

Article

Comparative Analysis of Morphological, Histological, and Metabolic Differences of *In Vitro*- and *Ex Vitro*-Grown *Panax ginseng*

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Abstract

Ginseng (*Panax ginseng*) is highly sensitive to heat stress caused by climate change; thus, the introduction of heat-tolerant cultivars is essential. However, the stable dissemination of heat-tolerant cultivars remains limited due to low propagation efficiency. Plant tissue culture has been introduced as an alternative approach, yet *in vitro*-grown ginseng often exhibit low survival rates during acclimatization, thereby restricting their practical application. This study was conducted as a fundamental investigation to address this limitation by comparing the morphological, histological, physiological, and metabolic differences between ginseng plants grown *in vitro* and *ex vitro*. The results demonstrated that *in vitro*-grown ginseng had stems and roots that were approximately 30% shorter, less prominent taproot development, and more than 30% lower root fresh weight. These plants also contained about 50% lower chlorophyll content and 52% higher stomatal density compared with *ex vitro*-grown ginseng. Histologically, *in vitro* plants exhibited narrow intercellular spaces, underdeveloped root cambium, and lignin deposition in cell walls. Metabolically, *in vitro*-grown ginseng was clearly distinguishable based on ginsenoside content and volatile compound profiles. The comprehensive findings of this study provide baseline information for future research and can be utilized to enhance the practicality of tissue culture-based micropropagation of ginseng.

Keywords: *Panax ginseng*; *in vitro* culture; histological analysis; ginsenosides; volatile compounds



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1. Introduction

Ginseng (*Panax ginseng*), a herbaceous perennial crop cultivated in temperate regions, is highly valued for its medicinal properties. Its major secondary metabolites, ginsenosides, exhibit diverse bioactivities—including antioxidant, anticancer, and anti-inflammatory effects—which contribute to its widespread use in East Asia, particularly in South Korea [1]. However, ginseng is highly susceptible to heat stress, and the rising incidence of extreme heat events due to global warming has threatened its stable production [2]. According to the Ginseng Statistics Yearbook published by the Ministry of Agriculture, Food and Rural

Affairs (MAFRA, Korea) [3], the cultivation area of ginseng decreased from 15,160 ha in 2020 to 11,745 ha in 2023, representing a reduction of approximately 22.5%. To mitigate this issue, heat-tolerant ginseng cultivars have been developed. Nevertheless, their adoption among farmers remains limited [4,5]. This constraint is largely attributed to ginseng's intrinsically low reproductive capacity, which significantly hampers the dissemination of elite cultivars. Ginseng primarily propagates through seeds, but it takes more than three years from sowing to reach the flowering stage [6]. Although seed yield per plant varies among cultivars, it typically does not exceed 40 seeds [7]. These challenges underscore the need for alternative propagation strategies to overcome current limitations and ensure the sustainable production of elite ginseng cultivars.

Plants possess cellular totipotency, the remarkable ability of a single cell to regenerate an entire organism—a property that is actively harnessed in tissue culture for clonal propagation. Plant tissue culture has emerged as a powerful technique for cultivating plants under sterile conditions and enabling large-scale propagation [8]. Given the intrinsic limitations of seed propagation, tissue culture currently represents the only realistic strategy for the large-scale dissemination of elite ginseng cultivars. Furthermore, it is widely applied in various areas such as the production of aseptic plants, genetic transformation, and genome editing [9].

Research on ginseng tissue culture, initiated by the pioneering work of Butenko [10], has since advanced considerably in key areas including micropropagation [11], anther culture [12], and gene editing [13]. Micropropagation of ginseng is primarily achieved through somatic embryogenesis, which facilitates the production of large numbers of tissue-cultured plantlets [14]. However, a major challenge in ginseng micropropagation lies in the low acclimatization rate, with only 30–40% of plantlets reported to survive upon transfer to *ex vitro* conditions [15]. Thus, low acclimatization success remains the primary barrier preventing the practical application of tissue culture in ginseng. Although recent studies have reported improved survival rates exceeding 70% through the induction of *in vitro*-grown taproots [16], acclimatization remains a critical bottleneck. This limitation is likely due to environmental differences between *in vitro* and *ex vitro* conditions, highlighting the need for further understanding of this transition. Understanding these limitations is essential not only for improving acclimatization success but also for ensuring the practicality of tissue culture as a propagation strategy for ginseng.

In vitro environments differ markedly from natural growing conditions, characterized by high humidity, limited light, and restricted gas exchange. As a result, *in vitro*-cultivated plants often exhibit substantial differences compared to their *ex vitro* counterparts [17,18].

Previous studies have reported morphological differences in various traits, including stem length, number of stems, leaf size, root weight, root diameter, and root hair development [19,20]. Histological analyses have revealed variations in cell organization and wall thickness, presumably attributable to the enclosed culture environment and limited gas exchange [21]. The limited light intensity and artificial light spectrum in *in vitro* environments are known to disrupt normal photosynthetic responses [22,23]. Additionally, differences in stomatal structure, shape, and density have been observed compared to *ex vitro*-grown plants [24]. Such conditions also influence the biosynthesis of physiologically active compounds in plants. While some studies have examined differences in the content and composition of ginsenosides in *in vitro*-cultured ginseng [4], limited attention has been given to antioxidant compounds, such as total phenolics, and to volatile metabolites.

To support the practical application and dissemination of ginseng micropropagation techniques via tissue culture and to establish precise *in vitro* cultivation methods, it is essential to gain a comprehensive understanding of the differences between plants grown *in vitro* and *ex vitro*. In ginseng, only a few studies have sporadically reported differences

in morphology, histology, physiology, or metabolites, and to our knowledge, no study has systematically compared these traits together in relation to the acclimatization problem. Therefore, this study investigates the morphological, physiological, histological, and compositional differences between ginseng cultivated under *in vitro* and *ex vitro* conditions, with the aim of elucidating how *in vitro* environments affect plant survival and quality. The findings offer insights into the underlying causes of low survival rates in *in vitro*-cultured seedlings and provide a basis for overcoming this limitation. Ultimately, this work may contribute to the advancement of propagation technologies, promoting the stable and efficient distribution of elite ginseng cultivars.

2. Materials and Methods

2.1. Plant Materials

The ginseng cultivar ‘Geumpoong’ was used in this study. Seeds were collected in July 2023 from the experimental field of the Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science, affiliated with the Rural Development Administration (RDA), South Korea. The seeds were subjected to dehiscence and cold stratification following the method described by Lee et al. [25].

2.2. In Vitro Cultivation of Ginseng

To initiate *in vitro* culture, approximately 1000 ginseng seeds were used. Seed coats were removed, and the seeds were immersed in 2% (*v/v*) sodium hypochlorite for 20 min, followed by treatment with 70% (*v/v*) ethanol for 1 min and two rinses with sterile distilled water. To complete the sterilization process, the seeds were again treated with 2% (*v/v*) sodium hypochlorite for 20 min and rinsed four to five times with sterile distilled water. The surface-sterilized seeds were cultured *in vitro* following the method described by Lee et al. [26]. Briefly, zygotic embryos were excised and cultured on germination medium consisting of half-strength Murashige and Skoog ($\frac{1}{2}$ MS) medium containing 2% sucrose and 0.8% agar (pH 5.7). All zygotic embryos germinated except for contaminated ones. After germination, seedlings were cultured on a growth medium composed of one-third strength Schenk and Hildebrandt ($\frac{1}{3}$ SH) medium containing 2% sucrose and 0.5% gelrite (pH 5.7), and maintained for approximately five months in a tissue culture room. Each seedling was grown on 50 mL of medium dispensed in Incu Tissue (65.40 × 65.40 × 98.20 mm) (InCu Tissue, SPL Life Sciences, Pocheon, Korea). Cultures were maintained at 23 °C ± 2 °C under a 16/8 h light/dark cycle with white fluorescent light at an intensity of 24 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

2.3. Ex Vitro Cultivation of Ginseng

For *ex vitro* cultivation, 1000 intact ginseng seeds were sown in plastic boxes (0.5 × 0.3 × 0.3 m) filled with artificial soil (Golden Root Ginseng Soil, Nongkyung Co, Jincheon, Korea) composed of peat moss and perlite at a 3:1 (*v/v*) ratio. The average soil pH was 6.17, as determined by soil analysis. The planting density for *ex vitro* conditions was adjusted to match the same unit area. Irrigation was performed once per week by applying 2 L of water per box to maintain field capacity, and the boxes were placed in a greenhouse. Germination rate was 92.38%. No additional nutrient supplementation was applied to the soil. Plants were cultivated for five months.

2.4. Experimental Design and Replication

All experiments were conducted under a completely randomized design (CRD). For each cultivation condition (*in vitro* and *ex vitro*), 1000 seeds were used. For growth measurements, 100 plants per treatment were randomly sampled. Chlorophyll content and stomatal analyses were conducted using ten randomly selected plants per treatment. Metabolite and

histological analyses were performed with three biological replicates per treatment. No subplots were included in this study.

2.5. Histological Analysis of Aerial and Underground Parts

Histological analysis was performed on the leaves and roots of ginseng plants cultivated *in vitro* and *ex vitro* for 60 days. For root samples, transverse sections were obtained from the proximal 0.5–1.5 cm region of the primary root directly connected to the shoot. For leaf samples, the central leaflet of each plant was selected, and rectangular segments were excised to include the widest region of the lamina, spanning from the midrib at the midpoint toward the leaf apex. Paraffin sectioning was conducted following the procedure reported by Hong et al. [2] to obtain cross-sectional images of the tissues. Fresh leaf and root tissue segments were fixed overnight at 4 °C in phosphate-buffered saline (PBS, pH 7.0) containing 3% glutaraldehyde and 3% formaldehyde. The fixed tissues were dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, and 100%), with each step repeated once for 1 h. Samples were embedded in paraffin and sectioned at 5–10 µm thickness using a microtome. The sections were dewaxed, stained with 1% Safranin-O (Sigma, St. Louis, MO, USA) and 0.5% Astra Blue (Santa Cruz Biochem, Dallas, TX, USA), and then dehydrated in a reverse ethanol series. Bright-field and polarized light images were acquired using a Slideview scanner and a BX53 microscope (Olympus, Tokyo, Japan).

2.6. Chlorophyll Content Analysis

Chlorophyll a and b contents in leaves harvested from *in vitro*- and *ex vitro*-grown ginseng were measured according to the method described by Lichtenthaler [27]. Ten leaves from each treatment were cut into 5 mm × 5 mm pieces, and each sample was extracted with 95% ethanol at 80 °C for 20 min using a solvent volume 50 times the fresh weight. To prevent photodegradation, samples were kept in the dark after extraction. Absorbance was measured at 648 nm (A_{648}) and 664 nm (A_{664}) using a Multiskan GO Microplate Spectrophotometer (Thermo Scientific, Waltham, MA, USA), and chlorophyll concentrations were calculated using the following equations:

$$\text{Chl a} = 13.36 \times A_{664} - 5.19 \times A_{648} \quad (1)$$

$$\text{Chl b} = 27.43 \times A_{648} - 812 \times A_{664} \quad (2)$$

$$\text{Total Chl} = 5.24 \times A_{664} + 22.24 \times A_{648} \quad (3)$$

2.7. Stomatal Traits Analysis

To investigate changes in stomatal characteristics under different cultivation environments, stomatal size and density were analyzed. The central leaflet of each plant was selected, and the stomata on the abaxial leaf surface were exposed by carefully scraping the central lamina with a scalpel, avoiding the midrib. For each treatment, ten leaves were collected, and three impressions per leaf were prepared. Ten stomata were measured per impression to determine stomatal length and width, pore length, and aperture (µm). The stomatal length-to-width ratio was also calculated. Stomatal density was calculated as the average of the three regions per leaf. Observations were conducted using a Leica DM3000 microscope (Leica Microsystems, Wetzlar, Germany).

2.8. Growth Measurements

After 60 days of cultivation, *in vitro*- and *ex vitro*-grown ginseng plants reached the developmental stage shown in Figure S1. A total of 100 plants from each treatment were harvested, and their aerial parts were separated for growth measurements. Stem length and diameter, and leaf length and width were measured using a ruler and caliper. Fresh weights

were recorded, and dry weights were measured after lyophilization using a freeze-dryer (FDU-2200, Eyela, Tokyo, Japan).

After five months of cultivation, once the aerial parts had senesced, roots were harvested from both *in vitro*- and *ex vitro*-grown plants. For each treatment, 100 roots were collected, and root traits including length, diameter, fresh weight, and dry weight were assessed.

2.9. Freeze-Drying

For the analysis of ginsenosides and volatile compounds, ginseng plants cultivated for five months under *in vitro* and *ex vitro* conditions were subjected to freeze-drying. *In vitro*-grown plants were carefully separated from the culture medium, while *ex vitro*-grown plants were washed to remove soil residues and gently blotted dry with tissue paper. The aerial parts were removed, and only roots were used. The samples were freeze-dried for 48 h using a freeze dryer (FDU-2200, Eyela, Tokyo, Japan) and subsequently ground into a fine powder for analysis.

2.10. Ginsenoside Profiling

Freeze-dried ginseng powder (0.2 g) was extracted with 2 mL of 70% methanol–water (*v/v*) by vortexing and ultrasonication at 50 °C for 30 min. Samples were centrifuged using an Eppendorf Centrifuge 5427 R (Eppendorf SE, Hamburg, Germany) at 15,000 rpm for 3 min. The supernatant was purified using a solid-phase extraction (SPE) cartridge (Agilent Technologies, Santa Clara, CA, USA), then filtered through a 0.45 µm PTFE membrane.

Ginsenosides were quantified using HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with a Halo[®] RP-Amide column (4.6 × 150 mm, 2.7 µm; Wilmington, DE, USA) at 50 °C. The mobile phases were (A) 10% acetonitrile and (B) 90% acetonitrile, with a gradient flow (0.6 mL/min). The gradient program was set as follows: 0.0 min (27% B), 0–6.0 min (28% B), 6.0–10.0 min (28% B), 10.0–30.0 min (34% B), 30.0–33.0 min (70% B), 33.0–35.0 min (80% B), 35.0–45.0 min (100% B), 45.0–50.0 min (100% B), 50.0–51.0 min (27% B), and 51.0–60.1 min (27% B). Peaks were detected at 203 nm using a photodiode array (PDA) detector. Standards were obtained from ChromaDex (Irvine, CA, USA) and Biosynth (Staad, Switzerland).

2.11. Volatile Compound Analysis

Volatile profiles were analyzed using HS-SPME–GC–MS (Agilent 8890 GC/5977B MSD with Gerstel MPS 2XL). Samples (2 g) were ground in liquid nitrogen and mixed with 3 mL distilled water and 0.025 µg/mL of 2-methylbutyl 2-methylbutyrate as an internal standard in a 20 mL vial.

A DVB/CAR/PDMS SPME fiber (50/30 µm; Supelco) was preconditioned at 250 °C for 10 min. Vials were incubated at 50 °C for 40 min, and volatiles were adsorbed at 50 °C for 20 min while agitating at 250 rpm. Desorption was performed at 240 °C for 10 min in splitless mode.

Separation was carried out on a DB-WAX column (30 m × 0.25 mm, 0.25 µm film; Agilent) with helium as the carrier gas. Oven temperature was programmed from 35 °C (2 min) to 225 °C. Mass spectra were scanned over 35–550 *m/z*. Compound identification was based on NIST 2014 library matches and retention indices using a C7–C30 alkane standard. Volatile levels were quantified as peak area ratios relative to the internal standard. Analyses were performed in triplicate.

2.12. Statistical Analysis

The experimental layout followed a randomized complete block design. Growth data were analyzed using R software (v4.3.3; The R Foundation). Depending on variance equal-

ity, either a two-sample *t*-test or Welch's *t*-test was applied. One-way ANOVA followed by Tukey's HSD test was used for post hoc comparisons. Principal component analysis (PCA) was performed using SIMCA-P 17.0 (Umetrics, Umeå, Sweden), and heatmaps were generated via MetaboAnalyst 6.0 (<https://www.metaboanalyst.ca/> accessed on 4 March 2025).

3. Results

3.1. Phenotypic Differences in Ginseng Under Different Cultivation Environments

After two months of cultivation, ginseng plants cultivated under *in vitro* and *ex vitro* conditions exhibited distinct phenotypic differences (Figure S1). In the aerial parts, *ex vitro*-grown ginseng had uniformly shaped, symmetrical leaves with a bright green coloration (Figure 1a). In contrast, the leaves of *in vitro*-grown ginseng varied in color and size and often exhibited slightly curled margins with an oblong shape.

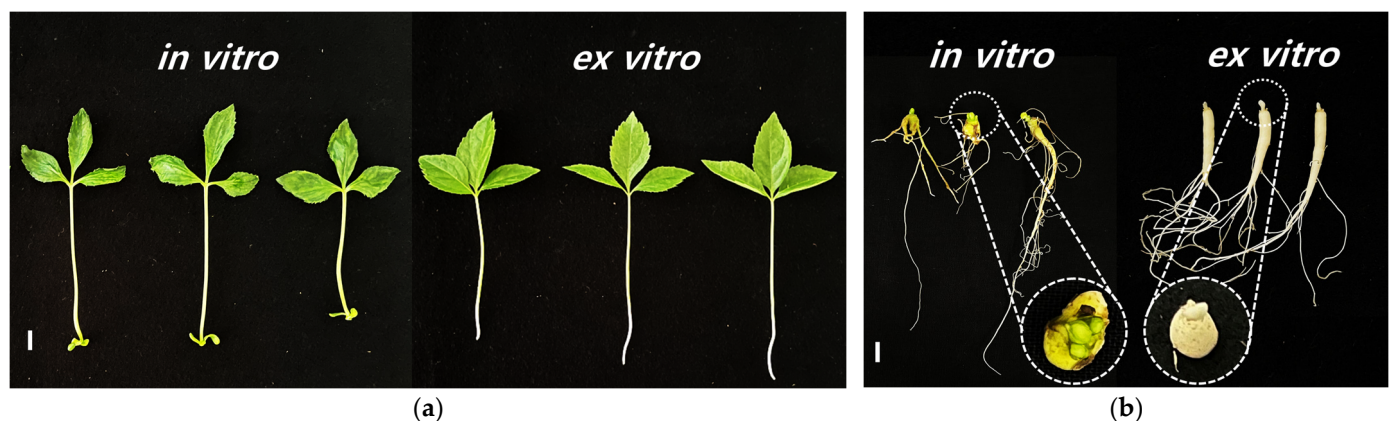


Figure 1. Phenotypic characteristics of ginseng grown under *in vitro* and *ex vitro* conditions. (a) Shoot morphology at 60 days after sowing. (b) Root and rhizome morphology at 5 months after sowing. Scale bar = 1 cm.

After five months of cultivation, clear phenotypic differences were also observed in the underground parts (Figure 1b). *Ex vitro*-grown ginseng formed a distinct taproot with abundant lateral roots, whereas *in vitro*-grown ginseng exhibited atypical root morphology as an adaptive response to *in vitro* culture conditions, characterized by the absence of a clearly defined taproot and limited lateral root development. Additionally, *ex vitro*-grown ginseng typically formed a single rhizome, in contrast to *in vitro*-grown ginseng, which often developed two to four rhizomes per plant.

3.2. Histological Differences in Ginseng Under Different Cultivation Environments

Histological differences were evident between leaves of ginseng cultivated under *in vitro* and *ex vitro* conditions (Figure 2). *In vitro*-grown ginseng showed irregularly shaped epidermal cells, while those of *ex vitro*-grown plants were symmetrically arranged (Figure 2a). The leaf cross-sectional thickness averaged 185.6 μm *in vitro* and 155.3 μm *ex vitro*, indicating that *in vitro*-grown leaves tended to be thicker. Mesophyll cells in *in vitro*-grown leaves were evenly distributed and uniform in size. By contrast, *ex vitro*-grown leaves exhibited an irregular mesophyll cell size and arrangement, larger intercellular spaces, and a looser tissue structure (Figure 2b). The cell walls of *in vitro*-grown leaves appeared to contain more lignin, as indicated by more intense red staining.

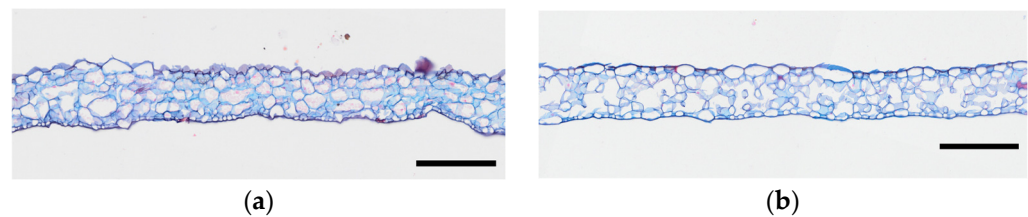


Figure 2. Transverse sections of ginseng leaves grown under (a) *in vitro* and (b) *ex vitro* conditions. Scale bar = 200 μm .

Substantial histological differences were also observed in the roots (Figure 3). *In vitro*-grown ginseng had a relatively thin epidermis with indistinct boundaries and irregular cell arrangement (Figure 3a). In contrast, *ex vitro*-grown ginseng exhibited a thicker epidermis with clearly defined boundaries and uniform cell organization (Figure 3b). The epidermal thickness averaged 49.20 μm *in vitro* and 66.37 μm *ex vitro*, while the mean width per epidermal cell was 15.67 μm and 16.86 μm , respectively. In the vascular tissues, *in vitro*-grown roots showed a disorganized structure with poorly differentiated xylem and phloem and irregular xylem vessel size and arrangement (Figure 3c). *Ex vitro*-grown roots, on the other hand, exhibited a well-organized vascular structure with a clear triarch xylem pattern and distinct xylem-phloem boundaries (Figure 3d).

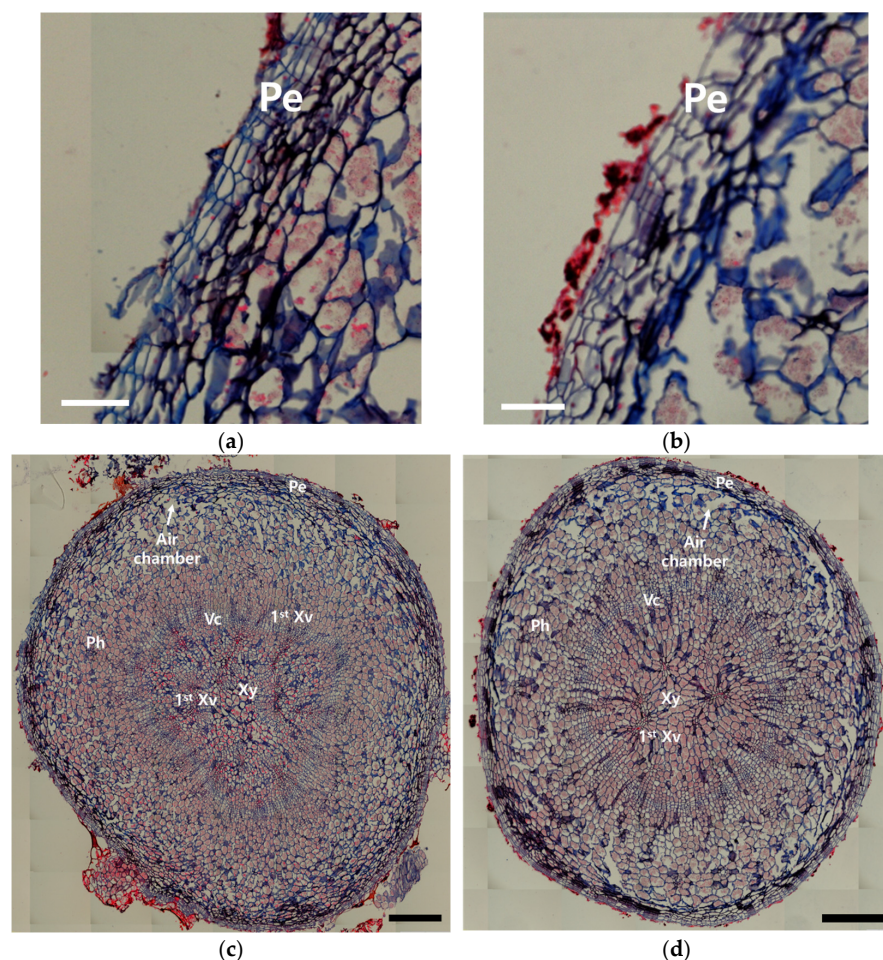


Figure 3. Histological characteristics of ginseng roots grown under *in vitro* and *ex vitro* conditions. (a) Periderm structure of *in vitro* roots; (b) periderm structure of *ex vitro* roots; (c) transverse section of *in vitro* roots; (d) transverse section of *ex vitro* roots. Scale bars: (a,b) = 100 μm ; (c,d) = 500 μm . Pe, periderm; Ph, phloem; Vc, vascular cambium; Xy, xylem, 1st Xv, primary xylem vessel.

The cambium in *in vitro*-grown roots was underdeveloped, with blurred margins and minimal signs of secondary growth. In contrast, the cambium in *ex vitro*-grown roots was clearly located between the xylem and phloem and exhibited active secondary development. Red staining, indicative of lignin accumulation, was more intense and irregularly distributed in *in vitro*-grown roots, while in *ex vitro*-grown roots, lignin was concentrated in the xylem and surrounding cell walls. Additionally, *in vitro*-grown roots had a denser tissue structure with smaller and uniformly distributed intercellular spaces, and the proportion of intercellular area relative to the total root cross-sectional area was 1.20%. In contrast, *ex vitro*-grown roots had larger and more irregular intercellular spaces that formed broader air cavities, with the proportion measured at 5.34%.

3.3. Chlorophyll Content Under Different Cultivation Environments

Significant differences in photosynthetic pigment content were observed between *in vitro*- and *ex vitro*-grown ginseng leaves. The chlorophyll a (Chl a) content in *in vitro*-grown leaves was 0.54 ± 0.28 mg/g, which was significantly lower than the 1.07 ± 0.22 mg/g measured in *ex vitro*-grown leaves (Figure 4a). Similarly, the chlorophyll b (Chl b) content was lower in *in vitro*-grown leaves than in *ex vitro*-grown leaves (Figure 4b). As a result, the total chlorophyll content was significantly reduced in *in vitro*-grown ginseng compared to its *ex vitro* counterpart (Figure 4c).

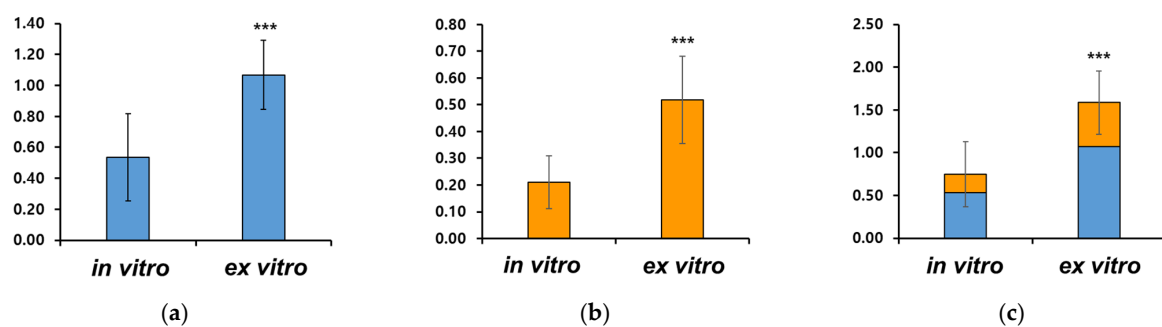


Figure 4. Comparison of chlorophyll content in ginseng leaves grown under *in vitro* and *ex vitro* conditions. (a) chlorophyll a, (b) chlorophyll b, and (c) total chlorophyll contents (mg/g) (***, $p < 0.001$). Data are presented as mean $\pm$ standard deviation ($n = 10$). Statistical significance was determined using a two-sample *t*-test or Welch's *t*-test. *** $p < 0.001$.

3.4. Stomatal Characteristics Under Different Cultivation Environments

A comparison of stomatal characteristics between *in vitro*- and *ex vitro*-grown ginseng leaves revealed notable differences (Figure 5). Stomatal density was approximately 1.5 times higher in *in vitro*-grown leaves compared to *ex vitro*-grown leaves (Table 1). *In vitro*-grown stomata were also larger, showing increased stomatal length, width, pore length, and aperture.

Table 1. Stomatal characteristics of ginseng leaves grown under *in vitro* and *ex vitro* conditions.

Treat	Stomata Density (cm ⁻²)	Stomata Length (μm)	Stomata Width (μm)	Pore Length (μm)	Pore Aperture (μm)	Stomata Length/Width
<i>in vitro</i>	3918 $\pm$ 936	55.3 $\pm$ 7.3	35.6 $\pm$ 6.0	29.3 $\pm$ 6.3	8.8 $\pm$ 4.4	1.6 $\pm$ 0.3
<i>ex vitro</i>	2571 $\pm$ 595	34.4 $\pm$ 4.5	22.3 $\pm$ 2.6	17.0 $\pm$ 3.4	4.5 $\pm$ 1.6	1.6 $\pm$ 0.2
	***	***	***	***	***	ns

Values are expressed as mean $\pm$ standard deviation ($n = 10$). Statistical significance was determined using a two-sample *t*-test or Welch's *t*-test. *** $p < 0.001$; ns, not significant ($p \geq 0.05$).

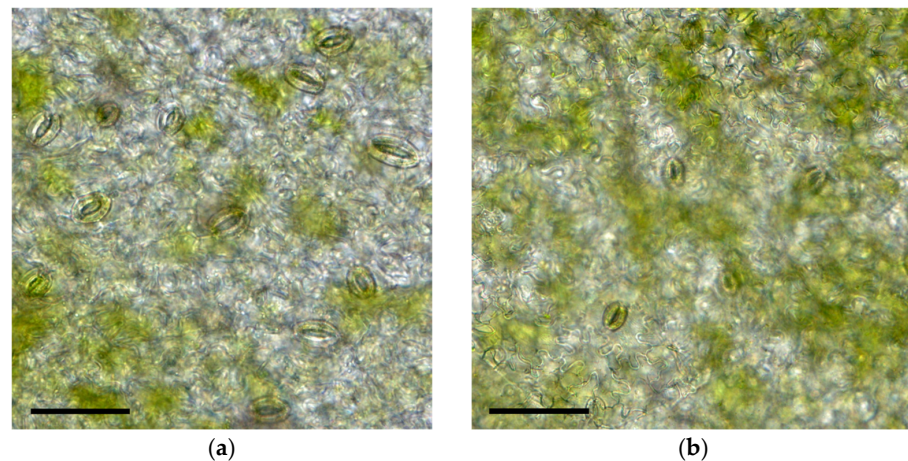


Figure 5. Stomatal morphology on the abaxial surface of ginseng leaves grown under (a) *in vitro* and (b) *ex vitro* conditions. Scale bar = 100 μm .

Notably, stomata in *in vitro*-grown leaves remained open, while those in *ex vitro*-grown leaves were mostly closed or partially open. However, there was no statistically significant difference in the stomatal length-to-width ratio between the two groups.

3.5. Growth Differences Under Different Cultivation Environments

Significant differences in aerial growth were observed between *in vitro*- and *ex vitro*-grown ginseng, except for in fresh weight (Table 2). The dry weight of aerial parts was significantly higher in *ex vitro*-grown plants compared to those grown *in vitro*. Stem length was greater in *ex vitro*-grown ginseng, while stem diameter was thicker in *in vitro*-grown plants. Leaf length and width were likewise greater in *ex vitro*-grown ginseng. Similarly, root growth following aerial senescence differed significantly between the two cultivation environments (Table 3). Both fresh and dry root weights were higher in *ex vitro*-grown ginseng. Root length was also greater in *ex vitro*-grown plants, whereas root diameter was thicker in those grown *in vitro*.

Table 2. Growth characteristics of the aerial parts of ginseng plants grown under *in vitro* and *ex vitro* conditions at 60 days after sowing.

Treat	Fresh Weight (mg)	Dry Weight (mg)	Stem Length (cm)	Stem Diameter (mm)	Leaf Length (cm)	Leaf Width (cm)
<i>in vitro</i>	187.8 $\pm$ 27.2	29.1 $\pm$ 11.0	4.7 $\pm$ 1.7	1.08 $\pm$ 0.26	2.9 $\pm$ 0.6	1.3 $\pm$ 0.3
<i>ex vitro</i>	181.3 $\pm$ 68.5	40.6 $\pm$ 5.9	7.0 $\pm$ 0.7	0.94 $\pm$ 0.16	3.9 $\pm$ 0.4	1.9 $\pm$ 0.2
	ns	***	***	***	***	***

Values are expressed as mean $\pm$ standard deviation ($n = 100$). Statistical significance was assessed using a two-sample *t*-test or Welch's *t*-test. *** $p < 0.001$; ns, not significant ($p \geq 0.05$).

Table 3. Growth characteristics of the underground parts of ginseng plants grown under *in vitro* and *ex vitro* conditions at 5 months after sowing.

Treat	Fresh Weight (mg)	Dry Weight (mg)	Root Length (cm)	Root Diameter (mm)
<i>in vitro</i>	396.5 $\pm$ 115.7	109.4 $\pm$ 31.9	10.4 $\pm$ 3.6	4.68 $\pm$ 1.10
<i>ex vitro</i>	602.7 $\pm$ 73.6	192.9 $\pm$ 23.6	16.5 $\pm$ 3.5	3.66 $\pm$ 0.64
	***	***	***	***

Values are expressed as mean $\pm$ standard deviation ($n = 100$). Statistical significance was assessed using a two-sample *t*-test or Welch's *t*-test. *** $p < 0.001$.

3.6. Ginsenoside Analysis

Ginsenoside content and composition were compared between *in vitro*- and *ex vitro*-grown ginseng based on underground parts harvested after five months of cultivation. A total of seven ginsenosides (Rb2, Rc, Rd, Re, Rg1, Rf, and Rh1) were identified in *ex vitro*-grown ginseng. In contrast, only six (Rb2, Rc, Rd, Re, Rg1, and Rf) were detected in *in vitro*-grown samples, with Rh1 exclusively present in the *ex vitro* group (Table 4).

Table 4. Contents of individual ginsenosides ($\mu\text{g/g}$ dry weight) in the underground parts of ginseng grown under *in vitro* and *ex vitro* conditions.

		Retention Time	In Vitro	Ex Vitro	
PPD ($\mu\text{g/g}$)	Ginsenoside Rb2	25.69	162.97 $\pm$ 22.88	872.97 $\pm$ 9.96	***
	Ginsenoside Rc	24.13	195.91 $\pm$ 3.88	694.72 $\pm$ 30.62	***
	Ginsenoside Rd	31.31	110.07 $\pm$ 3.98	529.26 $\pm$ 7.90	***
	Ginsenoside Rg3	35.59	nd	nd	-
	Ginsenoside Rg5	36.62	nd	nd	-
	Ginsenoside Rh2	37.15	nd	nd	-
PPT ($\mu\text{g/g}$)	Ginsenoside Re	4.62	279.47 $\pm$ 9.18	359.31 $\pm$ 6.92	***
	Ginsenoside Rf	14.60	162.96 $\pm$ 6.37	163.55 $\pm$ 9.27	ns
	Ginsenoside Rg1	4.82	414.72 $\pm$ 33.62	256.14 $\pm$ 17.93	**
	Ginsenoside Rh1	22.76	nd	44.02 $\pm$ 4.40	-
	Quinqueoside R1	29.01	nd	nd	-

Values are expressed as mean $\pm$ standard deviation (SD) from three independent replicates. nd, not detected. Statistical significance was determined using a two-sample *t*-test or Welch's *t*-test. *** $p < 0.001$; ** $p < 0.01$; ns, not significant ($p \geq 0.05$).

Among individual ginsenosides, protopanaxadiol (PPD)-type compounds (Rb2, Rc, and Rd) accumulated at higher levels in *ex vitro*-grown ginseng. In contrast, Rg1—a protopanaxatriol (PPT)-type ginsenoside—was more abundant in *in vitro*-grown ginseng, while Re was significantly higher in the *ex vitro* group. Rf was detected at similar levels in both groups, and Rh1 was exclusively found in *ex vitro*-grown plants. Ginsenosides Rg3, Rg5, Rh2, and quinqueoside R1 were not detected in either condition.

The total content of PPD-type ginsenosides was significantly higher in *ex vitro*-grown ginseng compared to *in vitro*-grown plants (Table 5). In contrast, PPT-type ginsenoside levels did not differ significantly between the two environments. Consequently, the PPD/PPT ratio was markedly higher under *ex vitro* conditions (2.55 ± 0.08) than under *in vitro* conditions (0.55 ± 0.03). Furthermore, the total ginsenoside content was significantly greater in *ex vitro*-grown ginseng.

Table 5. Summary of ginsenoside classes and total content in underground parts of ginseng grown under *in vitro* and *ex vitro* conditions.

Treat	PPD	PPT	PPD/PPT	Total Ginsenoside Content (mg/g)
<i>in vitro</i>	468.95 $\pm$ 20.6	857.2 $\pm$ 33.7	0.55 $\pm$ 0.03	1326.1 $\pm$ 42.2
<i>ex vitro</i>	2097.0 $\pm$ 23.9	823.0 $\pm$ 33.5	2.55 $\pm$ 0.08	2920.0 $\pm$ 56.2
	***	ns	***	***

Values are expressed as mean $\pm$ standard deviation (SD) from three independent replicates. Statistical significance was determined using a two-sample *t*-test or Welch's *t*-test. *** $p < 0.001$; ns, not significant ($p \geq 0.05$).

3.7. Volatile Compound Profiles

A total of 61 volatile compounds were identified from the underground parts of ginseng cultivated under *in vitro* and *ex vitro* conditions (Figure 6, Table 6). Overall, a

greater number of compounds were detected in *ex vitro*-grown ginseng. Among the volatiles common to both conditions, most exhibited higher concentrations in the *ex vitro* samples (Figure 6 and Figure S2).

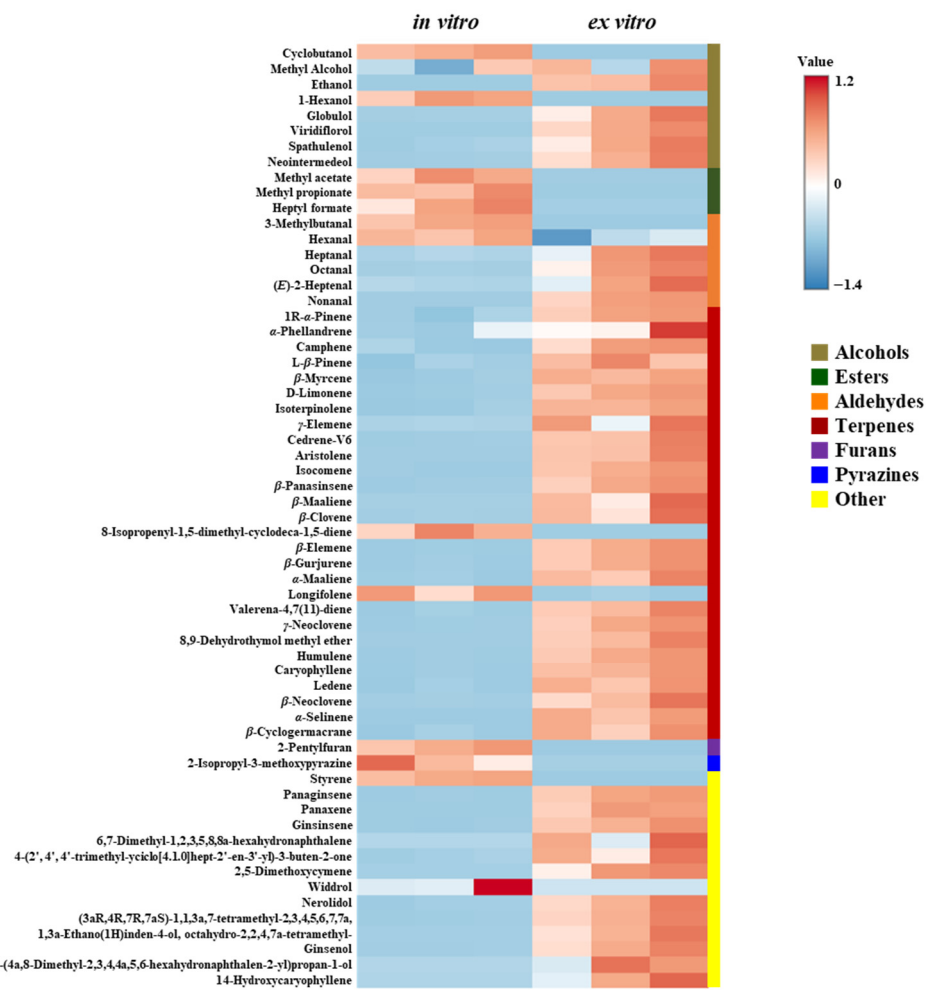


Figure 6. Heatmap visualization of volatile compound distribution in the underground parts of ginseng grown under *in vitro* and *ex vitro* conditions. Each row represents a compound, and columns represent biological replicates from each treatment group. Color intensity indicates relative abundance after normalization.

Table 6. Volatile compound profiles in the underground parts of ginseng cultivated under *in vitro* and *ex vitro* conditions.

		RT ^A	RI ^B	In Vitro	Ex Vitro	Identification ^C
Alcohols						
1	Cyclobutanol	5.34	-	0.09 ± 0.01	Nd ^D	MS, TI
2	Methyl Alcohol	8.78	899	0.53 ± 0.06	0.59 ± 0.07	MS, TI
3	Ethanol	9.91	936	nd	0.24 ± 0.03	MS, TI
4	1-Hexanol	22.66	1355	0.11 ± 0.02	nd	MS, TI
5	Globulol	33.99	2087	0.17 ± 0.01	2.85 ± 0.96	MS, TI
6	Viridiflorol	34.23	2111	nd	0.24 ± 0.05	MS, TI
7	Spathulenol	34.69	2153	0.12 ± 0.01	0.54 ± 0.14	MS, TI
8	Neointermedeol	34.86	2169	0.14 ± 0.01	1.24 ± 0.30	MS, TI
Esters						
9	Methyl acetate	7.07	823	0.06 ± 0.01	nd	MS, TI
10	Methyl propionate	8.95	905	0.08 ± 0.01	nd	MS, TI
11	Heptyl formate	25.22	1459	0.06 ± 0.02	nd	MS, TI

Table 6. Cont.

		RT ^A	RI ^B	In Vitro	Ex Vitro	Identification ^C
Aldehydes						
12	3-Methylbutanal	9.24	914	0.07 ± 0.01	nd	MS, TI
13	Hexanal	14.49	1079	0.52 ± 0.03	0.24 ± 0.10	MS, TI
14	Heptanal	17.81	1184	0.17 ± 0.01	0.56 ± 0.22	MS, TI
15	Octanal	20.91	1290	0.43 ± 0.02	2.28 ± 0.67	MS, TI
16	(E)-2-Heptenal	21.94	1328	0.06 ± 0.00	0.13 ± 0.04	MS, TI
17	Nonanal	23.76	1391	nd	0.10 ± 0.02	MS, TI
Terpenes						
18	1R- α -Pinene	12.52	1019	0.94 ± 0.10	2.47 ± 0.26	MS, TI
19	α -Phellandrene	12.68	1024	0.10 ± 0.05	0.19 ± 0.01	MS, TI
20	Camphene	13.96	1063	0.31 ± 0.03	0.81 ± 0.12	MS, TI
21	L- β -Pinene	15.17	1100	0.26 ± 0.03	0.81 ± 0.10	MS, TI
22	β -Myrcene	17.02	1159	0.47 ± 0.03	1.62 ± 0.08	MS, TI
23	D-Limonene	18.18	1196	0.31 ± 0.02	1.50 ± 0.17	MS, TI
24	Isoterpinolene	20.75	1285	0.09 ± 0.01	0.34 ± 0.02	MS, TI
25	γ -Elemene	25.93	1490	0.04 ± 0.00	0.39 ± 0.04	MS, TI
26	Cedrene-V6	26.35	1510	0.13 ± 0.01	1.75 ± 0.35	MS, TI
27	Aristolene	26.81	1535	nd	0.97 ± 0.16	MS, TI
28	Isocomene	26.87	1538	0.25 ± 0.02	3.53 ± 0.44	MS, TI
29	β -Panasinene	26.97	1544	1.30 ± 0.07	15.32 ± 2.65	MS, TI
30	β -Maaliene	27.31	1562	nd	0.62 ± 0.21	MS, TI
31	β -Clovane	27.50	1573	0.06 ± 0.01	0.95 ± 0.30	MS, TI
32	8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene	27.81	1590	0.05 ± 0.01	nd	MS, TI
33	β -Elemene	28.01	1601	0.72 ± 0.07	11.26 ± 1.77	MS, TI
34	β -Gurjurene	28.18	1612	0.59 ± 0.05	6.97 ± 1.05	MS, TI
35	α -Maaliene	28.36	1623	0.08 ± 0.01	0.80 ± 0.15	MS, TI
36	Longifolene	28.43	1628	0.23 ± 0.03	1.45 ± 0.29	MS, TI
37	Valerena-4,7(11)-diene	28.59	1638	0.52 ± 0.07	3.79 ± 0.67	MS, TI
38	γ -Neoclovane	29.06	1669	2.66 ± 0.20	28.83 ± 4.77	MS, TI
39	8,9-Dehydrothymol methyl ether	29.32	1686	nd	0.98 ± 0.18	MS, TI
40	Humulene	29.41	1692	0.95 ± 0.08	10.99 ± 1.55	MS, TI
41	Caryophyllene	29.51	1698	0.64 ± 0.06	7.63 ± 0.85	MS, TI
42	Ledene	29.74	1716	0.18 ± 0.03	1.40 ± 0.18	MS, TI
43	β -Neoclovane	29.88	1726	0.28 ± 0.03	3.62 ± 0.98	MS, TI
44	α -Selinene	30.20	1751	nd	5.24 ± 0.53	MS, TI
45	β -Cyclogermacrane	30.31	1759	0.35 ± 0.08	2.82 ± 0.47	MS, TI
Furans						
46	2-Pentylfuran	19.17	1230	0.11 ± 0.01	nd	MS, TI
Pyrazines						
47	2-Isopropyl-3-methoxypyrazine	24.80	1441	0.11 ± 0.04	nd	MS, TI
Others						
48	Styrene	20.00	1259	0.05 ± 0.00	nd	MS, TI
49	Panaginsene	24.44	1425	0.12 ± 0.01	1.95 ± 0.29	MS, TI
50	Panaxene	24.88	1444	nd	1.27 ± 0.19	MS, TI
51	Ginsinsene	25.11	1454	0.27 ± 0.03	4.98 ± 0.76	MS, TI
52	6,7-Dimethyl-1,2,3,5,8,8a-hexahydronaphthalene	25.43	1468	nd	0.50 ± 0.09	MS, TI
53	4-(2', 4', 4'-trimethyl-yciclo [4.1.0]hept-2'-en-3'-yl)-3-buten-2-one	25.60	1475	0.07 ± 0.02	1.03 ± 0.34	MS, TI
54	2,5-Dimethoxycymene	31.85	1885	nd	0.25 ± 0.08	MS, TI
55	Widdrol	32.82	1974	0.02 ± 0.00	nd	MS, TI
56	Nerolidol	33.51	2041	0.02 ± 0.00	0.31 ± 0.07	MS, TI
57	(3aR,4R,7R,7aS)-1,1,3a,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa [a]naphthalen-4-ol	33.89	2078	0.02 ± 0.00	0.34 ± 0.08	MS, TI

Table 6. Cont.

		RT ^A	RI ^B	In Vitro	Ex Vitro	Identification ^C
58	1,3a-Ethano(1H)inden-4-ol,	35.07	2188	nd	0.33 ± 0.09	MS, TI
59	octahydro-2,2,4,7a-tetramethyl- Ginsenoside	35.17	2198	0.13 ± 0.01	2.53 ± 0.65	MS, TI
60	2-(4a,8-Dimethyl-2,3,4,4a,5,6- hexahydronaphthalen-2- yl)propan-1-ol	35.54	2229	nd	0.13 ± 0.08	MS, TI
61	14-Hydroxycaryophyllene	38.07	2414	nd	0.27 ± 0.15	MS, TI

^A Retention time on the DB-WAX capillary column; ^B Retention indices were determined on DB-WAX capillary column using n-alkanes (C7–C30) as external reference; ^C Compound identification based on MS (mass spectra matched with NIST library) and TI (tentatively assigned using spectral data and literature reports); nd, not detected.

Terpenes represented the largest chemical class, comprising 28 compounds, followed by alcohols (8), aldehydes (6), esters (3), furans (1), pyrazines (1), and other miscellaneous compounds (14). In *ex vitro*-grown ginseng, major alcohols included globulol, spathulenol, and neointermedeol, while γ -neoclovene and β -panasinsene were the dominant terpenes. In contrast, *in vitro*-grown ginseng showed higher levels of methyl alcohol and 1-hexanol among alcohols, and caryophyllene and β -panasinsene among terpenes.

Among aldehydes, octanal and heptanal were predominant in *ex vitro*-grown samples, whereas hexanal and octanal were abundant in *in vitro*-grown ginseng. Interestingly, ester compounds such as methyl acetate and methyl propionate were detected only in *in vitro*-grown roots. Similarly, 2-pentylfuran (a furan) and 2-isopropyl-3-methoxypyrazine (a pyrazine) were uniquely found in the *in vitro* group. Among the miscellaneous compounds, ginsenoside and ginsenoside were present in both groups but were notably more concentrated in *ex vitro*-grown ginseng.

4. Discussion

Ginseng is inherently difficult to propagate via conventional seed-based methods due to its low reproductive efficiency [7]. Micropropagation via tissue culture offers a potential solution, although widespread use is restricted by acclimatization difficulties [13,14]. This stress is induced by artificial *in vitro* conditions, including high humidity, limited gas exchange, and low light intensity [28,29]. Therefore, a deeper understanding of the physiological, histological, and metabolic characteristics of *in vitro*-grown ginseng is essential for overcoming current bottlenecks in ginseng micropropagation.

Morphological differences were evident between ginseng grown *in vitro* and *ex vitro*, particularly in their root structures. Compared to *ex vitro*-grown ginseng, *in vitro*-grown ginseng exhibited a degree of leaf asymmetry (Figure 1a), which may be attributable to low-intensity, unidirectional lighting conditions. According to Shilpha et al. [30], when ginseng was exposed to lighting from either the side or the top alone, leaf morphology was distorted, whereas balanced development was promoted under simultaneous lateral and overhead lighting. Root morphology exhibited a more marked difference than the aerial parts: *in vitro*-grown ginseng developed shortened taproots and excessive lateral roots (Figure 1b). These atypical root structures are consistent with previous findings [14,26] and are generally attributed to *in vitro* conditions such as high sucrose concentrations, hormonal imbalances, and limited gas exchange factors known to contribute to the low survival rate during acclimatization [16,31].

Histological comparisons revealed marked histological differences in ginseng tissues under *in vitro* and *ex vitro* conditions. *In vitro*-grown leaves had densely packed mesophyll cells with minimal intercellular spaces, whereas *ex vitro* leaves exhibited more developed intercellular spaces (Figure 2). These tissue traits are presumed to represent adaptive

responses to *in vitro* culture conditions—such as excessive humidity, low light intensity, and restricted gas exchange [32]. Similar tissue responses were reported in *Ilex paraguariensis* under poor gas exchange conditions [33]. The intense red staining in *in vitro* leaves suggests greater lignin accumulation, likely as a stress response under sealed and artificial culture conditions [34].

In root tissues, *in vitro*-grown ginseng exhibited a thinner epidermis, irregular vascular organization, and underdeveloped cambium with minimal secondary growth (Figure 3). Comparable traits have been observed in *Forsythia* and *Malus domestica*, which showed reduced vascular development under *in vitro* conditions [35], and in *Eucalyptus grandis*, which exhibited poorly differentiated xylem [36]. These abnormalities may result from suppressed transpiration and altered mineral availability in the *in vitro* environment [21]. Lee et al. [26] also reported that *ex vitro*-grown ginseng roots exhibited a triarch primary xylem pattern, whereas *in vitro*-grown roots showed a polyarch arrangement. Our findings confirmed increased lignification in *in vitro* roots, which may reduce tissue elasticity and hinder taproot development [34,37]. Inhibiting lignin biosynthesis has been shown to enhance storage root development in *Ipomoea batatas* [38], *Rehmannia glutinosa* [39], and *Panax notoginseng* [40]. To the best of our knowledge, no previous studies have directly demonstrated a link between root anatomical structures and ginsenoside or other metabolite profiles; therefore, further integrated studies are required to clarify the potential relationships between anatomy and metabolic regulation in ginseng.

Chlorophyll a and b contents were significantly higher in *ex vitro*-grown ginseng than in *in vitro* ginseng (Figure 4), likely due to natural light conditions promoting chlorophyll synthesis. In contrast, artificial lighting and limited gas exchange *in vitro* may suppress chlorophyll biosynthesis [17,41,42]. This inhibition under high humidity and low light has also been reported in crops such as apple and cherry [41].

In vitro plants often exhibit photomixotrophic growth due to limited photosynthesis and dependence on external carbon sources, which can suppress chlorophyll synthesis and induce morphological abnormalities [32]. Ševčíková et al. [22] reported species-specific responses under such conditions, with decreased chlorophyll levels in potato and strawberry, no change in tobacco, and increases in rapeseed. This variation suggests that *Panax ginseng* responds in a manner similar to plants that show chlorophyll reduction under such conditions.

In addition to chlorophyll differences, stomatal traits also differed notably. *In vitro*-grown ginseng had 52.4% higher stomatal density than *ex vitro* ginseng (Table 1), likely due to elevated humidity promoting stomatal development [33]. For instance, in walnuts, reducing humidity via KCl treatment led to a 23% decrease in stomatal density after 2 weeks and a fourfold reduction after 4 weeks [43]. Limited CO₂ *in vitro* may also stimulate increases in stomatal number and size as adaptive mechanisms [17]. Stomatal size—including length, width, and aperture—was significantly larger *in vitro*, while the length/width ratio remained stable, indicating uniform expansion. Abnormally large and fully open stomata were frequently observed, reflecting adaptations to humidity and CO₂ limitations [42,43].

Environmental conditions strongly influenced overall ginseng growth and biomass accumulation. *Ex vitro*-grown ginseng exhibited longer stems and larger leaves, whereas *in vitro*-grown ginseng showed limited shoot growth (Table 2). These growth patterns are consistent with the effects of directional lighting and restricted gas exchange under *in vitro* conditions, which are known to reduce photosynthetic efficiency and resource allocation [32,44]. Although the fresh weight of aerial parts was comparable between treatments, dry weight was lower in *in vitro* plants, suggesting lower accumulation of structural biomass under high humidity and limited gas exchange [42,45].

Significant differences in root biomass were also observed. *Ex vitro* plants exhibited 1.5- and 1.7-fold greater fresh and dry root weights, respectively (Table 3), while *in vitro* roots showed reduced elongation and dry mass accumulation. These patterns may reflect suppressed metabolic activity and nutrient assimilation under low-oxygen and high-humidity conditions. Under such environments, fresh weight may be retained through excess water absorption, while dry matter production is reduced [45]. *In vitro*-grown ginseng may rely more heavily on external carbon sources, showing traits of photomixotrophic growth [22]. While this mode supports development in sealed systems, it may shape root development trajectories differently than in *ex vitro* conditions [46,47]. For example, *Paulownia fortunei* grown under photomixotrophic conditions exhibited reduced photosynthetic performance and shorter root systems [48]. These findings illustrate how altered physiological responses under *in vitro* conditions can lead to growth patterns distinct from those observed under *ex vitro* cultivation.

Ginsenoside profiling revealed significantly higher total contents in *ex vitro*-grown ginseng, particularly for PPD-type compounds such as Rb2, Rc, and Rd. This resulted in a 4.64-fold increase in the PPD/PPT ratio compared to *in vitro* plants (Tables 4 and 5). Multivariate analyses further confirmed this trend, showing elevated levels of Rh1 in *ex vitro* plants and higher Rg1 levels in *in vitro* samples. These findings suggest that environmental fluctuations in light and temperature stimulate PPD-type ginsenoside biosynthesis, possibly as a stress-responsive adaptation [49]. This difference may be due to the stable light and temperature conditions *in vitro*, whereas *ex vitro* conditions involve natural fluctuations that potentially enhance ginsenoside biosynthesis [50]. In contrast, constant high humidity in *in vitro* systems may suppress PPD accumulation, as reported in both *P. ginseng* and *P. quinquefolius* under water-saturated conditions [51,52].

PPT-type ginsenosides exhibited more variable accumulation patterns. Re was higher in *ex vitro* plants, Rg1 was more abundant under *in vitro* conditions, Rf remained unchanged, and Rh1 appeared only in *ex vitro* roots. These differences may reflect compound-specific regulation via environmental factors such as light, genotype, and developmental stage [53,54]. For example, Rg1 content in 5-year-old ginseng roots showed a significant correlation with soil water potential, unlike Re or Rf [45,52], highlighting intra-class variability in environmental responsiveness. These findings underscore the complexity of PPT-type ginsenoside regulation and support the need for further studies to clarify how cultivation conditions shape their biosynthetic pathways. In particular, no previous studies have directly explained the contrasting accumulation patterns observed here, such as higher Rg1 *in vitro* and higher Rb2 *ex vitro*. Therefore, further investigations are required to elucidate the metabolic basis of these differences.

Volatile compound composition in ginseng roots varied significantly between cultivation conditions. *Ex vitro*-grown ginseng exhibited greater diversity and higher concentrations of volatiles, particularly terpenes, compared to *in vitro* plants (Table 6, Figure 6). This may reflect the stimulatory effects of environmental variability—such as fluctuating temperature, adequate oxygen, and biotic interactions—on volatile biosynthesis [55]. Consistent with previous studies, some volatiles were detected exclusively in *ex vitro* plants, while several ester compounds appeared either solely or in greater abundance under *in vitro* conditions. Similar patterns have also been observed in other species, such as *Petiveria alliacea*, where terpenes were more abundant under *ex vitro* conditions, while esters increased under *in vitro* cultivation [56]. Such patterns may result from unique features of the *in vitro* environment, including high humidity, limited gas exchange, and elevated carbon availability, which are known to influence volatile metabolic pathways [57]. For example, Talaverano et al. [58] reported that esters such as ethyl butyrate and isoamyl

acetate decreased under drought stress in grapevine, suggesting that their presence in *in vitro* ginseng may be favored by moisture-rich conditions.

While prior studies have primarily examined volatile differences by cultivar and plant age [59,60], this study provides the first comparative analysis in *in vitro* and *ex vitro* environments. These findings offer foundational insight into how cultivation systems shape volatile composition in ginseng, and support future efforts to optimize environmental conditions for desired metabolite production. The results of this study reveal significant differences and suggest the possibility of a sequential causal linkage among these traits. For example, under *in vitro* conditions, high humidity and limited gas exchange may reduce chlorophyll content and increase stomatal density and size, thereby lowering photosynthetic efficiency and affecting assimilate accumulation. These physiological changes may in turn influence root development and structure, ultimately leading to differences in metabolite accumulation, particularly ginsenoside composition. Overall, these observations suggest that morphological, histological, and metabolic characteristics may be interconnected, providing important insights into the acclimatization and metabolic regulation of ginseng, and indicating the need for further studies to clarify the underlying mechanisms.

5. Conclusions

This study comprehensively compared *in vitro*- and *ex vitro*-grown ginseng in terms of morphological, histological, physiological, and compositional characteristics. Clear differences were observed in the morphology and tissue structure of ginseng depending on the cultivation environment. In particular, *in vitro* conditions markedly influenced chlorophyll content and stomatal characteristics. *Ex vitro*-grown samples had greater content and diversity of ginsenosides and volatile compounds. These findings provide valuable insights into the effects of *in vitro* culture environments on the growth, development, and metabolite profiles of ginseng, and serve as a useful baseline for future studies aimed at refining *in vitro* culture systems to improve acclimatization and propagation stability.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15092222/s1>, Figure S1: Phenotypic characteristics of *in vitro* and *ex vitro* ginseng 60 days after sowing. Scale bar = 1 cm; Figure S2: Principal component analysis (PCA) biplot showing the differentiation of volatile compound profiles between *in vitro*- and *ex vitro*-grown ginseng roots. IN1~3; *in vitro*-grown ginseng, EX1~3; *ex vitro*-grown ginseng.

Author Contributions: Designed the experiments, J.-W.L. and K.H.M.; performed methodology and investigation (*in vitro* culture, chlorophyll content, stomatal analysis), and wrote the original draft, S.-J.K.; carried out ginsenoside analysis and metabolite profiling, J.S. and Y.J.; conducted histological and stomatal analyses, J.H.; analyzed data and contributed to *ex vitro* culture, J.-U.K. and J.Y.H.; contributed to data analysis and interpretation, S.C.K.; provided methodology, supervision, and writing—review and editing, J.S. and J.-W.L.; contributed to conceptualization, project administration, reviewing and approving the manuscript, and funding acquisition, K.H.M. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

The following abbreviations are used in this manuscript:

PPD	Protopanaxadiol
PPT	Protopanaxatriol
PCA	Principal Component Analysis

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