MESOPHYLL STRUCTURE AND CHLOROPLAST DENSITY IN PANAX GINSENG LEAVES FROM THE SIKHOTE-ALIN MTS

YULIYA KHROLENKO*, OLGA BURUNDUKOVA, ELENA BURKOVSKAYA, AND YURI ZHURAVLEV

Institute of Biology and Soil Science, Russian Academy of Sciences, Stoletiya St. 159, 690022 Vladivostok, Russia

Received January 12, 2012; revision accepted August 15, 2012

This study investigated the quantitative anatomy of photosynthetic tissues (leaf mesostructure) of wild ginseng Panax ginseng C.A. Mey. (Araliaceae) plants from different natural habitats. The structural and functional traits of the photosynthetic apparatus shown to be especially elastic were mesophyll cell volume (Cv >40%) and traits related to filling of the leaf with cells and plastids (Cv ≥21%). P. ginseng possesses relatively few cells per leaf area (44.6–107.2 10^3/cm^2) and chloroplasts (1.7–4.9 10^6/cm^2). Also low are the values of such integral indexes as relative surface of mesophyll cells (Ames/A, 2.78–5.28) and relative surface of chloroplasts (Achl/A, 1.9–3.2). The leaf mesostructure of wild ginseng shows traits of a plant typically found in shady forest habitats. The photosynthetic apparatus of ginseng adapts to various habitat conditions on the level of leaf mesostructure, through structural transformations of mesophyll tissue, such as changes in the number and size of cells and chloroplasts and also the integral surface indexes Achl/A and Ames/A.

Key words: Panax ginseng, natural habitat, adaptation, mesophyll structure, quantitative leaf anatomy.

INTRODUCTION

Panax ginseng C.A. Mey. is a relict of Tertiary flora, ranked as a Category 1 endangered species (Kharkevich and Kachura, 1981) and listed in the Red Book of the Russian Federation (1988). The Russian Far East is the only location on Earth where P. ginseng can be found growing in natural habitats. To develop a strategy for protecting this endangered species, researchers must characterize its polymorphism by various methods. Mokronosov and Borzenkova (1978) offered a comprehensive approach, defining the functional activity of plants at the tissue and cellular organization levels of the plant's photosynthetic apparatus. Preliminary work on leaf mesostructure has addressed peculiar features of leaf mesostructure, of the photosynthetic apparatus in the Primorsky and Korean varieties of P. ginseng (Zhuravlev et al., 1994), the age variability of leaf mesostructure (Khrolenko and Burundukova, 2001), and the photosynthetic apparatus of ginseng in relation to its ecological strategy (Burundukova et al., 2008). Chinese researchers (Xu et al., 1994) have highlighted the influence of variation in light intensity on P. ginseng leaf mesostructure. However, most of these investigations have used cultivated plants which presumably do not preserve the diversity of features inherent in the species.

Here we investigated the mesostructure of the leaf photosynthetic apparatus of wild-grown individuals of P. ginseng. We use these results to suggest a site for in situ conservation.

MATERIALS AND METHODS

PLANT MATERIAL

We collected P. ginseng samples from their natural habitat in August in 1999–2005. The territory covered in this study is within the Chuguevsky District of the Primorye Region, close to the villages of Shumny and Lenino (Fig. 1). The plants were collected on the southern, western and southwestern slopes of the Ussyriysky Ararat mountain range, the southeastern spurs of the Medvezhy range, and on the grounds of the Verkhneussuriysky Research Station. According to Sudakov (2004), slopes with western and southwestern exposures provide the most favorable growth conditions for ginseng.

*e-mail: khrolenko@biosoil.ru
GROWTH CONDITIONS OF WILD P. GINSENG

At sites where P. ginseng grows wild, crown density is 0.6–0.8, and shrub layer projective cover varies from 40% to 60%. After leaf-fall, 50–60% of the solar radiation penetrates broadleaf forest (Tsel’niker, 1978). Forests with leafy tree crowns have the lowest PAR transmission (4%–20%). In the natural habitats of P. ginseng, PAR transmission varies from 5% to 15%.

The topsoil of the study area is complex and diverse but generally is brown soil. In the Chuguevsky District, P. ginseng is found in the middle montane zone at 300–600 m a.s.l. (Sudakov, 2004), in accord with the temperature regime of its natural habitat. Ginseng shoots start growing later than shoots of many other plants (mid to late May), when frosts have ended (Khrolenko et al., 2007). Spring frosts end earlier in middle montane belts than in lower belts, a phenomenon connected with the temperature inversions characteristic of the southern Far East (Grushwitsky, 1940).

PHYTOCENOTIC SURROUNDINGS

In the study area, ginseng was found in coniferous-broadleaf and pine-dark-coniferous forests. In P. ginseng habitats, the herb layer is mosaic and its total projective cover is 40–60%. The following species were found in proximity to P. ginseng: Oxalis acetosella L., Maianthemum bifolium (L.), F. W. Schmidt, Maianthemum intermedium Worosch., Mitella nuda L., Carex ussuriensis, Circaea alpina L., Galium davuricum Turcz. ex Ledeb., Galium paradoxum Maxim., Viola collina Bess. and Viola selkirkii Pursh ex Goldie. Plant names follow Kharkevych (1985–1996).

MESOSTRUCTURE ANALYSIS

Panax ginseng is a perennial polycarpic herb with a taproot system and whorl shoots during early ontogeny, and an elongated monocyclic monocarpic herb with sympodially replaced shoots in the adult period (Khrolenko et al., 2007). The generative whorl includes 3–4 palmately compound leaves, mostly pentamorous and less commonly tetramorous. The specific structure of the aboveground shoot and its structural pattern are determined at the bud stage and realized during the vegetative period, so the leaves follow that developmental plan. Only a terminal leaflet was collected from every generative plant. Leaves were collected from 10–15 plants in each population.

The quantitative anatomy of the mesophyll was examined according to Mokronosov and Borzenkova (1978). Further details can be found in Nobel (1977) and Araus et al. (1986). Quantitative traits can be calculated on the basis of direct measurements of cells and chloroplasts using the geometric equations describing a cylinder, sphere, rotation ellipsoid, etc.

Leaflet area was calculated by the equation $S=\frac{2}{3}AB$, where $A$ is leaf blade length and $B$ is leaf blade width.

Discs (15 per sample, diameter 7 mm) were cut from the middle part of the leaf blades and fixed in 3.5% glutaraldehyde solution in phosphate buffer (pH 7). Transverse sections of fixed leaves were made by hand. Sections were examined with a Zeiss Axioskop-40 light microscope and photographed with a Zeiss AxioCam (HRs) digital camera using AxioVision ver. 4.8.3. Some measurements (leaf thickness, chloroplast length and width, cell length and width) were taken from microscope images. P. ginseng leaf mesophyll contained only spongy cells.

To determine the number of cells per unit of leaf area, fixed discs of known area were macerated by heating briefly in 50% KOH at 80–90°C. The cells were counted in a Goryaev hemocytometric chamber under a light microscope.

To determine the number of cells per unit of leaf area, fixed discs of known area were macerated by heating briefly in 50% KOH at 80–90°C. The cells were counted in a Goryaev hemocytometric chamber under a light microscope.

The number of chloroplasts per unit of leaf area was calculated by multiplying the number of
chloroplasts per cell by the number of cells per unit of leaf area.

Chloroplast volume was determined by two methods. Chloroplast form was considered as a rotation ellipsoid. Chloroplast volume was determined by the equation $V = 4/3\pi l d^2$, where $l$ is half the length and $d$ is half the width of a chloroplast. The largest and medium axes of the ellipsoid were measured on images of transverse sections. The third and the shortest axis was taken for $1 \mu m$, excluding it from calculations (Goryshina, 1989). The third axis, chloroplast thickness, cannot be measured properly using the light microscope. For comparisons with the database of Mokronosov (1978), plastid volume was defined by a second method taking chloroplast thickness as equal to its width. Such methods (according to the nomograms of Mokronosov) increase the volume of plastids, as the usual proportion of thickness to width is 0.5–0.7.

The cell surface area and cell volume of mesophyll cells with complex shape were determined by a method based on stereological analysis of cell projections (Ivanova and Pyankov, 2002).

The relative surface area of mesophyll cells ($A_{mes}/A$) was determined by the equation $A_{mes}/A = (N_{cell}S_{cell})$, where $N_{cell}$ is the number of cells per unit of leaf area and $S_{cell}$ is cell surface area.

The relative surface area of chloroplasts ($A_{chl}/A$) was determined by the equation $A_{chl}/A = (N_{chl}S_{chl})$, where $N_{chl}$ is the number of chloroplasts per unit of leaf area and $S_{chl}$ is the surface area of the plastid.

Cell counts in macerates were done in 20 replications in 90 squares of Goryev's camera, chloroplast counts per cell were done in 30 replications, and chloroplast parameters were measured in 30 replications. In this approach the standard error does not exceed 5% (Mokronosov and Borzenkova, 1978). The results were processed using Statistica ver. 10. One-dimensional analysis was used to test the significance of the results. Maximum, minimum and mean modal values and variation coefficients ($C_v$, %) were calculated. The level of variability was estimated by the coefficients of variation using Mamaev's (1972) empirical scale. The results are presented as arithmetical means with standard errors and standard deviations. The Mann-Whitney test was applied to determine the significance of differences.

RESULTS AND DISCUSSION

In their early work, Vorobieva (1960) and Grushwitsky (1961) demonstrated that the leaf anatomical structure of *P. ginseng* is typical of shade-tolerant mesophytic plants: the leaf blade is very wide and thin; the stomata are sparse (25–40 per $1 \text{mm}^2$) and situated only on the underside of the leaf. The epidermal cells are large, with very wavy anticlinal walls, and the cells of the upper epidermis contain very large but few chloroplasts. The leaf mesophyll consists of 3–5 cell layers of spongy parenchyma; palisade parenchyma is not developed. Our data on leaf mesostructure (Fig. 2) complete this characterization. We observed individual variability in the mesostructure of the assimilation apparatus. Mesophyll cell volume was the most variable ($C_v > 40\%$) mesostructural feature of the leaf blade. The features with higher and high levels of variability ($C_v \geq 21\%$) were leaflet area, number of cells and chloroplasts per $1 \text{cm}^2$ of leaf, and the indexes for total surface area of mesophyll cells and total surface area of chloroplasts. The most stable features were leaf thickness ($81–126 \mu m$, $C_v = 11\%$) and the volume and number of plastids per cell ($C_v \leq 14\%$). The one-dimensional analysis indicated that the mean values of the majority of features of specimens collected from the Medvzhy range differed significantly from the specimens collected from the Ussuriysky Ararat range and the Verkhne-Ussuriysky Research Station (Tab. 1). Plants from the Medvzhy range and the Ussuriysky Ararat differed in most of the nine features. The slopes of south-exposed sites receive more warmth; snow melts faster there and the sites are drier, as on the slopes of the Medvzhy range where plants were collected. These plants had significantly more cells per leaf surface area and more plastids per cell, giving them higher $A_{chl}/A$ indexes than for the specimens from the other two localities. Samples from the Ussuriysky Ararat range and the Verkhne-Ussuriysky Research Station differed in three of the nine features, two of which are connected with quantitative traits of chlorenchyma. The mesophyll cells of plants from the latter two localities were larger than in those from the Medvzhy range (Tab. 1, Fig. 2). This difference probably is connected with the moister edaphic conditions of the slopes and consequent better watering of the plants and their mesophyll cells. More generally, the mesostructural variability of *P. ginseng* from the various locations in the Chuguevsky District is connected to differences in habitat conditions.

We also compared *P. ginseng* mesostructure with that of plants from different ecological groups (mesophytes, xerophytes, sciophytes; Mokronosov, 1978 and his group; Goryshina, 1989). Data from these comparisons are given in Table 2. Mokronosov (1978) gave leaf mesostructure data on more than 350 species of vascular plants and presented examples of amplitude deviation and modal classes for the more important features. Also shown are peculiarities of *P. ginseng* as compared to plants from different ecological groups that have a dorsoventral type of leaf structure similar to *P. ginseng*.
Fig. 2. Box plots of mesophyll structure parameters of *Panax ginseng* leaves from natural habitats (Mdz – Medvezhy; Vrh – Verkhneussursky; Uss – Ussurysky). Square symbols in each box indicate the mean value, the bottom and top of the box the standard error, and the whiskers the standard deviation.
Panax ginseng has comparatively large chloroplasts (diameter 6.1–8.1 μm). Chloroplast volume reaches the highest limit of this feature’s variation: about half of the studied species have chloroplast size ranging from 30 to 60 μm³. Large chloroplasts are typical of shade-requiring plants (sciophytes), from 100 to 240 μm³ in size (Mokronosov, 1978).

The number of chloroplasts in P. ginseng cells can be considered average: in vascular plants it varies from 3 to 400, the modal class being 10–30 chloroplasts per cell. About 80% of vascular plant species contain no more than 70 per cell (Mokronosov, 1978).

Panax ginseng has a relatively high cell volume for a species with a dorsoventral leaf structure. Its number of cells per unit of leaf area is low (Tab. 2). The number of chloroplasts per 1 cm² of leaf area varies generally from 2 to 80–90×10⁶/cm², the modal class being 5–10×10⁶/cm². The low value of this feature is typical of plants from shady habitats. About 70% of the species examined have an A[chl/A] index of 4–12. Some sciophytes studied from forest habitats have low A[chl/A] index values (Tab. 2). Among the species examined, P. ginseng has the lowest number of chloroplasts per unit of leaf area, and has the lowest A[chl/A] index, but shows the highest variability in chloroplast volume.

We compared our results for P. ginseng with two representative groups of species – meadow mesophytes and herbaceous plants of forest-steppe-oak-wood (Belgorodskaya oblast). Sciophytes growing under broadleaved forest canopy have more cells (140–420×10³) and chloroplasts (4–8×10⁶) per 1 cm² in their leaves, but lower chloroplast volume (31–57 μm³) and fewer chloroplasts per cell (19–40) (Goryshina, 1989) than P. ginseng.

The leaf mesophyll of sciophytic species usually is differentiated. There is frequently at least one

| TABLE 1. Significance of differences between pairs of three natural habitats of Panax ginseng by the Mann-Whitney test |
|-----------------|----------------|----------------|----------------|
| Variable        | Mdz-Vrh | Mdz-Uss | Vrh-Uss |
| Leaf area, cm²  | –       | –       | –       |
| Leaf thickness, μm | –   | *       | **      |
| Number of cells per unit leaf area, 10³/ cm² | – | – | * |
| Volume of cells, 10³μm³ | – | – | – |
| Number of chloroplasts per cell | – | – | ** |
| Number of chloroplasts per unit leaf area, 10³/ cm² | – | – | * |
| Volume of chloroplast, μm³ | – | – | – |
| A[chl]/A | –       | –       | –       |
| A[chl]/A | –       | –       | –       |

Mdz – Medvezhy; Vrh – Verkhneussuriysky; Uss – Ussuriysky; – not significant; *<0.05; ** P<0.01.

| TABLE 2. Quantitative traits of photosynthetic tissues of selected vascular plants |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Species                        | Variable        | N[chl] cell    | *V[chl] μm³     | **V[chl] μm³    | N[cell]10³/cm² | N[chl]10⁶/cm²  | A[chl]/A |
| Panax ginseng                  |                  | 35-52          | 46-72           | 115-250         | 8-48           | 44.6-107.2     | 1.7-4.9 | 1.9-3.2 |
| Asarum europaeum               |                  | 37-40G         | 57G             | 227M            | 52.4M          | 74.4M          | 2.8M | 0.8-2M |
| Convallaria majalis            |                  | 25M            | –               | 90M             | 9.1M           | 97.6M          | 2.4M | – |
| Viola mirabilis                |                  | 21-24M         | –               | 100M            | 10.3M          | 169.2M         | 3.6M | – |
| Maianthemum bifolium           |                  | 68-87M         | –               | 35M             | 141M           | 28.6M          | 2.3M | 0.8-2M |
| Oxalis acetosella              |                  | 18M            | –               | 23M             | 5.2M           | 124.4M         | 4.6G | – |
| Aegopodium podagraria          |                  | –              | –               | –               | –              | 4.3G           | 4.9G | – |
| Arisaema japonicum             |                  | 56V            | –               | –               | 18.3V          | 87V            | 4.9V | 2.8V |
| Epimedium koreanum             |                  | 75V            | –               | –               | 20.3V          | 119V           | 8.9V | 3.6V |

index* – first method; index** – second method; M – according to Mokronosov (1978); G – according to Goryshina (1989); V – according to Voronkova (1997); – no data; see Materials and Methods for other details.
layer of palisade cells which commonly are not cylindrical but rather oval or conical. This is not the case in ginseng.

*Oxalis acetosella* L., considered the most shade-tolerant species among the herbaceous plants, is similar to *P. ginseng* in number of plastids per leaf volume (Tab. 2). Another sciophyte, *Asarum sieboldii* Miq. resembles *P. ginseng* in number of plastids per cell, *A*<sub>chl</sub>/A index, and chloroplast volume (Tab. 2).

Among the herbaceous plants that share forest habitats with *P. ginseng*, *O. acetosella* and *Asarum sieboldii* Miq. have been recorded more frequently (Vasilyev, 1956; Grushwitsky, 1961). In 26 geobotanical descriptions of *P. ginseng* findings we analyzed, for *A. sieboldii* the frequency was 15%, and for *O. acetosella* 50%. Experienced ginseng gatherers call these and some other species satellites of *P. ginseng*, and use them as indicators of the possible presence of ginseng.

Voronkova et al. (1997) described the structural and functional peculiarities of leaves and seeds for 14 protected plant species of the Primorye Region, two of them sciophytes: *Arisaema japonicum* Blume and *Epimedium koreanum* Nakai (Tab. 2). The quantitative anatomy of photosynthetic tissues identifies *A. japonicum* as a typical plant of shady habitats; this species does not grow outside forest censuses, which are characterized by moderate light, temperature and humidity. These species resemble *P. ginseng* in the quantitative traits of their mesophyll structures.

A comparison of quantitative traits of the *ginseng* leaf mesostructure with those of plants from other ecological groups indicates that *P. ginseng* is a sciophyte with few cells and chloroplasts per unit of leaf area, and with low membrane indexes *A*<sub>mes</sub>/A and *A*<sub>chl</sub>/A. These traits are peculiar to plants from extremely shady habitats.

We examined the leaf mesostructure of *P. ginseng* growing in the Chuguevsky District of the Primorye Region. The plants showed variability of leaf mesostructure features which dependent on their habitat conditions. The structural and functional traits of the assimilative apparatus that we showed to be especially elastic are mesophyll cell volume and quantitative traits related to filling of the leaf space with cells and plastids. The differences in photosynthetic apparatus traits between those populations may reflect the plant’s capacity to use available resources in their natural habitats. The *A*<sub>mes</sub>/A and *A*<sub>chl</sub>/A indexes indicate differences in internal conductance for CO<sub>2</sub> and water vapor; increases of these parameters mean increasing leaf gas exchange and water use efficiency, and vice versa; plants that have maximal integral indexes grow in habitats at the ecological optimum for the given species (Pyankov et al., 1998: Ivanova and Pyankov, 2002).

The habitats from which *P. ginseng* plants showed the highest *A*<sub>mes</sub>/A and *A*<sub>chl</sub>/A indexes correspond to the ecological optimum of *P. ginseng*. These traits may thus be used as highly sensitive markers. The natural habitats of *P. ginseng* are very similar, but the Medvezhy range plants had significantly more cells per unit of leaf area, more plastids per cell, and consequently higher *A*<sub>chl</sub>/A indexes than the specimens from the other two populations.

In 2000 the Conference of the Parties to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) placed *P. ginseng* (wild plant roots) in Annex II. The Convention calls for appropriate in situ and ex situ conditions to be ensured. In situ conservation of ginseng in its natural habitat involves maintaining extant populations, establishing nature reserves and other measures. These are important elements of strategies to maintain the gene pool of ginseng. We believe that the habitat of the Medvezhy range should be a site of in situ conservation.

**ACKNOWLEDGEMENTS**

This research was supported by a grant from the Far East Branch of the Russian Academy of Sciences (RAS, project no. 11-04-98515-r_yos-tok_a), a grant from the Russian Foundation for Fundamental Research (no. 12-I-OBN-060), and a Molecular and Cell Biology (MCB) grant from the RAS Presidium.

**REFERENCES**

ARAUS JL, ALEGRE L, TAPIA L, CALAFELL R, and SERRET MD. 1986. Relationships between photosynthetic capacity and leaf structure in several shade plants. American Journal of Botany 73: 1760–1770.

BURUNDUKOVA OL, IVANOVA LA, IVANOVA LA, KIROLenko YA, BURKOVSKAYA EV, and ZHURAYLEV YN. 2008. Mesostructure of the ginseng photosynthetic apparatus in relation to ecological strategy of the species. Russian Journal of Plant Physiology 2: 246–248.

GORISHINA TK. 1989. Photosynthetic Apparatus and Environment. LGU, Leningrad.

GRUSHWITSKY IV. 1940. Inversion phenomenon of flora in Ussuriysky region. Botanical Journal USSR 1: 52–67.

GRUSHWITSKY IV. 1961. Ginseng: The Aspects of Biology. Nauka, Leningrad.

IVANOVA LA. and PYANKOV VI. 2002. Structural adaptation of the leaf mesophyll to shading. Russian Journal of Plant Physiology 3: 467–480.

KHARKEYCHY SS, and KACHIURA NN. 1981. Rare Plant Species of the Soviet Far East and their Conservation. Nauka, Moscow.

KHARKEYCHY SS [ed.]. 1985–1996. Vascular Plants of the Soviet Far East, vols. 1–8. Nauka, Moscow.
Khrolenko YA, and Burundukova OL. 2001. Age differences in leaf mesostructure in cultivated Panax ginseng C.A. Mey. Rastitelnye resursy 3: 54–59.

Khrolenko YA, Burundukova OL, Bezdeleva TA, Mizarok TI, and Zhuravlev YN. 2007. Age stages in the ontogeny of cultivated Panax ginseng C.A. Mey. Biology Bulletin 2: 120–125.

Mamayev SA. 1972. Subspecies Forms in Variability of Tree Plants. Nauka, Moscow.

Mokronosov AT. 1978. Mesostructure and Functional Activity of Photosynthesis Apparatus. UrGU, Sverdlovsk.

Mokronosov AT, and Borzenkova RA. 1978. Procedure of quantitative estimation of the structure and functional activity of photosynthesizing tissues and organs. Bulletin of Applied Botany, Genetics and Plant Breeding 3: 119–133.

Nobel PS. 1977. Internal leaf area and cellular CO₂ resistance: photosynthetic implications of variations with growth conditions and plant species. Plant Physiology 40: 137–144.

Pyankov VI, Ivanova LA, and Lambers H. 1998. Quantitative anatomy of photosynthetic tissues of plants species of different functional types in boreal vegetation. In: Lambers H, Poorter H, van Vuuren MMI [eds.], Inherent Variation in Plant Growth. Physiological Mechanisms and Ecological Consequences, 71–87. Backhuys Publishers, Leiden, The Netherlands.

Red Book of the Russian Federation: Plants. 1988. Rosagropromisdat, Moscow.

Sudakov YN. 2004. Environment of wild-grown ginseng (Panax ginseng C.A. Mey.) in forest of mountain range Ussuriysky Ararat. Komarovskie chteniya. Dalnauka, Vladivostok 50: 134–147.

Tsel’niker YL. 1978. The Physiological Basis of Tolerance of Tree Plants to Shadow. Nauka, Moscow.

Vasilyev NG. 1956. Black fer-broadleaf forests of southern Primorye. Ph.D. dissertation, Vladivostok.

Vorobieva PP. 1960. Development and some anatomic-physiological features of ginseng to lighting. Proceedings to research of ginseng and schisandra. LGU, Leningrad 4: 64–86.

Voronkova NM, Burundukova OL, Zhuravlev YN, Nesterova SV, and Aban’kina MN. 1997. Structural-functional features of some rare plant species. Komarovskie chteniya. Dalnauka, Vladivostok. 44: 72–88.

Xu Ke-Zhang, Zhang Zhi-An, Wang Ying-Dian et al. 1994. Effect of light intensity on microstructure and ultrastructure of Panax ginseng leaves under field condition. Acta Botanica Sinica 36: 23–27.

Zhuravlev YN, Burundukova OL, Koren OG, Zaytseva YA, and Kovalева EV. 1994. Panax ginseng C. A. Meyer: Biodiversity evaluation and conservation. The challenges of the 21st century. Proceedings of the International Ginseng Conference, 21–23 September 1994, 162–168. Vancouver.