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Effects of macro elements on biomass and ginsenoside production in cell suspension culture of Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv.)

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Abstract. We investigated the effects on ginseng cell and ginsenoside production when macro element concentrations were manipulated in the culture media. Biomass growth was greatest in the medium supplemented with 0.5 strength NH_4NO_3, whereas ginsenoside accumulation was highest (6.5 mg/g DW). At levels of 1.0 strength KNO_3, cell growth was maximum, but 2.0 strength of KNO_3 led to the greatest ginsenoside content (6.1 mg/g DW). High concentrations of $MgSO_4$ were most favorable for both cell growth and ginsenoside accumulation (up to 5.5 mg/g DW). Cell growth and ginsenoside content also increased in proportion to the concentration of $CaCl_2$ in the medium, with the greatest accumulation of ginsenoside (5.7 mg/g DW) occurring at a 1.5 strength.

Keywords: macro element, suspension culture, conical flask, *Panax*.

1. Introduction

Vietnamese ginseng was found at highland of Central Vietnam in 1973, and was regarded as a new species as *Panax vietnamensis* Ha et Grushv. (1985). This is the most southern distribution of *Panax* genus (Araliaceae). It is a secret medicine of the Sedang ethnic group as a miraculous, life-saving plant drug used for the treatment of many serious diseases and for enhancing body strength in long journeys in high mountains.

In recent years, plant cell culture technology has successfully applied to the production of many useful secondary metabolites, including pharmaceuticals, pigments, and other fine chemicals [1,2]. Ginsenosides also have been derived through cell culture [3-6], although the high fluctuation in ginsenoside content achieved via culturing is a large obstacle to commercialization.

Therefore, in this paper, we established cell suspension culture of ginseng cell and some attempts have been made to increase biomass and ginsenoside yield of Ngoc Linh ginseng cell culture by the effects of different macro element.

2. Materials and Methods

Induction of callus

Fresh mountain ginseng roots were collected from Ngoc Linh mountain, Quang Nam province. Selected root were washed with a detergent solution for 5-10 min and then rinsed with running tap water for 5-10 min.

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They were rinsed with sterilized water after being soaked in 70% aqueous EtOH for 0.5-3 min under reduced pressure, further sterilized with 1% sodium hypochloride for 10-30 min, and then rinsed repeatedly with sterile distilled water. The sterilized roots were cut into sections of 2-10 mm and then were inoculated into MS solid medium [7] containing 30 g/L sucrose, 1 mg/L 2,4-D, and 0.1 mg/L kinetin. After 1 month callus were induced. The callus were subcultured into above medium after every 20 days for proliferation of callus. After 5 times of subculture into the solid medium the callus were inoculated into liquid medium (same with above).

Stock cell culture and culture condition

Suspended cells of *P. vietnamensis* were initiated through callus induction from the cultivated plant root [8]. The cell line was maintained in MS liquid medium supplemented with 3 mg/L indole-3-butyric acid (IBA), 0.1 mg/L of kinetin and 30 g/L sucrose. The pH was adjusted to 5.8 before autoclaving.

Cells were cultivated in 300 ml conical flasks with a working volume 100 ml on a rotary shaker in darkness at a rotation speed of 105 rpm and a culture temperature of 25°C. Cells cultivated for 15 days were used in the experiment and the inoculum size 6 g/flask (fresh weight). The other cultural conditions were done as described by [9].

Determination and analyses

Extraction and determination of ginsenoside production were determined as reported previously [8,9].

Determination of cell growth rate

Fresh weigh (FW) was measured after the water was absorbed from the root surfaces. To measure dry weight (DW), cells were over-dried at 60°C until reached a constant mass. The cell growth rate was then calculated as:

Growth rate = harvested DW (g)/inoculated DW (g).

3. Results and discussion

Effects of macro elements on biomass and ginsenoside production

Table 1 and Figure 1 show how growth and yield of ginseng cell were affected by the concentrations of macro elements in the MS medium. Biomass production was greater when 0.5 and 1.0 strengths of NH_4NO_3 were used, with the highest yields (4.6) resulting from the 0.5 strength level. Ginsenoside accumulation also was influenced by macro-element supplements (Fig. 2), increasing at the lower concentration. In fact, the greatest ginsenoside production (10.3 mg/g DW) was obtained when 0.5 strength level of NH_4NO_3 from the culture medium.

Table 1. Biomass growth of ginseng cell was affected by concentration of macro elements in the MS medium. Cultures were maintained in 300 ml conical flasks for 4 weeks

Concentration of macro element		Biomass growth		
		Fresh wt. (g/L)	Dry wt. (g/L)	% dry wt.
NH_4NO_3	0.0	111 b[z]	8.4 b	3.3
	0.5	155 a	10.6 a	4.1
	1.0	147 a	10.3 a	3.9
	1.5	129 b	8.7 b	3.5
	2.0	114 b	8.4 b	3.3
KNO_3	0.0	78 d	5.9 d	2.9
	0.5	115 b	8.6 b	3.8
	1.0	150 a	10.5 a	4.0
	1.5	125 b	9.0 ab	3.4
	2.0	101 cd	8.4 b	3.2
$MgSO_4$	0.0	90 c	8.5 b	3.1
	0.5	134 ab	9.6 ab	3.5
	1.0	138 ab	9.8 ab	3.6
	1.5	152 a	10.4 a	3.8
	2.0	118 b	8.8 b	3.4
$CaCl_2$	0.0	113 b	8.4 b	3.4
	0.5	152 a	10.5 a	3.9
	1.0	149 a	10.4 a	4.0
	1.5	155 a	10.7 a	4.1
	2.0	157 a	10.8 a	4.2

[z]Mean separation by Duncan's multiple range test at $p \leq 0.05$

A 1.0 strength of KNO_3 resulted in the maximum DW (10.5 g), and growth yield (4.35), while the 2.0 strength led to the greatest ginsenoside content (6.1 mg/g DW). Higher strengths (1.0, 1.5, and 2.0) of $MgSO_4$ were more favorable for both cell biomass growth and ginsenoside accumulation, as seen by the highest cell biomass DW (10.4 g) and ginsenoside content (5.5 mg/g DW). Cell growth and ginsenoside accumulation also increased with higher $CaCl_2$ concentrations; the greatest ginsenoside content (5.7 mg/g DW) was achieved at a 1.5 strength in the medium. Overall, ginseng cell growth and ginsenoside production required higher concentrations of KNO_3, $MgSO_4$, and $CaCl_2$ than those normally used in culture media. In contrast, however, at low concentration of NH_4NO_3 enhanced ginsenoside accumulation.

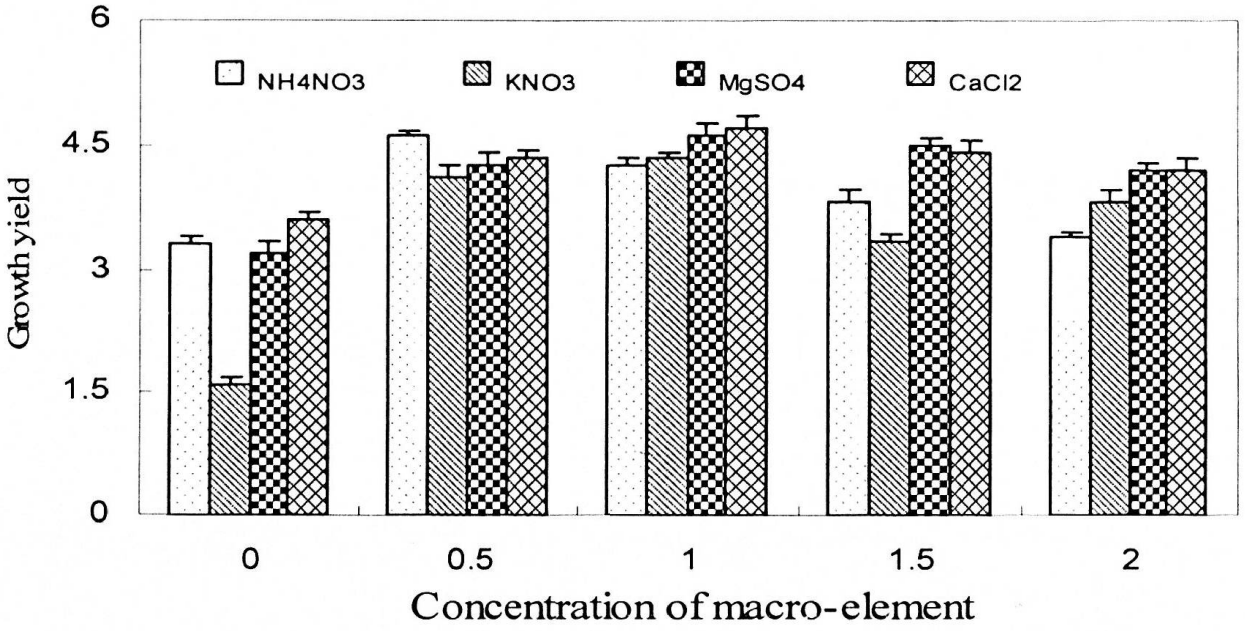

Fig. 1. Growth yield of ginseng cell was affected by concentration of macro elements. Values are the quotient of the root dry weight after 4 weeks of culture and the cell dry weight of the inoculum.

Depletion of nitrogen or phosphate is associated with limited cell growth and a concomitant increase in the level of secondary metabolism [10,11] demonstrated the effect of phosphate limitations on the accumulation of cinnamoyl putrescines in tobacco cultures, while [12] concluded that the lack of phosphate stimulated secondary metabolite biosynthesis. In cell suspension cultures of *P. ginseng* and *P. notoginseng*, a low initial concentration of phosphate in the medium sufficiently promoted both cell growth and ginsenoside accumulation [4,13], a result that is similar to our own. Likewise, [1] reported that NH_4^+ in the culture medium inhibited ginsenoside accumulation in *P. notoginseng* cell suspension cultures and that maximum ginsenoside production was obtained when NH_4^+ was absent. Therefore, optimizing macro element concentrations, especially for nitrogen and phosphate, in the culture media is a key step toward higher production of secondary metabolites in plant cell, tissue, or organ culture.

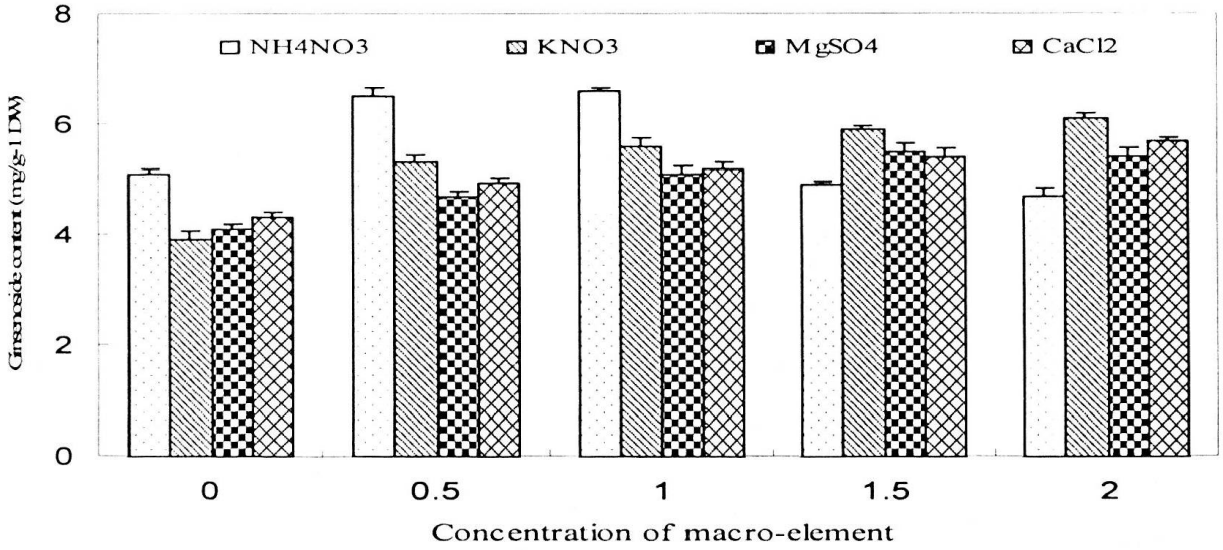

Fig. 2. Ginsenoside content in ginseng cell after 4 weeks of culture as affected by concentration of macro elements.

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References

[1] J.J. Zhong, S.J. Wang., Effects of nitrogen source on the production of ginseng saponin and polysaccharide by cell cultures of *P. quinquefolium*, *J. Pro. Biochem.* 33 (1998) 671.

[2] J.W. Gao, J.M. Lee., Effect of oxygen supply on the suspension culture of genetically modified tobacco cells. *Biotechnol. Prog.* 8 (1992) 285.

[3] T. Furuya, T. Yoshikawa, T. Ishii, K. Kajii., Studies on plant tissue cultures. Part 37. Effects of auxins on growth and saponin production in callus cultures of *P. ginseng*. *Planta Med.*, 47, 3 (1983) 183.

[4] S. Liu, J.J. Zhong., Simultaneous production of ginseng saponin and polysaccharide by suspension cultures of *P. ginseng*: Nitrogen effects, *J. Enzyme and Microbial Technology* 21 (1997) 518.

[5] S. Liu, J.J. Zhong., Phosphate effect on production of ginseng saponin and polysaccharide by cell suspension cultures of *P. ginseng* and *P. notoginseng*. *Process Biochem.* 33 (1998) 69.

[6] C.O. Akalezi, S. Liu, Q.S. Li, J.T. Yu, J.J. Zhong, Combined effects of initial sucrose concentration and inoculum size on cell growth and ginseng saponin production by suspension cultures of *P. ginseng*. *Process Biochem.* 34 (1998) 639.

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

[8] Thanh, L.T. Son, K.Y. Paek., Induction and proliferation of callus of Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv): Effects of plant growth regulators, *Journal of Science, Natural Sciences and Technology* 23, No.1S (2007) 167.

[9] N.T. Thanh, H.N. Murthy, Y.K. Woon, J.S. Cheol, E.J. Hahn, K.Y. Paek., Effect of oxygen supply on cell growth and saponin production in bioreactor cultures of *P. ginseng* C.A. Meyer, *J. Plant Physiology*, Germany, 163 (2006) 1337.

[10] M.M. Yeoman, C.L. Yeoman., Tansley Review No. 90, Manipulating secondary metabolism in cultured plant cells. *New Phytol.*, 134 (1996) 553.

[11] K.H. Knobloch, J. Berlin., Phosphate mediated regulation of cinnamoyl putrescine biosynthesis in cell suspension cultures of *Nicotiana tabacum*. *Planta Med.* 42 (1981) 167.

[12] S.H. Mantell, H. Smith., Cultural factors that influence secondary metabolite accumulation in plant cell and tissue cultures, In: S. H. Mantell, H. Smith, eds, *Plant Biotechnology,* Society for Experimental Biology Seminar Series 18, Cambridge University Press, Cambridge, 1983, pp. 75-108.

[13] Y.H. Zhang, J.J. Zhong, J.T. Yu., Effect of nitrogen sources on cell growth and production of ginseng saponin and polysaccharide in suspension cultures of P. notoginseng. *Biotechnol. Prog.* 12 (1996) 567.

Ảnh hưởng của các nguyên tố đa lượng đến sự tăng trưởng sinh khối và sự tích lũy sản phẩm ginsenoside trong nuôi cấy tế bào lỏng của Sâm Ngọc Linh (*Panax vietnamensis* Ha et Grushv.)

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Để sản xuất sinh khối và sản phẩm trao đổi chất thứ cấp ginsenoside, các thí nghiệm nuôi cấy tế bào lỏng của Sâm Ngọc Linh (*Panax vietnamensis* Ha et Grushv.) đã được tiến hành nghiên cứu ảnh hưởng của các nguyên tố đa lượng trong môi trường nuôi cấy. Sinh khối thu được lớn nhất khi bổ sung NH_4NO_3 với nồng độ 0.5, trong khi đó hàm lượng ginsenoside thu được (6.54 mg/g TL khô). Nồng độ 1.0 của KNO_3 tối ưu cho sự sinh trưởng của tế bào, còn sản phẩm ginsenoside thu được lớn nhất (6.1 mg/g DW) ở nồng độ 2. Nồng độ $MgSO_4$ thay đổi từ 0.5 - 2.0 nhìn chung ảnh hưởng không có y nghĩa đến sự sinh trưởng của tế bào và sự tổng sản phẩm ginsenoside (5.57 mg/g TL khô). Sinh khối tế bào và thành phần ginsenoside tăng trưởng đáng kể khi bổ sung $CaCl_2$ vào môi trường, với sự tích lũy sản phẩm ginsenoside thu được (5.75 mg/g TL khô) ở nồng độ 1.5.

Từ khóa: Nguyên tố đa lượng, nuôi cấy tế bào lỏng, bình tam giác, *Panax*.