



Research article

Cytohistological study of the leaf structures of *Panax ginseng* Meyer and *Panax quinquefolius* L.



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ABSTRACT

Background: Both *Panax ginseng* Meyer and *Panax quinquefolius* are obligate shade-loving plants whose natural habitats are broadleaved forests of Eastern Asia and North America. *Panax* species are easily damaged by photoinhibition when they are exposed to high temperatures or insufficient shade. In this study, a cytohistological study of the leaf structures of two of the most well-known *Panax* species was performed to better understand the physiological processes that limit photosynthesis.

Methods: Leaves of ginseng plants grown in soil and hydroponic culture were sectioned for analysis. Leaf structures of both *Panax* species were observed using a light microscope, scanning electron microscope, and transmission electron microscope.

Results: The mesostructure of both *P. ginseng* and *P. quinquefolius* frequently had one layer of non-cylindrical palisade cells and three or four layers of spongy parenchymal cells. *P. quinquefolius* contained a similar number of stomata in the abaxial leaf surface but more tightly appressed enlarged grana stacks than *P. ginseng* contained. The adaxial surface of the epidermis in *P. quinquefolius* showed cuticle ridges with a pattern similar to that of *P. ginseng*.

Conclusion: The anatomical leaf structure of both *P. ginseng* and *P. quinquefolius* shows that they are typical shade-loving sciophytes. Slight differences in chloroplast structure suggests that the two different species can be authenticated using transmission electron microscopy images, and light-resistant cultivar breeding can be performed via controlling photosynthesis efficiency.

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1. Introduction

Ginseng (genus *Panax*; “cure-all” in Latin) is one of the most important perennial medicinal plants that belong to the family Araliaceae, and it has been cultivated for its highly valued medicinal properties for > 2000 yrs in East Asian countries like China, Japan, and Korea [1,2].

The chromosome number of Korean ginseng (*Panax ginseng* Meyer) and American ginseng (*Panax quinquefolius* L.) has been reported to be $2n = 48$ [3–5], and the plants are tetraploid ($2n = 4x$). Some *Panax* species such as *Panax notoginseng* (Burk.)

F. H. Chen have half the number of chromosomes ($2n = 24$). *P. ginseng* has an estimated genome size of 3.12 Gbp per haploid chromosome equivalent [6], whereas *P. quinquefolius* has an estimated genome size of 4.91 Gbp [7]. *P. ginseng* and *P. quinquefolius* (the closest relative of *P. ginseng* that recently diverged in the *Panax* lineage) [8] have been cultivated in shaded areas of Eastern Asia and forest canopies of Eastern North America, respectively [9,10]. However, information on the physiological differences between both ginseng species, especially cytohistological leaf structures that may affect photosynthetic activity, is not well characterized.

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Ginseng plants prefer north- and east-facing sites on well-drained slopes under a forest canopy with 70–90% shade. In Asia, ginseng plants have been traditionally cultivated under an impermeable straw hatch. In Korea, production was found to be higher when *P. ginseng* plants were grown in an experimental plot shaded with straw hatch than with a polyethylene net [11]. Several studies have tried to develop better shading materials for growing ginseng in Korea [12,13], China [14], North America, and Ontario, Canada [15]. A physiological process that might limit photosynthesis was shown to be the result of smaller quantum yield of oxygen evolution in intact leaves of ginseng than in that of pea and spinach [16]. In this study, to investigate the differences between *P. ginseng* and *P. quinquefolius*, several morphological characteristics such as surface structure of the cuticle layer, number of stomata, and appressed versus nonappressed thylakoids were observed.

2. Materials and methods

2.1. Plant materials and growth conditions

Different-aged *P. ginseng* cultivars “Chunpoong”, “Gumpoong”, “Yunpoong”, and “K-1” and *P. quinquefolius* were kindly provided by the National Institute of Horticultural and Herbal Science (NIHHS) of the Rural Development Administration (RDA), Eumsung, Korea. Leaf tissue for stomata number counting was obtained from 3-wk-old ginseng plantlets. In addition, the ginseng plants were hydroponically grown in perlite and peatmoss at $23 \pm 2^\circ\text{C}$ under white fluorescent light ($60\text{--}100 \mu\text{mol}/\text{m}^2/\text{s}$) in a controlled greenhouse. Ginseng cultivars grown in soil and under a polyethylene fabric cloth were obtained from a ginseng field (Suwon, Korea).

2.2. Light microscopy and scanning electron microscopy

For light microscopy, samples embedded in LR White resin (London Resin Co., London, UK) were thin-sectioned, stained with 0.1% toluidine blue, and examined using a light microscope (Zeiss, Axiolba, Germany). For scanning electron microscopy, the samples were fixed with a mixture of 2% glutaraldehyde and 2% paraformaldehyde and were postfixed in 50mM cacodylate buffer (pH

7.2) containing 1% OsO_4 . After dehydrating the samples with a series of alcohols (30% for 40 min, 50% for 40 min, 70% for 40 min, 80% for 40 min, 90% for 40 min, and 100% for 40 min twice), the samples were dried with a HCP-2 critical point dryer (Hitachi, Tokyo, Japan), coated with gold in an Emitec K550 ion sputter, and observed using a scanning electron microscope (S-2400; Hitachi, Tokyo, Japan).

2.3. Transmission electron microscopy

Two-yr-old ginseng leaf tips were fixed with a mixture of 2% glutaraldehyde (v/v) and 2% paraformaldehyde (v/v) in 0.05M cacodylate buffer (pH 7.2) at room temperature for 4 h. The samples were washed using the same buffer and postfixed with 1% OsO_4 in 0.05M cacodylate buffer at room temperature for 1 h. The fixed samples were washed with the buffer and then dehydrated in an ethanol series of 30–100%. The samples were embedded in LR White resin at 50°C for 24 h, and ultrathin sections (80–100 nm thick) were prepared using an ultramicrotome with a diamond knife. The thin sections were stained with uranyl acetate and lead citrate and then examined using a transmission electron microscope (JEM-1400; Jeol, Tokyo, Japan).

3. Results and discussion

3.1. Original cultivation areas and characteristics of *P. ginseng* and *P. quinquefolius*

USA and Canada, ranging from the southern part of Canada (Ontario and British Columbia) to Central Alabama and from the east coast to the west of the Mississippi River, are the major producers of *P. quinquefolius* [17]. In Korea, major *P. ginseng* cultivars have been cultivated in Daejeon and Chuncheon between the latitudes of 36°N and 38°N [13] (Fig. 1). A previous study showed that *P. quinquefolius* is more tolerant against greater light intensity than *P. ginseng* [18]. Because plants that grow at higher latitudes receive greater light intensity than plants that grow at lower latitudes [14], this feature of *P. quinquefolius* seems possible. Cytohistological leaf structures of *P. quinquefolius* from Ontario and several *P. ginseng* cultivars [19,20], Yunpoong, Chunpoong, Gumpoong, and K-1, were analyzed. Yunpoong is a high-yielding cultivar [21] with two to three more

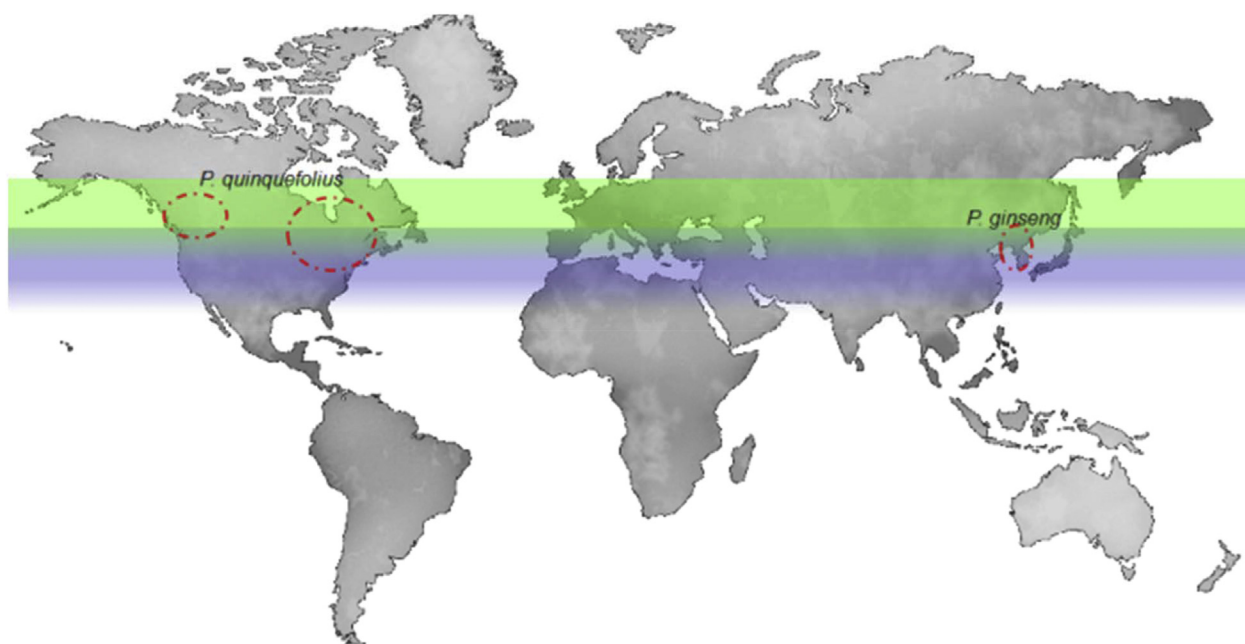


Fig. 1. Major *Panax ginseng* and *Panax quinquefolius* cultivation areas located worldwide.

proliferated axillary shoots than the other *P. ginseng* cultivars [19]. Yunpoong has a relatively short stem length and a higher number of flower buds and seeds per plant [19]. Chunpoong is one of the best-fit cultivars for producing red-ginseng materials, with the longest main root length and an appropriate root diameter [19] for forming a human body shape. The lower half of the stem of Chunpoong is typically purple, and it has more curled leaves and exhibits a higher percentage of leaf burning than Yunpoong [19]. Disease resistance, especially against root rust, is the hallmark of the cultivar Gum-poong [21]. K-1 was recently registered as a high saponin producer and a high-yielding cultivar [21]. For detailed cytohistological observations on each ginseng species, *P. ginseng* cultivars and *P. quinquefolius* were grown in Suwon, Korea, under the same environmental conditions, and the leaf tips were analyzed (Fig. 2A).

3.2. Abaxial and adaxial leaf-surface structures

Both ginseng species showed similar adaxial leaf-surface structures (Fig. 2A, left), and they had the same patterns of

cuticle ridges (Fig. 2B). Two different ginseng species also exhibited similar numbers of stomata located in the abaxial surface of the leaves grown under the same environmental conditions (Fig. 2C). In a given diameter (1.28 mm × 0.94 mm), 3-wk-old *P. quinquefolius* with other analyzed *P. ginseng* cultivars did not show any significant difference in stomata number (Figs. 2C and 2D). A previous study showed that the density of stomata in young snake tomato leaves increased to 28% and 58% compared to the semi-old and old leaves [22]. Evaluation whether ginseng leaf also show a decreased number of stomata with an increase in age needs to be analyzed in the near future. *Sebastiania myrtilloides* (Euphorbiaceae) is a good example of a plant that shows differential changes in leaf surface area between sunny and shady habitats; the number of stomata per leaf in the shady habitat was significantly lower than that in the sunny habitat [23]. The similar structure of abaxial and adaxial leaf in *P. quinquefolius* and *P. ginseng* cultivars suggests that both species are evolved for shady habitat.

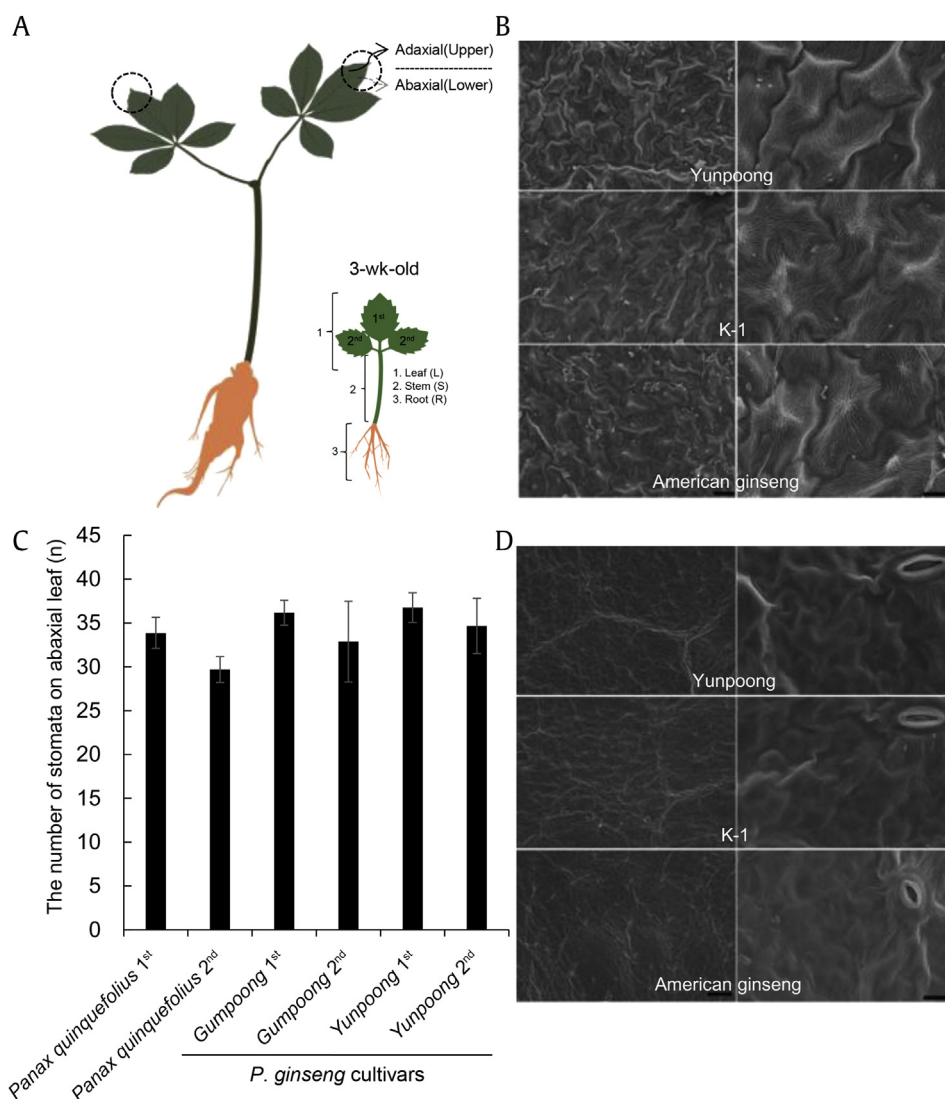


Fig. 2. Surface structure of ginseng leaves. (A) Schematic diagram of 2-yr-old ginseng and leaf tips used for surface structure analysis (left). Diagram of 3-wk-old ginseng plantlet (right). Five-leaf structure is additionally emerged as per age with no morphological changing in other organs. (B) Scanning electron microscopy image showing the adaxial (upper) surface of a ginseng leaf with wavy anticlinal cuticle ridges. Bar, 20 μ m (left) and 10 μ m (right). (C) In a diameter (1.28 mm × 0.94 mm) of 3-wk-old ginseng leaves, the total number of stomata is similar among *Panax quinquefolius* and three *Panax ginseng* cultivars in 1st and 2nd leaves depicted in Fig. 2A (right). Eight randomly selected different diameter images were used to count. (D) Scanning electron microscopy image showing the abaxial (lower) surface of a ginseng leaf with wavy epidermis and similar numbers of stomata. Bar, 20 μ m (left) and 10 μ m (right).

3.3. Internal leaf structure with spherical palisade cells

The internal space in leaves is composed of mesophyll cells, which are further divided into adaxial (upper) palisade and abaxial (lower) spongy cells in many dicotyledonous plants [24]. The skeleton of ginseng leaf shows reticulate venation with an elongated large vein, the midvein, per leaf. The midvein along the median longitudinal axis of the leaf in shaded field-grown 3-yr-old *P. ginseng* cultivars and 4-yr-old *P. quinquefolius* shows similar structural features with vascular bundle sheath cells in the center and collenchyma cells on both sides (Fig. 3A). The leaves of the four selected *P. ginseng* cultivars and *P. quinquefolius* consisted of a layer of epidermis in the upper and lower sides; a layer of spherical palisade cells, which are distinct when compared with other sun-type plant species such as *Capsicum annuum* and *Arabidopsis thaliana* [25]; and two or three layers of spongy cells (Fig. 3A). The structural features were confirmed using hydroponically cultured 3-yr-old *P. ginseng* cultivars and *P. quinquefolius* (Fig. 3B); this suggests that ginseng species of different ages have similar morphological internal leaf structures. One interesting aspect to be considered in ginseng leaf structure is that spherical palisade cells, rather than cylindrical palisade cells, are oriented perpendicular to the leaf surface [25]. Phototropins (PHOT1 and PHOT2) are blue-light receptors [26,27]. PHOT2 mediates blue-light-dependent development of cylindrical palisade cells [25]. When *phot2* is mutated, spherical palisade cells, as observed in the ginseng leaves (Fig. 3), are formed. When plants are grown under high-light conditions, leaves develop palisade tissues in which cylindrical cells are perpendicularly aligned to the epidermis to maximize the efficiency of photosynthesis [28–30]. Further characterization of blue-light receptors in ginseng is required, especially to understand how ginseng species have evolved to adapt to the environment as shade-loving plants.

3.4. Chloroplast and thylakoid structures

Mesophyll cells of leaves have many chloroplasts, which contain the specialized light-absorbing green pigments, chlorophylls. Thus, the most active photosynthetic tissue in higher plants is the mesophyll in leaves. The most striking aspect of the structure of the chloroplast is the extensive system of internal membranes known as thylakoids. Chlorophyll is found within the thylakoid membrane system, which is the site of the light reaction of photosynthesis. Transmission electron microscopy images showed that the ultrastructure of the chloroplasts in *P. quinquefolius* was ellipsoidal, whereas that of the chloroplasts in the *P. ginseng* cultivars was spherical (Fig. 4A). In barley, the shape of the chloroplasts is ellipsoidal in the light and enlarged and round in the dark [31]. Thus, *P. quinquefolius* used in this study may have originated from a higher-latitude area than the *P. ginseng* cultivars, and it has evolved for less densely shaded habitats. We cannot exclude the possibility that *P. quinquefolius* used in this study might be acclimated to a given condition and may not represent its original habitat. Sun-type chloroplasts have more nonappressed thylakoids, and shade-type chloroplasts have more appressed thylakoids that comprise almost the entire chloroplast [32,33]. Overall, the transmission electron microscopy ultrastructural images showed that both ginseng species were typical shade-adapted plants (Fig. 4). The chloroplast ultrastructure of the ginseng leaves showed large well-developed grana stacks that extended across the entire chloroplast (Fig. 4). The thylakoid membrane stacks of Yunpoong and Chunpoong were more loosely flattened than those of *P. quinquefolius* (Fig. 4B). The thylakoid structure of Gumpoong was more similar to that of *P. quinquefolius* (Fig. 4A) than that of Yunpoong and Chunpoong.

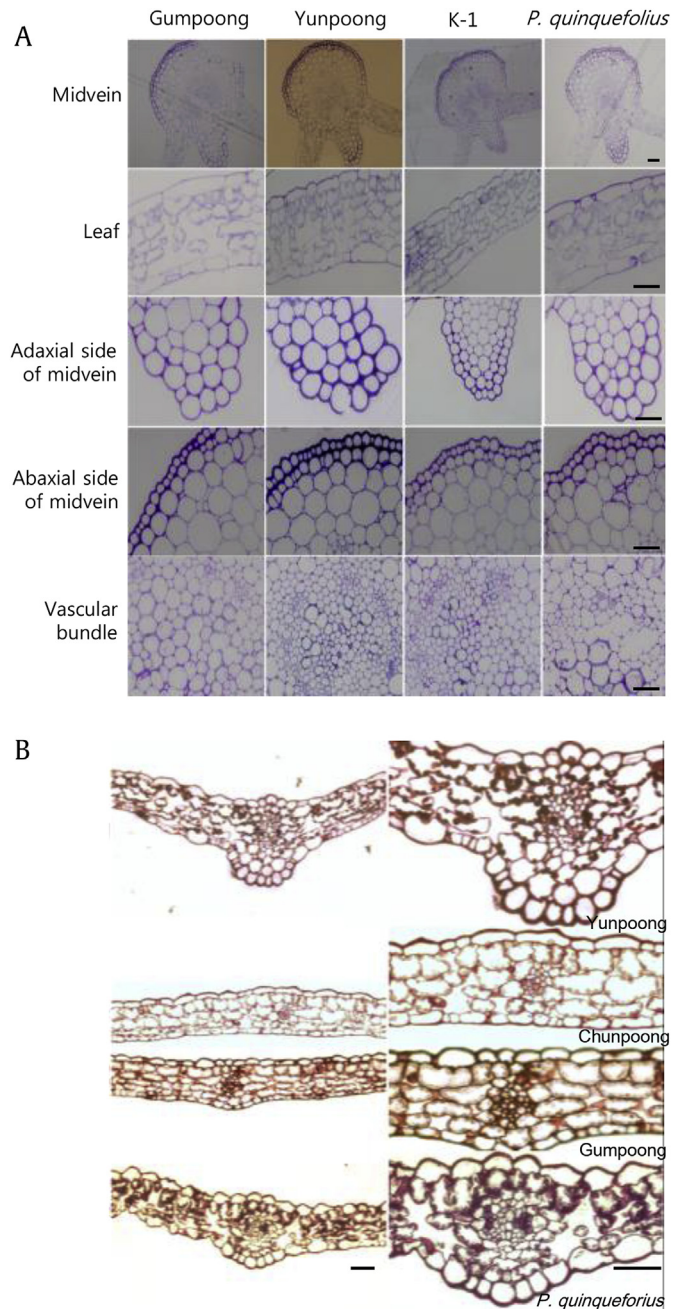


Fig. 3. Internal leaf structures of ginseng. (A) Midvein, leaf (circled uppermost part of leaf from Fig. 2A), adaxial and abaxial side of the midvein, and vascular bundle structure of soil-grown *Panax ginseng* cultivars and *Panax quinquefolius* observed using light microscopy. Bar, 5 μ m (uppermost row) and 20 μ m. (B) Mesostructures of ginseng leaves from 3-yr-old hydroponically cultured ginseng species. Bar, 50 μ m.

4. Conclusions

P. ginseng cultivars are derived from three lines, Jakyung, Chungkyung, and Hwangsook, by pure-line selection [20], and they are registered in the Korea Seed and Variety Service (<http://www.seed.go.kr>). Among nine cultivars, leaves of several superior *P. ginseng* cultivars, Yunpoong for a high-yielding variety from the Jakyung line, Chunpoong for red-ginseng manufacture from the Chungkyung line, and Gumpoong for disease resistance from the Hwangsook line were cytohistologically compared with the leaves of *P. quinquefolius*. As an obligate shade-loving plant, ginseng needs

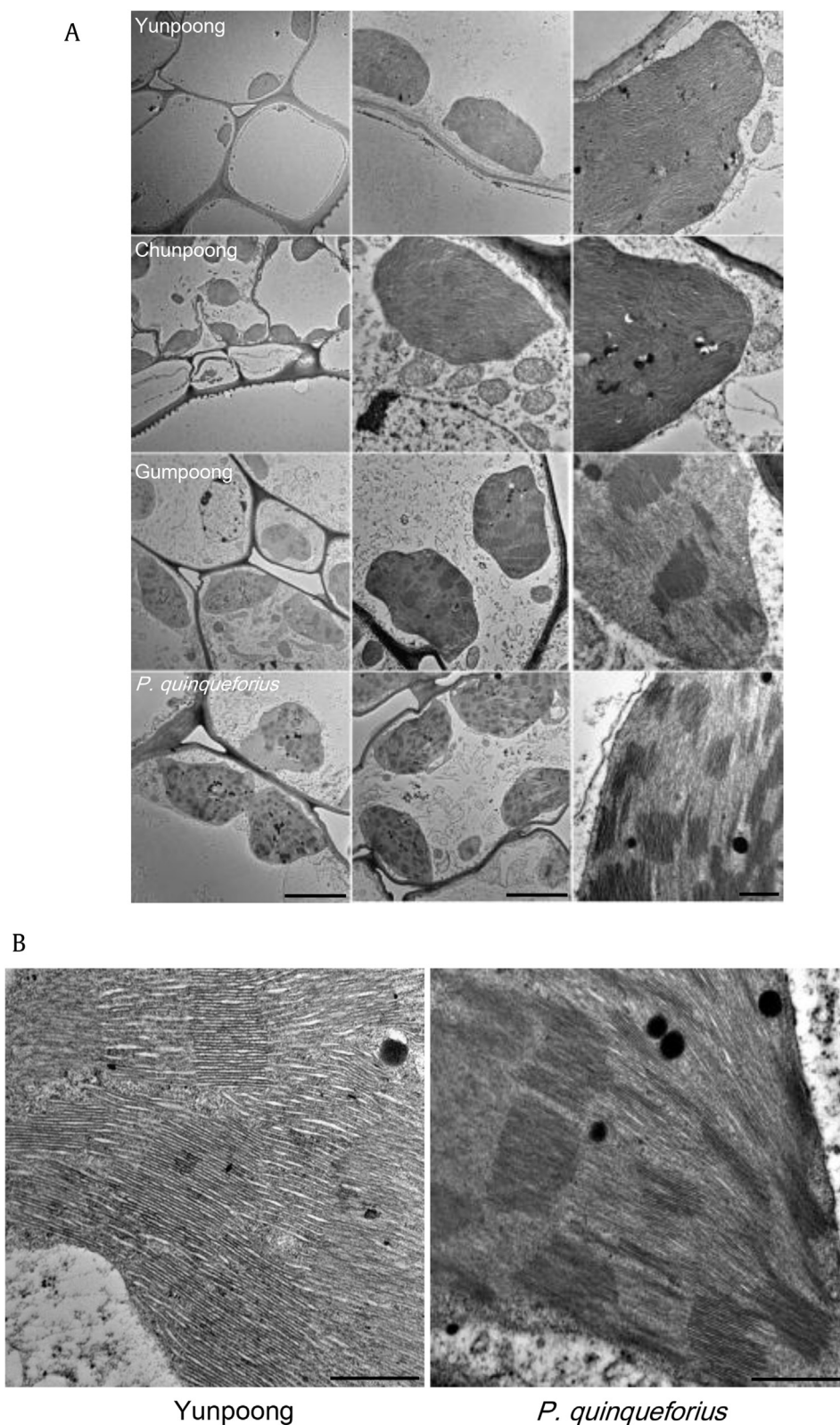


Fig. 4. Chloroplast and magnified thylakoid membrane structures. (A) Chloroplast morphology of three *Panax ginseng* cultivars and *Panax quinquefolius* is different. Bar, 5 μm . (B) Magnified chloroplast structure shows differently stacked thylakoid membranes. Bar, 500 nm.

to be grown under an artificial cloth or under densely shaded forests. The most popularly used shade cloth in Korea is a lightweight knitted polyethylene fabric that protects plants from the sun. Thus, breeding ginseng cultivars to be grown under natural sunshine

without any artificial shading will greatly reduce total cultivation expenses. Crossing two different ginseng species can result in more light-resistant cultivars. *P. quinquefolius* generally shows a more light-adapted phenotype than several *P. ginseng* cultivars. The

anticlinal walls of the adaxial surface of leaf epidermal cells were wavy and showed similar patterned cuticle ridges in *P. quinquefolius* and *P. ginseng* cultivars (Fig. 2B). The microscopic characteristics of the surface view were the same as those of a previously characterized 5-yr-old *P. quinquefolius* plant [34]. The abaxial surface of the leaf epidermis was also wavy in the two different ginseng species with similar number of stomata (Figs. 2C and 2D). The density of stomata in leaves in a sunny habitat is significantly higher than that in leaves in a shady habitat [23]. Highly dividing cells from leaf tips showed similarly structured forms in the midvein, both sides of epidermis cells in the midvein, and vascular bundles (Fig. 3A). The most striking features in the mesostructure of the ginseng leaves were the one layer of palisade cells, which are commonly cylindrical but were rather spherical and oval (Fig. 3B). The leaf mesophyll consisted of three or four cell layers of spongy parenchymal cells (Fig. 3) in *P. quinquefolius* and *P. ginseng* cultivars. Overall surface and internal leaf structures suggest that *P. ginseng* and *P. quinquefolius* are both sciophytes with few cells and chloroplasts per unit leaf area. This finding corresponds with that of a previous study on *P. quinquefolius* grown in Russia [35]. An important feature of shade plants is their chloroplast size and ultrastructure. The density of grana stacks in shade plants is always higher than the density of those in chloroplasts from plants exposed to full sunlight [32,33,36,37]. The shape of chloroplasts from *P. ginseng* was rounder than the ellipsoidal chloroplasts from *P. quinquefolius* (Fig. 4). A previous study showed that light-illuminated chloroplasts are ellipsoidal rather than round and enlarged during the dark period [31]. *P. quinquefolius* seemed more adapted to sunny conditions than the *P. ginseng* cultivars. The thylakoid membranes in *P. quinquefolius* were also appressed tightly with distinctly apparent grana stacks and decreased stroma volume. However, the most popular *P. ginseng* cultivars, Yunpoong and Chunpoong, showed relatively loosely stacked grana extended over the entire chloroplast body (Fig. 4B). Thus, the two different ginseng species are shade-loving sciophytic plants that share most ultrastructural features such as stacks of grana and stroma. The results obtained in this study can be useful not only for breeding light-adaptable cultivars but also for authentication of different widely cultivated species.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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