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ORIGINAL RESEARCH PAPER

Anatomical and morphological features of seedlings of some Cactoideae Eaton (Cactaceae Juss.) species

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Abstract

Three-month-old seedlings of 11 species of the subfamily Cactoideae (*Melocactus bahiensis*, *Melocactus curvispinus*, *Echinopsis eyriesii*, *E. mirablis*, *E. peruviana*, *Oreocereus celsianus*, *Rebutia flavistyla*, *Rebutia minuscula*, *Astrophytum myriostigma*, *Mamillaria columbiana*, and *M. prolifera*) have been studied. These plants exhibit a uniseriate epidermis, covered by a thin cuticle. Except for *E. peruviana* and *A. myriostigma*, no hypodermis could be detected. The shoots of all studied specimens consist mainly of cortex parenchyma with large thin-walled cells. The pith parenchyma is composed of much smaller cells. Due to the fact that the cortex parenchyma comprises the largest portion of the cross-sectional area, it can be concluded that it is the main water-storing tissue. The extent of vascular tissue development varies. Collateral vascular bundles are present in the stele. The studied seedlings contain various ergastic substances, in particular inclusions of calcium oxalate (all studied species), starch (*Mammillaria prolifera*, *E. mirabilis*, and the genus *Melocactus*), inulin-like inclusions, and occasionally lipid drops (some *Echinopsis* species).

Thus, it was found that all studied plants have a highly specialized anatomical and morphological structure. At the same time, the epidermis and hypodermis are poorly developed. Accordingly, the adaptation to arid conditions of the examined seedlings involves an increased growth of the water-storing tissue and the production of ergastic substances.

Keywords

Cactoideae; seedlings; stem anatomy; morphology

Introduction

Cactaceae is a specialized family, whose members are perennial stem succulents. According to the acknowledged classification, this family includes four subfamilies, 130 genera and 1870 species [1]. Due to their ecological and biological characteristics, Cactaceae plants are sensitive to environmental changes [2]. Today, more than 1400 species of the family are entered into The IUCN Red List of Threatened Species [3].

Representatives of the largest subfamily, Cactoideae Eaton, are the most specialized succulents among four Cactaceae subfamilies. Their characteristic morphological features are the complete reduction of leaves, the presence of areoles, and the green succulent stem. The specific morphological characteristics of this subfamily occur already in the early stages of ontogeny [2,4]. Therefore, Cactoideae seedlings possess a thickened succulent hypocotyl with a conical or spherical shape and diversely formed cotyledons: subulate, triangular, in the form of small tubercles, or even completely reduced [5,6].

Most anatomical studies concerning the subfamily Cactoideae are focused on adult plants [7–11]. According to Terrazas and Arias [10], about 350 species of the subfamily have already been investigated.

Most studies of the early stages of Cactoideae ontogeny concern their morphology, germination characteristics, and environmental factors that influence the germination [12–14]. The first investigations on the anatomical structure of cactus seedlings were carried out by Fraine [5] who reviewed the structure of the members from eight genera. Mauseth [15] described the anatomy and development of seedling shoot apical meristems of 27 species from 17 genera, but he did not pay attention to seedling organization. The structures of members of the genera *Cephalocereus*, *Cereus* [11], *Stenocereus* [16,17], *Pachycereus* [18], epiphytic cacti of the genera *Lepismium* and *Rhipsalis* [19], *Epiphyllum* [20] as well as changes in the anatomical and morphological structures of *Echinocactus* and *Polaskia* influenced by water deficit [21] were investigated later. Although studies of the anatomical structure of Cactoideae seedlings started in the early twentieth century, the number of investigated taxa is very limited. It is a well-known fact that the anatomical, morphological, and physiological characteristics of seedlings and young plants play an important role in the plant's ability to adapt to environmental conditions [17]. Mayr [22] stressed that each stage of ontogeny has structural and physiological characteristics that are essential for the adaptation of organisms during the stage of development considered. Accordingly, the degree of adaptation to unstable environmental conditions is various at different stages of the existence [23]. Thus, it is necessary to identify the specificity of adaptation at various stages of ontogeny in order to determine the adaptive features of organisms [24].

The aim of our work is to study the anatomical and morphological structures of the stems of Cactoideae seedlings and to define their adaptation features at the early stages of development.

Material and methods

The investigations were carried out on 3-month-old seedlings of 11 species from three tribes of Cactoideae: Cereeae [*Melocactus bahiensis* (Britton & Rose) Luetzelb., *Melocactus curvispinus* Pfeiff.], Trichocereeae [*Echinopsis eyriesii* Pfeiff. & Otto, *Echinopsis mirablis* Speg., *Echinopsis peruviana* (Britton & Rose) Friedrich & G. D. Rowley, *Oreocereus celsianus* (Salm-Dyck) A. Berger ex Riccob, *Rebutia flavistyla* F. Ritter., *Rebutia minuscula* K. Schum.), and Cacteae [*Astrophytum myriostigma* Lem., *Mamillaria columbiana* (Werdermann ex Backeberg) Dugand, *Mamillaria prolifera* (Mill.) Haw.]. Representatives of most of the species studied here grow naturally on stony or sandy soil in arid and semiarid regions of Central and South America (deserts, caatinga, campo rupestre, pampa, puna, etc.). Only the *Rebutia* species grows on rocky outcrops in yunga forest (*R. minuscula*) or near rivers and riverbeds (*R. flavistyla*).

Seedlings were grown from seeds under greenhouse conditions in the O. V. Fomin Botanical Garden. The conditions in the greenhouse were designed to resemble natural ones. The dry period with low air temperature (min 12–15°C) and short day (min 8 hours) from October to April is followed by the damp period with air temperature up to 45°C (from April to October) and day length up to 16.5 hours. The germination substrate consisted of sand (two parts), soil (one part), and peat (one part).

For the investigations, five typical seedlings of each species were selected. Until sectioning, the plants were fixed and stored in 70% ethanol during 5 to 7 days. The cross-sections were made by hand from the middle part of the stem (Fig. 1), and next stained with safranin, Lugol's reagent (to detect starch), or methylene blue (to detect mucilage) [25]. The observations were carried out by means of a XSP-146TR light microscope, equipped with a PowerShot A630 (Canon) digital camera and controlled with image capture software. The measurement was performed using ImageJ image processing program. Statistical analysis of the results was performed using the software package Statistica 8.0 (StatSoft, USA). The data were calculated using Student's *t*-test. The differences were considered statistically significant at p value <0.05.

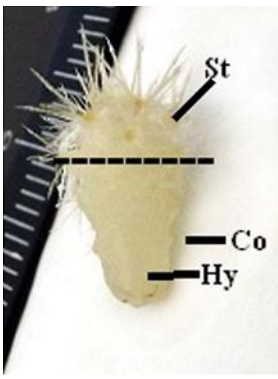

Fig. 1 *Mamillaria prolifera* seedling (after fixation) showing the section for anatomy (dotted line). St – stem; Co – cotyledons; Hy – hypocotyl.

The morphometric parameters and the size of the plants were analyzed in accordance with Vasilyev [26] and Gaidarzhy [27].

Results

All investigated plants have a well-developed hypocotyl, which is about half the length of the plant, whereas the epicotyl is poorly developed.

Melocactus bahiensis

Height – 6.7 ±0.3 mm, width at the base – 2.7 ±0.2 mm, and diameter of the widest part of the stem – 6 mm. The epidermis is uniseriate. The outer wall of the epidermal cells is thick with a thin cuticle (Tab. 1). Occasionally, small prismatic crystals of calcium oxalate can be found in the epidermal cells. The chlorenchyma consists of three to four layers of closely spaced isodiametric cells (the average area of a single cell is 27 456 ±2721 μm^2), but it is not always well developed. Starch is abundantly present in the pith and in the cortex parenchyma. The starch grains are compound with an average diameter of 11.2 μm. Calcium oxalate druses are rare in the cortex parenchyma (mainly around areolas). Their average diameter is about 64.6 μm. The stele has seven to nine vascular bundles. Xylem is well-developed and consists of seven to nine thin-walled elements with different diameters. The structure of phloem is similar in all studied seedlings. In particular, the primary phloem is not collapsed and the caps of sclereids are absent (Fig. 2).

Melocactus curvispinus

Height – 6.4 ±0.2 mm, diameter at the base – 2.6 ±0.1 mm, and diameter at the widest part of the stem – 6.3 ±0.1 mm. The epidermis is uniseriate (Fig. 3a). The outer wall of the epidermal cells is thick, covered by thin cuticle (Tab. 1). Small crystals of calcium oxalate are found in the epidermal cells. The thickness of the cortex parenchyma is three times wider than the pith diameter, while its cell area is quite larger than such index of pith parenchyma (Tab. 1). The stele has seven to nine open collateral vascular bundles. The number of xylem elements ranges from one to six. Large druses (the average diameter is about 140 μm) were observed near the vascular bundles. These plants are characterized by high contents of starch, which occurs in pith and cortex parenchyma throughout the stem cross-section. The starch grains in parenchyma cells are compound, on average 11.4 μm in diameter. They are united into large conglomerates in starch sheath cells (Fig. 3b).

Echinopsis eyriesii

Height – 17 ±0.9 mm, diameter of the widest part of the stem – 10 ±0.2 mm, diameter at the base – 3 ±0.2 mm. The epidermis is uniseriate with a thick outer cell wall, covered by thin cuticle (Tab. 1). Small crystals of calcium oxalate are found in the epidermis cells. The chlorenchyma consists of three to five layers of isodiametric cells (the average area of a single cell is 31 798 ±3884 μm^2). The cortex parenchyma thickness is almost three times bigger than the pith diameter. Parenchyma cells are significantly larger than those of the pith (Tab. 1). The cortex on the tops of the ribs contains mucilage cells. Numerous small crystals of calcium oxalate are present in the pith. The cortex parenchyma exhibits small druses and prismatic crystals of different sizes around the vascular bundles and in the ribs. There are 8–9 open collateral vascular bundles with 4 to 20 tracheary elements.

Echinopsis mirabilis

Height – 6.8 ±0.2 mm, diameter of the widest part of the stem – 5.9 ±0.1 mm, diameter at the base – 3.2 ±0.1 mm. The epidermis is uniseriate, the outer wall of the epidermal cells is thick, with a thin cuticle (Tab. 1). Parenchyma cells of the primary cortex are about two times larger than the pith parenchyma cells. The cortex parenchyma (closer to the vascular bundles) contains a large number of druses of diverse sizes. Single small crystals of calcium oxalate are located in the parenchyma. Starch grains are few and small, located in the pith and between the vascular bundles. There are 8–10 open collateral vascular bundles in the stele; they have the most complex structure among all the studied plants. There are from 10 to 30 conductive elements in the xylem.

Echinopsis peruviana

Height – 14.6 ±1.0 mm, diameter of the widest part of the stem – 6.8 ±0.5 mm, diameter at the base – 4.2 ±0.1 mm. The epidermis is uniseriate and the thickest among the studied species (Tab. 1). The outer walls of epidermal cells are thick with a thin cuticle and rare papillae. The hypodermis is uniseriate with calcium oxalate prismatic crystals. The chlorenchyma consists of 4–5 layers of isodiametric cells (the average area of a single cell is 23465 ±1022 μm^2). The cortical parenchyma is twice as wide as the pith diameter and the same trend is also observed for parenchyma cells. The cortex parenchyma contains calcium oxalate crystals of different shapes and sizes. Numerous crystals and crystal sand are also present in the pith (Fig. 4). Lipid drops and inulin-like inclusions are found in the parenchyma. There are 9–10 open collateral vascular bundles in the stele, which are well developed. The number of tracheary elements with a different diameter ranges from 4 to 25.

Oreocereus celsianus

Height – 13.6 ±0.8 mm, diameter at the base – 3.3 ±0.3 mm, diameter of the widest part of the stem – 6.4 ±0.3 mm. The epidermis is uniseriate with a very thick outer cell wall and a thin layer of cuticle (Tab. 1). The epidermal cells contain small prismatic crystals of calcium oxalate. The chlorenchyma consists of 4–5 layers of isodiametric cells (the average area of a single cell is 16928 ±876 μm^2), but it is not always well developed. The cortex parenchyma cells are significantly larger than those of the pith (Tab. 1). Small crystals of calcium oxalate of different shapes and sizes as well as crystal sand occur quite frequently in the pith parenchyma cells, while in the cortex parenchyma their amount is much lower. In the sub-epidermal parenchyma cells, druses with a diameter of 96 to 118 μm sometimes occur. The stele has 9–11 open well-developed collateral vascular bundles. The number of xylem elements ranges from 7 to 14 (Fig. 5).

Rebutia flavistyla

Height – 7.3 ±0.3 mm, diameter at the base – 2.1 ±0.1 mm, diameter at the widest part of the stem 5 ±0.2 mm. The epidermis is uniseriate with a thin outer wall of the cells (Tab. 1, Fig. 6a). The thickness of the cortex parenchyma is two times larger than the pith parenchyma diameter. The cortex parenchyma cells are also bigger than the pith parenchyma cells (Tab. 1). The cortex parenchyma cells contain small prismatic crystals of calcium oxalate. The central cylinder of the cortex has 6–10 open collateral vascular bundles arranged around the parenchymatous pith. Most of the vascular bundles are poorly developed and consist of 1–4 xylem elements (Fig. 6b).

Rebutia minuscula

Height – 7.7 ±0.3 mm, diameter at the base – 2.1 ±0.1 mm, and diameter of the widest part of the stem 5.9 ±0.2 mm. These plants have a uniseriate epidermis with a moderately thick outer cell wall, covered by thin cuticle (Tab. 1). Small prismatic crystals of calcium oxalate were found in the epidermal cells. In the outer cortex, discontinuous palisade chlorenchyma is present and its cell area is on the average 14 975 ±770 μm^2. The cortex parenchyma cells are almost five times bigger than the pith parenchyma cells (Tab. 1), but the thickness of the primary cortex and diameter of the pith are approximately equal (Tab. 1). There are 9–10 collateral vascular bundles in the stele. The number of xylem elements in the bundle ranges from 3 to 11.

Astrophytum myriostigma

Height – 12.1 ±0.3 mm, diameter of the widest part of the stem – 6.4 ±0.2 mm, diameter at the base – 2.3 ±0.1 mm. The epidermis is uniseriate with papillae. The outer wall of the epidermal cells is very thick, covered by relatively thin cuticle (Tab. 1). Stellate multicellular trichomes are present on the surface of plants (Fig. 7a). The hypodermis is collenchymatic, composed of cells with irregularly thickened primary walls, varying from one (between the ribs) to two (on the tops of the ribs) layers of cells. The hypodermal cells contain calcium oxalate crystals, prismatic ones or druses, and some of the latter are very large and fill the whole cross-sectional area of the cells and even protrude in the epidermal cells (Fig. 7b). The chlorenchyma is not always well developed, sometimes consists of 1–2 layers of palisade cells (the average area of a single cell is 9662 ±867 μm^2). The thickness of the cortex parenchyma is almost six times bigger than the diameter of the pith. The cortex parenchyma cells are also considerably bigger than those of the pith. There are 17–19 open collateral vascular bundles in the stele. The number of xylem elements ranges from one to eight (usually 1–4).

Mammillaria columbiana

Height – 7.8 ±0.1 mm, diameter of the widest part of the stem – 5 ±0.3 mm, and diameter at the base – 1.9 ±0.2 mm. The epidermis is uniseriate with a moderately thick outer wall and a thin cuticle (Tab. 1). The thickness of the cortex parenchyma is almost two times bigger than the diameter of the pith and the same trend is also observed for parenchyma cells (Tab. 1). The parenchyma cells contain prismatic crystals and druses of calcium oxalate. Druses, which are found in the sub-epidermal cortex, have a quite distinct appearance (Fig. 8a,b). The stele has 7–9 open collateral vascular bundles with three to eight xylem elements.

Mammillaria prolifera

Height – 12.1 ±0.9 mm, diameter of the widest part of the stem – 6.4 ±0.2 mm, diameter at the base – 2.5 ±0.1 mm. The epidermis is uniseriate with moderately thick outer cell walls and it is covered by a thin cuticle (Tab. 1). The thickness of the cortex parenchyma is larger than the diameter of the pith parenchyma. The cortex parenchyma cells are bigger than the pith parenchyma cells, but this difference is least among the examined species (Tab. 1). The cortex contains single mucilage cells. Inulin crystals were found along the vascular bundles and in some places in the parenchyma. There are also compound starch grains in the cortex and pith, whose diameter varies from 7.7 ±0.2 mm to 8.3 ±0.3 mm. The stele consists of 8–12 open collateral vascular bundles; the number of xylem elements in a cluster is from 1 to 10.

Tab. 1 Quantitative anatomical characteristic of the studied Cactoideae seedlings (mean ±standard error of the mean).

Species	Epidermis thickness, μm (n = 100)	Thickness of the outer wall of epidermal cells, μm (n = 100)	Cortex thickness, μm (n = 20)	Area of the cortex parenchyma cell, μm² (n = 100)	Diameter of the pith, μm (n = 20)	Area of the pith parenchyma cell, μm² (n = 100)
Melocactus bahiensis	26.9 ±0.6	4.5 ±0.2	2400 ±31	31327 ±1118	467 ±35	11661 ±1109
Melocactus curvispinus	23.4 ±0.4***	3.6 ±0.1***	1720 ±23	16568 ±781	653 ±11	13396 ±1073***
Echinopsis eyriesii	28.1 ±0.7##	3.9 ±0.2$^{\#\#\#}$	2667 ±35	29468 ±1352$^{\#\#\#}$	987 ±58	13102 ±585$^{\#\#}$
Echinopsis mirabilis	27.9 ±0.7	3.5 ±0.1$^{\#\#\#}$	1680 ±83	22199 ±2225^{+++}	639 ±11	10398 ±747^{+}
Echinopsis peruviana	31.5 ±1.1	3.1 ±0.1	1747 ±74	23614 ±669	867 ±48	10683 ±634
Oreocereus celsianus	28.0 ±0.5	5.4 ±0.2	1680 ±58	19922 ±973	1067 ±35	9347 ±353
Rebutia flavistyla	27.5 ±0.8	1.8 ±0.1*	1860 ±104	39013 ±1725***	933 ±30	9536 ±749***
Rebutia minuscula	27.2 ±0.6	2.2 ±0.1	1240 ±46	13106 ±510	1280 ±46	2714 ±144
Astrophytum myriostigma	27.0 ±0.5	5.5 ±0.2	2240 ±46	14065 ±654	400 ±23	3684 ±272
Mammillaria columbiana	21.0 ±0.8	2.5 ±0.1	1453 ±35	28795 ±1822***	787 ±35	12008 ±598***
Mammillaria prolifera	21.2 ±0.6	2.3 ±0.1	1960 ±46	19027 ±787	810 ±25	18802 ±1428

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ – significant differences relative to other species of the same genus. $^{\#}$ $p \leq 0.05$; $^{\#\#}$ $p \leq 0.01$; $^{\#\#\#}$ $p \leq 0.001$ – relative to *Echinopsis peruviana*. $^{+}$ $p \leq 0.05$; $^{+++}$ $p \leq 0.001$ – relative to *Echinopsis eyriesiir*. n – sample size.

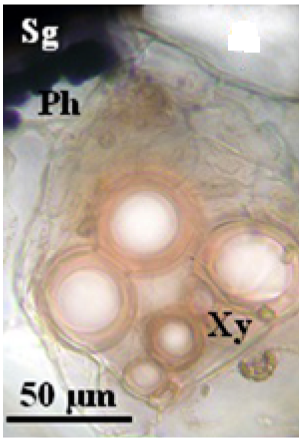

Fig. 2 Vascular bundle of *Melocactus bahiensis*. Xy – xylem; Ph – phloem; Sg – starch grains.

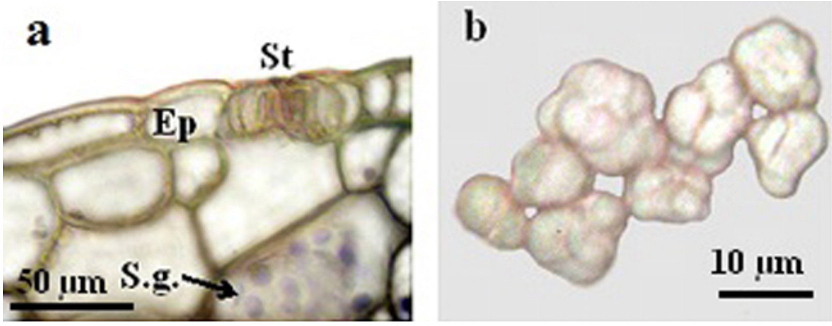

Fig. 3 Microphotography of *Melocactus curvispinus*. **a** Epidermis with a thick outer wall of the cells, covered by thin cuticle. **b** Starch grains isolated from starch sheath cells. St – stomata; Ep – epidermis; S.g. – starch grains.

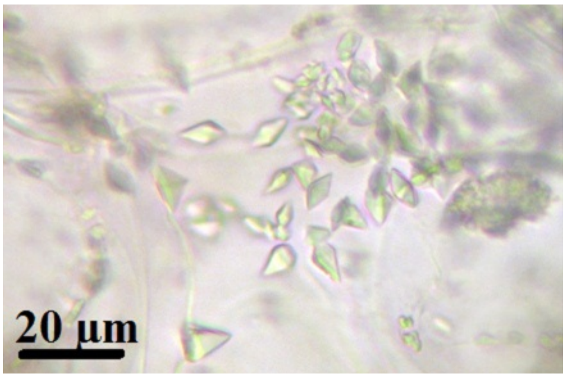

Fig. 4 Crystal sand in the pith of *Echinopsis peruviana*.

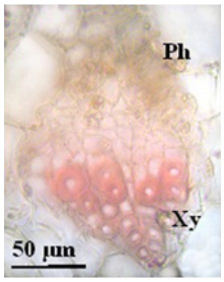

Fig. 5 Vascular bundle of *Oreocereus celsianus*. Xy – xylem; Ph – phloem.

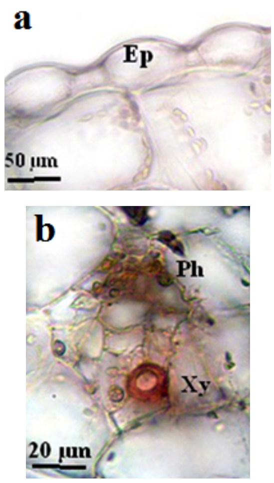

Fig. 6 Microphotography of *Rebutia flavistyla*. **a** Epidermis with a thin outer wall of the cells, covered by thin cuticle. **b** Vascular bundles. Ep – epidermis; Xy – xylem; Ph – phloem.

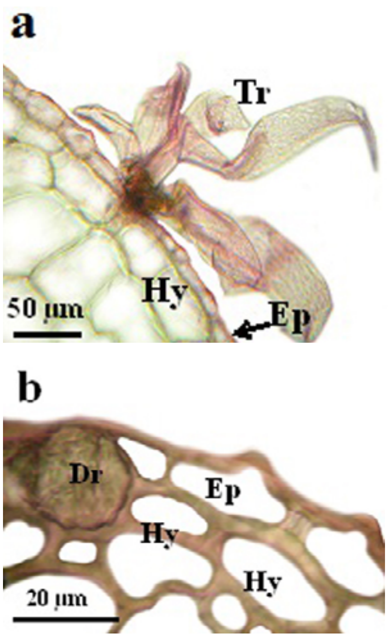

Fig. 7 The integumentary system of *A. myriostigma* seedlings. **a** Stellate multicellular trichome on the rib. **b** Papillate epidermis, hypodermis with calcium oxalate druses on the tops of the ribs. Ep – epidermis; Hy – hypodermis; Tr – trichome; Dr – druse.

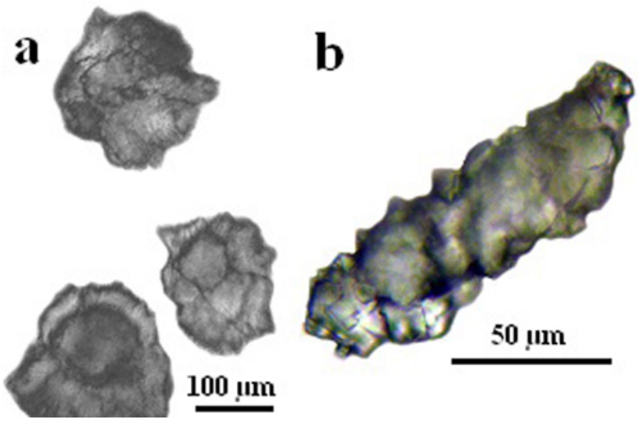

Fig. 8 Druses of *Mammillaria columbiana* seedlings. **a** Isolated from subepidermal cortical parenchyma cells. **b** Isolated from parenchyma cells.

Discussion

In our work, all cactoidean seedlings studied have a uniseriate epidermis with a thin cuticle. According to Vasilyev's classification [26], the thicknesses of the outer walls of the epidermal cells ranges from thin (*R. flavistyla*) to very thick (*A. myriostigma*, *O. celsianus*). The epidermal cells in the paradermal plane are large with flexuous or sinuous anticlinal walls [28]. However, the small sized epidermal cells are typical for plants growing in dry regions [29]. A uniseriate epidermis with a thin cuticle and flexuous or sinuous anticlinal walls were described for seedlings of other Cactoideae species [11,16–20]. In cactacean species, the sinuous anticlinal cell wall is an adaptation that allows the epidermal cell to expand and contract according to water loss or absorption, and thus it gives more strength to the epidermis [30].

Adult plants of the subfamily Cactoideae also have typically a uniseriate epidermis, which in some species has a thickened outer wall and a thick cuticle. Moreover, these plants have a multiseriate hypodermis [9,19,31,32]. In this work, a uniseriate hypodermis with calcium oxalate prismatic crystals and druses was found only in two species – *E. peruviana* and *A. myriostigma*. The latter has a well-pronounced hypodermis with thickened cell walls on the tops of the ribs. Other authors reported the presence of a uniseriate hypodermis in the stem of seedlings from several species of the tribe Pachycereeae [16,18] and the genera *Rhipsalis*, *Lepismium* [19], *Cereus* [11], and its absence in *Epiphyllum* seedlings [20]. The number of layers of the hypodermis and the cell wall thicknesses may influence the rigidity and xeromorphy of the stems. The hypodermis can affect the penetration of solar radiation to the underlying chlorenchyma and represents a path through which gases must diffuse [32].

Cortex parenchyma carries out at least two important functions related to xeric adaptations – photosynthesis and water storage. The outer cortex is called chlorenchyma or palisade cortex (in some species). It is green and photosynthetic. The chlorenchyma of Cactoideae adult plants is commonly characterized by multiple layers of palisade cells [9,31,32]. However, in the majority of species studied here, seedlings have a poorly developed chlorenchyma which consists of isodiametric cells, and palisade cells sometimes were only observed in *A. myriostigma* and *R. minuscula* seedlings. Other authors also reported the absence of palisade parenchyma in seedlings of different Cactoideae species, but indicated the presence of chlorophyllous cells in the cortex parenchyma [16,18–20].

The cortex parenchyma in all species studied here consists of big thin-walled cells and takes the largest part of the stem. The pith parenchyma consists of much smaller cells and takes the lesser part in the cross section of the stem. Mucilage cells were not found in the parenchyma of most of the investigated plants, except for *Mammillaria prolifera* and *E. eyriesii*. According to several authors, mucilage is hydrophilic, affects water relations and is an important adaptation to dry environments [31,32]. Large and thin-walled parenchyma cells occurred in the stems of the seedlings of other Cactoideae species, but in addition, some of these species had single mucilage cells [16,18–20]. Thus, the cortex parenchyma of seedlings, as in adult plants [32], performs the basic function of water storage.

The vascular tissues are differently developed in the seedlings studied here. There are only open collateral vascular bundles, which occur in the stele. Their number ranges from 6 to 19 in different plants. The number of conductive elements in the xylem ranges from 1–4 (*R. flavistyla*) to 10–30 (*E. mirabilis*). The collapsed phloem and the caps of sclereids are absent from seedlings of all species studied here. Thus, the xylem plays the primary role of giving mechanical support in the seedling stem. Adult plants of the subfamily Cactaceae are characterized by the occurrence of narrow phloem, caps of primary phloem fibers or sclereids outside conductive phloem, and rays, which are generally wider than in typical dicotyledons (an adaptation that facilitates water storage) [31,32]. In addition to wide rays, other adaptations of cactus xylem for water storage are that the fiber cell walls are thin, which increases lumen volume, and that the vessels and paratracheal parenchyma constitute a large fraction of the volume of the axial system [32].

The studied seedlings have various ergastic substances. All species were characterized by calcium oxalate inclusions of different shapes and sizes and with different locations in the tissues. Thus, in the 3-month-old seedlings oxalate crystals were found in the cortex (all studied species) and in the epidermis (the genus *Melocactus*, *R. minuscula*, *O. celsianus*, and *E. eyriesii*). Druses were found in the genus *Melocactus*, *Mammillaria columbiana*, *E. mirabilis*, *E. eyriesii*, *O. celsianus*, and *A. myriostigma*.

Terrazas Salgado and Mauseth indicated that cacti can accumulate enormous quantities of calcium oxalate. For example, up to 85% of the dry weight of *Cephalocereus minuscula* can be calcium oxalate [32]. Hartl et al. [33] showed the presence of calcium oxalate crystals in 245 out of 251 Cactaceae species. This family is also characterized by wide variation in crystals morphology. Crystals play several roles in the plant body, such as controlling the ion balance, defense, support, absorption and reflection of light [31], and may play a role in controlling herbivory [34]. Although not all the functions of calcium oxalate crystals in plant cells are perfectly clear, oxalate crystals in the epidermis probably mainly reflect light [31], while in the parenchyma they participate in osmotic pressure regulation, improving the adaptation to xeric conditions [31]. In addition, the occurrence of crystals in the epidermal cells often has taxonomic value [31,32,34]. For example, Gibson and Horak used the occurrence of crystals in the epidermis and hypodermis as a character, among others, to corroborate the division of Pachycereeae in two subtribes [34].

Seedlings of only four species among the 11 studied here (*Mammillaria prolifera*, *E. mirabilis*, and the genus *Melocactus*) have high contents of starch in the cortex and pith parenchyma cells (Fig. 3a). Since *Mammillaria prolifera* and *Melocactus bahiensis* have a relatively small number of calcium oxalate crystals, it is possible that the accumulation of starch grains regulates the osmotic pressure inside the cell in this species. Results of other scientists confirm this view. For example, Nobel and Bobich [35] described the following mechanism of internal water redistribution between the water-storage parenchyma and chlorenchyma. When *O. ficus-indica* is exposed to drought, the osmotic pressure in the water storage parenchyma becomes lower than in the chlorenchyma due to a polymerization of sugars leading to the formation of starch grains in the water-storage parenchyma. Gibson and Horak reported an inverse relationship between starch content and the presence of mucilage cells in the parenchyma of the cactus stem [34].

Furthermore, *Mammillaria prolifera*, *E. eyriesii*, and *E. peruviana* seedling are characterized by the presence of inulin-like inclusions. *Echinopsis peruviana* plants have single lipid drops. Those reserve nutrients (including starch) are necessary for the survival of the plants on poor soils.

Astrophytum myriostigma plants have stellate multicellular trichomes and a papillate epidermis. The latter were also found occasionally in the epidermis of *E. eyriesii* and *E. peruviana*. Trichomes and papillae take part in plant adaptation to arid conditions; in particular, they absorb sunlight and protect the mesophyll from overheating [36].

Some of the anatomical parameters studied may have taxonomic value. For example, the cortex and pith parenchyma cells in *R. flavistyla* are significantly larger than those in *R. minuscula*, but the pith diameter in *R. minuscula* is greater than in *R. flavistyla*. *Melocactus* seedlings have a large amount of starch and complex starch grains of almost the same diameter. *Melocactus bahiensis* plants have a significantly thicker epidermis and outer wall of the epidermal cells than *Melocactus curvispinus*. *Astrophytum myriostigma* is characterized by stellate trichomes, papillae, and hypodermis with calcium oxalate crystals.

Conclusions

It is ascertained that seedlings of all species studied here have a highly specialized anatomical and morphological structure. At the same time, the protective tissues are comparatively poorly developed. Accordingly, the adaptation to arid conditions at the early growth stage is based mainly on the strong development of water-storing tissue and the synthesis of ergastic substances, whose quantity and chemical composition depend on the taxonomic affiliation of the plants.

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Anatomiczne i morfologiczne cechy siewek wybranych gatunków Cactoideae Eaton (Cactaceae Juss.)

Streszczenie

Badano trzymiesięczne siewki 11 gatunków należących do podrodziny Cactoideae (*Melocactus bahiensis*, *Melocactus curvispinus*, *Echinopsis eyriesii*, *E. mirablis*, *E. peruviana*, *Oreocereus celsianus*, *Rebutia flavistyla*, *Rebutia minuscula*, *Astrophytum myriostigma*, *Mamillaria columbiana*, *Mamillaria prolifera*). Stwierdzono, że wszystkie badane rośliny posiadają wysoce wyspecjalizowaną strukturę anatomiczną i morfologiczną. Siewki okryte były jednowarstwową epidermą z cienką kutykulą. Za wyjątkiem *E. peruviana* oraz *A. myriostigma*, nie stwierdzono hipodermy. W łodygach wszystkich badanych okazów największą część pola przekroju poprzecznego stanowił miękisz korowy z dużymi komórkami o cienkich ścianach, miękisz rdzeniowy zbudowany był ze znacznie mniejszych komórek. W walcu osiowym wytworzone były kolateralne wiązki przewodzące, jednak ilość tkanek przewodzących była różna. Badane siewki zawierały różne substancje ergastyczne, w szczególności kryształy szczawianu wapnia (wszystkie badane gatunki), skrobię (*Mammillaria prolifera*, *E. mirabilis* oraz rodzaj *Melocactus*), substancje podobne do inuliny i czasami krople lipidowe (niektóre gatunki *Echinopsis*).
Na podstawie wyników pracy można wnioskować, że przystosowanie badanych siewek do suchych warunków polega na wzmożonym rozwoju tkanki wodnej (głównie w miękiszu korowym) oraz wytwarzaniu substancji ergastycznych.