0273-2289/98/7502–3—0261$10.00

Production of Ginseng Saponin and Polysaccharide by Cell Cultures of *Panax notoginseng* and *Panax ginseng*

Effects of Plant Growth Regulators

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ABSTRACT

The effects of various combinations of the two kinds of phytohormones, auxin and cytokinin, on cell growth and production of ginseng saponin and polysaccharide were investigated in suspension cultures of *Panax notoginseng*. It was found that a high concentration of kinetin (KT) (7 mg/L) seriously inhibited cell growth, but that of benzyl adenine (BA) did not. Under 0.7 mg/L of cytokinin (i.e., KT and BA), 2,4-dichlorophenoxy acetic acid (2,4-D) at 0.2 mg/L was better for the cell cultures than that at 2 or 20 mg/L; and for both naphthalene acetic acid (NAA) and indole acetic acid (IAA), 20 mg/L was their best level for the cell cultures. The highest cell concentration of 11.9 g/L (by dry wt) was obtained with the combination of 0.2 mg/L of 2,4-D and 0.7 mg/L of BA. The highest saponin content of 13.9% was achieved under 2.0 mg/L IAA and 0.07 mg/L KT; its highest production, i.e., 1.13 g/L, was obtained at 0.2 mg/L of 2,4-D and 0.7 mg/L of KT. Under 20 mg/L NAA and 0.7 mg/L KT, the highest polysaccharide content and production were reached, i.e., 16.4% and 1.86 g/L, respectively. In this work, the effects of phytohormones on *P. ginseng* cell cultures were also studied. A high saponin production of 1.78 g/L was observed at 10 mg/L of indole butyric acid and 0.1 mg/L of BA, and the highest production of polysaccharide (1.95 g/L) was reached with the combination of 10 mg/L NAA and 0.1 mg/L KT.

Index Entries: Plant growth regulator (phytohormone); *Panax notoginseng; Panax ginseng;* plant cell suspension culture; ginseng saponin; ginseng polysaccharide; useful metabolite production.

INTRODUCTION

Panax notoginseng and *Panax ginseng,* both of which belong to the Araliaceae family, are two famous traditional Chinese medicinal plants. Saponin is their main useful secondary metabolite, which can regulate the metabolism and immune function of the human body and has protective effects against many toxicant-induced hepatotoxicities in humans and experimental animals *(1).* Ginseng polysaccharide (a primary metabolite, mostly pectin) has recently been reported to possess antitumor and immunological activities *(2).* Compared with numerous publications on saponin production (e.g., *3,4*), reports on ginseng polysaccharide formation are very few, except those regarding chemical and medicinal studies *(2,5).* At present, the supplies of *P. notoginseng* and *P. ginseng* from wild sources are running short. Production of these two useful metabolites by plant tissue and cell cultures are considered to be a long-term, cost-effective approach to meeting the popular market demand.

Plant growth regulators (phytohormones) are compounds that can regulate the growth, differentiation, and metabolism of plant cells *(6–11).* Auxin and cytokinin are the two types of most frequently used phytohormones. Both the ratio of auxin to cytokinin and their individual levels in medium are very important in the regulation of cell metabolism. It is well known that different cell lines have big differences in physiological and metabolic responses to phytohormones *(6,8,11).* Thus, it is necessary to investigate the effects of phytohormones for a specific case in detail. In addition, the author is unaware of any reports until now, regarding the effects of phytohormones on ginseng polysaccharide formation by cultured cells. In this article, the effects of various combinations of auxin and cytokinin on *P. notoginseng* and *P. ginseng* suspension cells were investigated. The results obtained here are considered to be beneficial to large-scale efficient production of both saponin and polysaccharide by suspension cultures of ginseng cells.

MATERIALS AND METHODS

Plant Cells, Medium, and Culture Conditions

Suspended cells of *P. notoginseng* and *P. ginseng* were maintained in Murashige and Skoog (MS) medium, supplemented with 2 mg/L of 2,4-dichlorophenoxyacetic acid (2,4-D), 0.7 mg/L kinetin (KT), and 30 g/L

sucrose (for *P. notoginseng*) *(12)*, or with 1 mg/L 2,4-D, 0.1 mg/L KT, and 30 g/L sucrose (for *P. ginseng*) *(13)*, respectively. The cells were subcultured every 14 (*P. notoginseng*) or 25 d (*P. ginseng*) in 250-mL Erlenmeyer flasks containing 50 mL medium, on a rotary shaker (at 110 rpm) in darkness at culture temperature of 25°C. The medium pH was adjusted to 5.8 before sterilization. The inoculation size was 2 and 2.5 g fresh cells per flask for *P. notoginseng* and *P. ginseng* cultures, respectively.

Determination of Dry Cell Weight, Ginseng Saponin, and Polysaccharide

The cells were harvested by filtration under vacuum, and washed with a large amount of distilled water, then dried at 50°C for sufficient time, and the dry cell wt was recorded when it became a constant value.

For measurements of the metabolites that are stored intracellularly, a 100-mg aliquot of the ground dry cell powder was soaked in 5 mL *n*-butanol saturated by water at 4°C overnight, then treated for 30 min by ultrasound. After centrifugation, the supernatant was assayed for saponin, and the cell residual was dried for ginseng polysaccharide analysis. Ginseng saponin was determined by TLC-colorimetric method, as described previously *(12)*. The authentic ginsenoside saponin was purchased from the Drugs and Biological Products Identification Institute of China (Beijing). After being hydrolyzed by 10% sulfuric acid in boiling water for 4 h, ginseng polysaccharide was assayed by the carbazole–sulfuric acid method, with galacturonic acid (Sigma, St. Louis, MO) as the standard *(13)*.

RESULTS AND DISCUSSION

Effect of Phytohormones on Cell Growth of *P. notoginseng*

The effects of cytokinin (kinetin and benzyl adenine) concentration on cell growth were investigated at 2 mg/L auxin, i.e., 2,4-D, naphthalene acetic acid (NAA), and indole acetic acid (IAA), respectively. As shown in Fig. 1, a relatively high concentration (7 mg/L) of KT seriously inhibited the cell growth, and the cell did not grow under that condition. In the case of benzyl adenine (BA), the cells grew quite well, even at a high concentration of 7 mg/L. At 0.07 and 0.7 mg/L of KT or BA, it seems the dry cell wt made no obvious difference.

The effects of auxin (2,4-D, NAA, and IAA) level in medium on the cell growth were also studied at 0.7 mg/L cytokinin. For NAA and IAA, the dry cell wt was increased with an increase of their concentration in medium, and high dry cell wts were reached at their level of 20 mg/L. For 2,4-D, the cell growth was inhibited at its higher medium level; and the

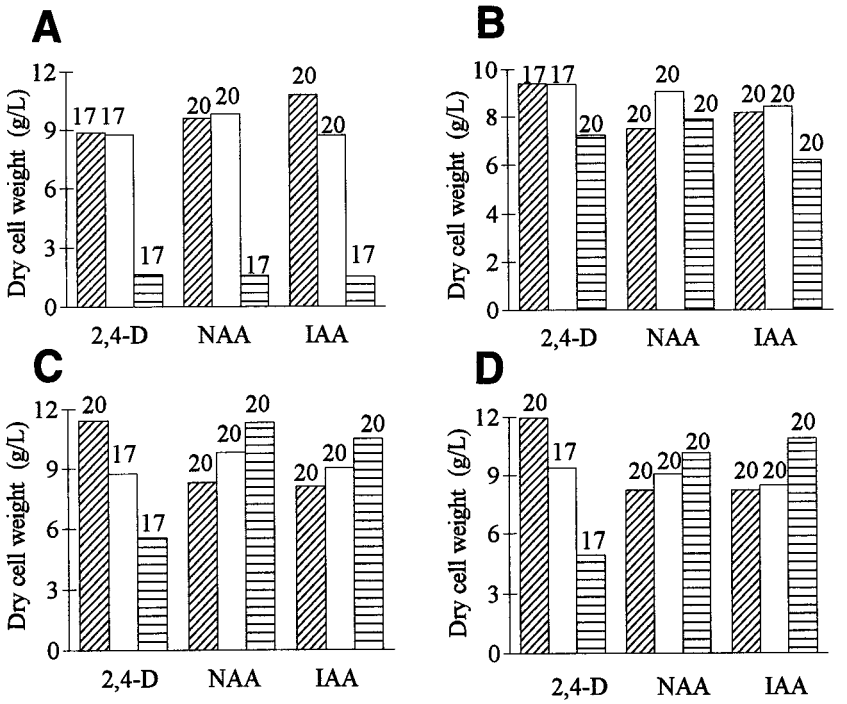

Fig. 1. Effects of auxin and cytokinin on cell growth of *P. notoginseng*. **(A)** KT = 0.07 (oblique line bar), 0.7 (white bar), and 7 mg/L (horizontal line bar) at 2 mg/L of auxin; **(B)** BA = 0.07 (oblique line bar), 0.7 (white bar), and 7 mg/L (horizontal line bar) at 2 mg/L of auxin; **(C)** auxin = 0.2 (oblique line bar), 2 (white bar), and 20 mg/L (horizontal line bar) at KT = 0.7 mg/L; **(D)** auxin = 0.2 (oblique line bar), 2 (white bar), and 20 mg/L (horizontal line bar) at BA = 0.7 mg/L. The numbers at the top of bars indicated the cultivation time (d) for each case.

phenomenon was different from other work, in which high auxin levels are often good for cell growth (*7*). The highest dry cell wt of 11.9 g/L was obtained with the combination of 0.2 mg/L of 2,4-D and 0.7 mg/L of BA after 20 d of cultivation.

Effect of Phytohormones on Saponin Biosynthesis by *P. notoginseng* Cells

Under 2 mg/L auxin (2,4-D, NAA, and IAA), both the saponin content (Fig. 2) and production (calculated by multiplying its content by the cell mass [direct data are not shown] which is the same as below) were decreased with an increase of KT concentration. When the auxin was 2,4-D or IAA, the saponin content and production were also decreased with an increase of BA level. However, when the auxin was NAA, there was little difference among the three values of saponin content at various concen-

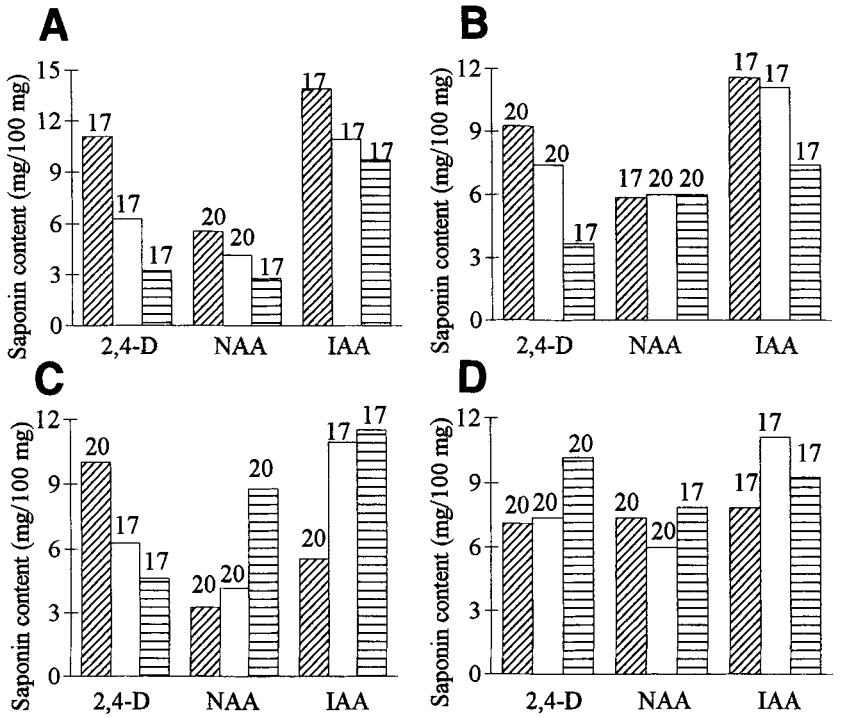

Fig. 2. Effects of auxin and cytokinin on saponin content of *P. notoginseng* cells. The cultivation conditions of Fig. 2A–D were the same as those in Fig. 1.

trations (i.e., 0.07, 0.7, and 7 mg/L) of BA; the saponin production was higher at 0.7 mg/L of BA (0.55 g/L) than that at 0.07 (0.39 g/L) or 7 mg/L (0.48 g/L) of BA, which was chiefly because of higher cell concentration under the former case.

The effects of auxin (2,4-D, NAA, and IAA) concentration on the saponin accumulation by *P. notoginseng* cells were also studied at 0.7 mg/L of cytokinin (KT and BA). Under 0.7 mg/L KT, both the saponin content and production were decreased with an increase of 2,4-D concentration; however, they were increased with an increase of NAA or IAA concentration. At 0.7 mg/L BA, the saponin content was increased with the increase of 2,4-D concentration, but the total production decreased; when the auxin was NAA, a low content and production (0.55 g/L) of saponin was observed at 2 mg/L NAA, and a high saponin content and production (0.73 g/L) was found in the case of 20 mg/L NAA; for IAA, a high saponin content appeared at its concentration of 2 mg/L; its high production (0.82 g/L) was achieved in the case of 20 mg/L IAA. It was understood that the highest saponin content of 13.9% was obtained under 2 mg/L IAA and 0.07 mg/L KT, which is much higher than that of native or classically cultured *Panax* spp. (ca. 3–10% by dry wt) *(14)*; and its highest total produc-

tion of 1.13 g/L was reached at 0.2 mg/L 2,4-D and 0.7 mg/L KT. In other plant cell cultures, as described by Dornenburg and Knorr (*7*), high auxin levels are often deleterious to secondary metabolite accumulation.

Effect of Phytohormones on Polysaccharide Accumulation by *P. notoginseng* Cells

For the polysaccharide content (Fig. 3), there was not much difference under various cytokinin concentrations (0.07, 0.7, and 7 mg/L of KT or BA) under 2 mg/L auxin (i.e., 2,4-D, NAA, and IAA). However, a high cytokinin concentration (i.e., 7 mg/L) was unfavorable for polysaccharide production (data can be calculated from Figs. 1 and 3), which was caused by significantly lower cell mass under the conditions discussed above.

Under 0.7 mg/L of KT or BA, the general tendency for the changes of polysaccharide content and production was as follows: They were decreased with an increase of 2,4-D level (0.2–20 mg/L), but increased with an increase of NAA or IAA concentration (0.2–20 mg/L). The highest content (16.4 mg/100 mg dry weight) and production (1.86 g/L) of polysaccharide were obtained at 20 mg/L NAA under 0.7 mg/L KT.

Effect of Phytohormones on *P. ginseng* Cell Cultures

In this work, the effects of plant growth regulators on the cell growth and metabolite production by suspension cultures of *P. ginseng* cells were also examined. Preliminary experiments indicated that perturbation of auxin level in medium was more important to the cell cultures than that of cytokinin (data not shown). Thus, here an auxin (2,4-D, NAA, or indole butyric acid [IBA]) level was altered from 0.1 to 10.0 mg/L; cytokinin (KT or BA) concentration was set at 0.1 mg/L as that in subcultures.

As the results summarized in Table 1 show, it was found that a relatively lower concentration (i.e., 0.1 mg/L) of NAA and IBA was unfavorable for the cell growth and production of ginseng saponin and polysaccharide in the cell cultures. With an increase of NAA concentration from 0.1 to 10 mg/L, both the dry cell wt and production of saponin and polysaccharide were increased. For IBA as the cytokinin, the highest saponin and polysaccharide production was found at the phytohormone combination of 1 mg/L IBA and 0.1 mg/L KT, as well as at 10 mg/L IBA and 0.1 mg/L KT. In the case of 2,4-D, the phytohormone combination of 1 mg/L 2,4-D and 0.1 mg/L cytokinin (KT or BA) was found to be optimal for saponin and polysaccharide production in the suspension cultures. The highest saponin production of 1.78 g/L was obtained at 10 mg/L IBA and 0.1 mg/L BA, and the highest polysaccharide production of 1.95 g/L was achieved at 10 mg/L NAA and 0.1 mg/L KT.

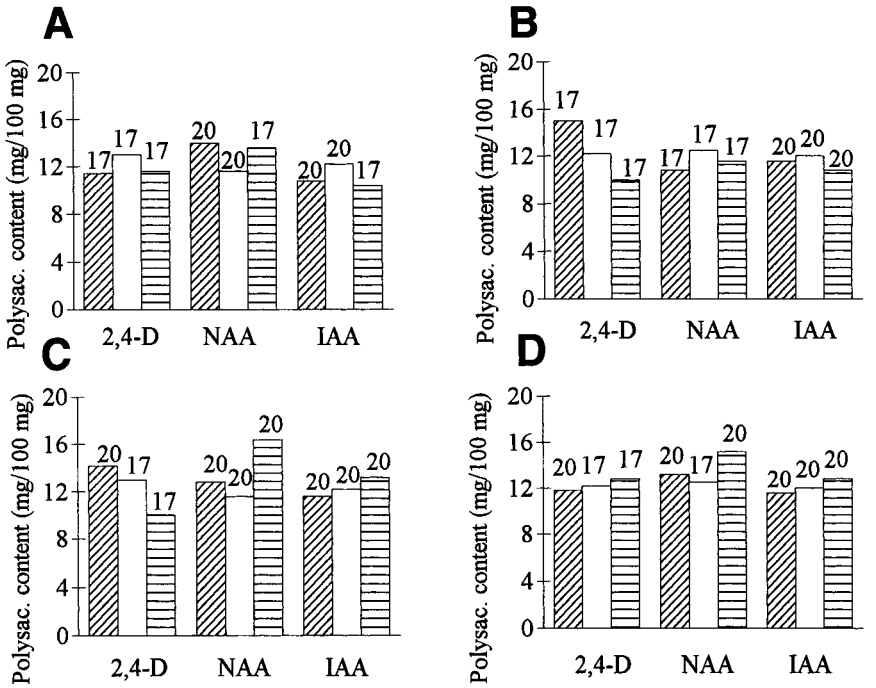

Fig. 3. Effects of auxin and cytokinin on polysaccharide content of *P. notoginseng* cells. The cultivation conditions of Fig. 3A–D were the same as those in Fig. 1.

Table 1
Effects of Auxin and Cytokinin on Dry Cell Weight, Saponin Production, and Polysaccharide Production in Suspension Cultures of *P. ginseng* (Unit g/L)

	2,4-D (mg/L)			NAA (mg/L)			IBA (mg/L)		
	0.1	1	10	0.1	1	10	0.1	1	10
Dry cell weight									
KT 0.1 mg/L	5.88[a]	9.5	6.88	5.26[a]	9.38	12.18	7.0[a]	10.54	9.74
BA 0.1 mg/L	7.56[a]	9.6	6.12	4.92[a]	9.04[a]	11.2	8.4[a]	9.4	12.02
Saponin production									
KT 0.1 mg/L	0.70[a]	1.05	0.89	0.46[a]	1.00[a]	1.69	0.81[a]	1.37[a]	1.07
BA 0.1 mg/L	0.81	1.16	0.80	0.59	1.13	1.46[a]	0.74[a]	0.87[a]	1.78
Polysaccharide production									
KT 0.1 mg/L	1.13[a]	1.86	1.27	0.97	1.39	1.95	0.95	1.43	1.33[a]
BA 0.1 mg/L	1.09	1.77	0.94	0.77	1.44	1.43	1.24[a]	1.64[a]	1.01

[a] The cultivation time was 35 d; it was 33 d for the other cases.

ACKNOWLEDGMENTS

The author thanks the Ministry of Education (formerly: State Education Commission) of China for partial financial support and Q.-M. Xiong for his excellent assistance during the experiments.

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