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Influence of air temperature on the histological characteristics of ginseng (*Panax ginseng* C. A. Meyer) in six regions of Korea

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Abstract

Ginseng (*Panax ginseng* C.A. Meyer) is a well-known oriental traditional herb that grows naturally on the Korean peninsula, in Manchuria (northeast of China), and the Maritime province of Siberia. The quality of ginseng is usually estimated according to its cultivation region in Korea. We analyzed the air temperature of six well-known ginseng cultivation regions and investigated the hardness and crude saponin content of the cultivated ginseng. According to the Korea Meteorological weather reports, the air temperature of Jinan and Ganghwa is lower than that of Geumsan. The cambial growth, tissue density, and xylem hardness of ginseng grown in the cooler regions (i.e., Ganghwa and Jinan) were greater than those in the other four regions. The crude saponin content of ginseng of Ganghwa and Jinan was 1.2 fold higher than that of Gimpo and Geumsan. From these results, we concluded that the lower summer air temperature contributes to the growth of ginseng and enriches its crude saponin content.

Keywords: *Panax ginseng*; air temperature; histological characteristic; hardness; crude saponin content.
Abbreviation; OCT-optimal cutting temperature; gf-gram force; KFDA- Korea Food and Drug Administration.

Introduction

Ginseng (*Panax ginseng* C.A. Meyer) which belongs to the family Araliaceae is a rare and slow-growing perennial plant. Ginseng is a well-known oriental traditional medicinal herb that has been used as a noble medicine for thousands of years (Zhuravlev et al., 2008). Recently, ginseng has also gained popularity in the West and studies show that it promotes health and increases general body vigor (Coleman et al., 2003; Ellis and Reddy, 2002). Ginseng grows naturally between 33°N and 48°N, which corresponds to the subarctic and temperate climate regions in Korea (between 33°7′N and 43°1′N), Manchuria (between 43°N and 47°N), and the Maritime province of Siberia (Choi et al., 2007; Korea Rural Development Administration, 2009). Plants are affected by various environmental factors including climate and soil factors (such as nutrients, hydrogen ion and moisture content, and microbial populations). Climatic factors usually include air temperature, precipitation, and amount of sunshine. The air temperature-related physiological characteristics of *Panax ginseng* have been extensively reviewed in the literature (Mahfuzur and Zamir, 2005). These studies indicate that ginseng does not favor high temperature, therefore culture methods have been developed to avoid the effect of large increases in the air temperature of the arable land (Park, 1979). Especially, leaf burning phenomenon of ginseng was observed when the air temperature remained above 30ºC for one week. The condition was prolonged greater than one week, whole leaf burning or bleaching might occur due to the destruction of pigments in leaf tissues (Lee, 1988; Lee et al., 2010). Wild ginseng grows under trees in deep mountains, and prefers an extremely cold climate. The optimal temperature is between 10 ℃ and 20 ℃ during the leafing phase and between 21 ℃ and 25 ℃ during the flowering and fruiting phases (Mork et al., 1981). The ginseng cultivation conditions are unusual and since ginseng is a perennial plant, accumulated damage over time affects the plant's growth and development. High temperature damages ginseng by causing a cessation of photosynthesis, drying of leaves, and early defoliation (Ohh, 1981). Leaf spot disease, anthracnose, and root rot are also caused by high temperature (Mahfuzur and Zamir, 2005). When the temperature exceeds 21 ℃, the incidence of leaf spot disease greatly increases (Ohh and Park, 1980). At temperatures above 30 ℃, the photosynthesis rate decreases, which increases the respiration rate and degrades the growth and development of ginseng (Korea Rural Development Administration, 2009). Because ginseng is a nodule (root) crop, its overall growth and development are influenced by root growth. Traditionally, compact-tissue ginseng has been evaluated as having the best growth. The cultivation environment, which possibly causes the production of large ginseng roots, can also produce compact-tissue ginseng (Park et al., 1993). In addition, the efficacy and bioactive components of ginseng may differ depending on the cultivation region (Hwang et al., 2005). No study has investigated the correlation between climate and the morphology of ginseng. In this study, the air temperature in six well-known Korean ginseng cultivation regions is analyzed and some histological characteristics, including the main root hardness and the crude saponin content of ginseng grown for six years from these regions are investigated.

Results

Analyzed data of air temperature

The annual averages of the highest and lowest air temperatures

Table 1. Annual averages of the highest air temperatures (°C) of the six regions.

Year	Ganghwa	Geumsan	Gimpo	Jinan	Pocheon	Punggi
2004	24.6	25.4	24.3	23.8	24.3	24.7
2005	24.1	25.0	24.5	23.3	24.3	24.5
2006	25.5	25.9	25.8	24.1	25.5	25.5
2007	25.1	26.0	25.3	24.2	24.8	25.1
2008	24.5	26.5	24.2	24.8	24.5	25.3
2009	24.2	24.0	24.2	22.8	24.4	23.2
Average	24.7	25.5	24.7	23.8	24.6	24.7

Table 2. Annual averages of the lowest air temperatures (°C) of the six regions.

Year	Ganghwa	Geumsan	Gimpo	Jinan	Pocheon	Punggi
2004	–3.5	–2.5	–5.0	–3.5	–4.7	–2.8
2005	–4.7	–3.9	–6.2	–4.7	–6.2	–2.5
2006	–1.9	–0.5	–2.7	–1.3	–2.6	–0.6
2007	–1.3	–1.4	–3.0	–1.8	–2.2	0
2008	–3.0	–2.3	–4.2	–3.3	–3.5	–1.5
2009	–3.0	–3.4	–4.6	–3.5	–3.5	–2.9
Average	–2.9	–2.3	–4.3	–3.0	–3.8	–1.7

in the last 6 years were analyzed (Tables 1 and 2). The annual average air temperature was highest in August and lowest in January. Every temperature record was referred from the Korea Meteorological Administration weather reports (http://www.kma.go.kr/). Geumsan and Jinan had the highest and lowest average temperature (25.5 °C and 23.8 °C), respectively. The lowest average temperature in the six regions was about –3 °C. The northern regions (i.e., Ganghwa, Gimpo, and Pocheon) were colder than the southern regions (i.e., Geumsan, Jinan, and Punggi). The number of days with a mean air temperature above 21 °C was counted (Table 3). The Geumsan region showed the highest percentage of the days (25.02%), which was about a quarter of a year. Ganghwa, Gimpo, Pocheon, and Punggi showed a percentage of approximately 22–24%. The Jinan region showed the lowest percentage of the days, i.e., 18.68% (68.2 days). The number of days with a peak air temperature above 30 °C was counted (Table 4). The Geumsan and Ganghwa regions showed the highest and lowest percentage of the days (12.69% and 4.52%), respectively.

Histological characteristics of ginseng

The cross sections of the main root of ginseng showing the extent of cambial growth and tissue density are shown in Fig. 2. The extent of cambial growth differed slightly depending on the regions: it was more in the samples from Jinan, Pocheon, and Punggi than in the other 3 regions. The tissue density of ginseng cultivated in Ganghwa, Gimpo, Jinan, and Pocheon regions was higher than that cultivated in the other regions, and the thickness of xylem was thicker in the samples from Ganghwa, Jinan, and Punggi regions than in the samples of the other two regions.

Hardness of the main root of ginseng

The hardness of the main root of ginseng that was collected from each region is given in gf (gram force) (Fig. 3). The ginseng of Ganghwa and Jinan was harder than that of other regions.

Crude saponin content in ginseng

The crude saponin content of ginseng is shown in Fig. 4. Ganghwa ginseng had a higher crude saponin content than the ginseng from the other regions. Ganghwa ginseng contained

Fig 1. Locations of ginseng plantations from which the ginseng was collected in this study (Global positioning system [GPS] information and altitude).

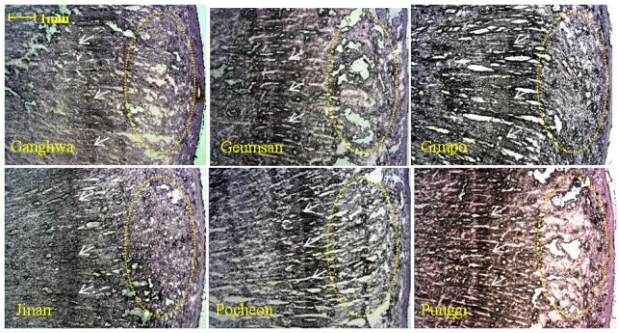

Fig 2. Light micrograph of ginseng root cross-section, taken from plants cultivated at six regions of Korea. The yellow circle can be distinguished by the thickness and compaction of the xylem. Differences in development of each ginseng root's

cambium can be distinguished as shown in the arrows indicated.

59.75 ± 0.87 mg of crude saponin in 1-g dried ginseng.

Correlation coefficient of crude saponin content and hardness of ginseng

A moderately positive correlation was observed between the crude saponin content and the hardness of ginseng (r = 0.672) (Fig.5).

Discussion

Plants belonging to the same species but occurring in different geographical zones may differ significantly in their content of particular secondary metabolites, both qualitatively and quantitatively (Szakiel et al., 2011). The suitable temperature for ginseng differs according to the growth phase: germination phase, 7–15 °C; leafing phase, 10–20 °C; and flowering and fruiting phases, 21–25 °C (Yang, 1974). Ginseng is usually cultivated by stratifying seeds so that the growth phase does not require much temperature maintenance. In this study, we analyzed the air temperatures in six well-known ginseng cultivation regions. The highest and lowest air temperatures in the six regions from 2004 to 2009 ranged from 23 °C to 26 °C and from –5 °C to –1 °C, respectively. Jinan showed the lowest average air temperature at 23.8°C. Jinan, Ganghwa, and Pocheon regions were cooler than the other three regions. The most popular ginseng cultivation region, Geumsan, showed the highest average air temperatures at over 25 °C (Table 1). The percentage of the days with a mean air temperature above 21 °C was over 20% in 5 regions; only Jinan region showed a percentage of less than 20% at 18.68%. Geumsan and Jinan are only about 40 km apart, but the difference in the average number of days with a mean air temperature of more than 21 °C between Geumsan and Jinan was large at 23 days. Ganghwa, Jinan, and Punggi regions showed fewer days than the other regions (Table 3). The number of days with a peak air temperature above 30 °C was less in Ganghwa, Gimpo, and Jinan regions than in Geumsan, Pocheon, and Punggi regions. Ganghwa and Gimpo regions, in particular, have an oceanic climate due to their proximity to the sea and are cooler than the other regions. Jinan also showed fewer days with a peak air temperature above 30 °C than the other regions (Table 4). This was attributed to Jinan's location at a higher altitude than the other regions. High temperature adversely affects soil moisture and microbial populations, which leads to secondary influence (Park, 1980). A temperature maintained over 21 °C can give leaf spot disease and reduce photosynthesis of ginseng (Ohh and Park, 1980). During the high-temperature summer period from July to August, when a high temperature of above 30 °C persists for over 7 days, ginseng shows reduced photosynthesis and early defoliation due to leaf-burning disease (Korea Ginseng and Tobacco Research Institute, 1996). This reduced photosynthesis degrades the whole ginseng, and eventually influences the root growth of ginseng, which is a nodule (root) plant. Ecological condition influenced on the annual and seasonal dynamics of the structure of ginseng main root (Liu et al., 1990). Ginsenosides, the active principles of ginseng, are present in the periderm and cortex, outside the root cambium, but not in the xylem or pith of ginseng roots (Kubo et al., 1980; Tani et al., 1981). Therefore, we focused on the density of ginseng xylem and pith. The thickness of xylem was more in Geumsan, Jinan, and Punggi regions and the density of xylem was higher in Ganghwa, Gimpo, Jinan, and Pocheon regions than in the other regions. A layer of cambium is a source of cells, known as lateral meristems, for secondary growth (Taiz and Zeiger, 1998). Cambial growth rate was higher in Jinan, Pocheon, and Punggi regions. Analysis of the air temperature and histological characteristics showed that the ginseng in the cooler regions had thicker xylem and more compact tissue than that in the other regions (Fig. 2). To evaluate the histological characteristics, we used a durometer to measure the hardness of ginseng. Our observation indicated that ginseng with a thick cross section of xylem had a greater hardness than that showing thin xylem. The ginseng of Geumsan, Jinan, and Punggi region belonged to this category. To measure the crude saponin content of all the ginseng samples under the same condition, ginseng collected from all the regions was dried to the same water content of 15%. Ganghwa and Jinan ginseng had higher crude saponin content than the ginseng samples cultivated in the other regions. Because the major ingredients of ginseng, ginsenosides and saponin, are contained in the cortex, ginseng with thick cortex and hard tissue showed a high crude saponin content. As aforementioned, the air temperature of Ganghwa and Jinan was lower than that in the other regions (Tables 1, 3 and 4). Ginseng of Ganghwa and Jinan regions was harder than that of the other four regions (Fig. 3). This implies that ginseng from cooler regions, which can withstand hot summers, has a higher crude saponin content (Fig. 4). A moderately positive correlation was observed between the crude saponin content and the hardness of ginseng (r = 0.672) (Fig. 5). The results indicated that a lower temperature promoted hardness and crude saponin content of ginseng.

Materials and methods

Materials

Five-years-old root samples of ginseng were collected from two locations in each of six regions in Korea: Ganghwa (between 37°44′46″N 126°30′03″E to 37°54′26″N 126°30′43″E), Geumsan (between 36°00′45″N 127°30′38″E to 36°06′19″N 127°31′48″E), Gimpo (between 37°40′29″N 126°37′05″E to 37°39′26″N 126°40′48″E), Jinan (between 35°48′07″N 127°25′50″E to 35°49′52″N 127°26′45″E), Pocheon (between 37°53′11″N 127°11′25″E to 37°54′26″N 127°12′39″E), and Punggi (between 36°52′53″N 128°31′30″E to 36°52′25″N 128°32′24″E) (Fig. 1).

Air temperature data

The air temperature of the six regions was analyzed using Korea Meteorological Administration weather reports from 2004 to 2009 (http://www.kma.go.kr/). The mean air temperature and the highest air temperature were analyzed and the numbers of days with a mean air temperature above 21 °C and a peak air temperature above 30 °C were counted.

Observation of histological characteristics of ginseng

Before cutting, fresh ginseng roots were kept under constant temperature and humidity. The roots were embedded in an optimal cutting temperature (OCT) compound (Sakura Finetek, Torrance, CA, USA) and cut into 30-µm-thick sections using a cryostat (Leica CM 1850; Leica Microsystems, Heidelberg, Germany) at –20 °C. The cross sections were stained with hematoxylin and eosin and observed under a microscope (Olympus BX41TF; Olympus, Tokyo, Japan) at 12.5× magnification. The observation was focused on the cambia of the ginseng roots, the density of root tissues, and the thickness of the xylem.

Measurement of main root hardness

Hardness can be used as a criterion for determining the quality of ginseng. Since there were no suggested methods for measuring the hardness of ginseng previously, we established

Table 3. The number of days with a mean air temperature above 21 °C.

Year	Ganghwa	Geumsan	Gimpo	Jinan	Pocheon	Punggi
2004	78	95	84	70	86	87
2005	87	99	92	80	92	95
2006	64	82	72	56	71	75
2007	91	95	88	70	84	82
2008	93	97	92	70	99	80
2009	81	80	79	63	83	73
Average	82.3	91.3	84.5	68.2	85.8	82.0
Percentage of days a (%)	22.56	25.02	23.15	18.68	23.52	22.47

a Percentage of days was calculated by dividing the average number of days by 365 (assuming 365 days in a year).

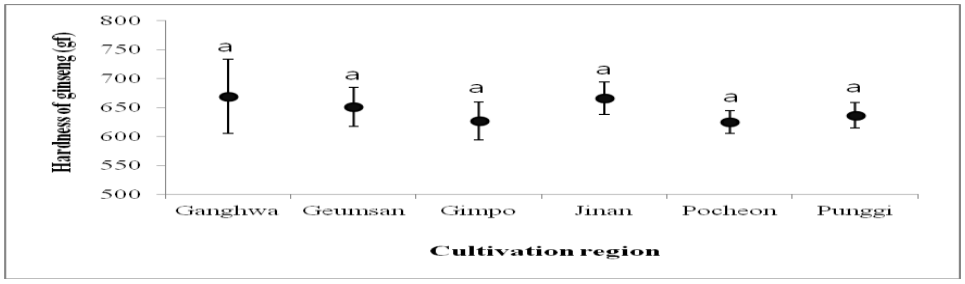

Fig 3. Hardness (mean ± SD) of the main root ginseng cultivated at six regions of Korea. Means with different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 4. The number of days with a peak air temperature above 30 °C.

Year	Ganghwa	Geumsan	Gimpo	Jinan	Pocheon	Punggi
2004	22	43	26	29	28	42
2005	19	52	28	20	30	39
2006	20	47	25	20	23	30
2007	15	56	35	17	31	36
2008	13	54	27	28	46	41
2009	10	26	29	15	35	23
Average	16.5	46.3	28.3	21.5	32.2	35.2
Percentage of days a (%)	4.52	12.69	7.76	5.89	8.81	9.63

a Percentage of days was calculated by dividing the average number of days by 365 (assuming 365 days in a year).

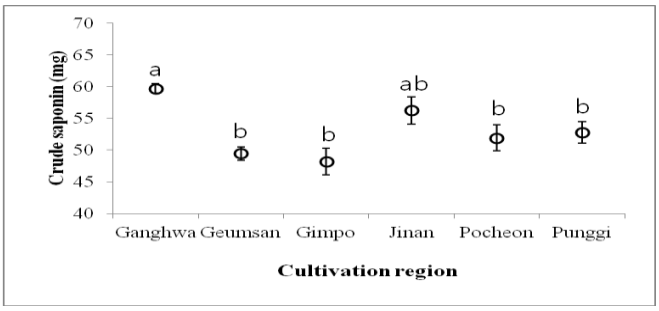

Fig 4. Crude saponin content (mean ± SD) in 1 g of dried ginseng cultivated at six regions of Korea. Means with different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

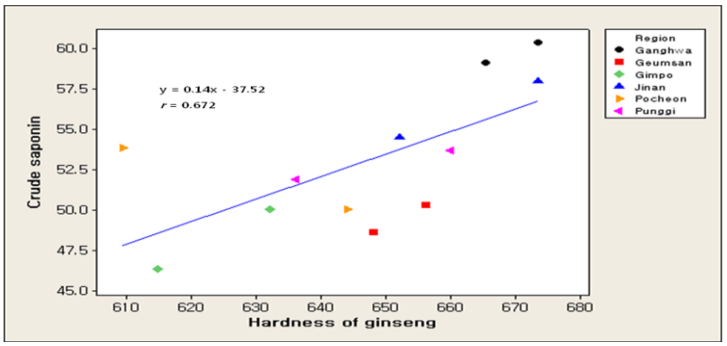

Fig 5. Correlation coefficient between crude saponin and hardness of ginseng cultivated at six regions of Korea.

our own using a durometer; the unit is gf (gram force). To measure the hardness of the ginseng root, it was kept in a chamber maintained at 20 ℃ and 80% humidity. After 72 hours, the hardness of the main root was randomly measured at three points using a durometer (Teclock GS-706N, Type A; Teclock, Nagano, Japan).

Analysis of crude saponin content

Crude saponin refers to the total crude ginsenoside quantified via fractionation of the solvent. The ginseng's crude saponin content was analyzed according to the Food Code of Korea Food and Drug Administration (KFDA) Food Code (KFDA, 2007). Fresh ginseng was dried in a hot-air drying machine at 65 ℃ for 48 hours. When the water content reached 15%, the dried ginseng was extracted with 70% ethyl alcohol for 24 hours. The extract was then extracted twice with water-saturated *n*-butanol at 70–80 ℃ for 1 hour and then concentrated by decompression. For fat removal, the concentrated extract was washed with ethyl ether (Lee et al., 2004).

Statistical analysis and the correlation coefficient of crude saponin content and hardness

The data on the crude saponin content and the hardness of ginseng presented are mean ± standard deviation (SD) from three separate experiments, each performed with six replicate measures. The data were evaluated with one-way analysis of variance (ANOVA) using SPSS (Chicago, Ill., USA). The differences between the means were determined using Duncan's multiple-range test. $P < 0.05$ was considered statistically significant. The Pearson correlation coefficient of the crude saponin content and hardness was analyzed using the statistical program MINITAB ver. 14.20.

Conclusions

The histological characteristics of *Panax ginseng* cultivated in six regions of Korea were investigated. Climatic factors are important for crop growth. We focused on the correlation between air temperature and the histological characteristics of ginseng. The numbers of days with a mean air temperature above 21℃ and a peak air temperature above 30℃ were determined from a standard index to assess the proper air temperature bounds. Based on these bounds, Ganghwa and Jinan were cooler than the other four regions. Ginseng of the two regions exhibited various good histological characteristics, such as hardness and crude saponin content. From our results, we concluded that a lower summer air temperature contributes to xylem compaction and hardness of ginseng, and in consequence, enriching crude saponin content and helping growth of ginseng.

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