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Saponin Production in Tissue Culture of Ginseng (*Panax ginseng* C.A. Meyer)

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Abstract□ Ginseng root explants and calli were cultured on modified Murashige and Skoog's media supplemented with different concentrations of organic or inorganic compounds and plant growth requlators to clarify the effects of chemical compositon and plant growth regulators in the medium on the growth of ginseng calli and the production of ginseng saponin. For optimum growth of ginseng calli, the concentrations of 2,4-D and sucrose were in the range of 1 to 5 mg/*l* and 1 to 3%, respectively. And it was clarified that sucrose, nitrogen, phosphate, calcium, magnesium, plant growth regulators and their concentrations influcenced the relative biosynthesis of saponin in tissue cultures of *Panax ginseng*.

Keywords□ *Panax ginseng* C.A. Meyer, tissue culture, saponin production, 2,4-D.

Introduction

Ginseng is a perennial medicinal plant belonging to Araliaceae family and *Panax* genus. Ginseng seedlings grown on the nursery bed for about 17 months are transplanted during late in March and early in April in our country. 4- or 6-year old roots are harvested and cultivation of the ginseng plant in the field requires 5 to 7 years. As the ginseng cultivation through the conventional methods is carried out in the field, it is subjected to the climatic condition. And ginseng cultivation works also require large facilities and manpower besides needing unusually long duration of years. Therefore, the production of one of the active components, ginseng saponin, by *in vitro* grown culture of *Panax ginseng* has been studied by a number of investigators.[11-15,18]

Callus and cell suspension cultures have been established from somatic tissues of *Panax ginseng* C.A. Meyer. Tissues amenable to such culture include root,[2-10,17,18] stem,[2,7] cotyledon,[7] hypocotyl and epicotyl.[7] Butenko *et al.*[2] first found conditions for sterilizing them, and determined the composition of nutrient medium, such as inorganic salts, carbohydrates, and physiologically active compounds, which fulfilled the requirements of ginseng root for growth on solid and liquid media in darkness and in light. In general, the growth of ginseng roots on solid agar medium is rapid. From a piece of tissue weighing 100-120 mg, one obtains 1-2 g of tissue after 1 month of culture. The productivity of ginseng root tissue in culture is considerably higher than that of cultivated plants. Furuya *et al.*[11-15] isolated the sapogenin, panaxatriol, from *Panax ginseng* callus and in 1973 isolated panaxadiol and oleanolic acid from *Panax ginseng* callus cultures. It is interesting that the kind and amount of saponins in ginseng callus are about the same as those in the ginseng root.

Therefore, this study was undertaken to establish ginseng tissue culture for the production of useful metabolite, saponin.

Materials and Methods

Induction of callus

Six-year old ginseng roots were surface-steri-

lized for 20 minutes in 2% sodium hypochlorite solution and rinsed 4 times with sterile distilled water. For the callus induction, the segments of root with cambial cells were cultured on the basal medium with 1 mg/*l* kinetin and 3 mg/*l* 2,4-D in the dark at 25 °C for 40 days. The basal medium used was a modified formulation from Murashige and Skoog and hereafter designated as MS medium. The modifications were: 0.5 mg/*l* nicotinic acid, 0.5 mg/*l* pyridoxine HCl, 0.1 mg/*l* thiamine HCl, 100 mg/*l* myo-inositol, 2 g/*l* glycine, 30 g/*l* sucrose, and 9 g/*l* agar. The pH was adjusted to 5.8 before autoclaving. To clarify the effect of individual constituents on the growth of callus and the production of saponin, ammonium nitrate and potassium nitrate as nitrogen sources, potassium dihydrogen phosphate as phosphate source, magnesium sulfate as magnesium source, calcium chloride as calcium source, 2,4-D and kinetin as growth regulators, and sucrose as carbon source were tested in combination and in different concentrations. On 40 days after culture, the average fresh weight of callus in 100 m*l* Erlenmyer flasks was determined. The callus harvested was dried in drying oven at 60 °C.

Determination of saponin

Saponin contents in dried callus were determined by the method of Ando *et al.*[1]. The last butanol layer was evaporated *in vacuo* to dryness until no butanol smell was detectable. The residue was weighed as crude saponin.

Results and Discussion

Formation and growth of callus

Ginseng calli were usually obtained by culturing explants on a solid medium containing a high concentration of salts, organic constituents and high auxin. About 15 days after culture, ginseng calli were visible arising at the injured cambial cells of the cut surface of explant. Regardless of the parts they arose, calli were formed more profusely on the media containing 2,4-D. The tissues were soft and friable and comprised a wide variety of cell shapes and sizes. In general, a temperature of 25 to 27 °C is employed for *in vitro* culture. However, the ginseng callus grew vigorously and fast at 20 °C, while at 15 and 25 °C it grew slowly.

Plant growth regulators, in intact plant, act to regulate and coordinate processes which lead to normal development. Growth, as well as differentiation of tissue and cells is affected by plant growth regulators. The addition of plant growth regulators to tissue culture medium is necessary for ginseng callus culture.[7,13] The commonly used auxins are 2,4-dichlorophenoxyacetic acid (2,4-D), naphthaleneacetic acid (NAA), and indole-3-acetic acid (IAA). In *Panax ginseng* C.A. Meyer, 2,4-D is the most effective auxin in both induction and growth of callus.

In order to determine the optimum levels of 2,4-D for the induction of callus, the segments of root were inoculated on the Murashige and Skoog's basic medium supplemented with different concentrations of 2,4-D. The concentration of 2,4-D for optimum growth of ginseng callus was in the range of 1 to 5 mg/*l* (Fig. 1). On the other hand, cytokinins such as kinetin, BA and 2iP strongly suppressed proliferation of callus.

The addition of an organic carbon source, such as sucrose, to ginseng tissue culture media is absolutely necessary for all tissues. Therefore, the requirement of sucrose for the growth of callus was tested in the presence of different concentrations of

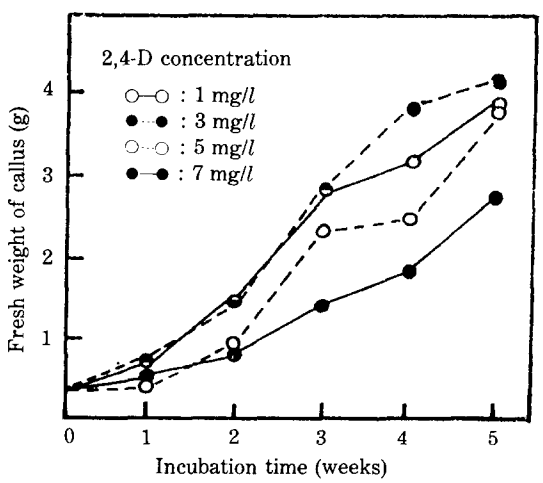

Fig. 1. Effect of 2,4-D on the growth of ginseng callus

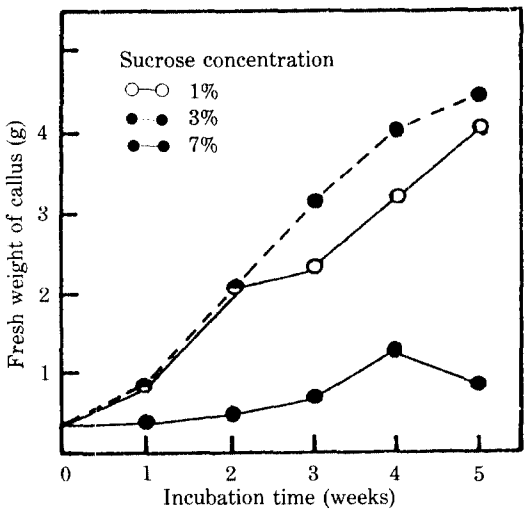

Fig. 2. Effect of sucrose on the growth of ginseng callus.

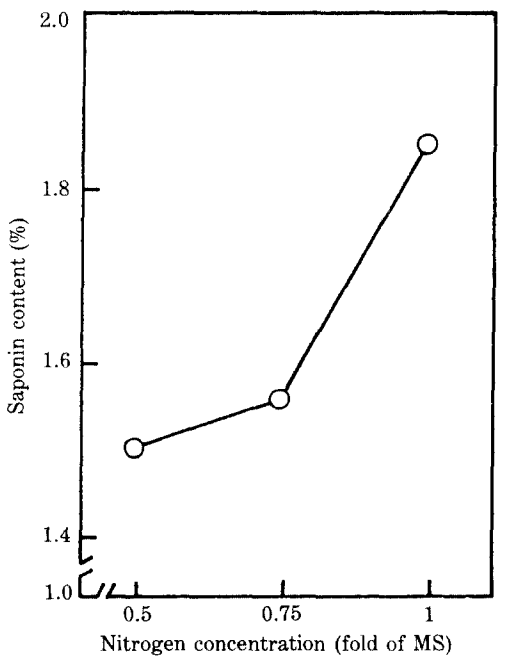

Fig. 3. Effect of nitrogen on the saponin content of ginseng callus.

sucrose. Fig. 2 shows the fresh weights of calli cultured on the media supplemented with different concentrations of sucrose. The concentration of sucrose for optimum growth of ginseng callus was in the range of 1 to 3%. Whereas higher concentration, 7% sucrose, caused growth retardation, the dry weights were greater than those where the concentration of sugar was lower.

Production of saponin from ginseng callus cultures

During approximately two decades of study it has been demonstrated that plant tissue culture can produce many useful metabolites; that cell strains can be selected for the high production of some alkaloids and pigments; that some plant tissue cultures can be stored for long periods; and that plant cells can be grown in fermentors. However, it remains to be solved that some compounds of interest are not produced in detectable or adequated amounts; selected strains are not always stable; growth may be undesirably long and costly; and some compounds may be affected by culture conditions.

Therefore the effects of macro-elements, sucrose and plant growth regulators on the production of saponon through ginseng callus culture was tested in the presence of different concentrations of macro-elements, sucrose and plant growth regulators. Fig. 3 shows saponin contents of ginseng calli cultured on MS media containing different concencultured on MS media containing different concentrations of nitrogen. The saponin contents of callus was increased by increasing nitrogen source in the media (Fig. 3). On the other hand, phosphorus and magnesium decreased the saponin conwere decreased when phosphorus and magnesium in the medium were raised from 0.5-fold to 1.5-fold of MS medium (Figs. 4 and 5). Further increases in the total phosphorus and magnesium increased the formation of this compound. Fig. 6 shows the effects of calcium concentrations on the saponin synthesis of ginseng calli. The formation of saponin was inhibited when the concentration of calcium in the medium was less or greater than 1.5-fold of MS medium (Fig. 6).

The effects of the sucrose concentration on the yield of secondary products in plant cell cultures has been examined by several groups. For example, increasing sucrose 2 to 4% increased the polyphenols per culture in *Rosa sp.* suspension cultures[10], while increasing sucrose from 2 to 5% decreased

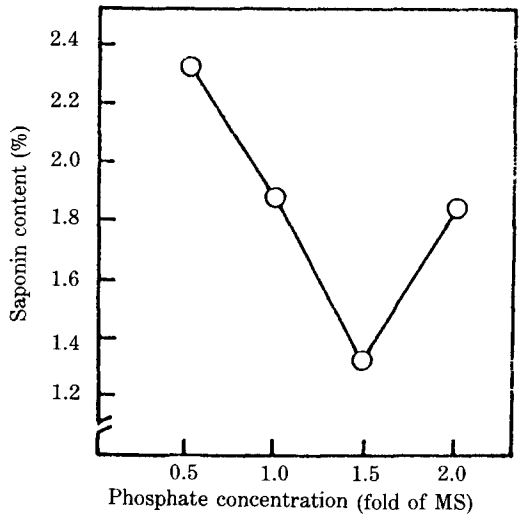

Fig. 4. Effect of phosphate on the saponin content of ginseng callus.

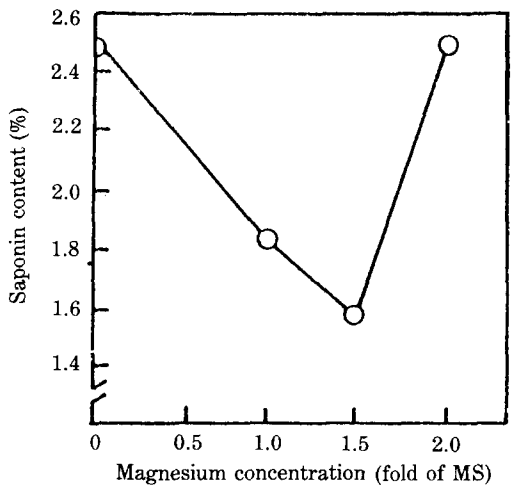

Fig. 5. Effect of magnesium on the saponin content of ginseng callus.

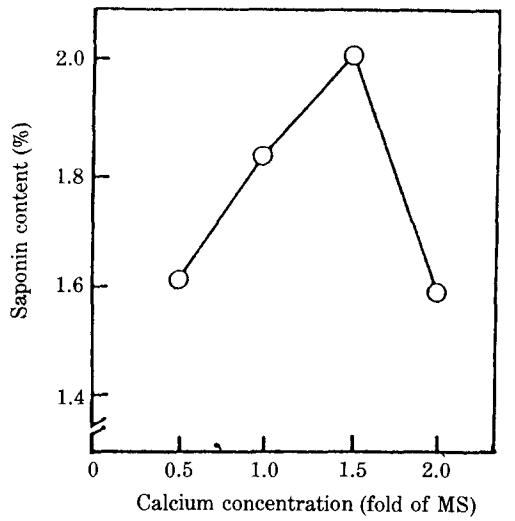

Fig. 6. Effect of calcium on the saponin content of ginseng callus.

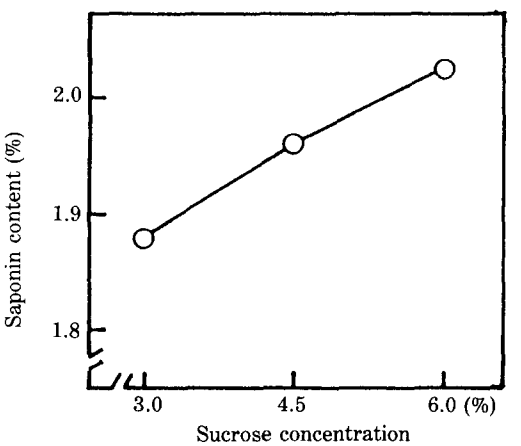

Fig. 7. Effect of sucrose on the saponin content of ginseng callus.

the ubiquinone per gram of dry cells in *Nicotiana tabacum* L. cv. BY-2[16]. Therefore, ginseng calli were cultured on the media containing different concentrations of sucrose to determine optimal concentration for the production of saponin from ginseng callus tissues. The yield of saponin of ginseng callus increased with increasing sucrose from 3 to 6% (Fig. 7).

The majority of plant cell parts, when excised and placed in tissue culture, require an exogenous

Table 1. Effects of 2,4-D and kinetin on saponin content of ginseng callus

Concentration (mg/*l*) of		Total saponin of callus (%)
2,4-D	Kinetin	
5	0	3.14
10	0	5.46
5	1	3.62
10	1	8.78

*Saponin contents of roots and leaves of ginseng are 4.75 and 10.0%, respectively.

supply of auxins and/or cytokinins for growth and cell division. The greatest difference between tissues from different species in terms of their behavior in tissue culture lies at the levels of auxins and/or cytokinins required for the growth and secondary product formation. Therefore, their effects on ginseng saponin formation from ginseng callus culture was tested in the presence of different concentrations of 2,4-D and kinetin. Table 1 shows the saponin contents of ginseng calli cultured on modified MS media with 2,4-D and/or kinetin. The saponin synthesis in the callus culture of ginseng was enhanced by 2,4-D and kinetin. The total saponin content of callus grown on the medium with 10 mg/*l* 2,4-D and 1 mg/*l* kinetin was about two times in comparison with that of the 6-year old ginseng roots commercially used as herbs (Table 1). On the other hand, Furuya *et al.*[13-15] reported that the content of saponin in ginseng callus cultured on the meida containing 2,4-D, IBA or kinetin was low (0.08-1.49%).

In these experiments, we found that macro-elements, sucorse and plant growth regulators influenced the relative biosynthesis of saponin in the ginseng tissue culture and that the ability of ginseng tissue culture to produce medicinal compound by cultured tissue was apparently higher than that of the intact plants. Although some promising data have already been obtained, more efforts are needed in improving biosynthetic rate of saponin, medicinal compound, in ginseng tissue culture by biochemical and genetic regulation of secondary metabolism.

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