J Ginseng Res xxx (2016) 1–6

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research article

Ginseng authenticity testing by measuring carbon, nitrogen, and sulfur stable isotope compositions that differ based on cultivation land and organic fertilizer type

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ARTICLE INFO

Article history:
Received 23 July 2015
Received in Revised form
17 November 2015
Accepted 9 March 2016
Available online xxx

Keywords:
cultivation land
ginseng authenticity
isotope ratio mass spectrometry
organic fertilizer
Panax ginseng

ABSTRACT

Background: The natural ratios of carbon (C), nitrogen (N), and sulfur (S) stable isotopes can be varied in some specific living organisms owing to various isotopic fractionation processes in nature. Therefore, the analysis of C, N, and S stable isotope ratios in ginseng can provide a feasible method for determining ginseng authenticity depending on the cultivation land and type of fertilizer.
Methods: C, N, and S stable isotope composition in 6-yr-old ginseng roots (Jagyeongjong variety) was measured by isotope ratio mass spectrometry.
Results: The type of cultivation land and organic fertilizers affected the C, N, and S stable isotope ratio in ginseng ($p < 0.05$). The $\delta^{15}N_{AIR}$ and $\delta^{34}S_{VCDT}$ values in ginseng roots more significantly discriminated the cultivation land and type of organic fertilizers in ginseng cultivation than the $\delta^{13}C_{VPDB}$ value. The combination of $\delta^{13}C_{VPDB}$, $\delta^{15}N_{AIR}$, or $\delta^{34}S_{VCDT}$ in ginseng, except the combination $\delta^{13}C_{VPDB}-^{34}S_{VCDT,}$ showed a better discrimination depending on soil type or fertilizer type.
Conclusion: This case study provides preliminary results about the variation of C, N, and S isotope composition in ginseng according to the cultivation soil type and organic fertilizer type. Hence, our findings are potentially applicable to evaluate ginseng authenticity depending on cultivation conditions.

1. Introduction

Ginseng (*Panax ginseng* Meyer), a perennial plant, is well known as a representative specialty in Korea for its medicinal properties because of the presence of various bioactive compounds such as saponins, polyacetylenes, polysaccharides, phenolics, and volatile isoprenoids [1–3]. China, Korea, Canada, and USA are the major ginseng producers, comprising > 99% of the global ginseng production [4]. In general, ginseng production and quality are affected by various physical, chemical, and microbial properties of the soil. In particular, ginseng cultivation for 4–6 yrs usually decreases soil fertility. Thus, the continuous cropping of ginseng at the same place is not recommended because of a decrement of ginseng production and quality of the cultivation area caused by a continuous cropping injury associated with *Cylindrocarpon destructans*. Therefore, management of the soil used for ginseng cultivation is a crucial step in the production of high quality and high yield of ginseng [5,6].

In Korea, the total ginseng cultivation area and production decreased from 19,702 ha and 27,460 tons in 2009 to 14,652 ha and 20,978 tons, respectively, in 2014 because of the lack of new ginseng cultivation areas [7]. Thus, stable production of high quality ginseng is of interest to both ginseng farmers and consumers in Korea. Recently, paddy-converted fields have attracted attention in Korea as a potential solution to both the lack of new cultivation areas and appearance of various disorders caused by continuous ginseng cultivation. In general, paddy-converted fields decrease the levels of *Cylindrocarpon destructans* (a pathogen source of disorders in continuous ginseng cultivation) and toxins associated with the

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p1226-8453 e2093-4947/$ – see front matter
http://dx.doi.org/10.1016/j.jgr.2016.03.004

inhibition of ginseng growth [8,9]. Furthermore, the saponin content, yield, and quality of ginseng cultivated in paddy-converted fields were not different from those of ginseng cultivated in upland fields by using conventional ginseng cultivation methods. Therefore, the demand for paddy-converted fields is expected to increase in ginseng farming in Korea in the future [6,9].

Meanwhile, prolonged cultivation of ginseng in the same location requires a steady supply of nutrients to obtain a high yield of high quality ginseng. Certain organic materials (not meaning organically cultivated), including manure, food waste, and rice straw compost, have been usually applied as organic fertilizers, whereas the application of chemical fertilizers has been banned during the ginseng cultivation period by the Ginseng Industry Act [10]. Because of the difference in soil properties compared to those of upland fields, ginseng cultivation in paddy-converted fields requires careful application of organic fertilizers. In general, the application of slow-release organic fertilizer types at the managing stage of preplanting in a paddy-converted field is preferred to avoid the occurrence of various physiological disorders during the entire ginseng cultivation period [1,5].

Given the unique natural abundance of hydrogen (H), carbon (C), nitrogen (N), oxygen (O), or sulfur (S) in some living organisms, the analysis of its stable isotope composition has been applied to determine the authenticity of various foods. To date, this method has been successfully applied to determine the authenticity of various agricultural products (i.e., geographical origin: rice, vegetables, olive oil, juice, honey, wine, nut, tea; whether it is of organic origin: beef, milk; others: discrimination of cow milk vs. buffalo milk) [11–17]. In a prior study [18], the H isotope composition in ginseng was used to discriminate the geographical origin effectively between Korea and China. In particular, the negative value of N isotope composition in ginseng collected at Incheon, Korea was indicative of the application of synthetic fertilizer, possibly urea.

Few studies [18,19], to our knowledge, have investigated the authenticity of ginseng using the analysis of light element isotope composition. Therefore, in the present study, we have measured the difference and variation of C, N, and S stable isotope ratios in 6-yr-old ginseng root depending on ginseng cultivation conditions (i.e., cultivation soil type and fertilizer type/amount). The preliminary results reported from this case study can be potentially applicable to assessing ginseng authenticity with respect to cultivation environment.

2. Materials and methods

2.1. Ginseng materials

Six-yr-old ginseng roots (Jagyeongjong variety) were obtained from the Department of Herbal Crop Research, Rural Development Administration in Eumseong, Korea. The ginseng was cultivated in upland and paddy-converted fields from March 2009. The paddy-converted field had been used as a paddy field until 2006, when it was converted into the upland to manage the lack of new ginseng cultivation areas (upland) in Korea. Physical and chemical properties of the paddy-converted field were managed in the pre-planting preparation stage via the cultivation/decomposition of soilage crop such as sudangrass prior to ginseng cultivation [9]. Prior to ginseng seedling transplant, three types of organic fertilizers (cattle manure, food waste, and rice straw compost) were applied to the upland and the paddy-converted fields. Each organic fertilizer was applied at the level of 1 ton/1,000 m^2, 2 tons/1,000 m^2, and 4 tons/1,000 m^2. After the organic fertilizer application, 1-yr-old ginseng seedlings were transplanted to the upland and the paddy-converted fields in March 2009. The seedlings were planted with the planting density of 30 cm × 20 cm (the space between ginseng plants in a row × the space between the rows). The roots of the 6-yr-old plants were collected and stored at −70°C until required for the analysis. The land management, including applications of various agrochemicals (e.g., pesticide), was carried out using the standard ginseng farming method [20].

2.2. Sample preparation for the analysis of C, N, and S isotope composition

Collected ginseng roots were lyophilized at −45°C for > 3 d and pulverized before the isotope ratio mass spectrometry (IRMS) analysis. The pulverized ginseng was enclosed in a tin capsule (5 mm × 9 mm; Costech Analytical Technologies Inc., Valencia, CA, USA). About 5 mg of ginseng powder was used for simultaneous measurements of C and N stable isotope composition and about 20 mg for the measurement of S stable isotope composition. Finally, the encapsulated ginseng samples were placed in a desiccator before being used in the IRMS analysis.

2.3. Measurement of C, N, and S stable isotope compositions in ginseng by IRMS

The C and N stable isotope compositions ($\delta^{13}C_{VPDB}$ and $\delta^{15}N_{AIR}$) in ginseng ($n = 3$ per each treatment) were determined by using a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS; Sercon Ltd., Crewe Cheshire, UK) linked to a PDZ Europa ANCA-GSL elemental analyzer (Sercon Ltd.). The S stable isotope ratio ($\delta^{34}S_{VCDT}$) in ginseng ($n = 3$ per treatment) was measured by using a vario ISOTOPE cube (Elementar, Hanau, Germany) and a pre-concentration unit interfaced with a continuous-flow Sercon 20-22 IRMS (Sercon Ltd.). Detailed analytical conditions were described previously [16]. The samples' $\delta^{13}C_{VPDB}$, $\delta^{15}N_{AIR}$, and $\delta^{34}S_{VCDT}$ were calculated as follows:

$$\delta, ‰ = (r_{sample}/r_{standard}) - 1, \quad (1)$$

where r is the $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$ ratio and r_{sample} and $r_{standard}$ from the samples of interest and the standard, respectively.

Thus, the ^{13}C, ^{15}N, and ^{34}S enrichment in ginseng were expressed against the international or established laboratory reference standards [Vienna PeeDee Belemnite (VPDB) for $\delta^{13}C_{VPDB}$; atmospheric (air) N_2 for $\delta\ ^{15}N_{AIR}$; Vienna Canyon Diablo Troilite (VCDT) for $\delta^{34}S_{VCDT}$].

For the quality control of the IRMS measurements, we simultaneously analyzed several replicates of our laboratory standards that were compositionally similar to our ginseng samples. These laboratory standards had been previously calibrated against the selected standard reference materials (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, and USGS-41 for $\delta^{13}C_{VPDB}$ and $\delta^{15}N_{AIR}$; IAEA-S-1, IAEA-S-2, or IAEA-S-3 for $\delta^{34}S_{VCDT}$) [21]. Based on the measurements of our laboratory standards (USGS41 for $\delta^{13}C_{VPDB}$ and $\delta^{15}N_{AIR}$; hair for $\delta^{34}S_{VCDT}$), the analytical precision was ± 0.1‰ for $\delta^{13}C_{VPDB}$, ± 0.1‰ for $\delta^{15}N_{AIR}$, and ± 0.2‰ for $\delta^{34}S_{VCDT}$. In addition, the long-term reproducibility (± standard deviation) was ± 0.2‰ for $\delta^{13}C_{VPDB}$, ± 0.3‰ for $\delta^{15}N_{AIR}$, and ± 0.4‰ for $\delta^{34}S_{VCDT}$.

2.4. Statistical analysis

Statistical analysis was conducted using a general linear model procedure of the statistical analysis program (SAS version 9.3; SAS Institute Inc., Cary, NC, USA). The experimental design, including ginseng cultivation and sample collection, was a completely randomized design conducted in triplicate. The least significant difference test was based on a 0.05 probability level.

Please cite this article in press as: Chung I-M, et al., Ginseng authenticity testing by measuring carbon, nitrogen, and sulfur stable isotope compositions that differ based on cultivation land and organic fertilizer type, Journal of Ginseng Research (2016), http://dx.doi.org/10.1016/j.jgr.2016.03.004