mL medium using a 200 mL Erlenmeyer flask. In order to investigate the effect of sucrose and WPM medium concentration on root growth in the suspension culture of yellowtail tree, various concentration of sucrose [(0, 10, 30 and 50) g L^{-1}] was added to ½ WPM medium supplemented with 3 mg L^{-1} IBA. Same with previous, 0.1 g of adventitious roots was inoculated into a 100 mL medium to investigate the growth. The inoculated Erlenmeyer flask was incubated for 4 wk in a dark room at 110 rpm in a shaking incubator (1 rpm =1/60 Hz).

2.5. Mass production using bioreactor system

In order to confirm that the mass growth of adventitious root was appropriate, the cells were cultured in a 18-L bioreactor plastic container. WPM liquid medium (15 L) supplemented with sucrose 30 g L^{-1} and IBA 3 mg L^{-1} was adjusted to pH 5.7 and sterilized autoclave at 121 °C for 20 min. Adventitious roots (30 g) were inoculated on laminar air flow and cultured for 4 wk at (22 ± 1) °C.

2.6. Statistical analysis

The significance of the difference between the pretreatment of this study was the statistical program SAS (statistical analysis system, version 9.3, SAS Institute Inc.). Duncan's multiple range test (DMRT) was performed at $P < 0.05$.

3. Results and discussion

3.1. Callus induction of D. morbifera using various plant growth hormone treatment

After 5 wk, WPM medium show significant callus generated from leaves explant instead of MS medium. The WPM medium showed almost 50 % generated callus, while most of the explants were withered in the MS medium and cannot form any callus at all (table 1). Previous report showed that endangered Lily hybrids and kind of *Lilium cernum* Komarvo. callus were formed using the medium without any additional plant growth regulator/ PGR [14]. After it was confirmed by the callus formation, WPM medium which showed highest callus formation rate was mixed with the auxin-based hormone, 2.4-D and cytokinin-based hormone, BA. The observation of callus formation was done after 5 wk (table 1). Previous report showed that *Iris sanguinea* [15], *Freesia hybrida* [16], and *Bupleurum latissimum* Nakai [17] callus formation was promoted in higher BA concentration [18]. After 3 wk, it was observed higher callus formation rate in the 2.4-D 1 mg L^{-1}, compared to the treatment with 2.4-D 0.5 mg L^{-1} and BA 1 mg L^{-1} group (table 1 and figure 2).

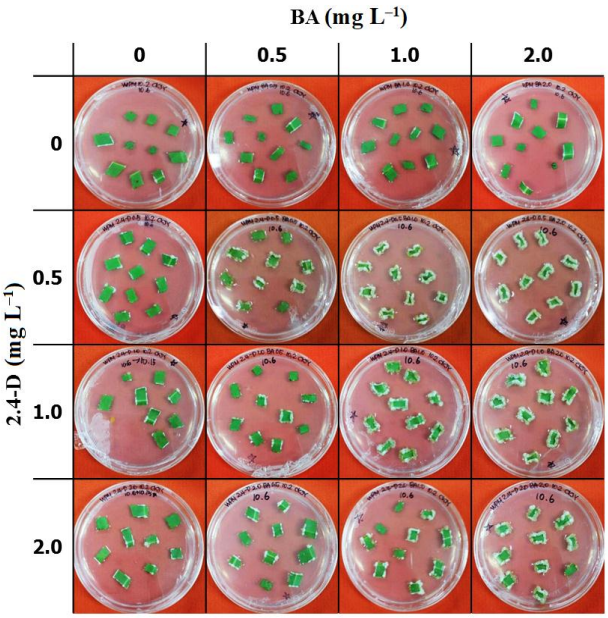

Figure 2. Callus induction of *D. morbifera* on WPM with various PGRs after 5 wk of culture

Previously, 100 % callus formation was reported by mixing cytokinin and kinetin hormone with concentration of 3.0 mg L^{-1} and [(0.1, 0.5, and 1.0) mg L^{-1}], respectively. Lower kinetin concentration was reported to increase the callus formation rate and its weight [19]. This is the first time comprehensive experiment was carried out using *D. morbifera* leaf segments. BA hormone in WPM showed higher callus formation rate than 2.4-D. Especially 0.5 mg L^{-1} of 2.4-D and 2 mg L^{-1} of BA that resulted 100 % fastest callus formation rate, in 2 wk.

Table 1. Effect of media and PGRs concentration on callus growth of *D. morbifera* during 5 wk.

Media	PGRs (mg L^{-1})		Callus formation (%)				
	2.4-D	BA	wk				
			1	2	3	4	5
MS	-	-	0.0 ± 0.0^{d}	0.0 ± 0.0^{h}	0.0 ± 0.0^{h}	0.0 ± 0.0^{f}	$0.0 \pm 0.0^{f*}$
WPM	-	-	0.0 ± 0.0^{d}	3.3 ± 2.7^{gh}	10.0 ± 0.0^{gh}	23.3 ± 2.7^{e}	50.0 ± 8.7^{de}
	0.5	-	0.0 ± 0.0^{d}	10.0 ± 8.1^{fgh}	40.0 ± 8.1^{def}	76.6 ± 2.7^{bcd}	93.3 ± 2.7^{ab}
	1.0	-	0.0 ± 0.0^{d}	16.6 ± 9.8^{efgh}	33.3 ± 7.2^{ef}	60.0 ± 4.1^{d}	80.0 ± 4.1^{bc}
	2.0	-	16.6 ± 7.2^{bc}	30.0 ± 4.1^{cdefg}	70.0 ± 4.1^{bc}	73.3 ± 5.4^{cd}	93.3 ± 5.4^{ab}
	-	0.5	0.0 ± 0.0^{d}	10.0 ± 8.1^{fgh}	53.3 ± 11.8^{cde}	63.3 ± 13.6^{d}	66.6 ± 15.1^{cd}
	-	1.0	0.0 ± 0.0^{d}	10.0 ± 8.1^{fgh}	30.0 ± 0.0^{fg}	40.0 ± 4.7^{e}	46.6 ± 2.7^{e}
	-	2.0	10.0 ± 4.7^{cd}	20.0 ± 8.1^{efgh}	30.0 ± 4.7^{fg}	33.3 ± 2.7^{e}	43.3 ± 2.7^{e}
	0.5	0.5	16.6 ± 2.7^{bc}	36.6 ± 5.4^{bcdef}	83.3 ± 7.2^{ab}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	0.5	1.0	20.0 ± 4.7^{bc}	60.0 ± 8.7^{b}	93.3 ± 2.7^{a}	96.6 ± 2.7^{a}	100.0 ± 0.0^{a}
	0.5	2.0	50.0 ± 8.1^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	1.0	0.5	10.0 ± 4.7^{cd}	23.3 ± 7.2^{defgh}	40.0 ± 4.7^{def}	60.0 ± 0.0^{d}	100.0 ± 0.0^{a}
	1.0	1.0	26.6 ± 2.7^{b}	50.0 ± 4.7^{bcd}	60.0 ± 8.1^{cd}	86.6 ± 5.4^{abc}	100.0 ± 0.0^{a}
	1.0	2.0	16.6 ± 2.7^{bc}	$33.3 \pm 5.4b^{cdef}$	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	2.0	0.5	20.0 ± 4.7^{bc}	$40.0 \pm 9.4b^{cde}$	70.0 ± 4.7^{bc}	76.6 ± 2.7^{bcd}	80.0 ± 0.0^{bc}
	2.0	1.0	30.0 ± 4.7^{b}	56.6 ± 5.4^{bc}	83.3 ± 5.4^{ab}	86.6 ± 7.2^{abc}	90.0 ± 4.7^{ab}
	2.0	2.0	26.6 ± 2.7^{b}	50.0 ± 9.4^{bcd}	90.0 ± 4.7^{ab}	93.3 ± 2.7^{ab}	100.0 ± 0.0^{a}

*Different alphabets on the bars indicate statistical significance at $P < 0.05$ by Duncan's multiple range test.

3.2. Adventitious root induction of D. morbifera by optimizing medium with auxin treatment

Additional auxin hormones (IAA, IBA, NAA) were given to various medium (MS, WPM and B5) and 8 wk after, adventitious root induction from callus was observed. It clears that type and concentration of auxin made significant differences. MS and B5 medium did not induce adventitious roots (table 2). In contrast, WPM medium with 3 mg L^{-1} of any additional auxin was showed induction of adventitious roots. Previous report of direct adventitious root induction of *D. morbifera* took 4 wk, while it took only 10 d in *Codonopsis pilosula* [20]. Different plant or sources might show different response time of induction.

The present study showed the highest induction rate was in the treatment IBA hormone treatment in WPM medium after 8 wk (table 2). Similar report stated that adventitious root was induced from the leaf of *Echinacea purpurea* (L.) Moench with IBA than NAA and IAA hormone treatment [21]. For every petri-dish which contain IBA 3 mg L^{-1}, five pieces adventitious root were induced, with an average 16.42 mm (table 2. and figure 3A.). *C. pilosula* also showed the average number of induced adventitious root at most 25.8, with IBA usage during the treatment. The growth of adventitious root length was 21.40 mm in average [20]. In general, IBA has strong action to induce adventitious root formation and length growth in comparison with other auxin. This results similar with previous report on *Panax ginseng* adventitious root induction [22, 23]. One reported hormonal treatment in adventitious root induction described that 3 mg L^{-1} concentration of hormone was selected rather than 5 mg L^{-1} hormone treatment, because it has shorter incubation period. After hormone treatment has elapsed since the case of a solid medium, circulation did not occur very well and higher ethylene concentration was accumulated, especially in NAA treatment of ethylene. This increasing amount of ethylene was significantly inhibited the growth of adventitious roots [24].

Table 2. Effect of media and auxins concentrations on adventitious root growth of *D. morbifera* after 8 wk of culture.

Media	PGRs (mg L^{-1})		Callus formation (%)				
	2.4-D	BA	Wk				
			1	2	3	4	5
MS	-	-	0.0 ± 0.0^{d}	0.0 ± 0.0^{h}	0.0 ± 0.0^{h}	0.0 ± 0.0^{f}	$0.0 \pm 0.0^{f*}$
WPM	-	-	0.0 ± 0.0^{d}	3.3 ± 2.7^{gh}	10.0 ± 0.0^{gh}	23.3 ± 2.7^{e}	50.0 ± 8.7^{de}
	0.5	-	0.0 ± 0.0^{d}	10.0 ± 8.1^{fgh}	40.0 ± 8.1^{def}	76.6 ± 2.7^{bcd}	93.3 ± 2.7^{ab}
	1.0	-	0.0 ± 0.0^{d}	16.6 ± 9.8^{efgh}	33.3 ± 7.2^{ef}	60.0 ± 4.1^{d}	80.0 ± 4.1^{bc}
	2.0	-	16.6 ± 7.2^{bc}	30.0 ± 4.1^{cdefg}	70.0 ± 4.1^{bc}	73.3 ± 5.4^{cd}	93.3 ± 5.4^{ab}
	-	0.5	0.0 ± 0.0^{d}	10.0 ± 8.1^{fgh}	53.3 ± 11.8^{cde}	63.3 ± 13.6^{d}	66.6 ± 15.1^{cd}
	-	1.0	0.0 ± 0.0^{d}	10.0 ± 8.1^{fgh}	30.0 ± 0.0^{fg}	40.0 ± 4.7^{e}	46.6 ± 2.7^{e}
	-	2.0	10.0 ± 4.7^{cd}	20.0 ± 8.1^{efgh}	30.0 ± 4.7^{fg}	33.3 ± 2.7^{e}	43.3 ± 2.7^{e}
	0.5	0.5	16.6 ± 2.7^{bc}	36.6 ± 5.4^{bcdef}	83.3 ± 7.2^{ab}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	0.5	1.0	20.0 ± 4.7^{bc}	60.0 ± 8.7^{b}	93.3 ± 2.7^{a}	96.6 ± 2.7^{a}	100.0 ± 0.0^{a}
	0.5	2.0	50.0 ± 8.1^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	1.0	0.5	10.0 ± 4.7^{cd}	23.3 ± 7.2^{defgh}	40.0 ± 4.7^{def}	60.0 ± 0.0^{d}	100.0 ± 0.0^{a}
	1.0	1.0	26.6 ± 2.7^{b}	50.0 ± 4.7^{bcd}	60.0 ± 8.1^{cd}	86.6 ± 5.4^{abc}	100.0 ± 0.0^{a}
	1.0	2.0	16.6 ± 2.7^{bc}	$33.3 \pm 5.4b^{cdef}$	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
	2.0	0.5	20.0 ± 4.7^{bc}	$40.0 \pm 9.4b^{cde}$	70.0 ± 4.7^{bc}	76.6 ± 2.7^{bcd}	80.0 ± 0.0^{bc}
	2.0	1.0	30.0 ± 4.7^{b}	56.6 ± 5.4^{bc}	83.3 ± 5.4^{ab}	86.6 ± 7.2^{abc}	90.0 ± 4.7^{ab}
	2.0	2.0	26.6 ± 2.7^{b}	50.0 ± 9.4^{bcd}	90.0 ± 4.7^{ab}	93.3 ± 2.7^{ab}	100.0 ± 0.0^{a}

*Different alphabets on the bars indicate statistical significance at $P < 0.05$ by Duncan's multiple range test.

In IBA 5 mg L^{-1}, as shown in table 2, the number and length of adventitious roots was reduced compared to treatment with IBA 3 mg L^{-1} concentration. Concentrated IBA process in the result of inducing adventitious roots from *D. morbifera* tree seeds [25] is the length elongation of adventitious roots is inhibited was reported that callus formation and liabilities do adventitious root induction

studies can also adventitious roots, adventitious root length and live weight reduction was reported [26]. The concentration of the hormone may appear different depending on the kind of cultures. In addition, Lee et al. [23], may result in a chromosomal abnormality in the case a long time culturing the plant tissue on the medium containing a high concentration auxin cultured cells. In continuous culture with the IBA 5 mg L^{-1} or higher concentration, the callus of *Eleutherococcus* was formed. In general, optimum IBA concentration for growth was reported to be a concentration of less than 5 mg L^{-1} the optimum concentration [27].

Table 3. Effect of IBA concentration on 2nd adventitious root growth of *D. morbifera* after 4 wk of culture.

IBA concentration (mg L^{-1})	No. of adventitious root/segment	Length of adventitious root/segment (mm)	Fresh weight/segment (mg)	Dry weight/segment (mg)
0	5.70 ± 0.97[d]	2.45 ± 0.67[d]	5.90 ± 0.47[d]	0.44 ± 0.02[d]
1	9.10 ± 1.42[c]	3.85 ± 0.34[c]	13.26 ± 0.54[c]	1.94 ± 0.10[c]
2	13.60 ± 0.91[b]	8.81 ± 0.55[b]	66.58 ± 3.71[b]	6.90 ± 0.35[b]
3	25.10 ± 0.79[a]	15.24 ± 0.35[a]	155.14 ± 2.32[a]	14.54 ± 0.25[a]

* Different alphabets on the bars indicate statistical significance at $P < 0.05$ by Duncan's multiple range test.

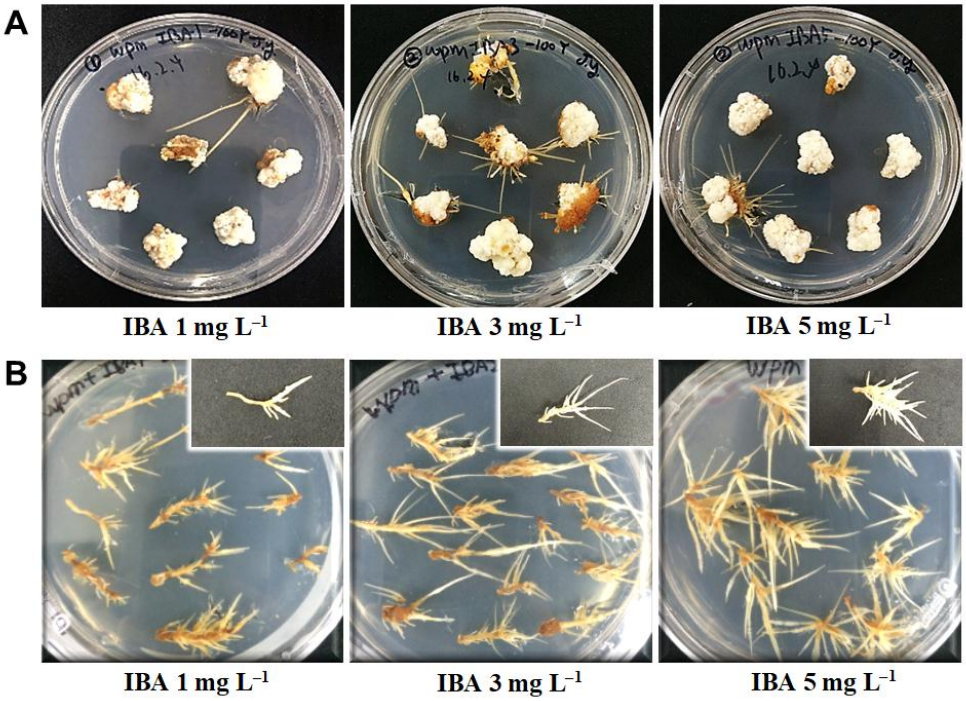

Fig. 3. Formation of *D. morbifera* adventitious root on WPM medium including IBA hormone concentration after 8 wk of culture. A. WPM media without hormone. B. WPM media including 1 mg L^{-1} of IBA, C. WPM media including 3 mg L^{-1} of IBA, D. WPM media including 5 mg L^{-1} of IBA.

3.3. Adventitious root induction of D. morbifera using several IBA concentration

Adventitious roots were induced at diverse rate on WPM with additional (0, 1, 2, and 3) mg L^{-1} IBA as shown on table 3, in which the IBA 3 mg L^{-1} give highest callus formation rate. The number of the

adventitious root induction was 25.10 ± 0.79; the longest induced adventitious root was 15.24 ± 0.35 (figure 3B). Fresh weight was (155.14 ± 2.32) mg and after it completely dried for 3 d, its dry weight was (14.54 ± 0.25) mg.

3.4. Effects of IBA concentration in the suspension culture of adventitious root of D. morbifera
In order to do mass culture of adventitious roots, the mass of adventitious roots subcultured WPM medium supplemented with sucrose 30 g L^{-1} and (0, 1, 2, 3) mg L^{-1} IBA concentration. It cultured using 200 mL Erlenmeyer flask and checked after 4 wk (table 4).

Table 4. Effect of IBA concentration on adventitious root growth of *D. morbifera* after 4 wk of suspension culture

IBA concentration (mg L^{-1})	No. of adventitious root/segment	Length of adventitious root/segment (mm)	Fresh weight/segment (mg)	Dry weight/segment (mg)
0	5.70 ± 0.97[d]	2.45 ± 0.67[d]	5.90 ± 0.47[d]	0.44 ± 0.02[d]
1	9.10 ± 1.42[c]	3.85 ± 0.34[c]	13.26 ± 0.54[c]	1.94 ± 0.10[c]
2	13.60 ± 0.91[b]	8.81 ± 0.55[b]	66.58 ± 3.71[b]	6.90 ± 0.35[b]
3	25.10 ± 0.79[a]	15.24 ± 0.35[a]	155.14 ± 2.32[a]	14.54 ± 0.25[a]

* Different alphabets on the bars indicate statistical significance at $P < 0.05$ by Duncan's multiple range test.

During suspension culture, little amount of callus appeared in the cutting area of the roots. Browning was appeared on the apical region of adventitious root. This phenomenon showed similar symptoms and physiological activity with *C. pilosula* adventitious roots [28]. When liquid medium compared to the solid medium, the growth culture will be faster, and at the same time, it facilitates new media and the replacement of the addition of nutrients. The liquid medium has the advantage to make the mass production possible [20, 29]. However, the erlenmeyer flask used in the liquid culture vessel is limited in size, thus, during suspension culture, it will inhibit the growth and give stress to the cell cultures [30]. The weight of roots was increased with increasing IBA concentrations, as shown in figure 3. Meanwhile, when the IBA concentration was lower, the adventitious roots tend to be elongated (table 4). Fresh weight and the dry weight profile was able to confirm that IBA 3 mg L^{-1} gave the highest results (figure 4A, 4B). Therefore, in order to feed the adventitious root growth through the liquid suspension culture, IBA 3 mg L^{-1} treatment were used for next step.

3.5. Effects of sucrose concentration in the suspension culture of adventitious root of D. morbifera
Investigation of sucrose effect on growth of *D. morbifera* adventitious root showed no significant difference in selected sucrose concentration. When treated with WPM + sucrose 30 g L^{-1} + IBA 3 mg L^{-1}, average fresh weight was 5.11 g 100 mL^{-1} while dry weight was the highest with 0.58 g 100 mL^{-1}. Meanwhile, when treated with ½ WPM + sucrose 50 g L^{-1} + IBA 3 mg L^{-1}, average fresh weight was 4.49 g 100 mL^{-1} and its dry weight was 0.52 g 100 mL^{-1} (figure 4B). In previous report, adventitious root fresh weight after treated with 30 g L^{-1} sucrose in MS media was 6.53 g 100 mL^{-1} [28].

The osmotic pressure of the cultured cells is increased on culture medium with high concentration of sucrose and also the water-absorbing process is suppressed. High concentration of sucrose is preferable as it used as substrate for the biosynthesis of secondary metabolites. The increasing secondary metabolite content due to higher sucrose content has been reported [31]. As such, sucrose will not only be used as cell wall constituents such as an energy source needed for metabolism to act as modulators of the cultures [28]. It was possible to confirm that the approximately biomass is 5 to 6 times higher compared to the initial appropriate amount adventitious root proliferation (figure 4B). This result is considered to be economic usage of *Codonopsis* adventitious culture, remembering the findings that reported six times increased of metabolites [30]. The content of the ½ WPM medium is the best to give fast-proliferated tissue culture yield. When viewed by the naked eye after incubation at

4 wk it can be seen that the WPM medium with additional 30 g L^{-1} of sucrose did not show any differences in the growth of the adventitious roots, compared with sucrose 50 g L^{-1} treatment (figure 4A).

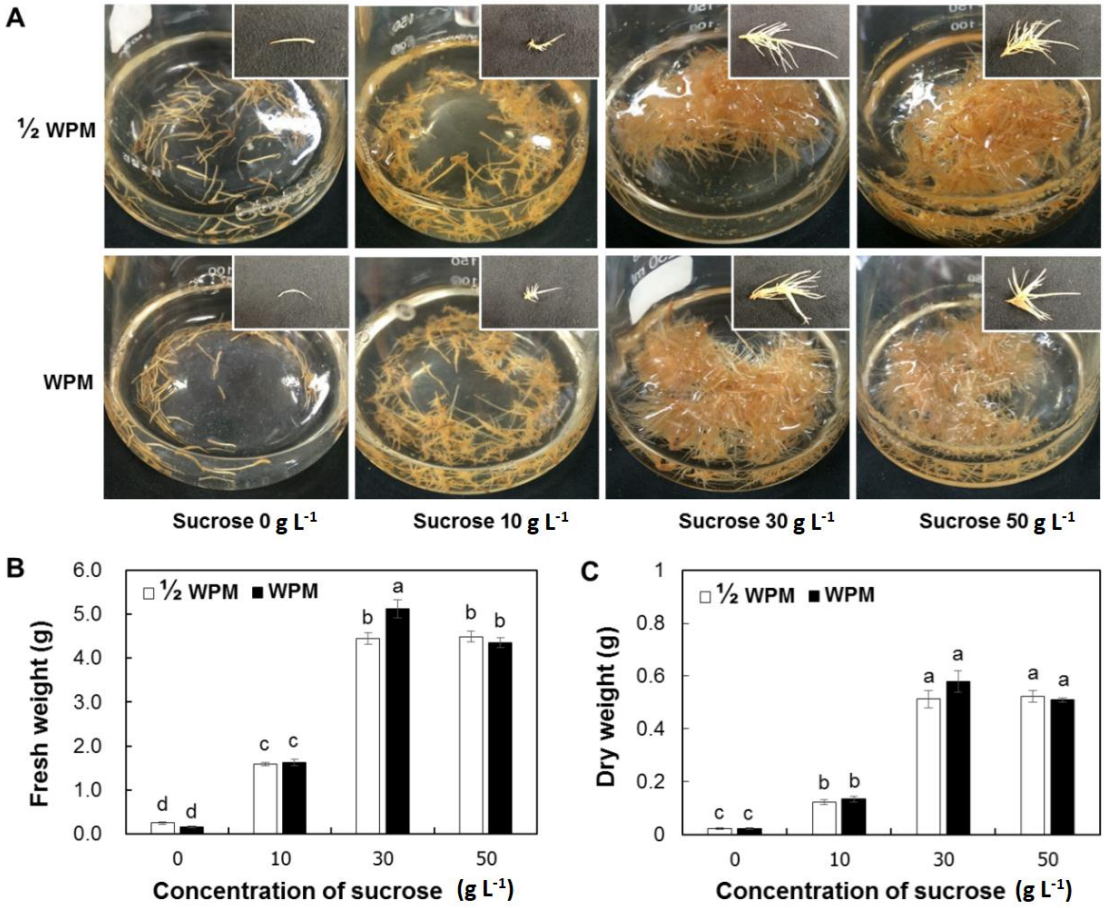

Figure 4. Effect of sucrose concentration on adventitious root growth of *D. morbifera* after 4 wk of culture (A) Morphological appearance, (B) fresh weight and (C) dry weight.

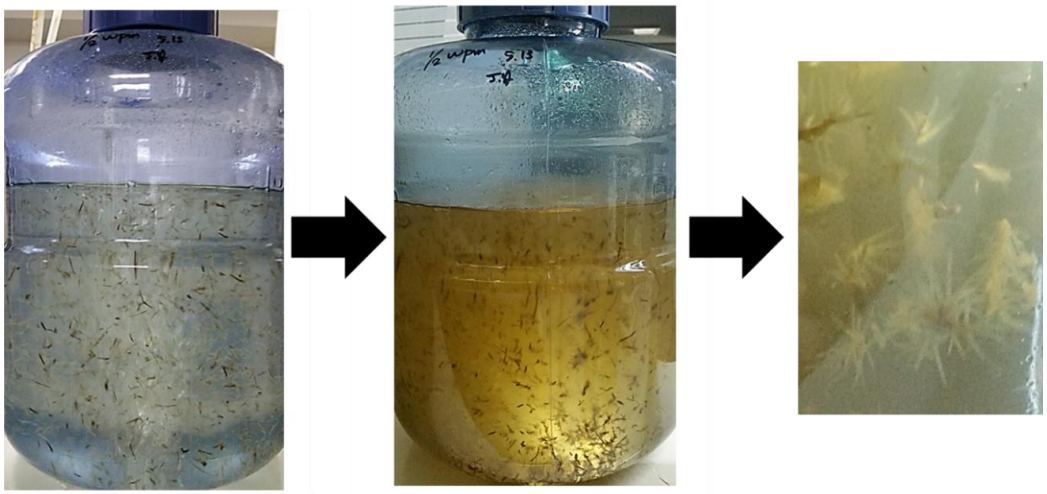

Figure. 5. The growth of adventitious root of *D. morbifera* in liquid culture. Photo of culture was taken in 18.3 L bioreactor containing ½WPM supplemented with 30 g L^{-1} sucrose and 3 mg L^{-1} IBA after 2 wk incubation.

3.6. Mass culture of the adventitious root of D. morbifera using bioreactor system

Utilization of ½ WPM medium with additional IBA 3 mg L^{-1} and sucrose 30 g L^{-1} in bioreactor system was able to confirm the mass propagation of adventitious root of *D. morbifera* after 4 wk of culture (figure 5). Plants that use ginseng roots as a medicinal needs a long time and much effort to the production of roots. Moreover, when taking the roots to harvest, the production itself stops. Therefore, utilizing the large biological incubator by applying the adventitious root induction for mass-propagation method is one of the ways to overcome these disadvantages [32–34].

4. Conclusion

This experiment was done by using a wild 100 yr *D. morbifera* leaves as explants. Adventitious roots induced from callus was carried out for the purpose of mass production. The callus formation was higher 50 % on WPM medium compared to MS medium. WPM medium supplemented with sucrose 30 g L^{-1} was the best formulation to induce callus from leaf explant. WPM medium with additional 2.4-D and BA hormones with various concentration [(0, 0.5, 1, 2) mg L^{-1}] by a single, or mixed both was done, resulting 100 % callus formation after 2 wk on 2.4-D 0.5 mg $L^{-1}$and BA 2 mg L^{-1}. The MS, WPM, and B5 medium with additional auxin like IBA, IAA, NAA and sucrose 30 g L^{-1} were tested in order to induce the adventitious root from the callus. The results showed that the MS and B5 medium is not good enough to induce adventitious root formation for the *ex vitro* leaves explants.

However, 4 wk later (total in 8 wk), the adventitious roots were induced in WPM medium. More details, there were 5.19 adventitious roots formed, with average length was 16.42 mm from explant cultured in WPM with additional 3 mg L^{-1} IBA. After several subculture process using same media formulation, mass propagation of adventitious roots was started using liquid media (WPM+ 3 mg L^{-1} IBA). For optimize the culture condition, ½ WPM medium and WPM medium were used with various additional sucrose (0, 10, 30, 50) g L^{-1}.

Experimental results showed that on the lower or no sucrose (0 and 10 g L^{-1}), the growth of adventitious root growth was poor, compared to 30 g L^{-1} and 50 g L^{-1} of sucrose. However, the yield after were not significant between ½WPM or WPM supplemented with 30 g L^{-1} of sucrose. The same results also observed on ½ WPM or WPM supplemented with 50 g L^{-1} of sucrose. Considering the economic aspects, ½ WPM with additional 30 g L^{-1} of sucrose is the appropriate media to be used for mass propagation. Through these findings, adventitious root derived from callus of wild *D. morbifera* can be obtained and mass-cultured to provide a material required for the industry.

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