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Anatomical characteristics of leaves of five Poaceae weeds in horticultural systems: *Axonopus compressus* (Sw.) P.Beauv., *Digitaria ciliaris* (Retz.) Koeler, *Digitaria sanguinalis* (L.) Scop., *Eleusine indica* (L.) Gaertn., and *Setaria plicata* (Lam.) T.Cooke

Khusnul Khotimah, Sri Darmanti* and Muhammad Luqman Hakim

*Biology Department, Science and Mathematics Faculty, Diponegoro University, Jl. Prof. Soedarto, SH. Street, Tembalang, Semarang, Central Java 50275, Indonesia. *E-mail: darmantisri@yahoo.co.id*

Abstract

Weeds are a group of plants which are undesirable because they can reduce the growth and productivity of horticultural crops. Weeds belonging to the Poaceae family, such as *Axonopus compressus* (Sw.) P.Beauv., *Digitaria ciliaris* (Retz.) Koeler, *D. sanguinalis* (L.) Scop., *Eleusine indica* (L.) Gaertn., and *Setaria plicata* (Lam.) T.Cooke, have been reported to disrupt various horticultural cultivated fields. Leaf anatomy is a crucial aspect that requires study because it is closely related to adaptation, taxonomy, evolution, and the ability of a plant species to interact with horticultural crops. This study examined the anatomical characteristics of the leaves of these five weed species. Exploration methods were used to obtain leaf samples. Cross-section preparations were made using a semi-permanent method with free-hand sectioning techniques. The parameters studied were the leaf anatomy of the five weed species. The results showed that the leaf anatomy of the five species consists of epidermal tissue, mesophyll, xylem, phloem, bundle sheath cells, and sclerenchyma. All five species belong to the C_4 group with Kranz leaf anatomy. Bulliform cells and stomata, as derivative epidermis, are found in all five weed species. Non-glandular trichomes were found in *D. sanguinalis* and *S. plicata*. The anatomical structure of the five weed species shows adaptations for efficient carbon use in dry environments. These findings support an integrated weed management approach by identifying key anatomical characteristics that can inform the development of weed control strategies in horticultural agricultural fields, such as efforts to enhance herbicide penetration efficiency.

Key words: Kranz anatomy, stomata, bundle sheath cell, C_4 plant, bulliform cell

Introduction

Weeds are one of the determinants of successful horticultural production (Murtilaksono *et al.*, 2023). Marfield (2022) defines weeds as plants or plant populations that grow at a specific time and place and can cause significant damage, either directly or in the long term, based on a comprehensive analysis. Weeds are the most commonly found group of organisms among all plant pests that attack agriculture. Weeds are reported as the primary cause of crop failure (34%), followed by insects (18%) and pathogens (16%) (Matloob *et al.*, 2020). Weeds are also commonly found in other locations, such as city parks (Üstüner, 2017) and flower gardens (Im *et al.*, 2017). Weeds compete with horticultural crops for nutrients, water, CO_2 and light. Weeds also have higher tolerance to various environmental conditions compared to other plants (Hutabarat *et al.*, 2021).

The Poaceae family is a common weed due to its rapid growth and high competitiveness (Kefi *et al.*, 2022). These weeds utilize the C_4 metabolic pathway, enabling them to grow under drought-stress conditions. The presence of these weeds creates competition with horticultural crops for limited growth factors such as sunlight, water, nutrients, O_2, and growing space, thereby causing disruption (Ngawit and Fauzi, 2021).

Weeds from the Poaceae family are one of the plant groups reported to cause significant disruption to horticultural crops. Murtilaksono *et al.* (2023) reported that *Axonopus compressus* (Sw.) P.Beauv., *Eleusine indica* (L.) Gaertn., and *Digitaria sanguinalis* (L.) Scop. from the Poaceae family are reported to be disturbing the horticultural land of mustard leaves, large chilli peppers, cayenne pepper, and tomatoes in West Tarakan, Indonesia. *D. sanguinalis* has also been reported to reduce potato productivity by 35-76% (Basinger *et al.*, 2019). *D. ciliaris* has been reported to interfere with tomato cultivation (Nurfitasari and Sebayang, 2025) and *Setaria plicata* (Lam.) T.Cooke has disrupted coffee cultivation areas (Definiati *et al.*, 2023).

Knowledge of a weed's leaf anatomy is a crucial aspect, especially when developing control strategies. Plant anatomy has been extensively studied because it is closely related to adaptation, taxonomy, and the evolution of plants. One important aspect of anatomy to study is leaf structure because of its role in photosynthesis, transpiration, and gas exchange (Harun *et al.*, 2022). Research by Muller *et al.* (2009) reported the role of leaf thickness and chloroplast arrangement in photosynthetic acclimation in *Aucuba japonica*. Research by Martínez-Sagarra *et al.* (2017) characterized the leaf anatomy of 68 plants from the Poaceae family, which can serve as a basis for species identification. Chatterjee *et al.* (2016) reported that the anatomical diversity of *Oryza sativa* leaves indicates genetic control over anatomical traits, with significant variations in mesophyll and vein structure reflecting evolutionary adaptation.

Several studies have investigated the leaf anatomy of the Poaceae family, including Aliscioni *et al.* (2016) on the leaf anatomy of

93 species of the *Setaria* Genus, Ali *et al.* (2022) on the leaf anatomy of four species from the Poaceae family in Iraq, and Rafique *et al.* (2021) compared the leaf anatomy of 22 species from the Poaceae family in Pakistan. However, research on the leaf anatomy of the Poaceae family, which has potential as weeds, is still limited. Therefore, this study aims to investigate the leaf anatomical structure of the weeds *A. compressus, D. ciliaris, D. sanguinalis, E. indica*, and *S. plicata*. This characterization can be utilized for biodiversity inventory purposes and to enhance scientific knowledge. This information, such as the location of stomata and the presence of trichomes, can also be considered in assessing herbicide penetration in weeds, allowing for informed planning of herbicide applications in integrated weed management.

Materials and methods

This study was conducted from January to June 2025. Leaf samples were collected from the Diponegoro University Education Reservoir in Semarang, Central Jawa Tengah, Indonesia. Preparation and microscopic observation were carried out at the Plant Structure and Function Biology Laboratory, Biology Study Program, Faculty of Science and Mathematics, Diponegoro University.

The equipment used in this study included slides, coverslips, a light microscope, OptiLab and OptiLab Viewer 4 software, a laptop, scalpels, beaker glasses, dropper pipettes, a luxmeter, a thermometer, an anemometer, and a hygrometer. The materials required for this study were leaf samples consisting of *A. compressus, D. ciliaris, D. sanguinalis, E. indica,* and *S. plicata* leaves. Other materials required included distilled water, 70% alcohol, safranin dye, and glycerin.

Preparation and identification of weed species: The weed species were selected as research objects through literature studies and direct observation at the research location. Weed species were identified using the plant identification key from Backer and Backuizen (1968) and confirmed through consultation with taxonomy experts.

Weed sample selection: The leaf samples studied were leaves from plants that were relatively uniform in size between replicates of each weed species. The samples selected were the third leaves counted from the fully opened leaves from the tip (Maricle *et al.*, 2009).

Preparation of leaf cross-sections: Cross-section leaf preparations were made using the third leaf in the middle of the leaf blade, including the leaf midrib. Three replicates were prepared for each weed species using plants from three different sources. One leaf was taken from each plant to make the preparation. Cross-section leaf preparations were made using a modified free-hand section technique (Sass, 1958). Leaf samples were taken and sliced as thinly as possible. Next, the cross-section leaf slices were soaked in 70% alcohol for 10 minutes to dissolve the chlorophyll. The slices were then soaked in safranin for 2 minutes, followed by three rinses with distilled water, each for 1 minute. The slices were placed on a slide, then dripped with glycerin and gently covered with a cover slip.

Anatomical observations on leaves and data analysis: Observations were made on cross-section leaf preparations using a light microscope connected to OptiLab and assisted by OptiLab Viewer 4 software. The preparations were observed under 10x magnification. The results of the observations, which consisted of the anatomical structure of the leaves, were analyzed descriptively.

Results and discussion

The cross-section of the leaves of *A. compressus, D. ciliaris, D. sanguinalis, E. indica,* and *S. plicata* from top to bottom consists of upper epidermal tissue, mesophyll, xylem and phloem that form vascular tissue, bundle sheath cells, sclerenchyma, and lower epidermal tissue (Fig. 1).

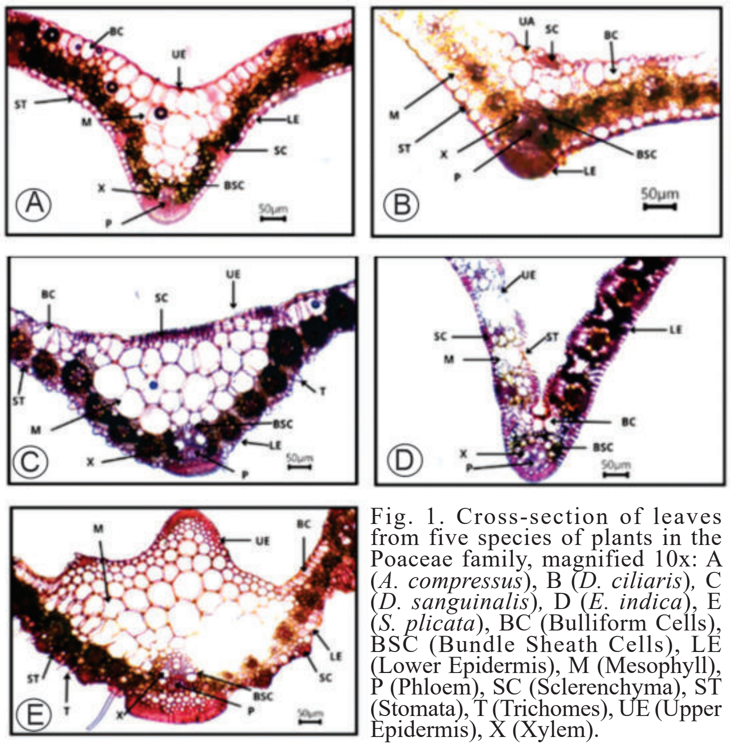

Fig. 1. Cross-section of leaves from five species of plants in the Poaceae family, magnified 10x: A (*A. compressus*), B (*D. ciliaris*), C (*D. sanguinalis*), D (*E. indica*), E (*S. plicata*), BC (Bulliform Cells), BSC (Bundle Sheath Cells), LE (Lower Epidermis), M (Mesophyll), P (Phloem), SC (Sclerenchyma), ST (Stomata), T (Trichomes), UE (Upper Epidermis), X (Xylem).

The epidermal tissue forms the outermost layer of the leaf, thereby protecting the tissues beneath it. This is consistent with Nadira *et al.* (2021), the epidermis consists of the upper epidermis (adaxial) and the lower epidermis (abaxial). Bulliform cells are one of the epidermal derivatives observed in the five plant species studied (Fig. 2). This is consistent with the findings of Rafique *et al.* (2021), who reported that all 22 species studied from the Poaceae family possess bulliform cells. These bulliform cells are located on the upper surface of the leaf, which can cause the leaf to curl when in a water-deficient condition, thereby reducing evaporation. This aligns with the opinion of Harun *et al.* (2022) that the modifications of the epidermis found in the Poaceae family, in the form of bulliform cells, play a role in reducing excessive evaporation and storing water.

Non-glandular trichomes found on *D. sanguinalis* and *S. plicata* are also epidermal derivatives. Trichomes are visible on both the upper and lower surfaces of the leaves, as shown in Fig. 3. This is consistent with the research by Jattisha and Sabu (2015), who reported the presence of hair-like trichomes on the leaf surface of *D. sanguinalis* and Aliscioni *et al.* (2016) on *Setaria* sp. These trichomes play a role in inhibiting evaporation. According to Amada *et al.* (2017), trichomes can enhance water use efficiency by slowing down air movement around the leaves, making it difficult for gases like water vapour and CO_2 to move. This helps leaves conserve water without significantly reducing photosynthesis, as water is more easily evaporated than CO_2 that is absorbed.

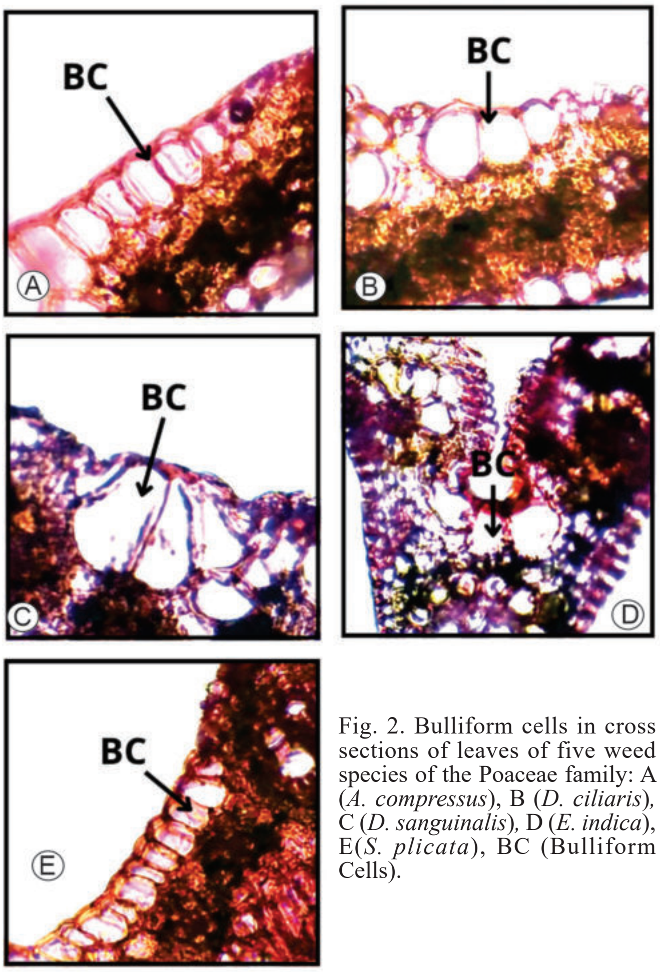

Fig. 2. Bulliform cells in cross sections of leaves of five weed species of the Poaceae family: A (*A. compressus*), B (*D. ciliaris*), C (*D. sanguinalis*), D (*E. indica*), E(*S. plicata*), BC (Bulliform Cells).

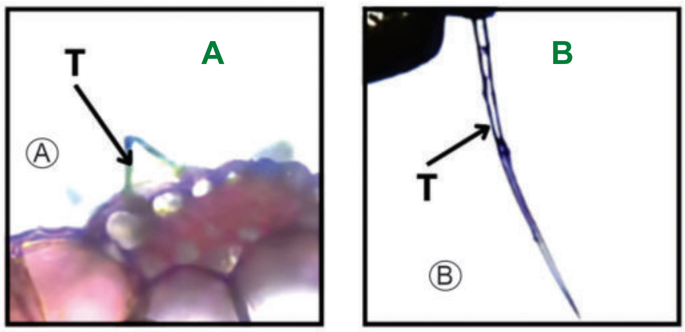

Fig. 3. Non-glandular trichomes on a cross-section of a leaf: A (*D. sanguinalis*), B (*S. plicata*), T (Trichome).

The stomata of *A. compressus, D. ciliaris, D. sanguinalis, E. indica,* and *S. plicata* are arranged in rows and parallel to the leaf veins. All five weed species have Graminoid-type stomata scattered across both surfaces of the leaves (amphistomatic). This type is a special form of the parasitic type, characterized by two halter-shaped guard cells flanked by two subsidiary cells. This is consistent with the research by Nitawaro *et al.* (2024), who reported that eight bamboo species from the Poaceae family have Graminoid-type stomata with halter-shaped guard cells and subsidiary cells that are parallel to each other. This finding is also consistent with the results of research on Graminoid stomata type by Rahman and Sultana (2021) on *E. indica*.

The mesophyll tissue is a parenchyma tissue that contains chlorophyll. This tissue is located between the upper and lower epidermis. In the five weed species studied, this tissue did not undergo differentiation to maintain flexibility and storage capacity. The mesophyll tissue is where carbon fixation occurs. This finding is consistent with the results of Liu *et al.* (2024), who studied 242 C_4 plant species and found no evidence of mesophyll differentiation in response to dry environments. These adaptations include the absence of mesophyll differentiation into palisade and spongy parenchyma.

The vascular tissues of the five plant species studied are conjoint collateral closed, consisting of xylem and phloem (Fig. 4). Xylem is composed of thickened cells and plays a role in the transport of water and minerals. Meanwhile, phloem is smaller than xylem and consists of living cells that play a role in the transport of photosynthetic products. This aligns with Rahman *et al.* (2022) that xylem consists of various types of cells, both living and dead. Its main components are tracheids and trachea elements, which function in transporting water and minerals from the roots to the leaves. Phloem also consists of various types of cells, but the sieve elements are a crucial component that transports photosynthetic products.

The anatomy of the leaves of *A. compressus, D. ciliaris, D. sanguinalis, E. indica,* and *S. plicata* shows the characteristics of C_4 plants with a distinctive Kranz anatomy. This structure is characterized by the presence of vascular tissue surrounded by bundle sheath cells and mesophyll tissue. This structure also shows a separation between the location of carbon fixation and the

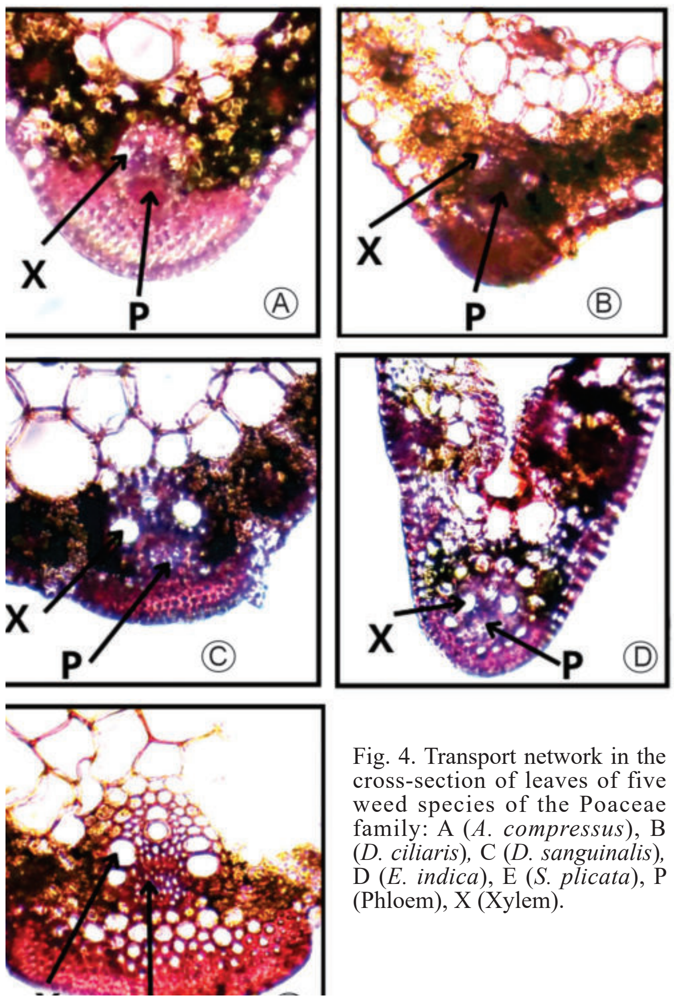

Fig. 4. Transport network in the cross-section of leaves of five weed species of the Poaceae family: A (*A. compressus*), B (*D. ciliaris*), C (*D. sanguinalis*), D (*E. indica*), E (*S. plicata*), P (Phloem), X (Xylem).

Calvin cycle. Carbon fixation occurs in the mesophyll, while the Calvin cycle occurs within the bundle sheath cells. This structure is important for protecting the vascular tissue and supporting the photosynthesis process. This aligns with the findings of Lundgren *et al.* (2019), who reported that *Alloteropsis semialataa* C4 plant, possesses a specialized leaf anatomy known as Kranz anatomy. This structure consists of two concentric layers of bundle sheath cells surrounded by mesophyll.

The spatial separation between initial carbon fixation in mesophyll cells and the Calvin cycle in bundle sheath cells can minimize photorespiration, a process that reduces photosynthetic efficiency commonly observed in C_3 plants. This aligns with the view of von Caemmerer and Furbank (2016) that the C_4 photosynthetic pathway is a CO_2 efficiency mechanism occurring in two types of cells. CO_2 from the atmosphere diffuses into mesophyll cells, where it is converted into HCO_3 by carbonic anhydrase. HCO_3 is then fixed by phosphoenolpyruvate carboxylase (PEPC) to produce a four-carbon acid that diffuses into the bundle sheath cells. This acid is decarboxylated to supply CO_2 to ribulose bisphosphate carboxylase oxygenase (Rubisco). This increases the partial pressure of CO_2 (pCO2) in the bundle sheath cells, enabling Rubisco to function optimally.

The presence of the Kranz anatomical structure in these five weed species indicates that they can adapt well to various environmental conditions, including dry areas (Maricle *et al.*, 2009). This adaptation allows them to grow faster and compete with other plants for resources, such as water, nutrients, and light (Matloob *et al.*, 2020). The size of the bundle sheath cells reflects the photosynthetic activity occurring within them. The larger the size of the bundle sheath cells, the higher the photosynthetic activity. This aligns with the research of Lundgren *et al.* (2019), which suggests that the leaves of C_4 plants can perform photosynthesis efficiently. This efficiency is supported by the large size of the bundle sheath cells, which can accommodate the necessary photosynthetic organelles. Additionally, the proximity between the mesophyll and the bundle sheath cells is necessary to enable rapid metabolite transfer.

The largest diameter of the bundle sheath cell was found in *E. indica* (9.47 µm). This can be attributed to the species adaptation to diverse environments. *E. indica* is known as a weed that is easily found in various soil conditions, ranging from dry to moist, and open to semi-shaded. This supports the relatively rapid growth rate of *E. indica*, enabling it to dominate the land in a short period. This is consistent with the research by Rafique *et al.* (2021), which reported that *E. indica* is one of several species of plants in the Poaceae family that have large bundle sheath cell sizes.

Sclerenchyma tissue was found in all five plant species observed. This finding is consistent with those of Aliscioni *et al.* (2016), who reported that 93 species of the *Setaria* genus possess sclerenchyma tissue. This tissue can be found on both the upper and lower surfaces of the leaf. Sclerenchyma tissue plays a role in providing strength and mechanical support to the leaf. According to Barclay (2015), sclerenchyma have thick, lignified secondary walls, which make them strong and water-resistant. Sclerenchyma tissue can be a vascular sheath in monocots. The vascular sheath on grass leaves can form a vascular sheath extensions when it extends to the epidermis.

An anatomical analysis of five economically important Poaceae weeds, such as *A. compressus, D. ciliaris, D. sanguinalis, E. indica,* and *S. plicata*, provides critical insights into horticultural production systems. The identification of Kranz anatomy, which features C_4 photosynthetic pathways and drought-adaptive structures, such as bulliform cells and trichomes, explains the competitive success of these weeds in horticultural fields and makes it difficult to eradicate. Weeds that are difficult to control can cause problems, such as herbicide resistance. Integrated weed management is needed to handle these issues by reducing competition between crops and weeds (Choudhary *et al.*, 2022). According to Chacko *et al.* (2021), integrated weed management is a systematic approach that combines various methods, such as cultural practices, mechanical methods, and herbicides, to control weed populations. One way to improve herbicide efficiency by increasing its penetration into weeds is to understand the type and location of stomata and the presence of trichomes. Santos *et al.* (2021) reported that stomata influence the entry of herbicides into plants. Herbicides can be applied to both surfaces of leaves on plants with amphistomatic stomata by evaluating the optimal opening time.

The species *A. compressus, D. ciliaris, D. sanguinalis, E. indica,* and *S. plicata* belong to the C_4 group, characterized by Kranz leaf anatomy, which features bundle sheath cells and mesophyll surrounding the vascular tissue. The bundle sheath cells serve as the site of the Calvin cycle. The presence of bulliform cells and Graminoid-type stomata in all five weed species contributes to efficient water use. This structure represents the adaptation of five weed species to dry conditions, particularly in terms of carbon efficiency. It explains the competitive success of these weeds in various horticultural fields, such as fruit and vegetable gardens. Therefore, integrated weed management is necessary to solve this problem. Anatomical structures, such as the location of stomata and the presence of trichomes, are important information for developing weed control strategies in horticultural crop protection.

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