

SOME ASPECTS OF MORPHO-ANATOMICAL FEATURES OF THE INVASIVE SPECIES *Eleusine indica* (L.) Gaertn.

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Abstract

Eleusine indica (L.) Gaertn. – goose grass (Poaceae) (syn. *Cynosurus indicus* L., *Eleusine distans* Moench, *E. gracilis* Salisb.) is an invasive species in Romania as well as in several countries in Europe, Asia, Central and South America, the Caribbean and many Pacific islands. In our country this species is found in ruderal locations or alongside town roads. A single plant may produce more than 50,000 small seeds, which can be easily dispersed by wind and water. According to different sources, *Eleusine indica* (L.) Gaertn. is native to Africa. It is also considered a very extensive plant and often a difficult weed, drought-tolerant and herbicide-resistant. In 2018, *Eleusine indica* (L.) Gaertn. was studied at the Bucharest University of Agronomic Sciences and Veterinary Medicine. Our study targeted the roots, stems, leaves, inflorescence and caryopsis. Morphological and anatomic analyses were made on the plant material collected from ruderal locations in Bucharest, District 4 (Romania).

Key words: anatomy, *Eleusine indica*, morphology, vegetative organs.

INTRODUCTION

Eleusine indica (L.) Gaertn. - goose grass (Poaceae family), which grows in fields and open grounds and is found very often during the rainy season in its native areas. According to different sources, *Eleusine indica* (L.) Gaertn. is originally native to Africa. A single plant may produce more than 50,000 small seeds, which can be easily dispersed and viable seeds persist in the upper soil for only 2-5 years (Standifer, 1984; Rojas-Sandoval & Acevedo-Rodríguez, 2014). In our country it is an invasive species developing in ruderal locations. *Eleusine indica* (L.) Gaertn. is a worldwide weed of the tropics and one of the world's serious weedy grasses; at the same time, it has medicinal uses. In the last years there is a continuous enrichment of Romania's flora with new alien plant species (Anastasiu & Negrean, 2006; 2008; Anastasiu et al., 2009; 2011; Oprea & Sârbru, 2010; Sîrbu & Oprea, 2011). According to Anastasiu and Negrean (2005), Romania's alien flora represents some 13% of the country's total flora, which was

estimated by Ciocârlan (2009) to 3,335 species; after the year 2000 a number of 47 new alien plant species were recorded in Romania (Sîrbu & Oprea, 2011; Anastasiu & Negrean, 2011). This species was reported in Romania for the first time in Iași (Răvărut & Mititelu, 1960). In our country the presence of this species was also signalled in Crișana, northwestern Romania, (Negrean & Karacsonyi, 1984), Wallachia (Negrean & Constantin, 1999; Oprea et al., 2004), Galați (Sîrbu, 2011), Oltenia (Răduțoiu & Stan, 2013).

Eleusine indica is resistant to herbicides (Valverde et al., 1993; Baird, 1997; Doll, 2000; Jung et al., 2014; Mueller et al., 2011; Vargas et al., 2013; Jalaludin et al., 2010; An Jing et al., 2014; Cha Thye San, 2014; Penescu et al., 2018).

In the native regions *Eleusine indica* is used as a local medicinal plant with antioxidant (Al-Zubairi et al., 2011; Iqbal et al., 2012; Sagnia et al., 2014), antimicrobial (Sagnia et al., 2014) and anticancer (Al-Zubairi et al., 2011; Amarpreet; 2014; Hansakul; 2009) properties. The whole plant, especially the root, is

depurative, diuretic, anti inflammatory (De Melo, 2005; Sagnia et al., 2014), febrifuge, laxative and sudorific (Chopra et al. 1956; Nguyen et al., 1989; Nagathein, 1977), antiviral (Iberahim et al., 2016), used for liver disorders and convulsion as well as with antipyretic (Iqbal et al., 2011; Kirtikar & Basu, 1935; Pattanayak et al., 2017) properties. Anatomic studies were carried out by: Babu et al., 2014; Rubina et al., 2007; Hafliger and Schoz, 1981; Bibi et al., 2007; Metcalfe, 1961; Metcalfe and Clifford, 1968; Niteesh Kumar et al., 2017; Saw, 2011. Fahn (1965) studied the stomata of some Poaceae and Cyperaceae members. Numerous reports on foliar anatomy and micro morphology are used for delimiting the different groups or tribes in the Poaceae family (Brown, 1958; Ellis, 1987; Amarasinghe & Watson, 1990; Ahmad et al., 2011).

MATERIALS AND METHODS

The plant and the fresh material, roots, stems, leaves and inflorescence which were used for the macroscopic and micro-morphologic studies was collected in October-November 2017 from District 4 of Bucharest, Romania. For the microscopic studies, free hand sections of the fresh root, culm (internode), leaf and leaf-sheath of *Eleusine indica* (L.) Gaertn. were prepared using a razor blade. The present paper intends to illustrate the morphological features of the plant. Cross-sections performed in the botany laboratory were used to highlight anatomical characteristics. The sections were clarified with chloral hydrate for 24 hours and stained with alau carmine and iodine green suitable to optical microscopic techniques. Observations and images of the anatomical structures in root, stem and leaves were made with the optical microscope Leica DM1000 LED, a Leica DFC295 video camera and a Leica S8 APO stereo microscope. A Sony digital camera belonging to the microscopic and plant anatomy laboratory of the same research center was also used. Voucher specimens were deposited in the University of Agronomic Sciences and Veterinary Medicine of Bucharest's Herbarium. The taxonomic status of this plant was checked against different literature sources (Hafliger et al., 1981).

RESULTS AND DISCUSSIONS

Macroscopic characteristics

Morphological features of *Eleusine indica* harvested in Bucharest, District 4 (Romania). *Eleusine indica* (L.) Gaertn. (Figure 1) is an annual erect plant, with the culm having 15-40 cm in length, 0.6 mm in diameter, glabrous, branched in the upper part, not just at the base, the nodes 0.51-3.46 mm thick, the stem 3.5-6 mm in diameter with alternate linear leaves, basal leaves 25 cm long and the leaf-sheath 5.5 cm, medium leaves with lamina 20 cm long and the leaf-sheath 5 cm, and the last leaf below the inflorescence has a lamina 14.5 cm long and the leaf-sheath 8.5 cm; the leaf width is 0.7 mm for both basal and upper leaves. Leaf-sheaths are coriaceous on the adaxial side, glabrous on the abaxial side, with short-ciliate along the edges. In the area between the leaf-sheath and the lamina (at the base of the lamina) 3.1 mm long hairs (Figure 2) are observed, the ligula is short 0.5-1 mm and membranous.



Figure 1. Habitus

The stem has branches at the second and third node from the ground surface, both shoots being fertile, with 5.5 cm long inflorescences. In the case of some other specimens, from near the ground develops three shoots 5.9-17 cm in height. The stem shows some shoots from the last nodule under the inflorescence, and the leaf on this stem has a total length of 38 cm (30 cm the lamina and 8 cm the sheath).

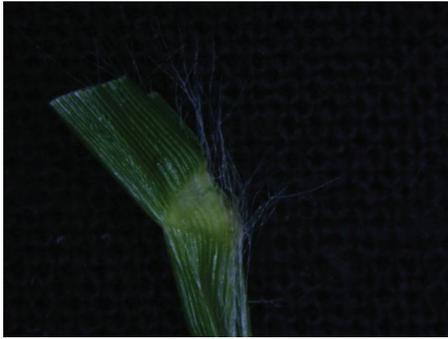


Figure 2. Leaf-sheath with hairs

The plants were harvested in October and November 2018 while some specimens were in the blooming phase and other individuals were in the fructification phase.

The inflorescence consists of 3-8 racemes, each 5-9 cm long, arranged more-or-less digitately, though one raceme may be inserted about 0,8-1 cm below the rest; the spikelets are elliptic, sessile, alternate on the rachis, laterally compressed, have 2-5 flowers and are arranged on two rows. The spikelets are 5.1-5.3 mm long and 2.1-2.6 mm wide (Figure 3).

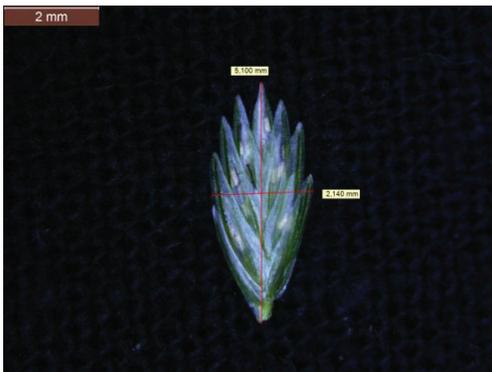


Figure 3. Spikelet

Glumes are shorter than the spikelet, unequal, the lower glume is small (1.1-3.0 mm) and 1-veined: the upper glume is longer than the lower, lanceolate, 2.1-4.1 mm long, 5-7 nerved, lemmas are broadly lanceolate, 2.3-3.1 mm long, membranous, 3-nerved, the palea is broadly lanceolate to narrowly ovate, 1.7-2.4 mm long. The flowers are bisexual with 3 stamens, short anthers, a glabrous ovary, 2 styles, free, with stigmas 0,8-0,9 mm long, plumose, reddish. Caryopses are ellipsoidal,

black and 1.04-1.8-2 mm long, conspicuously, embryo basal, nearly circular; the hilum is basal and punctiform. The anatomical analysis of the caryopsis was supplemented by observations under a scanning electron microscope (Figure 4).

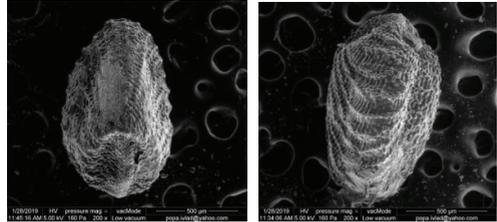


Figure 4. Caryopsis

The microscopic characters were studied as determined by Metcalfe and Chalk, 1950.

The branches of the stem (Figure 5) in the analyzed specimens are atypical because the literature states that branching is at the base of the plant (Nobis et al., 2011).



Figure 5. Stem branched

Microscopical characters

Root anatomy

Cross-section observations: the roots are about 1.5-1.8 mm in diameter; the epiblem with numerous unicellular root hairs; the hypodermis is one layered thick, parenchymatous, and the cells are polygonal in shape; the ground tissue is thin-walled, parenchymatous, consists of cortex, endodermis, pericycle, vascular bundle and

pith; the cortex consists of 6-7 layers, polygonal parenchymatous cells, with intercellular spaces; the endodermis is the innermost layer of the cortex and forms a definite 1-layered ring, the cells are parenchymatous; the pericycle layer is ring-like, one-layered; the vascular bundles are of the radial type, polyarch, xylem strands alternate with phloem strands, xylem and phloem are oval to round, the protoxylem occurring near the periphery and the metaxylem inwards; the parenchymatous cells in the centre is the pith.

Culm anatomy (Figure 6)

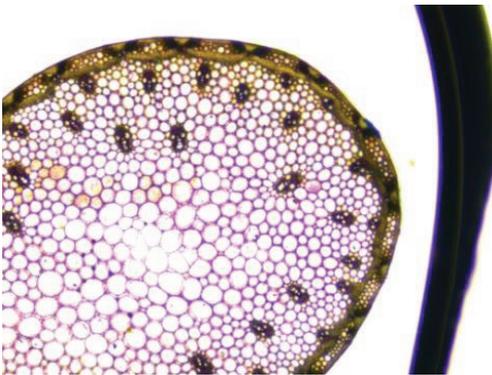


Figure 6. Stem cross-section

Cross-section observations: culms are about 3.5-6 mm in diameter, oval, with the cuticle layer thin, 0.49-0.5 μ thick; the epidermal cells are one-layered, rectangular in shape; the ground tissue consists of two types of cell, sclerenchymatous (4-9 layers, at the periphery) and parenchymatous (oval or rounded, intercellular spaces present); the vascular bundles are scattered in the center of culm, with smaller bundles near the periphery; the outline of the vascular bundles is oval or rounded, the bundle sheath is complete, sclerenchymatous, around each bundle; the vascular bundle presents xylem (vessel elements, fiber and xylem parenchyma) towards the centre and phloem (sieve tube elements, companion cells and phloem parenchyma) towards the epidermis (Figure 7).

The microscopical characters of the culm are: vascular bundle 44.8-75.20 μ m, parenchyma cells 11.5-57.5 μ m, vessel metaxylem 14.7-13.25 μ m.

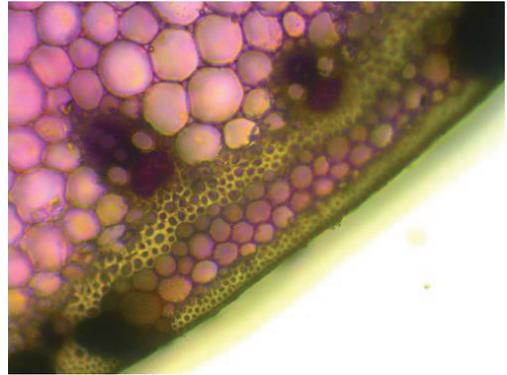


Figure 7. Stem cross-section (detail)

Leaf anatomy - lamina (Figure 8)

Anatomical analyses show that the leaves are amphistomatic with graminaceous type stomata, microhairs, macrohairs, collateral vascular bundles in the mezophyll with a Kranz structure.

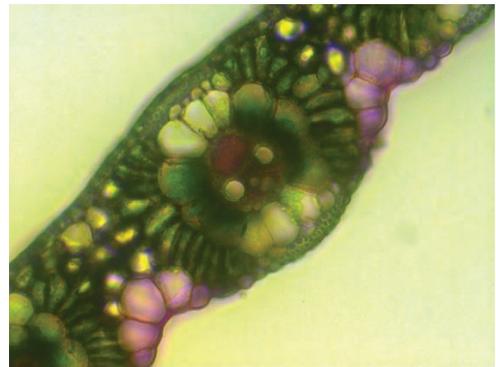


Figure 8. Lamina cross-section

The adaxial side features wide rounded ribs, separated by shallow V-shaped furrows.

The epidermal cells are arranged in parallel rows for both surfaces, with sinuous anticlinal walls; the cells are short and long. The short cells, located intercostally, are filled with silica bodies. The long cells, found between the veins, are elongated. The stomata are of the graminaceous type, present on the upper and lower epidermis. The upper and lower epidermis (Figures 9, 10) are one-layered with rectangular cells, with microhairs and macrohairs.

The bulliform cells (motor cells), with associated colorless cells, are present in the furrows of the upper epidermis; they have an oval or circular shape, are present in narrow

groups penetrating the mesophyll, between two consecutive vascular bundles. The vascular bundles are of the close collateral type. The chlorenchyma cells are radially arranged around the vascular bundles.

The vascular bundles feature sclerenchyma strands, on both the abaxial and adaxial sides. The xylem is found towards the upper side and the phloem towards the lower side. The xylem is composed of vessel elements, fibers and xylem parenchyma. The phloem is made up of sieve tube elements, companion cells and phloem parenchyma.

Adaxial intercostal zone: adaxial intercostal long cells with slightly sinuous walls, 6-9 rows of long cells between two costal zones, 1-2 stomatal rows between two costal zones; microhairs and macrohairs present.

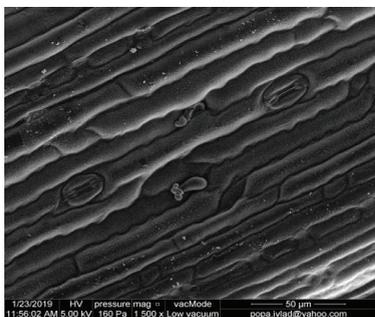


Figure 9. Upper epidermis with stomata and microhairs

Costal zone: saddle-shaped silica bodies, long cells with sinuous walls along with silica bodies. Abaxial intercostal zone: abaxial intercostal long cells with thin to moderately thick sinuous walls, 8-9 rows of long cells between two costal zones, 1-2 stomatal rows between two costal zones; microhairs present.

Costal zone: saddle-shaped silica bodies, long cells with sinuous walls along with silica bodies.

The results of cells' measurement in the leaf's cross-section: 10.65-13.49 μm thick upper epidermis, 0.5-0.6 μm thick cuticle, 135.79-231.7 μm thick mesophyll, 26.38-58.84 μm sclerenchyma sheath; 3.25-51.30 μm parenchyma cells, 10.01-10.35 μm thick lower epidermis, vascular bundles 78.8-80.7 μm . On the lower epidermis there are stomata 24.44-25.49 μm in length, long cells 163.7 μm in length and 15.97 μm in width, the short cells are 21.43-34.57 μm long and 8.10-8.18 μm wide.



Figure 10. Lower epidermis with stomata and microhairs

The present study revealed that micro-morphological features of the leaf surfaces have a considerable identification.

Friere et al., (2005) reported that *Eleusine indica* is characterized by pear-like microhairs. However, Bibi et al. (2007), Ahmad (2009), Ahmad et al. (2011) did not find microhairs but saddle-shaped silica bodies. The leaf's epidermal features can help elucidate taxonomic problems (Prat, 1936; Metcalfe, 1960; Ellis, 1979; Palmer & Tucker, 1981; Palmer et al., 1985; Davila & Clark 1990; Cai & Wang, 1994; Mejia-Saules & Bisbey 2003; Prat & Vignal, 1968; Sanchez, 1974). These leaf epidermal characters are of great value in grass systematics and characterization of broad groups within the grasses, particularly subfamilies and tribes.

According to Prat (1936), the types of microhairs and silica bodies are highly useful in systematic studies. The anatomical analysis of the leaves was supplemented by observations performed under a scanning electron microscope (Figures 11, 12).

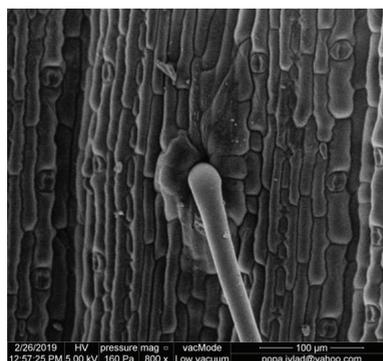


Figure 11. Upper epidermis with stomata and macrohairs



Figure 12. Upper epidermis and macrohairs

Midrib anatomy

The microscopic features of the midribs are as shown in Figure 13.

Cross-section observations: the cuticle is about 0.40μ thick; the epidermal cells are rectangular in shape; the parenchyma cells have a hexagonal to polygonal shape, 3-5 layers; the vascular bundles are of the closed collateral type, each being circular with outer and inner bundle sheaths.

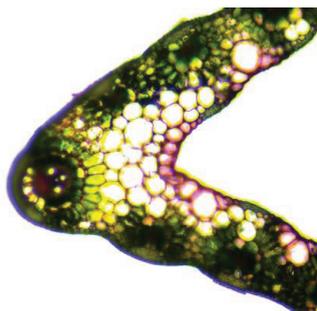


Figure 13. Midrib

The outer sheath is composed of parenchymatous cells, the inner sheath has sclerenchyma phloem lying towards the outer leaf side, the xylem is composed of vessel elements, fiber and xylem parenchyma. The phloem is composed of sieve tube elements, companion cells and phloem parenchyma.

Leaf-sheath anatomy

The cross-section shows that the cuticle is present on the epidermis of both leaf sides. The ground parenchymatous cells are arranged in 4-10 layers, compact, thin walled, hexagonal, variable in size. The vascular bundles are scattered, of the closed collateral type, oval to

circular, the bundle sheath is complete. The xylem is composed of vessel elements, fibers and xylem, while the phloem is made up of sieve tube elements, companion cells and phloem parenchyma. The microscopic characteristics of the leaf-sheath are shown in Figures 14 and 15.

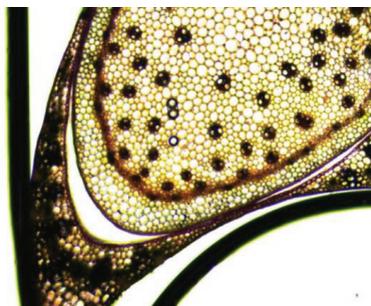


Figure 14. Cross-section in stem and leaf-sheaths

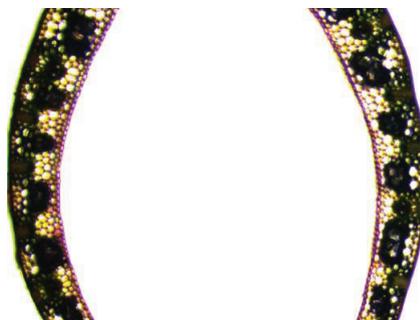


Figure 15. Cross-section of leaf-sheaths

CONCLUSIONS

This study is the first to report a detailed analysis of the morpho-anatomical features of *Eleusine indica* specimens growing in Romania.

The microscopic features (epidermis, stomata, cuticle, ornamentation and roughness) are in use since the beginning of the last century and information on foliar micromorphology can shed more light on structural features and their possible functional attributes.

We are of the opinion that the presently described micromorphological and anatomical characteristics will be quite useful for further studies and can be employed to obtain a key to set apart different species of the genera and different genera of the tribe.

The following characteristics were observed: the epidermis surface, the intercostal cells, the long and short cells, the orientation, the microhairs in both epidermis, the mesophyll, the vascular bundles. The distribution of the short cells' silica bodies along with their shape are bear significance for taxonomic purposes. It is the first time when macrohairs hair is seen in the leaf in the upper epidermis, as an adaptation to the conditions of our country.

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