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Effect of Substrate on the Production of Korean Ginseng (*Panax ginseng* C.A. Meyer) in Nutrient Culture

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Abstract

To overcome a decrease of Korean ginseng production caused by successive cropping, we have tried to develop a nutrient culture system for Korean ginseng production. For determining the optimal substrate, mixture of sand and TKS-2 (S+T), peatmoss (P), reused rockwool (RR), and granular rockwool (GR) were investigated. The overall physico-chemical properties of RR fell into the reported optimal range for the ginseng cultivation. However, bulk density of S+T was a little higher than that of soil in Korean ginseng fields. The top fresh weight of the ginseng was high in RR and S+T substrates. The root fresh and dry weights in the RR were remarkably greater than that in the conventional soil (CS) of Korean ginseng fields. In terms of ginseng quality, the vitamin C content of ginseng root in nutrient culture was higher than that in CS. However, the contents of crude saponin and total ginsenosides in ginseng between in the nutrient culture and in the soil culture did not show any significant differences.

Key words: crude saponin, ginsenoside, rockwool, vitamin C, nutrient culture

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Introduction

Ginseng, one of the best selling botanical supplements in Korea, China, Europe and America, is an important economic and medicinal plant throughout the world (Briskin, 2000). Above all, the most phytomedicinal interest for ginseng came from the triterpene saponins, known as the ginsenosides; Rb_1, Rb_2, Rc, Rd, Re, Rf, and Rg_1 (Court, 2000). Most commercial ginseng root is produced by soil cultivation, primarily in Korea and in Northeastern China, but *Panax ginseng* produced in Korea has a worldwide reputation in medicinal effect (Mahady et al., 2000).

However, recently ginseng production in Korea has been faced with lots of difficulties such as a decrease in Korean ginseng yield from successive cropping, resulting in root rot by *Cylindrocarpon destructans* and *Fusarium solani*, and in other physiological diseases. In the future, Korean ginseng production may decline sharply, if these difficulties are not overcome. In order to avoid this risk, we need to establish a new technique for ginseng production in nutrient culture, to enable disease-free and healthy Korean ginseng mass-production. The main advantages of nutrient culture over conventional soil culture are the ease of sterilizing the medium, high-density planting, and efficient nutrient regulation. Also, ginseng production can be badly damaged from weather conditions, but this threat can be escaped in nutrient culture (Park et al., 1998). Therefore, by applying nutrient culture we may realize to reduce injury by successive cropping and an increase in production. Nutrient culture on ginseng was little studied which it is recently reported the effects of GA_3 on the leaf emergence and of NO_3^- to NH_4^+ ratio on the growth, and the growth on the various substrates and on the different ionic strength (Han, 1998; Park et al., 1999).

In this study, to improve the production of Korean ginseng, we have attempted to develop the optimal substrate for the production of Korean ginseng in nutrient culture, comparing with conventional soil culture.

Materials and methods

1. Plant materials

This experiment was conducted in the greenhouse at Korea University, Seoul, Korea from March 2001 to September 2001. The plant material was 1-year-old Korean ginseng (15.3 cm length, 0.51 cm diameter, 1.15 g fresh weight), which was transplanted in the beds (50×31.5×22 cm) containing water (25 L) for water culture (WC) in which plant roots were suspended in a liquid medium and four different substrates (25 L); 4:1 (by volume) mixture of sand and TKS-2 (Torfkultursubstrat, Germany) (S+T) (Han, 1998), peatmoss (P) (Park et al., 1999), reused rockwool (RR, tomato-grown substrate for 6 years, which was crushed in 3 mm size by mill), and granular rockwool (GR), respectively, with 45 in 3 cm depth (Lee, 1996). Before transplanting, the seedlings were soaked into GA_3 50 ppm solution for 3 hours to break the bud dormancy of 1-year-old root and then thoroughly rinsed with distilled water (Park et al., 1999).

The composition of all nutrient solutions was as follows: macro element (NO_3-N 6.0, NH_4-N 0.5, PO_4-P 1.5, SO_4-S 1.0, K 4.0, Ca 2.0, and Mg 1.0 $me \cdot L^{-1}$), and micro element (FeEDTA 3.0, Mn 0.5, B 0.5, Cu 0.02, Mo 0.05, and Zn 0.05 $mg \cdot L^{-1}$). All plants were irrigated with 270 m*l* the nutrient solution per day. The solution EC was maintained at 0.5 $mS \cdot cm^{-1}$ in the leaf expanding stage and then 1.0 $mS \cdot cm^{-1}$ in the fully leaf-expanded stage (Park et al., 1999). For WC system, solutions were aerated 45 minute per hour and exchanged every 15 days.

The light intensity in the greenhouse was 300±50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (70 percent shading) at 24±2°C in spring season and 120±30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (90 percent shading) at 32±2°C in summer season and at that time the temperature of root zone was maintained at 16±2°C and 26±2°C, respectively.

To compare the effect of substrate by nutrient culture on Korean ginseng growth, ginseng grown in the conventional soil (CS) by traditional culture was used.

2. Physico-chemical properties of substrate

The substrates were air dried, thoroughly mixed and passed through a 2-mm sieve. Sample preparation and extraction procedures were followed by Maurice et al. (1995). The bulk density was analyzed using the core method as proposed by Stolte (1997). In the filtrate (1:10 dilution), pH (Orion Model 520A meter, Orion Research) and EC (HI 9033 conductance meter, Hanna Instrument) were measured. Exchangeable cations were determined with atomic absorption spectrophotometer (3280HPSF, Hewlett Packard) after extraction by 1N CH_3COONH_4 (pH 7.0).

3. Plant growth

Stem length, shoot fresh weight, chlorophyll content, root length, fresh weight, dry weight, and vitamin C were measured on 22 weeks after planting in harvest time. Chlorophyll content was analyzed by N,N-dimethylformamide (DMF) extraction method (Inskeep et al., 1985). The vitamin C content was determined by 2,6-dichlorophenolindophnol method by fluorometer (AOAC, 1995).

4. Analysis of crude saponin and ginsenoside content

The roots were cut into pieces, dried in the forced-air dry oven at 70°C and then ground. The crude saponin content was analyzed by gravimetric analysis through 1-butanol extract of dry powder (Ando et al., 1971). The crude saponin was applied to HPLC (ALC-244) for the ginsenoside content. The Lichrosorb NH_2 column (Merck Co., 10 μm, 4 mm I.D. 250 mm) using a mixed solvent of acetonitrile:water:1-butanol (80:20:10, v/v) at a flow rate of 1.0 m*l* min^{-1} was used. The ginsenoside standard was obtained from Korea Ginseng and Tobacco Research Institution.

5. Experimental design and statistical analysis

The experiment plot was a randomized complete block design with three replications. All data were subjected to Duncan's multiple range test.

Results and Discussion

1. Physico-chemical properties of substrates

In the physical property of substrates, S+T (sand:TKS-2=4:1, v/v) had 4–9 fold higher bulk density (1.07 $g \cdot cm^{-3}$) than peatmoss (P) (0.12 $g \cdot cm^{-3}$), reused rockwool (RR) (0.27 $g \cdot cm^{-3}$), and granular rockwool (GR) (0.29 $g \cdot cm^{-3}$) (Table 1). It is reported that bulk density of soil in conventional ginseng culture is ca. 0.80 $g \cdot cm^{-3}$. Lee et al. (1995) concluded that root weight of ginseng

Table 1. Physico-chemical properties of substrates used in the experiment.

Treatment[z]	Bulk density ($g \cdot cm^{-3}$)	pH		EC ($dS \cdot m^{-1}$)		Exchangeable cations ($cmol \cdot kg^{-1}$)					
						Ca^{2+}		K^+		Mg^{2+}	
		B[y]	A	B	A	B	A	B	A	B	A
S+T	1.07 a	5.91 b	6.21 ab	0.18 c	0.06 c	0.77 c	0.50 c	0.07 c	0.26 c	0.25 c	0.20 b
P	0.12 c	3.44 c	4.22 c	1.75 a	1.14 a	10.3 a	6.37 a	6.43 a	5.39 a	3.60 a	3.21 a
RR	0.27 b	6.06 ab	6.82 ab	1.29 b	0.36 b	2.81 b	1.03 b	2.52 b	1.11 b	1.61 b	0.34 b
GR	0.29 b	7.97 a	7.58 a	0.15 c	0.20 b	1.30 bc	0.97 b	0.06 c	0.83 b	0.07 d	0.35 b

[z] S+T: Sand:TKS-2 = 4:1; P, Peatmoss; RR, Reused Rockwool; and GR, Granular Rockwool.
[y] B, before culture; and A, after culture.
[x] Means separation within columns by Duncan's multiple rage test at P=0.05.

was significantly decreased with bulk density increased over 0.80 $g \cdot cm^{-3}$, and then suggested that lowering bulk density of the soil is essential for ginseng root growth. For chemical properties of substrates, the pH, EC, and exchangeable cation were measured before and after cultivation, respectively (Table 1). Court (2000) reported that few ginsengs survive in alkaline (> pH 7.0) and acid (< pH 5.0) soils. In addition, Korea Ginseng and Tobacco Research Institute (1996) showed that ginseng could grow well in the range of pH 5.0 to 6.5. Therefore, the pH of RR (5.91) and S+T (6.06) was within the optimum ranges of ginseng cultivation before and after cultivation. In the EC, S+T (0.18 $dS \cdot m^{-1}$) and GR (0.15 $dS \cdot m^{-1}$) were adequate for a low salinity-loving (0.05–0.2 $dS \cdot m^{-1}$) ginseng. On the other hand, RR was greatly different before (1.29 $dS \cdot m^{-1}$) and after cultivation (0.36 $dS \cdot m^{-1}$). EC of RR was adjusted into optimal range in the process of cultivation, in spite of higher EC than optimal range. For improving early growth of ginseng, EC of RR should be adjusted before planting to prevent injury of high salinity. Park et al. (1998) recommended a method to irrigate fresh water of lower conductivity in substrate for reducing EC. In addition, higher EC in substrates showed higher exchangeable cations such as calcium, potassium, and magnesium ions. RR, shown optimum range of EC, also had optimum range of exchangeable cations, which is fell into previous data reported by Korea Geinseng and Tobacco Research Institute (1996): Ca^{2+} (2.0–6.0 $cmol \cdot kg^{-1}$), K^+ (0.2–0.8 $cmol \cdot kg^{-1}$), and Mg^{2+} (1.0–4.5 $cmol \cdot kg^{-1}$).

2. Evaluation of Korean ginseng growth in nutrient culture

In the shoot growth of ginseng, the S+T showed best results in stem length (Table 2). Park et al. (1996) reported that top growth of root vegetables was affected depending on the substrates. The shoot fresh weight was significantly high in S+T and followed by RR. It is possible to measure directly the status of root such as carrot, beet, and ginseng through the shoot fresh weight correlated with the root weight. Korea Ginseng and Tobacco Research Institute (1996) reported a significantly positive correlation between the shoot and root fresh weight. By measuring shoot growth, we could predict that the root growth in S+T and RR would be good.

The chlorophyll content of Korean ginseng was the best in RR substrate (Table 2). It might be resulted from proper physico-chemical properties of RR, which had optimum range of EC. RR could supply sufficient nutrients to ginseng (Shannon et al., 1999). Although ginseng is a shade plant, the plant uses solar energy to synthesize organic compounds that cannot be formed without the input of energy. Therefore, the formation of a large storage organ (root) leads to demand carbohydrates resulted from photosynthesis (Korea Ginseng and Tobacco Research Institute, 1996).

In the root growth of ginseng, RR substrate was the best for root length, fresh weight, and dry weight (Table 2). Especially, the root fresh and dry weight in the RR were higher than those in CS. This result was associated with shoot growth of ginseng and physico-chemical properties of substrates (Kim et al., 1995; Lee et al., 1995). Soil applications of dolomitic limestone and P fertilizer before seeding American ginseng affected root weight gain. Root size was the greatest with the intermediate liming rate and with the high P fertilizer rate (Konsler et al., 1990a).

Table 2. The effect of various substrates and water culture on the growth of Korean ginseng on 22 weeks after planting.

Treatment	Shoot			Root			
	Stem length (cm)	Fresh weight (g · plant^{-1})	Chlorophyll content (mg · g^{-1})	Length (cm)	Dry weight (g · plant^{-1})	Fresh weight (g · plant^{-1})	Vitamin C content (mg · 100g^{-1} fw)
S+T	10.2 a[z]	1.83 a	2.13 bc	13.7 b	1.22 ab	4.64 b	18.6 a
P	7.6 b	1.22 c	2.03 bc	13.3 b	1.00 c	4.19 b	16.7 a
RR	8.8 ab	1.50 b	2.56 a	16.7 a	1.39 a	5.62 a	16.1 b
GR	8.2 b	1.21 c	2.29 ab	13.7 b	1.13 bc	4.30 b	14.1 c
WC[y]	6.4 b	0.86 d	1.92 c	14.3 b	0.66 d	2.61 c	7.3 d
CS[x]	8.9 ab	1.25 c	2.36 ab	18.2 a	1.19 abc	4.40 b	13.3 c

[z] Means separation within columns by Duncan's multiple rage test at P=0.05.
[y] Water culture
[x] Conventional soil culture

Fig. 1. Comparison of root growth in S+T (left) and RR (right) on 22 weeks after planting.

Formation of root hairs in the S+T substrate was inferior to RR (Fig. 1). We concur with Lee et al. (1995) who concluded that high bulk density caused formation of root hairs to prevent. On the other hand, root hairs of ginseng in RR substrate were noticeably formed. This enabled remarkable root growth, because root hairs increase the surface area over which water and nutrient absorption can take place (Taiz et al., 1991).

3. Evaluation of Korean ginseng quality in nutrient culture

Vitamin C : The vitamin C contents of the ginseng root in S+T, P, and RR was 18.6, 16.7, and 16.1 mg · 100 g^{-1} FW, respectively and those contents were higher than in CS (13.3 mg · 100 g^{-1} FW) (Table 2). Generally, one- to two-year-old Korean ginseng is preferred as a salad of preference in Korea. But, vitamin C in ginseng has not been regarded as important as ginsenoside. However, Li et al. (2000) observed interactions between ginseng saponins and vitamin C. Low amounts of saponins can generate significant degree of antioxidant effects in the presence of low but physiologically essential amounts of vitamin C. Moreover, vitamin C can mainly act as excellent antioxidant, so it can scavenge many types of free radicals including singlet oxygen, superoxide, and hydroxy radicals not only in plant but also in human (Padh, 1994). Although saponin is a major active constituent in ginseng, vitamin C in Korean ginseng is an important factor of quality for using a salad plant.

Crude saponin and ginsenosides : In Table 3, the contents of crude saponin and total and individual ginsenosides were shown. The contents of crude saponin in S+T, P, RR, GR, and CS were 4.16, 4.62, 4.26, 4.48, and 3.92 mg · 100 g^{-1} DW, respectively. However, there were no significant differences each other. All ginsenosides and the total contents were not also affected by nutrient culture and soil culture. A study conducted by Kim et al. (1987) to get basic information on saponin contents according to ginseng root growth showed that crude saponin and total ginsenoside contents was 4.83, and 2.00 mg · 100 g^{-1} DW, respectively. These data also suggested that ginseng production by nutrient culture was little different in saponin content comparing with conventional soil culture. Consequently, ginseng in RR shown the highest root weight contained more ginsenoside con-

Table 3. Comparison of crude saponin, and individual and total ginsenosides in 2-year-old Korean ginseng roots in the nutrient culture and soil culture.

Treatment	Crude saponins ($g \cdot 100g^{-1}$ dw)	Root ginsenoside ($g \cdot 100g^{-1}$ dw)							
		Rb_1	Rb_2	Rc	Rd	Re	Rf	Rg_1	Total
S:T	4.16 a[z]	0.34	0.21	0.19	0.13	0.43	0.08	0.14	1.51 a
P	4.62 a	0.31	0.22	0.18	0.13	0.32	0.07	0.13	1.36 a
RR	4.26 a	0.35	0.24	0.20	0.17	0.43	0.07	0.17	1.63 a
GR	4.48 a	0.28	0.22	0.19	0.14	0.34	0.10	0.15	1.41 a
WC	3.53 a	0.37	0.21	0.17	0.10	0.33	0.02	0.11	1.31 a
CS	3.92 a	0.36	0.29	0.25	0.18	0.31	0.06	0.14	1.59 a

[Z]Means separation within columns by Duncan's multiple rage test at P=0.05.

tent than that in other substrates. Saponins were reported in all plant parts of *Panax* species at different concentration. In particular, their root hairs had the highest saponin yield per unit weight (Court, 2000). A number of root hairs in RR substrate simply indicated that ginseng in RR substrate is salubrious not only in terms of root mass but also medicinal effect. These results certainly showed a new possibility of ginseng culture in nutrient culture. Soil-applied dolomitic limestone and fertilizer affected the level of certain root and leaf ginsenosides in 4-year-old American ginseng (Konsler et al., 1990b). The production site of ginseng influenced the ginsenoside contents of roots and leaves (Li et al., 1996). Thomas et al. (1999) suggested that soil nutrient levels might play a major role in the synthesis of ginsenosides, and pointed out the importance of soil nutrient levels in the accumulation of the biologically active ginsenosides in leaves and roots of ginseng through correlation between minor elements and ginsenoside contents of ginseng. Further study on the nutrient solution for ginseng is needed to enhance ginseng's value by increasing the amount of ginsenosides.

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한국인삼 양액재배시 배지의 영향

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적 요

한국의 인삼재배시 연작장해로 인한 생산량 감소가 문제가 되고 있다. 이를 극복하기 위하여 본 연구에서는 한국인삼 재배를 위하여 양액재배 시스템을 적용하였다. 최적의 양액재배용 배지를 선발하기 위하여 모래, TSK-2 (S+T), 피트모스 (P), 재활용 암면 (RR)과 입상 암면 (GR) 등의 다양한 배지를 단독 및 혼합처리구로 사용하였다. RR 배지의 경우 전반적인 물리화학적 특성은 기존에 보고된 인삼재배지의 토양특성과 유사하였다. S+T 배지는 가밀도가 인삼용 토양보다 높았다. 인삼의 지상부 생체중은 RR 배지와 S+T 배지에서 높았다. 인삼 뿌리의 생체중 및 건물중은 RR 배지에서 가장 높았다. 인삼의 뿌리의 품질적 측면에서 vitamin C 함량은 양액재배로 생육시킨 인삼이 토양에서 생육한 인삼보다 높았으나, crude saponin과 ginsenosides 함량에서는 차이를 나타내지 않았다.

주제어 : 조사포닌, ginsenoside, 암면, 비타민 C, 수경재배