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A preliminary study on suitability of growing ginseng (*Panax ginseng* Meyer) in the Western Himalayan region

Swati Walia[1,2], Pawan Kumar[3], Dinesh Kumar[1,3], Rakesh Kumar[1,2]**

[1]*Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India*
[2]*Agrotechnology Division, CSIR – Institute of Himalayan Bioresource Technology, Council of Scientific and Industrial Research, Palampur, India*
[3]*Chemical Technology Division, CSIR – Institute of Himalayan Bioresource Technology, Council of Scientific and Industrial Research, Palampur, India*
**Corresponding authors: dineshkumar@ihbt.res.in; rakeshkumar@ihbt.res.in*

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Abstract: *Panax ginseng* Meyer is one of Asia's most popular medicinal plants, with triterpene saponins as principal bioactive compounds. The present study investigates the possibility of ginseng cultivation in Lahaul & Spiti, Himachal Pradesh, India in the Western Himalayas focusing on growth characteristics, and ginsenoside content in the roots. Plant growth parameters increased with an increase in the crop age and reached maximum maturity at the age of five years along with the production of a good amount of seeds and roots. Root fresh and dry weight of the five-year-old plant was 142.6 g and 45.5 g, respectively, which almost doubled as compared with the four-year-old plant. The HPLC analysis of *P. ginseng* roots leads to the identification of 14 compounds representing 31.81 ± 2.89 mg/g of total ginsenoside contents, where Rb1, Rg2 and Re were found to be major ginsenosides with 7.53 ± 0.37, 7.04 ± 0.61 and 3.77 ± 0.26 mg/g content. Protopanaxadiol (PPD) and protopanaxatriol (PPT) represent the major classes of ginsenosides present in the ginseng roots with a 0.98 ratio of PPD/PPT. Our studies revealed that the soil and climate of the Lahaul and Spiti district of Himachal Pradesh State in the Western Himalayas are suitable for the cultivation of *P. ginseng* with good content of ginsenosides in five-year-old roots.

Keywords: perennial herb; environmental condition; adaptation behaviour; secondary metabolite; biomass

Panax ginseng Meyer (family Araliaceae) has been used in oriental medicine for over 2000 years having a long history as a general tonic promoting health, and it is believed to be a panacea and to promote longevity. It is distributed in 35 countries, mainly in Eastern Asia (especially in Japan, Korea, northeastern China, Bhutan, and East Siberia) and in Northern America, usually in cooler climates (Ryu et al. 2012). Also, very valuable wild species of ginseng are found in India, Nepal, and Myanmar with characteristically long growing periods. In India, it is found in the hills of Arunachal Pradesh, Manipur, Meghalaya, and Nagaland (Mao et al. 2009). The bioactive compounds present in *P. ginseng* mainly consist of ginsenosides, polysaccharides, phenolics, and polyacetylenes. Saponin triterpenoid glycosides called "ginsenosides" (or panaxosides) are the main active ingredients in the roots of Asian ginseng, responsible for its medicinal properties (Pannacci et al. 2016). Ginseng tastes sweet, has the ability to keep the body warm, and has protective effects on heart, kidney, liver, lungs, and spleen (Kim et al. 2018). It acts as an adaptogen and is used to increase physical endurance, boost the immune system, erectile dysfunction, impotence and male fertility, bleeding disorders, disorders of pregnancy and childbirth (Ratan et al. 2021). All ginsenosides have been reported to exhibit various biological activities such as anti-cancer, heart-

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protective, neuroprotective, anti-diabetic, antiageing, antitumor, antifungal, anti-obesity, radioprotective, and antiamnestic ones (Pannacci et al. 2016).

P. ginseng commonly known as Asian Ginseng, is a slow-growing, glabrous, perennial herb reaching a height of 30–60 cm at the age of 5 years with thick corpulent roots. The plant bears three to five palmately compound stalked leaves with a cluster of white to greenish-white flowers in an umbel. It forms oval berries turning red when ripe, each containing two to three greyish-white wrinkled seeds (Kim et al. 2018). The roots tend to attain a marketable height (7.5 to 20.5 cm long and 1 to 2.5 cm thick) and weight (28 g) after three years of growth and are forked in mature plants. The total world ginseng production is 80 080 t per year, and its global demand, including ginseng root and refined goods, valued 2 084 million USD (Baeg and So 2013). Wild ginseng is relatively rare and even increasingly endangered due to the high demand for the product in recent years, which has led to the wild plants being sought out and harvested faster than the new ones can grow to reach maturity. Therefore, predicting the distribution of plants is necessary for their conservation and cultivation.

Originated in cold mountainous areas of northeastern China to Russia and Korea, ginseng is a typical temperate medicinal plant that requires a relatively cold temperature for optimum growth and development (Kim et al. 2018). The production of ginseng biomass and quality can be greatly improved through enhanced biological/soil health (Liu et al. 2022). Due to its higher price and greater demand for the world market, ginseng is one of the most interesting crops to be cultivated in the agricultural area. So the main aim of this study was to observe for the first time the growth, adaptation behaviour and its ginsenoside content, which is recognised as an important factor in determining the quality of ginseng in the Western Himalayas.

MATERIAL AND METHODS

Site description

A field experiment was conducted in the farmer's field at Sansha village in Lahaul and Spiti (3 800 m a.s.l., 32°61'92"N, 77°37'84"'E) district of Himachal Pradesh (HP), India, during 2011–2016. The soil of the study site was coarse sandy loam in texture and classified as Udorthents as per the taxonomic system of soil classification with rock types quartzite, slate, phyllite, limestone, shale, schists and conglomerate. The basic properties of the topsoil (0–15 cm) before the experiment were as follows: pH_{H_2O} acidic in reaction (pH 6.48), high level of organic carbon (1.22%) with 0.38 mS/cm electrical conductivity. Available nitrogen (87.22 ppm) low, while available potassium (129.86 ppm) and available phosphorus (25.50 ppm) were high. Weather data was collected from weather towers located in the Centre for High Altitude Biology (CeHAB), Ribling Keylong, Lahaul and Spiti, HP, a research unit of CSIR-Institute of Himalayan Bioresource Technology, Palampur, HP and the data is depicted in Table 1.

Experimental details

Ginseng was propagated through seeds. Healthy seeds of Asian ginseng were grown in Lahaul & Spiti, HP, India in 2006 for 3–4 years. Seeds of plants grown in the experimental location were collected and used for the current experiment. Before sowing, the seeds were exposed to long storage time in a damp medium (moist sand, old sawdust or forest soil) with warm/cold treatment; a method known as stratification. The stratified seeds were sown during November 2011. One-year-old healthy rooted plantlets were transplanted in rows to maintain a distance of 15 to 20 cm between October to November 2012. The growing plants were provided with 50% shade by using HDPE green shade net. Proper ventilation is necessary for ginseng, so North and East directions were kept open to ensure free ventilation. In winters, mulching was done by covering the beds with a 4–6 cm layer of forest leaves, stalks, hay or other coarse litter to protect the underground roots from freezing temperatures and frost injury. Beds were kept free from weeds by regular weeding and hoeing. Three to four irrigations were given during the summer months (May to August) every year during the crop growth. Different growth stages of ginseng cultivation in the Western Himalayas are depicted in Figure 1.

Growth and yield analysis

Following the experimental procedure, the experiment was executed in three replicates to increase the precision, and the experimental field was differentiated into three plots. Plot size was 3 m^2 with 50 plants per plot spaced at 30 × 20 cm spacing. Growth data *viz.* plant height, plant spread, number of leaflets and number of leaves was recorded for different age group ginseng plants by selecting 5 plants per plot

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Table 1. Comparison of meteorological data at Lahaul & Spiti, Himachal Pradesh (HP), India, with those at Nagano, Japan

Month	Lahaul & Spiti, Himachal Pradesh, India*						Nagano, Japan**		
	developmental stage of ginseng (cultivation practices)	temperature (°C)		soil temp. (°C)	rainfall (mm)	relative humidity (%)	temperature (°C)		soil temp. (°C)
		max.	min.				max.	min.	
January	dormancy	–2.4	–9.4	–0.5	9.8	56.2	4.4	–6.9	1.8
February	dormancy	–0.2	–8.7	0.1	7.3	58.6	4.2	–7.1	2.4
March	dormancy	–1.3	–9.1	–0.2	8.6	57.4	8.3	–3.5	3.9
April	buds/seeds start to develop, leaf expanding, flower bud bolting	7.4	–1.9	1.2	115.7	68.6	16.4	2.4	9.2
May	flowering	13.7	2.6	8.2	34.3	65.9	22.9	7.3	13.4
June	fruit development	17.8	6.7	14.3	14.2	62.0	23.6	12.3	18.3
July	fruit harvesting, seed stratification (until early august)	21.2	10.8	18.4	4.8	64.9	26.9	16.5	21.8
August	GA_3 treatment, stratification	20.8	10.2	18.3	12.8	65.4	28.9	17.8	24.3
September	root harvesting	18.3	6.8	15.6	5.5	60.3	24.1	14.2	21.7
October	senescence start	15.4	3.3	12.5	1.4	46.7	17.6	6.5	13.6
November	dormancy (seeding)	7.5	–2.4	3.9	48.7	37.5	12.6	0.6	8.1
December	dormancy (transplant of roots)	3.5	–5.5	0.8	5.2	35.6	7.7	–4.7	1.6

*CeHAB Ribling farm, Lahaul and Spiti, HP, India (5-year average); **Kitamimaki experimental station, Nagano, Japan (10-year average) (Sugino et al. 1995)

Figure 1. Growth cycle observed during the cultivation of *Panax ginseng* in Lahaul & Spiti district of Himachal Pradesh

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(in total 15 plants from three plots). However, root parameters *viz.* root length, number of rootlets, root diameter and root weight were recorded from 4- and 5-year-old plants (keeping the same sampling size, i.e. 5 plants/plot). The plant height was measured from the soil level to the plant tip. Plant spread was recorded in North-South (N-S) and East-West (E-W) directions. Survival rate was also calculated after five years according to the equation: SR = N5/N1 × 100%, where: SR – survival rate; N5 – number of ginseng plants in the 5th year, and N1 – number of ginseng plants in the 1st year. Harvesting of ginseng roots was done during the month of September in 2016 and 2017 from four- and five-year-old plants, respectively. The root samples were collected, and the root parameters were determined.

Ginsenoside extraction

For ginsenoside extraction, three plants were randomly taken from the experimental field. Within several days from collecting plants in plastic bags, samples were rinsed with tap water to remove soil, blotted dry, and then air-dried in a hot air oven for 48 h at 35–40 °C. The rhizome and fibrous secondary roots were collected after drying, and for analysis, the sample was prepared by measuring and grinding to a fine powder. Powdered samples were stored in airtight glass scintillation vials at room temperature.

Preparation of sample solution. Five hundred milligrams of material of each sample was sonicated in 5 mL LC-MS grade methanol for 45 min and filtered through a 0.25 µm syringe filter. Finally, 100 mg/mL solution was used for the testing.

Preparation of standard solution. Fourteen standards (Rb1, Rb2, Rb3, Rc, Rd, Re, Rf1, Rg1, Rg2, Rg3, Rh2, Ro, Rr1, F1) were selected for the preparation of the standard stock solution. Each sample was prepared in a concentration of 1 mg/mL. The standard solution was further serially diluted to get the regression equation.

LC-IMS-QTOF-MS conditions. Qualitative and quantitative analysis was done using an Agilent UHPLC-IMS-QTOF system. The analytical Agilent RRHD C18 column (2.1 mm × 150 mm, 1.8 µm) was used with the gradient elution of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). The separation was achieved with elution started with 0 min, at 5% B; 0–0.5 min, 5% B; 0.5–8 min, linear gradient from 5% B to 65% B; 8–11 min, 65% B; 11–29 min, 85% B; 29–30 min, 95% B, run in the same concentration till 35 min, then again the mobile phase run in initial conditions, from 35 to 36 min, 5% B and post-run time was 4 min in initial conditions, 5% B. Elution was performed at a solvent flow rate of 0.21 mL/min. The column temperature was set at 30 °C. MS conditions – source – Dual AJS-ESI, gas temperature – 350 °C, gas flow (L/min) – 12, nebuliser (PSIS) – 30, sheath gas temperature – 325 °C, sheath gas flow – 12 L/min, capillary voltage – 3 100, nozzle voltage (V) – 500, fragmentor set – 400.

Ginsenoside analysis. The analysis was conducted based on BPC and XIC chromatogram method on a highly sensitive instrument LC-IMS-QTOF-MS. The identification of ginsenosides was carried out by comparing the retention time and mass with the reference standards, while quantitative analysis was performed using EIC (extracted ion chromatogram). The calibration curves of reference standards were created using an area under the EIC of each standard in different dilutions. The calibration curve was found linear in the concentration range of 12.5–200 µg/mL. The analysis was performed in triplicate.

Statistical analysis

The statistical significance of the differences was determined using Duncan's multiple tests and one-way analysis of variance, evaluating significant differences at $P = 0.05$. All data were at the 5% significance level and were reported as mean ± standard deviation.

RESULTS

Developmental stages and meteorological data

In ginseng, the active photosynthetic period was only recorded for about 5–6 months in a year, i.e. from April to September. During this period, different activities were observed like seed/bud development, leaf expanding, flower bud bolting, flowering, fruit development and root development. In October, senescence started, which was followed by a long period of dormancy, i.e. from November to March. After this dormancy period, only one shoot with a definite number of branches and leaves develops every year. The ginseng plant showed a slow growth rate, due to which roots were found to be matured after five years, and the final harvesting was done after six years from germination. Meteorological observations were also recorded during the growth cycle of ginseng for six consecutive years, the average values of which are presented in Table 1. Monthly

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average data of different weather parameters was recorded from September 2013 to September 2017. The maximum temperature ranged from –2.3 to 21.2 °C, whereas the minimum temperature ranged from –9.8 to 10.4 °C during 2013–2017. Throughout the year, average soil temperature ranged from –0.5 to 18.4 °C, mean relative humidity ranged between 35.6% and 68.8%, while the average total rainfall received during the year was 268.77 mm.

Plant growth parameters

A perusal of the data presented in Table 2 revealed that the growth parameters of ginseng increased with the increase in plant age. Plant height ranged from 12.5 to 38.2 cm during 2015 and 9.0 to 47.0 cm during 2016. The number of leaflets also increased continuously reaching a maximum from one to four leaflets in four-years-old and five in five-years-old plants in 2015 and 2016, respectively. A similar trend was observed in the number of leaves and plant spread in both directions. The number of leaves ranged from 12.2 to 23.0 in 2015 and 12.4 to 25.2 in 2016. The plant spread in the N-S direction ranged from 9.2 to 32.9 and 9.2 to 45.4 cm in 2015 and 2016, respectively; however, in the E-W direction, it ranged from 9.7 to 30.2 cm in 2015 and 10.6 to 43.4 cm in 2016. Plants established well in the Western Himalayas as the plant growth increased with the age of the plants and started producing a good amount of seeds in the five-year-old crop. The average survival rate recorded after five years from three plots was 85.3 ± 1.2%, which was quite good in the cold desert conditions of Lahaul & Spiti.

Root growth and biomass

The growth and yield parameters of *P. ginseng* root increased with the increase in the age of the crop (Figure 2). Root length was 32.4 cm in a 4-year-old and 43.5 cm in a 5-year-old crop, while root diameter was 2.2 cm and 4.1 cm in 4- and 5-year-old crops, respectively. The number of rootlets also increased with the plant age (7.0 rootlets in four-year-old and 14.0 in five-year-old plants). A similar trend was seen in the case of the fresh root and dry weight. Root fresh weight was 98.7 g/plant in 4-year and 142.6 g/plant in 5-year-old crops, while root dry weight was 29.6 g/plant and 45.5 g/plant in 4- and 5-year-old crops, respectively. Root fresh weight increases by 44.4% in five-year-old plants as compared with four-year-old plants. Root growth parameters increased with the age of the plants and started producing a good amount of roots in the five-year-old crop. Root weight doubles in the period between the 4th and 5th year of life.

Analytical performance of HPLC

Total 14 ginsenosides, i.e. Rb1, Rc, Rb2, Rb3, Re, Rd, Rg1, Rf1, Rg2, Rg3, Rh2, Ro, Rr1, and F1, in the extracts were classified by comparing retention times with authentic standard ginsenosides obtained from standard chromatograms. The relationships between the analyte concentrations and measured signals for 14 ginsenosides are listed in Table 3. The correlation coefficients confirmed the linearity of the calibration curves (Table 3). The accuracy of the injection was achieved by evaluating the differences in the peak area of 14 ginsenoside levels. All the correlation

Table 2. Growth parameters of *Panax ginseng* of different crops during 2015 and 2016

Age of plant	Plant height (cm)		Number of leaflets/plant		Number of leaves/plant		Plant spread (cm)			
							N-S		E-W	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
1 year old	12.5 ± 1.6	9.0 ± 2.3	1.0 ± 0.0	1.0 ± 0.0	12.2 ± 0.8	12.4 ± 0.9	9.2 ± 2.1	9.2 ± 2.4	9.7 ± 1.3	10.6 ± 1.1
2 years old	19.7 ± 1.1	14.2 ± 1.9	2.0 ± 0.6	2.4 ± 0.5	16.8 ± 2.4	16.6 ± 2.4	15.9 ± 0.9	17.4 ± 3.0	16.5 ± 1.6	17.8 ± 3.3
3 years old	32.8 ± 2.5	27.0 ± 3.5	3.4 ± 0.5	4.6 ± 0.5	22.6 ± 2.3	23.4 ± 2.7	23.7 ± 1.8	26.0 ± 3.3	24.5 ± 1.4	25.2 ± 1.8
4 years old	38.2 ± 2.0	45.2 ± 4.9	4.6 ± 0.5	5.0 ± 0.0	23.0 ± 1.6	24.2 ± 1.5	32.9 ± 3.0	40.2 ± 3.1	30.2 ± 3.4	42.0 ± 6.4
5 years old*		47.0 ± 2.9	*	5.0 ± 0.0	*	25.2 ± 0.4	*	45.4 ± 3.9	*	43.4 ± 3.0

N-S – north-south; E-W – east-west; *Five-year-old plants were not available in 2015 for data; mean value ± standard deviation (n = 5)

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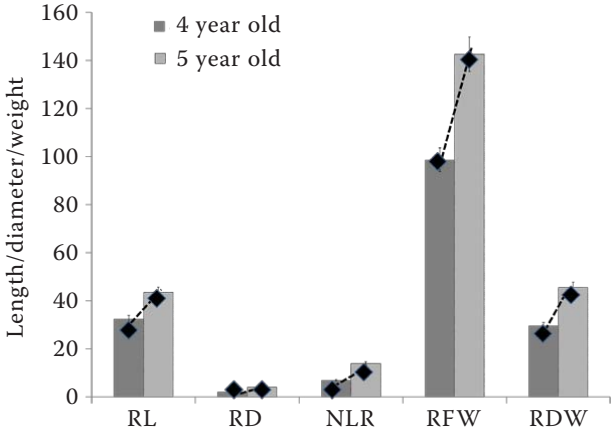


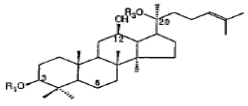
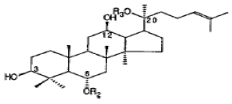
Figure 2. Graphical representation of root parameters *viz*. RL – root length (cm); RD – root diameter (cm); NLR – number of lateral roots; RFW – root fresh weight (g/plant); RDW – root dry weight of *Panax ginseng* in 4- and 5-year-old crop. The vertical bars represent the standard error of means (n = 5)

coefficients were high, indicating good linearity. All these data indicate that this method is accurate and sensitive.

Ginsenoside content

To provide the basic information on the components of five-year-old roots of ginseng, the content and the composition of ginsenosides were analysed by HPLC (high-performance liquid chromatography). Ginsenoside standards were prepared on the ginseng ginsenosides; the results were directly compared with the standard ginsenosides for confirmation through the HPLC analysis (Figure 3). *P. ginseng* root analysis led to the identification of 14 compounds representing 31.81 ± 2.89 mg/g of total ginsenoside content, which was further classified into different classes such as protopanaxadiol (PPD) with 14.76 ± 2.18 mg/g (including ginsenosides Rb1, Rc, Rb2,

Table 3. The linear regression equations, correlation coefficient and ginsenoside content of 14 ginsenosides in five-year-old roots of *Panax ginseng*

Ginsenosides		Linear ranges (μg/mL)	Regression equation	R^2	LOD (ng)	LOQ (ng)	Content (mg/g)
Protopanaxadiol (PPD)	Rb1	12.5–200	y = 7 277x + 23 827	0.991	0.042	0.128	7.53 ± 0.37
	Rc	12.5–200	y = 15 341x + 33 687	0.996	0.011	0.035	0.10 ± 0.01
	Rb2	12.5–200	y = 20 546x + 24 027	0.999	0.023	0.069	2.35 ± 0.36
	Rb3	12.5–200	y = 28 784x + 19 941	0.997	0.020	0.059	2.07 ± 0.46
	Rd	12.5–200	y = 46 133x + 54 338	0.994	0.027	0.082	2.30 ± 0.94
	Rg3	12.5–200	y = 22 012x – 50 178	0.987	0.020	0.059	0.36 ± 0.06
	Rh2	12.5–200	y = 71 935x + 70 544	0.994	0.151	0.458	nd
Protopanaxatriol (PPT)	Re	12.5–200	y = 38 388x + 99 139	0.994	0.014	0.044	3.77 ± 0.26
	Rg1	12.5–200	y = 79 103x + 40 472	0.976	0.041	0.123	1.22 ± 0.22
	Rf1	12.5–200	y = 32 189x + 51 682	0.986	0.040	0.121	2.91 ± 0.05
	Rg2	12.5–200	y = 10 949x + 65 812	0.978	0.047	0.144	7.04 ± 0.61
	Ro	12.5–200	y = 31 997x – 11 370	0.999	0.057	0.172	0.93 ± 0.06
Others	Rr1	12.5–200	y = 41 231x + 76 571	0.981	0.111	0.335	0.11 ± 0.01
	F1	12.5–200	y = 16 921x + 66 628	0.986	0.071	0.214	1.14 ± 0.08
Total ginsenosides (mg/g)					31.81 ± 2.89		
Protopanaxadiol (PPD) (mg/g)					14.76 ± 2.18		
Protopanaxatriol (PPT) (mg/g)					14.95 ± 0.69		
Others (mg/g)					2.18 ± 0.01		
PPD/PPT					0.98		
Rb1/total ginsenosides					0.23		
Re/total ginsenosides					0.12		
Rg2/total ginsenosides					0.22		

Y and X are the peak area and the concentration of analytes, respectively. R^2 – correlation coefficient; nd – not determined; ng – nanogram; LOD – limit of detection; LOQ – limit of quantification. Mean value ± standard deviation (n = 3)

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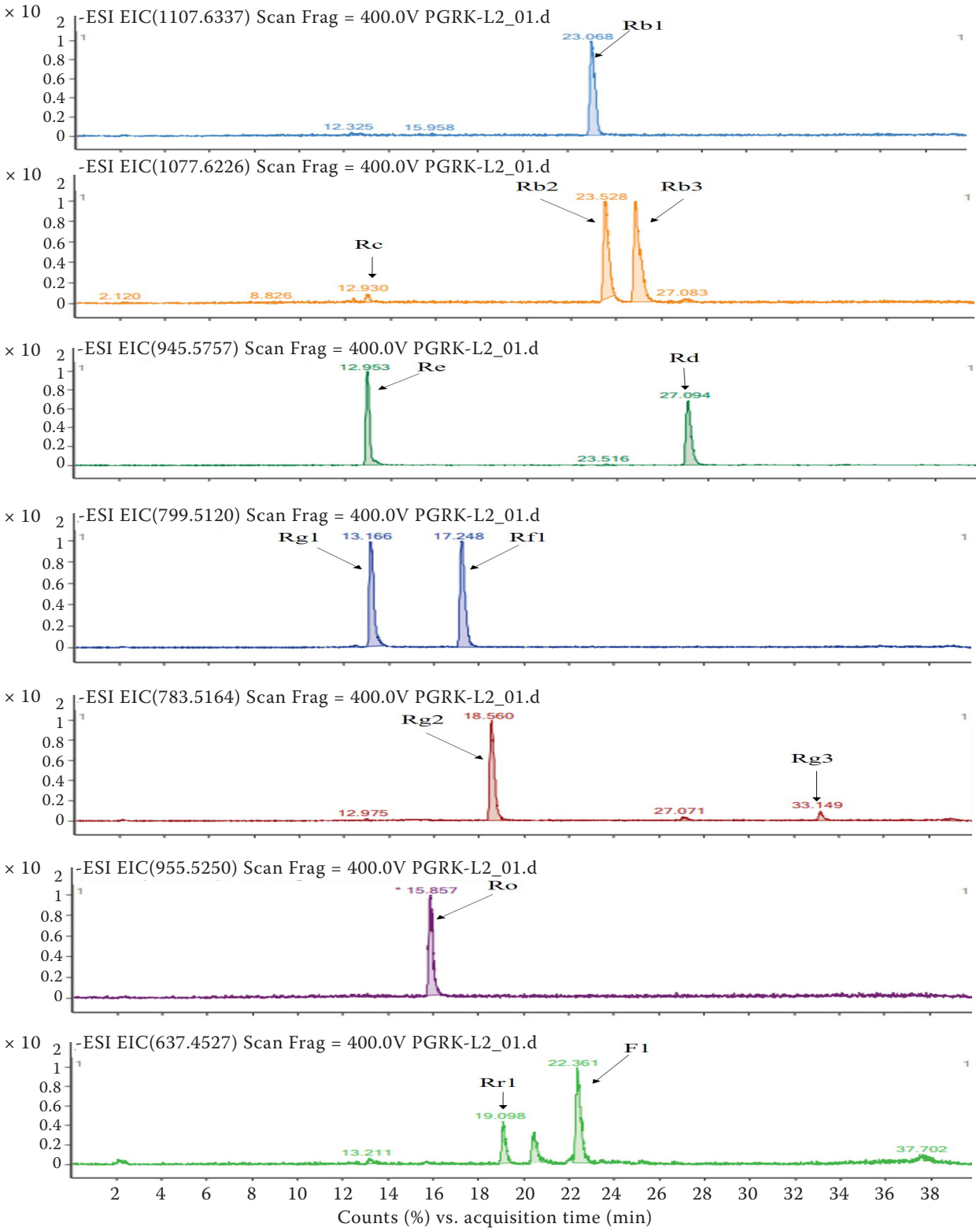

Figure 3. Extracted ion chromatograms (XIC) chromatogram of ginsenosides

Rb3, Rd, Rg3, Rh2), protopanaxatriol (PPT) with 14.95 ± 0.69 mg/g (including ginsenosides Re, Rg1, Rf1, Rg2) and others with 2.18 ± 0.01 mg/g (including Ro, Rr1, F1) as given in Table 3. The ratio of PPD/PPT was 0.98. Rb1, Rg2, and Re were found to be major ginsenosides in the five-year-old roots of

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P. ginseng with 7.53 ± 0.37, 7.04 ± 0.61, and 3.77 ± 0.26 mg/g content, respectively. The ratio of Rb1, Rg2, and Re to total ginsenosides was 0.23, 0.22, and 0.12, respectively. Other ginsenosides, such as Rb2 (2.35 ± 0.36 mg/g), Rf1 (2.91 ± 0.05 mg/g), Rd (2.30 ± 0.94 mg/g), Rb3 (2.07 ± 0.46 mg/g), F1 (1.14 ± 0.08 mg/g) and Rg1 (1.22 ± 0.22 mg/g) were also present in a good amount. However, ginsenosides Ro (0.93 ± 0.06 mg/g), Rg3 (0.36 ± 0.06 mg/g), Rr1 (0.11 ± 0.01 mg/g) and Rc (0.10 ± 0.01 mg/g) were present in the least amount, while Rh2 was not detected.

DISCUSSION

Ginseng grows natively as a shade plant beneath trees in the mountainous area of temperate China, Russia, Japan, and Korea. The possibility of cultivating ginseng in Lahaul & Spiti district of HP was figured out by carefully analysing the microclimate differences by comparing the meteorological data of Nagano, Japan (long experience in growing ginseng) and CeHAB Lahaul & Spiti, HP, India (Table 1). Ginseng requires a relatively low light intensity and an optimum air temperature of 16–28 °C for normal growth. High air temperature above 35 °C readily damages plant growth. The maximum temperature and minimum temperature recorded in Lahaul & Spiti is an advantage for ginseng growth. Low soil temperature recorded from November to February is also advantageous for its growth in Lahaul & Spiti, as the low soil temperature is necessary for breaking bud dormancy in the basal portion of the stem above the main root and for promoting seed germination. The average air humidity of 40% seems to be optimum for plant growth; however, the cold desert region of Lahaul & Spiti has insufficiently high relative humidity throughout the growth period. Since the high air humidity, together with the high temperature, usually causes severe problems of damage by pests and diseases, but in the Lahaul & Spiti region, the temperature is optimum throughout the year, and it seems not to have any disadvantages over its growth. In the case of soil properties, loamy soil makes the best soil conditions due to its good drainage and appropriate moisture content for ginseng growth, which is similar to Lahaul & Spiti with sandy loam soil. However, the soil pH (6.48) is slightly higher than the optimum soil pH (5.0). Environmental conditions are quite similar in the Lahaul & Spiti region as compared with Nagano, Japan (Sugino et al. 1995), so there is a good possibility of cultivating high-quality ginseng plants in the cold desert region of Lahaul & Spiti, HP, India.

Growth parameters increased with the increase in the age of plants during 2015 and 2016. This is because ginseng in the early growth cycle, absorbs a lot of nutrients to provide adequate food and energy for growth and development (Ma et al. 2016). Soldati and Tanaka (1984) also reported similar results showing that growth parameters increase with the increase in the age of the crop and was seen maximum in 4- and 5-year-old crops. Choi (2008) also reported an increase in growth parameters with the increasing age in Korean *P. ginseng* plants. Ma et al. (2016) reported in *P. ginseng* cultivated in a forest that, morphological characteristics increased with the years of growth. The height of *P. wangianus*, *P. japonicus* and *P. vietnamensis* aerial shoots showed a similar pattern in growth relative to the plant age (Shu 2007). Kołodziej et al. (2006) reported that there were three to five leaflets in *P. quinquefolium* two-year-old plant, 10 leaflets from three to six years which keep on increasing and reached 20 leaflets in 10- to 11-year-old plants. These growth trends are as per the studies of Guo et al. (2010). Venugopal and Ahuja (2011) also reported an increase in different growth parameters like plant height, number of leaflets and leaf area with an increase in the plant age.

Root growth parameters increased with the age of the plants and started producing a good amount of roots in the five-year-old crop (Figure 2). These results are as per the study of Soldati and Tanaka (1984), reporting major development of plants between the 4^{th} and 5^{th} year of life with almost double root weight during this period. While Ma et al. (2016) demonstrated an opposite trend reporting a decrease in the root length of main and lateral roots with an increase in the age of *P. ginseng*. Venugopal and Ahuja (2011) reported that rhizome diameter increased with an increase in the age of ginseng plants with 0.3 cm to 0.7 cm and 2.0 to 3.5 cm rhizome diameter in 1–10 years and 50 years old plants, respectively. Others also reported a similar trend in different *Panax* species (Guo et al. 2010, Lee and Mudge 2013).

Currently, about 30 ginsenosides have been isolated and identified in the cultivated and wild types of *P. ginseng*. According to the reports of Cho et al. (2014), in dry ginseng Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 were mainly detected in ginsenosides. Previously, two papers reported that *P. ginseng* roots had the highest amounts of Rb1 (Lee et al. 2017, Dai et al. 2020), while Wang et al. (2006) reported Re as the main constituent in ginseng roots. Environment plays an important role in the

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accumulation of secondary metabolites. As per the study of Zhang et al. (2017), ginsenosides Rb1, Rb2, RC, and Rg2 are the major extract constituents at a normal temperature, while less polar ginsenosides such as Rg3, Rg6, F4, Rs5, Rs4, Rg5, and Rk1 are the unique extract constituents at higher temperatures (> 120 °C). So, big difference in the percentage of components can be due to new cultivation sites with different environmental conditions, which might have caused the difference in the accumulation of different ginsenosides (Chen et al. 2020). The quality requirements for ginseng products, according to the domestic health functional food law, specify that the concentrations of Rb1 and Rg1 should be 0.8–34.0 mg/g (Cho et al. 2014). Choi et al. (2007), while studying the chemical characteristics of the field and mountain-cultivated ginseng roots, recorded 6.80 and 7.24 mg/g Rb1 in five-year-old roots, respectively, which was similar to the content recorded in our study. These results were in line with the studies of Kołodziej et al. (2006) and Ahn et al. (2013) while studying the accumulation of ginsenosides in ginseng roots. Similarly, Dai et al. (2020) also recorded various ginsenosides like Rb1, Rb2, Rg1, and Rg2 in the same range as recorded in our study. Wang et al. (2016), while studying the ginsenosides in roots of different age, recorded quite lower content of Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 when compared with our findings. These results indicate that the content of Rb1, Rg2, Re, Rb2, Rf1, Rd, Rb3, F1, Rg1, and total ginsenosides was rather higher in five-years-old roots of *P. ginseng* cultivated in the Lahaul & Spiti region of Himachal Pradesh.

The accumulation of ginsenosides in ginseng is variable and can be influenced by the surrounding environment, including soil fertility, temperature, light, and humidity. As shown in Table 3, the average content of total ginsenosides from Lahaul & Spiti, India, grown ginseng (31.81 ± 2.89 mg/g) is 93.02% higher than in China grown ginseng (16.48 ± 1.24 mg/g, n = 113) and 51.11% higher than in Korea grown ginseng (21.05 ± 1.57 mg/g, n = 106) when compared with the meta-analysis data reported by Chen et al. (2019). The PPD/PPT ratios of Lahaul & Spiti, India, grown ginseng was less than 2.0 (Table 3), although the ratio of PPD/PPT in most of the studies from China and Korea grown ginseng is more than 2.0 (Chen et al. 2019). However, Lahaul & Spiti, India, grown ginseng is grown in similar growth conditions, including the cold winter, temperate summer, and weakly acidic soil, to those of the natural ginseng habitat in Northeast China and Korea; however, there are some differences growing characteristics in Lahaul & Spiti, including high-intensity light radiation. It was reported that the total ginsenoside content increased significantly until the light transmission rate increased by 20%, but the PPT-type ginsenosides increased more than the PPD-type ginsenosides, leading to the ratio of PPD/PPT eventually decreasing. So to some extent, we have reason to believe that Lahaul & Spiti, India, has the unique geographical environment that encourages elevated ginsenoside (especially PPT-type ginsenoside) content compared to ginseng grown in the natural habitat in Northeast China and Korea. Environmental conditions are highly suitable in the cold desert region of Lahaul & Spiti, Himachal Pradesh, so there is a good possibility of cultivating high-quality ginseng plants. Ginseng is established well in the Western Himalayas as growth parameters increase with the increasing age of the plant and reach maximum maturity at the age of five years along with the production of a good amount of seeds and roots. The results also indicated that the content of ginsenosides was quite higher in five-year-old roots of ginseng, and it can be concluded that the soil and climate of the Lahaul and Spiti districts of Himachal Pradesh are suitable for the cultivation of *P. ginseng*. As per the analysis of the potential scale of the area similar to the studied village, an area of around 201 084 ha, including the Udaipur and Lahaul valley of Lahaul and Spiti district of HP, can be brought under ginseng cultivation. Different areas with similar environmental conditions as those of Lahaul and Spiti can also act as potential sites for ginseng cultivation. The environmental conditions prevailing in the inner temperate areas of Chamba, Kullu and Kinnaur districts of Himachal Pradesh, India, also show great potential for producing ginseng, so with the applicability of this study, rural people in these potential areas can be benefited, and the livelihood of tribal farmers present in these regions can be enhanced.

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