韓藥作誌(Korean J. Medicinal Crop Sci.) 25(4) : 217 – 223 (2017)

ISSN(Print) 1225-9306
ISSN(Online) 2288-0186
http://www.medicinalcrop.org
http://dx.doi.org/10.7783/KJMCS.2017.25.4.217

인삼 품종별 경흔적 분포 및 홍삼의 진세노사이드 종류에 따른 연근 판별

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Distribution of Stem Vestige according to Ginseng Cultivars and Determination of Root Age by Ginsenoside Types of Red Ginseng

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ABSTRACT

Background: In Korea, 6-year-old ginseng root is economically more important than 4 or 5-year-old roots. In general, the root age is determined by counting the number of stem vestiges. However, this method does not accurately estimate ginseng root age.
Methods and Results: In this study, the stem vestige counting method was used to survey a total of 18,395 fresh ginsengs cultured in 2014, and 2015, to determine the accuracy of this method. The proportion of 6-year-old roots, with more than four stem vestiges, was 46.1% in 2014. For the cultivar Chunpoong cultivated in Eumseong and Goesan countries in 2015, the proportion of more than four stem vestiges was 55.9%, and 43.5%, respectively. The proportion of more than four stem vestiges for the Gumpoong cultivated in Eumseong and Yangpyeong countries was 67.0%, and 35.1%, respectively, whereas that for the cultivar Yunpoong was 36.0% and 61.0%, respectively. Moreover, it was confirmed that differences in the levels of Rg1 will enable root age determination.
Conclusions: Root age determination by the stem vestige test was found to differ depending on the environmental and cultivation conditions. To determine the age of ginseng roots, a comprehensive method, such as counting stem vestiges and evaluating differences in ginsenoside levels, should be applied.

Key Words **:** *Panax ginseng* C. A. Meyer, Cultivar, Ginsenoside, Rhizome, Root Age, Stem Vestiges

INTRODUCTION

Ginseng (*Panax ginseng* C. A. Meyer) is a perennial medicinal crop that is widely cultivated in the Asian region. Ginseng has been shown to improve the immune system (Park *et al*., 2015), reduce fatigue, enhance blood circulation and antioxidative effect, improve memory. It also helps to maintain body homeostasis. The effect is greater for the 6-year-old root than for the 4 to 5-year-old root (Oliynyk and Oh, 2013), therefore, the 6-year-old roots command a higher price. For this reason, there have been cases where 4 or 5-year-old roots were disguised as 6-year-old root, for the purposes of false distribution. To prevent the falsification of age, the National Agricultural Products Quality Management Service (NAQS) has recommended that age be determined using the stem vestige test (MAFRA, 2016).

A single stem of a ginseng develops once a year, and a vestige is created when it senescences. A 6-year-old root usually has 4 recognizable stem vestiges, because the

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Received 2017 June 22 / 1st Revised 2017 July 21 / 2nd Revised 2017 August 8 / 3rd Revised 2017 August 16 / Accepted 2017 August 17

2 stem vestiges generated in the first two years are usually not observable, as they are buried within the epidermis as the tuber during early growth. Therefore, a root with 4 stem vestiges is 6-year-old (MAFRA, 2016; Lee *et al*., 2007).

The stem can develop one or more in any given year, depending on the cultivation conditions, and this reduces the reliability of the stem vestige test. Several alternative methods have been suggested for determining the age of ginseng: the number of secretory duct layer using histochemical staining method (Lee *et al*., 2001) which confirms the number of secretory duct layer in dyed samples of cut root using an electron microscope; annual ring method (Lee *et al*., 1996) which confirms the number of annual rings in the cut root; ginsenoside analysis (Court *et al*., 1996; Lee *et al*., 2004; Li and Wardle, 2002) for analyzing specific saponin content for each age group; and age discrimination of ginseng using metabolomics approach (Kim *et al*., 2012; Li *et al*., 2012; Yang *et al*., 2012; Han *et al*., 2013).

These methods all require physical damage to the fresh root, therefore cannot be applied to the whole plant, limiting their usefulness. A major problem arising from the stem vestige method is the underestimation of root age if the stem vestiges are not all easily visible, which can reduce the profitability of the industry at all levels, from the farm to the export market, which eventually leads to loss in the national level.

This study compared ginseng plants by region, cultivar and the changes in the number of stem vestiges before and after the manufacture of red ginseng, in order to confirm the accuracy of age determination test of the current stem vestige.

The present study investigated whether the characteristics of stem vestiges vary among three different ginseng varieties across different regions.

Moreover, the existing methods have focused on the root age identification of rhizomes while lacking results for red ginseng. Therefore, this study sought to verify whether the stem vestige method was suitable for root age identification of red ginseng. Furthermore, this study analyzed whether the contained volume of specific ginsenoside in particular varieties enabled the root age identification.

Table 1. Plant materials used in this study.

Cultivars	Year			Collection region
	2014	2015		
	KGC[1)]	KGC	RDA	
Landrace	7,597	–[2)]	–	Nationwide
Chunpoong	–	453	220	Goesan, Eumseong
Yunpoong	–	659	100	Eumseong
Gumpoong	–	850	100	Yangpyeong, Eumseong
Total	7,597	1,962	420	9,979

3 cultivars (Chunpoong, Yunpoong and Gumpoong) cultivated in Eumseong were controlled by RDA, and cultivars cultivated in Goesan and Yangpyeong were controlled by KGC. The purity of the cultivars was maintained over 95%. [1)]KGC; institute that investigated this study. In order to identify the characteristics of stem vestige of cultivars cultivated in different regions in 2015, we divided them into two regions. [2)]–; non investigated.

MATERIALS AND METHODS

1. Plant materials

9,979 fresh ginseng (*Panax ginseng* C. A. Meyer) roots used in this study were collected from different geographical regions in South Korea from 2014 to 2015 (Table 1). In 2014, stem vestige number was surveyed in 7,597 violet-stem Landrace of 6-year-old fresh ginseng and was again surveyed on these plants after their manufacture into red ginseng. In 2015, the Korea Ginseng Corporation (KGC) surveyed 453 Chunpoong and 850 Gumpoong (Lee *et al*., 2015), a cultivar with a high rate of occurrence of 1 stem development per year (Lee *et al*., 2015), and 659 Yunpoong that has a high occurrence rate of 2 stems development per year (Kwon *et al*., 2000).

In the same year, the Rural Development Administration (RDA) surveyed 220 Chunpoong, 100 Yunpoong, and 100 Gumpoong cultivars. All the materials used in this study are 6-year-old fresh ginseng.

2. Investigation of stem vestige in rhizome

The determination method of stem vestige age was conformed to the guidelines for the Inspection and Management of Ginseng recommended in the MAFRA (2016). The root part of ginseng that was used for the survey was rhizome. The number of stem vestige remaining in the rhizome was measured, and the shape and number of the stem vestige are shown in Fig. 1.

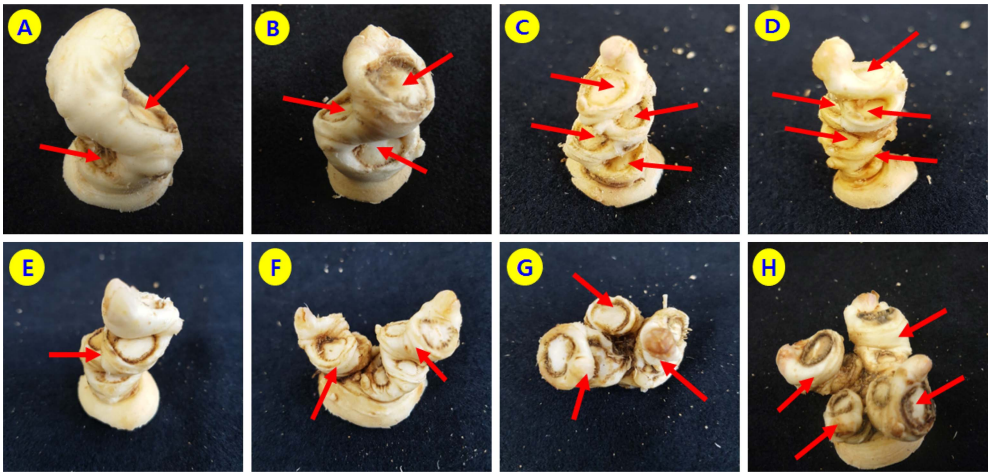

Fig. 1. Stem vestiges in rhizome of 6-year-old root. A; 2 stem vestiges, B; 3 stem vestiges, C; 4 stem vestiges, D; 5 stem vestiges, E; 1 rhizome, F; 2 rhizomes, G; 3 rhizomes, H; 4 rhizomes.

The root age of ginseng was measured by adding 2 to the stem vestige in a root according to the Lee *et al.* (2007) method.

3. Analysis of ginsenoside

4, 5 and 6-year-old ginsengs grown in the Biology Resource Research Center of KGC were cultivated. For the samples used in the analysis, the number of stem vestige according to the root age was clear and 60 g, 80 g, and 100 g were used for the 4, 5, and 6-year-old ginseng weights, respectively.

After red ginseng preparation, fine roots were completely removed and used for ginsenoside analysis so that the ratio of main roots and lateral roots was 87% : 13%. Red ginseng samples were ground in 40 mesh and analyzed. Ten milliliters of 50% methanol was added to 0.5 g of sample and extraction was performed in an ultrasonic cleaner (60 Hz, Wiseclean, Seoul, Korea) for 30 min. After ultrasonic extraction, centrifugal separation (Legand Mach 1.6R, Thermo Fisher Scientific Inc., Waltham, MA, USA) was performed for 10 min at 3,000 rpm. The supernatant solution was filtered using a syringe filter (0.2 ㎛, Acrodisk, Gelman Sciences Inc., Ann Arbor, MI, USA) and used as the test solution.

The UPLC test was conducted using a ACQUITY UPLC system (Waters, Millford, MA, USA). The chromatographic separation was carried out on ACQUITY BEH C18 column (2.1 × 50 ㎜, 1.7 ㎛, Waters, Milford, MA, USA) at 40℃. The gradient elution system consisted of water (A) and acetonitrile (B) using following program; 0 - 0.5 min (15% B), 14.5 min (30% B), 15.5 min (32% B), 16.5 min (40% B), 17 min (55% B), 20 min (55% B), 21 min (90% B), 23 min (90% B), 25 min (15% B) and 27 min (15% B). The flow rate was 0.6 ㎖/min, sample injection volume was 2.0 ㎕ and detection wavelength was 203 ㎚. The standard materials of ginsenoside was obtained from the ChromaDex (Irvine, CA, USA) and acetonitrile, MeOH were purchased from Sigma-Aldrich (Sigma-Aldrich Co., St. Louis, MO, USA). Water used in this experiment was purified by a Milli-Q gradient system (Merck Millipore, Billerica, MA, USA) and the resistance value was measured as 18 MΩ prior to use.

4. Statistical analysis

The results were analyzed via Student's *t*-test using the SAS sofware package (SAS v9.2, SAS Institute Inc., Cary, NC, USA), and the difference was proven to be statistically significant when the *p*-value was under 0.05

RESULTS

1. Investigation of stem vestige in fresh and red ginseng

In the 2014 survey of 7,597 fresh roots, it was found that the proportion of fresh ginseng (*Panax ginseng* C. A. Meyer) with over 4 stem vestiges was 46.2% in a root, whereas the proportion of root with less more than 3 stem vestiges was 53.8% (Table 2). The occurrence of

Table 2. Distribution of stem vestiges of fresh ginseng and red ginseng.

(Unit; %)

	Number of total stem vestige in a individual								
	1	2	3	4	5	6	7	8	above 9
Fresh ginseng[1)]	0.5	14.3	39.0	24.0	13.0	6.2	2.1	0.6	0.3
Red ginseng	0.8	5.8	22.6	39.8	15.6	10.3	3.3	1.1	0.7

[1)]This samples were investigated in the KGC's task force team in 2014.

root with more than 2 stems in fresh sample of the 2014 was 99.5%. The proportion of root with 4 or more stem vestiges after their manufacture into red ginseng was 70.8%, and the proportion of root with 3 or less stem vestiges was 29.2% (Table 2).

This is probably due to the fact that stem vestiges that were not seen in the fresh ginseng state appeared during the process of red ginseng with contraction and drying.

2. Investigation of stem vestige in ginseng cultivars

In 2015, the number of stem vestiges was surveyed in the cultivars Chunpoong and Gumpoong, which have high occurrence rates of 1 stem per year. The Chunpoong was cultivated in two separate places of Goesan and Eumseong. The proportion of root with 4 or more stem vestiges cultivated in Goesan was 43.5% and the proportion with 3 or less stem vestiges was 56.5%. The proportion of root with 4 or more stem vestiges cultivated in Eumseong was 55.8%, while the proportion with 3 or less stem vestiges was 44.2% (Table 3).

Lee *et al*. (2007) reported that the proportions of Chunpoong and Gumpoong cultivars with 4 stem vestiges are respectively 66.3% and 70.0%, while the occurrence rates in ginseng plants with more than 2 stem vestiges are 17.9% and 8.2%, respectively. In this study, the comparative values of 25.6% and 21.8% were obtained, which are relatively small. This was because the occurrence rates of Chunpoong and Gumpoong with more than 2 stems had increased to 52.2% and 44.5% respectively (data not shown). The Yunpoong, with a high occurrence rate of more than 2 stems, with more than 4 stem vestiges took up 61.0% and those with 3 or less stem vestiges took up 39.0% in Yangpyeong, and took up 64% and 36%, respectively in Eumseong.

In a study by Lee *et al*. (2007), the occurrence rate of Yunpoong with more than two stems was 56.8%, which is similar to the result of 53.3% obtained in the present study (data not shown). As the occurrence rate of plants with more than 2 stems increases, the stem vestige test becomes less accurate for determining age of root.

The RDA cultivated Chunpoong, Gumpoong and Yunpoong at the ginseng experiment field of National Institute of Horticultural and Herbal Science Institute, Eumseong. The ratios of Chunpoong and Gumpoong with more than 4 stem vestiges were respectively 55.8% and 67.0%, while the ratio was 39.0% for Yunpoong (Table 3). Lee *et al.* (2007) reported that the stem vestige test result for Yunpoong was 48.0%, which is greater than the result obtained in the present study. This is seemingly due to the influence of cultivation conditions.

As a consequence, the proportions of ginseng roots that were determined as 6-year-old were 46.2% in 2014, which can be deemed as similar. The 6-year-old Chunpoong ginseng plants cultivated in Goesan and Eumseong in 2015 were 43.5% and 55.8%, respectively. Whereas that in the 6-year-old Gumpoong was 35.1% in Yangpyeong, and 67.0% in Eumseong (Table 3). It is indicated that

Table 3. Difference in the stem vestige number for 3 cultivars by different regions.

Cultivar	Location	Number of stem vestige in a root						
		2	3	4	5	6	7	8
Chun-poong	Goesan	3.3[1)]	53.2	25.6	13.0	3.3	1.3	0.3
	Eumseong	1.8	42.4	52.9	2.9	0.0	0.0	0.0
Gum-poong	Yangpyeong	14.4	50.5	21.8	10.2	2.7	0.4	0.0
	Eumseong	4.0	29.0	67.0	0.0	0.0	0.0	0.0
Yun-poong	Yangpyeong	6.4	32.6	33.4	17.1	7.4	2.0	1.1
	Eumseong	8.0	28.0	39.0	23.0	2.0	0.0	0.0

[1)]proportion of root (%).

Table 4. Significant difference in the content of ginsenosides in the Chunpoong.

(Unit; ㎎/g, drybasis)

Ginsenosides \ Years	4	5		4	6		5	6	
Rg1	1.18±0.0	2.05±0.2	**	1.18±0.0	2.89±0.2	**	2.05±0.2	2.89±0.2	*
Re	0.90±0.1	1.01±0.1	ns[1)]	0.90±0.1	1.68±0.3	*	1.01±0.1	1.68±0.3	**
Rf	0.48±0.0	0.45±1.1	ns	0.48±0.0	0.60±0.0	ns	0.45±1.1	0.60±0.0	ns
Rh1	0.06±0.1	0.07±0.0	ns	0.06±0.1	0.11±0.0	ns	0.07±0.0	0.11±0.0	ns
Rg2	0.26±0.2	0.29±1.1	ns	0.26±0.2	0.46±0.5	*	0.29±1.1	0.46±0.5	*
Rb1	2.45±0.1	2.66±1.1	ns	2.45±0.1	4.04±0.2	**	2.66±1.1	4.04±0.2	**
Rc	0.72±1.1	0.78±0.0	ns	0.72±1.1	1.23±0.6	**	0.78±0.0	1.23±0.6	**
Rb2	0.49±1.1	0.52±0.5	ns	0.49±1.1	0.92±1.3	**	0.52±0.5	0.92±1.3	**
Rd	0.16±0.1	0.16±0.0	ns	0.16±0.1	0.22±0.0	ns	0.16±0.0	0.22±0.0	ns
Rg3	0.06±0.0	0.07±0.0	ns	0.06±0.0	0.12±0.0	ns	0.07±0.0	0.12±0.0	ns
Total	7.40±1.0	8.05±0.8		7.40±1.3	7.69±1.5		8.05±1.3	7.69±1.1	

Mean values ± SD from 30 red ginseng samples analysis. ns; Non significant. *t*-test was used to compare 4 and 5 years, 4 and 6 years, and 5 and 6 years (*$p < 0.05$, **$p < 0.01$).

Table 5. Significant difference in the content of ginsenosides in the Yunpoong.

(Unit; ㎎/g, drybasis)

Ginsenosides \ Years	4	5		4	6		5	6	
Rg1	2.92±0.2	3.78±0.1	**	2.92±0.2	5.24±0.7	**	3.78±0.1	5.24±0.7	**
Re	1.38±1.2	1.08±0.0	ns[1)]	1.38±0.2	2.04±0.2	**	1.08±0.0	2.04±0.2	**
Rf	0.97±0.5	0.72±0.3	ns	0.97±0.5	1.38±1.2	**	0.72±0.3	1.38±1.2	**
Rh1	0.11±0.2	0.09±0.3	ns	0.11±0.2	0.18±1.2	ns	0.09±0.3	0.18±1.2	ns
Rg2	0.39±0.2	0.30±0.2	ns	0.39±0.2	0.55±0.4	ns	0.30±0.2	0.55±0.2	ns
Rb1	4.72±0.3	3.96±1.0	**	4.72±0.3	7.90±0.0	**	3.96±1.0	7.90±0.0	**
Rc	1.21±1.0	1.05±0.3	ns	1.21±1.0	1.74±0.2	**	1.05±0.3	1.74±0.2	**
Rb2	1.06±0.1	0.88±0.8	**	1.06±0.1	1.49±0.0	ns	0.88±0.8	1.49±0.0	**
Rd	0.35±0.0	0.23±0.4	ns	0.35±0.0	0.39±0.1	ns	0.23±0.4	0.39±0.1	ns
Rg3	0.16±0.4	0.11±0.0	ns	0.16±0.4	0.17±0.2	ns	0.11±0.0	0.17±0.2	ns
Total	13.27±1.2	11.21±1.5		13.27±2.3	13.80±2.0		11.21±1.5	13.80±1.7	

Mean values ± SD from 30 red ginseng samples analysis. ns; Non significant. *t*-test was used to compare 4 and 5 years, 4 and 6 years, and 5 and 6 years (*$p < 0.05$, **$p < 0.01$).

there can be a difference in the proportion of plants determined to be 6-year-old, even within the same cultivar, due to different region and cultivation conditions. Thus, new method to determinate age of ginseng root has to be developed.

3. Characteristics of ginsenoside content by cultivars

Regarding the changes in Rg1 content among the ginsenosides by root age, Chunpoong contained 1.18 ㎎/g at 4 years of age, 2.0 ㎎/g at 5 years of age, and 2.89 ㎎/g at 6 years (Table 4). Yunpoong contained 2.92 ㎎/g, 3.78 ㎎/g, and 5.24 ㎎/g at 4, 5, and 6 years, respectively (Table 5); Gumpoong contained 2.36 ㎎/g, 2.66 ㎎/g, and 2.89 ㎎/g at 4, 5, and 6 years, respectively (Table 6), displaying statistically significant differences.

There was also a significant difference in the Rb1 of the Yunpoong. However, the content of 5-year-old was lower than that of 4-year-old. While it was difficult to ascertain the identity of ginseng based on the ginsenoside content by root age, this study revealed that root age could be identified using Rg1 content.

Future research should further verify the possibility of root age identification using ginsenoside content based on the region of origin or root age. However, root age identification inferred from the total Rg1 content was not consistent these results were in line with those reported by Han *et al*. (2013) and Li *et al*. (2012).

The proportion of ginseng plants determined to be 6-

Table 6. Significant difference in the content of ginsenosides in the Gumpoong.

(Unit; ㎎/g, drybasis)

Ginsenosides \ Years	4	5		4	6		5	6	
Rg1	2.36±0.0	2.57±0.2	**	2.36±0.0	2.79±0.0	**	2.57±0.2	2.79±0.0	**
Re	0.84±0.1	0.72±0.5	ns[1)]	0.84±0.1	1.60±0.4	**	0.72±0.5	1.60±0.4	**
Rf	1.22±0.2	0.48±1.0	**	1.22±0.2	0.62±0.7	**	0.48±1.0	0.62±0.7	ns
Rh1	0.09±0.0	0.09±0.1	ns	0.09±0.0	0.11±0.1	ns	0.09±0.1	0.11±0.1	ns
Rg2	0.25±0.2	0.22±0.0	ns	0.25±0.2	0.43±0.3	ns	0.22±0.0	0.43±0.3	ns
Rb1	3.78±1.0	3.26±0.0	ns	3.78±1.0	3.18±0.4	ns	3.26±0.0	3.18±0.4	ns
Rc	1.14±0.0	0.99±0.0	ns	1.14±0.0	0.93±0.2	ns	0.99±0.0	0.93±0.2	ns
Rb2	0.80±0.1	0.73±0.3	ns	0.80±0.1	0.93±0.1	ns	0.73±0.3	0.93±0.1	ns
Rd	0.39±0.0	0.32±0.2	ns	0.39±0.0	0.24±0.0	ns	0.32±0.2	0.24±0.0	ns
Rg3	0.12±0.0	0.11±0.0	ns	0.12±0.0	0.09±0.1	ns	0.11±0.0	0.09±0.1	ns
Total	10.90±1.2	9.32±1.5		10.90±1.3	10.92±2.1		9.32±1.5	10.92±2.1	

Mean values ± SD from 30 red ginseng samples analysis. ns; Non significant. *t*-test was used to compare 4 and 5 years, 4 and 6 years, and 5 and 6 years (*$p < 0.05$, **$p < 0.01$).

year-old using the naked-eye inspection of stem vestiges showed large differences between the survey years and the stem development characteristics differed from region to region (within cultivar). Overall, the proportion of ginseng plants determined to be 6-year-old did not exceed 70%. The stem number of a ginseng plant is a quantitative trait in which multiple genes are related (Kim *et al*., 2015). The number of stems can vary, depending on the shading type, micro environment in field, soil fertility and texture, cultivar, early shatter, and other factors; therefore, it can become unclear that we know the development of stem vestiges, which hinders the accurate determination of the ginseng plant's age. In addition, some ginsengs can develop more than 2 stems at the yielding period of 4-year-old ginseng, and this can be falsely presented as a 6-year-old ginseng for the purposes of distribution. Since the number of stem vestige in ginseng roots varies depending on the type of cultivar, cultivation area and environment, it is impossible to use this method for environmental change, various varieties, dormant ginseng root and ginseng with poor growth. However, root age identification based on ginsenosides complex because it varies significantly by region and age.

Falsification of ginseng age can tarnish the reputation of international trade reliability as well as consumer confidence in the product. This could potentially cause serious harm or even destroy the infrastructure of the ginseng industry. Therefore, until a new method for root age identification is developed, good agricultural practices should be utilized to record root age for preventing any confusion in distribution order.

ACKNOWLEDGMENTS

The 2014 survey was conducted by the fresh and red ginseng task force team. Special thanks to those who provided necessary data. And, this work was carried out with the support of cooperative research program for agriculture science and technology development(PJ01018701). Rural Development Administration, Republic of Korea.

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