Scientific Papers. Series B, Horticulture. Vol. LXVI, No. 1, 2022
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

COMPARATIVE LEAF AND FLOWER MORPHO-ANATOMICAL STUDY OF WILD AND CULTIVATED GOJIBERRY (*LYCIUM BARBARUM* L.) IN ROMANIA

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Abstract

Goji berry (Lycium barbarum L.) is widely used as food and medicine in Asian countries and recently had a dramatic gain in popularity on American and European continents. Due to their complex composition and recommendations in traditional Chinese Medicine, goji berry is also one of the most studied species in the recent years. The species grow wild in Romania, being appreciated for its bush density for hedges and fences. Initially, imported L. barbarum varieties were used by goji berry growers for commercial plantations, while in the last year five new varieties were homologated. The morpho-anatomical structure of leaves and flowers of the wild and cultivated goji berry from the Bucharest area was compared, to determine important traits that could be relevant for goji breeders but also for taxonomists. Morphological differences were found regarding the leaves shape, position, and leaves width. The wild L. barbarum has cuticle-covered leaves, highly developed vascular bundles and vascular bundle sheaths were present in the mesophyll. The palisade cells appeared to be very large. These characteristics of the leaf's anatomy are also relevant in the context of biotic stressors, as eriophyid mites, that are one of the most important pests of goji berry shrubs.

Key words*: wild and cultivated Lycium barbarum, leaf anatomy, mesophyll, vascular bundle.*

INTRODUCTION

Goji is the generic name for different plant species from the genus *Lycium*, belonging to the Solanaceae family. It represents one of the most popular products of traditional Chinese medicine that is also used outside of China (Wetters et al., 2018). The genus *Lycium* includes more than 80 distinct species, distributed in temperate or sub-tropical regions (Levin & Miller, 2005).

The exact origin of *L. barbarum* is not known, but its natural habitat is between Southeast Europe and Southwest Asia. While the wild *L. barbarum* is also known as fences sea buckthorn, the "goji" name can be considered a trading name, given initially by the North American ethnobotanist Bradley Dobos, being the species with the most significant commercial value (Tabără, 2020a, 2020b). Moreover, given that *L. barbarum* L. is a naturalized species in most countries of the world, this has hindered the process of differentiation between the species and especially between the subspecies of this shrub. *L. barbarum* is also found in Romania, being observed in the Dobrogea area, including the Danube Delta (Doroftei, 2009), in the region of Moldova, in Oltenia, where it was described as an adventitious plant with a pronounced invasive character (Răduţoiu & Stan, 2013) and in the city of Timișoara (Coste & Arsene, 2003).

The invasive potential of this species was also observed in 34 localities in Oltenia (Răduţoiu & Băloniu, 2021). Also, *L. barbarum* is found in the spontaneous flora of the Republic of Moldova, being an allochthonous and naturalized plant that was introduced from China for ornamental purposes and penetrated into natural ecosystems, acquiring a potentially invasive character (Palancean, 2015).

While originally grown as a health food in Asia, *L. barbarum* and its berry are now known worldwide for its health benefits, including a high fiber, potassium, magnesium, iron, vitamin E, vitamin C, carotenoids, and beta-carotene content (Niro et al., 2017). *L. barbarum* plant has been consumed for over 2500 years with no toxicity being reported (Donno et al., 2015). The fruit, leaf, root bark of many species of the genus *Lycium* have long been used as local foods and/or medicines. Recently, *Lycium* fruits, have become increasingly popular in the western world because of their nutritional properties (Amagase and Farnsworth, 2011; Mencinicopschi, 2013a, 2013b; Asănică et al., 2016; Qian, et al., 2017), they are even advertised as "superfood" in Europe and North America (Chang & So, 2015). Phytochemical studies indicate that the richness in numerous constitutions of different classes, such as polysaccharides, carotenoids, flavonoids, alkaloids, amides, terpenoids, endows *Lycium* species with a variety of biological activities (Yao et al., 2011; Qian, et al., 2017;). Plant-based products are important sources of both food and medicine. Whether a plant is used as food or medicine depends on a wide range of factors but is not necessarily due to its pharmacological or nutritional properties (Leonti, 2011; Jennings, et al., 2015). *L. barbarum* has special medicinal properties, so in recent years this plant has gained more and more attention from consumers due to its antioxidant properties and for improving vision (Shen et al., 2012), anticancer and immunomodulatory effects (Gan et al., 2004; Tang et al., 2012), antioxidant activity (Henning et al, 2014; Benchennouf et al., 2017; Shi et al. 2017), hepatoprotective activity (Ahn, et al., 2014), hypoglycemic properties (Guowen et al. 2010), neuroprotective effects (Ho et al., 2010). Especially polysaccharides, zeaxanthin dipalmitate, vitamins, betaine, and mixed extracts were reported to be responsible for anti-aging, improving eyesight, and anti-fatigue effects (Wu and Guo, 2015; Yao et al., 2017).

A protective property of goji extracts on retina cells has been proved in the early stage of the degeneration of the retina. It is proposed that absorbing the light zeaxanthin and luteolin present in goji fruit displays an inhibitory effect on neuron apoptosis (Ni et al., 2013), antioxidant and antimicrobial activities (Mocan et al., 2014).

In the present paper, we compared for the first time the morphology of the leaf and the flower and the anatomical structure of the leaf lamina of the wild and cultivated species. These studies highlight morpho-anatomical characteristics of the species. As the leaves are the plant organs with the highest plasticity and receptivity to changes in the environment, we focused on the leaf anatomy.

MATERIALS AND METHODS

The wild goji leaves and flowers were gathered form the spontaneous flora, from the Morii Lake shore (Ilfov county) and the cultivated goji leaves and flowers were taken from the experimental field of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania. From both sites, samples were collected during the flowering period, from August to October 2021. A collector number was given, and the specimens were dried according to standard herbarium methods. The specimens are kept in the herbarium of USAMV Bucharest. The leaves were sectioned by hand using razor blades to make semipermanent and permanent slides for microscopic studies. The sections were cleansed with chloral hydrate for 24 hours, then washed and stained with carmine alaunate and green iodine (Georgescu et al., 2015). Analyses and observations of the cross-sections were performed at the Research Center for Studies of Food Quality and Agricultural Products, at USAMV Bucharest. Photos were taken and measurements were made using the Leica DM1000 LED microscope, equipped with a Leica DFC295 video camera and with a Leica S8 APO Stereomicroscope, as well as a Sony photo camera. Photos were taken using the light microscope at different magnifications.

RESULTS AND DISSCUSIONS

Leaves morphology

Studies related to leaf morphology and micromorphology were made by Săvulescu et al., 2019: Luchian et al, 2019, 2020, 2021; Toma et al., 2021; Vârban et al., 2021. The

leaves of the studied shrubs were solitary or arranged in bundles 5-16 (rosettes) especially in the wild *L. barbarum* (Figure 1). The solitary leaves that had an alternating disposition predominated on the new shoots that emerged in the second half of the growing season. The wild goji had the leaves grouped in bundles of 5-16 leaves each, on the branches of previous years or at the base of the yearly shoots. The shape of the leaves was obovate (Figure 2), elliptical in wild goji and mainly lanceolate or elliptical and leathery in the cultivated goji (Figure 3). The wild plants had leaves with a blade of 5-9 cm long, 1.5-2.5 cm wide and the petiole 0.5-1.5 cm long, while the leaves of cultivated plants were 2.5-5.5 cm long, 0.4-2 cm wide and a petiole 0.3-0.4 cm long.

Figure 1. Obovate leaves arranged in bundles in the wild *Lycium barbarum*

Figure 2. Obovate leaves and flower of wild *Lycium barbarum*

Figure 3. Lanceolate leaves of cultivated *Lycium barbarum*

Flower morphology

Studies related to flower morphology were made by Anghelescu et al., 2021. On the analyzed goji shrubs, all the developmental stages of flowers and fruits were found simultaneously. The flowers of the shrubs appear in the nodes area, being solitary or in inflorescences of 3-5 flowers each. The flowers have a calyx with 2-3-4 sepals, pubescent (the calyx predominates with 2 sepals, sometimes a sepal is bent at the top), the corolla is rotated, having 5-6-7 petals (predominantly corolla flowers with 5 petals) of one-color, from lilac to an intense purple (Figures 4-7). The petals of wild goji have a corolla tube of 0.9 cm, and the lacini are 0.5-0.8 cm and 0.4-0.5 cm wide, the diameter of the open flower can reach 1.9-2 cm. In wild and cultivated goji, the five anthers open longitudinally, from the top to the base (Figures 8-9). In the wild species the anthers have filaments 1-1.4 cm long, and in the cultivated one the length of the filaments is 1-1.6 cm long. Towards the end of their developmental cycle, the flowers gradually lose their color and become beige.

Figure 4. Hairy calyx, with two sepals on wild *Lycium barbarum*

Figure 5. Two-lobed calyx on wild *Lycium barbarum*

Figure 6. Wild *Lycium barbarum* corolla with 5-6 petals

Figure 7. Corolla tube of a wild *Lycium barbarum* flower

Figure 8. Flower of cultivated *Lycium barbarum*

The corolla tube has hairs at the top, towards the lacins, both in the wild and cultivated *L. barbarum*. Also, the stamens filaments have hairs towards the base, in the form of a tuft, in both cultivated and wild goji (Figure 10). All goji flowers have darker stripes on the petals. The petals of wild goji have multicellular hairs, rarely arranged on the edge of the petal, and in the cultivated species multicellular hairs are observed, abundant (Figures 11-12).

Figure 9. Stamen's detail of anthers, with longitudinal opening

Figure 10. Hairs in the upper part of the corolla tube and at the base of the stem filaments on wild *L. barbarum*

Figure 11. Rare hairs on a wild *Lycium barbarum* petal

Leaves anatomy

Research on the anatomy of the genus Lycium has been done by Jobert et al., 1984; Norverto, 2000; Selvi et al., 2009; Tabără, 2020a; Konarska, 2018; Amanova & Duschanova, 2021.

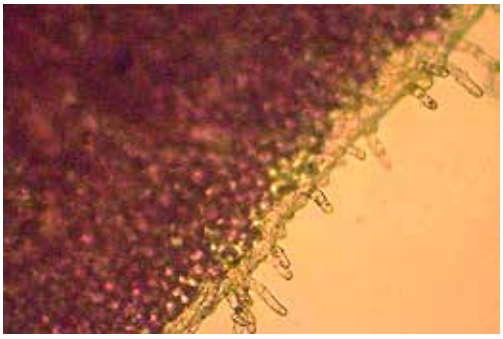

Figure 12. Dense hairs on a cultivates petal of *Lycium barbarum*

The anatomy of the leaves was studied in sections, on fresh or fixed preparations. On the transverse sections taken from the middle part of the leaf the following were observed: the epidermis is composed of a single layer of cells, and the cells are rectangular. The cells of the upper epidermis are larger than the lower epidermis ones, the walls of the cells are corrugated, and the epidermal cells are covered with a cuticle. Stomata are presented on both surfaces (amphistomatic leaves) and the stomata are more on the abaxial surface than on the adaxial surface. Stomata is generally present on both surfaces of the leaves, these findings also reported Metcalfe and Chalk, 1979.

Both epidermises are covered by the cuticle. In the wild goji, the upper epidermis was 11.3-11.5 µm, the cuticle 5.5-6 µm, and the lower epidermis 8.3-8.5 µm and the cuticle 5 µm. In cultivated goji, the upper epidermis was 15 µm and a cuticle of 5-5.9 µm, the lower epidermis was 6-8 µm and the cuticle of 4.8-5 µm.

The leaves of wild and cultivated goji possessed bifacial and amphistomatic structure with well-developed adaxial and abaxial epidermis (Figures 13-16). Upper epidermis of wild goji present elongated trichomes (Figure 17). The mesophile is arranged 1-2 layers of palisade on wild goji and 2-3 layers of palisade in cultivated goji (Figures 17-19). The shape of the palisade parenchyma in cross section is cylindrical. Over abaxial epidermis spongy parenchyma is disposed consisting of 2–4 layers circular or ovoidal cells. Adaxial phloem is absent from the smaller lateral veins. Middle vein on leaf is surrounded by parenchymatic cells, vascular bundles are bicollateral and

usually cells and upper and lower parts are accompanied by collenchyma (Figure 20). The thickness of wild goji leaf was between 150-172 µm, and in cultivated goji the leaf thickness was 450-470 µm.

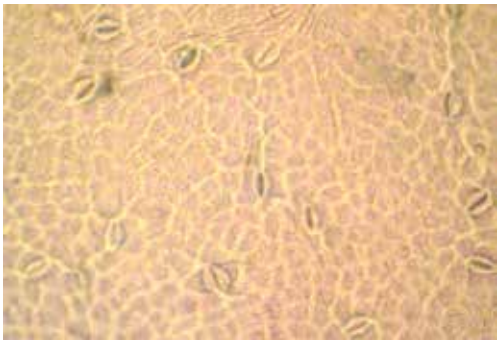

Figure 13. Upper epidermis in cultivated *Lycium barbarum*

Figure 14. Lower epidermis in cultivated *Lycium barbarum*

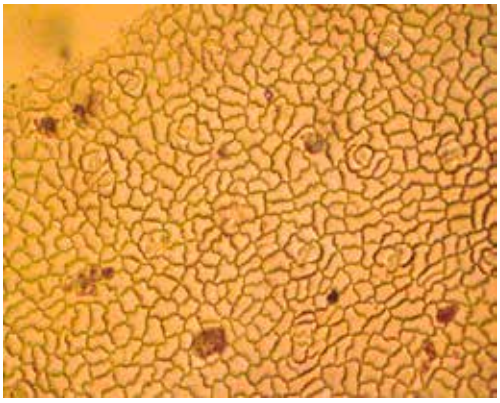

Figure 15. Upper epidermis in wild *Lycium barbarum*

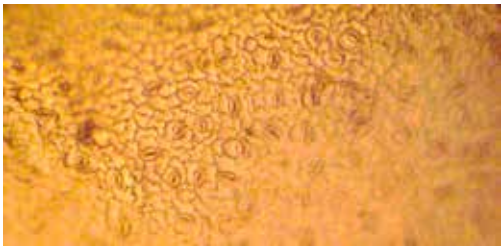

Figure 16. Lower epidermis on wild *Lycium barbarum*

Figure 17. Leaf-upper epidermis with trichomes, on wild *Lycium barbarum* leaf

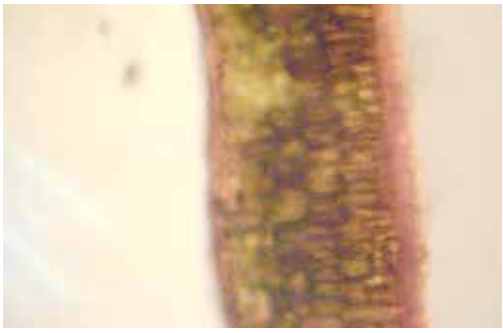

Figure 18. Cross section of a Lycium barbarum leaf - wild

Figure 19. Leaf anatomy on cutivated *Lycium barbarum*

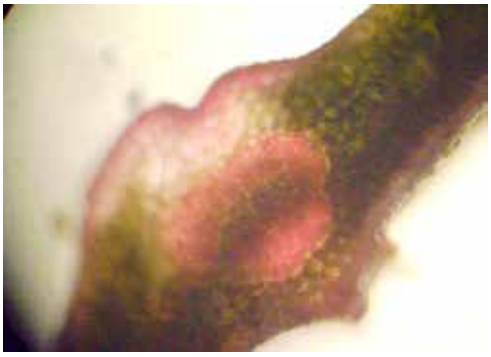

Figure 20. Midrib - bicollateral vascular bundle on wild *Lycium barbarum*

CONCLUSIONS

The anatomical studies carried out on the Lycium genus are just at the beginning, more aspects requiring in depth studies.

The micromorphological and anatomical characteristics are of great interest and significance to the discussion of the taxonomy of the species.

The petals of wild goji have multicellular hairs, rarely arranged on the edge of the petal, and in the cultivated species multicellular hairs are observed, abundant.

On the sampled wild goji, the upper epidermis is thinner than in cultivated goji and display elongated trichomes. The mesophile is arranged 1-2 layers of palisade on wild goji and 2-3 layers of palisade in cultivated goji.

The thickness of wild goji leaf was between 150-172 µm, and in cultivated goji the leaf thickness was 450-470 µm. These aspects will be further analyzed on multiple samples, collected from different places, to exclude the influence on climatic factor on the leaves.

The anatomical studies we performed on leaf cross-sections as well as on leaf surface sections demonstrate for the first time the anatomy of the vegetative organs of specimens growing wild in Romania. The microscopic observations made on leaves show structural-anatomical characters, with role in species adaptability and will be further analyzed on samples of *L. barbarum* collected from different places, to see the influence of climatic factors on the anatomical characteristics of leaves.

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