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EFFECTING OF SUCROSE CONCENTRATIONS AND INOCULUM DENSITY ON ADVENTITIUOS ROOT GROWTH IN CELL SUSPENSION CULTURE OF Panax vietnamensis AND INITIALLY GROWTH IN A BIOREACTOR

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Abstract

It has been demonstrated that the difference of initial sucrose concentrations (0, 3, 5, 7, 9 %) effected on adventitious root growth in cell suspension culture of Panax vietnamensis. The maximum adventitious root growth was obtained in a concentration of 5% sucrose. The density of initial explants also effected to the course of growth. The concentration of 30 mM.2% inoculum density was optimal, and further incresase of inoculum density led to a specific decrease of the rate root growth. Adventitious root of Panax vietnamensis developed well in 5 - liter bioreactors containing 2 liters of SH medium (working medium) supplemented with 5 mg l-1 IBA, and 0,02 mg/l BA. The growth rate of root was 5.6 folds after 45 days in this condition.

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1. Introduction

This is a specific variety of Vietnamese ginseng, called Sâm Ngoc Linh (Panax vietnamensis Ha et Grushv). It was found in high mountains of Vietnam central at the height from 1200 - 2100 metres above sea level. The southern central area such as Ngoc Lum Heo and Ngoc Am (Quang Nam) and highlands such as Ngoc Linh (Dakto), Tra Mi are key distributions of Panax. It is considered a highly valuable medicine used for the treatments of various diseases, exciting nervous systems, stimulating memories and immune systems, enhancing vilality and hormone, supporting anti-oxidant and anti-depression, regulating heart blood circulation, reducing blood cholesterol content and body inflammation. As a result, Ngoc Linh ginseng has been exploring dramatically and it becomes scarce in nature and highly extintive if no actively protective treatments have been desired.

In fact, in recent years, various researches on mass propagation in nature conditions have been conducted. However, the pharmatical market demand of Ngoc Linh ginseng has been so big that powder and axtracts from the mountain collection has not been enough due to its extreme slow growth. That is the result why more reseaches have been recently attracted to speed up Ngoc Linh ginseng procdution. In addition to cell tissue culture in vitro, cell suspension culture have been made. The methods were also applied to other varieties of ginseng such as Panax ginseng (Yu, 2000 v Thanh et al., 2005), Panax notonginseng (Zhang et al., 1995). In reality, researches on adventitious root growth of sm Ng?c Linh have been very few and need being continued in order to find out a potential alternative to a huge commercial makets worldwide of gingseng.

2. Materials and Methods

1. Materials Adventitious roots of Ngoc Linh ginseng from callus at 0.5 - 1cm.

2. Culture condition: The samples were cultivated in darkness at room temperature 2520C.

3. Methods: - Generating and quick propagation of aventitious roots SH medium (Schenk and Hildebrandt) supplemented 5mg/l IBA and 8% agar (data report is not preferred) has been used as induction medium for generating and quick propagation of Ngoc Linh aventitious roots from callus.

- The effect of sucrose concentrations to culture medium

Aventitious root lines at different density rates 2, 4, 6, 8 % were mainted SH medium (Schenk and Hildebrandt) supplemented 5mg/l IBA with various sucrose concentrations. The root growth rate was checked after 45 days of cultivation.

- Cultivation in bioreactors Aventitious roots were cut into small sections

of 1-1.5cm and then cultivated in 5 - liter bioreactors containing 2 liters of SH medium (working medium) supplemented 5 mg l-1 IBA, and 5% sucrose concentration. Root lines were mantained in darkness at room temperature 25 20C. Fresh weight and dry weight were done after 45 days of cultivation.

- The effect of cultivated density Aventitious roots were cultivated in 100 ml conical flasks with SH medium (working medium) supplemented 5 mg l-1 IBA, and 50 g sucrose.

3. Results and Discussion

3.1 Results

After 45 days of culture, the final fresh weight and dry weight were increased considerably with the increase of initial sucrose concentrations from 0 to 5%. Higher sucrose concentrations (7-9%) seemed to repress aventitious root growth (Table 1). In fact, culture condition at 5% sucrose concentration is optimized for the aventitious root growth of Ngoc Linh gingseng (3378 mg fresh weight and 344 mg dry weight).

Table 1: The effect of sucrose concentrations to the root growth of Ngoc Linh gingseng (Panax vietnamensis). The data collected after 45 days of culture in 100 ml conical flasks with a 20 ml working volume of SH medium.

Sucrose concentrations %	**Fresh weight (mg)**	Dry weight (mg)
0	1034^{d}	49^{c}
3	2514^{c}	278^{b}
5	3378^{a}	344^{a}
7	2859^{b}	336^{a}
9	2309^{c}	326^{a}
CV%	5,13	4,68
$LCD_{0,05}$	225,90	22,74

The effects of cultured density Different culture densities increasing from 2, 4, 6, 8 % were investigated. After 45 days, the results indicated that culture at 2% resulted in highest growth rate; and other increasings of cultured densities were observed in relatively decreasing growth rate. Truly, the optimum culture density for aventitious root growth of Ngoc Linh gingseng was at 2%, 5.3 times higher than initial samples (Table 2).

Table 2: The effect of different culture densities to the growth of adventitious roots of Ngoc Linh gingseng (Panax vietnamensis). The data was collected

after 45 days of culture in 100 ml conical flasks with a working volume of 20 ml SH medium.

Densities%(w/v)	Fresh weight(mg)	Dry weight(mg))	Growth rate
2	1732^{d}	190^{d}	4.3^{a}
4	3010^{c}	298^{c}	3.8^{b}
6	3740^{b}	349^{b}	3.1^{c}
8	4198^{a}	395^{a}	2.6^{d}

The culture in bioreactors Aventitious roots started increasing in length and inducing callus after 10 days of culture. New adventitious roots changed in color, relatively light yellow, after 21 days. And after 45days, fresh weight of 45 g was obtained, making an increase of 5.6 times higher than initial samples.

3.2 Discussion

3.2.1 Effect of sucrose concentrations Sucrose has been used as optimim carbon source to plant cell and tissue cultures (Giaquinta, 1980; Strickland et al. 1987). Sucrose supplies carbons to the process of plant growth as well as energy to cell life. Similarly, sucrose also provides essential carbons to the formation of bi-products from metabolism. In a sufficient medium with essential nutritions, an increase of sucrose concentraton potentially affected to an increase of biomass and the ratio of the metabolism progress for bi-products (Rokem v Goldberg, 1985).

Within this experiment, increasing of innital sucrose concentrations from 0 to 5% was studied and shown that after 45 days of culture fresh weight and dry weight were increased accordingly (Table 1).

It gives the same result as culture of Panax ginseng (Choi et al., 1994a, 1994b; Akalezi, 1999). On the other hand, further increase of sucrose concentrations from 7 to 9% led to a heavily negative effect to root growth rate. It can be said that penetration potential of cell membrance made a certain negative effect to root growth and to secondary metabolite cell production (Narayan, 2002). When studying Panax notoginseng, Zhang (1995), it was also said that higher sucrose cncentrations (above 5%) inhibited rooth growth significantly.

3.2.2 The effect of different culture densities The initial culture density showed a considerable effect to the developing of cell suspension. A lower initial culture density lessened cell division. It is likely that the substance from cells of cell suspension was developing that stimulated cell growth. In fact, the initial cell density plays a specific role, namely 'an adapter', promoted the growth of cell suspension (Henshaw et al., 1966; Torees, 1989). Consequently, culture

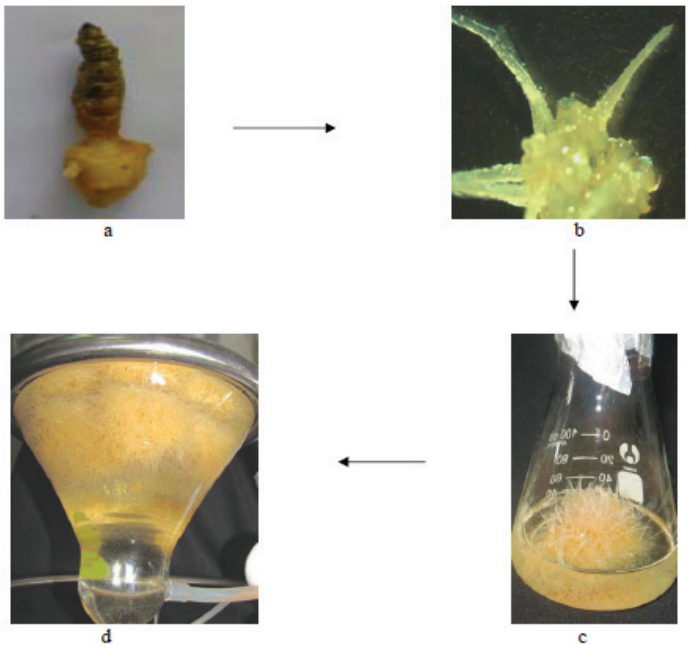
density is tremendously important in the process of sub-cultures of plant cells and similariry affected to the progress of bi-products (Rokem v Goldberg, 1985; Liang et al., 1991). On the contrary, a relatively high initial culture density will lessen the growth of cell suspension due to competition between nutritions and oxygen source from culture medium (Henshaw et al, 1966; Torees, 1989). Based on the experiment, it can be concluded that optimal density to root growth is made by 2%; and it is apparent that the growth steadily decreases at higer culture densities. The result is similar to the report of the above named authors.

3.2.3 Culture of Panax vietnamensis adventitious roots in bioreactors

Traditional plant cell culture methods for mass production of gingseng still encounters various constraints because of uncapable adjustment of physical and chemical factors in the cultured flasks and of expensively manual labour cost. Therefore, application of culture in bioreactors is getting more beneficial in in-vitro culture, is much easier to respectively adjust to optimal conditions for the growth of adventitious roots (increasing of gas exchange and of output of metabolic production process), is able to produce an adequate yield throughout the year and makes a lower production cost (Levin et al., 1988; Preil et al., 1988, 1991; Sharma, 1992; Christie et al., 1995; Honda et al., 2001; Paek et al., 2001). In principle, culture of gingseng in bioreactor chains, essential nutritions can be regularly provided and supplemented to adventitious roots especially when the culture medium becomes exhausted and unables to supply adequate nutrition to the samples. For this reason, culture in bioreactors is beneficial to better growth of samples (Yao v Zhong, 1999). In addition, culture in bioreactors, due to a continual gas supplement source, adventitious roots are able to get relatively higher oxygen than that from a traditional cell culture. As a result, root systems grew up vigorously. Suplement of oxygen source affected to the growth and secondary metabolic synthesis in various plant cultures including Catharanthus roseus (Leckie et al., 1991) v Perilla frutescens (Zhong et al., 1993).

Cell cultures in liquid medium Thalictrum minus to produce berberine, cells that synthesized berberine obtained oxygen content two times higher than that did not (Kobayashi et al., 1991). Cultures in liquid medium and continual supplement of gas provide an adequate oxygen content for the growth and secondary metabolic synthesis.

The experiment showed that after 10 days of culture, adventitious roots started developing in size and inducing callus, and that after 21 days of culture, new adventitious roots became light yellow. After 45 days of culture, fresh weight of adventitious roots were ontained by 45 g and growth rate is 5.6 times higher than the samples. After that, such adventitious got older and seemed to change in colour, getting darker. Then, subculture needed to be done respectively.



Picture 1: The process of biomass production of roots of Ngoc Linh gingseng.

a) Fresh mountain gingseng roots collected from Ngoc Linh Mountain; b) Adventitous Ngoc Linh gingseng roots developed from callus; c) Roots cultured in 100 ml conical flasks; d) Roots cultured in bioreactors.

4. Conclussion

Adventitous roots of Ngoc Linh P. Vietnamesis cultured in liquid medium after 45 days, our results are as follows:

- The optimal concentration of sucrose for adventitious root growth was by 5%;

- The optimal density for adventitious root growth was by 2% and other concentrated densities inhibited adventitious root growth.

- After 45 days of culture, adventitous roots of Ngoc Linh gingseng cultured in 5-liter bioreactors with a working volume of 2 liter SH medium supplemented 5mg/l IBA developed 5.6 times higher than innital samples.

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