EFFECT OF SOME FACTORS ON ADVENTITIOUS ROOT INDUCTION FROM *IN VITRO* LEAF AND CALLUS OF VIETNAMESE GINSENG (*PANAX VIETNAMENSIS* HA ET GRUSHV.)

Duong Tan Nhut, Le Nu Minh Thuy, Pham Trung Tuyen, Hoang Xuan Chien

Tay Nguyen Institute of Biology

SUMMARY

Biomass technology of plant cells based on cellular differentiation has been widely applied in many fields such as pharmaceuticals, functional foods, food additives, agricultural and forestry. It both made materials for production and conserved plant genetic resources. A larger number of plant-derived active ingredients were produced by biomass technology Ginseng products made from *in vitro* ginseng roots containing saponin are very popular worldwide. Many studies have been conducted to produce ginseng root biomass from adventitious and secondary roots. In this study, the formation of adventitious roots of Vietnamese ginseng (*Panax vietnamensis*) from *in vitro* calli and leaf tissues were compared. Culture media was also optimized for producing root biomass in bioreactor. After eight weeks of culture, all leaf explants on SH media supplemented with 3 mg/l NAA, 30 g/l sucrose, and 8 g/l agar produced high adventitious roots with an average number of 24.73 roots per explant. These adventitious roots could be used for rapid multiplication in bioreactor systems for extracting saponins in commercial purposes. The regeneration system of adventitious roots from callus and *in vitro* leaf could be applied for transformation study of *Panax vietnamensis*.

Keywords: *adventitious roots, bioreactor, callus, leaf in vitro, Panax vietnamensis*

INTRODUCTION

Vietnamese ginseng (*Panax vietnamensis* Ha et Grushv.), an endemic to Vietnam, has high content of triterpenoid saponin (among the giseng species that have the highest dammarane, 12–15%), and low level of oleanolic acid. Majonoside-R_2, which accounts for almost 50% of total saponin content in Vietnamese ginseng, plays a major role in the antidepressant, anti-stress, and memory-improving effect of this plant (Dong *et al.*, 2007).

The natural supply of Vietnamese ginseng is very limited as these plants only grow on Mount Ngoc Linh (in the Central Highland of Vietnam), and their ginsenoside level will not be sufficiently accumulated until they reach four to six years of age. The use of *in vitro* culture systems including bioreactor to produce biomass of medicinal plants within a short time is one of the outstanding solutions. Adventitious and hairy roots have been used for *in vitro* culture of ginseng (Asaka *et al.*, 1993; Choi *et al.*, 2000). Adventitious root culture is particularly useful for biomass production for the fast growth and consistent yield of natural products (Carvalho *et al*, 1998). This type of research, however, is still limited in Vietnamese ginseng. Therefore, the objective of this study is to optimize the conditions for producing biomass of Vietnamese ginseng from roots in *in vitro* conditions to provide materials for medicinal products. We determined the appropriate culture explants and optimized the bioreactor conditions for producing high quantity and quality of roots.

MATERIALS AND METHODS

Materials

In vitro leaf and callus transverse thin cell layers of Vietnamese ginseng were used as culture explants.

Culture media was based on the MS (Murashige, Skoog, 1962) and SH (Schenk, Hildebrandt, 1972) formulas, supplemented with 30 g/l sucrose, 8 g/l agar and plant growth regulators at different concentrations, pH was adjusted to 5.7–5.8 before autoclave. Explants were cultured in 250-ml vessels each of which contains 30 ml media.

Methods

In vitro adventitious root formation

Effect of culture media

Callus explants were put on different media, including MS, ½ MS (half-strength macroelements and full-strength microelements), MS ½ (half-strength macro- and microelements), SH supplemented with 3 mg/l NAA.

Culture media with optimized salt concentration were supplemented with NAA (0.5, 1.0, 2.0, and 3.0 mg/l) and IBA (0.5, 1.0, 2.0, and 3.0 mg/l). Different sources of carbohydrate, including fructose, glucose, and sucrose, were also tested for their effect on the culture.

Effect of explant source

Optimized culture media was used to evaluate the effect of explants from *in vitro* leaf tissue and calli on the formation of adventitious roots

Culture conditions

Adventious root formation was performed in dark, at 25 ± 2°C and 40–45% relative humidity. Experiments were triplicated, and adventitious root formation rate and numbers were recorded after eight weeks of culture. Dunca's multiple range test ($P = 0.05$) for the data was performed with the statistical analysis software SPSS 16.0.

RESULTS AND DISCUSSION

Effect of culture media salts on adventitious root formation from calli

After eight weeks of culture, adventitious root formation rate on SH media was higher than that on MS media (65% to 45%) while no adventitious roots were observed on ½ MS and MS ½. Number of adventitious roots per explant was also higher on SH media (Table 1).

Table 1. Effect of different culture media salt formula on the adventitious root formation from calli of Vietnamese ginseng after eight weeks of culture. * Different letters (a, b, and c) indicates significant difference ($P = 0.05$) with Duncan's test.

Treatments	Adventitious root formation	
	Formation rate (%)	Number of roots per explant
MS	40	4.00b*
½ MS	0	0c
MS ½	0	0c
SH	65	8.75a

The ratio of NH_4^+:NO_3^- might play the critical role for the high adventitious root formation rate on SH media. This ratio is very low in SH media due to the lack of NH_4NO_3, while the content of that in MS media is 1 650 mg/l. In addition, the MS media have low concentration of $NH_4H_2PO_4$ (300 mg/l), and high concentration of KNO_3 (2,500 mg/l). The low ratio of NH_4^+:NO_3^- (1:9.5) with 382 mg/l nitrogen was demonstrated to be optimal for improved fresh weight and saponin biosynthesis in *in vitro* cultured Korean ginseng (*P. ginseng*) Similar results were obtained in notoginseng (*P. notoginseng*) (Jung *et al.*, 2005).

Vitamins also contribute to the better results observed on SH media The amount of vitamins in SH media is higher than that in MS media, particularly thiamin-HCl (50 fold) and *myo*-inositol (10 fold). Park *et al.* (2000) reported the use of SH media for efficient culture of Korean ginseng adventitious roots in bioreactors resulted in high growth and number of roots. Kim *et al* (2003) also used SH media for Korean ginseng root culture. From these results, we applied SH media for subsequent experiments.

Effect of NAA and IBA on adventitious root formation from calli

Auxin is well known not only for inducing rooting (Nemeth, 1986) but also for its effect on improved root fresh weight, and both NAA and IBA are normally used for root induction (George, Sherington, 1984). Auxin is a critical factor affecting the organogenesis, especially adventitious root formation, as it controls the cellular elongation and division. Nevertheless, plant's response to auxin is often species- or variety-specific, and there is a

threshold of auxin level beyond which cellular growth and development are inhibited (Rakhakrishana *et al.*, 2001). Therefore, it is necessary to determine the appropriate type and concentration of auxin for adventitious root formation.

After eight weeks of culture, the highest adventitious formation rate (100%) and number of roots were recorded on media with NAA at 3 mg/l compared to those on media with IBA (Table 2). Our results agree with those of Bonfill *et al* (2002) that adventitious roots formed on media supplemented with NAA are longer, thicker, and more abundant while those on media with IBA are thinner and more likely to die after subculture. Thus, SH media with 3 mg/l NAA was used for subsequent experiments.

Table 2. Effect of NAA and IBA at different concentrations on the adventitious root formation from calli of Vietnamese ginseng after eight weeks of culture. * Different letters (a, b, and c) indicates significant difference (P = 0.05) with Duncan's test

Concentration (mg/l)	NAA		IBA	
	Adventitious formation rate (%)	No. of adventitious roots / explant	Adventitious formation rate (%)	No. of adventitious roots / explant
0.5	45	1.05c*	35	0.25c
1.0	55	1.55c	80	2.83b
2.0	95	4.25ab	80	2.14b
3.0	100	7.80a	90	4.05ab

Effect of carbohydrate sources on the adventitious root formation from calli

The highest adventitious root formation rate was observed on media with sucrose while no roots were formed on fructose-supplemented media. The number of roots on sucrose-supplemented media was almost 30-fold higher than that on media with glucose (Table 3).

Sucrose, fructose, and glucose are three major carbohydrate sources for *in vitro* plant tissue culture. According to Khuri and Moorby (1995), the best and most common carbohydrate sources for *in vitro* culture is sucrose at the concentration of 2 – 5%; the sugar mainly accumulate in the roots and is quickly absorbed. In contrast, the use of glucose and/or fructose may cause insufficient hexose absorption in *in vitro* plants. If occurring during the initial phase of the culture, it may interrupt with the root formation in several species including Korean ginseng (Inomata *et al*, 1993), *Catharanthus roseus* (Jung *et al.*, 1992), *Datura stramonium* (Holmes *et al.*, 1997), and *Erythrorhizon* (Sim, Chang, 1997).

Table 3. Effect of carbohydrate sources on the adventitious root formation from calli of Vietnamese ginseng after eight weeks of culture. * Different letters (a, b, and c) indicates significant difference (P = 0.05) with Duncan's test

Carbohydrate sources	Adventitious root formation rate (%)	No. of adventitious roots / explant
Sucrose	100	11.95a*
Glucose	30	0.40b
Fructose	0	0c

The presence of hexoses in culture media is often associated with an increase in enzymatic activities of the cell wall, (Wender *et al.*, 1990), and this has been observed in *in vitro* adventitious root formation from calli of Korean ginseng (Odnevall, Bjork, 1989). On the other hand, glucose and fructose indirectly inhibit enzymatic activities (phosphofructokinase, α-glucosidase) during *in vitro* culture (Isla *et al.*, 1991). With sucrose as the optimal carbohydrate source, our results for Vietnamese ginseng are similar to those of Kim *et al.* (2005) for Korean ginseng.

Adventitious root formation from calli and leaf tissue

Responses to exogenous auxins are different

one explant to another, and these differences come from the type and age of the explants, and the type of exposure time of the auxin (Nemeth, 1986). Our results showed that all leaf and callus explants gave higher number of adventitious roots from calli (Table 4, Figure 1). Results from this study are different from those of notoginseng by Gao *et al* (2005), in which side roots gave callus formation after ten days and adventitious roots after 35 days of culture, while leaf petioles gave callus formation after 14 days and adventitious root formation after 45 days of culture, and no differentiation and adventitious roots were observed from leaves, shoots, and roots.

Compared to calli, leaf explants are more favourable as they do not require a formation period that is quite long in the case of calli. The time for adventitious root formation from leaf tissue is also shorter (40 days) than that from calli (50 days)

Table 4. Effect of explant sources on the adventitious root formation of Vietnamese ginseng after eight weeks of culture * Different letters (a, b, and c) indicates significant difference (P = 0 05) with Duncan's test

Explant sources	Adventitious root formation rate (%)	No. of adventitious roots / explant
Callus	100	11 13ab*
In vitro leaf	100	24 73a

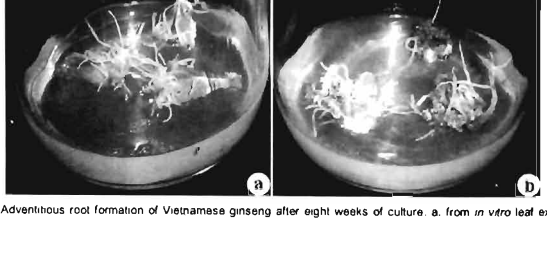

Figure 1. Adventitious root formation of Vietnamese ginseng after eight weeks of culture. a. from *in vitro* leaf explants; b. from calli

CONCLUSION

The optimized condition for *in vitro* adventitious root formation in Vietnamese ginseng is the culture of leaf tissue on SH medium supplemented with 3 mg/l NAA and 30 g/l sucrose. Our study proves the feasibility of generating Vietnamese ginseng adventitious roots in a simple tissue culture system.

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REFERENCES

Asaka I, Li L, Hirotani M, Asada Y, Furuya T (1993) Production of ginsenoside saponin by culturing ginseng (*Panax ginseng*) embryonic tissues in bioreactors. *Biotechnol Lett* 15: 1259-1264

Bonfill M, Cusidó RM, Palazón J, Piñol MT, Morales C (2002) Influence of auxins on organogenesis and ginsenoside production in *Panax ginseng* calluse. *Plant Cell Tiss Org* 68: 73-78.

Carvalho EB, Curtis WR (1998) Characterization of fluid flow resistance in root cultures with a convective flow tubular bioreactor. *Biotechnol Bioeng* 60: 375-384.

Choi SM, Son SH, Yun SR, Kwon OW, Seon JH, Paek KY (2000) Pilot-scale culture of adventitious roots of

ginseng in a bioreactor system. *Plant Cell Tiss Org* 62: 187-193.

Dong NT, Luan TC, Huong NTT (2007) *Ngoc Linh Ginseng and some medicinal plants belong to Ginseng family*. Science and Technology Publishing House.

Gao X, Zhu C, Jia W, Gao W, Qiu M, Zhang Y, Xiao P (2005) Induction and characterization of adventitious roots directly from the explants of *Panax notoginseng*. *Biotechnol Lett* 27: 1771-1775.

George EF, Sherington PD (1984) *Plant propagation by tisue culture* Exegetics Ltd Eversley England: 709.

Holmes P, Li SL, Green KD, Ford-Lloyd BV, Thomas NH (1997) *Drip-tube technology for continuous culture of hairy-roots with integrated alkaloid extraction*. In Doran PM, ed. Hairy-roots Culture and Applications. Harwood Academic. 201-208.

Inomata S, Yokoyama M, Gozu Y, Shimizu T, Yanagi M (1993) Growth pattern and ginsenoside production of *Agrobacterium*-transformed *Panax ginseng* roots. *Plant Cell Rep* 12: 681-686.

Isla MI, Vattuone MA, Sampietro AR (1991) Modulation of potato invertase activity by fructose. *Phytochemistry* 30: 423-426.

Jung HK, Eun JC, Hoon-Il O (2005) Saponin Production in Submerged Adventitious Root Culture of *Panax ginseng* as Affected by Culture Conditions and Elicitors. *Asia-Pac J Mol Biol* 13(2): 87-91.

Jung KH, Kwak SS, Kim SW, Lee H, Choi CY, Liu JR (1992) Improvement of the catharanthine productivity in hairy-root cultures of *Catharanthus roseus* by using monosaccharides as a carbon source. *Biotechnol Lett* 14: 695-700.

Khuri S, Moorby J (1995) Investigation into the role of sucrose in potato: Estima microtuber production *in vitro*. *Ann Bot* 75: 295-303.

Kim YS, Hand EJ, Yeung EC, Peak KY (2003) Lateral root development and saponin accumulation as affected by IBA or NAA in adventitious root cultures of *Panax ginseng* C A. Meyer. *In Vitro Cell Dev Biol Plant* 39: 245-249.

Kim JH, Chang EJ, Oh HI (2005) Saponin Production in Submerged Adventitious Root Culture of *Panax ginseng* as Affected by Culture Conditions and Elicitors. *Asia-Pac J Mol Biol* 13(2): 87-91.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15: 473-497.

Nemeth G (1986) Induction of rooting. In: Bajaj YPS, eds. *Biotechnology in Agriculture and Forestry. Tree I*. Springer. Berlin Heidelberg New York Tokyo Vol 1: 49-64.

Park SJ, Kim SM, Kim MH, Kim CS, Lee CH (2000) Development of a prototype continuous flow dryer using far infrared ray and heated-air for white gingseng *J Korean Soc Agr Mac* 25(2): 115-122.

Odnevall A, Bjork L (1989) Differentiated tissue cultures of *Panax* ginseng and their response to various carbon sources. *Biochem Physiol Pft* 185, 403-413.

Schenk RU, Hildebrandt AC (1972) Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Can J Bot* 50. 199-204

Radhakrishna T, Murthy TGK, Chandran K, Banyopadhyay A (2001) Somatic Embryogenesis in *Arachis hypogaea*: revisited. *Aust J Bot* 49: 753-759.

Sim SJ, Chang HN (1997) Shikonin production by hairy-roots of *Lithospermum erythrorhizon* in bioreactors with in situ separation. In Doran PM, ed. *Hairy-roots – Culture and Applications* Harwood Academic Publishers, Australia. 201-208.

Wendler R, Veith R, Dacer J, Stitt M, Komor E (1990) Sucrose storage in cell-suspension cultures of *Sacharum* sp. (sugarcane) is regulated by a cycle of synthesis and degradation *Planta* 183: 31-39

ẢNH HƯỞNG CỦA MỘT SỐ YẾU TỐ LÊN KHẢ NĂNG HÌNH THÀNH RỄ BẤT ĐỊNH TỪ MÔ SẸO VÀ LÁ *IN VITRO* CỦA CÂY SÂM NGỌC LINH (*PANAX VIETNAMENSIS* HA ET GRUSHV.)

Dương Tấn Nhựt*, Lê Nữ Minh Thủy, Phạm Trung Tuyến, Hoàng Xuân Chiến

Viện Sinh học Tây Nguyên

TÓM TẮT

Công nghệ sinh khối tế bào thực vật dựa trên cơ sở tính toàn năng và tính biệt hóa của tế bào thực vật đã được ứng dụng rộng rãi trong nhiều lĩnh vực: dược phẩm, sản phẩm chức năng, chất phụ gia thực phẩm, nông

* *Author for correspondence:* Tel: *+84-63-3831056;* Fax: *+84-63-3831028,* E-mail: *duongtannhut@gmail.com*

nghiệp, lâm nghiệp và vừa tạo ra nguyên liệu, vừa giúp bảo tồn nguồn gen quý hiếm. Rất nhiều các hoạt chất nguồn gốc từ thực vật có giá trị kinh tế cao là sản phẩm của sinh khối tế bào thực vật. Sinh khối rễ sâm *in vitro* có chứa các hợp chất saponin đã và đang được sử dụng làm nguồn nguyên liệu để sản xuất rộng rãi các sản phẩm về sâm trên thế giới. Đã có nhiều nghiên cứu được thực hiện với mục đích sản xuất sinh khối rễ sâm từ nguồn nguyên liệu ban đầu là rễ bất định và rễ thứ cấp. Trong nghiên cứu này, sự hình thành rễ bất định đã được so sánh giữa hai nguồn mẫu có nguồn gốc từ mô sẹo và lá *in vitro* sâm Ngọc Linh. Nuôi cấy này nhằm tạo nguồn nguyên liệu ban đầu cho sản xuất sinh khối rễ trong hệ thống bioreactor. Sau 8 tuần, các mẫu cấy từ lá *in vitro* được nuôi cấy trên môi trường SH bổ sung 3 mg/l NAA, 30 g/l sucrose và 8 g/l agar cho tỉ lệ hình thành rễ bất định cao (100%) và số lượng rễ đạt 24,73 rễ/mẫu. Những rễ bất định này có thể sử dụng để nhân nhanh trong các hệ thống nuôi cấy bioreactor để tách chiết saponin. Hệ thống tái sinh rễ bất định từ mô sẹo và lá *in vitro* có thể ứng dụng cho công tác nghiên cứu chuyển gen vào cây sâm Ngọc Linh.

Từ khóa *bioreactor, lá in vitro, mô sẹo, Panax vietnamensis, rễ bất định*