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Anatomical and micromorphological analysis of root, stem and leaf of *Echinochloa crus-galli* (L.) Beauv.

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SUMMARY

The aim of this research was to investigate and characterise the anatomical structure of vegetative organs in *Echinochloa crus-galli* (L.) Beauv., commonly known as cockspur or barnyardgrass. *E. crus-galli* is a member of the Poaceae family and a common weed species which occures in many crops. For the purposes of this research, fully developed plants were collected from the experimental field of the Institute of Vegetable crops in Smederevska Palanka. Several methodes of microslide preparation were employed to investigate the anatomy of root, stem, and leaf. Cockspur has high water requirements and belongs to the hygrophilous weed species (F_4), which is anatomically reflected by the presence of large intercellular spaces (aerenchyma) present in the stem and root. Generally speaking, the anatomical characteristics of *E. crus-galli*, especially considering not only lignified endodermis, but also presence of lignified exodermis in roots, share similarities with anatomical characteristics of rice (*Oryza sativa* L.), in whose fields this species is considered one of the most troublesome weeds. The anatomical structure of the stem is typical of monocots, featuring a single-layered epidermis on the surface, a hypodermis composed of chlorenchyma and sclerenchyma, and collateral vascular bundles. The collateral vascular bundles were distributed in a pattern characteristic of the Poaceae family, and scattered throughout the stem's main body, surrounded by well-developed parenchyma tissue. The leaves are characterized by Kranz anatomy, which is typical of plants with C_4-type photosynthesis. The leaf surface is characterized by the presence of short, non-glandular trichomes on both the adaxial and abaxial leaf surfaces, while silica cells are located exclusively in the adaxial epidermis.

These morpho-anatomical characteristics could be an significant factor in the absorption of foliar-applied herbicides, making their understanding crucial for effective chemical control.
Keywords**:** cockspur, barnyardgrass, stomata, C_4 plant, silica cells.

INTRODUCTION

Echinochloa crus-galli (L.) Beauv. is one of the most troublesome weeds in rice fields worldwide, but it could also be present in many crops, such as vegetable and field crops. This species is an annual grass that overwinters in the form of seeds. The seeds could remain viable for up to 15 years (Maun and Barret, 1986), although germination and emergence do not occur every year, due to specific requirements for environmental conditions. Over the past years, even decades, due to global warming and climate change, this species has become more dominant in agricultural fields worldwide. In some countries and crops, this species has become an invasive weed species. *E. crus-galli* is an extremely competitive species in terms of competition for nutrients, light, water and other resources available for plant uptake (Khanh et al., 2007). Invasiveness of this species is based on its broad physiological tolerance, as it is a C_4 plant. In Serbia, Vrbnicanin et al. (2004) classified this species as an adventive invasive weed species, while Andjelkovic et al. (2014) conducted research around the area of the Pančevo moor and pointed out that this species became highly invasive. Additional reasons that may explain why *E. crus-galli* could become more dominant in Serbia as well include the rising ammount of nitrogen fertilizers used in early spring before planting, transplanting or even field cultivation, combined with irrigation (especially in vegetable crops) or heavy rainfall on poorly drained soils in spring. These conditions can serve as the impulse for germination and emergence.

According to the indicator values for plant classification by ecological factors given by Ellenberg (1974) *E. crus-galli* belongs to Eutropic category, with the index of 7.7. This places it closer to category 8—species that are pronounced indicators of nutrient-rich conditions—than to category 7, which includes species typically found at nutrient-rich sites more often than average ones, and only rarely at nutrient-poor sites(Vegetation Science Group and European Vegetation Survey, 2024). In terms of substrate humidity, this species is categorized in the mesic category, with an index between 5 and 6, as plants of habitats with mesic humidity (Vegetation Science Group and European Vegetation Survey, 2024). This species is also a C_4 plant, which means that it initially fixes carbon dioxide into four-carbon sugar compounds before entering the Calvin cycle, making its photosynthesis more efficient in hot, dry climates compared to C_3 plants (Hatch and Slack, 1966).

The aim of this study was to determine the anatomical structure of vegetative organes of *E. crus-galli* from a local population, with emphasis on leaf structure, as the main organ responsible for herbicide uptake and translocation.

MATERIAL AND METHODS

Plant material was sampled in September 2024 from the experimental field of the Institute for Vegetable crops Smederevska Palanka (N 44°21'25.0"; E 20°57'06.9"). Plant material contained 10 individual plants collected from the field during the vegetative phase of the life cycle.

Anatomical analysis. After thoroughly washing the plants to remove dirt and mud, the roots, stems, and leaves were carefully dissected using a razor blade. Thin sections of these plant parts were then placed onto microscope slides and prepared for microscopic observation. Two types of temporary microscope slides were prepared: 1) sections were observed without staining, simply mounted in a drop of water and covered with a cover slip, or 2) sections were histochemical stained using Phloroglucinol-HCl (Blaschek et al., 2020) for the localization of lignified tissues, or with Safranin and Astra Blue (Neto et al., 2019) to distinguish lignified and cellulose cell walls, as well as to stain chloroplasts and test for C4 photosynthesis in grasses.

Micromorphological analysis. To examine the distribution of stomata and the shape and arrangement of epidermal cells, epidermal peels were taken or epidermal impressions were made by using clear nail varnish and tape from both the adaxial and abaxial leaf surfaces.

All slides were observed using light microscope (LEICA DM2000) equipped with a digital camera (LEICA DFC320) connected to computer software (IM1000) for image capture and analysis. The preparation of misroslides, microscopy and image analysis were conducted at the Faculty of Agriculture University of Belgrade, in the Laboratory for functional anatomy of plants, within the Department of Agrobotany.

RESULTS AND DISCUSSION

Root. *E. crus-galli* posseses a fibrous root system, a characteristic typical of the Poaceae family (Metcalfe, 1960). In root cross-section, three zones are observable: the root epidermis (rhizodermis), the cortex and the central cylinder (stele) (Figure 1). The rhizodermis is composed of a single layer of cells that elongate to form root hairs. The outermost part of the cortex is composed of several layers ofcells known as exodermis, with lightly lignified cell walls. Part of the cortex below the exodermis forms a wide zone characterized by large intercellular spaces, which create the aerenchyma.

Previous research (Imaz et al., 2012) has also observed aerenchyma in the root systems of other *Echinochloa* species, particularly in plants grown under flooded conditions where the root system is exposed to hypoxia. A similar structure is also found in rice. Cortical aerenchyma could have a crucial role in radial water transport and in reducing root hydraulic conductivity. The root system is in relation with the natural habitat and represents a kind of defense mechanism during dry season to combat water scarcity. Under water-deficient conditions, the reduction of radial hydraulic conductivity helps conserve water in the surrounding soil, a phenomenon known as „water banking" (Feng et al., 2016). This reduction in hydraulic conductivity during water scarcity ensures that water is retained in the soil around the root zone, supporting continuous root growth throughout the growing season. The innermost part of the cortex consists of a single layer of endodermal cells with strongly lignified radial and inner tangential cell walls. Casparian strips formed in these cells act as efficient barriers to the apoplastic water transport pathway. In the central cylinder, adjacent to the endodermis, lies the pericycle, which is composed of a single layer of cells. Next to the perycicle is a polyarch radial vascular bundle.

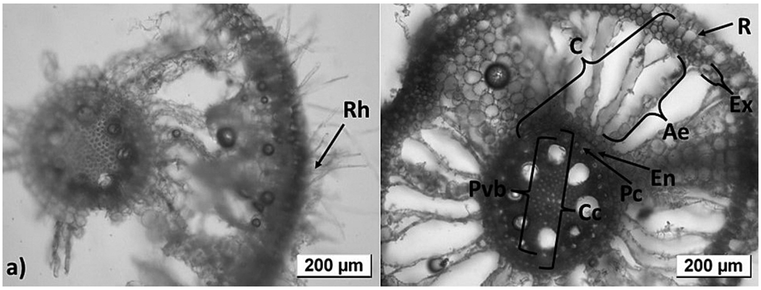

Figure 1. Cross-section of the root system unstained (a) and stained by Phloroglucinol-HCl (b): Rh = Root hair, R = root epidermis, Ex = exodermis, C = cortex, Ae = aerenchyma, En = endodermis, Pc = pericycle, Cc = central cortex, Pvb = polyarch vascular bundle.

Compared to unstained root sections, sections treated with Phloroglucinol-HCl exhibited red coloration, indicating the localization of lignin in the cell walls. In addition to the xylem and mechanical tissues located at the center of the root, lignification was also observed in the cell walls of both endodermal and exodermal cells (Figure 1). It is well established that drought-resistant genotypes exhibit increased deposition of suberin in the endodermis (Henri Henry et al., 2012), along with enhanced lignification (Hazman and Brown, 2018).

Elevated lignin content in the root system is typically associated with enhanced root strength and the ability to penetrate compacted soils (Chimungu et al., 2015). Lignification of the exodermal cells, on the other hand, is a form of adaptation to hypoxic conditions. The deposition of suberin and lignin in cell walls forms a barrier to apoplastic transport, preventing radial oxygen loss (Lux et al., 2004; Armstrong, 2013; Yamauchi et al., 2021), and minimising radial water loss under drought conditions (Peralta Ogorek et al., 2024).

According to Ejiri et al. (2021), radial oxygen loss prevents the loss of oxygen molecules during radial transport, effectively conserving oxygen and ensuring a steady supply to the root apex. In species that exhibit radial oxygen loss, oxygen is directed toward the root apex, where it supports serves essential functions: (i) respiration of the root apex, (ii) detoxification of toxic substances reduced in waterlogged soils, and (iii) preventing the influxof potentially toxic compounds from highly reduced soils.

Stem. The stem of *E. crus-galli* lies horizontally on the ground, with the base of the stem exhibiting a red-purple coloration (Figure 2).

Figure 2. *E. crus-galli* seedling.

In longitudinal section, the stem appears partially hollow, with transverse partitions at the internodal regions (Figure 3).

Figure 3. Longitudinal stem section of *E. crus-galli*.

The internodes of *E. crus-galli* are intermittently hollow, in contrast to other grasses, such as wheat (*Triticum aestivum* L.), whose stems are completely hollow except at the nodes (Hamman et al., 2005), or maize (*Zea mays* L.), whose stems are filled with parenchyma tissue throughout both the nodes and internodes (Ottesen et al., 2022).

Anatomically, the stem of *E. crus-galli* exhibits a typical grass-like structure. In cross-section, it is generally circular or oval, with three distinct layers visible: the epidermis, hypodermis, and ground tissue (Figure 4). The epidermis consists of a single layer of cells (Javelle et al., 2010) (Figure 4a). The hypodermis consists of both chlorenchyma and sclerenchyma (Figure 4d).

The assimilation tissue (chlorenchyma) is surrounded by a well-defined, discontinuous ring of mechanical tissue, 1-2 cells wide. The majority of the stem consists of ground tissue, composed of large, rounded parenchymal cells with thin walls. In the central cavities of the stem, patches of stellate parenchyma may also be present (Figure 4b). Closed collateral vascular bundles are located within the stem (Figure 4a-d), arranged from the hypodermis toward the central region. These vascular bundles are smaller in the peripheral parts of the stem, embedded in chlorenchyma or sclerenchyma, while those in the central part are larger (Figure 4a). Each vascular bundle comprises protophloem, metaphloem, protoxylem, and metaxylem, with a large protoxylem lacuna, and is usually encircled by a sclerenchymatous bundle sheath (Figure 4d). In certain cross-sections of the stem, the basal parts of leaves, still attached to the stem, may also be observed (Figure 4a, c).

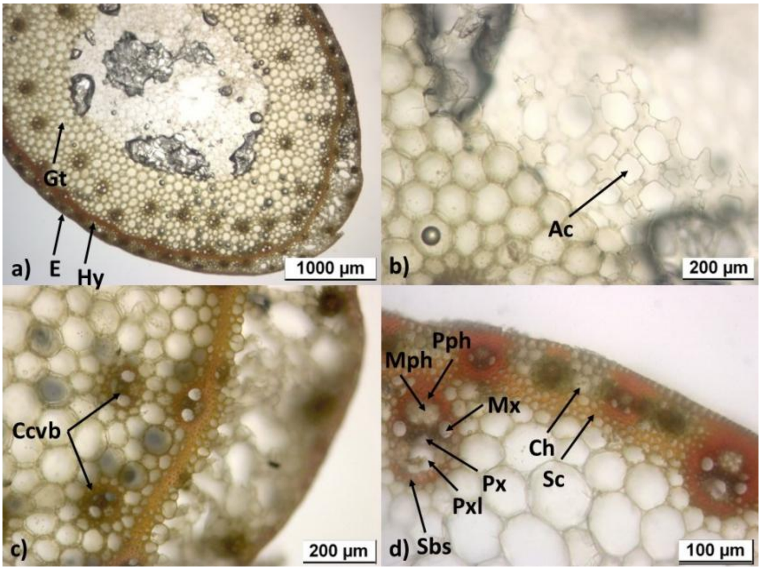

Figure 4. Stem cross-section of E. *crus-galli* with visible (a): E = epidermis, Hy = hypodermis, Gt = ground tissue, (b): Ac = air cavities, (c): Ccvb = colateral closed vascular bundle, (d): Ch = chlorenchyma, Sc = sclerenchyma, Pph = protophloem, Mph = metaphloem, Px = protoxylem, Mx = metaxylem, Pxl = protoxylem lacuna and Sbs = sclerenchymatous bundle sheath.

Leaves. The leaf blade is dorsoventral, with a single-layered epidermis on its surface (Figure 5a). The adaxial surface is typically smooth, with prominent ribs over the largest vascular bundles (Figure 5). A cuticle covers the outer surface of the epidermal cells (Figure 5a). Long cells bearing papillary trichomes are present in the epidermis, and stomata are found on both the adaxial and abaxial surfaces. The guard cells are bone-shaped and are surrounded by two triangular subsidiary cells (Figure 5b). Buliform cells in the epidermis are arranged in irregular clusters.

The chlorenchyma in the mesophyll is distinctly radiate and is not differentiated into palisade and spongy tissue (Figure 5a). The vascular bundles within the mesophyll consist of xylem and phloem elements. The xylem contains two large vessels positioned laterally within

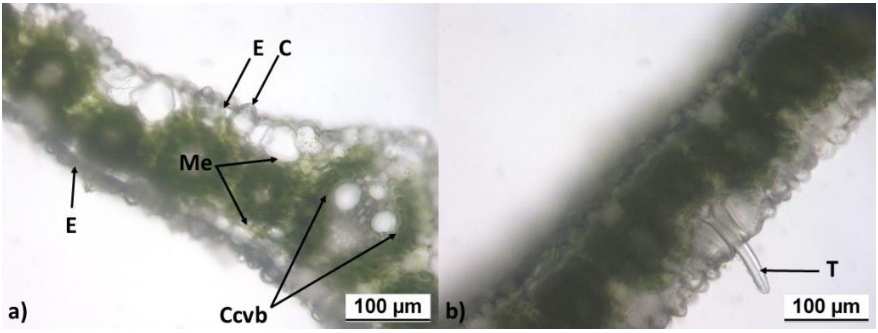

Figure 5. Leaf cross-section of *E. crus-galli* (a): E = epidermis, C = cuticle, Me = mesophyll, Ccvb = collateral closed vascular bundle, (b): T = trichome.

each bundle (Figure 6b), while the phloem is located below the vessels (Figure 6b). In leaf cross-sections stained with Phloroglucinol-HCl, cell walls rich in lignin are clearly visible, as they are stained red (Figure 6a). The vascular bundle sheath forms a single layer, completely encircling the vascular bundles. Most of the smaller vascular bundles lack sclerenchyma or have only slightly developed adaxial and abaxial strands. Conversely, larger vascular bundles have well-developed adaxial and abaxial strands.

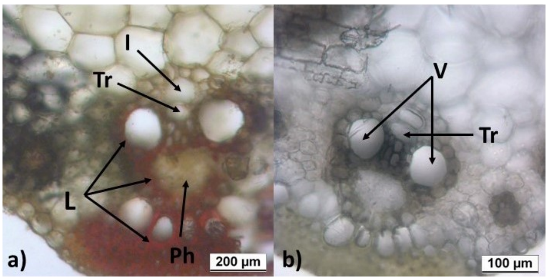

Figure 6. Leaf vascular bundle in Phloroglucinol-HCl a): I = intercellular, L = lignified cell walls, Ph = phloem, Tr = tracheids b): Tr = tracheids, V = vessel.

Echinochloa crus-galli belongs to the C_4 group of plants, in which the first compound formed during photosynthesis contains four carbon atoms (Lundgren et al., 2014; EHRAC, 2021). The leaves exhibit Kranz structure, characterized by a concentric radial arrangement of mesophyll and chloroplast-rich bundle sheath cells surrounding the vascular bundles (Botha et al., 1998) (Figure 7). Staining with Astra Blue and Safranin revealed that all chloroplast-rich cells surrounding the vascular bundles were stained red (Figure 7b). In contrast, in C_3 grasses, the first layer of the bundle sheath remains unstained when treated with the same histochemical dye combination.

According to Arber (1934) species belonging to Panicoideae subfamily have only parenchymatous bundle sheath surrounding the vascular bundles, while species from Pooideae

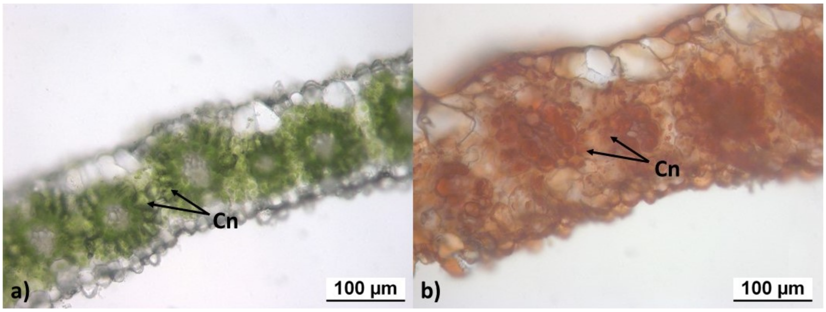

Figure 7. Leaf cross-section in water a) and leaf cross section in Astra blue and Safranin b): Cn = chloroplast-rich cells which surround the vascular bundle.

subfamily have a thick-walled mestome sheath. The unique leaf anatomy of C_4 plants, known as Kranz anatomy (German: Kranz „wreath") facilitates a more efficient photosynthetic pathway. In C_4 plants, the initial phase of photosynthesis, including carbon dioxide fixation, is separated from the rest of the photosynthesis process (Gowik and Westhoff, 2010). Compared to C_3 plants, C_4 plants are better adapted to dry conditions, high temperatures and low carbon dioxide availability. The vascular sheath cells are large and contain a special type of chloroplasts, known as agranal chloroplasts (Figure 7), where carbon dioxide fixation occurs. These sheath cells of the conductive bundle are surrounded by ordinary mesophyll cells, which contain normally structured chloroplasts, where the remainder of the photosynthesis process takes place. Esau (1965) considered the bundle sheath to function as an endodermis, and in some mestome sheaths, Casparian strips have been identified (van Fleet, 1950). The presence of a Casparian strip or extensive suberization in the bundle sheath serves to restrict the apoplastic transport of solutes.

Micromorphological studies revealed that the epidermis is predominantly composed of slightly elongated epidermal cells. Long cells between the veins have non-sinuous walls, while a few narrower and slightly longer cells feature sinuous walls. The *Gramineae* type of stomata (S) are arranged in rows, parallel to the lateral edges of the leaf. Rare nonglandular trichomes

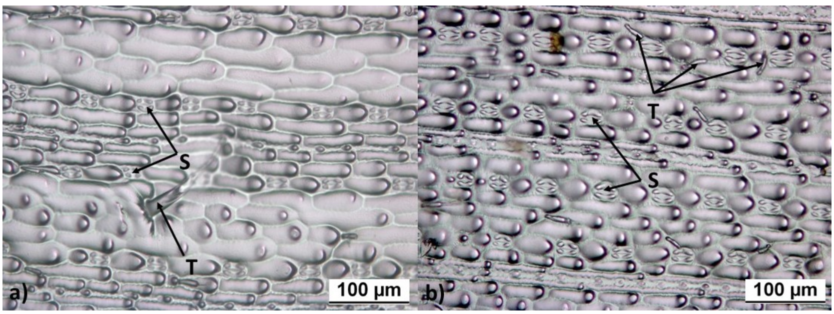

Figure 8. Epidermis of *E. crus-galli* on a) adaxial leaf side: S = stomata, T = trichome and b) abaxial: S = stomata, T = trichoma.

(T) are present on both the adaxial and abaxial surfaces (Figure 8), with greater abundance on the abaxial side (Figure 8). These trichomes are relatively uniform in diameter along their length. Oblique papillae with thickened tips are present on most long cells, with a single papilla arising from each cell.

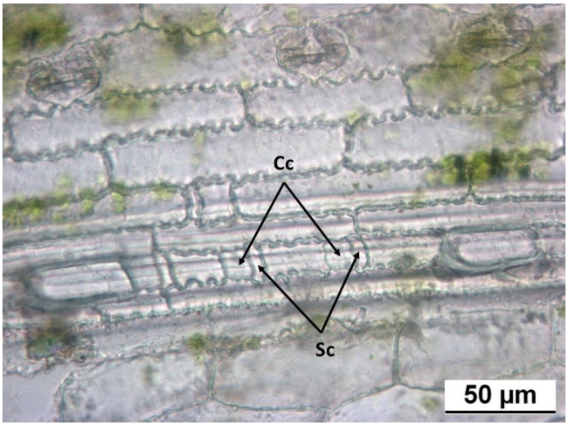

Figure 9. Epidermis on adaxial side: Sc = silica cells, Cc = cork cells.

In addition to the long cells elongated in the direction of leaf growth, short cells are also present in the epidermis. These short cells typically occur in pairs, with one cell having a suberinized cell wall (referred to as a cork cell) and the other containing a silicon dioxide crystal (referred to as a silica cell) (Figure 9). These short cells are positioned over the veins.

CONCLUSION

Weed characteristics, such as anatomy and micromorphology, can vary depending on multiple factors, including genetic potential and the effects of different stressors. However, weed species typically exhibit a remarkable ability to adapt and thrive in diverse environmental conditions as a survival mechanism. The anatomical and micromorphological analysis of *E. crus-galli* reveals several features critical for its adaptation to various habitats, such as soil water availability. Its root structure is particularly well-suited for both wet and dry conditions. Lignified exodermal root cells form a protective barrier that enhances water conservation and efficient utilization under dry conditions, while the well-developed aerenchyma facilitates adequate oxygenation in waterlogged soils. Visible intercellular spaces are present in both the root and stem, with the stem being partially hollow and featuring transverse partitions at the internodal regions. Leaves of *E. crus-galli* possess a Kranz structure around the vascular bundle in leaf tissues, since this grass weed species belongs to the C_4 type of plants, which implies that the photosynthesis is more intensive compared to C_3 plants. Furthermore, it could be speculated that silica cells present on the adaxial leaf side could lead to reduced herbicide uptake and efficacy. Since herbicides may be applied pre- or postemergence, they can be absorbed by the root, stem or leaves. The effectiveness of herbicides is often influenced by the anatomical and micromorphological characteristics of the leaves, as well as the biochemical processes occurring within each plant organ. As a key vegetative organ, the leaves, with their specific anatomical and micromorphological traits, play a crucial role in herbicide efficacy due to the interaction between the herbicide and the leaf surface. Therefore, the leaves were studied most intensively due to their variability and importance for species survival under stress conditions, including herbicide treatment. For example, *E. crus-galli* can develop *non-target site* resistance that reduce herbicide absorption and translocation to the site of action (Vrbničanin, 2020). Based on previous research and available data in our observations,future research should focuse on more detailed investigations and analysis of leaf surface characteristics, with specific emphasis on trichome, stomata and silica bodies, as leaf characteristics critical for herbicide efficacy.

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Anatomska i mikromorfološka analiza korena, stabla i lista vrste *Echinochloa crus-galli* (L.) Beauv.

REZIME

Cilj ovog istraživanja bio je da se ispita i okarakteriše anatomska građa vegetativnih organa vrste *Echinochloa crus-galli* (L.) Beauv., poznate kao korovsko proso ili koštan. Ova biljka pripada porodici Poaceae i predstavlja čestu korovsku vrstu koja se javlja na parcelama na kojima se gaje mnoge gajene biljke. Za potrebe ovog istraživanja, sakupljene su potpuno razvijene jedinke sa oglednog polja Instituta za povrtarstvo u Smederevskoj Palanci, a za anatomsko ispitivanje korena, stabla i listova primenjeno je nekoliko različitih metoda pripreme mikroskopskih preparata.

Korovsko proso ima visoke potrebe za vodom i pripada higrofilnim vrstama korova (F_4), što se u anatomskoj građi ogleda kroz dobro razvijen aerenhim, tj kroz prisustvo krupnih međućelijskih prostora u stablu i korenu. Generalno govoreći, u pogledu nekih anatomskih karakteristika, poput prisustva ne samo lignifikovanog endodrmisa već i lignifikovanog egzidermisa, *E. crus-galli* deli sličnosti sa anatomskom građom pirinča (*Oryza sativa* L.), na čijim poljima se ova vrsta smatra jednim od najproblematičnijih korova. Anatomska građa stabla je tipična za monokotile, sa jednoćelijskim epidermisom na površini, hipodermisom građenim od hlorenhima i sklerenhima, dok su kolateralni provodni snopići razbacani po najvećem delu stabla i okruženi dobro razvijenim parenhimskim tkivom. U anatomskom pogledu listovi se odlikuju tzv Krancovom anatomijom tipičnom za biljke sa C_4 tipom fotosinteze. Površina listova se odlikuje prisustvom kratkih nežlezdanih dlaka koje se javljaju se na obe površine lista, adaksijalnoj i abaksijalnoj, dok su ćelije sa kristalima silicijum-dioksida prisutne isključivo u adaksijalnom epidermisu. Navedene morfo-anatomske karakteristike mogu biti značajan faktor u apsorpciji folijarno primenjenih herbicida, pa bi razumevanje njihove uloge moglo biti od velike važnosti za uspešnu hemijsku kontrolu.

Ključne reči: korovsko proso, veliki muhar, stome, C_4 biljka, silicijumske ćelije.