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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.

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.

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

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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 ± 2°C under white fluorescent light (60–100 μmol/m^2/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 Daejon 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 proliferated axillary shoots than the other *P. ginseng* cultivars [19]. Yunpoong has a relatively short stem length and a higher number of

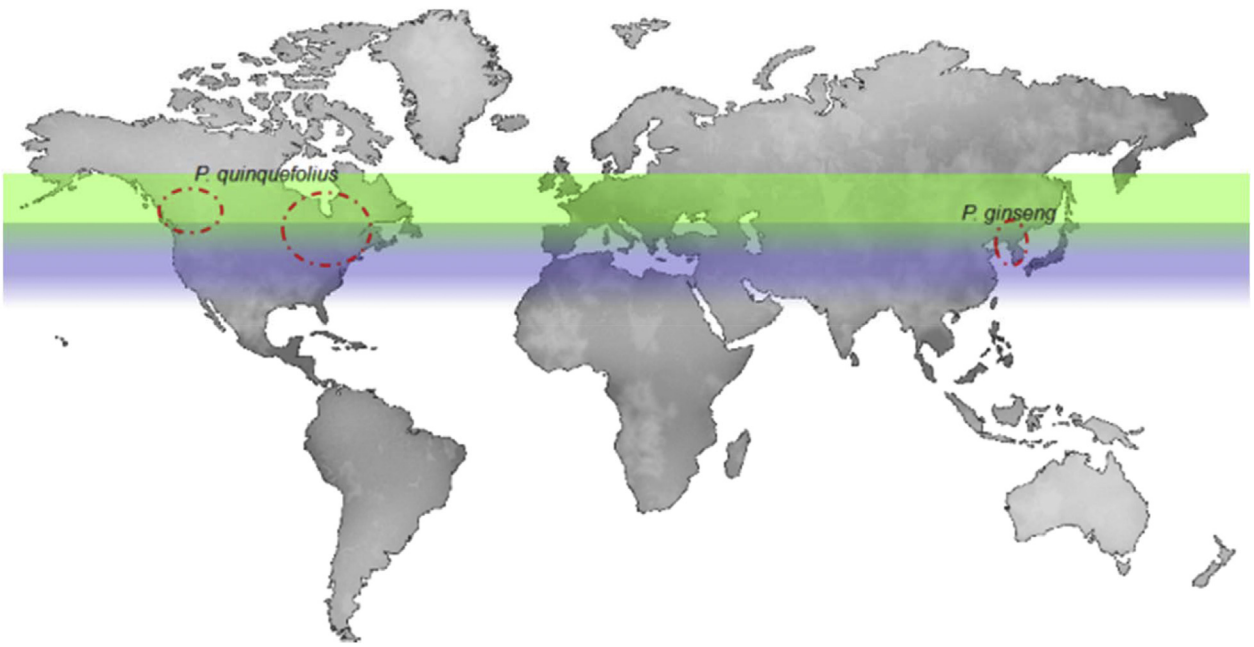

Fig. 1. Major *Panax ginseng* and *Panax quinquefolius* cultivation areas located worldwide.

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