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Calcium oxalate crystals in the stem of *Sida hermaphrodita* (L.) Rusby (*Malvaceae*)

Kryształy szczawianu wapnia w łodydze *Sida hermaphrodita* (L.) Rusby (*Malvaceae*)

SUMMARY

Observations of calcium oxalate crystals of the stem of an energetic plant *S. hermaphrodita* (L.) Rusby from the *Malvaceae* family, were performed using LM, DIC, and CLSM microscopes. The transversal and longitudinal sections showed the presence of calcium oxalate crystals in the parenchymal tissue distributed in various layers of the stem. The crystals occurred only in the form of druses. In the innermost part of the stem, i.e. in the pith, the calcium oxalate crystals occurred singly in individual cells. In the parenchyma cells separating sclerenchyma fibres and adjacent to the xylem, the crystals were observed individually in single cells, but the cells containing druses formed rows consisting of even several cells. The cortex contained the different-size druses scattered randomly within the cells. Druses differ in shape and size but they do not protrude beyond the cells although they very often fill them completely. The functions of calcium oxalate crystals are discussed.

Key words: calcium oxalate crystals, *Malvaceae*, *Sida hermaphrodita*, stem

STRESZCZENIE

Przy użyciu mikroskopu świetlnego z kontrastem Nomarskiego i konfokalnego, przeprowadzono obserwacje kryształów szczawianu wapnia w łodydze energetycznej rośliny *Sida hermaphrodita* (L.) Rusby z rodziny *Malvaceae.* Przekroje poprzeczne oraz podłużne wykazały obecność kryształów szczawianu wapnia w tkance parenchymatycznej znajdującej się w różnych warstwach łodygi. Kryształy występowały wyłącznie w postaci druzów. W najgłębszej części łodygi, tj. w rdze-

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Data: 28/11/2026 12:50:32

niu, kryształy szczawianu wapnia występowały pojedynczo w poszczególnych komórkach. W komórkach parenchymatycznych oddzielających włókna sklerenchymatyczne i przylegających do ksylemu, obserwowano pojedyncze kryształy w poszczególnych komórkach, a komórki zawierające druzy formowały rzędy składające się z kilku komórek. Kora zawierała różnej wielkości druzy rozmieszczone przypadkowo w komórkach. Druzy różniące się kształtem i wielkością często wypełniają całkowicie komórkę, ale nie wystają poza nią. W pracy dyskutowane są funkcje kryształów szczawianu wapnia.

Słowa kluczowe: *Sida hermaphrodita*, *Malvaceae,* kryształy szczawianu wapnia, łodyga

INTRODUCTION

The genus Sida includes about 200 species of annual plants, perennials, and shrubs. One of the species belonging to this genus, the Virginia mallow (*Sida hermaphrodita* (L.) Rusby), was introduced in Poland in the fifties of the last century. The first initiated studies were focused on *S. hermaphrodita* as a fibre plant. Subsequent studies expanded our understanding of the value of the use of this species. They demonstrated the possibility of using Sida as a fodder plant as well as for honey production, bioremediation, restoration, and as a pharmaceutical raw material. It also has a number of features ensuring potential use as pulp in paper industry. Apart from all these proposals of the use of the Virginia mallow, it carries a great promise to be a valuable energy plant (1, 7).

Calcium salts are a commonly occurring component of plant cells. This element is present in the ionic or bound form. In most plants, calcium is deposited in the form of calcium salts, usually as insoluble calcium oxalate crystals. It occurs as a mono- or dihydrate salt and rarely as a trihydrate. The monohydrate form is more stable and the most common in plants. Calcium oxalate has been identified in more than 215 plant families, including *Malvaceae* (11, 15). The crystals are formed in almost all parts of the plant: flowers (5), leaves (3, 6, 11, 16), stem (13), and roots (4). The amount of accumulated oxalates is dependent on the anatomical part of the plant. The largest amounts can be found in lower leaves, lower quantities in upper leaves, stems, and seeds, and the smallest amounts are present in the roots. Excess calcium accumulates predominantly in vacuoles and cisterns of the endoplasmic reticulum but also in the cell wall. Calcium oxalate crystals may be considerably smaller than the cells containing them, fill the cells completely, or cause perforations of their walls (12).

Cells in which crystals are formed may be arranged randomly within the tissue. Specialized secretory cells, so-called, idioblasts, often have a number of distinguishing characteristics. Among others, they can contain an increased cell nucleus, modified plastids, a highly developed ER and Golgi apparatus, increased levels of rRNA and a unique composition of the vacuole content. The cytoplasm of these cells is rich in various organelles, membranes, and vesicles. The cell wall is also modified, because it is irregular and much thinner in some areas. Mucus, a component of the cytoplasm swells, hence perforation and ejection of the crystals outside the cell occurs (3).

MATERIALS AND METHODS

The material comprised stems of *S. hermaphrodita* (L.) Rusby. The 1-cm thick stem fragments were taken at a height of 1.5 m and fixed in a mixture of ethanol and acetic acid (Carnoy's fixative). The used mixture was composed of 96% ethanol and acetic acid mixed at a ratio of 3:1. Then, the material was dehydrated in increasing concentrations of ethanol solutions. The material was soaked in a mixture of anhydrous ethanol and benzene. The final stage of the procedure consisted of embedding the material in paraffin for a few days (up to one week). The obtained paraffin blocks were cut on a rotary microtome into 9-micron thick sections. After removing the paraffin, the sections mounted on the glass slides and stained with safranin and fast green.

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The material fixed in Carnoy's mixture (without embedding in paraffin) was cut on the sledge microtome. The sections obtained were stained with safranin and fast green. The preparations were observed under light microscope (LM) and differential interference contrast *microscope* (DIC). For the observations under confocal laser scanning microscope (CLSM), the sections were stained with 1% eosin Y water solution.

The images were taken using Nikon Coolpix 4500 and Olympus DP72 cameras.

RESULTS AND DISCUSSION

Calcium oxalate crystals are characterized by a great diversity of morphology. They take such different shapes that they can constitute a useful diagnostic feature in the taxonomy in many unrelated groups of plants. Calcium oxalate most commonly occurs in the form of crystals forming different size and spatial shape groups. The basic morphological structures can be considered as crystal sand, raphides, styloids, prisms, and crystalline druses. Styloids are large sized, pillar-like crystals. They usually occur singly, and have blunt or sharp ends. They are very common in the cells and tissues of the representatives of the family *Iridaceae*. Raphides, which are characteristic of monocots, take the form of crystalline needles, which are usually gathered in single bundles. Typical bundles reach a considerable size and they may be grooved. Prismatic crystals are short, irregular prisms. Druses are spherical, stellar clusters and polymorphic aggregates of crystals. This form of calcium oxalate is often found in dicots. Druses are very common in young shoots in the rays of the core of many trees. Crystalline sand is tiny crystals loosely scattered in cells. This form of calcium oxalate can be found in plants of the *Solanacaeae* family, such as the potato (*Solanum tuberosum*) or Deadly Nightshade (*Atropa belladonna*) (3, 5, 6).

Calcium oxalate crystals were observed in the stem of the energetic plant *Sida hermaphrodita* (Figs. 1 and 2). The stem of *S. hermaphrodita* was sectioned transversely at a height of 1.5 metres. The following layers were clearly recognizable in the stem: the epidermis, primary cortex, xylem, and pith (Fig. 3). In the innermost portion of the stem, the pith is composed of different-size parenchymal cells, which very often contain calcium oxalate crystals (Figs. 4 and 5). In this part of the *S. hermaphrodita* stem, the calcium oxalate crystals occurred singly in individual cells. In turn, in the parenchyma cells separating sclerenchyma fibres, the crystals were also observed individually in single cells, but in this case, the cells containing druses formed rows consisting of even several cells (Fig. 6). In the described tissue, it is possible to notice extremely regular arrangement of cells containing the calcium oxalate crystals. On the other hand, in the cortex, different-size druses were scattered randomly within this parenchymal tissue (Fig. 7). The longitudinal section of the stem of *S. hermaphrodita* showed the presence of druses in the series of parenchyma cells adjacent to the tracheary ele-

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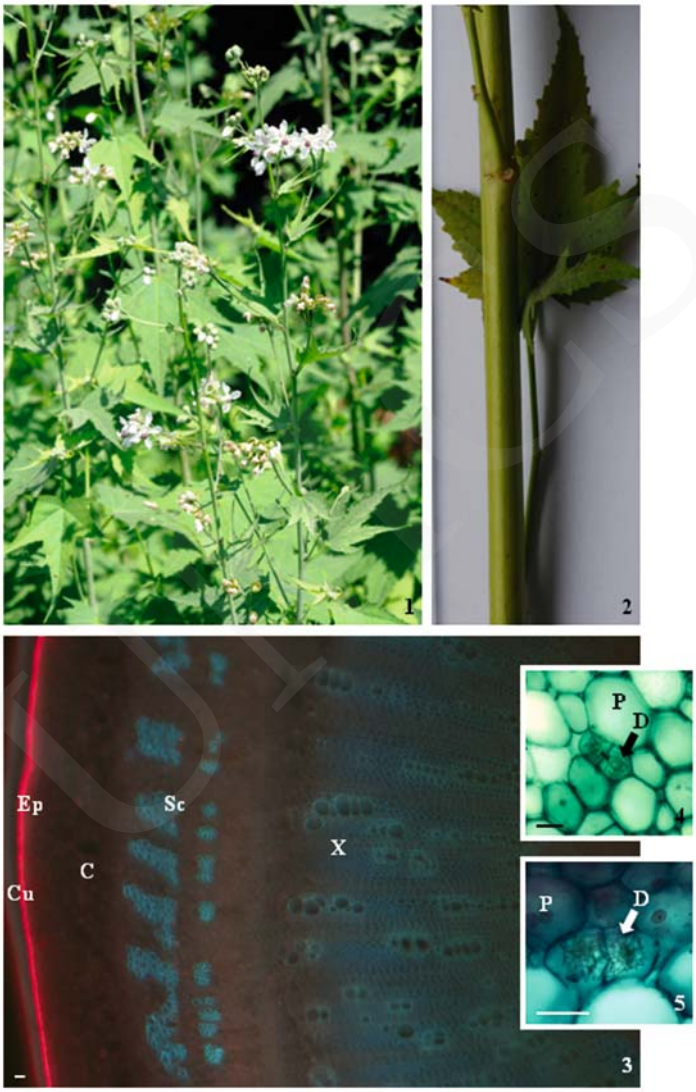

Tab. I

Fig. 1. *Sida hermaphrodita* (L.) Rusby. Plants growing in an experimental field of the University of Life Sciences in Lublin.

Fig. 2. Fragment of the green stem of *S. hermaphrodita* with a thickness of 1 cm.

Figs. 3–5. Cross section of the stem of *S. hermaphrodita*. The figure shows three typical layers: epidermis (Ep) with the cuticle (Cu), cortex (C), sclerenchyma fibres (Sc) and vascular tissue with fluorescent xylem (X). CLSM. Figs. 4 and 5 show diffuse parenchyma (P) and cluster crystals of calcium oxalate (D). Fig. 4. LM. Fig. 5. DIC. Scale bar = 20 µm

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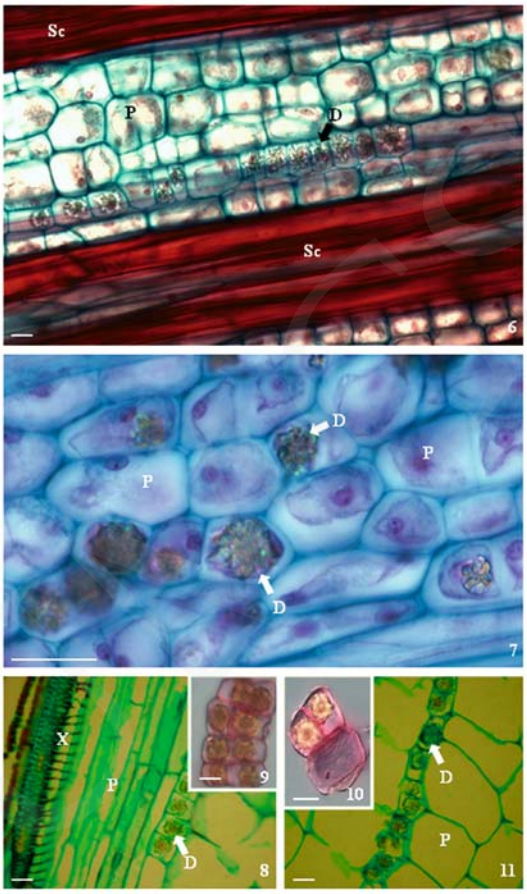

Tab. II

The stem of *S. hermaphrodita*.

Fig. 6. Tangential section. Fragments of the stem showing sclerenchyma fibres (Sc) and parenchyma cells (P) with druses (D). Staining with safranin and fast green. DIC. Scale bar = 20 µm

Fig. 7. Cross section. The anatomical structure of a fragment of the cortex. The figure shows various sizes of parenchyma cells (P) containing multifarious groups of calcium oxalate cells (D). Staining with safranin and fast green. DIC. Scale bar = 20 µm

Fig. 8. The stem of *S. hermaphrodita* – longitudinal section. The fragment shows xylem elements with visible helical thickenings (X) and parenchyma cells (P) with series of druses (D). The elongated parenchyma cells on the left from the row containing druses started to differentiate into another kind of tissue. Staining with safranin and fast green. LM. Scale bar = 20 µm

Figs. 9 and 10. Longitudinal section. Druses in the cells arranged in rows and in the neighbouring cells after mitotic cell division. Staining with safranin and fast green. LM (Fig. 9) and DIC (Fig. 10). Scale bar = 20 µm

Fig. 11. The stem of *S. hermaphrodita* – cross section. Fragment shows parenchyma cells (P) with a row of cells containing druses (D). Staining with safranin and fast green. LM. Scale bar = 20 µm

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ments of the xylem (Fig. 8). In this parenchymal part of the stem, calcium oxalate crystals were sporadically present in the cells arranged in two rows of cells (Figs. 9 and 10). The cross section of the described parenchymal tissue revealed long rows of cells containing druses, smaller than neighbouring cells (Fig. 11).

Observations of the cross-section of the *S. hermaphrodita* stems indicated the presence of calcium oxalate crystals in the parenchymal tissue. The crystals take the form of druses, which are irregular clumps and aggregates of smaller crystals. Druses are present in various layers of the *S. hermaphrodita* stem. They are present in a crumb structure associated with the vascular bundles, as well as in cells of the parenchymal cortex. Most of the druses are present in groups of cells arranged linearly along the xylem fibres. Druses differ in shape and size but they do not protrude beyond the cells although they very often fill them completely. However, the crystals in the *S. hermaphrodita* stem are usually located in cells with slightly smaller dimensions. As mentioned above, druses can fill the entire cell but their sizes are clearly differentiated. In the *S. hermaphrodita* stem, small crystals (about 4 microns) and those with a considerable size of 15–20 microns were observed. In order to describe special features, and to specify the precise type and localization of calcium oxalate crystals further studies are required. In the cells of the *S. hermaphrodita* stem, crystals of calcium oxalate were only observed in the form of druses and never in the other forms of calcium oxalate.

The size, location, and other properties of the calcium oxalate crystals in plants are the result of physical, chemical, and biological factors, such as temperature, light intensity, pressure, pH, and ion concentration (10). Crystals form from endogenously synthesized oxalic acid and calcium charged from the environment. Since the main source of oxalic acid is ascorbic acid, many studies have shown that the formation of CaOx (calcium oxalate crystals) is related to the mechanism for regulating the level of free calcium in cells. It has been shown that there is a relationship between the level of free calcium and the amount and size of crystals. As the amount of metal ions in the cell increases, the number of generated crystals also increases. However, the presence of calcium oxalate, its morphological form, and the distribution is controlled genetically. The formation of crystals is not a simple process of salt precipitation. The formation of these structures is a combination of genetic and environmental factors (8).

The prevalence and diversity of calcium oxalate occurring in plants tend to define its biological role. These salts may play an important role in many vital functions of the cell. They are a reservoir of excess calcium in the cell and are involved in the regulation of its level. CaOx crystals play a defensive function in deterring animals that feed on plants. The characteristic sharp fragments of crystals are an effective, passive defence against preying insects (5). Research conducted

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Data: 28/11/2026 12:50:32

on the stem of *Pistia stratiotes* provides information on active defence against herbivores and allelopathic activity (14). Calcium oxalate crystals also act as helpers in detoxifying heavy metals. Experimental analysis of the chemical composition of these structures present in the cells of *Malva parviflora* proved that the crystals contain dopant ions of aluminium, cadmium, and lead. The investigations also showed that the presence of heavy metals affects the biological mechanism of the formation of the crystals. Based on experiments investigating the presence of crystals in the leaf tissues of *Phaseolus vulgaris*, it was hypothesized that the emergence of heavy metals reduces the formation of these structures in cells (16). Calcium oxalate crystals may possibly have an impact on photosynthesis. This is due to the spatial structure and the possibility of reflection of light in different directions. This can result in low light levels reaching the deepest underlying cells. Research concerning druses located in the palisade parenchyma of *Peperomia glabella* leaf confirmed this hypothesis. It undoubtedly results from the studies showing that there is a relationship between the position and the number of crystals and the intensity of light (9). The durable crystal structure of CaOx reinforces cell walls. This may be evidenced by the typical location of these structures in the cells of the epidermis of different plants, strengthening the cells forming the vascular layer covering the seed (2). The calcium oxalate crystals in the stem of *Sida hermaphrodita* have similar functions like in the other groups of plants. Virginia mallow has a significant height so the presence of crystals has an important role for strengthening of the stem.

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