Genotypic and Organ Variation in Ginsenoside Contents from American Ginseng Populations

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ABSTRACT. Variation in ginsenoside content was investigated as a function of population/genotype, plant organ, and age using four geographically isolated wild populations and one landrace population of american ginseng (*Panax quinquefolius* L.). The contents of individual and total ginsenosides were affected by the main and two-way interactions between population, organ, and age. Ginsenoside Re was not detected in roots of the wild population plants but was found in leaves and in both organs of the landrace population. A positive relationship between root age and total root ginsenosides was detected in two wild populations. Individual root ginsenosides were highly correlated with certain leaf ginsenosides in wild populations rather than in landrace populations. Therefore, the results suggest that certain leaf ginsenosides would be applied for potential biomarkers to estimate individual root ginsenosides. Principal component analysis (PCA) scores plot indicates that all wild populations were segregated from the single landrace population. However, cluster analysis indicates that differences existed between organs, and between the wild and landrace populations. Overall, the result suggests that the variation of individual and total ginsenoside contents would be influenced by a combination of population, plant organ, and root age.

American ginseng contains the secondary active pharmaceutical compounds, triterpene saponins, known as ginsenosides (Attele et al., 1999; Li, 1995; Proctor and Bailey, 1987). More than 30 kinds of ginsenosides have been isolated and structures reported in ginseng (Chuang and Sheu, 1994; Luchtefeld et al., 2004; Mahady et al., 2000). Individual or total ginsenosides have one or more specific beneficial functions and effects on human physiology and health, such as antitumor, antistress, central nervous system stimulation, and adaptogenic effects (Attele et al., 1999; Mahady et al., 2000; Qi et al., 2011; Sivakumar et al., 2005).

Ginsenosides have been classified into three categories, panaxadiols, panaxtriols, and oleanic acid, based on molecular structure, especially type and number of sugar moieties, and number and site of hydroxyl group (Attele et al., 1999). In american ginseng, the two major groups are 20(S)-protopanaxadiol and 20(S)-protopanaxatriol (Li et al., 1996; Wang et al., 2005, 2006). The most abundant ginsenosides reported in american ginseng root are ginsenosides Rg1 and Re, belonging to 20(S)-protopanaxatriol, and ginsenosides Rb1, Rc, Rb2, and Rd, belonging to 20(S)-protopanaxadiol (Corbit et al., 2005; Qi et al., 2011). Ginsenosides Rg1, Re, and Rb1 are higher than ginsenosides Rc, Rb2, and Rd in wild american ginseng populations (Assinewe et al., 2003; Lim et al., 2005; Schlag and McIntosh, 2006). However, the composition and contents of root ginsenosides in wild ginseng populations varies greatly (Assinewe et al., 2003; Lim et al., 2005), compared with landrace populations with respect to cultivation method,

season, and location (Chung et al., 2012; Lee et al., 2011; Lim et al., 2005; Smith et al., 1996). Even within a single ginseng farm, the contents of ginsenosides varied greatly (Hong et al., 2005). The storage root of american ginseng is the only organ that is used widely and commercially to meet the high demand for the consumption of pharmaceutical products and health supplements, but all the organs of american ginseng contain ginsenosides (Konsler and Shelton, 1990). The contents and composition of individual ginsenosides vary highly among ginseng organs (Jackson et al., 2003; Kim and Choi, 1987; Konsler and Shelton, 1990; Li and Mazza, 1999; Li et al., 1996; Wang et al., 2006). Among roots of individual plants, there is significant variation in the compositions and contents of individual and total ginsenosides (Smith et al., 1996). Similarly, among the roots of 6-year-old asian ginseng (*Panax ginseng* Meyer) plants, there was variation in the contents of the individual ginsenosides (Nam et al., 1998). Furthermore, the contents and compositions of ginsenosides in root are strongly affected by seasonal variation (An et al., 2002; Jochum et al., 2007; Kim et al., 1981; Li and Wardle, 2002), storage root age (Court et al., 1996; Mudge et al., 2004; Soldati and Tanaka, 1984), soil fertility (Konsler and Shelton, 1990), soil nutrients (Lee and Mudge, 2013a), and water deficit (Lee and Mudge, 2013b). Leaf ginsenosides are also influenced by the maturity of the foliar organ (Li et al., 1996). The contents of six major ginsenosides and total ginsenoside show considerable genotypic variation among geographically isolated wild ginseng populations (Assinewe et al., 2003; Konsler et al., 1990; Lim et al., 2005; Mudge et al., 2004). Also, the compositions of ginsenoside differ between root and foliar organs within the landrace american ginseng populations (Li and Mazza, 1999; Li et al., 1996). In addition, the contents of root ginsenosides were highly correlated with the estimated ginseng root age

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(Court et al., 1996; Mudge et al., 2004; Qu et al., 2009; Shi et al., 2007; Soldati and Tanaka, 1984), although not in all cases (Qu et al., 2009; Shi et al., 2007).

Ginsenoside Re and total ginsenosides in leaves may serve as phytochemical biomarkers for root ginsenosides because the accumulation pattern of ginsenoside Re and total ginsenoside in root was closely associated with those in leaf (Assinewe et al., 2003; Wang et al., 2006). Therefore, the primary objective of this study was to test the hypothesis that the contents of particular ginsenosides in leaves might be linked with the corresponding ginsenosides, the other ginsenosides in the roots from wild and landrace american ginseng populations or both. Additional objectives were to determine the contents and compositions of leaf and root ginsenosides between geographically isolated wild and landrace populations, to elucidate the effect of a storage root age on ginsenoside accumulation from wild ginseng populations. An additional objective was to evaluate the usefulness of non- or less destructively harvested leaf ginsenosides as biomarkers for root ginsenosides for screening for high quality ginseng population among geographically isolated wild populations of american ginseng.

Materials and Methods

SAMPLE COLLECTION AND PREPARATION. Several geographically isolated wild populations of american ginseng were identified and individual plants were tagged. The selected wild populations were growing under the dense shade of deciduous hardwood trees on northwest-, north-, northeast-, or east-facing slopes within Cornell University's Arnot Teaching and Research Forest (ATRF), near Van Etten, NY (Chemung County). Among the selected populations, four populations were identified. Each population was physically separated from all other populations by at least 1.5 km distance. The location of each population was recorded with a global positioning system. Soil samples from 10 cm depth of soil from soil surface were taken within 30 cm distance from the identified wild ginseng plants on 8–11 Aug. Ten soil samples per population were pooled and were then air-dried for 7 d. Nutrient elements were analyzed by inductively coupled plasma spectrophotometry at the Plant and Soil Nutrient Analysis Laboratory, Department of Crop and Soil Sciences, Cornell University, Ithaca, NY. Twelve individual ginseng plants, which had three prongs and five leaflets per prong, for total of 15 leaflets were randomly taken from each wild ginseng population and then shoot growth characteristics were evaluated. The roots were wrapped with a damp paper towel, put into a ziplock plastic bag, transferred to the laboratory, and then stored at 4 °C on 15 and 17 Aug. Twelve landrace ginseng plants, which originated from Wisconsin and seeded in 2000 at the forest garden of Cornell University's ATRF were taken on 17 Aug. After transfer to the laboratory, ginseng plants were washed with tap water to remove soil and then blotted with a paper towel to remove remaining water. After completing the measurement of shoot and root fresh weight (FW), leaf areas were recorded with a leaf area meter (LI-3100; LI-COR, Lincoln, NE) to obtain leaf area index (LAI). The age of each ginseng plant was estimated by counting the annual bud scars along the rhizome (Lim, 2005). Root diameter and total root length were also measured. Shoot tissues were dried at 70 °C for 3 d, for the measurement of shoot dry weight (DW) and analysis of leaf ginsenosides. Roots were placed in a forced-air food dehydrator at 35 °C for 7 d for

the measurement of root DW and the analysis of root ginsenosides. Dried ginseng roots and leaves were prepared by grinding to a powder with a tissue grinder (AG-2005; Angel Electronic, Seoul, Korea) and passed through a 60-mesh sieve screen for the analysis of ginsenosides.

GINSENOSE EXTRACTION AND ANALYSIS. Ginsenoside analysis followed the procedure of Lee and Mudge (2013a). One hundred milligram dried ground ginseng leaf and root tissue was extracted in 30 mL of 70% methanol (MeOH). The MeOH extract was vacuum-evaporated at 38 °C with a rotary evaporator (011; BUCHI Analytical, New Castle, DE), redissolved in 5 mL of 100% MeOH, and dried with a rotary evaporator. The residue was redissolved in 500 µL of 16% acetonitrile. Samples of 15 µL were analyzed by high-performance liquid chromatography [HPLC (2690 Separations Module HPLC; Waters Corporation, Milford, MA)], with a photodiode array detector (996, Waters) as an ultraviolet detector at 203 nm. Empower Pro software (Build 1154, Waters) was used for solvent gradient, peak identification, and integration. A reversed phase C₁₈ column [packing material LiChrosorb RP18, particle size 5 µm, length 250 mm, internal diameter 3 mm (HPLC Column; Varian, Lake Forest, CA)] was used with a guard column (Reversed Phase ChromSep Guard Column SS; Varian). A composition gradient of the two eluents of the mobile phase (A) 0.14% phosphate buffer and (B) 100% acetonitrile was 0–20 min, 84% to 82% A, 16% to 18% B; 20–60 min, 82% to 60% A, and 18% to 40% B. The flow rate was 1.15 mL·min⁻¹. As an internal standard, *m*-cresol (Sigma-Aldrich Chemical Co., St. Louis, MO) was added into each sample to confirm the injection volume and retention time per injection. Ginsenoside standards included ginsenosides Rg1, Re, Rb1, Rc, Rb2, and Rd (Indofine Chemical Co., Hillsborough, NJ). Individual ginsenosides from the extracts were identified and quantified by retention time and peak areas as compared with those of authentic ginsenoside standards.

STATISTICAL ANALYSIS. Each dependent variable (plant growth characteristics, and leaf and root ginsenosides) was individually analyzed using analysis of variance [ANOVA (SAS version 8.02; SAS Institute, Cary, NC)]. Following analysis of variation, mean separations of plant growth characteristics, and leaf and root ginsenosides were performed using Duncan's multiple range tests at *P* = 0.05 based on the mean square error for each dependent variable. Linear regression analysis was performed to determine the relationship between total ginsenosides and estimated storage root age by using the SigmaPlot (version 10.0; Systat Software, San Jose, CA). Pearson correlation coefficient analysis was performed to determine the relationships between plant growth characteristics and root and leaf ginsenosides. Pearson correlation coefficients were obtained by subjecting the data set using the correlation coefficient procedure (PROC CORR) in SAS (version 8.02). Red and blue colors indicated a positive and negative correlation coefficient between factors. To test whether leaf ginsenoside could contribute to the accumulation of root ginsenoside, analysis of covariance (ANCOVA) was performed using ANCOVA procedure (PROC ANCOVA) by controlling leaf ginsenosides.

To provide an overview of population diversity on the contents of ginsenosides, a PCA was applied to visualize population diversity and segregation between ginseng populations in ginseng organs based on the composition and contents of ginsenosides. The mean values of each ginsenoside from all

populations were transformed by mean centering and SD scaling and then PCA was performed using The Unscrambler (version 10.0.1; Camo, Trondheim, Norway). For the validation test, random cross validation method was applied by using 20 segments with three samples per segment. In addition, for the evaluation of phylogenetic relationships, the transformed data set was also used for hierarchical cluster analysis by using The Unscrambler (version 10.0.1), with hierarchical complete linkage clustering method and squared Euclidean as distance measure.

Results

Soil nutrients were mostly greater in wild populations than in landrace population. However, soil S, Fe, Mn, and Al contents were higher in landrace population than in wild populations. Soil acidity was about one unit lower in wild populations than in landrace population, whereas the organic matter was $\approx 1.5\%$ higher in wild populations than in the landrace population (Table 1).

Of plant growth characteristics, shoot DW, root diameter, root FW and DW, and estimated ginseng root age were higher in wild populations than in landrace population. However, sympodium height (stalk length from soil surface to base of petiole), leaf area, shoot FW, and total root length were not different among wild and landrace populations (Table 2).

Correlation analysis indicated that all plant growth parameters were positively correlated with each other but that leaflet number was not significantly correlated with any other plant

growth parameters in wild populations (Fig. 2A). By contrast, certain plant growth parameters, i.e., plant height, root length, root diameter, and leaf number were negatively correlated in landrace population (Fig. 2B).

The compositions and contents of six individual and total ginsenosides from wild and landrace populations showed differences between leaf and root (Table 3). In leaves, individual and total ginsenosides showed a significant variation among populations except for ginsenoside Rb1. Leaf ginsenosides Re, Rd, and total ginsenoside were greater in landrace population than in wild populations. Root ginsenosides Rg1 and Rb1 were higher in wild populations than in landrace population but root ginsenosides Re and Rd were lower in wild populations. Intriguingly, in roots, ginsenoside Re was not detected in any wild populations but present only in landrace population. As a main effect, the contents of ginsenosides Rc, Rb2, Rd, and total were affected by population. All ginsenosides were affected by organ (leaf and root) but only ginsenosides Rg1, Rb1, and total were affected by the estimated storage root age. There were significant two-way interactions between ginsenosides Rg1 and Rb2 for population and organs ($P \times O$), ginsenoside Rc and total ginsenoside for ginseng population and root age ($P \times A$), and ginsenosides Rg1, Rb1, Rc, and total ginsenoside for root age and organs ($A \times O$). There was no statistically significant three-way interaction.

To compare the effect on ginsenosides of wild populations (correctively, $n = 48$) with the landrace population ($n = 12$), ginsenoside Rb2 was higher in wild populations than in landrace population in leaf ginsenosides, whereas ginsenosides

Table 1. Soil nutritional characteristics of wild and landrace (or cultivated) populations of american ginseng.

Population ^z	K	Ca	Mg	P	S	Fe	Mn	Zn	Cu	Al	Soil pH	OM (%) ^y
	(mg·kg ⁻¹)											
P1	103.8 ^x	3568.7	151.4	14.2	10.1	33.1	148.0	7.0	6.1	65.3	5.3	12.7
P2	95.5	526.9	58.0	9.49	8.7	81.3	65.8	3.9	2.7	186.1	4.6	7.4
P3	103.3	1467.5	94.8	4.8	9.4	82.4	58.3	4.6	1.3	135.9	4.9	11.6
P4	74.2	665.8	78.9	6.1	18.7	112.6	59.8	4.8	1.9	196.8	4.5	8.5
Mean ^w	94.2	1557.2	95.8	8.6	11.7	77.3	83.0	5.1	2.9	146.0	4.8	10.1
P5	52.1	182.9	38.7	6.6	255.6	163.1	121.5	3.3	0.5	316.4	3.9	8.4

^zFour wild populations (P1, P2, P3, and P4) were consecutively assigned based on finding order. Each wild population obtained from within Cornell University's Arnot Teaching and Research Forest (ATRF, Van Etten, NY) was physically separated from all other populations by at least 1.5 km. P5 represents the landrace (or cultivated) population, which was cultivated at ATRF by using the seeds of cultivated ginseng landrace from Wisconsin.

^yOrganic matter.

^xValues were pooled from 10 individual soil samples.

^wMean value of four wild ginseng populations.

Table 2. Plant growth characteristics of four wild and one landrace populations in american ginseng.

Population ^z	Sympodium length (cm)	Leaf area (cm ²)	Shoot FW (g)	Shoot DW (g)	Root length (cm)	Root diam (mm)	Root FW (g)	Root DW (g)	Root age (yr)
P1	18.7 ^y	183.0	3.1	1.0 a ^x	19.4	15.6 a	6.1 a	2.6 a	13.3 ab
P2	21.8	194.6	4.0	0.7 ab	22.0	13.6 ab	6.0 a	2.1 a	16.1 a
P3	18.3	234.8	5.0	0.8 a	19.4	12.5 b	5.9 a	2.0 a	10.9 b
P4	16.7	166.4	4.1	0.7 ab	21.0	14.6 ab	7.1 a	2.3 a	12.9 ab
P5	18.4	174.1	3.4	0.5 b	15.1	10.1 c	2.8 b	0.9 b	4.0 c

^zFour wild populations (P1, P2, P3, and P4) were consecutively assigned based on finding order. Each wild population obtained from within Cornell University's Arnot Teaching and Research Forest (ATRF, Van Etten, NY) was physically separated from all other populations by at least 1.5 km. P5 represents the landrace population, which was cultivated at ATRF by using the seeds of landrace ginseng population from Wisconsin.

^yValues are the means of 12 replications per population ($n = 12$).

^xMeans accompanied by the same letters are not significantly different in Duncan's multiple range tests at $P = 0.05$.

FW = fresh weight; DW = dry weight.

Table 3. The contents of individual and total ginsenosides from leaf and root organs of four wild and one landrace populations in american ginseng.

	Ginsenosides (g·kg ⁻¹ DW) ^y						
Population ^z	Rg1	Re	Rb1	Rc	Rb2	Rd	Total
Leaf							
P1	2.5 c ^x	6.6 c	3.1	3.7 d	10.6 b	14.7 b	41.1 b
P2	4.1 ab	7.6 c	3.8	4.8 b	10.4 b	11.3 c	42.1 b
P3	3.7 b	7.4 c	4.0	4.3 c	7.7 c	10.5 c	37.5 b
P4	4.4 a	9.5 b	4.0	6.3 a	13.5 a	15.9 b	53.5 a
P5	3.7 b	12.0 a	4.7	4.0 cd	7.4 c	22.7 a	54.4 a
Root							
P1	15.9 a	0.0 b	11.2 abc	2.9 b	0.5	1.6 bc	32.1 abc
P2	14.2 a	0.0 b	13.0 ab	3.8 a	0.5	1.5 bc	33.0 ab
P3	11.0 b	0.0 b	8.6 c	3.4 ab	0.5	1.0 c	24.4 c
P4	16.2 a	0.0 b	14.4 a	3.2 ab	0.5	1.9 b	16.2 a
P5	2.4 c	6.5 a	9.6 bc	3.6 ab	0.5	2.6 a	25.3 bc
Significance							
Population (P)	NS	NS	NS	*	***	*	**
Organ (O)	***	***	***	**	***	***	***
Age (A)	**	NS	**	NS	NS	NS	*
P × O	*	NS	NS	NS	***	NS	NS
P × A	NS	NS	NS	*	NS	NS	*
O × A	***	NS	**	*	NS	NS	**
P × O × A	NS	NS	NS	NS	NS	NS	NS

^zFour wild populations (P1, P2, P3, and P4) were consecutively assigned based on finding order. Each wild population obtained from within Cornell University's Arnot Teaching and Research Forest (ATRF, Van Etten, NY) was physically separated from all other populations by at least 1.5 km. P5 represents the landrace population, which was cultivated at ATRF by using the seeds of cultivated ginseng landrace from Wisconsin.

^yValues are the means of 12 replications per population ($n = 12$).

^xMeans accompanied by the same letters are not significantly different only within a column for leaf or root, respectively, in Duncan's multiple range tests at $P = 0.05$.

NS, *, **, ***Not significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Re, Rd, and total ginsenoside were greater in the landrace population. By contrast, ginsenosides Rg1, Rb1, and Rc did not show any difference between wild and the landrace population. On the other hand, root ginsenoside Rg1, and total ginsenoside were greater in wild populations than in landrace population, whereas root ginsenoside Re was higher in landrace population than in wild populations. Of individual ginsenosides, ginsenoside Rg1 was the highest in root from overall populations, whereas ginsenoside Rd was the greatest in leaves from all populations.

The effect of the organ difference on the compositions and contents of ginsenosides within each population was evaluated. The content and composition of ginsenosides from wild and landrace populations, respectively, were significantly different between leaf and root organs. Within the four wild populations, ginsenosides Rg1 and Rb1 were greater in root than in leaf, whereas ginsenoside Rb1 was higher in the root than in the leaf only within the landrace population. Ginsenoside Re, Rb2, and Rd were greater in leaves than in roots for both wild and landrace populations. The contents of total ginsenoside from both wild and landrace populations were significantly greater in leaf than in root tissues. In a 4-year-old landrace population, total ginsenoside content was ≈ 2 -fold higher in leaf than in root.

As the contents of leaf and root ginsenosides were determined, it became pertinent to test whether leaf ginsenosides were associated with accumulation of root ginsenosides (Table 4). No significant effect of leaf ginsenosides on chemotypic responses of root ginsenosides was found, but the effects of population and

Table 4. Significance levels for effects of population, covariate leaf ginsenoside, and interactions on the contents of individual and total root ginsenosides from four wild and one landrace populations of american ginseng.

Factors	Root ginsenosides						
	Rg1	Re	Rb1	Rc	Rb2	Rd	Total
Population (P)	NS	NS	NS	NS	NS	***	NS
Leaf ginsenosides (LG)	NS	NS	NS	NS	NS	NS	NS
P × LG	NS	NS	NS	NS	NS	**	NS

NS, **, ***Not significant or significant at $P \leq 0.01$ or 0.001, respectively.

interaction between population and leaf ginsenoside variables were detected only in the case of ginsenoside Rd (Table 4).

Polynomial regression analyses were performed between total ginsenoside contents of leaf/root and the estimated ginseng storage root age as independent variables, only from wild populations (Fig. 1). The estimated ginseng root age was negatively correlated with total leaf ginsenoside but only in population 1 [$P = 0.038$ (Fig. 1A)], whereas no quadratic or cubic regression effect appeared in population 1. However, two positive relationships between estimated ginseng root age and total root ginsenosides were detected in wild populations 2 and 4 [$P = 0.004$ and $P = 0.005$, respectively (Fig. 1B)]. Linear, quadratic and cubic regression analyses were detected in population 2, whereas linear and quadratic regression responses only appeared in population 4.

To evaluate the relationship among plant growth parameters, individual and total ginsenosides between root and foliar

Fig. 1. Relationship between estimated storage root age and total ginsenoside contents from leaf (A) and root (B) organs of four wild populations in American ginseng ($n = 12$). Regression equations for total leaf ginsenoside (A) are $y = -0.834x + 52.192$ ($r^2 = 0.364$, $P = 0.038$) for population 1 (P1, black line), $y = 0.041x + 41.420$ ($r^2 = 0.008$, $P = 0.787$) for population 2 (P2, gray line), $y = 0.376x + 33.383$ ($r^2 = 0.103$, $P = 0.308$) for population 3 (P3, red line), and $y = -0.091x + 54.716$ ($r^2 = 0.037$, $P = 0.547$) for population 4 (P4, blue line). Regression equations for total root ginsenoside (B) are $y = 0.506x + 0.506$ ($r^2 = 0.109$, $P = 0.295$) for population 1 (P1, black line), $y = 1.409x + 10.379$ ($r^2 = 0.589$, $P = 0.004$) for population 2 (P2, gray line), $y = -0.090x + 25.351$ ($r^2 = 0.003$, $P = 0.863$) for population 3 (P3, red line), and $y = 0.698x + 27.157$ ($r^2 = 0.563$, $P = 0.005$) for population 4 (P4, blue line). Four wild populations (P1, P2, P3, and P4) were consecutively assigned based on finding order. Each wild population obtained from within Cornell University's Arnot Teaching and Research Forest in Van Etten, NY was physically separated from all other populations by at least 1.5 km. DW = dry weight.

organs, a Pearson correlation analysis was performed based on the responses of plant growth characteristics and the contents of individual and total ginsenosides between the two organs from wild and landrace populations, respectively (Fig. 2). In wild populations, plant growth characteristics except for leaflet number were positively correlated with each other. With the exception of ginsenoside Re, root ginsenosides were positively correlated with each other. Ginsenoside Re was not detected in wild populations. However, most leaf ginsenosides were positively correlated with each other but some leaf ginsenosides, such as Rb1 and Rg1 were negatively correlated (Fig. 2A). On the other hand, in the landrace population, plant FW and DW were strongly positively correlated with each other but

certain plant growth characteristics, such as leaf number, plant height, root length, and root diameter, were negatively correlated with each other. Root age did not correspond to the other parameters because root age in landrace population was identical as those seeds were seeded and harvested at the same date. Most root ginsenosides were positively correlated except that root ginsenoside Rg1 was negatively correlated with other root ginsenosides. By contrast, leaf ginsenosides were differently associated with each other (Fig. 2B).

It is most likely that plant growth characteristics more highly positively correlated with root and leaf ginsenosides in landrace population than in wild populations. Especially, leaf ginsenoside Rd and leaf total ginsenoside were positively correlated with plant height, shoot FW, LAI, and shoot and leaf DW. However, the positive correlation between root and leaf ginsenosides was much stronger in the wild population than in the landrace population. That is, root ginsenosides Rg1, Rb1, Rd, and total ginsenoside were positively correlated with leaf ginsenosides except for leaf ginsenosides Rg1 and Rb1 in wild populations. In the landrace population, there were only two significant correlations between leaf and root ginsenosides; leaf ginsenoside Rg1 was positively and negatively correlated with root ginsenoside Rg1 and Re, respectively.

Principal component analysis models were performed to provide the overall responses of all the ginsenosides to the given ginseng populations, depending on organ types. For leaf ginsenoside, principal components 1 (PC-1) and 2 (PC-2) accounted for 44% and 17% of variance, respectively (Fig. 3A). For root ginsenoside, PC-1 and PC-2 accounted for 51% and 25% of variance, respectively (Fig. 3B). In both of PCA scores plots, the landrace population was clearly separated from all wild populations. However, ginsenosides were more closely linked with each other in leaves (Fig. 3A) than in roots (Fig. 3B).

Hierarchical cluster analysis was also performed to evaluate the similarities among wild and landrace populations based on the results of individual and total ginsenosides contents. Two clusters (clusters A and B vs. clusters C and D) were identified in terms of organ types, root and leaf (Fig. 4). Within each organ, two clusters were observed. In leaves, populations 1, 2, and 3 as the first cluster (cluster B) were most tightly located, whereas populations 4 and 5 belonged to the second cluster (cluster A). In roots, populations 1, 2, and 4 as the first cluster (cluster D) were most closely clustered, whereas populations 3 and 5 as the second cluster (cluster C) were grouped.

Discussion

The content and composition of individual ginsenosides in American ginseng are affected by both environmental cues and genotypic variations (Lim et al., 2005, 2006; Mudge et al., 2004). In previous work, the metabolic diversity of ginsenoside contents and compositions derived from genotypic variations was mainly considered, based on a geographically broad range as a sampling approach (Assinewe et al., 2003; Dong et al., 2003; Jackson et al., 2003; Mudge et al., 2004; Schlag and McIntosh, 2006). However, high ginsenoside variation was apparent within small populations, such as a single farm unit (Hong et al., 2005). These studies suggest that chemotypic diversity of ginsenoside profiles might occur within a small scale with high resolution regarding a natural diversity of ginseng populations. Therefore, we screened four wild

Fig. 2. Pearson correlation coefficient (r) matrixes on plant growth characteristics, root and leaf ginsenosides of four wild (A) and one landrace (B) populations in american ginseng. Red and blue colors indicate positive and negative correlations between parameters. Sample number for the correlation test was 48 ($n = 48$) for four wild populations and 12 ($n = 12$) for one landrace population.

american ginseng populations separated by only a relatively short physical distance to test the hypothesis that the chemotypic variation of ginsenoside contents and compositions might be detected in wild populations with a small scale range.

Although we used wild ginseng populations with a small scale range, it appeared that the compositions and contents of leaf and root ginsenosides were considerably different among all wild populations and between wild and landrace populations. In wild populations, roots had the highest contents of ginsenosides Rg1 and Rb1. The results are in agreement with studies that found that Rg1 was the most abundant ginsenoside in roots, even within a broad (low resolution) sampling area (Assinewe et al., 2003; Lim, 2005; Lim et al., 2005; Schlag and McIntosh, 2006). By contrast, root ginsenoside Re was not detected in wild populations in this study, whereas root ginsenoside Re in previous work was broadly distributed within more widely distributed wild populations (Lim et al., 2005; Schlag and McIntosh, 2006). This difference might be derived from the reason that the wild populations in this study would be more isolated than the wild populations in the studies of Lim et al. (2005) and Schlag and McIntosh (2006), based on the different response patterns of root ginsenosides Re and Rg1. Also, it is assumed that these inverse responses of ginsenosides Re and Rg1 might result from the absence, downregulation of ginsenoside Re or both, compared with ginsenoside Rg1. On the other hand, there might be a competition on the accumulation of ginsenosides Rg1 and Re because both ginsenosides share the same biosynthetic pathway by presumable upregulation

of cytochrome P450 and glycosyltransferase, followed by biosynthesis of protopanaxadiol (Kim et al., 2006; Liang and Zhao, 2008). This result suggests that root ginsenoside Re might be used as a phytochemical biomarker for detecting the certain authentic wild american ginseng population, as opposed to introduced populations (e.g., wild simulated plantings in unmanaged forest settings). Moreover, natural american ginseng populations would be conserved in terms of the absence of root ginsenoside Re from wild populations with a small scale range. Also, based on the results of the PCA models, these wild populations were clearly distinguished from landrace population. Furthermore, the relative abundance of individual ginsenosides is similar in wild and landrace populations (Assinewe et al., 2003; Li et al., 1996; Lim, 2005; Lim et al., 2005). The content of total root ginsenosides was $\approx 30 \text{ g} \cdot \text{kg}^{-1}$ (3%) DW in both wild and landrace populations, which is consistent with the other results (Li and Mazza, 1999; Li et al., 1996; Lim et al., 2005). However, Assinewe et al. (2003) reported that total root ginsenoside from wild ginseng populations was 5.8%, which was ≈ 2 -fold higher than the results presented here.

The composition (relative abundance) of individual leaf ginsenoside wild and landrace populations were different. Leaf ginsenosides Re and Rd were greater in landrace population than in wild populations. The difference of these two leaf ginsenosides could contribute to the higher accumulation of total leaf ginsenosides in landrace population. The relative abundance of leaf ginsenosides in this study is consistent with the result of Assinewe et al. (2003). That is, leaf ginsenosides

Fig. 3. Two-dimensional scores plots of the principal component (PC) analysis of leaf (A) and root (B) ginsenosides from four wild and one landrace populations in american ginseng. Each population represents the mean of 12 replications ($n = 12$).

Re, Rb2, and Rd had a higher content than leaf ginsenosides Rg1, Rb1, and Rc. In this study, total leaf ginsenosides in both wild and landrace populations was $\approx 50 \text{ g} \cdot \text{kg}^{-1}$ (5%) on the basis of DW. This result is consistent with that of Li and Mazza (1999) and Li et al. (1996), although Assinewe et al. (2003) reported that leaf ginsenoside was only 3.3%. It is assumed that the difference in leaf ginsenoside contents between wild and landrace populations could be related to different environmental factors, including soil nutrient and soil moisture content.

Alongside the genotypic variation of ginsenoside compositions among wild populations having a high resolution, the organ difference on the compositions and contents of leaf and root ginsenosides was also observed within wild and landrace populations, respectively. That is, the relative abundance of leaf and root ginsenosides showed different response patterns among the different wild populations. The relative abundance of leaf ginsenosides but not of root ginsenosides in this study was fairly consistent with that from the 4-year-old wild american ginseng reported by Assinewe et al. (2003). This difference might be due to reduction, absence of ginsenoside Re in this study or both, compared with the results of Assinewe

et al. (2003). This response in root ginsenoside Re is also reported by Lim et al. (2005), who reported the inverse relationship between Re and Rg1 from eight wild populations. Furthermore, the ratio of root ginsenoside Rg1 and Re was highly diverse within wild or landrace populations (Schlag and McIntosh, 2006). In this study, only root ginsenoside Rg1 but not root ginsenoside Re was detected. In addition, to the accumulation of ginsenosides from wild populations, the most abundant leaf ginsenosides from landrace population were Rd, Re, and Rb2 in that order, consistent with the results of Li et al. (1996), and Li and Mazza (1999). In the case of the least abundant leaf ginsenosides, there was less variation in ginsenosides Rg1, Rb1, and Rc, compared with other studies (Li and Mazza, 1999; Li et al., 1996). Furthermore, Li and Wardle (2002) reported that the two most abundant leaf ginsenosides were ginsenosides Rd and Re but the two least abundant ginsenosides were Rb1 and Rc. In this study, the three most abundant root ginsenosides in landrace population were Rb1, Re, and Rd. The result was similar to the previous reports (Li and Mazza, 1999; Li and Wardle, 2002; Li et al., 1996). The content of total leaf ginsenoside was higher than that of total root ginsenosides in a 6-year-old asian ginseng (Kim and Choi, 1987).

We evaluated the effect of the storage root age on the contents of total ginsenosides in both leaf and root. Total leaf ginsenosides did not show any significant positive correlation with estimated storage root age, whereas total root ginsenosides was positively correlated with estimated storage root age in two of four wild populations (Fig. 1). Mudge et al. (2004) observed that root ginsenoside Rb1 from six of eight wild populations increased with the storage root age. Furthermore, Shi et al. (2007) and Qu et al. (2009) reported that the contents of total root ginsenosides increased with increase in a storage root age. However, as in this result, the contents of total leaf ginsenosides were unaffected by the estimated ginseng root age (Qu et al., 2009; Shi et al., 2007). It is likely that the composition and content of leaf ginsenosides in wild populations might be more affected by adverse environmental stimuli, including soil nutrient and light levels in the understory of deciduous forests than genotypic factors (Fournier et al., 2003; Konsler et al., 1990). Even in the ANOVA results, age was less influenced by the ginsenoside variables than the organ factor (Table 2). Considering the organ factor for the interaction effect, the age factor was less significant in the contents of individual and total ginsenosides than organ factor because the main effect on age was only detected in ginsenosides Rg1, Rb1, and total ginsenosides, whereas all the individual and total ginsenosides were significantly influenced by organ factor. Furthermore, leaf ginsenosides did not influence accumulation of root ginsenosides based on the result of the ANCOVA, in which leaf ginsenosides were analyzed as a covariant (Table 3). These results indicate that leaf ginsenosides may accumulate independently of root ginsenosides. Thus, it is highly considered that the contents of leaf ginsenosides might be more affected by abiotic factors than by genotypic factors. Nevertheless, the higher abundance of leaf ginsenoside (Qu et al., 2009; Shi et al., 2007; Starratt et al., 2001; Wang et al., 2006) might make it medicinally useful (Xie et al., 2004), although ginseng leaf has been considerably less used for the commercial application than ginseng root.

The significance of the main and all interaction effects on ginsenoside accumulation, shown in Table 3, indicates that the

Fig. 4. Dendrogram of hierarchical cluster analysis (HCA) using hierarchical complete linkage clustering method of leaf and root ginsenosides from four (P1, P2, P3, and P4) wild and one (P5) landrace populations in american ginseng. In HCA, clustering of samples is based on their similarity. Each component (each population) represents the mean value of 12 samples ($n = 12$). White and gray boxes indicate root and leaf, respectively. Four wild populations (P1, P2, P3, and P4) were consecutively assigned based on finding order. Each wild population obtained from within Cornell University's Arnot Teaching and Research Forest (ATRF, Van Etten, NY) was physically separated from all other populations by at least 1.5 km. P5 represents the landrace ginseng population that was cultivated at ATRF by using the seeds of cultivated ginseng landrace from Wisconsin.

main effects of population and organ were similar except for the significance of ginsenosides Rg1 and Re on the organ factor, whereas the main effects of population and age differed considerably. All two-way interaction effects on population $\times$ organ, population $\times$ age, and organ $\times$ age were different from each other for accumulation of ginsenosides. Lim et al. (2005) found that the effects of population and age were considerably different at 0–2 years after transplanting. By contrast, root age effect was not statistically detected at 1 year after transplanting, whereas only the population effect appeared in all individual and total ginsenosides (Lim, 2005). The interaction effects of population and age were apparent with all ginsenosides, except for ginsenoside Re (Lim, 2005) but in this study, we observed the interaction effect of populations and age on ginsenoside Rc and total ginsenosides.

To test the hypothesis that a certain ginsenoside in leaf might be related with the corresponding, other ginsenosides accumulation in root from the wild and cultivated ginseng populations or both, respectively, we performed the Pearson's correlation procedure on both leaf and root ginsenosides to predict root ginsenoside contents from the estimation of leaf ginsenoside levels. In wild populations, within all possible correlation combinations, the most significant combination was root ginsenoside Rd with leaf ginsenoside Rb2. On the other hand, in landrace population, only two combinations showed the significant correlation of leaf ginsenoside Rg1 with root ginsenosides Rg1 ($r = 0.943$, $P < 0.0001$) and Re ($r = -0.766$, $P = 0.004$), respectively (Fig. 2B). This result is consistent with the result of Li et al. (1996), revealing that the correlations between leaf and root ginsenosides from nine cultivated ginseng populations were very low. In the 4-year-old american ginseng, total root ginsenoside was positively correlated with leaf ginsenosides Rd, Re, and Rb2 (Konsler et al., 1990). This

indicates that certain individual leaf ginsenosides could be used as indicators for the estimation of individual, total root ginsenosides without destructive root sampling or both.

In conclusion, there was considerable genotypic variation in the compositions and contents of ginsenosides between leaf and root from wild and landrace populations. The relative abundance of individual ginsenosides between leaf and root differed between wild and landrace populations. Total ginsenoside was higher in leaf than in root from both wild and landrace populations. Storage root age was not a good predictor of total leaf ginsenosides but it was positively correlated with total root ginsenosides in certain wild populations. Also, there were certainly the main and two-way interaction effects on all individual ginsenosides and total ginsenosides, whereas there was no three-way interaction effect. The correlation analysis revealed that leaf ginsenosides Rd and Rg1 had moderate and

strong correlation with root ginsenosides Rb2 and Rg1 from wild and landrace populations, respectively. Furthermore, PCA and cluster analysis revealed that these wild populations differed from the landrace ginseng population. Leaf ginsenosides might not be affected by the estimated root age. Nevertheless, certain leaf ginsenoside might be useful as a potential phytochemical biomarker to estimate root ginsenosides in commercial settings, to screen the wild ginseng populations, and to select landrace populations for higher yield of ginsenosides.

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