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Cell suspension culture *Panax ginseng* C. A. Meyer: Role of plant growth regulators and medium composition on biomass and ginsenoside production

Nguyen Trung Thanh[1,*], Kee Yoeup Paek[2]

[1]*Faculty of Biology, Hanoi University of Science, VNU, 334 Nguyen Trai, Hanoi, Vietnam*
[2]*Department of Horticulture, Chungbuk National University, 361-736 Cheongju, Korea*

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Abstract. We established cell suspension culture on ginseng and some attempts have been made to increase ginsenoside yield of ginseng cell culture through manipulation various culture factors and process variable. The maximum biomass yields of cell suspension culture of ginseng was obtained in medium containing 2,4-D as compared to IBA or NAA. However, ginsenoside production was much higher in IBA or NAA containing medium and 7 mg/L IBA was determined to be optimal for cell growth (10.1 mg/L DW) and ginsenoside production (7.2 mg/g DW). Addition of cytokinin (BA and kinetin) did not affect cell growth but ginsenoside production was increased when the medium supplemented with 0.5 mg/L BA or 0.5 mg/L kinetin. Half and full strength MS medium were found to be equally suitable for both biomass as well as ginsenoside production. At various sucrose concentration investigated, 30 g/L sucrose enhanced biomass yield as well as ginsenoside production and further increase of sucrose concentration upto 70 g/L led to a decrease in ginsenoside accumulation and biomass production. The maximum growth and ginsenoside production was obtained for nitrogen concentration of 30 mM.

Keywords: Auxin, cytokinin, sucrose, MS strength and nitrogen.

1. Introduction

Ginseng (*Panax ginseng* C.A. Meyer) which belong to the Araliaceae, is one of the most variable oriental herbs. It has been used as a healing drug and health tonic in countries as Korea, China and Japan since ancient times [1]. Until now, ginseng has reported to contain saponins, antioxidants, peptides, polysaccharides, fatty acids, alcohol and vitamin [2]. The saponins known as ginsenosides are widely believed to the major bioactive compounds of ginseng.

Recently, the production of secondary metabolites using plant cells has been the subject of extended research [3]. The plant cell cultures has been considerd as a potential alternative for the efficient production of ginseng and its active ingradients, such as ginsenoside in terms of product quality. A

* Corresponding author. Tel.: 84-4-38582178.
E-mail: thanhntsh@gmail.com

number of reports of ginseng cell culture were published time to time [4-6]. However, there is still a need to the productivity of the tissue culture process in order to be economically competitive with field cultivation of ginseng. A number of physical and chemical factors that could influence secondary metabolite in plant cell cultures have been found [2,7]. Optimization of the hormone concentration and combination are often effective. For ginseng cell growth, 2,4 D is most commomly used in routine culture maintenance [6]. But use of this suspected carcinogen often create health and safety concerns. Alterations in the environmental factors such as nutrient levels, light, and temperature may also effective in increasing productivity.

In this paper, we established cell suspension culture of ginseng cell and some attempts have been made to increase biomass and ginsenoside yield of ginseng cell culture.

2. Materials and methods

Stock cell culture and culture condition

Stock suspension cells of *P. ginseng* were maintained in MS medium. The cultural conditions were done as described by [8].

Determination and analyses

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

Experimental design and data analysis

All experiment were repeated three times with 3 replicates. Data were subjected to Duncan's multiple range test using SAS program (Version 6.12, SAS Institute Inc., Cary, USA).

3. Results and discussion

To understand the culture characteristics of the suspended cells of ginseng in shake flask, the effect of plant growth regulators (2,4-D or IBA or NAA combination with BA or kinetin) on cell growth, ginsenoside production by cultured cells were focused on. The maximum biomass yield of cell suspension culture of ginseng was obtained in medium containing 2,4-D as compared to IBA or NAA. However, ginsenoside production was much higher in IBA or NAA containing medium (Table 1). Considering these results, 7 mg/L IBA was determined to be optimal for the cell growth (10.1 g/L DW) and ginsenoside (7.29 mg/g DW total ginsenoside) of ginseng suspension culture. Addition of cytokinins (BA and kinetin) did not affect cell growth but ginsenoside productivity (particularly Rb group) was increased when the medium supplemented with 0.5 mg/L BA or 0.5 mg/L kinetin (Table 2). These results obtained in this present study is quite interesting because almost all suspension culture of ginseng cells reported until now were claimed to require 2,4-D which is a potent herbicide and carcinogen and therefore unsuitable for pharmaceutical and food industries [10]. From this point of view, our system is apparently favourable for the process scale up for commercial ginsenoside production by ginseng cells without addition of the chemical 2,4-D.

Table 1. Effect of different auxin on cell growth and ginsenoside production

Auxin	Concentration (mg/L)	Fresh wt. (g/L)	Dry wt. (g/L)	Ginsenoside (mg/g DW)		
				Rg	Rb	Total
2,4-D	1	328 a[z]	11.9 a	1.81	2.35	4.16
IBA	1	144 d	7.5 d	2.16	3.05	5.21
	3	170 c	8.8 cd	2.09	3.49	5.58
	5	178 c	9.1 c	2.43	3.31	5.74
	7	216 b	10.1 b	2.61	4.69	7.29
	9	226 b	10.5 b	1.42	4.22	5.64
NAA	1	132 d	7.1 e	2.85	5.33	8.18
	3	134 d	7.3 de	3.28	5.58	8.76
	5	152 cd	7.8 d	2.61	4.83	7.44
	7	164 c	7.6 cd	2.16	4.08	6.24
	9	188 c	9.7 b	2.16	2.45	4.61

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

Table 2. Effect of different cytokinins (along with 7mg/L IBA) on cell growth and ginsenoside production

Cytokinins	Concentration (mg/L)	Fresh wt. (g/L)	Dry wt. (g/L)	Ginsenoside (mg/g DW)		
				Rg	Rb	Total
	0	192 b[z]	10.1 b	1.88	2.78	4.66
BA	0.1	230 a	11.2 ab	1.81	2.75	4.55
	0.5	252 a	11.5 a	1.75	5.33	7.08
	1.0	243 a	11.1 ab	1.79	3.53	5.32
Kinetin	0.1	225 ab	11.1 ab	1.16	4.62	5.78
	0.5	240 a	11.7 a	1.49	5.82	7.32
	1.0	242 a	11.4 a	1.56	3.59	5.15

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

Table 3 shows the effects of different strength of MS medium on biomass and ginsenoside production. Half and full strength MS medium were found to be equally suitable for both biomass as well as ginsenoside production. High salt strength (2.0) inhibited cell growth ginsenoside production. Such a phenomenon was also described in provious cultures of ginseng adventitious roots [11].

Table 3. Effect of different sucrose concentration on cell growth and ginsenoside production

Sucrose concentr. (g/L)	Fresh wt. (g/L)	Dry wt. (g/L)	Ginsenoside (mg/g DW)		
			Rg	Rb	Total
10	27 d[z]	2.9 d	0.32	0.66	0.98
30	180 a	10.8 a	2.17	4.39	6.56
50	98 b	8.4 b	1.07	2.25	3.32
70	52 c	5.7 c	0.09	1.58	1.67

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

At various sucrose concentrations investigated, 30 g/L sucrose enhanced biomass yield (180 g/L FW, and 10.8 g/L DW), and ginsenoside production (total ginsenoside production upto 6.56 mg/g DW). Further increase of sucrose concentration upto 70 g/L led to a decrease in ginsenoside accumulation and biomass production (Table 4). On the contrary of our results, several authors suggested that a relatively high sucrose level was benificial to secondary metabolite synthesis [12]. For example, Weselake et al. [13] reported that the triacylglycerol content of the cells of oil seed rape could be increase about 8-fold on a fresh weight basis when sucrose concentration in the growth medium was raise from 2 to 22% (w/v). Choi et al. (1994a, b) found that the optimal concentration of sucrose for cell growth was between 30 and 50 g/L and upto 70 g/L sucrose inhibited cell growth, while the ginsenoside content showed a steady increase with sucrose concentration of upto 60 g/L. Based on our results it can be concluded that high sucrose level and secondary metablite production is not a general phenomenon and depends on plant species.

Table 4. Effect of different strength of MS medium on cell growth and ginsenoside production

Sucrose concentr. (g/L)	Fresh wt. (g/L)	Dry wt. (g/L)	Ginsenoside (mg/g DW)		
			Rg	Rb	Total
10	225 a[z]	9.9 a	1.45	4.88	6.33
30	185 b	10.3 a	2.27	4.45	6.73
50	153 c	9.4 a	0.98	3.57	4.55
70	98 d	6.8 b	0.46	3.45	3.91

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

The effect of the initial nitrogen concentration in the medium for cell growth and metabolite production was studied in *P. ginseng* cell cultures. The initial nitrogen level was adjusted to 30, 60, 90 and 120 mM. The kinetics of growth (based on dry weight) in various cultures is shown in (Table 5). It is apparent that growth was inhibited at a high initial N concentration. The highest dry weight reached 11.6 g/L at an initial nitrogen concentration of 30 mM. The highest ginsenoside production was (7.46 mg/g DW) at initial medium nitrogen concentration of 30 mM after 25 days of culture.

Table 5. Effect of different nitrogen concentration on cell growth and ginsenoside production

nitrogen concent.(mM)	Fresh wt. (g/L)	Dry wt. (g/L)	Ginsenoside (mg/g DW)		
			Rg	Rb	Total
30	206 a[z]	11.6 a	2.85	4.62	7.47
60	182 b	10.8 a	2.17	4.48	6.65
90	112 c	8.1 b	0.65	4.11	4.76
120	76 d	6.1 d	0.23	4.32	4.56

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

In cell cultures of *P. quinquefolium*, [13] reported that the final dry cell weight was relatively low with the low nitrogen concentration. Maximum cell dry weight obtained (15 g/L) at a total initial nitrogen concentration of 40 mM and the cell growth was inhibited at a high initial nitrogen concentration of 80 mM. Similarly, the accumulation of total saponin and polysaccharide were also influenced by initial nitrogen concentration in the medium. The maximum production of ginseng saponin and polysaccharide obtained (1.5 g/L and 2.19 g/L) at the initial nitrogen concentration of 40 mM.[14]. In the simultaneous production of ginseng saponin and polysaccharide by suspension cultures of *P. ginseng*, [15] reported that production of ginseng saponin was related with the total nitrogen concentration. The result suggested that a low nitrogen concentration was beneficial for the stimulation of total saponin production.

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Nuôi cấy huyền phù tế bào Nhân sâm *Panax ginseng* C.A. Meyer: Ảnh hưởng của các chất kích thích sinh trưởng và một số thành phần trong môi trường đến sinh khối và sản phẩm ginsenoside

Nguyễn Trung Thành[1], Kee Yoeup Paek[2]

[1]*Khoa Sinh học, Trường Đại học Khoa học Tự nhiên, ĐHQGHN, 334 Nguyễn Trãi, Hà Nội, Việt Nam*
[2]*Khoa Cây trồng, Trường Đại học Quốc gia Chungbuk, 361-736 Cheongju, Hàn Quốc*

Tế bào Nhân sâm đã được nuôi cấy trong môi trường lỏng MS để sản xuất sinh khối và sản phẩm trao đổi chất ginsenoside. Đối với auxin, sinh khối thu được lớn nhất ở 2,4-D so sánh với IBA và NAA. Nhưng sản phẩm ginsenoside tích lũy cao ở IBA và NAA. Kết quả thu được là (7.2 mg/g trọng lượng khô, tổng số ginsenoside) và sinh khối là 10.1 g/L trọng lượng khô ở nồng độ 7 mg/L IBA. Còn đối với cytokinin khi nồng độ tăng từ 0.1 đến 1.0 mg/L (BA và kinetin) đã không ảnh hưởng đến sự sinh trưởng của tế bào, nhưng tăng có ý nghĩa đối với sản phẩm ginsenoside khi môi trường MS được bổ sung 0.5 mg/L IBA hoặc NAA. Nồng độ môi trường MS với 50 hoặc 100% là thích hợp cho sự tích lũy sinh khối tế bào và tổng hợp sản phẩm ginsenoside. Nồng độ đường 30 g/L là tối ưu cho sự sinh trưởng của tế bào và sự tổng hợp sản phẩm ginsenoside. Sinh khối tế bào và sản phẩm ginsenoside đã thu được lớn nhất ở nồng độ 30mM nitrogen.

Keywords: Auxin, cytokinin, sucrose, nồng độ môi trường MS.