Morphological and Anatomical Features of Leaf and Stem of Dioscorea Nipponica Makino

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Abstract: In this article *Dioscorea nipponica* Makino. information on the anatomical structure of the leaf and stem of the species, the location, structure and functions of each tissue. The results of this study support the taxonomy of *D. nipponica* species based on anatomical structure. This study showed that the leaves and stems of *Dioscorea nipponica* have characteristics similar to those of Eudicot. Our observations showed that the leaf surface of *Dioscorea nipponica* is covered with solitary trichomes. The structure of the vascular bundle is represented by a definitive V-shaped arrangement of metaxylem vessels, tracheids and phloem units. Raphid crystals are present in the leaf mesophyll tissue. It was also found that the stem shape is undulating, parenchyma cells are saturated with granular substance, conducting tissues are arranged in 2 rows, conducting vessels are paired.

1 INTRODUCTION

Dioscorea nipponica Makino. - is a perennial herbaceous liana of the Dioscoreae family (Dioscoreaceae R.Br.), reaching a length of 4-5 m (Gubanov et al., 1976). The rhizome is horizontal, shallow from the soil surface, sparsely branched, brownish-brown (white or yellowish at the break), up to 1.5 m long and 2 cm in diameter, with traces of dead stems and numerous thin, rigid cord-like roots (Anthony and Ibok, 2021). Younger parts of the rhizome are lighter, yellowish, fleshy, with large buds; the outer layer of the rhizome is easily separated as a flaky thin dark-coloured corky layer. Decoction and tincture of the roots of Dioscorea nipponese folk medicine recommends atherosclerosis of cerebral vessels, coronary atherosclerosis, angina pectoris, hypertension, diabetes mellitus. Already after the first course of taking dioscorea reduces or disappears headache, tinnitus, fatigue. Further increases the removal of cholesterol with bile, improves the work of the heart, pancreas, liver, kidneys.

2 MATERIALS AND METHODS

Leaves and stems of *Dioscorea nipponica* were fixed in 70% ethanol according to a generally recognised technique and its anatomical structure was studied on a transverse section of the fixed material (Trankovsky, 1979).

The anatomical features were studied using preparations prepared by cutting the transverse section manually and using a Motic B1 microscope. The preparations were stained with safranin. Cells and tissues were measured using a MOB-15 micrometer.

Quantitative measurements of several traits: leaf diameter, seed coat thickness, seed coat thickness and

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endosperm thickness were performed according to the generally accepted method (Dospekhov, 1985).

Statistical analysis of data was calculated with a personal computer (MS Excel) using generally accepted methods. Microphotographs were taken using a digital camera, and mathematical analysis was performed using a Motic microscope.

3 RESULTS AND DISCUSSION

Dioscorea has a positive effect on sleep, memory, vision, depth of breathing, pulse rate. Acts as an anti-inflammatory agent, reduces blood clotting. Treats inflammation of the trigeminal nerve. Rhizomes of Dioscorea contain numerous compounds, but the main active substances are steroidal saponins (up to 8 %), derivatives of diosgenin, the main of which is dioscin. Diosgenin can be a starting product for the synthesis of hormonal drugs - cortisone, progesterone (Ki-Sun et al., 2020).

There are several stems; they are simple, glabrous, whorled, about 0.5 cm in diameter. Leaves are regular, petiolate, broadly ovate with a heart-shaped base. Flowers with simple corolla-shaped yellowish-greenish perianth. Fruits are three-nested, broadly elliptic bolls. Blooms in July-August; seeds ripen in August-September. In the medical industry, rhizomes and roots of Dioscorea nipponica are used to produce the drug polysponin.

Dioscorea nipponica Makino. Far Eastern species, grows in Primorsky Krai, southern parts of Khabarovsk Krai and in the south-east of Amur Oblast, in Primorsky Krai, southern parts of Khabarovsk Krai and in the south-east of Amur Oblast, distributed mainly in the north-eastern, northern, eastern and central regions of China. It also occurs in mixed forests, on mountain slopes, in ravines and along roadsides, at altitudes of 1,400-3,200 m in Guizhou, Sichuan, eastern Xizang and Yunnan provinces. Most often found in secondary plant communities occurring on clear-cutting and fire sites. Listed in the Red Data Book of Russia (Harkevich, 2012).

Dioscorea nipponiana was introduced from Russia to Uzbekistan in 1996 by Y.M. Murdahaev; today it grows only in the Tashkent Botanical Garden (Murdakhayev, 1990). We tudied morphological and anatomical structures of the leaf in the conditions of the Botanical Garden (Fig. 1.).

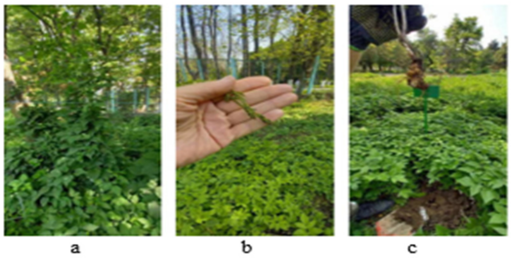

Figure 1: General view of Dioscorea nipponica Makino.: a - general view,b - flowers, c – rhizome.

The leaves are three and seven-lobed. Leaves are evenly arranged on the stem in an orderly fashion (Fig.2).

Figure 2: Morphological structure of the leaf of Dioscorea nipponica Makino.

Leaves are broadly heart-shaped in outline, with strongly projecting auricles; their veining is arcuate, most often 9 primary veins reach the leaf tip. The marginal veins ensure the strength of these "wind" leaves, which, obviously, is also served by anastomoses between primary veins, formed by veins of the 2nd order and creating a common reticulate-nerve veining. The leaf tip is strongly elongated into a drop-shaped tip. Such droplet spicules hanging downward from the leaf allow water runoff from its surface and water secreted by hydatodes. However, they exhibit an interesting peculiarity of internal structure. The whole length of the leaf tip is crossed here inside by a complex glandular system of cavities (pockets) with a slit-like exit to the surface of the drop spicule. The glandular epithelium of the cavities secretes mucus in them, in which nitrogen-fixing bacteria settle. It was also found that the nitrogen content in the drop spicules is higher than in the leaf lamina.

Features of leaf epidermis that revealed similarities between wild-type and micropropagated plants included amphistome state, presence of mucus, glandular unicellular trichomes with multicellular heads, polygonal cells with smooth walls, and type and shape of stomata. Minor variations included a thick cuticular wall with closed stomata in wild-type plants compared with thin-walled open stomata in in vitro plants. The opening of the stomata resulted in an increase in the average size of the stomata (7.68-0.38) µm and (6.14-0.46) µm on the adaxial side (Aina et al., 2011).

Examined under optical microscope, scanning electron microscope (SEM) and transmission electron microscope (TEM) for stem, leaf, petiole, tuber, root and flower of Dioscorea hispida Dennst. provided detailed information on the anatomical features that defined this species. The anatomical study showed that the leaves of Dioscorea hispida had similar features to eudicot plants, but the stem, tuber and flower resembled unicotyledonous plants. The leaf surface of Dioscorea hispida was covered with rough, bristly and spiny trichomes or hairy surface (Bu, 2015).

Structure of the leaf lamina of Dioscorea nipponica (1-drawing).

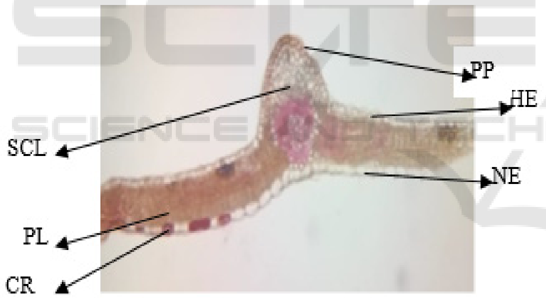

Figure 3: General view of a transverse section of a Dioscorea nipponica Makino. leaf: PP-Conducting bundle, HE- upper epidermis, NE-lower epidermis, SCL-sclerenchyma, PL-palisade, CR- raphid crystals.

The epidermis is a layer of cells that protects against harmful environmental influences and excessive water evaporation. The upper (adaxial) and lower (abaxial) epidermis have a single layer of cells, each covered by a cuticle. Often on top of the epidermis, the leaf is covered with a protective layer of waxy origin (cuticle). The stomata are restricted to the lower surface only (hypostomal). In the epidermis of Dioscorea nipponica leaves, anamocytic and anisocytic stomata, as well as single pilosebaceous trichomes with cuticular striated cell wall were found on the adaxial and abaxial epidermis. Also, raphide crystals are present in the epidermis.

The palisade (above, densely packed cells) and spongy (below, loosely packed cells) portions of the mesophyll, located between the upper and lower epidermal layers, are shown. The mesophyll, or parenchyma, is the inner chlorophyll-bearing tissue that performs the main function, photosynthesis. Network of veins formed by conducting bundles (conducting tissue) consisting of vessels and sieve-like tubes for the movement of water, dissolved salts, sugars and mechanical elements.

Stomata are special complexes of cells located mainly on the lower surface of leaves; they are used for evaporation of excess water (transpiration) and gas exchange. Epiderma is the outer layer of a multilayered cell structure that covers the leaf from all sides; the boundary area between the leaf and the environment. The epidermis performs several important functions: it protects the leaf from excessive evaporation, regulates gas exchange with the environment, excretes metabolic substances and, in some cases, absorbs water. Most leaves have a dorsoventral anatomy: the upper and lower surfaces of the leaf have different structures and perform different functions.

The epidermis is usually transparent (there are no or few chloroplasts in its structure) and is covered on the outside by a protective layer of waxy origin (cuticle), which prevents evaporation. The cuticle on the lower part of the leaf is generally thinner than on the upper part, and thicker in biotopes with arid climates compared to those where there is no moisture deficit. The epidermal tissue consists of the following cell types: epidermal (or motor) cells, defense cells, accessory cells, and trichomes. Epidermal cells are the most numerous, largest (11.2±0.3, 15.4±0.4) and least adapted (9.1±0.2, 11.1±0.4). The epidermis is covered with pores called stomata, which are part of a whole complex consisting of a pore surrounded on all sides by chloroplast-containing guard cells and two to four side cells lacking chloroplast.

This complex regulates evaporation and gas exchange of the leaf with the environment. As a rule, the number of stomata on the lower part of the leaf.

Most of the leaf interior between the upper and lower layers of the epidermis is parenchyma (the main tissue), or mesophyll. Normally, the mesophyll is formed by chlorophyll-synthesizing cells, so the synonymous name chlororenchyma is also used. The product of photosynthesis is called photosynthate.

Leaves are usually colored green due to chlorophyll, a photosynthetic pigment found in chloroplasts, the green plastids. Plants lacking or lacking chlorophyll cannot photosynthesize. The veins consist of xylem, the tissue used to conduct water and

Table 1: Quantitative parameters of Dioscorea nipponica leaf mesophyll.

№	Signs	Indicators, microns
1	Length of the upper epidermis	11,2±0,3
2	Width of upper epidermis	9,1±0,2
3	Length of lower epidermis	15,4±0,4
4	Width of lower epidermis	11,1±0,4
5	Palisade length	13,2±0,4
6	Palisade width	10,1±0,2
7	Sclerenchyma length	12,2±0,4
8	Sclerenchyma width	11,3±0,2
9	Parenchyma length	14,5±0,4
10	Parenchyma width	13,3±0,3

Figure 4: Transverse section of Dioscorea nipponica stem: A - general view, B - peel, C - pith: C - cortex, S - sclerenchyma, PP - conductive bundles, SG - granular matter.

dissolved minerals, and phloem, the tissue used to conduct organic matter synthesized by the leaves. Usually the xylem lies on top of the phloem. Together they form the main tissue called the leaf core. The lamina has 3-4 protruding veins running from the base to the apex. The cuticle of the lamina is soptically thickened.

The leaf has a layered epidermis, the soptic wall on the abaxial cells is convoluted and the adaxial cells have a straight wall. Stomata with irregular distribution and anomocytic type are found on the abaxial surface. Palisade tissue was clearly distinguished, elongated, compactly arranged and oriented vertically, with a transverse section on the adaxial side (13.2±0.4, 10.1±0.2), and spongy tissue was arranged either loosely or compactly with irregularly sized cells.

Dioscorea nipponica on transverse section is round to wavy in shape, internal cells and tissues are densely arranged (Fig. 4).

The stem is finely cellular, covered with cortex on the outside. The structure of the cortex (containing chloroplast) consists of one row of epidermis with cuticle and 4-5 rows of parenchyma cells under it. The parenchyma cells are followed by a ring of 5-6 rows of thin-walled sclerenchyma cells. After the sclerenchyma cells there are large and small conducting bundles. The bundles have pairs of large vessels.

The central core consists of parenchyma cells, which increase in size as you approach the centre. Among the parenchyma cells, granular filled cells can be observed.

Epidermal cells are 13 µm high and 10 µm wide. The table below summarises the size of all organoids of the stem (Table 2).

Table 2: Anatomical parameters of stem Dioscorea nipponica.

№	**Symbols**	**Indicators, µm**
1	Epidermal height	13,3±0,3
2	Epidermal width	10,1±0,2
3	Sclerenchyma length	11,2±0,4
4	Sclerenchyma width	9,3±0,2
5	Parenchyma length	12,5±0,4
6	Parenchyma width	11,3±0,3
7	Height of large conductive bundles	18,8±0,5
8	Width of large conductive bundles	15,4±0,4
9	Length of smaller conductive bundles	9,4±0,3
10	Width of smaller conductive bundles	8,1±0,2

The anatomical structure of the stem of Dioscorea nipponica is little studied than leaves, tubers, roots, rhizomes (Martin et al., 1963), (Cunyu et al., 2022), (Vı´tor et al., 2016), (Berdibaeva and Atabaeva, 2023).

We anatomically characterised the aerial stems of the genus Dioscorea and evaluated the possibility of using these anatomical characteristics to better understand the taxonomy, systematics and diversity of component species in the non-tropics. Air stem fragments from 23 species were collected for anatomical analysis using conventional cytohistological techniques (Berdibaeva, 2021).

4 CONCLUSIONS

Dioscorea has two layers of vascular bundles, with the outer layer containing sclerenchyma and the medulla having sclereids, in contrast the parenchyma had a sclerenchyma layer with vascular bundles. The sclerenchyma layer in the stem may increase mechanical strength.

In conclusion, we would like to report that the study of the anatomical structure of the cross-section of the stem Dioscorea nipponica is established, has a wavy shape, parenchyma cells have filled with granular substances, two-row arrangement of conductive bundles, the presence of paired vessels.

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