

Effects of Population and Age on Ginsenoside Content of American Ginseng (*Panax quinquefolium* L.)

Kenneth W. Mudge
Department of Horticulture
15 Plant Science
Cornell University
Ithaca, NY 14853 USA

Wansang Lim
Department of Horticulture
Cornell University
Ithaca, NY 14853, USA

Joseph P. Lardner
Department of Horticulture
Cornell University
Ithaca, NY 14853, USA

Robert L. Beyfuss
Cornell Cooperative Extension of Greene
County
906 Greene County Office Building
Cairo, NY, USA

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Abstract

American ginseng (*Panax quinquefolium*) is valued in traditional Asian medicine, and its use in Western countries has increased in recent years. The value of artificially-cultivated ginseng is much lower than that of woods-cultivated or wild-collected ginseng, but populations of the latter are threatened throughout its range. Our goal is to identify what factors influence the concentration of the pharmacologically active ginsenosides. Age-related increase in ginsenosides have been reported by several authors but variation among wild populations has not been considered. Ginsenoside analysis via HPLC was performed on roots collected from 8 different NY State populations. Statistical analysis of the effects of population and age on ginsenoside content indicated that there was a significant effect of both on ginsenoside Rc, a significant interaction between population and age for ginsenosides Rb1 and Rb2, and a significant three way interaction between population, age and root fresh weight for Re. Ginsenosides Rb1 and Rb2 increased with age for some populations, but not for other populations. We conclude that the prevailing generalization that ginsenosides increase with root age cannot be applied to wild North American ginseng without taking into consideration population differences. The effects of population on ginsenoside content is likely to be related not only to environmental differences among collection sites but also genetic differences since there was considerable (several fold) variation in the content of ginsenosides within single populations. It is likely therefore that selection and cloning of individual roots for higher ginsenoside content could be a useful strategy for improvement of this medicinal crop. These results also suggest that populations could be selected based on their sensitivity to age and fresh weight-related increase in ginsenosides concentration.

INTRODUCTION

Ginseng is a valuable medicinal herb that is used widely in traditional Asian medicine, and its use in Western countries is increasing. The value of this herb root-crop varies depending on several factors including whether it is cultivated or wild-collected, what production system is used to grow it under cultivation, and the age of the plant. Roots collected from the wild are worth approximately \$600 per pound dry weight, whereas woods cultivated ginseng is worth approximately 1/3 than amount, and intensively (artificial shade) cultivated ginseng is worth 10% or less as much as wild-collected ginseng. Although wild ginseng is becoming increasingly scarce, it is still collected in substantial quantities throughout most of its range in eastern North America. There are competing claims that roots collected from or grown in one region are of higher quality than roots collected from another region. Because criteria for determining quality and economic value are largely subjective it is difficult to assess the validity of such

claims. Furthermore, lack of objective quality criteria complicates the goal of long term deliberate genetic improvement of the crop through breeding or clonal selection.

Although the medicinal properties of American Ginseng are thought to be associated, at least in part, with a group of triterpene saponins known as ginsenosides, the ginsenoside content is not taken into consideration in assessing quality, i.e. there is no clear relationship between the price of ginseng and its ginsenoside content. Instead, root morphology (shape), and to some extent root age, are the primary determinants of quality and price. Although there is no direct determination of ginsenoside content in assessing quality and price, the fact that older roots are considered more valuable (per unit weight), does relate indirectly to ginsenoside content since there are several reports that ginsenoside content increases with age (Court, Reynolds, and Hendel, 1996; Jackson, Dini, Lavandier, Rupasinghe, and Proctor, 2001; Jackson, Rupasinghe, and Schooley, In Review for this volume; Soldati and Tanaka, 1984). Although genetic effects of ginsenoside content can be inferred from studies which have compared different cultivated populations (Jackson, Dini, Lavandier, Rupasinghe, and Proctor, 2001; Jackson, Rupasinghe, and Schooley, In Review for this volume; Li, et al. 1996), little is known about the extent of genetic influence on ginsenoside content among wild populations. This could be valuable information in future breeding and/or clonal selection programs.

Hence the objectives of this study was to compare ginsenoside content of several wild American ginseng populations from New York state and determine the extent to which genetic difference contribute to observe variation in ginsenosides among populations. This report will present the results from the first year of an ongoing three year study.

MATERIALS AND METHODS

Collection and Planting of Wild American Ginseng Populations

In the Fall of 2000, wild ginseng roots were collected from 8 different hardwood forested sites, within a contiguous five county region in south-central New York (Fig. 1). These included 3 populations from Chenango county, 2 populations from Deleware county, 1 population from Broome county, 1 population from Otsego county, and 1 population from Schoharie county. Approximately 150 plants were collected from each site, and each set is referred to as a population below. After collection, root age was estimated by counting annual bud scars (nodes) along the rhizome. Plants ranged in age because it was not feasible to predetermine root age before digging the plant, nor would it have been feasible to collect from the wild populations sufficient numbers of equal or closely-aged plants for the experiment. Most plants ranged in age from 3 to 12 years old, except one plant from population 1 was one year old, one plant from each of populations 1 and 3 was two years old, one plant from population 5 was 16 years old, and one plant from population 1 was 30 years old. Six to 15 plants, depending on population, were collected from each population for analysis of ginsenoside at the start of the experiment (Time₀), and approximately 40 of the remaining plants of each of the 8 populations were planted at each of three experimental woods garden sites (same 8 populations at each of 3 sites). These included Cornell University's Arnot Teaching and Research Forest near Van Etten, NY; Phetteplace Raw Furs near Norwich, NY and Sylvan Botanicals near Cooperstown, NY. The latter two experimental ginseng plantings were managed by cooperating ginseng growers, Bruce Phetteplace, and Scott Harris, respectively. A "woods cultivation" management system was used at each site, i.e. natural forest shade and minimal inputs other than bed preparation which consisted of rototilling and the addition of leaf mulch from local hardwoods (maple, oak, etc.).

Ginsenoside Analysis

At Time₀ (Fall, 2000) the number of replicate plants used for ginsenoside analysis, ranged from 6 to 15, depending on population. Subsequently, at the end of the first

growing season (September, 2001) an additional 10 plants from each of the 8 populations were harvested for ginsenoside analysis from each of the three experimental gardens. Similarly, plants will be harvested from each population at each garden, at the end of the second (2002) and third (2003) growing seasons. For each plant, the storage root was separated from the rhizome, and fibrous roots were removed. The storage root was air dried at 38C for 3 days, then ground to a powder with a coffee grinder. Powdered root samples were stored in tightly capped glass vials at room temperature for several months prior to analysis.

The method of Court, Hendel, Elmi, and Jama (1996) was used for analysis of ginsenosides. One hundred mg of dried, powdered root was extracted in 3 x 10ml of 70% methanol. After centrifugation, the supernatant was concentrated to dryness in vacuo and redissolved in acetonitrile for HPLC analysis on a reverse phase C₁₈ column using an acetonitrile/water gradient. A photo diode array detector was used to determine absorbance at 203 nm. Peak identification was based on coelution with know standards of Rb1, Rb2, Rc, Rd, Re, and Rg1. Absorption detector output was converted into mg of ginsenoside based on standard curves obtained from standards of each ginsenoside. Results are expressed at percent (w/w). Each root sample from each population was analyzed separately. Data was subjected to ANOVA (PROC GLM) using SAS statistical software, testing the model ginsenoside (%) = P (population) A (age) F (root fresh weight) P*A P*F A*F and P*A*F.

RESULTS

Ginsenoside levels for each of the six ginsenosides and each of the eight populations are shown in Figure 1. Unlike most other published reports of ginsenoside levels in American ginseng (e.g. Jackson, Rupasinghe, and Schooley, In Review for this volume), for most populations ginsenoside Re was present in higher concentration than Rb1. Table 1 shows the level of statistical significance (*, ≤ 0.05 , ** ≤ 0.01), or actual p value for the ANOVA analysis based on Type I sums of squares, for each of the three main effects (P, A, and F) and each two way and the single three way interaction in the model. Population had a significant effect on Rg1 levels, and there was a significant interaction between population and age for ginsenosides Rb1, Rb2, and Rd ($p=0.064$ for Rc). In the case of Re, there was a significant three way interaction among population, age and fresh weight.

Figure 2 shows the relationship between plant age and ginsenoside content for each of the eight populations for ginsenoside Rb1. As has generally been reported in the literature, Rb1 level increased with plant age for 6 of the 8 populations (positive sloping lines in Figure 2), but the rate of the age-related increase differed among populations. This can be seen from the differing slopes among the populations in Fig. 2, and is indicated by the significant interaction between population and age (Table 1). Populations 2 and 6, which are both from Chenango County, did not exhibit the age-related increase in Rb1 apparent in the other populations. Similar results (differing slopes among populations) were observed for the other ginsenosides (data not shown). Within populations, variation among individual roots (6 to 15 per population) was considerable, with as much as five-fold difference in ginsenoside content, for some populations, between the root with the lowest and the root with the highest content (data not shown).

DISCUSSION

Because it was not practical to collect roots of uniform age from the wild populations sampled in this experiment, it was necessary to take root age into consideration in the analysis of these results. Despite considerable variation in ginsenoside content among roots within each population, the results shown here indicate that the independent variable population contributed to the variation in ginsenoside content. Only in the cases of Rg1 was the contribution of population independent of age, whereas for Rb1, Rb2, and Rd the effect of population depended on the age of the plant as indicated by the significant interaction between population and age. Age-related increase

in ginsenoside concentrations is consistent with other reports (cited above), but there have been no previous reports that the rate of age-related increase differs among populations. The complementary interpretation, that the contribution of population to ginsenoside content depends on root age, should be considered in future attempts to select individual roots with higher than average ginsenoside content for cloning.

Although these results clearly show that at T_0 , the different New York populations of ginseng sampled in this study differed in ginsenoside content, it is too early in the experiment to conclude that these differences necessarily reflect genetic differences among populations, because the environments from which the roots were obtained were not uniform. The extent of the contribution of genotype to the observed population differences should become more apparent from the results obtained from subsequent years (T_1 , T_2), since the all eight populations were grown, since T_0 , within a more or less identical local environment, within each of the three experimental sites. At this point, it is reasonable to conclude that genetic differences probably contribute, along with environmental differences, to the population-related differences in ginsenosides observed at T_0 . The considerable variation in ginsenoside concentrations among replicate roots within populations supports this interpretation. If genetic differences in ginsenoside levels is verified in the future as this experiment progresses, and if a reliable method for clonal propagation of ginseng is available, then clonal selection of high ginsenoside producing individual plants may be a practical means of bringing about genetic improvement of this species. Attempts in our laboratory to clonally propagate American ginseng have been encouraging. We have successfully established callus from adult ginseng roots and induced somatic embryogenesis and subsequent shoot development, although ex vitro recovery of plantlets has not yet been achieved.

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Tables

Table 1. Statistical significance of population (P), age (A) and fresh weight (F) and interactions on ginsenoside content. Significance (p) levels: * $p \leq 0.05$ ** $p \leq 0.01$.

	P	A	F	P*A	P*F	A*F	P*A*F
Rg1	*						
Re	*	**	0.064		*		*
Rb1	0.091	**		*			
Rc	*	**		0.064			
Rb2	**	**		*			
Rd	**	**		*			
Total	0.081	**				**	
PD/PT	*	**	*				**

Figures

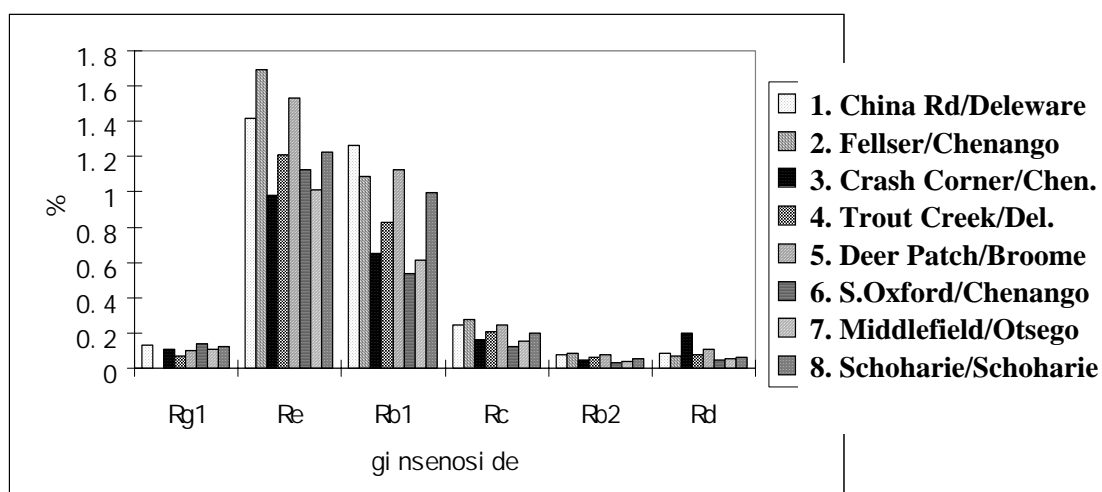


Fig. 1. Effect of population on ginsenoside content. Populations (bars from left to right for each ginsenoside) are identified by specific location / county within New York state.

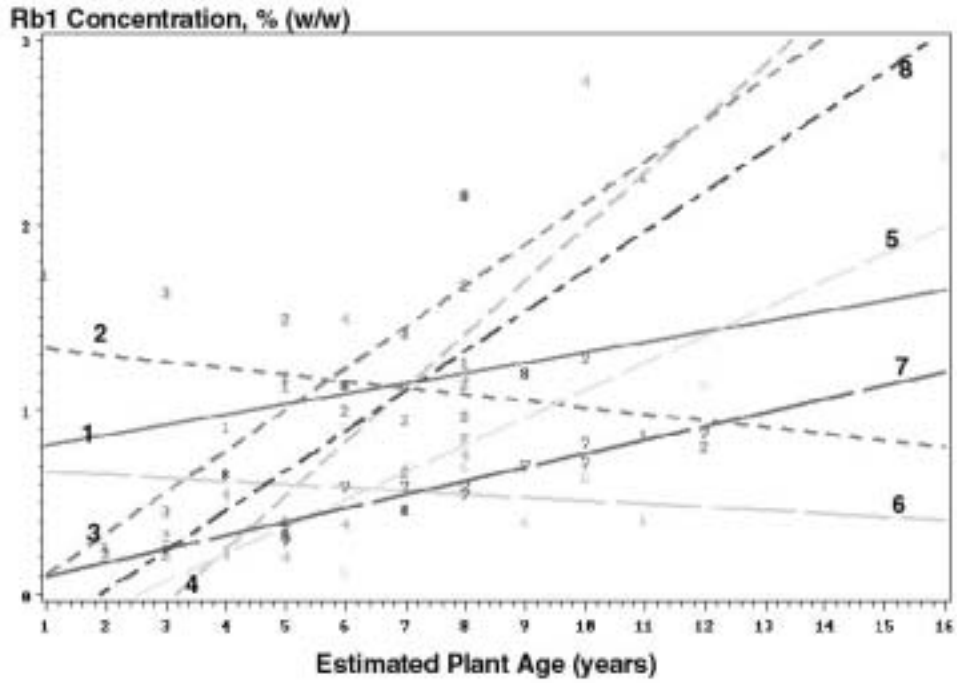


Fig. 2. Effect of plant age and population on ginsenoside Rb1 concentration. Large numbers within the figure denote the regression lines corresponding to the different populations identified in Fig. 1. Corresponding small numbers identify data points (replicates) from individual plants.