Revue de Cytologie et Biologie végétales – Le Botaniste
2005 - **29** (1/2): 23 - 29

LOWER EPIDERMIS STRUCTURE IN LEAVES OF *TROCHODENDRON ARALIOIDES* Siebold et Zucc.

by

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Summary

Leaf structure of *T. aralioides* is discussed and correlations between several characters are estimated. Leaves of *T. aralioides*, as well as average-sized leaves of other plants, have well developed transpiration surface and similar to theirs relative water transfer surface. At the same time, xylem of *T. aralioides* has not vessels, only tracheids; therefore, water transfer efficiency of this tissue is decreased. Very characteristic features of the epidermal tissue of *T. aralioides* are the presence of thick strongly cutinizated outer walls of cells of the epidermis, and the stomata, which are enclosed in cavities formed by cuticular outgrowth. It has been argued that such a structure of the epidermis decreases a risk of water deficit, which could occur in the leaves due to imperfection of xylem organization in this plant.

Key-words
Trochodendron aralioides, leaf, epidermis, stoma.

Structure de l'épiderme inférieur des feuilles de Trochodendron aralioides *Siebold et Zucc.*

Résumé

La structure des feuilles de Trochodendron. aralioides *est discutée et les corrélations entre plusieurs caractères sont estimées. Les feuilles de* T. aralioides, *ainsi que de nombreuses feuilles d'autres plantes développent des surfaces transpirantes. Le xylème de* T.aralioides *ne possède pas de vaisseaux mais seulement des trachéides, donc les transferts d'eau sont faibles. Une caractéristique de l'épiderme de* T. aralioides *est la présence d'une cuticule épaisse sur les cellules épidermiques mais aussi de stomates enfoncés entourés d'une excroissance de la cuticule. Il ressort de cette étude l'argument que la structure de cet épiderme diminue les risques de déficit hydrique qui compense dans les feuilles les imperfections du xylème chez cette plante*

Mots clés
Trochodendron aralioides, feuille, épiderme, stomate

1.-Introduction

Genus *Trochodendron* is a monotypic genus from family *Trochodendraceae* belonging to the order *Magnoliales* (HUTCHINSON, 1969) or, according to another classification, to its own order *Trochodendrales* (TAKHTAJAN, 1987). *Trochodendron aralioides* is an evergreen tree of 5 to 25 meters height with trunk diameter up 60 centimeters. Life duration of its leaves is about 2 - 4 years. At present, distribution area of this species is very limited. It is found in Japan, Korean peninsular and in Taiwan. However, it has wide ecological amplitude. *T. aralioides* grows in the lowland and mountainous beech forests, sometimes forms single species stands. It can also grow on rocks. In Taiwan this species is found in high mountains (altitude 2000 - 3000 s. m.). There is evidence suggesting that this species had a wide distribution area across all Holarctic before the glaciation (PERVUKHINA and IOFFE, 1962; TAKHTAJAN, 1980; LESNAYA Encyclopedia, 1986).

T. aralioides is regarded as one of the most archaic flowering plants. Its xylem lacks vessels and water transport is done by tracheids (BAILEY, 1953; CHEADLE, 1956; TAKHTAJAN, 1980). Tracheids of *T. aralioides* are considered to be more primitive than in majority of modern coniferous plants. They are very long with early xylem forming scalariformis borded pits. Tracheids of the late xylem are narrow and their pits acquire somewhat roundish contours. Primitive features of xylem organization in *T. aralioides* had stimulated much interest in this plant. There is a wide range of publications devoted to the structure of its vegetative and generative organs (BAILEY and NAST, 1945; PERVUKHINA and IOFFE, 1962; PERVUKHINA, 1970). Most detailed work on *T. aralioides* leaves is done by GOLYSHEVA (1976) with particular reference to

Character	Symbol	$\bar{x}$	σ	x_{min}	x_{max}	cv
Surface area of leaf blade, cm^2	*ab*	32.1	7.8	19.5	46.0	24.5
Number of cells per 1 mm^2 of lower epidermis	*nc*	2019.0	131.6	1800.0	2300.0	6.5
Number of stomata per 1 mm^2 of lower epidermis	*ns*	200.0	32.7	125.0	275.0	16.4
Length of stoma, μm	*ls*	29.3	0.9	27.3	30.7	2.9
Stomatal index, % (ratio of number of stomata to the total cell number in epidermis)	*si*	11.0	1.6	7.3	15.3	14.8
Mesophyll thickness, μm	*tm*	284.4	18.8	245.0	312.0	6.6
Coefficient of palisadity, % (ratio of palisade to mesophyll thickness)	*cp*	46.9	3.2	40.9	51.7	6.8
Density of venation, mm/cm^2 (length of veins per 1 cm^2)	*lv*	472.8	72.9	355.6	666.7	15.4
Cross section area of xylem in petiole, mm^2	*ax*	0.16	0.04	0.1	0.26	27.7
Cross section area of tracheids in petiole xylem, μm^2	*at*	160.5	22.8	120.0	210.0	14.2
Relative water transfer surface of leaf (ratio of leaf surface area to xylem surface area on petiole cross section)	*b/x*	19976.0	1419.0	17049.0	21905.0	7.1
Volume of mesophyll per 1 stoma, μm^3	*m/s*	0.0015	0.0003	0.0011	0.0023	20.7
Area of xylem on petiole cross section per 1 stoma, μm^2	*x/s*	0.26	0.05	0.17	0.41	18.2

x - arithmetic mean, x_{min} – minimum value, x_{max} - maximum value of characters, σ - standard deviation, cv – coefficient of variation

Table 1. – Quantitative characteristics of *T. aralioides* leaf structure

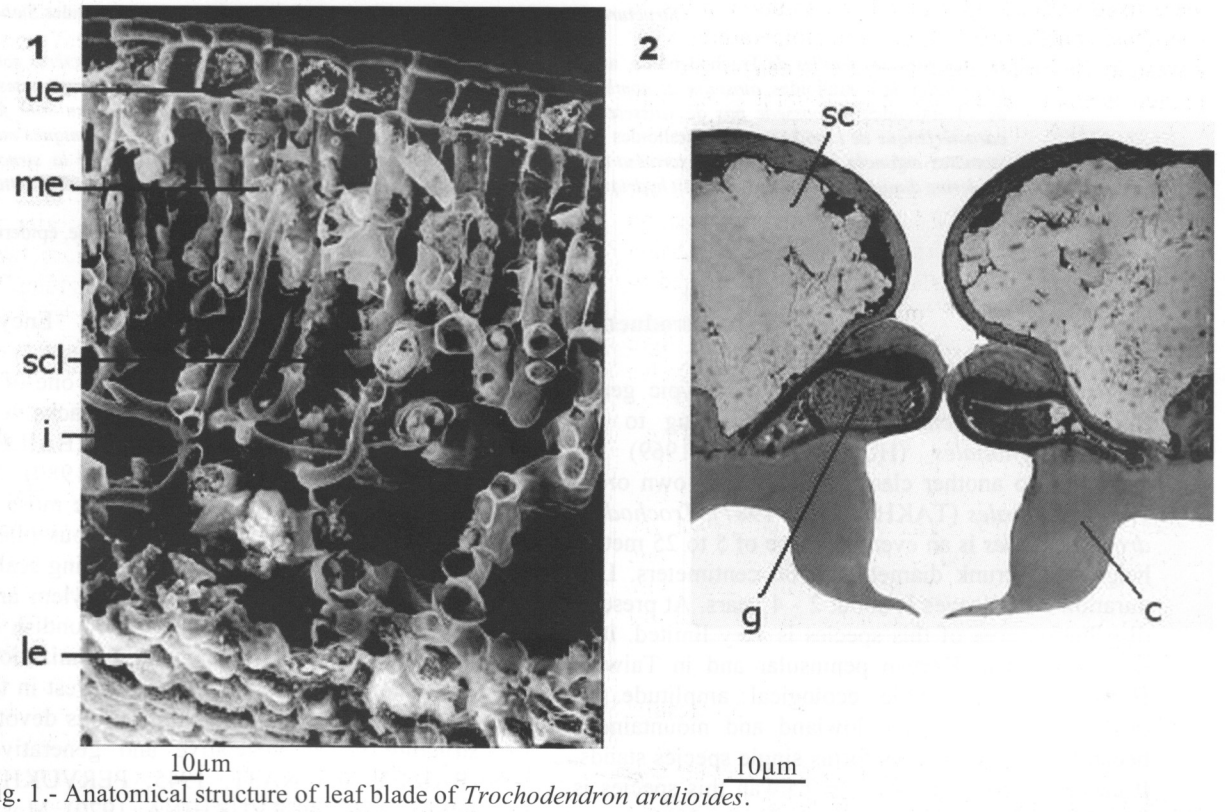

Fig. 1.- Anatomical structure of leaf blade of *Trochodendron aralioides*.
1.- cross section of leaf blade (scanning electron microscope), 2. - cross section through stoma (transmission electron microscope).
ue – upper epidermis, le – lower epidermis, me – mesophyll, i – intercellular space, scl – sclereid, g – guard cell, sc – subsidiary cell, c – cuticle.

organization of stomata. GOLYSHEVA pointed out to the presence of the varying number of polar and lateral cells nearby the guard cells of stomata. Thus the stomata can be classified as octocytic to tetracyctic with transition to anomocytic. With this type of stomata *T. aralioides* approaches ancestral Protoangiospermae, which are close to Gymnospermae in these characters. On the contrary, stomata of *T. aralioides* are considered by TAKHTAJAN (1980) as specialized. BARANOVA (1990) describes them as laterocytic.

It is well established, that water regime of plants depends not only on xylem formation, but also on structural features of other tissues, in particularly, leaf tissues. In this paper possible relationships between characters of *T. aralioides* leaf epidermis and primitive organization of xylem of this plant is discussed.

2.- Materials and methods

Leaves ot *T. aralioides* are collected from cultivated trees grown in the Botanical gardens "Southern Cultures" (Adler) and in the Komarov Botanical Institute (St. Petersburg). For light microscopy (for measures) the leaves were fixed in ethanol 70% . Cross sections of fragments of the middle part of leaf blade and petiole and also preparations of epidermis was carried out according to the traditional methods and techniques (LANGERON, 1949; PROZINA, 1960). Histological material was examined with light microscope MBI-3.

For scanning electron microscopy samples were fixed with 3% glutaraldehyde solution in 0,1 M cacodylate buffer (pH 7,4) at room temperature. After a wash in the buffer, the material was dehydrated in a graded acetone series, and critical-point dried with CO_2 (COHEN and GARNIER, 1971; GUILLAUMIN, 1980), then mounted on stubs and sputter-coated with a layer of gold (70 - 100 nm), for examination with a JSM – 35 scanning electron microscope, operating at 5 KV. Transmission electron microscope (Tesla BS - 500) was applied to study complex of cells forming stomata.

All measurements were done from the light microscope using drawing apparatus PA-6. Twelve characters studied which characterize structure of epidermis, mesophyll, and xylem of petiole. Qualitative description of these characters is given according to the scheme developed by VASILIEV (1988). In order to characterize variability of the characters we used coefficient of variation (cv), and Pearson coefficient (r) to estimate pair wise relationships between the characters. Sample size was 25 specimens.

3.- Results

Leaves of *T. aralioides* have an average size (table 1). The surface area of leaf blade is about 30 cm^2. Leaf blade is very thick, about 340 μm. Mesophyll is dorsiventral and multilayer (fig. 1.1). It has 3 layers of palisade and 6 - 7 of spongy cells. Spongy mesophyll is very loose. Coefficient of palisadity is an average (about 47%). A very characteristic feature of mesophyll is the presence of branching sclereids. Density of venation is not very high, not more than 470 mm/cm^2.

The surface of upper epidermis is smooth. The lower epidermis develops papillary outgrowths made of outer walls of its cells (fig. 2.1). Both upper and lower epidermis consists of average-size cells. In the upper epidermis there are more than 1200 cells per 1 mm^2, in the lower – more than 2000 cells. Outer walls of these cells are thick, strongly cuticlised. Their thickness together with cuticle at the end of the first year of leaf growth is 8.5 μm – for the upper epidermis cells; and more than 5 μm – for the lower epidermis cells. On the surface of leaf there are significant wax deposits (fig. 2.2 and 2.3). It is important that in evergreen plants development of cuticle continues during all period of leaf life (SCHIEFERSTEIN and LOOMIS, 1959). Thus, with the age of leaf its cuticle thickness also increases. Leaves are of hypostomatal type. Stomatal index is low (11%). An average number of stomata is 200 per 1 mm^2 of the unit area of the lower epidermis. The stomata are of laterocytic type, large. Their average length is about 30 μm. There are 1 - 2 unmodified epidermal cells adjacent to the poles of stomata. Each of the guard cells, as a rule, is accompanied by 2-3 of the lateral cells. A cross section through stomata shows that the guard cells are positioned not nearby the lateral subsidiary cells, but practically lay on them (fig. 1.2). Guard cells of all stomata develop vigorous cuticular outgrowths (fig. 1.2). Secondary stomata (according to terminology of DUNN *et al.* (1965), which are the most abundant of leaf stomata, these outgrowths are curved towards the aperture of a stoma. As the result, a cavity is formed, which is separated from the environment by the small hole (fig. 2.2,and 2,3). The outgrowths around the primary stomata form thick peristomatal rims (fig.2.3).

The petiole is about 2 times shorter than blade, thick. Its cross section area is more than 3 mm^2. Cortex is well developed and constitutes 80% of the total tissue volume. Cells of the cortex are lay loose. Intercellular spaces occupy about 20% cross section area of cortex. There are many sclereids. Phloem is less developed as compared to xylem. The ratio of their areas in petiole cross section is about 0.8.

The low level of variation ($cv<10\%$) has been revealed for length of stoma, total number of cells in epidermis per 1 mm^2 of the epidermis surface, mesophyll thickness, coefficient of palisadity, ratio of leaf blade surface area to xylem area on petiole cross section. Stomatal index, number of stomata per 1 mm^2 of the epidermis surface, area of tracheid cross sections in xylem of petiole per one stoma had shown an average level of variation ($10\%<cv<20\%$). High level of variation ($cv\geq20\%$) has been recorded for leaf surface area, mesophyll volume per one stoma and area of petiole cross section.

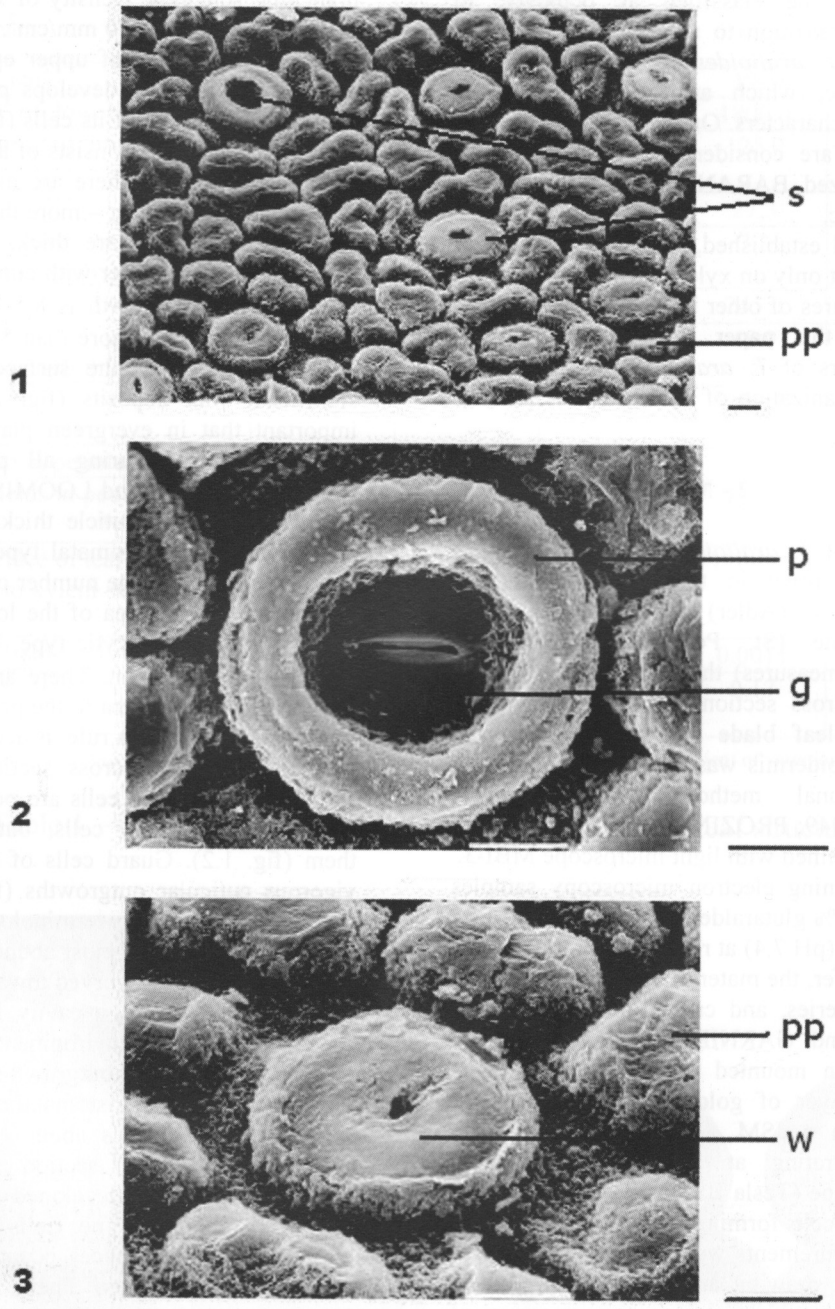

Fig. 3.- Surface of lower epidermis of *Trochodendron aralioides* (scanning electron microscope). 1 - general view of the surface, 2 – primary stoma, 3 – secondary stoma.

s – stoma, p – peristomatal rim, g – guard cells, w – cuticular wall of air cavity in which there are the guard cells of stoma (see fig. 1.2), pp – papilla.
Scala - 10 μm.

	ab	*nc*	*ns*	*ls*	*si*	*tm*	*cp*	*lv*	*ax*	*at*	*b/x*	*m/s*
nc	-095											
ns	-018	636										
ls	171	-168	-198									
si	011	295	924	-149								
tm	-164	-273	-110	-018	-015							
cp	-240	-125	-078	-092	-028	588						
lv	-040	364	-022	176	-194	-200	-161					
ax	957	025	040	159	018	-105	-233	-064				
at	147	-026	078	-008	108	-176	-058	-057	209			
b/x	-126	-342	-171	039	-016	-184	018	166	-401	-146		
m/s	030	-644	-901	161	-809	465	178	-006	005	-099	041	
x/s	068	-437	-887	162	-895	169	070	-053	141	-003	-298	837

Notes: Qualitative description of the different characters according to VASSILIEV (1988); Zero and dot as a decimal separator are omitted.

Table 2. – Pair wise correlation coefficients of leaf characters of *T. aralioides.*

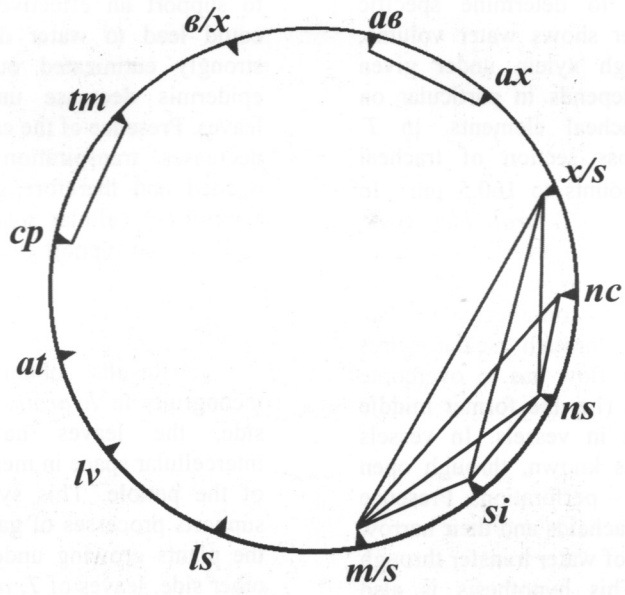

Fig. 3. - Correlation clusters of the leaf characters at the level of $|r| \geq 0.5$

Correlation analysis had shown that the characters form three clusters on level r>0.5 (table 2, fig. 3). First cluster consists of size of leaf blade and area of xylem on petiole cross section and establishes the following relationship: the larger the leaf blade, the more developed xylem in its petiole.

Mesophyll thickness and coefficient of its palisadity compose the second cluster of characters. Thus, the thicker mesophyll is the larger proportion of palisade tissues in its volume. And on the opposite, the thinner mesophyll is the lower the coefficient of its palisadity.

The third cluster of characters contains the total number of cells, number of stomata per 1 mm^2, proportion of stomata of the total number of epidermal cells, mesophyll volume per one stoma and, finally, surface area of xylem in petiole cross section per one stoma. Density of stomata in the epidermis depends on two factors. The first one is which part of the cells of this tissue is differentiated into stomata. The second one is the size of basic cells: the larger the cells situated between the stomata, the more distant are the stomata from each other, and the smaller the cells, the closer the stomata. The first factor is the most important during the first stages of morphogenesis, when there are mainly cells divisions taking place. During the later stages of morphogenesis, when cell divisions are superseded by their extensions, the second factor prevails (PAUTOV *et al.,* 2006). Density of stomata is highly correlated with mesophyll volume and area of xylem cross section in petiole (r = 0.88). Increase in number of stomata leads to decrease in area of xylem and mesophyll volume per one stoma. Both these characters have important functional role because of the degree of their development affecting transpiration and gas exchange.

Correlations between length of stomata, density of venation cross section area of tracheid in petiole xylem, relative conductive surface of leaf and other characters are not statistically significant.

4.- Discussion

These features of leaf structure can be partially explained, in our opinion, by organization of the water transport system in this plant. According to GUBER (quoted from CRAMER and KOZLOVSKYI, 1983), there are two ways to evaluate efficiency of this system. First, it is to calculate a relative water transfer surface area measured as transpiration to transfer surface area ratio. In our care: ap/aks. The value of this index in *T. aralioides* is 2 x 10^4. Available data show similar values in range of plants, which have a well developed leaf blade. For example, in *Populus* L. an average value of leaf relative transfer surface area is the same as in *T. aralioides* (PAUTOV, 2002). Second is another way to estimate efficiency of the water transport system is to determine specific conductivity. This parameter shows water volume, which is transferred through xylem under given conditions. Conductivity depends in particular on type and size of the tracheal elements. In *T. aralioides* the area of cross section of tracheal elements in leaf petiole amounts to 160.5 μm^2. In *Populus*, which has similar to *T. aralioides* value relative transfer surface area, an average value of cross section area in vessels is more than 600 μm^2. Situation with *T. aralioides* is complicated by fact that water is transferred through scalariformis tracheids not vessels. Water flow has to overcome resistance of pit membranes (i.e. the former middle laminae), which are absent in vessels. In vessels water is transferred, as it is known, through open holes in the walls of vessels – perforations. Presence of pit membranes between tracheids and their narrow diameter slow down the rate of water transfer through xylem of *T. aralioides*. This hypothesis is also confirmed by the fact known about the water transport in xylem of coniferous plants, which is also made of tracheids. Thus, specific conductivity, which is measured in the number of litres per hour under one bar of pressure along 1 m of distance with cross section area of 1 cm^2 amounts to 20 litres for coniferous plants and to 65 - 128 litres for deciduous broad-leaves trees. The speed of water transfer in xylem of coniferous plants is also lower than in deciduous broad-leaved trees.

It has been established that even when the water supply is sufficient, transpiration might exceed water influx in leaves of wooded plants. This causes water deficit, loss of turgor and competition for water between different parts of a tree. Water deficit in leaves also depends on external factors as well as on resistance to water flow through trunk and branches. Closing stomata can solve such situations, but this would decrease photosynthesis (KRAMER and KOZLOVSKYI, 1983).

Thus according to our own and other published results it can be argued that the leaves of *T. aralioides* is of an average size, i.e. they have well developed transpiration surface. Their relative water transfer surface area corresponds to that of the leaves of some other flowering plants. It is identical, in particularly, to the relative surface area found in Poplars, which, according to USMANOV (1975), can have a very high level of transpiration. At the same time, there are also reasons to believe that organization of xylem in *T. aralioides* is not efficient to support an effective water transport. All these could lead to water deficit in the leaves. Thick strongly cutinizated outer walls of cells of the epidermis decrease unregulated water losses in leaves. Presence of the cameras covering stomata also decreases transpiration even when stomata are opened, and, therefore, gas exchange is still possible. Large inter cellular spaces in both leaves and their petioles also support gas exchange.

5.- Conclusion

Results obtained in our study show incongruity in *T. aralioides* leaf structure. From one side, the leaves have very well developed intercellular space in mesophyll and in the cortex part of the petiole. This system of auriferous hollows supports processes of gas exchange and is typical of the plants growing under wet conditions. From the other side, leaves of *T. aralioides* have thick strongly cutinised outer walls of cells of the epidermis and their stomata are enclosed in cavities. These features provide protection from water losses, and they usually present in plants of the dry habitats. We think that such a structure of the epidermis decreases a risk of water deficit, which could occur in the leaves due to imperfection of xylem organization in this plant.

Acknowlegment

This study has been carried out with financial support of the Russian Fund of Basic Research (project N 04-04-48999).

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