

Botany

Bioecological Peculiarities and Microstructural Characteristics of the Leaf of *Rhododendron Brachycarpum* D.Don. ex G.Don.

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The paper presents the results of one of the research stages, namely, the general bioecological peculiarities and microstructural characteristics of the leaf of *Rhododendron brachycarpum* D.Don. ex G.Don., of East Asian origin, growing in the humid subtropics of Georgia, in particular, in the Batumi Botanical Garden. The authors actively conduct scientific research on the unexplored species of the rich collection of rhododendrons in the Batumi Botanical Garden collection. They have determined that *Rhododendron brachycarpum* is characterized by a rich content of biologically active substances. This led to the study of the issue presented in the paper because the knowledge of the bioecological and microstructural peculiarities of the plant is necessary for its practical use and accurate identification. *Rhododendron brachycarpum* goes through a full cycle of development, well adapted to local environmental conditions. The morphological characteristics are consistent with the description in the scientific literature. The quality of seed germination is relatively low, however, it can be propagated by stem cuttings. The microstructure of the important raw material – the leaf, including the main midrib of the leaf, the leaf epidermis, and the leaf mesophyll have been studied. © 2023 Bull. Georg. Natl. Acad. Sci.

Rhododendron brachycarpum, leaf microstructure

The heath family (*Ericaceae*) combines more than 4000 species of shrubs, grass, lianas and trees. Most of them are distinguished by decorative as well as medicinal properties. Among them, *Rhododendron* L. is noteworthy, representing six species in Georgia: *Rh. ungerii* Trautv., *Rh. ponticum* L., *Rh. caucasicum* Pall., *Rh. luteum* Sweet, *Rh. Sokhadze*,

Charadze et Davlian., *Rh. smirnowii* Trautv. Beside decorative values, these species are used in perfumery and for industrial purposes too. Their antioxidant, anti-inflammatory and antimicrobial activities are applied against various diseases in traditional and scientific medicine [1-6].



Fig. 1. Leaf of *Rhododendron brachycarpum* D.Don ex G.Don.

Among local and introduced species growing in subtropical climatic conditions of the floristic region of Ajara, particularly, in the collection of the Batumi Botanical Garden, the most distinguished ones are *Rhododendron ungerii* Trautv., *Rh. arborescens* (Pursh.) Torr., *Rh. arboreum* Smith f.roseum, *Rh. brachycarpum* D.Don. ex G.Don., *Rh. delavayi* Franch., *Rh. japonicum* A.Gray., *Rh. Macrosepalum* Maxim. In general, the representatives of the said genus are characterized by spreading over a large area and plasticity [6]. Evergreen, semievergreen and deciduous species of *Rhododendron*, its breeds and forms are highly decorative because of their habitus and original blooming. That is why they are considered to be important for landscape designing and interior decorating, best confirmed by an impressive palette of Rhododendrons represented in the Batumi Botanical Garden.

Unique species of *Rhododendron* growing in the collection of the Batumi Botanical Garden are less or even not studied at all, and almost unstudied, therefore, we have been researching them in recent years.

This work presents the results of one of the stages of the research, namely, general bioecological peculiarities of *Rhododendron brachycarpum* and microstructural peculiarities of a leaf, identifying its diagnostic characteristics, because without knowing the inner structure of

vegetative and generative parts of plants, it is impossible to understand the whole life of a plant and set the goal to obtain it thoroughly; Moreover, preciseness of critical taxa is strengthened by anatomic data, which is a reliable method for plant diagnostics together with the other parameters. Besides that, this species deserves attention due to its bioactive content being studied for the first time by our research team.

We have determined that the mentioned species is distinguished by a rich content of biologically active substances, characterized by high antimicrobial activity, which makes it credible in terms of further thorough research in the perspective of theoretical and practical use in pharmacy, medicine, ecology and other purposes.

Rhododendron brachycarpum is a strong, upright, 2-6 meters tall evergreen shrub or tree, mainly with 8-20 cm long and 3-5 cm wide lance-shaped leaves (Fig.1); upper surface is green and shiny; lower part is naked or thinly covered with grey-brownish trichomes; its stem is 1-3 cm long; 10-20 white-pinkish flowers are clustered in 10-12 cm diameter spherical catkins; the fruit is 8-10 mm long box. The plant is overspread in Korea and Japan from the sea level to subalpine zones. The species is quite frost-tolerant and therefore, appreciated in cold regions of Japan [1-6].

Materials and Methods

For the study of the general bioecological peculiarities used Beidemann method [7]. Leaves of *Rhododendron brachycarpum* were collected from plant introduced in the Batumi Botanical Garden in 1960 (the floristic region of Ajara). Transversal, longitudinal and surface slices cuticles of preparatory samples were done by a sharp razor from a live unfixed material, collected from a medial part of a leaf plate and midrib. Slices were kept in safranin solution for 24 hours and placed in glycerin on the slide. Observation of specimens was done using Carl Zeiss, Jeneval light microscope; digital images were taken by a camera Canon

Digital IXUS75 and post-processed using Adobe Photoshop CS5 software [8,9].

Results and Discussion

As a result of our observations over the years, vegetative development of *Rhododendron brachycarpum* under study comprises 8-10 months. The development of vegetative buds and the beginning of shoot growth begins in March, after a 1-2-week recess period of rest in June-July, complete the second growth in August. It is a species flowering in spring-summer. According to the observations of the last years, the duration of flowering is comprises in average 35-45 days, characterized by average degree of flowering. Fruits ripen mainly in September-October, in November, the process of sowing seeds in November-December, however, is characterized by a very weak quality of fruiting, therefore, in terms of propagation, in the conditions of the Batumi Botanical Garden, propagation by stem cuttings gave us results, and we recommend this method. Defoliation occurs in summer months and mostly ends by late August. Negative winter temperatures do not damage the plant and do not hinder its development.

The leaf of *Rhododendron brachycarpum* is bifacial, bare in terms of ventilation system, the structure is hypostamatic, the pulp has a dorsoventral structure. The leaf covering tissue is cutinized with equal-layered cuticle; adaxial epidermis is double-layered and abaxial – single-layered. In the ventral side of the leaf, there are tightly packed and tangentially slightly stretched smaller epidermal cells. However, in the dorsal side of the leaf, there is smaller nipple-like epidermal tissue. In the abaxial covering tissue, the differential stomata apparatus is sunk toward epidermis cells.

The basic texture of the leaf pulp of *Rhododendron brachycarpum* is composed of structural units of a rather thick spongy parenchyma; typical palisade parenchyma is represented by double-layered cells, separated by the cells with not typical palisade-like habitus. Significant amount of calcium oxalate

druse crystals is accumulated in the leaf mesophyll of short-fruit rhododendron, especially in the spongy parenchyma, the size of druse is really impressive (Fig. 2).

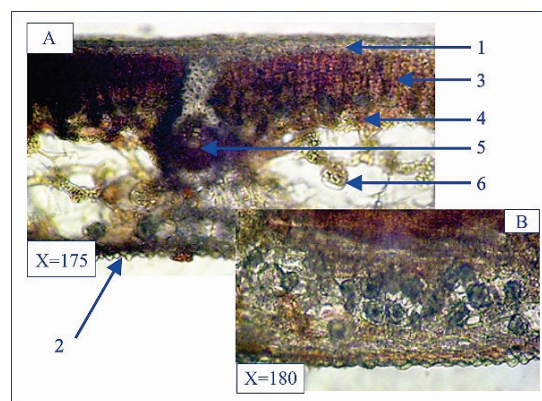


Fig. 2. Leaf microstructure of *Rhododendron brachycarpum* D.Don ex G.Don.: A. Dorsoventral mesophyll of a leaf; B. Druse of calcium oxalate crystals in spongy parenchyma of the leaf: 1. Upper epidermis; 2. Lower epidermis; 3. Palisade parenchyma; 4. Palisade-like parenchyma; 5. Vascular bundle; 6. Spongy parenchyma.

Vascular bundles of *Rhododendron brachycarpum* packed in the ventral part of the leaf pulp are surrounded by starch sheath and sclerenchymal cells; the vascular bundle differentiated in the leaf is complex and contains fibrovascular and reverse-collateral structures; rounded and weakly angular lumens of smaller caliber are arranged in the xylem (Fig. 2).

The covering tissue of the midrib of the leaf of *Rhododendron brachycarpum* is cutinized. The vascular structure is more or less crumbly, polygonal cells are represented. Lamellar collenchyma areas are shown in ventral and dorsal sides of the main vein. Vascular system concentrated in the costa is surrounded by mechanical tissue. The phloem is especially narrow-cellular, roundish lumens of cortex fibers and vascular vessels are arranged in the cortex, membrane of tracheary elements are spirally thickened. Radial rays differentiated in the xylem are short and single-layered (Fig. 3).

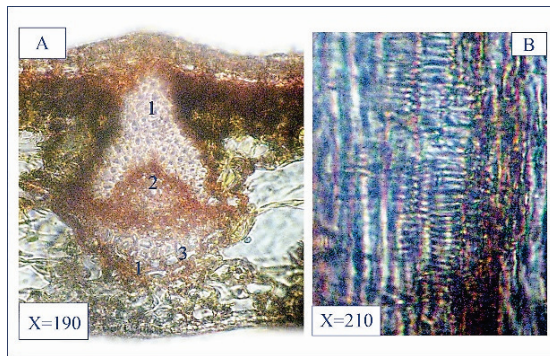


Fig. 3. Microstructure of the midrib of *Rhododendron brachycarpum* D.Don ex G.Don.: A. Vascular bundle with fibrovascular and reverse-collateral structure; B. Spirally thickened vascular vessels of membrane: 1. Mechanical cells; 2. Xylem; 3. Phloem.

Cells at the base of adaxial and abaxial epidermis of the leaf of *Rhododendron brachycarpum* are not lined but with curved and uneven structure; abundant blade apparatus arranged in the lower epidermis of the leaf is simple and anomocytic [8] (Fig. 4). The membrane of stomatal locking cells is rectilinear and thin; the clefts are mostly spindle-shaped, although sometimes oval (Fig. 5). Considering the direction of stomatal clefts toward the midrib of the leaf, the ventilation system is revealed to be chaotic. Significant amount of calcium oxalate crystals druse is accumulated in cells at the base of abaxial epidermis of the leaf of *Rhododendron brachycarpum* (Fig. 5).

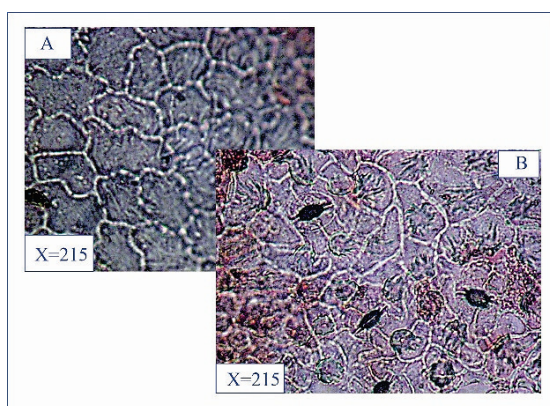


Fig. 4. Microstructure of the leaf epidermis of *Rhododendron brachycarpum* D.Don ex G.Don.: A. The texture of not lined, but curved and uneven tissue near the base of adaxial side; B. Ventilation system in not lined, but curved and uneven tissue at the base of abaxial side.

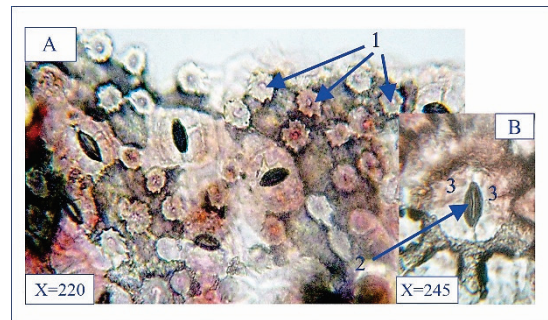


Fig. 5. Microstructure of lower leaf epidermis of *Rhododendron brachycarpum* D.Don ex G.Don.: A. Calcium oxalate crystals druse in abaxial covering tissue; B. Simple, anomocytic stomatal apparatus with a spindle-shaped stomatal cleft: 1. Druses; 2. Stomatal cleft; 3. Stomatal locking cells.

Conclusion

The study and knowledge of the bioecological and microstructural peculiarities of the plant is necessary from the point of view of practical use of the plant, for its accurate identification, based on diagnostic characteristics, from the point of view of use in medicine, pharmacy, perfumery, agronomical disciplines and other fields. Knowledge of the internal structure of a research object is extremely helpful for the study of the plant biology, as vital processes ongoing in the organism are linked with specific structural units. The diagnostic signs of the plant microstructure are the following: indumentum type, outline and interlocking features of epidermal tissue basic cells of an organ of interest; types of stomata in the covering tissue and their characteristics; leaf pulp architectonics and details of structural elements of vascular tissue differentiated in axial organs.

In the humid subtropical climate of Georgia, in particular, in the Batumi Botanical Garden, the *Rhododendron brachycarpum* is fully adapted and goes through a full cycle of development, provides healthy vegetative and generative raw materials, negative winter temperatures It does not harm it.

The study revealed the diagnostic characteristics of the internal structure of the leaf of *Rhododendron brachycarpum* D.Don ex G.Don.:

- Leaf is naked bifacial;
- Hypostomatic arrangement of stomata;
- Leaf mesophyll has a dorsoventral structure;
- Vascular bundle is complex with fibrovascular and reverse collateral structure;
- Significant amount of calcium oxalate crystal druses are revealed in abaxial covering tissue of the leaf and spongy parenchyma;
- Abundance of mechanical cells can be found in vascular bundles and midribs;
- Lumens of vascular vessels in the xylem are mostly round-shaped, their membrane is spirally thickened;
- Radial rays of cortex are short and single-layered;
- Cells at the base of adaxial and abaxial epidermis of the leaf are not lined, but curved and uneven;
- Stomatal ventilation apparatus is anomocytic;
- Stomatal locking cells are rectilinear and clefts are mostly spindle-shaped.

ბოტანიკა

Rhododendron Brachycarpum D.DON. ex G.Don.

ბიოეკოლოგიური თავისებურებები და ფოთლის მიკროსტრუქტურული მახასიათებლები

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(წარმოდგენილია აკადემიის წევრის ვ. პაპუნძის მიერ)

ნაშრომში მოცემულია საქართველოს ტენიანი სუბტროპიკული კლიმატის პირობებში, ბათუმის ბოტანიკურ ბაღში მოზარდი აღმოსავლეთაზიური წარმოშობის სახეობის – *Rhododendron brachycarpum* D.Don. ex G.Don., კვლევის ერთ-ერთი ეტაპის შედეგები, კერძოდ, ზოგადი ბიოეკოლოგიური თავისებურებები და ფოთლის მიკროსტრუქტურული მახასიათებლები. ავტორთა მიერ აქტიურად მიმდინარეობს ბათუმის ბოტანიკური ბაღის კოლექციაში არსებული

როდოდენდრონის მდიდარი კოლექციის შეუსწავლელი სახეობების სამეცნიერო კვლევა დადგენილია, რომ *Rhododendron brachycarpum* გამოირჩევა ბიოლოგიურად აქტიური ნივთიერებების მდიდარი შემცველობით, ახასიათებს მაღალი ანტიმიკრობული აქტივობა, რაც სარწმუნოს ხდის პერსპექტივაში ფარმაციის, მედიცინის, ეკოლოგიის, აგრარული მიმართულებით და სხვა დანიშნულებით მის გამოყენებას. ამან განაპირობა ნაშრომში წარმოდგენილი საკითხის შესწავლა, რადგან მცენარის ბიოეკოლოგიური და მიკროსტრუქტურული თავისებურებების ცოდნა აუცილებელია მისი პრაქტიკული გამოყენებისა და ზუსტი იდენტიფიკაციისთვის. *Rhododendron brachycarpum* განვითარების სრულ ციკლს გადის, კარგად არის ადაპტირებული გარემოს ადგილობრივ პირობებთან. ბიომორფოლოგიური მახასიათებლები შესაბამისობაშია სამეცნიერო ლიტერატურაში არსებულ აღწერილობასთან. თესლის წარმოქმნის ხარისხი შედარებით დაბალია, თუმცა გამრავლება შესაძლებელია ღეროს კალმებით. შესწავლილია მნიშვნელოვანი ნედლეულის – ფოთლის, მათ შორის, ფოთლის მთავარი ძარღვის, ფოთლის ეპიდერმისის და ფოთლის მეზოფილის მიკროსტრუქტურა.

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