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파종 방법에 따른 고려인삼의 대사체 비교

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Comparative Analysis of Metabolites in Roots of *Panax ginseng* Obtained from Different Sowing Methods

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ABSTRACT : Ginsenosides of roots in *Panax ginseng* were analyzed by metabolic-targeting HPLC using the partial least squares discriminant analysis (PLS-DA) and compared depending on sowing methods between direct seeding and transplanting method. Score plots derived from PLS-DA could identify the sowing method between the direct seeding and transplanting method in *P. ginseng* roots. The ginsenoside compounds were assigned as Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3, and Rd. Contents of Re, Rf, Rg2, Rb1, Rc, Rb3, and Rd of main roots produced from the transplanting method were relatively higher than those of samples produced from direct seeding method. Also, contents of Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3, and Rd of lateral roots from the transplanted samples were relatively higher than those of samples produced from direct seeding method. Therefore, HPLC with PLS-DA analysis can be a straightforward tool for identification of ginsenosides in main or lateral roots of *P. ginseng* obtained from two different seeding methods between direct and transplanting methods.

Key Words **:** *Panax ginseng* Root, *S*owing Method, Metabolic Targeting, HPLC

INTRODUCTION

Panax ginseng C. A. Meyer (Araliaceae) is a medicinal herb around the world. The ginseng products have been rapidly increased and expanded in the commercial market. Ginseng has been known to have ginsenoside compounds including protopanaxadiols and potopanaxatriols. Rb1, Rb2, Rb3, Rc, Rd, Rg3, and Rh2, were classified in the group of 20-(s)-protopanaxadiols, and Re, Rf, Rg1, Rg2, and Rh1 were identified as the group of 2-(s)-protopanaxatriols (Lü *et al.*, 2009; Cho *et al.*, 2010; Han *et al.*, 2013). Ginsenosides are the major components having biological activities and pharmacological effects in ginsengs (Keith and Mark, 2003; Nah *et al.*, 2007; Carlini, 2003; Xie *et al.*, 2005; Yun, 2003; Choi, 2008; Kim *et al.*, 1992, 2011; Lim *et al.*, 2010; Attele *et al.*, 2002).

Commonly, the shape and weight of ginseng roots are important properties evaluating the quality of ginseng. The shape of roots can determine the quality and cost of fresh ginseng in commercial markets. There are two methods

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cultivating ginseng, either direct seeding or transplanting method. The direct seeding can define the plant grown from seeds directly in the ground. Direct seeding cultivation can omit the step raising seeds, sorting, digging of young ginseng, and transplanting seeds (Lee *et al*., 2005). On the other hand, the transplanting method was to demonstrate the effect of growing condition on mature ginseng roots in the first year. In addition, it was expected to explore opportunities to manage beds and manipulate the shape of root in transplanted ginseng, which may be a common practice in future (Roy *et al*., 2008).

The metabolomics using target analysis can be an effective tool for performing metabolites analysis and profiling medicinal plants. Also, it has been used in the agriculture fields including the quality control of crops and the assessment of plant breeding (Okada *et al*., 2010). The typical equipment includes chromatography and spectroscopy, such as liquid chromatography (LC) and gas chromatography (GC) coupled with mass spectrometry (MS) and nuclear magnetic resonance (NMR), which are recommended for quality control in medicinal plant (Lan *et al*., 2010; Sangster *et al*., 2006; Van *et al*., 2009). LC-based equipment has higher resolution and sensitivity than NMR, and its use has been more various and convenient than GC. High performance liquid chromatography (HPLC) can be used for analyzing a wide range of metabolites, polar, thermo stable or non-derivatization samples (Wilson *et al.,* 2005). It can be applied in the fields of plant, clinical, and biomarker discovery (Warwick and David, 2005). There have been a few previous studies regarding to investigation of metabolomics in ginseng roots, such as the identification of different origins (Kang *et al*., 2008), the differentiation of cultivation ages (Yang *et al*., 2011), and the quality control of ginsengs (Yang *et al*., 2006). However, there are no report regarding to the differentiation of the metabolic compounds and the prediction of different sowing method in main or lateral roots of *P. ginseng* obtained from the same region.

Therefore, this study compared the metabolic targeting and prediction of main or lateral roots of *P. ginseng* obtained from different cultivation methods between direct seeding and transplanting method using an HPLC coupled with multivariate statistical.

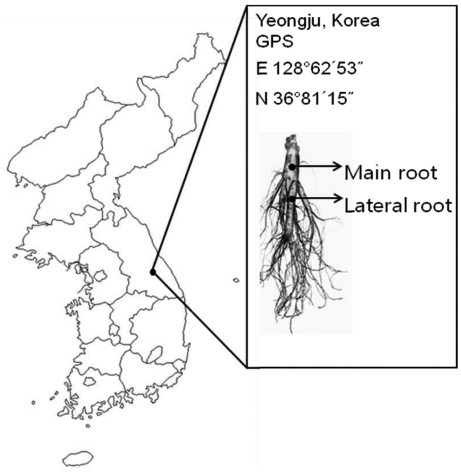

Fig. 1. Geographic location of Yeongju in Korea and parts of ginseng root.

MATERIALS AND METHODS

1. Solvents and Chemicals

The standards of ginsenoside, Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, and Rg2, were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile and methanol were obtained from J. T. Baker (Phillipsburg, NJ, USA) and S.K Chemicals (Ulsan, Korea), respectively.

2. Plant Materials

P. ginseng of 5 year-old ginseng root were collected from Yeongju province (GPS: E 128°62′53″N 36°81′15″) in Korea during October in 2007 (Fig. 1). Main and lateral roots (each 10 roots) of 5 year-old ginseng was obtained from direct and transplanting seeding method. The main and lateral part of ginseng root was divided as following Fig. 1. The root samples were freeze-dried and stored in a −70℃ frseezer prior to analysis.

3. Sample Preparation and Extraction

The main and lateral roots of ginseng were put in 25 mL centrifuge tubes (Corning, Union City, California, USA) and extracted with 15 mL of 99.8% methanol for 24 h at room temperature under the continuous shaking. Each sample was analyzed by HPLC after filtering with polyvinylidene fluoride syringe filter (0.45 ㎛, Whatman, Piscataway, NJ, USA).

4. HPLC Analysis

Methanol extracts of main and lateral roots in *P. ginseng* were separated and identified using HPLC equipped with an ultraviolet detector (Agilent 1100 series, Santa Clara, CA, USA). Sample separation was achieved using a 5 ㎛ Capcell pak C_{18} MGII column (150 ㎜ × 3.0 ㎜ I.D., Shiseido, Co. LTD, Tokyo, Japan). Injection volume was 20 μL, and UV absorbance was at 203 ㎚. The flow rate of mobile phase was 0.8 mL/min^{-1} and column oven temperature was 30℃. The Mobile phase was composed of a 100% acetonitrile (solvent A) and 100% water (solvent B). The gradient program of mobile phase was a linear gradient from 30% solvent A and 70% solvent B ($t = 0$ min) to 100% solvent A at $t = 70$ min.

5. Statistical Analysis

The 9 variables were normalized for principle component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). PLS-DA was performed with SIMCA-P software (version 13.0, Umetrics, Umea, Sweden). Data were analyzed statistically by *t*-test using SPSS software program (SPSS Inc., Chicago, IL, USA). A P-value < 0.05 was considered to be significant difference.

RESULTS AND DISCUSSION

1. Assignment of the Peaks in *P. ginseng* Root Samples

HPLC analysis of *P. ginseng* was carried for comparing ginsenosides of methanol extracts in main and lateral roots obtained from different cultivation methods between direct seeding and transplanting method. Nine peaks were assigned in main and lateral roots of *P. ginseng,* indicating ginsenosides, Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3, and each Rd was identified using ginsenoside standards. The nine peaks of HPLC were quantified depending on a retention time/assignment (Table 1).

Table 1. Retention time and assignment of nine peaks of HPLC chromatogram of methanol extracts main and lateral of *P. ginseng* roots.

No.	RT (min)*	Assignment
1	24.88	Rg1
2	26.45	Re
3	45.35	Rf
4	53.69	Rg2
5	55.97	Rb1
6	58.13	Rc
7	59.70	Rb2
8	61.19	Rb3
9	62.96	Rd

*RT; Retention Time.

2. Difference of Metabolic Compounds between Direct Seeding and Transplanting Method of *P. ginseng* Roots

To investigate the differences of metabolites in the main or lateral root of *P. ginseng* between samples produced from direct seeding and transplanting cultivation, *P. ginseng* root samples were analyzed using HPLC-based metabolic targeting technique. PLS-DA is a PLS regression method with a 'dummy' y-variable and used class information to maximize the group classification and/or biomarker selections (Eriksson *et al*., 2006). We conducted PLS-DA using the processed HPLC chromatogram data to differentiate cultivation method of *P. ginseng* root samples by using metabolic targeting. The chromatogram data were scaled to Pareto and mean-centered by SIMCA-P 13.0 software.

We excluded 1 and 2 outliers in the preliminary PCA of 10 samples of each *P. ginseng* main and lateral root samples, respectively (data not shown), and PLS-DA was thus performed using 9 and 8 samples for each *P. ginseng* main and lateral, respectively. Figure 2 (A) and (B) show the score plots derived from the PLS-DA of *P. ginseng* main and lateral roots obtained from direct seeding and transplanting method, respectively. Direct seeding and transplanting samples of *P. ginseng* main and lateral root could be clearly separated by using PLS component 1 and 2. The two PLS components accounted for 85.4% and 94.1% of the total variance in main and lateral roots of *P. ginseng*, respectively.

Figure 2 (A and B) show the PLS-DA score plots (PLS component 1 and PLS component 2) derived from the HPLC chromatogram data. Parameter in PLS-DA including R^2 and Q^2 could evaluate the models, and demonstrate the goodness of fit and ability of prediction. When the values of R^2 and Q^2 are close to 1.0, the model has good capacity and prediction (Eriksson *et al*., 2006). PLS-DA modeling between direct seeding and transplanting revealed R_X^2, R_Y^2, and Q^2 value of 0.95, 0.76, and 0.65 for main root and 0.96, 0.96, and 0.93 for lateral root, respectively.

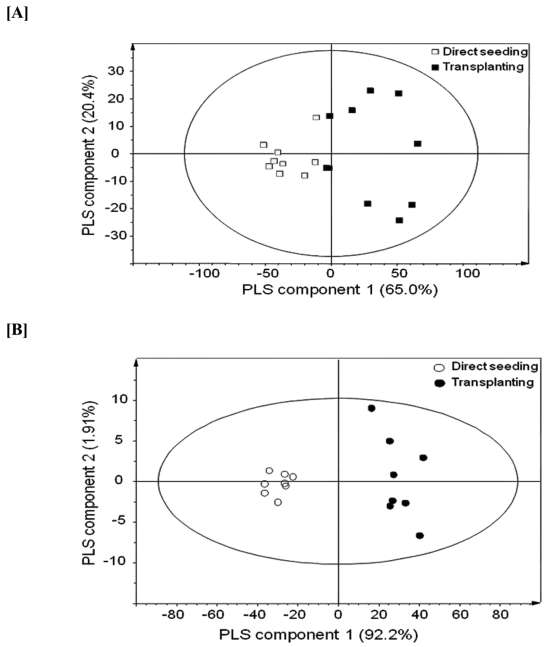

Fig. 2. PLS-DA derived score plots (A and B) obtained using HPLC chromatogram data of *P. ginseng* roots from different cultivation method demonstrating the separation between direct seeding and transplanting cultivation from main (A) and lateral (B). □; Main root by direct seeding, ■; Main root by transplanting, ○;Lateral root by direct seeding, ●; Lateral root by transplanting.

The PLS-DA score plot between direct seeding and transplanting in main and lateral roots of *P. ginseng* showed clear separation by PLS component 1 (Fig. 2A and B). The loading plot of *P. ginseng* main and lateral root from direct seeding and transplanting method showed that the main root of transplanting method contained higher amount of Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3, and Rd compounds than that of direct seeding cultivation.

Variable influence on projection (VIP) is a weighed sum of squares of the PLS taking into account the amount of Y-variance in each dimension. VIP values of 0.7-0.8 could be considered as important value for separation of each sample through a PLS model (Eriksson *et al*., 2006). As shown in Table 2, VIP values of the major contributing compounds in the score plots derived from PLS-DA (Fig. 2 (A and B)) were like follows; Re: 1.62, Rb1: 1.35, Rc: 1.31, Rb2: 0.84, Rf: 0.73, Rg1: 0.72, Rd: 0.70, in main root of *P. ginseng* samples, and Rg1: 2.01, Re: 1.23, Rb1: 1.15, Rf: 0.96, Rc: 0.72 in lateral root of *P.ginseng* samples.

To obtain clear information of the relative levels in each compound based upon the VIP analysis, *t*-test was performed and shown in Fig. 3. The levels of Re, Rf, Rg2, Rb1, Rc, Rb3, and Rd were significantly ($p < 0.05$ in all cases) higher in main root of transplanting method than direct seeding samples. The levels of Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3, and Rd were also significantly ($p < 0.05$ in all cases) higher in lateral root of transplanting cultivation than direct seeding samples.

This study found higher amount of Re representing protopanaxatriol and Rb1 and Rc representing proto panaxadiol in main root of *P. ginseng*, and Rg1, Re, and Rb1 representing protopanaxatriol in lateral roots of *P. ginseng*. Total amount of ginsenoside from main and lateral root was 1.9 times and 3.3 times higher in transplanting cultivation samples than direct seeding samples, respectively. According to the result comparing ginsenoside between direct seeding and transplanting method in the study of Li et al., total amount of ginsenoside in transplanting cultivation samples was 56.8% higher than direct seeding cultivation samples. The reason was

Table 2. VIP of various peaks of HPLC chromatogram of methanol extracts main and lateral of *P. ginseng* roots.

(A) Main root of *P. ginseng*

RT (min)*	VIP**	Ginsenosides
26.45	1.62	Re
55.97	1.35	Rb1
58.13	1.31	Rc
59.70	0.84	Rb2
45.35	0.73	Rf
24.88	0.72	Rg1
62.96	0.70	Rd
53.69	0.59	Rg2
61.16	0.38	Rb3

(B) Lateral root of *P. ginseng*

RT (min)*	VIP**	Ginsenosides
24.88	2.01	Rg1
26.45	1.23	Re
55.97	1.15	Rb1
45.35	0.96	Rf
58.13	0.72	Rc
55.97	0.61	Rb1
53.69	0.32	Rg2
61.19	0.27	Rb3
62.96	0.26	Rd

*RT; Retention Time, **VIP; Variable Influence on Projection.

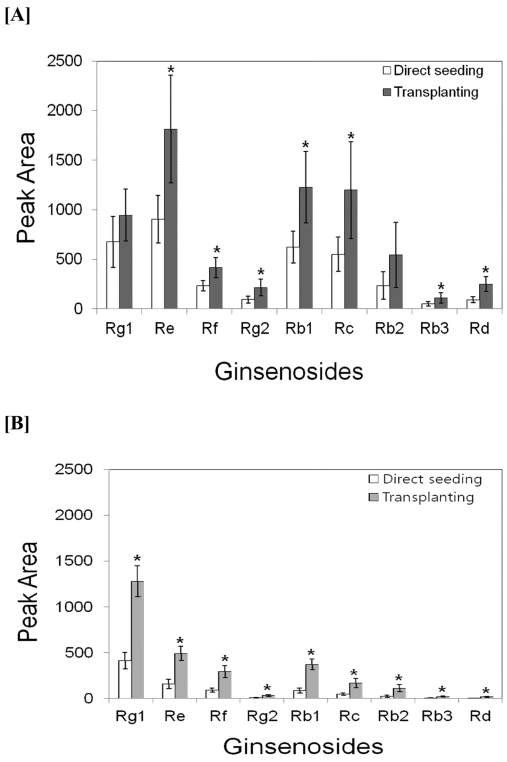

Fig. 3. Peak area of Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3, and Rd in *P. ginseng* root samples. *t*-test was performed to compare and assess statistical significance ($p < 0.05$). The error bars are expressed as the standard deviation. (A); Main root of *P. ginseng*, (B); Lateral root of *P. ginseng*.

demonstrated that the weight per individual ginseng obtained from samples of transplanting cultivation was heavier than that from direct seeding samples; especially lateral root in ginseng from transplanting cultivation was much more mature in comparison with that from direct seeding (Li *et al*., 2009). This result is consistent with Qu *et al*. (2009), showing higher amount of Re and Rb1 in main roots of American ginseng than those in root-hair of 5-years-old American ginseng. However, the amount of Re, Rb1, and Rc of root-hair in American ginseng was higher in order. Also, the amount of Rb1 and Rg1 of main roots in and Rb1 and Re of lateral root in *P. ginseng* was higher in Yunpoong ginseng cultivated in Daejeon, Korea (Li *et al*., 2009). Shi *et al*. (2007) compared the amount of ginsenoside in roots of 5-years-old *P. ginseng*, and confirmed that the amount of ginsenoside including Rg1, Rb1, and Re of main roots and Re, Rb1, and Rc of root-hair were higher in order.

This study investigated the amount of ginsenoside of main or lateral roots in *P. ginseng* cultivated by two different methods, such as direct seeding and transplanting method, and newly found that there was significant difference of ginsenoside between different cultivation methods according to multivariate statistical. The amount of ginsenoside of roots in *P. ginseng* was higher in transplanting method than direct seeding cultivation method.

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