Differential Modulation of Volatile Constituents in *Artemisia princeps* and *Artemisia argyi* Plants after Gamma Ray or Electron Beam Irradiation

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Abstract - The effects of gamma ray or electron beam irradiation on herbaceous medicinal plants were investigated in terms of the composition of volatile constituents using the aerial parts or leaves of *Artemisia princeps* Pamp. cv. Ganghwayakssuk and *Artemisia argyi* cvs. Namhaeyakssuk and Hwanghaessuk. The composition of volatile constituents in leaves was clearly distinguishable among the three *Artemisia* cultivars. However, the relative proportions of the major volatile constituents such as 1,8-cineole, α-pinene, camphene, santolina triene, and artemesia triene, were similarly changed in two or three cultivars by gamma ray or electron beam irradiation. In particular, the proportion of 1,8-cineole was increased up to 1.29- to 1.71-fold in the three cultivars after irradiation with gamma ray. These results suggest that gamma ray or electron beam irradiation can be applied to modulate the composition of volatile constituents in the leaves of *Artemisia* plants.

Key words : *Artemisia*, Gamma ray, Electron beam, Solid-phage microextraction, Volatile constituent

INTRODUCTION

Artemisia is an herbaceous medicinal plant, whose leaf has been used as a traditional oriental medicine to cure various diseases such as asthma, malaria, hepatitis, inflammation and infections by fungi, bacteria and viruses (Zheng *et al.* 2004). Therefore, many volatile constituents for various medicinal properties have been separated and identified from *Artemisia* leaves using a novel technique of solid-phase microextraction (SPME) as well as routine analysis methods such as steam distillation and solvent extraction (Choi *et al.* 2005; Koobkokkruad *et al.* 2008). However, since these constituents have different medicinal effects, and some of them are harmful to human health, their relative compositions need to be controlled by post-harvest processing for increasing the medical or industrial use of *Artemisia*.

Ionizing radiation generates reactive intermediates and stable products from the dissociation of water molecules, which include water radical cation ($H_2O\cdot^+$), excited water (H_2O^*), hydronium ion (H_3O^+), hydroxyl radical ($\cdot OH$), hydrogen ion (H^+), hydrated electron (e^-_{aq}), hydrogen radical ($H\cdot$), hydrogen peroxide (H_2O_2), hydroxyl ion (OH^-), superoxide radical anion ($O_2\cdot^-$), and molecular hydrogen (H_2) (Spinks and Woods 1990; Lee *et al.* 2009). Among these, $\cdot OH$, $H\cdot$, $O_2\cdot^-$, and H_2O_2 are biologically quite toxic and damaging to cellular components (Cadenas 1989; Esnault *et al.* 2010). However, these free radicals or reactive oxygen species are also the secondary determinants for the radiation-

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induced physiological responses (Esnault *et al.* 2010). Therefore, both the direct physicochemical and indirect reactive oxygen species-mediated effects of ionizing radiation on cellular components can be effectively utilized for radiation processing of biological materials (Koobkokkruad *et al.* 2008; Kim *et al.* 2009; Lee *et al.* 2011).

In the present study, we report that volatile constituents in aerial parts or leaves of *Artemisia* plants were differently identified from *Artemisia princeps* Pamp. cv. Ganghwayakssuk and *Artemisia argyi* cvs. Namhaeyakssuk and Hwanghaessuk. Moreover, it is suggested that gamma ray and/or electron beam irradiation could modulate the relative proportions of these constituents in leaves of *Artemisia* plants.

MATERIALS AND METHODS

1. Plant material and gamma ray or electron beam irradiation

Artemisia princeps Pamp. cv. Ganghwayakssuk and *Artemisia argyi* cvs. Namhaeyakssuk and Hwanghaessuk were cultivated in the same experimental field. *Artemisia princeps* Pamp. cv. Ganghwayakssuk and *Artemisia argyi* cv. Namhaeyakssuk were collected from islands Ganghwa and Namhae in Republic of Korea, respectively. Aerial parts or leaves of *Artemisia* plants were harvested and ground in liquid nitrogen to a fine powder. About one gram of the leaf powder was put in a glass vial and one day later irradiated with 100-kGy gamma ray at a dose rate of 6.25 kGy h^{-1} for 16 h or with 10-kGy electron beam. Irradiation was carried out using a ^{60}Co gamma (IR-221, MDS Nordion Inc., Kanata, Canada) or an electron beam irradiator (UELV-10-10S, EB-Tech Co. Ltd., Daejeon, Korea) at the Advanced Radiation Technology Institute in Jeongeup-si, Jeollabuk-do, Republic of Korea.

2. SPME-GC/MS analysis of volatile constituents

Glass vials containing the leaf powder were further incubated at room temperature for one day after irradiation. They were then subjected to GC/MS analysis using a solid-phase microextraction (SPME) fiber (Zheng *et al.* 2004). Although steam distillation and solvent extraction are routine analysis methods for volatile constituents, they require time-consuming procedures and relatively large amounts of samples and organic solvents. Therefore, the SPME method has been used for determination of volatile constituents in *Artemisia* leaves (Zheng *et al.* 2004; Choi *et al.* 2005).

In the present study, volatile constituents of the leaf powder in glass vials were analyzed using a SPME fiber assembly (50/30 m DVB/Carboxen™/PDMS StableFlex™, Supelco, Bellefonte, PA, USA) in a GC/MS system (6890N/5975B, Agilent Technologies, Santa Clara, CA, USA), which was equipped with a capillary column (HP5-MS 19091S-433, Agilent Technologies, Santa Clara, CA, USA) and a multifunctional autosampler and sample preparation robot (MPS Twister®, Gerstel GmbH & Co. KG, Mülheim van der Ruhr, Germany). The SPME fiber was exposed to the headspace of the sample vials for 10 min. After autoinjection, the bound analysts were desorbed from the fiber in a GC inlet at 250°C for 2 min and the volatiles chromatographed on the HP5-MS capillary column. The GC temperature was raised from initially 35°C to 180°C at a rate of 5°C min^{-1} and then to 325°C at a rate of 10°C min^{-1} and held constant for 5 min. The helium carrier gas flow was kept constant at 1.0 ml min^{-1}. Mass selective detection was accomplished by the 5975B MS interfaced to the GC, operated at a mass range of m/z= 50～350 amu.

RESULTS AND DISCUSSION

1. Difference in the composition of volatile constituents in three *Artemisia* cultivars

Artemisia plants have many volatile constituents with a variety of biological activities, exhibiting strong medicinal and/or allelopathic properties (Zheng *et al.* 2004; Yun 2009). However, it has been reported in various *Artemisia* species that the composition of volatile constituents is substantially changed depending on the growing season and/or habitat (Kim 1997; Yun and Choi 2003; Zheng *et al.* 2004). In the present study, *Artemisia princeps* Pamp. cv. Ganghwayakssuk and *Artemisia argyi* cvs. Namhaeyakssuk and Hwanghaessuk were cultivated in the same experimental field and harvested at the same time (Fig. 1). Therefore, the genotypic variations in relation to the composition of volatile constituents could be estimated and compared among the three *Artemisia* cultivars. The composition of volatile constituents in leaves was clearly distinguishable among the three *Artemisia* cultivars, even between *Artemisia argyi* cvs. Namhaeyakssuk and Hwanghaessuk, which belong to the same species (Fig.

Fig. 1. Cultivation of *Artemisia* plants in the experimental field. A, B and C represent *Artemisia princeps* Pamp. cv. Ganghwayakssuk and *Artemisia argyi* cvs. Namhaeyakssuk and Hwanghaessuk, respectively.

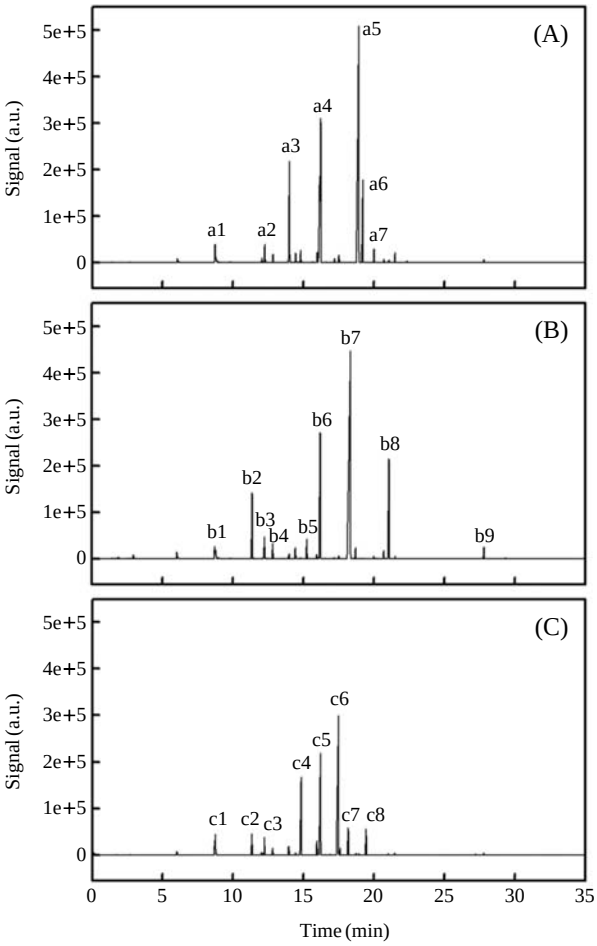

Fig. 2. Difference in the composition of volatile constituents of leaves among three *Artemisia* cultivars. Chromatograms were obtained by SPME-GC/MS analysis. Peaks with a character represent major volatile constituents in each cultivar. A, B, and C represent *Artemisia princeps* Pamp. cv. Ganghwayakssuk and *Artemisia argyi* cvs. Namhaeyakssuk and Hwanghaessuk, respectively.

2). The most abundant volatile constituent was β-thujone in Ganghwayakssuk, artemesia triene in Namhaeyakssuk, and artemesia ketone in Hwanghaessuk (Table 1). The 2-hexenal, α-pinene, and 1,8-cineole were commonly detected in the three cultivars. In contrast, the camphene or the santolina triene and artemesia triene was detectable only in Ganghwayakssuk and Namhaeyakssuk, or in Namhaeyakssuk and Hwanghaessuk, respectively. These results imply that the difference in the composition of volatile constituents among the three cultivars can be attributed to the genotypic variations more than the growing season and/or habitat. Moreover, the profiles of common and different volatile constituents among the three *Artemisia* cultivars could be used to evaluate and compare the influence of gamma ray or electron beam on major volatile constituents of *Artemisia* leaves.

2. Change in the composition of major volatile constituents in three *Artemisia* cultivars after irradiation with gamma ray or electron beam

The growing season and habitat of *Artemisia* plants are important factors to affect the composition of volatile constituents in leaves (Kim 1997; Yun and Choi 2003; Zheng *et al.* 2004). Moreover, it was recently reported that the relative abundance of isoprenoids and other volatile components in the aerial parts of *Artemisia annua* was substantially changed by different nutritional treatments (Malik *et al.* 2009). However, post-harvest treatments such as drying, freezing, or heating are general considerations for enhancement of effective constituents and improvement of function-

Table 1. Change in the composition of major volatile constituents in *Artemisia* leaves after irradiation of 100-kGy gamma ray at a dose rate of 6.25 kGy h^{-1} for 16 h or 10-kGy electron beam. A, B, and C represent *Artemisia princeps* Pamp. cv. Ganghwayakssuk and *Artemisia argyi* cvs. Namhaeyakssuk and Hwanghaessuk, respectively. Digits are the proportion of the respective to the total volatile constituents and are expressed as means ± S.E. of 2 ~ 4 independent experiments; N.D., not detected. In A-a3, A-a4, B-b6, B-b9, C-c4, and C-c5 peaks, the mean values without a common letter are significantly different at $P=0.05$ by Tukey's honestly significant difference (HSD) test

GC peak	Composition (%)			Constituent
	Control	Gamma ray	Electron beam	
A-a1	2.0 ± 0.5	1.4 ± 0.2	3.1 ± 0.6	2-hexenal
A-a2	1.8 ± 0.2	N.D.	2.1 ± 0.2	α-pinene
A-a3	10.8 ± 1.0a	1.0 ± 0.1b	11.5 ± 1.4a	β-phellandrene
A-a4	15.5 ± 0.3a	20.0 ± 0.3b	16.2 ± 0.6a	1,8-cineole
A-a5, A-a6	68.3 ± 1.1	76.4 ± 0.4	65.8 ± 2.2	β-thujone
A-a7	1.6 ± 0.2	1.2 ± 0.3	1.3 ± 0.1	camphene
B-b1	1.5 ± 0.2	3.1 ± 0.9	6.0 ± 1.5	2-hexenal
B-b2	4.8 ± 0.9	0.5 ± 0.2	6.5 ± 0.9	santolina triene
B-b3	1.9 ± 0.4	1.6 ± 0.2	2.6 ± 0.5	α-pinene
B-b4	1.0 ± 0.2	0.5 ± 0.2	1.2 ± 0.2	camphene
B-b5, B-b7, B-b8	73.7 ± 1.7	76.1 ± 1.7	69.2 ± 2.9	artemesia triene
B-b6	11.9 ± 0.9a	17.3 ± 1.1b	13.1 ± 0.5a	1,8-cineole
B-b9	5.3 ± 2.0a	0.9 ± 0.4a	1.5 ± 0.6a	β-caryophyllene
C-c1	3.7 ± 1.1	3.0 ± 0.6	3.2 ± 0.9	2-hexenal
C-c2	3.1 ± 0.4	0.2 ± 0.2	3.1 ± 0.6	santolina triene
C-c3	2.7 ± 0.1	0.9 ± 0.5	3.2 ± 0.4	α-pinene
C-c4	12.1 ± 2.1a	2.4 ± 0.8b	11.8 ± 1.0a	β-myrcene
C-c5	19.8 ± 2.6a	33.9 ± 0.1b	24.1 ± 0.5ab	1,8-cineole
C-c6	43.5 ± 2.0	41.7 ± 0.6	40.5 ± 1.3	artemesia ketone
C-c7	7.8 ± 1.4	9.1 ± 1.2	7.3 ± 0.5	artemesia triene
C-c8	7.4 ± 1.7	8.8 ± 1.3	6.8 ± 0.6	1,3-cyclopentadiene

ality and preservation in plant materials.

In the present study, the composition of major volatile constituents in *Artemisia* leaves was compared among the three cultivars, Ganghwayakssuk, Namhaeyakssuk, and Hwanghaessuk, before and after post-harvest irradiation with 100-kGy gamma ray or 10-kGy electron beam. As shown in Table 1, the gamma ray or electron beam irradiation caused noticeable changes in the relative proportions of major volatile constituents. The relative proportion of 1,8-cineole in volatile constituents, which was about 16% in Ganghwayakssuk, 12% in Namhaeyakssuk, and 20% in Hwanghaessuk, was substantially increased up to 20%, 17%, and 34% (1.29-, 1.45-, and 1.71-fold) after irradiation with gamma ray. This increase was also observed to some extent after treatment with electron beam. In contrast, the proportion of artemesia triene in Namhaeyakssuk, and Hwanghaessuk seemed to be increased by gamma ray but decreased by electron beam. Moreover, the gamma ray irradiation commonly decreased the relative proportions of α-pinene, camphene, and santolina triene in leaves of two or three cultivars. These results imply that the influence of gamma ray or electron beam irradiation on the composition of volatile constituents in *Artemisia* leaves could be more dependent on the constituents themselves than the species or cultivar. This supposition is partly supported by the fact that β-phellandrene, β-caryophyllene, and β-myrcene were steeply decreased up to 9%, 17%, and 20% of the control after irradiation with gamma ray, respectively, as these constituents were found to exist only in one of three cultivars tested. Furthermore, unlike 100-kGy gamma ray or 10-kGy electron beam, 0.5 ~ 10 kGy gamma ray failed to directly and shortly modulate the composition of volatile constituents in the three cultivars (data not shown).

CONCLUSION

In the present study, the obtained data indicated that gamma ray or electron beam irradiation could modulate the composition of volatile constituents in leaves of *Artemisia princeps* and *Artemisia argyi*, depending on the radiation dose or each constituent more than the species or cultivar. Therefore, it is suggested that such irradiation can be applied to

modulate the composition of volatile constituents in *Artemisia* leaves for specific industrial/medical purposes such as aroma therapy, air fresheners, antifungal or antibacterial agents, and so on.

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