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Study on *in vitro* Propagation of Japanese Honeysuckle (*Lonicera japonica* Thunb.) via the Callus Method

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Abstract: The purpose of this study was to propagate the japanese honeysuckle species (*Lonicera japonica* Thunb.) via callus formation. We described callus induction in young leaves and shoot tip explants of this species, their proliferation and shoot regeneration from the callus. Both explants were cultured on MS medium supplemented with growth plant regulators (2.4-D; NAA and BAP) for callus induction. Our results showed that callus formation from shoot tip explants was better than that from leaf explants with white in color and soft callus when cultured on MS medium containing 1.5 mg/l of BAP. Callus formation from this medium was 92.31% successful with an average length size of 1.8 cm. After four weeks of callus induction in a completely dark condition, calli were transferred for two weeks to brightly light conditions for callus proliferation on MS medium supplemented with 0.5 mg/l of BAP in which calli increased five times in size. Calli were luxuriant and pale green in color. Shoots were regenerated from the callus on MS medium containing 1 mg/l of BAP in which 100% of cultured callus pieces produced adventitious shoots with shoot numbers ranging from 14 to 20 per callus.

Keywords: Callus, *in vitro* propagation, *Lonicera japonica*, medicinal plant.

1. Introduction

The japanese honeysuckle (*Lonicera japonica*) is a species of woody plant (family Caprifoliaceae) native to eastern Asia including China, Japan and Korea. In Vietnam, *L. japonica* grows wild in mountainous areas, mainly in Cao Bằng, Bắc Kạn, Thái Nguyên, Quảng Ninh, Ninh Bình, Thanh Hóa, Nghệ An, Hà Tĩnh provinces, and also to be cultivated as an ornamental or medicinal plant. The flower blooms from April to October and emits a pleasant honey-like odour. The flowers and leaves of this species have a sweet-bitter taste[1].

L. Japonica. has long been used in Vietnamese traditional medicine. Liquid extracted from flowers, leaves and branches of *L. japonica* has been used for treating fever, cholera, dysentery, inflammatory diseases, arthritis and infectious diseases [1, 2].

There has been considerable research on the chemical composition of *L. japonica*. Shang et al. (2011) isolated more than 140 chemical

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compounds from this species including essential oils, organic acids, and flavonoids [3]. This species has also been shown to display a wide spectrum of biological and pharmacological activities such as antibacterial, antiviral [4, 5], antioxidant and inhibition of platelet activating factors [6]. *L. japonica* can act as an anti-inflammatory agent through regulation of NF-κB activation [7]. Rutin is one key compound identified in *L. japonica* shown to provide protection against ischemia and reperfusion (I/R) in a variety of experimental models and via multiple mechanisms [8]. *L. japonica* contains anti-complementary polysaccharides and poly-phenolic compound. The polyphenolic compounds inhibit the platelet aggregation, thromboxane biosynthesis and hydrogen peroxide induced endothelial injury [9]. This species is rich in iridoid secologanin and is a potentially useful model for the study of secologanin biosynthesis. Secologanin is a primary terpenoid intermediate in the biosynthesis of monoterpenoid indole alkaloids such as reserpine, ajmaline, ajmalicine and vinbiastine [10].

Some Vietnamese studies have focused on the chemical composition and anti-bacterial and cytotoxic activity of honeysuckle [11, 12], anti-inflammatory effects of saponins and flavonoids in honeysuckle extract [13] and the possibility of Xanthine oxidase enzyme inhibitor in honeysuckle extract [14].

It is an important medical plant, but the honeysuckle growing area in Vietnam is being reduced. In addition, *L. japonica* seeds have the problem of low germination rates and long seedling time. It has become difficult to provide adequate amounts to pharmaceutical companies, as well as plants for households. In this situation, tissue culture can be an efficient method of providing material to satisfy these demands. This paper presented of *in vitro* propagation via the callus method towards development of additional and alternative sources of material.

2. Materials and Methods

Young leaf and shoot tip samples were collected from *in vitro* grown *L. japonica* at the Centre of Life Science Research, Faculty of Biology, VNU University of Science. Leaf-base explants of 0.5 x 0.5 cm and shoot tips of 0.5 cm were excised from *in vitro* grown plants on a MS solid medium [15].

Study methods

Medium preparation. MS media with 0.7% (w/v) agar and 3.0% (w/v) sucrose was prepared. Plant growth regulator solutions of 6-benzylaminopurine (BAP), naphthalene acetic acid (NAA) or 2.4-dichorophenoxyacetic acid (2.4-D) were added in different concentrations into MS media and the medium pH adjusted to 5.7 before autoclaving at 121^0C for 15 min.

Callus induction. For successful callus induction, factors such as type of explants, plant growth regulators, culture media and culture conditions are important. Firstly, we tested the effect of plant growth regulators to forming calli on leaf explants. Leaf explants were grown in MS media supplemented with 0.5 - 2.5 mg/l of 2.4-D; 0.1 - 0.9 mg/l of NAA and 0.5 - 2.5 mg/l of BAP; and shoot tips grown in MS medium supplemented with 0.5 - 2.5 mg/l of BAP were used for callus induction. The callus culture was maintained in completely dark conditions. Based on the percentage of cultured explants in callus formation, the optimal concentrations of growth regulators for callus induction were identified. A large number of calli were produced from explants cultured only on this formulation.

Callus proliferation. After 4 weeks for callus induction, calli of 0.5 x 0.5 cm size were transferred to a proliferating MS medium supplemented with 0.5 - 2.5 mg/l of BAP for two weeks under a brightly light condition.

Shoot induction. Calli were cut into $1cm^3$ pieces and cultured on a shoot induction medium with three callus pieces per flask. The shoot induction medium was MS medium

supplemented with 0.5 - 2.5 mg/l of BAP. Based on the resulting percentage of successfully cultured callus pieces with shoots, with the best formulation for shoot induction identified. This formulation was used for inducing shoots from calli.

Culture conditions. The Japanese honeysuckle plants were grown in tissue culture under 16-h light/8-h dark condition at 25 ± 2^0C and subcultured every three weeks.

3. Results and Discussion

3.1. Callus induction

Results were shown in Table 1 which presented the effects of 2.4-D, NAA and BAP on callus induction using the leaf-base explants of *in vitro L. japonica* plant. The cut edge of leaf explants started to expand after seven days of inoculation, and the entire leaf explants expanded after 21 days of inoculation. Rapid callus formation occurred in four weeks after inoculation. No calli informed in explants cultured on the basal medium alone. Although callus formation frequencies of leaf explants cultured on media containing 2.4-D ranged from 16.67% to 80% and on media containing NAA could be up to 100%, callus formation was in bad quality with brownish color and viscous. The MS medium supplemented with 1 mg/l BAP showed the strongest induction ability with 80% of leaf explants producing white and soft callus and calli. Therefore, we concluded that the best results from leaves were obtained with the MS medium supplemented with 1 mg/l BAP for producing calli on the leaf explants in four weeks under dark condition. However, in using leaves for callus induction, callus quality and size were not adequate for shoot regeneration.

Table 1. Callus induction and morphogenesis of *L. japonica* leaf explants under dark condition

Concentration of 2.4 D (mg/l)	Concentration of NAA (mg/l)	Concentration of BAP (mg/l)	% leaf explants producing a callus	Callus induction	Callus Morphologies
0	-	-		-	
0.5			16.67	+	Brown, viscous
1			50	+	Brown, viscous
1.5			54.54	+	Brown, viscous
2			66.67	++	Yellow, viscous
2.5			80	+++	White, viscous
	0.1		46.15	+	Brown, viscous
	0.3		100	++	White, viscous
	0.5		92.86	++	Black, viscous
	0.7		84.61	++	Black, viscous
	0.9		57.14	+	Black, viscous
		0.5	80	++	White and soft
		1	80	+++	White and soft
		1.5	68.57	++	Yellow and soft
		2	50.77	+	Yellow and soft
		2.5	38.46	+	Brown and viscous

-: no induction; +: induction; ++: low production of callus; +++: medium production of callus; ++++: high production of callus

So we tested effect of the media with BAP on callus formation of *L. japonica* shoot tips. Results showed that calli started to develop from the cut edges of shoot tip explants after seven days cultured in completely dark conditions. After four weeks inoculation, calli were formed. Callus formation frequencies of shoot tip explants cultured on medium containing BAP at 0.5 - 2.5 mg/l ranged from 70.34% to 92.31% (Table 2). Best results were medium with a concentration of 1.5 mg/l BAP in which callus formation after four weeks was 92.31% with an average size of 1.8 cm (Fig. 1). Calli cultured in this medium had good quality, presenting as white and soft calli. We identified the preferred medium for callus induction of *L. japonica* as the MS medium supplemented with 1.5 mg/l of BAP on the shoot tips with 4 weeks of induction in completely dark conditions. Our results are consistent with research findings of effects of plant growth regulators on callus growth of *Lonicera* sp. from leaf, stem and root segments [16, 17].

Table 2. Callus induction and morphogenesis of *L. japonica* shoot tips in dark conditions

Concentration of BAP (mg/l)	% shoot tip explant producing a callus	Callus induction	Morphologies
0	-	-	-
0.5	70.34	++	Brown and viscous
1	84.23	+++	White and soft
1.5	92.31	++++	White and soft
2	6502	+++	Yellow and soft
2.5	50.12	++	Brown and viscous

-: no induction; +: induction; ++: low production of callus; +++: medium production of callus; ++++: high production of callus

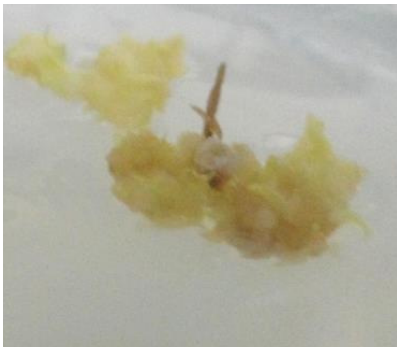

Fig. 1. Shoot tip explants of *L. japonica* cultured on media containing 1.5 mg/l of BAP after four weeks incubation in dark condition.

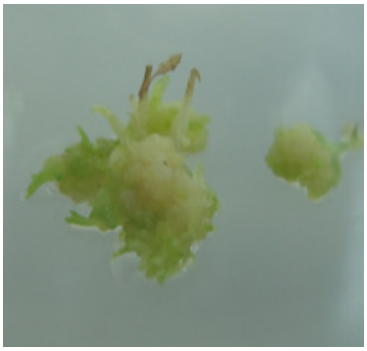

Fig. 2. Calli transferred to light condition after two days.

3.2. Callus proliferation

Calli using for shoot tips turned green and proliferated quickly (Fig. 2). Figure 3 showed that callus size was largest on MS medium supplemented with 0.5 mg/l of BAP. In this medium, calli increased quickly to five times in size in just over two weeks. Calli were luxuriant and pale green in color. In the MS medium supplemented with BAP at concentrations other than 0.5 mg/l, calli proliferated slowly. In the MS medium containing 2.5 mg/l BAP calli even turned brown and died.

3.3. Shoot induction

The first calli adventitious shoots appeared initially 12 weeks after transferring to shoot induction medium, with a size of 0.5 cm in length (Fig. 5). The shoots regenerated quickly two weeks after (Fig. 6). The best shoot formation condition was MS medium containing 1 mg/l BAP, in which 100% cultured callus pieces produced shoots with shoot numbers ranging from 14 to 20 per piece of callus. In the MS medium supplemented with 2.5 mg/l BAP, calli turned brown and died after six weeks of shoot culture. During the callus regeneration, the batch callus culture was continuously examined by taking a subculture at weekly intervals to prevent the cell death and browning of media. These findings were well supported by previous scientist, who also found that the induced callus regeneration by NAA with BA in the nodal explants of *Stevia rebaudiana* [18].

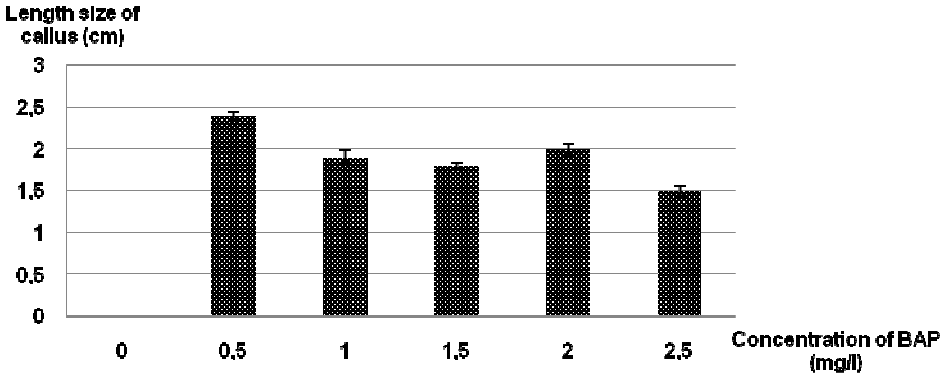

Fig 3. Effect of MS medium supplemented with BAP on callus proliferation after two weeks.

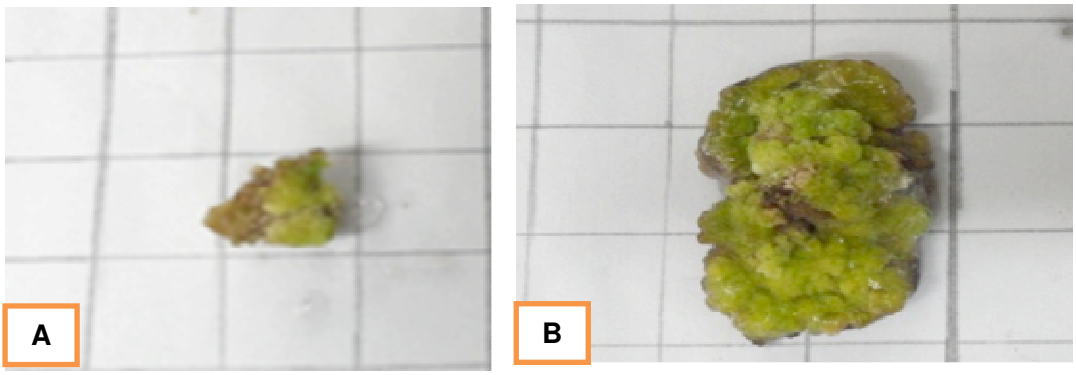

Fig. 4. Callus proliferation of *L. japonica* on MS medium supplement with 0.5 mg/l BAP from A to B after two weeks incubation.

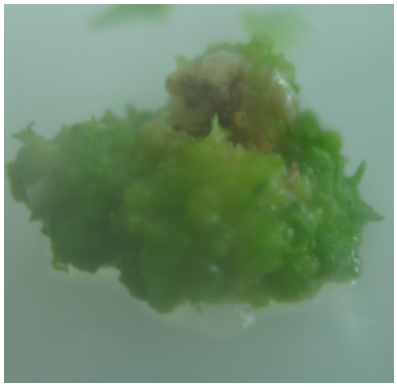

Fig. 5. Shoot formation of *L. japonica* on MS medium supplemented with 1 mg/l of BAP after 12 weeks incubation.

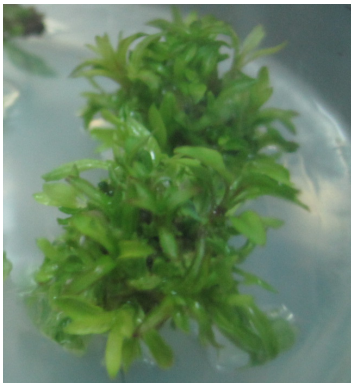

Fig. 6. Shoot regereration in explant cultured on MS medium containing 1 mg/l of BAP at two weeks later.

4. Conclusion

For callus formation, we identified MS medium supplemented with 1.5 mg/l of BAP using shoot tip explants of *L. japonica* in completely dark conditions for four weeks as the optimal culture condition. Callus formation from this medium was 92.31% successful with an average length size of 1.8 cm. For proliferation, we identified MS medium supplemented with 0.5 mg/l of BAP in which calli increased three times in size over two weeks. Finally, shoots were most effectively regenerated on a MS medium supplemented with 1 mg/l of BAP over 14 weeks in which 100% of cultured callus pieces produced adventitious shoots with shoot numbers ranging from 14 to 20 per callus.

Acknowledgements

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References

[1] Đỗ Tất Lợi, Những cây thuốc và vị thuốc Việt Nam, Nhà xuất bản Khoa học và Kỹ thuật, Hà Nội, 1991.

[2] Võ Văn Chi, Từ điển cây thuốc Việt Nam, Nhà xuất bản Y học, Hà Nội và TP Hồ Chí Minh, 1997.

[3] X. Shang, H. Pan, M. Li, X. Maio and H. Ding, *Lonicera japonica* Thunb.: Ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine. Journal of Ethnopharmacology, Vol. 138, issue 1 (2011) 1.

[4] A. Rahman and S.C. Kan, *In vitro* control of food-borne and food spoilage bacteria by essential oil and ethanol extracts of *Lonicera japonica* Thunb. Food Chemistry 116 (2009) 670.

[5] J. Xiong, S. Li, W. Wang, Y. Hong, K. Tang and Q. Luo, Screening and identification of the antibacterial bioactive compounds from *Lonicera japonica* Thunb., Food chemistry 138 (2013) 327.

[6] W. Ning, et al., Enhanced secondary metabolites production and antioxidant activity in postharvest *Lonicera japonica* Thunb. in response to UV radiation, Innovative Food Science & Emerging Technologies 13 (2012) 231.

[7] J.H. Lee, W.S. Ko, Y.H. Kim, H.S. Kang, H.D. Kim and B.T. Choi, Anti-inflammatory effect of the aqueous extract from *Lonicera japonica* flower is related to inhibition of NF-kappaB activation through reducing I-kappaBalpha

degradation in rat liver, International Journal of Molecular medicine 7 (2001) 79.

[8] R. Lanteri, R. Acquaviva, C. DiGiacomo, V.L. Sorrenti, G.Destri, M. Santangelo, L. Vanella and A. DiCataldo, Rutin in rat liver ischemia/reperfusion injury: effect on DDAH/NOS pathway, Microsurgery 27 (2007) 245.

[9] W.C. Chang and F.L. Hsu, Inhibition of platelet activation and endothelial cell injury by polyphenolic compounds isolated from *Lonicera japonica* Thunb. Prostag Leukotr *Ess*. 45 (1992) 307.

[10] K.H. Son, K.Y. Jung, H.W. Chang, H.P. Kim and S.S. Kang. Triterpenoid saponins from the aerial parts of *Lonicera japonica.* Phytochemistry. 35 (1994) 1005.

[11] Phan Minh Giang, Nguyễn Tuấn Minh, Phan Tống Sơn, Phytochemical investigation and study on cytotoxic and antimicrobial activites of *Lonicera japonica* Thumb., Caprifoliaceae, of Vietnam. Journal of Chemistry, Vol. 40, No. 3, (2002) 103.

[12] Phạm Minh Giang, Nguyễn Tuấn Minh, Nguyễn Minh Hằng, Phan Tống Sơn, Nghiên cứu hoạt chất sinh học từ cây kim ngân (*Lonicera japonica* Thunb., Caprifoliaceae) của Việt Nam. Kỷ yếu hội nghị khoa học và công nghệ Hóa hữu cơ toàn quốc lần thứ III, Hội hóa học Việt Nam, phân hội Hóa hữu cơ, (2005) 318.

[13] Lê Thị Diễm Hồng, Nguyễn Thị Hồng Nhiên, Nguyễn Thị Hồng Vân, Nguyễn Viết Thân và Nguyễn Xuân Thắng, Nghiên cứu tác dụng chống viêm mạn của saponin và flavonoid cây kim ngân (*Lonicera japonica* Thunb.), Tạp chí Dược học, số 378 (2007) 24.

[14] Nguyen Thi Thanh Mai, S. Awale, Y. Tezuke, Tran Le Quan, H. Watanabe and S. Kadota, Xanthine oxidase inhibitory activity of Vietnamese medicinal plants. Biol. Pharm. Bull. 27(9) (2004) 1414.

[15] T. Murashige and F. Skoog. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15 (1962) 473.

[16] D. Georges, J. Chenieux and S. Ochatt. Plant regeneration from aged-callus of the woody ornamental species *Lonicera japonica* cv. 'Hall's prolific'. Plant Cell Rep. 13 (1993) 91.

[17] X.Wang, J. Chen, Y. Li, Q. Nie and J. Li. An Efficient Procedure for Regeneration from Leaf-derived Calluses of *Lonicera macranthoides* 'Jincuilei', an Important Medicinal Plant. *Hortscience 4*4 (2009) 746.

[18] R.M. Patel and R.R. Shah. Regeneration of Stevia plant through callus culture. Indian Journal Pharmaceut. Sci. 71 (2009) 46.

Nghiên cứu nhân nhanh cây Kim ngân nhật (*Lonicera japonica* Thunb.) bằng phương pháp tạo mô sẹo

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Mục đích của nghiên cứu này là nhân nhanh cây Kim ngân nhật trong điều kiện phòng thí nghiệm thông qua phương pháp tạo mô sẹo. Mô sẹo được hình thành từ mảnh lá non và đỉnh chồi trong môi trường MS có bổ sung các chất kích thích sinh trưởng (2.4-D; NAA và BAP). Nguyên liệu tạo mô sẹo tốt nhất cho Kim ngân là chồi đỉnh cấy trên môi trường MS bổ sung 1.5 mg/l BAP trong điều kiện tối hoàn toàn. Sau bốn tuần tỷ lệ hình thành mô sẹo đạt 92.31%, và chiều dài trung bình của chúng đạt 1.8 cm. Sau đó các khối mô sẹo được cấy chuyển sang môi trường MS bổ sung 0.5 mg/l BAP để tăng sinh trong điều kiện sáng. Khối mô sẹo tăng sinh nhanh chóng lên năm lần so với ban đầu chỉ sau hai tuần nuôi cấy. Chồi được tái sinh tốt nhất trong môi trường MS bổ sung 1 mg/l với 100% các khối mô sẹo có khả năng tái sinh chồi, số lượng chồi trên một khối mô sẹo từ 14 đến 20 chồi.

Từ khóa: Callus, nhân nhanh, *Lonicera japonica*, cây thuốc.