Effect of Growth Conditions on Saponin Content and Ginsenoside Pattern of *Panax ginseng*

Mee-Kyoung Lee, Hoon Park and Chong-Hwa Lee
Korea Ginseng and Tobacco Research Institute Science Town, Daejeon Korea

Abstract : For the elucidation of significance of saponin as quality criterion of ginseng ginsenoside content(GC) and ginsenoside pattern similarity(GPS) by simple correlation were investigated in relation to red ginseng quality factors, age, plant part, harvest season, mineral nutrition, soil physical characteristics, growth light and temperature, shading material, growth location, physiological disease and crop stand through survey of ginseng plantations, field experiments, water culture and phytotron experiments. Effect of tissue culture was also reviewed. GC was negatively correlated with good quality of red ginseng and positively with bad quality. Age did not show any consistency with GC but GPS was less with the increase of age difference. GPS was less or not significant between tap root that is lowest in GC and epidermis highest, and significant between leaf and tap root. Harvest season marked with the lowest GC and pattern was also different. Nutrient imbalance, the increase of hazardous soil nutrient and physical condition to growth increased GC, but GPS was little different. The higher the growth light intensity and temperature the higher the GC but GPS was little changed. Root rust increased GC, but root scab decreased it. Sponge-like and inside cavity phenomena increased GC. Ginsenoside pattern of cultured tissues and rootlet showed great variation. These results strongly indicate that there are optimum saponin content and ginsenoside pattern and that these are accomplished under the optimum growth condition.

Introduction

Korea ginseng(*Panax ginseng* C.A. Meyer) is still the mysterious queen of medicinal herbs since a number of known physiologically active compounds are just a skin deep for the explanation of the reported physiological and clinical efficacies. The intensive chemical research on saponin, partly by chance and partly due to uniqueness in ginseng made it the king of physiologically active compounds and it became the quality criterion[1] in the dark without proper assessment only because there was no better alternative.

Thus it seems to be commonly accepted that the higher the saponin content the better the quality. Consequently ginseng tissue culture[2,3] and furthermore saponin production[4,5,6] by cell culture are attempted. The ratio of protopanaxatriol to protopanaxadiol(PT/PD) was proposed as a quality criterion in view of different physiological effects of two fractions[7] and PT/PD is still used for quality assessment, especially in tissue culture[6,8]. Recently the isolation technique of ginsenosides is remarkably developed and physiological activities are investigated at each ginsenoside level[9]. Since the physiological responses of ginsenosides are different case by case[9] the grouping into triol and diol is not always reasonable. Furthermore ginsenosides sorted into three groups[10] in view of molecular aggregation pattern in solution. Ginsenosides could be the best index compound to identify ginseng from other similar herbs since twenty eight ginsenosides are known[9]) and only six of them(two in Luffa and four in Gynostemma) are found in other plants[11,12].

But for the assessment of ginseng quality, the content and composition of ginsenosides should be considered in different way. Response of one group of ginsenosides is reverse to the response of the other group of ginsenosides but the mixture shows in one way response or no response at all[9]. These facts make it difficult to establish the quality index with these compounds on the basis of physiological activity. In this paper we assessed the significance of ginsenosides as a quality index in view of growth conditions and proposed ginsenoside pattern similarity together with saponin content as the quality index.

Materials and Methods

Plant sampling for plantation survey

Six years old ginsengs(2nd grade of fresh ginseng) were collected from ginseng plantations all over the country at harvest(September to October) for three years(1984-1986). Soil samples were also taken for chemical and physical analysis.

Shading material and crop stand were recorded at each plantation. Red ginseng grade and quality factors were investigated at Red Ginseng Processing Company. All samples were dried at 55℃ and powdered. Details are reported elsewhere[13,14,15].

Saponin analysis

Crude saponin content was measured by 80% methanol extract of dry powder including fine root and then butanol extract for plantation sample in 1984. Tap root in 1985 and cortex and epidermis in 1986 were used and extracted with butanol. Crude saponin was applied to high performance liquid chromatography(HPLC) for ginsenoside pattern. Detailes are reported elsewhere[14,15].

Age and plant part

Plant samples of each age were taken at Jeungpyeong Experiment Station. Tap root was separated into two parts, cortex-epidermis and xylem-pith and analyzed for ginsenosides by HPLC.

Physiological disease

Root samples of scab, rust, inside cavity, sponge tissue and low starch(negative iodine test) were collected from various fields and subjected to ginsenosides analysis and crude saponin determination. Details are reported elsewhere[14,16].

Seasonal change of saponin

Four years old roots were sampled 11 times from one month before emergence to conventional harvesting time(October 12th) and analyzed for ginsenoside pattern after drying[13].

Effect of growth light and temperature on saponin: Ginseng plants were grown under various light intensity(10-30% of whole light) in field condition for 5 years[17]. The 4th and 6th year ginseng plants were grown in pot with soil at various artificial light intensity and temperature in a phytotron[17,18]. Roots and leaves were used for ginsenoside analysis.

Effect of minerals on saponin

Ginseng seedlings were cultured with solutions of various nitrogen, phosphorus and potassium levels in pot with sand for one year[18]. Roots were analyzed for ginsenosides composition.

Effect of location

From plantation survey fields or means of fields in each location were compared for ginsenosides pattern[14].

Effect of tissue culture

GPS was investigated with cultured tissue or rootlet and field grown normal roots according to other's reports[5,6,8,19].

Ginsenoside pattern similarity(GPS)

Simple correlation coefficient between treatments with counterpart ginsenosides was used[20].

Result and Discussion

The relationship of saponin content and ginsenoside pattern to various growth conditions or the factors being determined by growth conditions are as follows.

Red ginseng quality

The quality of red ginseng depends on the quality of fresh ginseng[13]. The quality of the latter depends on growth condition for six years. It is prerequisite for any compound to be a quality criterion that the compound should well match to red ginseng quality. The correlation coefficients between red ginseng grade

Table 1. *Correlation(r) between saponin content of tap roots and red ginseng quality.*

	Heaven (H)	Earth (E)	H+E	Inside cavity	Inside white	White skin	Cracking
1984 (20) a	0.336	0.242	0.293	0.023	-0.241	0.565***	0.303
1985 (27) b	-0.055	-0.329*	-0.307	0.307	-0.023	0.074	-0.462**
1986 (28) c	-0.092	-0.299	-0.232	-0.068	0.160	0.046	-0.055

( ); number of fields, ***,**,*; p=0.01, 0.05 and 0.1
a: Whole root, b: Tap root, c: Cortex-epidermis of tap root

Table 2. *Ginsenosides content and pattern similarity of P. ginseng in relation to root age*

Part	Age	5	4	3	2	1
Tap root	GC(% DW)	0.77	0.79	0.94	0.93	0.93
	GSP	1.000	0.975****	0.986****	0.925***	0.340
	PT/PD	0.92	0.97	0.87	0.86	0.57
Cortex + Phloem	GC(% DW)	1.50	1.41	1.80	0.94	—
	GSP	1.000	0.959****	0.957****	0.840**	—
	PT/PD	0.81	0.85	0.77	0.64	—
Xylem + Pith	GC(% DW)	0.22	0.30	0.32	0.90	—
	GSP	1.000	0.990****	0.936***	0.883***	—
	PT/PD	1.74	1.59	1.49	1.43	—

****,***,**; p=0.001, 0.01 and 0.05

percentage(Heaven, Earth etc) or percent occurence of red ginseng quality factors such as inside cavity and inside white and crude saponin content were shown in Table 1. The higher crude saponin content the greater the percent occurence of white skin was with great significance(P=0.01) in 1984. Earth grade percentage of red ginseng significantly(P=0.1) decreased with the increase of crude saponin of tap root while percent occurence of cracking decreased(P=0.05) in survey of ginseng plantation in 1985. There was yearly variation due to the difference in weather of growth years.

It was rainy year in 1985 and 1986 while 1984 was dry year. Thus general tendency was same in the latter two years.

Age

Since saponin content is greatly different according to plant part, certain part such as cortex-epidermis or tap root may be the representative sample rather than whole root including fine root. Percentage of lateral root and fine root is more dependent on soil condition than on age. Sums of ginsenosides in tap root, inside and outside part of each age were seen in Table 2. Total ginsenoside content(GC) in cortex increased except 3 years old and decreased in xylem-pith with age increase. But GC of tap root did not increase with age.

In other reports[1,21-26] 5 cases did not show any consistent change with age(Fig.1) while 4 cases showed increasing trend with age(Fig.2). These results indicate that traditional high value of age is not due to high ginsenoside content.

The ratio of protopanaxatriol saponin to protopanaxadiol saponin(PT/PD) did not change consistently with age and was different in each case as shown in Fig.3. However GPS decreased with the increase of age difference in all parts resulting in insignificant GPS between 5th and lst year tap root(Table 2). The consistent decreases of GPS with age difference were also seen in all other cases(Fig.4)[21-25]. The

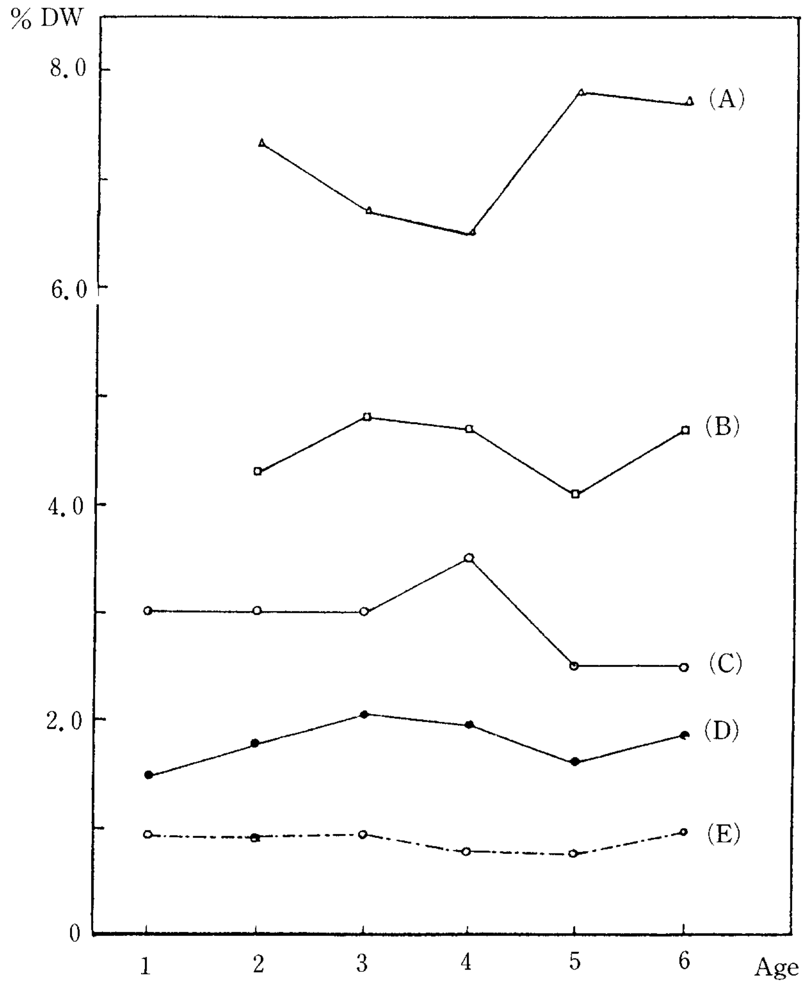

Fig.1. *Ginsenosides content of P. ginseng root in relation to age.*
(A): Lee *et al.*(1978), (B): Cho *et al.*(1977), (C): Namba *et al.*(1974), (D): Soldati and Tanaka(1984) Cultivated in Japan, (E): Authors(1982), o---o tap root without epidermis, (A), (B) and (C): Crude saponin content, (D) and (E): Sum of ginsenosides

indications are that GPS is better parameter for age related quality than ginsenoside content. There were two exceptions[26] in the general trend of decrease with age. Those were GPS of D in Fig.1 and Fig.2. But GPS of D in Fig.2 followed general trend from 5th to 2nd year roots. Thus extreme exception was one. GC of these two exceptional GPS were calculated from the figure reported[26] (personal communication 4 cm=0.5%). These GC were also the lowest as shown in Fig.1 and 2. E in Fig.1 is for tap root. Soldati and Tanaka recommended the 5th year for ginseng harvest only based on ginsenoside production[26]. But it does not seem to be right in view of GPS.

Plant part

Ginsenoside content and pattern similarity of each part to tap root were seen in Fig.5. Since GC was lowest in xylem-pith, the weight of epidermis was small, and GC in cortex-phloem was low, the GC in tap root was lowest. Pattern similarity to tap root was generally highest for cortex-phloem and least for epidermis or fine root that had high content of ginsenosides. Such

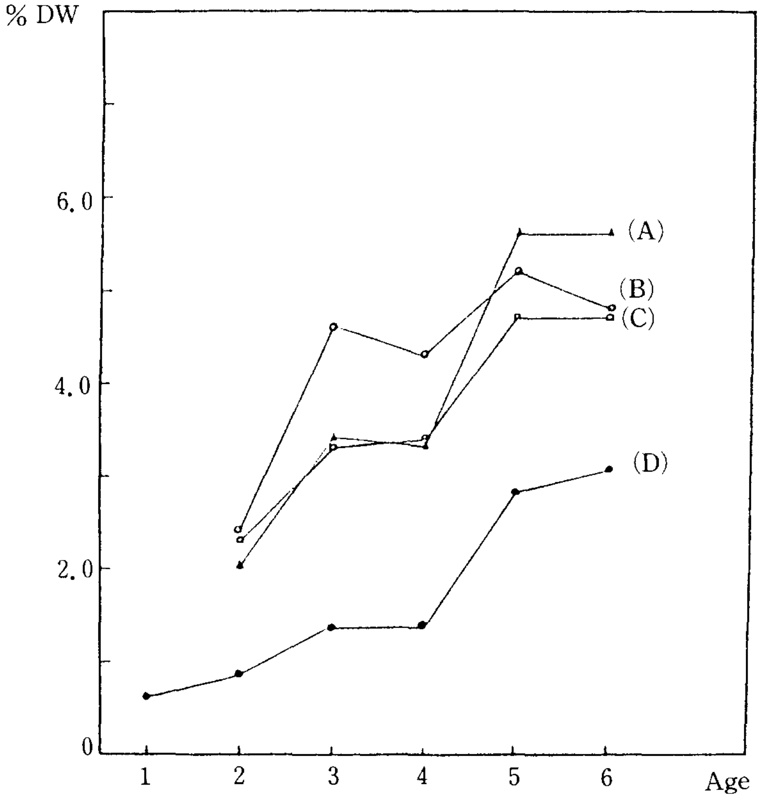

Fig.2. *Ginsenosides content of P. ginseng root in relation to age.*
(A): Kim *et al.*(1985), (B): Jang *et al.*(1983), (C): Kim *et al.*(1986), (D): Soldati and Tanaka(1984), cultivated in Korea

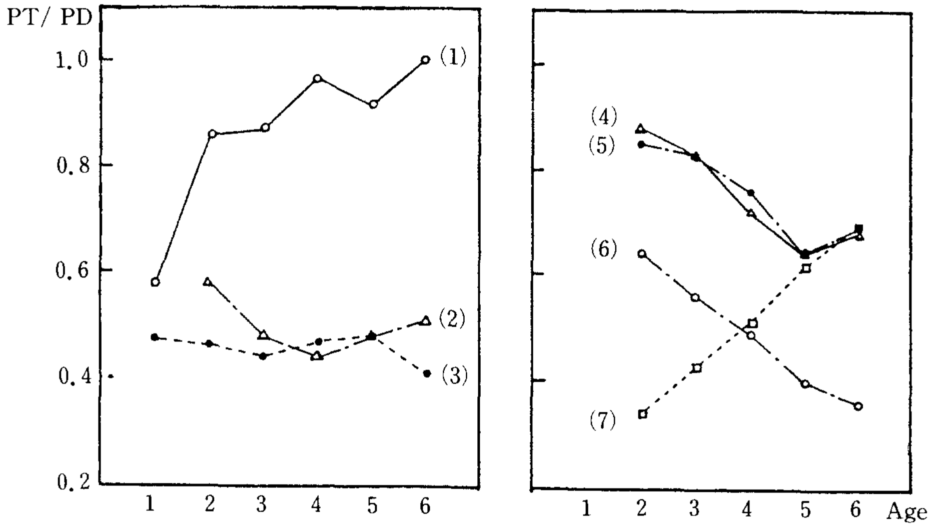

Fig.3. *Protopanaxatriol ginsenosides content(PT) to protopanaxadiol ginsenosides content(PD) ratio of P. ginseng root in relation to age.*
(1) Authors, tap root without epidermis(1982), (2) Jang *et al.*(1983)
(3) Namba *et al.*(1974), (4) Kim *et al.*(1986), (5) Kim *et al.*(1986)
(6) Kim *et al.*(1985), (7) Cho(1977), (3),(7): calculated from TLC densitogram.

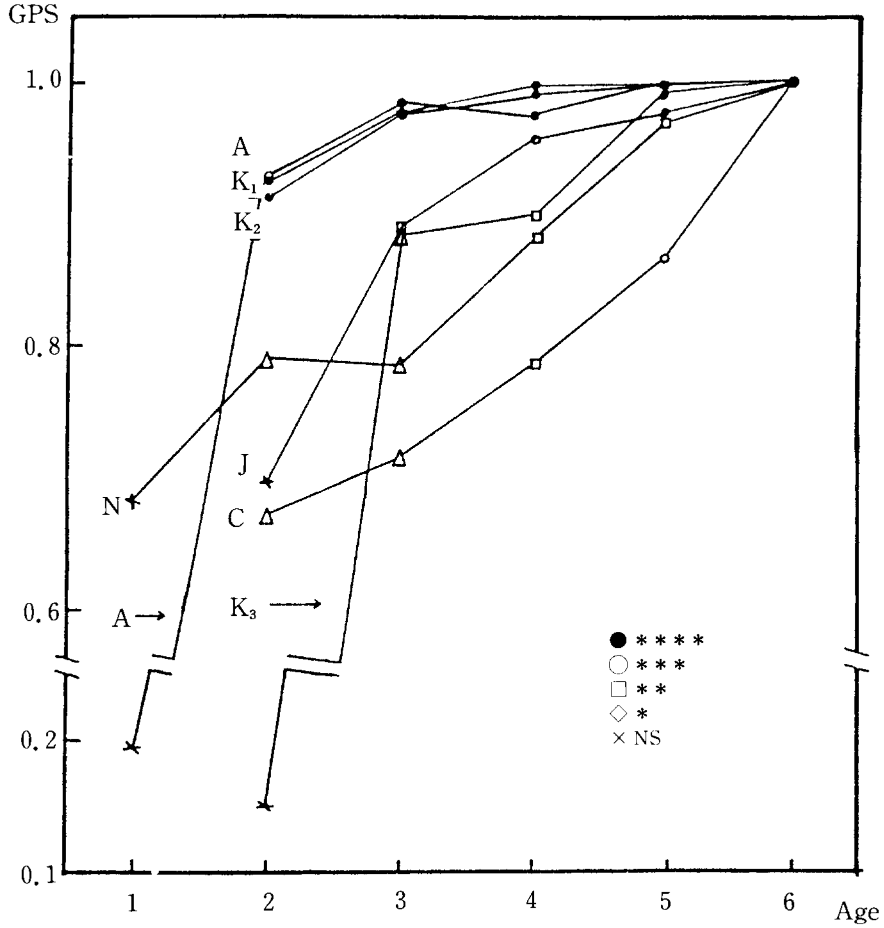

Fig.4. *Ginsenosides pattern similarity(GPS, r) of P. ginseng root in relation to age.*
****, ***, **, * ; p=0.001, 0.01, 0.05 and 0.1
A: Author's(1982), C: Cho, S.H.(1977), J: Jang, J.G. *et al.*(1983), K: Kim, M.W. *et al.*, 1 and 2(1986), 3(1985), N: Namba *et al.*(1974)

phenomenon seems to be universal as shown in Table 3 when calculated from other's reports[16,25,27,28]. In the case of white ginseng[29] that consisted of tap root and lateral root GPS was also very poor(r=0.180, n=7) with epidermis(peelings).

·PT/PD was greatly different among each part of root(Table 3). There was considerable difference among tap root. PT/PD trended to decrease with the increase of GC but it is not consistent between epidermis and fine root.

It is common sense that tap root is the primary object of ginseng root for medicinal use. According to the traditional red ginseng processing the fine roots are eliminated and the end portion of lateral root was also cut out. In white ginseng processing the fine roots and skin are eliminated. However based only on GC Tani *et al* thought the removal of peelings in white ginseng would result in poor quality[30]. For medicinal purpose tap and lateral roots have long been used together. These facts strongly suggest that ginsenoside pattern is more important than content. The high content does not seem to be always good.

Ginsenoside content of aerial parts and GPS to root calculated from author's and other reports[1,27-29,31-33] are shown in Table 4. GPS

Table 3. *Ginsenosides content and pattern similarity between tap and various parts of P. ginseng root .*

	Tap root	Xylem -pith	Cortex -phloem	Epidermis of tap root	Rhizome	Lateral root	Fine root	Whole root	Reference
GC (%FW)	0.51	0.34	0.60	4.18	–	1.13	1.77	0.73	Park et al (16)
GPS	1.000	0.958****	0.980****	0.253	–	0.455	0.095	0.889***	
PT/PD	1.04	1.33	0.93	0.84	–	0.60	0.45	0.75	
GC (%DW)	2.05	1.02	2.70	7.60	6.06	3.94	8.31	–	Kim et al (25)
GPS	1.000	0.964****	0.979****	0.730**	0.897***	0.880***	0.758**	–	
PT/PD	1.06	1.39	0.94	0.88	0.67	0.63	0.51	–	
GC (%DW)	1.35	–	–	–	–	3.53	6.15	–	Soldati and sticher (27)
GPS	1.000	–	–	–	–	0.663	0.375	–	
PT/PD	0.92	–	–	–	–	0.63	0.59	–	
GC. (Fresh)	1.00	–	–	–	1.74	2.32	–	1.08	Hong et al (28)
GPS	1.000	–	–	–	0.880****	0.832***	–	0.870***	
PT/PD	0.67	–	–	–	0.52	0.33	–	0.37	
GC (%DW) (White)	2.73	–	–	–	–	6.48	–	–	Hong et al (28)
GPS	1.000	–	–	–	–	0.818***	–	–	
PT/PD	0.73	–	–	–	–	0.33	–	–	

****, ***, **, *; p=0.001, 0.01, 0.05 and 0.1

was mostly insignificant and negative in spite of high GC. Between aerial part GPS was positive and highly significant. But as shown in Table 4, GPS between aerial parts appeared to be greatly variable according to seasonal change. The use of leaves and flower buds is traditionally very rare. Leaf saponin showed hemolysis while root saponin did not[1]. These facts also forcefully support the importance of ginsenoside pattern rather than content.

Harvest season

Investigation on seasonal change of ginsenosides is very rare[13,34]. Seasonal change of ginsenoside content in root was shown in Fig.6. Traditional harvesting season, October, marked as the lowest time of GC. PT/PD was not consistent in seasonal change(Table 5). GPS was greater when the sampling time was nearer to the harvest time(Table 5). GPS of root on May and June to that at harvest was significantly low in some cases(Table 5) but it was not great in comparison with other cases mentioned above such as plant part and age(Table 5). Since some scientists considered only ginsenoside content they recommended summer as best harvesting season[26,34]. But it does not appear to be like that in terms of GPS. In Korea all living things prepare for winter in autumn with maximum nourishment. Ginseng plant must not be exceptional.

Mineral nutrition

Ginseng seedlings were grown with nutrient solution different in nitrogen, phophorus and potassium level for one year. Ginsenoside con-

Table 4. *Ginsenosides content(GC) and pattern similarity(GPS) of aerial part of Panax ginseng.*

	Root	Leaf	Petiole	Stem	Flower-bud	Fruit	Seed	Reference
GC(% DW) June	3.95^a	9.77	1.24	0.90	–	–	8.88	Author's
GPS, r 12th	1.000	0.284(5)	0.636(5)	0.647(5)	–		-0.045(6)	unpublished
GC(% DW) Sept.	3.66^a	4.44	1.91	0.84	–	–	–	Author's
GPS 18th	1.000	-0.227(6)	0.116(7)	0.170(7)	–	–	–	unpublished
GC(% DW)	1.60^b	5.20	–	–	4.1	–	6.44	Yahara
GPS	1.000	-0.699(6)	–	–	-0.306(6)	–	-0.252(5)	et al. (33)
GC(% DW)	2.10^b	7.40			9,42	–	–	Bombardelli
GPS	1.000	-0.562(6)			-0.378(6)	–	–	et al. (31)
GC(% DW)	2.37^b	10.84	–	0.64	–	–	–	Choi et al. (32)
GPS	1.000	0.006(7)	–	0.145(7)	–	–	–	
GC(% FW)	1.08^a	0.72	–	–	–	0.62	0.32	Hong et al. (28)
GPS	1.000	0.514(3)	–	–	–	-0.078(7)	-0.517(4)	
GC(% DW) c	3.10^b	12.6	–	–	15.0^e	–	0.70	Namba
GPS d	1.000	0.328(6)	–	–	-0.273(6)	–	0.037(6)	et al. (1)
GC(% DW)	1.35^f	5.19	0.77	0.76	–	–	–	Soldati and 27
GPS	1.000	-0.346(6)	0.351(6)	0.153(6)	–	–	–	Sticher (27)
GPS d	1.000			-0.356(6)	-0.423(5)	–	–	Kim et al. (29)

a: whole root, b: white ginseng, c: crude saponin content, d: calculated from TLC densitogram, e: buds only, f: tap root only

Table 5. *Ginsenosides pattern similarity(GPS) in relation to harvest season.*

Month		Mar.	Apr.		May		June		July	Aug.	Sept.	Oct.
Day		24	7	23	3	19	7	29		10	5	12
GPS												
Xylem-pith	(n=7)	0.923*	0.978**	0.997**	0.984**	0.984**	0.984**	0.983**	–	0.977**	0.991**	1.000
Cortex-phloem	(n=7)	0.956**	0.995**	0.937*	0.985**	0.971**	0.968**	0.910*	–	0.983**	0.985**	1.000
Tap root	(n=7)	0.946*	0.996**	0.976**	0.989**	0.983**	0.986**	0.942*	–	0.982**	0.971**	1.000
Whole root^a	(n=6)	0.980**	0.988**	0.963*	0.956*	0.971*	–	0.989**	0.995**	0.993**	0.983**	1.000
		(20)	(5)	(20)	(5)	(20)		(20)	(20)	(20)	(20)	(20)
PT / PD												
Xylem-pith		1.69	2.39	2.38	2.00	1.96	1.77	1.92	–	1.88	2.02	2.60
Cortex-phloem		1.01	1.16	0.94	0.94	0.93	1.10	0.83	–	0.91	1.19	1.12
Tap root		1.22	1.35	1.15	1.11	1.10	1.21	0.97	–	0.98	1.33	1.24
Whole root^a		0.36	0.32	0.31	0.36	0.35	–	0.36	0.37	0.33	0.35	0.34
		(20)	(5)	(20)	(5)	(20)		(20)	(20)	(20)	(20)	(20)

a: Calculated from Kim *et al*[34]. ( ): Sample harvest day of whole root **, *; p=0.001 and 0.01

Table 6. *Effect of mineral nutrients on ginsenosides contents(mg/g dw) and pattern similarity of Panax ginseng root grown under solution culture.*

	-N	-P	-K	B	3K	3NPK	NPK	3P	3N
GC	15.51	14.72	14.65	14.09	11.72	11.02	10.35	10.13	8.85
	a	ab	ab	abc	bcd	cd	d	d	d
GPS	0.986*	0.974*	0.982**	0.992*	0.996*	0.978*	1.00*	0.992*	0.972*
P.D	8.92	8.25	8.40	7.82	6.39	5.95	5.74	5.52	4.47
	a	ab	a	abc	bcd	cd	d	d	d
P.T	6.59	6.47	6.25	6.25	5.33	5.06	4.61	4.61	4.38
	a	a	a	a	ab	ab	b	b	b
PT / PD	0.74	0.78	0.74	0.80	0.83	0.85	0.80	0.84	0.98
	b	b	b	ab	ab	ab	ab	ab	a
Root weight	5.49	5.11	5.14	3.53	4.94	5.79	6.16	5.80	5.09
(g dw/10)	a	a	a	b	a	a	a	a	a

B: tap water only
ab, etc: the same letter indicates no significance at $p=0.05$ by Duncan multiple range test
GC: ginsenoside content, GPS: ginsenoside pattern similarity
*significant at $p=0.001$

tent and GPS are shown in Table 6. GC was very high when one of N, P, K was eliminated from the solutions(-N, -P, -K in Table 6) and tap water(B in Table 6). When NPK were balanced, GC was medium. When either N or P was high, GC tended to decrease. Root growth had no relation to GC. There was no significant change in GPS. In the field condition the relationship between crude saponin content and mineral content in soil showed yearly variation(Table 7). The higher the soil phosphorus the higher the GC was, while the higher the nitrate nitrogen in soil the lower the GC was only in one year(1985). These results are not quite in accordance with the case of solution culture(Table 6) in which N or P did not exert any significant influence. Soil phosphorus is one of hazardous nutrients for ginseng growth in most plantation. The high phosphorus content might decrease soil water potential by salt accumulation under the shade roof during dry season. In 1986 ammonium nitrogen, manganese and electric conductivity(EC) of soil solutions increased crude saponin content while organic matter content(OM), magnesium, calcium and pH decreased crude saponin. High ammonium nitro-

Table 7. *Correlation(r) between soil chemical characteristics and saponin content.*

	pH	EC	OM	NO_3(N)	NH_4(A)	N+A	P
1984(16)	-0.058	0.185	0.009	0.172	0.306	0.194	0.051
1985(26)	0.028	-0.181	0.016	-0.416**	0.036	-0.343*	0.371*
1986(28)	-0.365*	0.343*	-0.464**	0.020	0.407**	0.353*	0.239
	K	**Ca**	**Mg**	**Fe**	**Mn**	**Cu**	**Zn**
1984(16)	0.166	-0.112	-0.019	0.177	0.213	-0.026	0.179
1985(26)	0.054	0.003	-0.260	0.263	0.319	0.133	0.142
1986(28)	-0.247	-0.489***	-0.378**	0.029	0.337*	-0.205	-0.312

( ): number of fields, ***,**,*: $p=0.01$, 0.05 and 0.1

gen and EC are harmful for ginseng growth and quality but high OM, Ca and Mg are beneficial. Physiological role of saponin in root is yet unknown. It is posturated that saponin exerts preventive machanism against hazardous condition. Thus saponin content is highest in epidermis and next in cortex as shown in Fig.5. The fact that unfavorable growth condition increases saponin content indicates that high saponin content is not always necessary to high quality of ginseng.

Soil physical factors

The relationship between crude saponin content and soil physical factors was shown in Table 8. Saponin content was in significant relation negatively with soil moisture content and positively soil air phase percentage. Yearly variation in physical factors was much less than in soil chemical factors(Table 7). Correlation coefficient was almost same in direction indicating that soil physical conditions exert consistent effect on saponin content. Soil moisture, liquid phase and porosity are three favorable factors for ginseng growth and red ginseng quality[13-15]. These factors were negatively correlated with saponin content(Table 8). Soil air phase percentage is generally great when moisture content is low. The above facts suggest that favorable condition for ginseng growth did not require much saponins. It is well in accordance with the case of unfavorable soil chemical condition as mentioned above(Table 7).

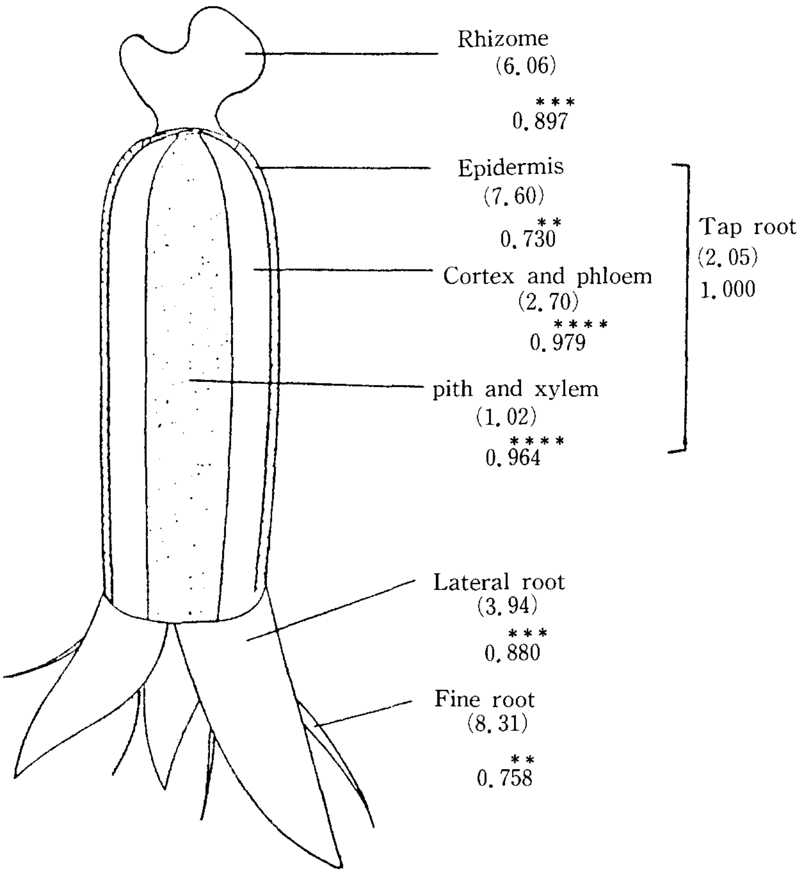

Fig.5. *Ginsenosides content(% dw) and pattern similarity(r) to that of tap root.*

Table 8. *Correlation(r) between saponin content and soil physical properties.*

	Mositure	Phase			Porosity	Bulk density	Bed height
		Solid	Liquid	Air			
1984(19)	-0.246	0.202	-0.238	0.079	-0.231	0.115	-0.065
1985(26)	-0.211	0.169	-0.119	-0.112	-0.265	0.271	0.047
1986(27)	-0.403**	-0.105	-0.265	0.372*	0.106	-0.132	-0.135

( ): number of fields **,*: p=0.05 and 0.1

Growth light and temperature

Effect of growth light on GC and GPS of ginseng leaves in a phytotron was shown in Table 9. When the growth light intensity increased GC greatly increased at 15℃ and 25℃ but pattern of ginsenoside did not changed. At all light intensities leaf ginsenoside content increased with the increase of growth temperature from 15℃ to 25℃. PT/PD was increased with the increase of growth light at 15℃ but decreased at 25℃. Thus the relation between GC and PT/PD is different according to growth temperature. Ginseng plant grown under natural light in a phytotron increased saponin content in leaves with the increase of light intensity from 5% to 30% of full sun light at 15℃ and from 10% to 20% at 20℃(Table 10). When the light intensity increased leaf water content decreased. Whether the increase of saponin content in leaves under high light intensity is due to water stress or sun light itself is uncertain. Saponin content was also higher at 20℃ than at 15℃(Table 10). Superoptimum light or temperature would be direct stress or indirect stress such as water stress as seen in Table 10. It could be generalized that saponin content is increased under stress condition even in aerial part. GC, GPS and growth of ginseng root at three air temperatures in a phytotron experiment were shown in Table 11. Effect of temperature on GC was not significant but the general trend was seen. When the root growth was best at 20℃, there appeared the lowest GC. A very hazardous temperature, 30℃, GC drastically

Table 9. *Ginsenosides content and pattern similarity of P. ginseng leaves at various growth light in a phytotron.*

Growth light (Klux)	Ginsenosides (mg/g FW)		Pattern similarity(r)		PT/PD	
	15°C	25°C	15°C	25°C	15°C	25°C
5	20.5	27.5	1.000	0.984*	0.972	1.215
10	28.4	35.5	0.996*	0.974*	1.056	1.084
15	30.2	50.0	0.980*	0.996*	1.142	1.018

4 years old, 3 months after emergence. *; p=0.001

Table 10. *Crude saponin content and water content in P ginseng leaves grown at various light intensities and temperatures.*

	15°C			20°C	
LTR(%)	5	15	30	10	20
Saponin (%FW)	2.11	4.47	5.12	6.34	6.90
Water content(%)	79,9	72.6	71.5	77.5	74.8

LTR: Light transmission rate

Table 11. *Ginsenosides content(GC) and pattan similarity(GPS) of roots grown at various temperature conditions in phytotron.*

	GC			GPS		
	15°C (17/15)[a]	20°C (23/18)	30°C	15°C	20°C	30°C
Xylem-pith (mg/g DW)	3.08	3.59	20.86	0.992**	1.000	0.968*
Cortex-phloem (mg/g DW)	19.74	13.86	23.59	0.998**	1.000	0.956*
Tap root (mg/g DW)	11.72	8.86	22.06	0.999**	1.000	0.981**
Root growth(%)	4.2	26.4	-31.5	—	—	—

a: (day/night) b: no emergence **,*: p=0.001 and 0.01

increased. The increase of GC at 30℃ was partly due to the decrease of dry matter but far beyond it. The GC increase was much greater in xylem-pith than in cortex-phloem. More experiments are needed for the further elucidation. In spite of drastic change of GC GPS was not much changed and only significantly decreased at 30℃ that is harmful for ginseng growth. At this temperature ginseng root did not emerge. Effect of growth light intensity on GC and GPS of root in field condition was shown in Table 12. GC increased consistently with the increase of light intensity but pattern was little changed. Thus the growth light intensity will not alter ginsenoside pattern of root in the allowable range of light intensity for ginseng leaves. PT/PD tended decrease with the increase of GC. In other field experiment growth light increased GC in all parts of the root, especially in xylem-pith, lateral root and epidermis(Table 13). Ginsenoside pattern change of a part by the change of growth light intensity can be easily

Table 12. *Ginsenosides content and pattern similarity in phloem and cortex of p. ginseng root at various growth light.*

LTR(%)	5	10	20	30
Content (mg/g dw)	13.6	16.3	17.7	19.1
Pattern similarity(r)	1.000	0.994*	0.996*	0.978*
PT/PD	1.22	1.15	1.15	1.00

6 years old root, *: p=0.001

Table 13. *Effect of growth light on ginsenosides content and pattern similarity.*

Growth light LTR	Ginsenosides (mg/g FW) 5%	15%	PT/PD 5%	15%	Similarity (r)
Xylem-pith	2.22	5.36	1.43	1.39	0.883***
Cortex-phloem	9.66	12.50	0.75	0.57	0.890***
Lateral root	8.84	16.80	0.61	0.43	0.788**
Fine root	19.27	21.68	0.31	0.29	0.917***
Epidermis	21.85	39.39	0.67	0.60	0.932***
Tap root	6.99	10.65	0.80	0.72	0.884***
Whole root	9.93	15.61	0.54	0.44	0.898***

4th year root, July 8th, ***,**; p=0.01 and 0.05
LTR: Light transmission rate

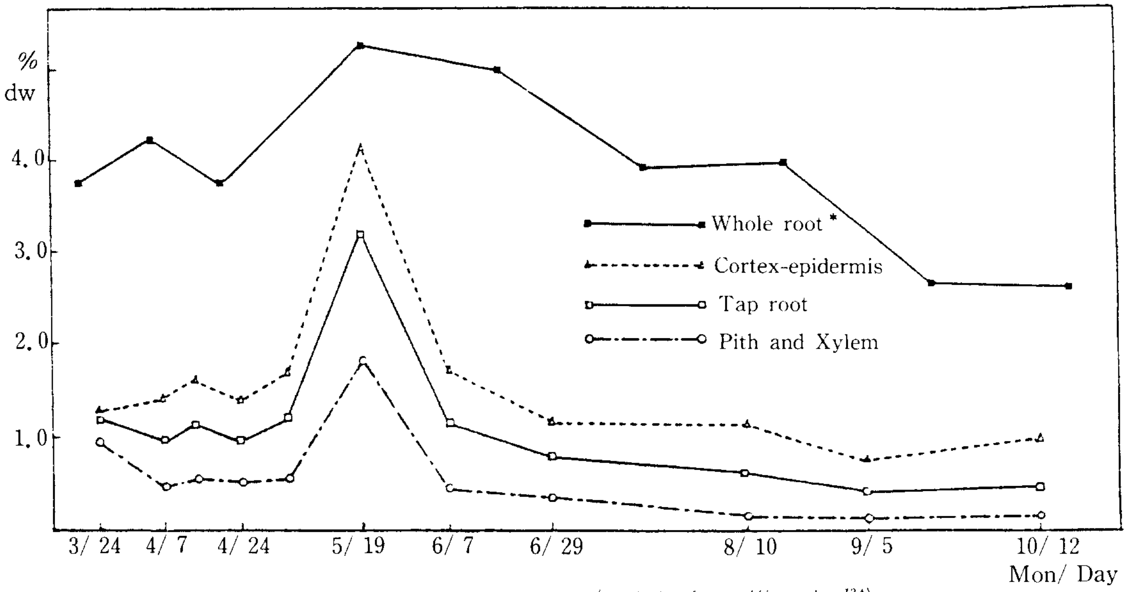

Fig.6. *Seasonal change of ginsenosides content(mg/g dw)* from Kim et al*[34].

learnt by GPS. The pattern change was not great. The greatest change occured in the lateral root. The trim of lateral root tip in the traditional red ginseng processing seems to be reasonable for keeping better ginsenoside pattern. GPS was affected differently by the change of light intensity. In Table 12 GPS between 5% and 20% of growth light was very high($p=0.001$) but in Table 13 it was lower($P=0.01$) even between 5% and 10%.

It may be due to the interaction of other environmental factors with light intensity due to field experiments.

Shading material

In the field condition the change of shading material induces the changes of various factors such as light intensity, air and soil temperature and soil moisture content. Primary concern choosing polyethylene shading net(PEN) by some growers is for labor saving. Great impact of PEN on ginseng plant is the better light environment in spite of the high risk of super-optimum temperature and water stress, and subsequent leaf blight damage. Effect of shading materials and other cultivation practice on GC and GPS in plantation survey was shown in Table 14. Either shading material or other cultivation practice affected little on GPS. In Korea ginsenoside pattern seems to be similar everywhere.

Physiological disease

When soil condition is poor such as severe dry or wet, root scab or root rust occur. GC and GPS of scabrous or rusty roots were shown in Fig.7. GC increased remarkably in xylem-pith of rusty root while it decreased in epidermis. GPS

Table 14. *Ginsenosides content(GC) and pattern similarity(GPS) in relation to cultivation method.*

	Conventional	Polyethylene net	Straw mulch	Rice precrop
GPS	1.000	0.9976****	0.9956****	0.9960****
PT/PD	1.103	1.104	1.192	1.197
GC(%DW)	0.95	1.05	0.96	0.85

Tap root, 1985, ****; $p=0.001$

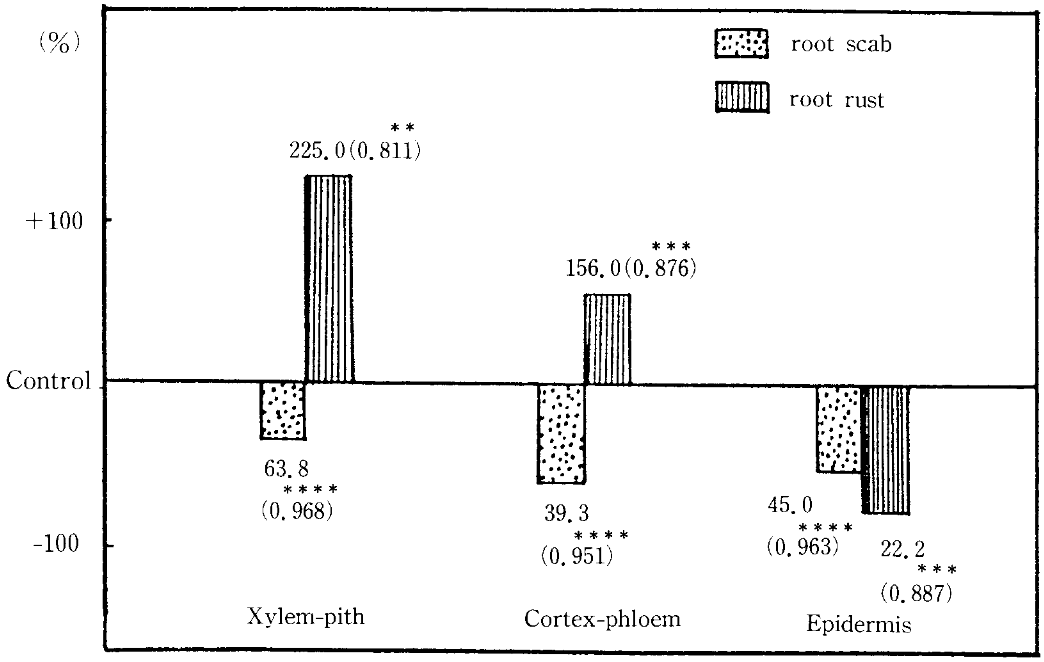

Fig.7. *Relative ginsenosides content and pattern similarity in tap roots with various physiological disease.*
( ): pattern similarity, n=7
, *, **** : p=0.05, 0.01 and 0.001

was significantly decreased at xylem-pith. GC decreased in all parts of scabrous root but the change of GPS was not significant. Scabrous root was the decreasing case of saponin in the environmental stresses. GC for good quality ginseng seems to be medium and not too high as rusty root and not too low as scabrous root. Scabrous or rusty roots are eliminated during selection of raw ginseng in red ginseng processing. Sponge-like tissue of tap root and inside cavity of fresh ginseng are kinds of physiological disorder. Some roots were characterized being negative to iodine test indicating low starch. Such kind of roots can not make red ginseng. All kinds of such problem roots showed higher saponin content than that of healthy root(Table 15). It was not due to dry matter decrease. The saponin content alone can not be a quality criterion.

Crop stand

The correlation between crude saponin content and crop stand was shown in Table 16.

Table 15. *Crude saponin contents of tap root in relation to tissue characters.*

		Normal tap root	Mild sponge	Severe sponge	Inside cavity	I_2 negative
Cortex	% dw	3.78	4.75	4.52	5.26	6.26
+						
Phloem	mg/cm^3	9.45	11.40	11.75	11.05	—
Xylem	% dw	1.76	2.72	2.06	2.64	2.90
+						
Pith	mg/cm^3	4.75	6.26	4.74	5.28	—

Table 16. Correlation(r) between saponin content of tap root and crop stand.

	Yield	Missing rate	Harvest date	Percent leaf fall
a 1984(20)	-0.191	0.330	-0.358	—
b 1985(26)	0.233	0.069	0.154	0.080
c 1986(24)	-0.026	0.192	-0.205	-0.227

( ): number of fields
a: Whole root, b: Tap root, c: Cortex-epidermis of main root

Table 17. Ginsenosides content and pattern similarity between callus and common root of Panax ginseng.

		Culture method					
		1-1	1-2	1-3	2-1	2-2	2-3
Content (mg/100g dw)		721	466	735	334	575	347
Similarity	n=5	-0.698	0.099	0.014	0.382	0.045	0.225
(r)	n=6	0.001	0.249	0.348	0.668	0.650	0.549
		3-1	3-2	3-3	4-1	4-2	KR
Content (mg/100g dw)		131	285	177	457	313	315
Similarity	n=5	0.398	0.306	0.241	-0.394	-0.155	1.000
(r)	n=6	0.729*	0.664	0.569	0.442	0.502	1.000

*; p=0.1, KR; common root from Korea

There was no significant relation but yearly variation. Positive correlation coefficient with missing rate is a positive trend that saponin content increases in undesirable condition. Negative trends between crude saponin content and harvest date in 1984 and 1986 indicate that saponin content decreases from the late August to the middle October. This result is well in accordance with the seasonal change of GC(Fig. 6).

Tissue culture

PD/PT has been used for quality index in ginseng tissue culture[3,8]. Recently comparison of thin layer chromatogram by eye balling is used[6,19]. For such fashion the report on ginsenoside composition of culture tissue is hardly seen. Ginsenoside content in calli cultured various culture mediums by Ushiyama[8] and the calculated GPS between callus and normal root are shown in Table 17. When six ginsenosides were compared, only method 3-1 showed significant similarity(P=0.1). Similarity was not significant when minor ginsenoside Rh was eliminated. When minor ginsenoside Ra was included, two methods(3-1 and 3-2) were significant(P=0.05). Treatment 1-1 was MS medium. It showed the highest negative GPS similar to leaf. It may be related to the growth of calli under light. Highest GPS(P=0.01) was shown in one case of suspension culture(Table 18). GC was not high at optimum condition at which dry matter production was maximum. Insignificant GPS between callus and root in most cases strongly indicates that tissue culture may not be successful for ginseng mimics even in terms of saponin. Ginsenoside pattern similarity among callus, rootlet differentiated from callus and normal root calculated from Ushiyama's data[8] is shown in Table 19. Callus-delivered rootlet was closer to root from Japan than to callus in terms of GPS. GPS between the callus-delivered rootlet and common root from

Table 18. *Ginsenosides content(GC) and pattern similarity(GPS) between root and callus.*

		Yang et al (1978)	Furuya et al (1983)	Chi et al (1985)
GC	Root	1.49 (5.60)	0.086 (0.53)	0.082
%FW(%DW)	Callus max.	0.19 (2.04)	0.109[b](2.16)	0.084[b](5.75)
	min.	—	0.014[b](0.34)	0.009[a](0.24)
	opt.	—	0.077[b](1.29)	0.057[b](3.90)
GPS	Root	1.000	1.000	1.000
	Callus	0.105(n=5)	0.880***(n=7)	0.702*(n=7)
	Root	1.18	0.70	1.18
PT/PD	Callus max.	1.62	3.21[b](1.84)[a]	0.77[a]
	min.	—	0.55[b](0.33)[a]	0.30[b]

a: static culture, b: suspension culture max., min.: in GC opt.: which is maximum in dry weight ***,*: p=0.01 and 0.1

Korea was not significant. Although GPS decreased significantly by eliminating minor ginsenoside Rh, GPS among callus, rootlet and root appeared to be reasonable(Table 19). GPS between callus and root is poorer than that between rootlet and root whether GPS is significant(with R in Table 19) or insignificant(with KR in Table 19). These facts strongly suggest that shape-compound relation-ship exists. The consistent shape-compound relationship can be well expected because the shape must be decided by the size of each organ, the chemical characteristics of which will be unique and consistent in a species. In traditional method of red ginseng quality control the root shape comes first.

Table 19. *Ginsenosides pattern similarity[a] among callus(C), tissue cultured rootlet(CR) and common root of P. ginseng.*

	C (335)	CR (479)	R (351)	KR (315)
	n=7			
C n=6		0.778**	0.878***	0.572
CR	0.744*		0.853**	0.519
R	0.822**	0.859**		0.817**
KR	0.379	0.409	0.718*	

***,**,*; p=0.01, 0.05 and 0.1
KR: Root from Korea in Japan market
R: Root from drug store in Japan
a: Calculated from Ushiyama[7]
( ): total ginsenoside (mg/100gDW)

Growth location

Effect of growth location on GC and GPS was shown in Table 20. Comparison was made between means of fields in each location. Ginsenoside patterns were almost same in spite of wide range of location. Among 13 fields GPS were not different(Table is not shown). The highest GPS was r=0.978(P=0.001) and lowest r=0.932(P=0.001). In other's reports[21,25,32] the effect of growth location on GPS was also very little as shown in Table 21. In Japan[1,35] however the growth location more significantly affected ginsenoside pattern(Table 21). It may be due to that Japan is not natural habitat for *P. ginseng.*

Table 20. *Ginsenosides content and pattern similarity of tap root in relation to growth location.*

	Pocheon	Gangwha	Kimpo	Baghak	Yangju
GC(%DW)	0.99	0.88	0.79	1.05	0.74
PT/PD	1.16	1.20	1.19	1.07	1.05
GPS	1.000	0.997****	0.981****	0.995****	0.990****

6 years old, ****; p=0.001

Table 21. *Effect of growth location on GPS of ginseng root.*

Location	GPS	Reference
Pocheon		
—Kimpo(Kp)	0.987****	Cho(1977)
—Kumsan(Ks)	0.963****	〃
Kp—Ks	0.987****	〃
Pocheon		
—Jeungpyung(Jp)	0.997****	Choi et al. (1984)
—Goesan(Goe)	0.996****	〃
—Jinchun(Jc)	0.987****	〃
Jp—Jc	0.996****	〃
Jp-Goe	0.998****	〃
Jeonju		
—Jeungpyung	0.999****	Kim et al. (1987)
Nagano(Japan)		
---Fukushima	0.966***	Namba et al. (1974)
---Aizo	0.764**	Otsuka et al. (1977)

****,***,**; significant at $p=0.001$, 0.01 and 0.05

It may be due to the uniformity of cultivation method. It is very good point to produce uniform quality ginseng in Korea.

Conclusion

Physiological activities of ginsenoside are so versatile that grouping into diol and triol is less meaningful. Ginsenoside pattern similarity(GPS) by simple correlation appears to be better criteria for quality assessment. Traditional quality criteria are well in accordance with keeping the high GPS. General belief that the higher the ginsenoside content the better the quality seems to be wrong. Under the best growth condition ginsenoside content is neither high nor low. Physiological diseases and unfavorable growth condition increased ginsenoside content. GPS showed great variation depending on age, part and least by location, mineral nutrition, light and temperature in Korea.

Tissue cultured rootlet showed closer pattern to the common root than the callus. The shape-compound relationship could be fully supported by GPS. Ginsenosides pattern and content of Heaven grade red ginseng should be the standard criteria of red ginseng quality.

Only the best growth condition guarantees the best quality.

References

1. Namba, T., M. Yoshizaki, T. Tomimori, K. Kobashi, K. Mitsui and J. Hase: Fundamental studies on the evaluation of the crude drugs III. Chemical and biological evaluation of ginseng and related crude drugs. *Yakugaku Zasshi* **94:** 252-260(1974).
2. Kita, K. and M. Sugii: Tissue culture studies on *Panax ginseng* C.A. Meyer I. On the cultural requirements of callus. *Yakugaku Zasshi* **89:** 1474-1476(1969).
3. Jhang, J.J., E.J. Staba and J.Y. Kim: American and Korean ginseng tissue cultures: Growth, chemical analysis and plantlet production. *In vitro* **9:** 253-259(1974).
4. Furuya, T., T. Yoshikawa, Y. Orihara and H. Oda: Saponin production in cell suspension cultures of *Panax ginseng*. *Planta Medica* **48:** 83-87(1983).
5. Furuya, T., T. Yoshikawa, Y. Orihara and H. Oda: Studies of the culture condition for *Panax ginseng* cells in jar fermentors. *J. Natural Products* **47:** 70-75(1984).
6. Chi, H.J. and H.S. Kim: The production of ginseng saponins with the cell culture of Korean ginseng plant. Korea *J. Pharmacogn.* **16:** 171-174(1985).
7. Han, B.H. and L.K. Woo: Chemical and biochemical aspects of dammarane triterpene glycosides of Koeran ginseng. *J. Pharm. Soc. Korea* **19:** 144-158(1975).
8. Ushiyama, K.: Culture of *Panax ginseng* root, In "Plant Biotechnology " Yamada, K. and Y. Okada ed. *Mordern Chemistry Supplement* **5:** 96 -104(1985).
9. *Ginseng'85-Its basic and clinical research*- Kumakai, A., H. Oura and H. Okuda ed. pp.270 Kouritsu Pub. Co. LTD. Tokyo, Japan.
10. Park, H., M.K. Lee and Q.H. Park: Pattern of molecular aggregation of ginsenoside in aqueous solution. *J. Kor. Agr. Chem. Soc.* **29:**

198-206(1986).
11. Takemoto, T., S. Arihara, T. Nakajima and M. Okuhir: Studies on the constituents of Gynostemma pentaphyllum Makino. I. Structures of Gypenoside I-XIV. *Yakugaku Zasshi* **103:** 173-185(1983).
12. Takemoto, T., S. Arihara, K. Yoshikawa, K. Kusumoto, I. Yano and T. Hayashi: Studies on the constituents of Cucurbitaceae Plants VI. On the saponin constituents of Luffa cylindrica Roem(1). *Yakugaku Zasshi* **104:** 246-255(1984).
13. Park, H., J.H. Yun, M.K. Lee, B.K. Cho, J.S. Byen and C.Y. Lee: Study on effect of cultural condition on quality of raw ginseng. pp.165 Korea Ginseng and Tobacco Reasearch Institute(1984).
14. Park, H., J.H. Yun, M.K. Lee, B.K. Cho, Y.J. Chang and C.Y. Lee: Study on effect of cultural condition on quality of red ginseng. *Ginseng Research Report* (cultivation), pp.215-466. Korea Ginseng and Tobacco Research Institute(1985).
15. Park, H., M.K. Lee, J.H. Yun, M.K. Lee, B.K. Cho and C.Y. Lee: Study on effect of cultural condition on inside cavity and inside white of red ginseng. *ibid* pp.377-454. Korea Ginseng and Tobacco Research Institute(1986).
16. Park, Q.H., M.K. Lee and N.G. Kim: Studies on the ginseng saponin biosynthesis. *ibid* pp.270-304. Korea Ginseng and Tobacco Res. Inst. (1981).
17. Lee, C.H., H. Park, M.K. Lee, B.K. Cho and H. S. Park: Studies on the ginseng plant metabolism. In "Studies on Ginseng Nutrition and Physiology ". pp.153-212. Korea Ginseng and Tobacco Research Institute(1983).
18. Lee, C.H. and M.K. Lee: Studies on ginseng saponin biosynthesis. *Ginseng Res. Report* (cultivation) pp.187-212. Korea Ginseng and Tobacco Research Institute(1982).
19. Yang, R., Y.C. Choi, H.J. Kim, S.C. Lee and S.H. Park: Study on the changes in saponins from ginseng callus by tissue culture. Part I. Comparison of saponins from callus tissue and from the root of ginseng plant. Korean *J. Food Sci. Technol.* **10:** 181-188(1978).
20. Park, H., M.K. Lee and C.H. Lee: Effect of nitrogen, phosphorus and potassium on ginsenoside composition of *Panax ginseng* root grown with nutrient solution. *J. Korean Agric. Chem. Soc.* **29:** 78-82(1986).
21. Cho, S.H.: Saponins of korean ginseng(*Panax ginseng* C.A. Meyer(part III)) Saponins of ginseng by the cultivating locations, sampling seasons, plant parts, growing stages and the processings. *J. Korean Agric. Chem. Soc.* **20:** 188-204(1977).
22. Lee, C.H., K.Y. Nam and K.J. Choi: Relationship between the age and chemical components of ginseng root's portion(*Panax ginseng* C.A. Meyer). Korean *J. Food Sci. Technol.* **10:** 263-268(1978).
23. Jang, J.G., K.S. Lee, D.W. Kwon, K.Y. Nam and J.H. Choi: Study on the changes of saponin contents in relation to root age of *Panax ginseng.* Korean *J. Food Nutrition.* **12:** 207-210(1983).
24. Kim, M.W., K.J. Choi, J.D. Park, S.C. Kim and S.R. Ko: Studies on isolation and purification of ginseng saponin. *Ginseng Res. Report* (Processing) pp.179-228. Korea Ginseng and Tobacco Res. Inst.(1985).
25. Kim, M.W., S.R. Ko, K.J. Choi and S.K. Kim: Distribution of saponin in various sections of *Panax ginseng* root and changes of its content according to root age. ***Korean*** *J. Ginseng Sci.* **11:** 10-16(1987).
26. Soldati, F and O. Tanaka: *Panax ginseng:* Relation between age of plant and content of ginsenosides. Planta Medica **49:** 351-352(1984).
27. Soldati, F. and O. Sticher: HPLC separation and quantitative determination of ginsenosides from *Panax ginseng,* Panax quinquefolium and from ginseng drug preparations. *Planta Medica* **38:** 348-357(1980).
28. Hong, S.K., E.K. Park, C.Y. Lee and M.U. Kim: High performance liquid chromatographic determination of ginseng saponins: *Yakhak Hoeji* **23:** 181-186(1979).
29. Kim, H.J., S.H. Nam, Y. Fukura and S.K. Lee: Studies on the ginseng saponins. The patterns of ginseng saponin in the commercial ginseng teas and each parts of ginseng plant. Korean *J. Food Sci. Technol.* **9:** 24-30(1977).
30. Tani, T., M. Kubo, T. Katsuki, M. Higashino, T. Hayashi and S. Arichi: Histochemistry II: Ginsenosides in ginseng(*Panax ginseng,* root). *J. of Natural Product* **44** : 401-407(1981).

31. Bombardelli, E., A. Bonati, B. Gabetta and E.M. Martinelli: Gas-liquid chromatographic method for determination of ginsenosides in *Panax ginseng*. *J. Chromatog.* **196:** 121-132(1980).
32. Choi, K.J., J.D. Park, S.C. Kim and S.R. Ko: Studies on isolation and purification of ginseng saponin. *Ginseng Res. Report* (processing). Korea Ginseng and Tobacco Res. Inst.(1984)
33. Yahara, S., K. Kaji and O. Tanaka: Further study on dammarane-type saponins of roots, leaves, flower-buds and fruit of *Panax ginseng* C.A. Meyer. *Chem. Pharm. Bull.* **27:** 88-92(1979).
34. Kim, S.K., I. Sskamoto, K. Morimoto, M. Sskata, K. Yamasaki and O. Tanaka: Seasonal variation of saponins, sucrose and monosaccharides in cultivated ginseng roots. *Planta Medica* **42:** 181-186(1981).
35. Otsuka, H., Y. Morita, Y. Ogihara and S. Shibata: The evaluation of ginseng and its congeners by droplet countercurrent chromatography(DCC). *Planta Medica* **32:** 9-17(1977).