STUDY OF ADVENTITIOUS ROOT FORMATION DERIVED FROM NODE OF *Morinda officinalis* How. CULTURED *IN VITRO*

Trinh Thi Huong[1]*, Ngo Thi Kim Mong[1],
Le Thanh Loc[1], Nguyen Thi Quy Co[2], Tran Trong Tuan[3]
[1]*Ho Chi Minh City University of Food Industry*
[2]*Binh Chanh - Cu Chi Management Board for Protection Forest*
[3]*Institute of Tropical Biology, VAST*
*Email: *trinhthihuongcsdl@gmail.com*
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ABSTRACT

Morinda officinalis is a precious medicinal plant with high economic value in Vietnam. In recent years, along with the tendency of breeding and preserving medicinal plants, the cultivation of biomass roots has been receiving attention. In this study, the role of IBA (indole-3-butyric acid), method of placing node on root induction were investigated. The results showed that SH (Schenk & Hildebrandt) medium supplemented with 2 mg/L IBA was suitable for adventitious root induction of *Morinda officinalis* cultured *in vitro*. The node explant placed vertically obtained the higher ratio of root formation (100%) and numbers of root (13.46 roots/sample) than node explant placed horizontally. The state of medium culture and the concentration of sucrose also play an important role in the proliferation of adventitious root. The results indicated that explants cultured on solid SH medium (agar 8.0 g/L) had higher growth rates than explants cultured on shaking liquid medium (without agar). In the experiment using sucrose, the medium supplemented with 45 g/L sucrose was suitable for adventitious root proliferation. The fresh weight of adventitious root achieved the highest value which was six times higher than initial used weight (1,2 g). The results of the study can be referred for the process of culturing *Morinda officinalis* root biomass at large scale, providing materials for the pharmaceutical industry.

Keywords: Adventitious root, indole-3-butyric acid, *Morinda officinalis*, node, sucrose.

1. INTRODUCTION

The roots of *Morinda officinalis* contain bioactive compounds that have high pharmacological value, so this species is considered as a valuable medicinal plant in traditional medicine. *M. officinalis* is mainly distributed in Laos, Vietnam, China, India and North Korea. It is a herbaceous plant, vines which can live for years. In Vietnam, *M. officinalis* is ranked first in the group of positive tonic herbs because the roots contain many valuable medicinal ingredients such as anthranoid, physcion, rubiadin, daucosterol, etc. The root extract of *M. officinalis* has various health effects, such as nourishing the kidney, reducing blood pressure, good for the brain, increasing appetite and improving sleeping quality. Due to the recently increasing demand for medicinal materials, the wild *M. officinalis* has been heavily exploited. The growth of *M. officinalis* in nature depends on ecological conditions, and it takes 3-5 years to harvest. Besides, it is also affected by insects, diseases, etc. Currently, the technology of culturing plant cell biomass has many advantages such as being able to actively control the process of producing large biomass in a short time without being affected by natural factors,

and the quality of biomass is stable. From this biomass, bioactive substances can be extracted as raw materials for the production of medicinal products, functional foods, and cosmetics. This technology has been creating valuable products for many areas of life, especially the pharmaceutical industry. In this situation, besides conducting propagation of *M. officinalis*, it is also necessary to develop the culture of plant cell biomass of *M. officinalis* to create a stable source of raw materials for the pharmaceutical industry. In particular, culturing adventitous root biomass is considered as a potential solution.

Adventitious roots are primarily derived from the cells surrounding vascular and medullary tissues. However, in some cases it is derived from epidermal cells [1]. Basically, the adventitious root has endogenous origin due to the differentiation of parenchymal cells located around the vascular tissue system by the action of auxin. During the process of differentiation, cells of the medullary and cortex region restore their ability to divide. However, only the certain cells, such as the phloem tissue of the meristem, are able to differentiate into the initial roots [2, 3].

During the cell and organ differentiation, the role of plant growth regulator is important. Auxin determines root differentiation, and sometimes auxin is considered as a root stimulant. If auxin concentration in the medium is too low, the explants should not stimulate rooting or slow rooting. There is a correlation between the positive polar movement of auxin and the root formation. This movement is an active transport process that occurs in the phloem parenchymal cells. When a sample of tissue is removed, their physiological properties are disordered, leading to redistribution of some substances, in particular auxin. Endogenous auxin is usually concentrated in the stem segment, the root formation position, and the amount of auxin in the stem will determine to promote or inhibit the process of forming new roots. Therefore, supplement of plant growth regulator helps to increase the ability of adventitious root formation [4].

In order to meet the high demand for medicinal materials of M.oficinalis and utilize the technology of root biomass culture, this study was conducted to investigate the influence of several factors of culture media including IBA concentration, the state of culture medium, sucrose concentration and placing the explants on the surface of the medium on the process of adventitious root formation and multiplication derived from nodes of *M. officinalis* How.

2. MATERIALS AND METHODS

2.1. Materials

The initial samples were nodes of the shoots of *M. officinalis* cultured in vitro.

The medium used in the experiments was SH medium (Schenk and Hildebrant, 1972) [5] supplemented with 30 g/L sucrose (except for investigation of sugar concentration's effect), 8.0 g/L agar, and the pH was adjusted to 5.8 before autoclaving.

2.2. Methods

2.2.1. Effect of IBA on the ability of inducing adventitious roots from node of M. officinalis

Nodes (1.5 cm in length) of *M. officinalis* were placed horizontally on SH medium supplemented with IBA at diferrent concentrations (1, 2, 3, 4, 5 mg/L). The control was the SH medium without IBA.

2.2.2. Effect of placed sample on the ability of inducing adventitious roots from node of M. officinalis

Nodes (1.5 cm in length) of *M. officinalis* were cultured on the SH medium supplemented with IBA (with the suitable concentration obtained from the experiment 2.2.1) in two ways: (1) The node explants were placed vertically (similar to the natural direction of the shoots) into the medium; (2) The node explants were placed horizontally exposured to surface of the medium.

2.2.3. Effect of the state of culture medium on the adventitious root multiplication of M. officinalis

0.2 g fresh weight of adventitious roots which were obtained from the two above experiments were inoculated on SH medium supplemented with IBA (at appropriate concentration from the experiment 2.2.1). The experiment investigated two different medium states that affected on the adventitious root multiplication. They were solid medium (addition of 8 g/L agar) and liquid shaking medium (without agar) with a shaking rate of 100 rpm.

2.2.4. Effect of sucrose concentration on the adventitious root multiplication of M. officinalis

Adventitious roots (0.2 g fresh weight) which were obtained from the two above experiments were inoculated on SH medium supplemented with IBA (at appropriate concentration from the experiment 2.2.1) and added sucrose with different concentrations of 15, 30, 45, 60 g/L.

2.2.5. Cultural conditions

All root induction and multiplication experiments were cultured in the dark under temperature conditions of 23 ± 2 °C, and average humidity of 55-60%. The shoots were cultured under lighting condition of 12 hours/day with light intensity of 2500-3000 lux.

2.2.6. Evaluation

For root induction: The ratio of rooting samples, number of roots/explant.

For root multiplication: The fresh weight root obtained after culture, multiplication coefficient.

$$Multiplication\ coefficient\ = \frac{The\ fresh\ weight\ root\ obtained\ after\ culture}{Initial\ fresh\ weight\ root}$$

2.2.7. Statistical analysis

Experiments were arranged completely randomly with three replicates. The criteria were observed after 4-6 weeks of culture. The data were analyzed statistically by Microsoft Excel and Statgraphics software at 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Effect of IBA on the adventitious root formation from node of *M. officinalis*

Adventitious roots are induced directly from different organs or indirectly through callus on the medium supplemented with auxin. In this experiment, effect of SH medium supplemented with IBA at concentrations of 0-5.0 mg/L on the ability of inducing adventitious root fomation from node was studied. After 6 weeks of culture, the results showed that 50% of node explants formed adventitious roots with average number of roots of 1.25 roots/explant in the control. When IBA was added to the culture medium at concentration of 1-4 mg/L, the rooting rate and number of roots/explant were higher than the control. The rooting rate (100%)

and the number of roots (5.58 roots/explant) were the highest at experiment using 2 mg/L IBA (Table 1). Morphological observation showed that the roots were long and branched at concentration of 2 mg/L IBA. In addition, new shoots were formed at concentrations of 1-3 mg/L IBA.

Table 1. Effects of IBA concentration on the adventitious root induction from node of *M. officinalis* after 6 weeks of culture

IBA (mg/L)	The rate of root formation (%)	Number of root/explant
0	50.00^{d}	1.25^{e}
1	91.67^{ab}	2.42^{c}
2	100.00^{a}	5.58^{a}
3	83.33^{bc}	3.25^{b}
4	75.00^{c}	1.83^{de}
5	58.33^{d}	0.67^{f}
P	*	*

**In the same column, means with different letters differ at a significant level of $p < 0.05$*

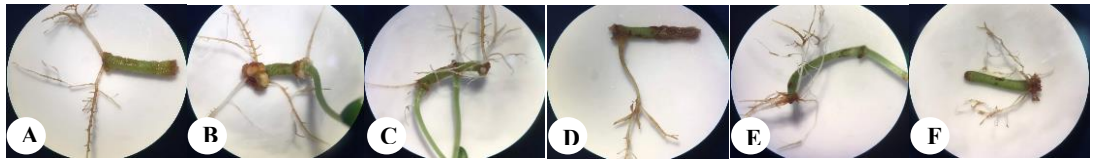

Figure 1. Effect of IBA concentration on the adventitious root induction from node of *M. officinalis* after 6 weeks of culture. A, B, C, D, E, F: 0, 1, 2, 3, 4, 5 mg/L IBA, respectively.

Auxin is a factor that determines root differentiation [4]. In plant cell tissue culture, IAA, IBA and NAA are main auxins used to stimulate rooting, and IBA is more effective for adventitious root culture than other auxins [6]. Trinh Thi Huong *et al*. (2012) reported that IBA (5 mg/L) had stronger influence on adventitious root formation of Ngoc Linh ginseng than NAA and IAA [7]. San José *et al*., (2012) also demonstrated that addition of 0.1 mg/L IBA to the culture medium helped to increase the secondary root formation of *Alnus glutinosa* compared to the control (without IBA) [8]. Concentration and kind of auxin added to the culture medium to induce adventitious root differ for different plant species. Ninh Thi Thao *et al*. (2016) also conducted the study of adventitious root culture derived from node of *M. officinalis* [9]. The results indicated that IBA was not suitable for adventitious root induction, and the medium for growth of adventitious roots was MS supplemented with 0.75 mg/L NAA; on this medium, the rate of root formation was 100% and the number of root reached 4.53 roots/sample.

3.2. Effect of placed sample on the adventitious roots formation from node of *M. officinalis*

Adventitious root formation is a profound change in histological activity, due to differentiation of parenchymal cells located around the vascular tissue system under the action of auxin. In most cases, it has endogenous origin, that means it starts from the center or in the conduction tissue [10]. In this study, node was cultured on SH medium supplemented with 2 mg/L IBA and placed on medium by two ways (vertical and horizontal). The results showed that explant placed vertically had number of root (13.46 roots/sample) higher than explant placed horizontally (3.54 roots/sample) (Table 2, Figure 2). The reason for this is the polar movement of auxin and the root formation correlated with each other when nodes were placed

vertically. This movement is an active transport process that takes place in the phloem parenchymal cell system of node when the node is in direct contact with the culture medium. As a result, the vertically placed explant absorbs nutrient and auxin better than horizontally placed explant.

Table 2. Effect of placed explant on the adventitious root formation from node of *M. officinalis* after 6 weeks of culture

How to place a sample	The number of root/sample
Node explants were placed vertically	13.46
Node explants were placed horizontally	3.54
P	*

**There is significant difference at 95% confidence level.*

Figure 2. Effect of placed explant on the adventitious root formation from node of *M. officinalis*
A: Node explants were placed horizontally after 6 weeks;
B: Node explants were placed vertically after 6 weeks (B1), and 10 weeks (B2).

3.3. Effect of culture medium on the adventitious root multiplication of *M. officinalis*

The adventitious roots obtained from the two previous experiments were used as materials to study of root multiplication on solid and liquid media. The results showed that on the solid medium the fresh weight of root was 0.38 g, and multiplication coefficient was 1.9 times. On the shaking liquid medium, however, the fresh weight of root was 0.29 g, and multiplication coefficient was 1.4 times. The reason is that on a shaking liquid culture medium the roots are submerged in the medium led to lack of oxygen, so the roots grown slowly (Table 3). Duong Tan Nhut *et al*. (2012) studied effects of several culture systems on multiplication of adventitious roots and secondary roots of Ngoc Linh ginseng (*Panax vietnamensis* Ha *et* Grushv.); the results also indicated that the semi-solid medium was more suitable for the multiplication of roots than the shaking liquid medium [11].

Table 3. Effect of the state of culture medium on the adventitious root multiplication of *M. officinalis* after 4 weeks

State of culture medium	Fresh weight of roots (g)	Multiplication coefficient (times)
Solid medium	0.38	1.9
Shaking liquid medium	0.29	1.4
P	*	

** There is significant difference at 95% confidence level.*

Morphological observation showed that roots swelled, and secondary roots were short; the roots began to brown and died after 2 weeks of culturing on the shaking liquid medium. However, on solid cultures, secondary roots tended to extended and penetrated the medium surface, formed white callus tissue surrounding the roots (Figure 3).

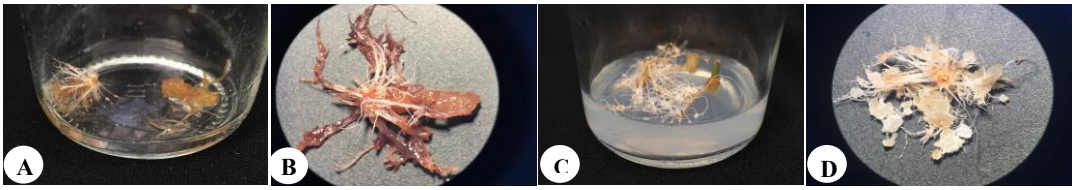

Figure 3. Effect of the state of culture medium on the adventitious root multiplication of *M. officinalis.* A,B: Shaking liquid medium; C,D: Solid medium.

3.4. Effect of sucrose concentration on the adventitious root multiplication of *M. officinalis*

In *in vitro* culture, sucrose is the source of carbon for plants, and the concentration of sucrose added in the medium is usually 30 g/L. However, in root biomass culture, the amount of sucrose added to the medium varies depending on the species. In this study, the fresh weight of roots did not increase in the control (without sucrose). When the concentration of sucrose increased from 15 to 45 g/L, the fresh weight of roots increased gradually. In particular, at the concentration of 45 g/L sucrose, the fresh weight of roots reached the highest (1.20 g), and multiplication coefficient was 6 times. Nguyen Trung Thanh and Paek Kee Yoeup (2008) studied multiplication of adventitious roots of *Panax ginseng* C.A. Meyer. The results also indicated that the concentration of 30 g/L sucrose was not suitable for multiplication of adventitious roots of *Panax ginseng* C.A. Meyer., and the most appropriate concentration of sucrose was 50 g/L [12]. When sucrose concentration increased to 60 g/L, the fresh weight of root decreased (1.01 g) (Table 4). This is because the high sucrose concentration causes osmotic pressure, which lead to inhibition of nutrient absorption of roots. According to Khuri and Moorby (1995), sucrose at a concentration of 2-5% was suitable for *in vitro* culture process, and it was quickly absorbed in the roots [13]. This experiment clearly showed the important role of sucrose in adventitious root growth. However, when sucrose was added to the culture medium at high concentration, root growth decreased. This is also similar to study on the roots of *Panax ginseng* C.A. Meyer by Hahn *et al*. (2003) [14]. Therefore, the appropriate concentration of sucrose for adventitious root growth of *M. officinalis* was 45 g/L.

Table 4. Effect of sucrose concentration on the adventitious root multiplication of *M. officinalis* after 6 weeks of culture

Sucrose (g/L)	Initial fresh weight of root (g)	Fresh weight of root (g)	Multiplication coefficient (times)
0	0.20	0.20^{d*}	1.0
15		0.87^{c}	4.35
30		1.07^{b}	5.35
45		1.20^{a}	6.0
60		1.01^{b}	5.05
P		*	

**In the same column, means with different letters differ at a significant level of $p < 0.05$.*

4. CONCLUSION

The appropriate concentration of IBA for adventitious root induction from the node of *M. officinalis* was 2 mg/L. Vertical culture of the node explants was more suitable for root formation than horizontal culture.

For the multiplication of *M. officinalis* root, the solid medium was more suitable than shaking liquid medium, and the appropriate sucrose concentration was 45 g/L.

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TÓM TẮT

NGHIÊN CỨU TẠO RỄ BẤT ĐỊNH TỪ ĐỐT THÂN CỦA CÂY BA KÍCH (*Morinda officinalis* How.) TRONG ĐIỀU KIỆN NUÔI CẤY *IN VITRO*

Trịnh Thị Hương[1]*, Ngô Thị Kim Mộng[1],
Lê Thành Lộc[1], Nguyễn Thị Quý Cơ[2], Trần Trọng Tuấn[3]
[1]*Trường Đại học Công nghiệp Thực phẩm TP.HCM*
[2]*Ban Quản lý rừng phòng hộ Bình Chánh-Củ Chi*
[3]*Viện Sinh học Nhiệt đới TP.HCM*
*Email: *trinhthihuongcsdl@gmail.com*

Cây ba kích là một cây dược liệu quý và có giá trị kinh tế cao của Việt Nam. Trong những năm gần đây, cùng với xu hướng nhân giống và bảo tồn các loài cây dược liệu thì nuôi cấy tạo sinh khối rễ cây ba kích cũng đang được quan tâm. Trong nghiên cứu này, vai trò của IBA (indole-3-butyric acid) và phương pháp đặt mẫu trong sự cảm ứng phát sinh rễ bất định đã được nghiên cứu. Kết quả nghiên cứu cho thấy, mẫu đốt thân được đặt đứng và nuôi cấy trên môi trường SH có bổ sung 2 mg/L IBA thích hợp cho sự cảm ứng tạo rễ bất định cây ba kích *in vitro*, với tỷ lệ mẫu tạo rễ đạt 100% và số rễ đạt 13,46 rễ/mẫu. Trạng thái môi trường nuôi cấy và nồng độ sucrose cũng đóng vai trò quan trọng đối với sự tăng sinh rễ bất định cây ba kích. Đối với quá trình tăng sinh rễ bất định, các mẫu được nuôi cấy trên môi trường SH có bổ sung 8 g/L agar có hệ số tăng sinh cao hơn so với các mẫu được nuôi cấy trong môi trường lỏng lắc (không bổ sung agar). Trong thí nghiệm nghiên cứu về nồng độ đường, môi trường có bổ sung 45 g/L sucrose thích hợp cho sự tăng sinh khối rễ bất định. Khối lượng mẫu tươi thu được sau 6 tuần nuôi cấy tăng gấp 6 lần so với khối lượng được sử dụng ban đầu (1,2 g). Kết quả đạt được của nghiên cứu là tiền đề cho quy trình nuôi cấy thu nhận sinh khối rễ ba kích ở quy mô lớn, nhằm cung cấp nguồn nguyên liệu ba kích một cách chủ động cho ngành công nghiệp dược.

Từ khóa: Cây ba kích, đốt thân, indole-3-butyric acid, rễ bất định, sucrose.