

ULTRASTRUCTURE OF ENDODERMIS AND STELE CELLS OF DEHYDRATED *POLYPODIUM VULGARE* L. RHIZOMES

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Polypodium vulgare L. rhizome cells tolerate water deficit stress to different degrees. This study examined the extent of ultrastructural changes in the endodermis and stelar elements in response to mannitol dehydration treatment. Cytological observations showed that the rhizomes possess structural adaptations to withstand drying by maintaining water inside the stele or activating mechanisms that mitigate stress. There are Casparian strips on the walls of the endodermis, and thicker cell walls of cortex parenchyma cells bordered with endodermal cells. Numerous electron-dense vesicles accumulate in dehydrated endodermal cells, making the organelles not visible. In parenchymatous cells of pericycle and vascular parenchyma cells, only nuclei with slightly condensed chromatin, smaller starch grains and vesicle formation were observed in the cytoplasm after dehydration. Changes in cell membrane ultrastructure were not identified. Incubation of the rhizome in abscisic acid prior to dehydration did not produce ultrastructural changes.

Key words: *Polypodium vulgare*, common polypody, dehydration tolerance, TEM analysis, abscisic acid.

INTRODUCTION

Only a small number of vascular plants can tolerate dehydration (Bewley, 1979). Some 60–70% of pteridophytes are dehydration-tolerant plants (Oliver et al., 2000), including *Polypodium vulgare* (Kappen, 1964). Common polypody (*Polypodium vulgare* L.) is an epiphytic phanerophyte that grows on trees and rocky slopes, and is well adapted to withstand drought, heat, frost and salinity.

The responses of plants to various environmental stresses, including dehydration, are determined by the reaction of their individual cells, in which the integrity of structure and function is affected. However, from cellular responses alone it is not possible to predict the whole plant's reaction to water stress. According to Proctor and Tuba (2002) there are two kinds of plant adaptations to desiccation. The first includes factors preserving membrane and macromolecule stability; the second includes structural adaptations and forms of protection against oxidative stress. Dehydration tolerance in plants also depends on exposure of vegetative tissues to exogenous ABA (Wang et al., 2002). Ultrastructural and biochemical experiments on dehydration toler-

ance have usually been performed on crop plants such as maize (Čiamporová, 1997; Čiamporová and Mistrik, 1993), wheat (Corbineau et al., 2004) and cauliflower (Barrieu et al., 1999), or on resurrection plants such as *Xerophyta humilis*, *Craterostigma vilmsii* and *Myrothamnus flabellifolius* (Farrant, 2000). Usually the changes in ultrastructure in response to water deficit stress are studied in roots and leaves. Brighigna et al. (2002) used TEM to observe interesting details after rewetting plants from a completely dried form of the resurrection pteridophyte *Selaginella lepidophylla*. All these papers indicate that some similarities might be found in the response of different organs and species to dehydration. Chromatin condensation, microvacuolation, membrane stability changes, polyribosome degradation and ultrastructural alterations in the endoplasmic reticulum and mitochondria are among the phenomena reported.

Desiccation-tolerant plants have usually been divided into two groups: homoiochlorophyllous plants, which retain their chlorophyll during drying, and poikilochlorophyllous plants, which lose it (Hambler, 1961). There is increasing evidence that the ultrastructural changes in response to water

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stress differ between homoiochlorophyllous and poikilochlorophyllous plants (Farrant, 2000). Although the central vacuole in leaf cells was divided into a number of small ones in both types of plant, in poikilochlorophyllous *X. humilis* the sub-cellular changes involved mainly chloroplasts (dismantling of the photosynthetic apparatus by vesiculation of thylakoids), while in homoiochlorophyllous *C. vilmsii* and *M. flabellifolius* there was cell wall folding and some degree of plasmalemma withdrawal. During plasmolysis in plant cells (i.e., under hyperosmotic stress), actin filaments and microtubules were also reorganized (Komis et al., 2001, 2002). Our previous paper (Zenkteler and Bagniewska-Zadworna, 2005) concerned changes in *P. vulgare* rhizome cortex parenchyma cells dehydrated in a hypertonic solution of mannitol. Plasmolysis, nuclei with a large amount of condensed chromatin, rearrangement of endoplasmic reticulum cisternae and changes in amyloplast ultrastructure, including reduction of the number of starch grains, occurred in these cells. However, cell wall folding was not frequent. As compared with untreated rhizomes, pretreatment with abscisic acid protected cells against ultrastructural changes and damage.

This work aimed to investigate changes in endodermis and stele cell ultrastructure in response to water deficit stress, and to determine whether any alterations in their ultrastructure are similar to those described earlier for cortex parenchyma cells.

MATERIALS AND METHODS

PLANT MATERIAL

Adult sporophytes of *Polypodium vulgare* L. were collected from the wild in Mosina near Poznan, Poland, at the end of the vegetative season. The leaves and roots were cut off and then the rhizomes were dehydrated in 20% mannitol solution (Sigma, St. Louis, U.S.A.) for 9 h. Prior to dehydration, half of the rhizomes were incubated for 24 h in 2 mg l⁻¹ abscisic acid (Sigma) solution; the rest of the rhizomes were incubated in water for 24 h.

LIGHT MICROSCOPY

For fixation (4% glutaraldehyde and 4% paraformaldehyde 1:1, Polysciences, Warrington, U.S.A.; pH 6.8; overnight with one change of solution) the material was cut into pieces < 0.5 cm long, then stored in 70% ethanol, dehydrated in a graded ethanol series (80–100%) followed by butanol treatment and finally infiltrated and embedded in Paraplast Plus (Sigma). Samples were sectioned 12 µm thick with a Jung RM 2045 rotary microtome (Leica,

Nussloch, Germany). Sections were double-stained with Safranin O and fast green. The slides were examined under light and fluorescence microscopes (Axioscope, Carl Zeiss, Jena, Germany). All size details and anatomical structure were studied from cross and longitudinal (radial and tangential) sections. Measurements were repeated 15–20 times, using an OK-15-KM calibrated ocular micrometer (PZO, Warsaw, Poland). The size values given for cells are averages of cells from rhizome steles of adult (3–4-year-old) sporophytes.

ELECTRON MICROSCOPY

Rhizome pieces 2 mm³ containing the stele were selected under a stereoscopic microscope (Educo, Warsaw, Poland) and then fixed with 4% glutaraldehyde and 4% paraformaldehyde (1:1; pH 6.8) for 2 h at room temperature, rinsed three times with cacodylate buffer (0.05 M) and post-fixed with 1% osmium tetroxide for 2 h at 4°C. The material was counterstained for 1 h with 2% uranyl acetate (Polysciences), dehydrated in a graded acetone series and embedded in low-viscosity Spurr's resin (Spurr, 1969). Ultrathin sections (0.1 µm) cut with an Ultracut S ultramicrotome (Leica-Reichert, Bensheim, Germany) were collected on Formvar-coated copper grids, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined with a JEM 1200 EX II transmission electron microscope (Jeol, Tokyo, Japan) operating at accelerating voltage of 80 kV. On average, 5 samples from at least three different rhizomes were investigated.

RESULTS

STRUCTURE OF RHIZOMES

In cross section, the parenchyma cortex of the *P. vulgare* rhizome is encircled by hypodermis (Fig. 1). The rhizome is polystelic; meristele elements are located in a single circle inside the cortex. In transverse section, 6 large and 3 small steles are observed. Each stele, oval or roundish in cross section, is surrounded by a unilayered endodermis (Fig. 2) consisting of tangentially elongated cells with Casparian strips in the radial (Fig. 3) and tangential (Fig. 4) walls. The pericycle consists of 1 or 2 layers of parenchymatous cells. The phloem is built of sieve elements and phloem parenchyma cells (Fig. 5). It encircles the xylem (protoxylem, metaxylem), located in the middle of the stele. In longitudinal sections the metaxylem shows scalariform tracheary elements. Protoxylem tracheary elements have spiral or ring-shaped thickenings of cell walls. Apart from tracheary elements, parenchyma cells are differentiated in the xylem.

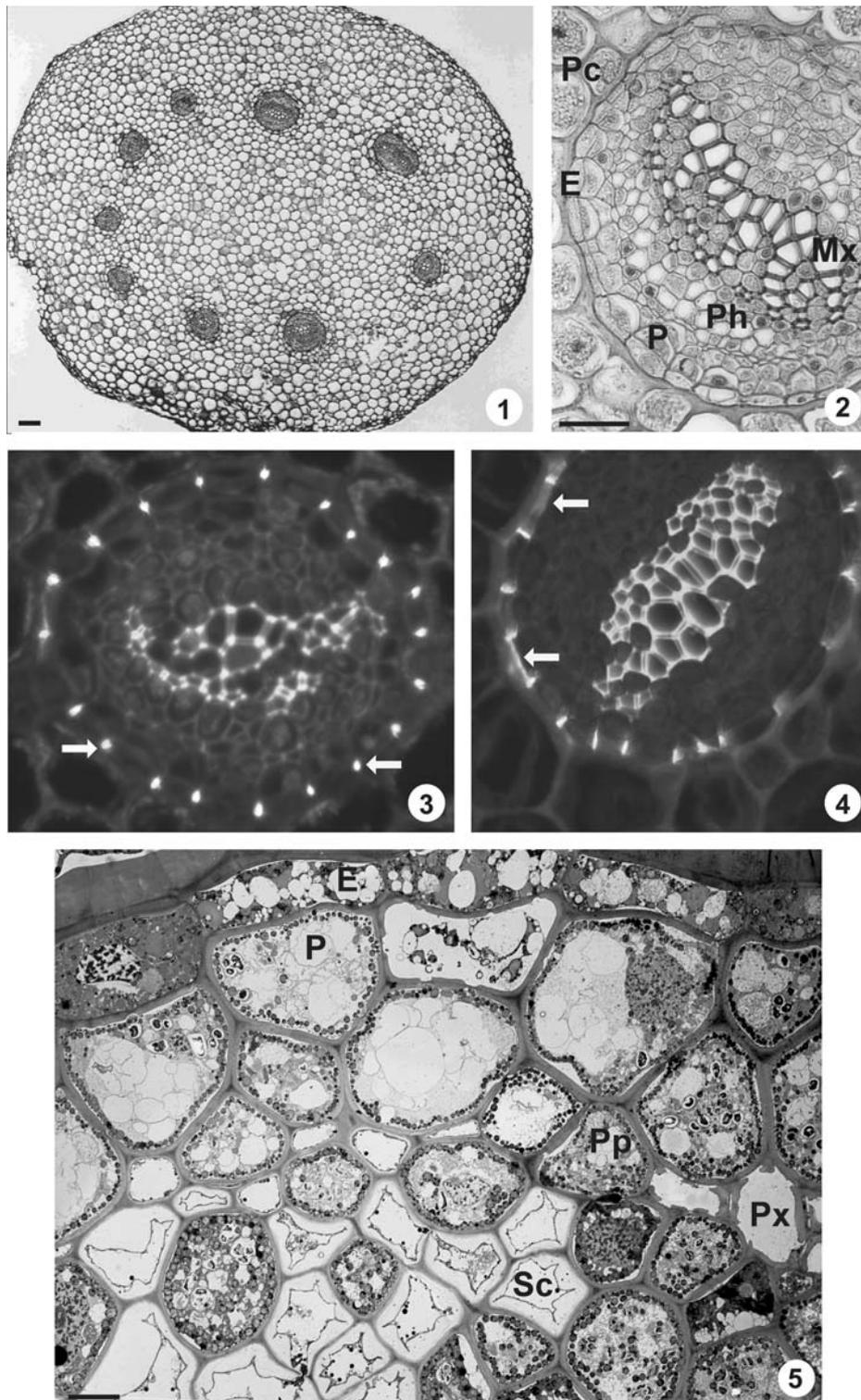


Fig. 1. Transverse section of *P. vulgare* rhizome with concentric arrangement of steles in cortex parenchyma. Bar = 100 μm . **Fig. 2.** Endodermis and stelar elements in *P. vulgare* rhizome. Bar = 50 μm . **Figs. 3, 4.** Casparian strips (arrows) in radial (Fig. 3) and tangential (Fig. 4) walls of endodermis (fluorescence microscopy). **Fig. 5.** Subcellular organization of rhizome stele (TEM). Pc – cortex parenchyma cell; E – endodermis; P – pericycle; Ph – phloem; Pp – phloem parenchyma cell; Sc – sieve cell; Px – protoxylem; Mx – metaxylem. Bar = 5 μm .

ULTRASTRUCTURE OF HYDRATED RHIZOMES (CONTROL)

The endodermal cells are radially flattened in cross section; their diameters reached (for average width of this cell) 4–6 μm (radially) and 10–17 μm (tangentially). The cell walls adjoining the pericycle are thin, unlike the walls adjoining the cortical parenchyma. Cortical parenchyma cell walls facing the endodermis are at least a third thicker than other walls of the same cell, and several times thicker than endodermal walls (Fig. 6), making a barrier for water efflux. The nucleus, with one nucleolus, is filled by a small mass of dense chromatin (not shown). The vacuolar system consists of several vacuoles (usually from 3 bigger to more than dozen smaller ones per section). Electron-dense, sometimes polymorphic material fills some of them. A characteristic feature of these cells is the presence of numerous vesicles with heterogeneous substance inside (Fig. 6). Rarely there are amyloplasts with two or three starch grains.

Stelar elements

The pericycle is one- or rarely two-layered, and its multangular parenchymatous cells are the biggest in the stele, usually 10–12 μm wide (radially) and 20–35 μm long. A transverse section through the stele shows typical ultrastructure of the pericycle. The cells are thin-walled, with numerous pits and plasmodesmata (Fig. 7). In the ribosome-enriched cytoplasm are a large number of organelles, including vacuoles, the nucleus, plastids, mitochondria and endoplasmic reticulum. The cells appear turgid; the vacuoles (from one or two larger to more than a dozen smaller ones per cell cross section) are rounded. Usually 2–3 elongated starch grains occupy the central parts of amyloplasts (Fig. 7). Plastoglobuli are only occasionally observed. The nucleus, with a relatively small amount of dense chromatin (Fig. 7), varies in size. Along plasma membranes there are numerous small electron-dense vesicles. In the cytoplasm, little spherical mitochondria, ribosomes and dictyosomes appear. Only a few short cisternae of endoplasmic reticulum are present near the nucleus (Fig. 7).

Phloem and xylem

Sieve cells of phloem (5–8 μm wide, 5–12 μm long) have irregularly thickened cell walls in cross section (Figs. 8, 9). The cytoplasm of sieve elements is characteristically devoid of several organelles, but is still limited by an intact plasma membrane. Nuclei, vacuoles, amyloplasts, Golgi bodies and endoplasmic reticulum cisternae are dispersed due to selective autophagy during the differentiation of sieve cells.

The sieve cells contain only a small number of vesicles (Fig. 8). Most of the bigger ones contain electron-dense material.

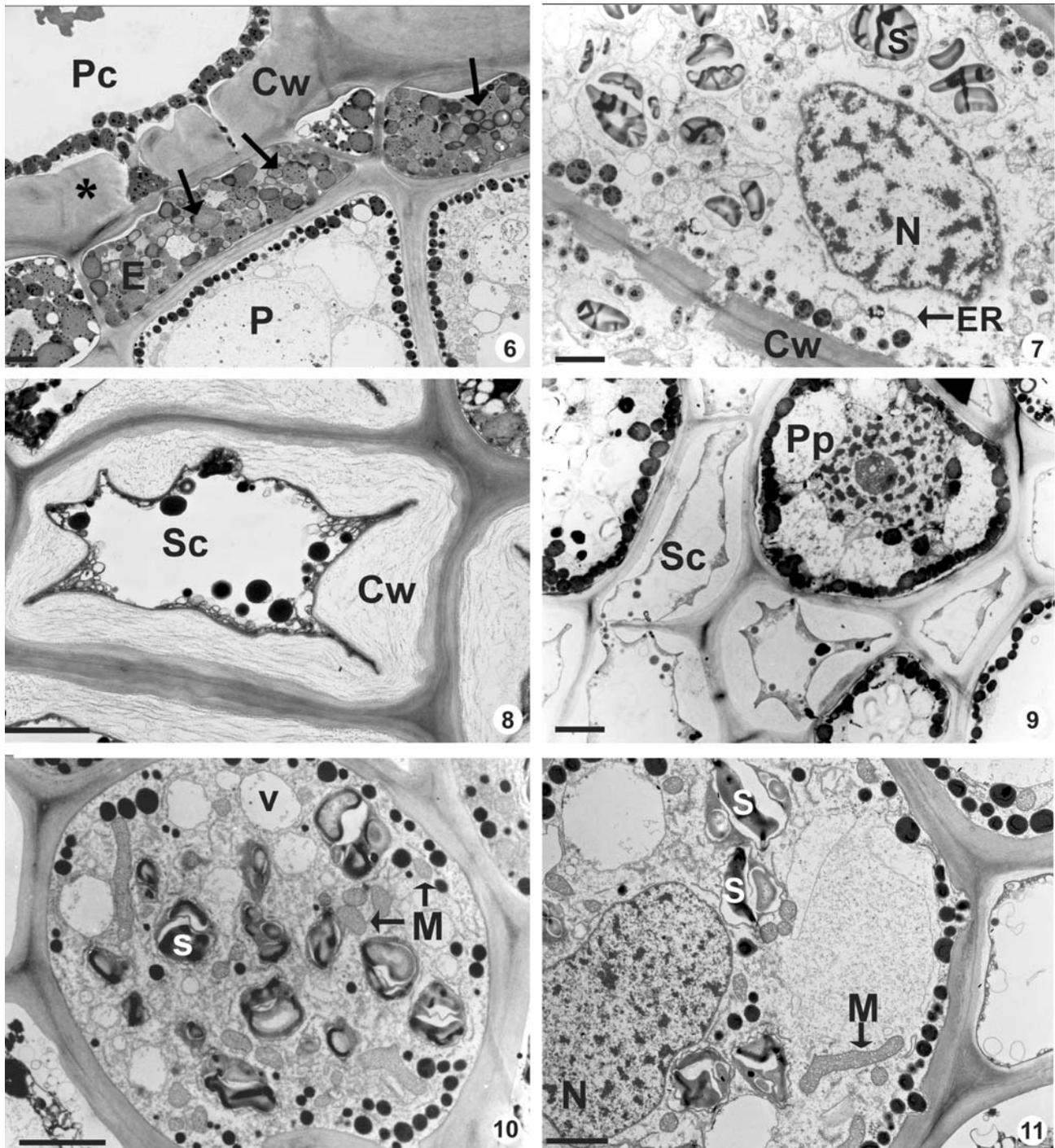
Vascular parenchyma cells are usually roundish, and highly variable in size (compare in Fig. 5), with small ones 5–7 μm in diameter in phloem and 5–10 μm in xylem, and large ones 10–18 μm in diameter. Cell walls delimit their protoplasts, and numerous organelles are found in the ribosome-rich cytoplasm (Figs. 9–11). The cells are highly vacuolated (usually 2–6 small vacuoles per cross section, but sometimes even 17–20). Amyloplasts with 2–5 small, elongated starch grains are dispersed in cell cross sections (Figs. 10, 11). The nuclear organization is true to type, with one packed nucleolus and a small amount of dense chromatin (Figs. 9, 11). Many electron-dense secretory vesicles occur in the cells, not necessarily at the plasma membrane (unlike other rhizome cells). Mitochondrial structure varies; they are frequently roundish or elongated in cell cross sections (Figs. 10, 11).

ULTRASTRUCTURE OF DEHYDRATED RHIZOMES

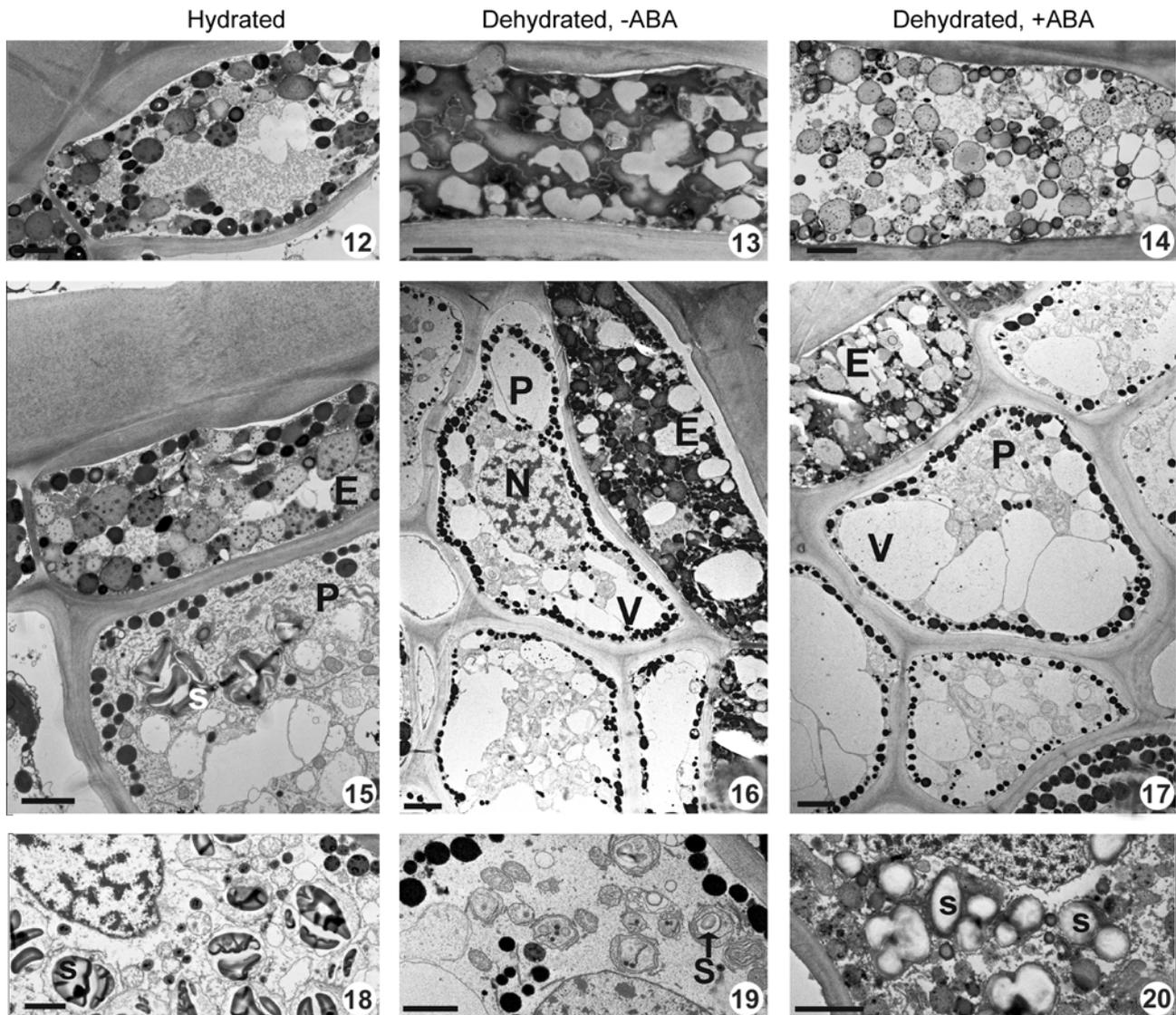
After dehydration, ultrastructural alterations (as compared to the control; Fig. 12) are evident in endodermal cells (Figs. 13, 14). Vacuole formation is intensified, marked by an increasing number of small vacuoles and secretory vesicles containing denser osmiophilic substances than in the control material (Fig. 13). Vesicles of very high electron density are always encountered as large aggregates in the cytoplasm. Some are connected together, forming larger vesicles varying in shape and size. These vacuoles and vesicles fill such a large surface of the analyzed cells that the rest of the organelles are not visible (Fig. 13). Such changes occur only in ABA-untreated rhizomes; treated ones do not show any ultrastructural alterations. The only exception is the lower degree of microvacuolation, but the new vacuoles are smaller, less electron-dense, and devoid of electron-dense content (Fig. 14).

Stelar elements

After mannitol dehydration treatment only a few changes are observed in the pericycle. Comparison with the control (Fig. 15) shows that plasmolysis does not occur in pericycle cells and that the continuity of the plasma membrane is not disturbed (Figs. 16, 17). Nuclei with dense chromatin are observed only occasionally in ABA-untreated rhizome cells, most of them being comparable to the control (Fig. 16). The number of mitochondria increases after dehydration, and only a few vesicles, likely resulting from fragmentation of large



Figs. 6-11. Subcellular organization of endodermis and stelar elements in *P. vulgare* rhizomes in hydrated state (control). **Fig. 6.** Endodermal cell. Numerous small vesicles (arrows) and vacuoles visible. Note thick cell wall (star) of cortex parenchyma cell in the vicinity of endodermis. **Fig. 7.** Starch grains in amyloplasts, short cisternae of endoplasmic reticulum and nucleus in pericycle. **Fig. 8.** Sieve cell. Characteristic irregular cell wall thickness visible. **Figs. 9-11.** Ultrastructure of vascular parenchyma cells. Note small vacuoles, nucleus, amyloplasts with starch grains and mitochondria of different sizes. Pc – cortex parenchyma cell; E – endodermis; P – pericycle; Sc – sieve cell; Pp – phloem parenchyma cell; Cw – cell wall; S – starch grain; N – nucleus; ER – endoplasmic reticulum; V – vacuole; M – mitochondrion. Bar = 2 μ m.

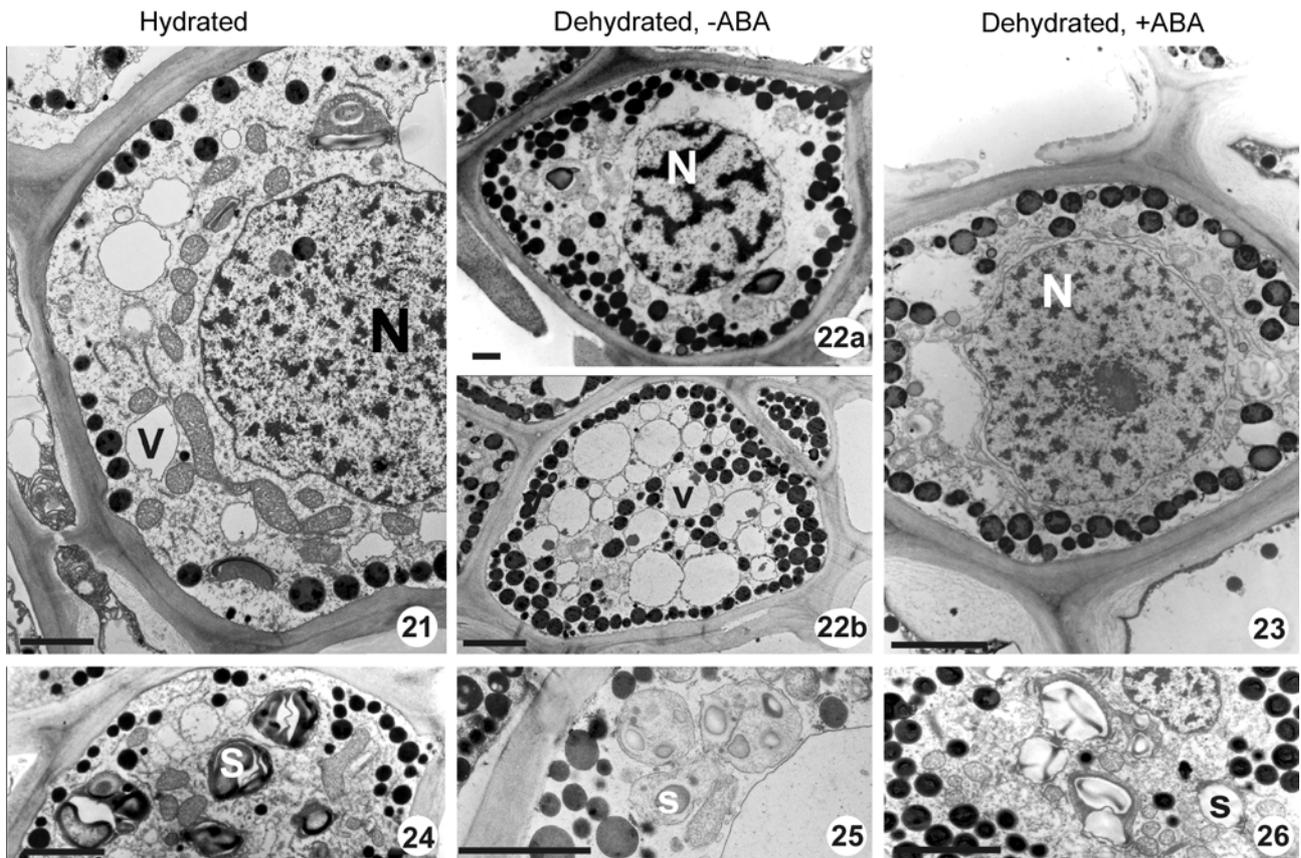


Figs. 12–14. Comparison of ultrastructure of endodermis in hydrated and dehydrated states of *P. vulgare* rhizomes. Note electron-denser deposits in endodermis of ABA-untreated rhizomes (Fig. 13). **Figs. 15–17.** Comparison of ultrastructure of pericycle in hydrated and dehydrated states. Note many vacuoles in pericycle in both ABA-treated and untreated cells. **Figs. 18–20.** Comparison of amyloplast internal organization in pericycle of hydrated and dehydrated rhizomes. Note smaller starch grains and plastoglobuli in amyloplasts of ABA-untreated rhizomes (Fig. 19). E – endodermis; P – pericycle; N – nucleus; V – vacuole; S – Starch grain. Bar = 2 μ m.

vacuoles, are visible. In dehydrated ABA-treated rhizomes, vacuole formation also occurs in very few of the examined pericycle cells (Fig. 17), but no other alterations are found. In some of the pericycle cells from ABA-untreated rhizomes, starch grains are smaller than in the control (Fig. 19 vs. 18), and amyloplasts contain structures resembling thylakoids and a few plastoglobuli. In ABA-treated rhizomes, starch grain sizes are similar to those of the control (Fig. 20).

Phloem and xylem

In sieve cells, dehydration does not result in ultrastructural alterations. After dehydration, nuclei with dense chromatin are present in only a small number of vascular parenchyma cells from ABA-untreated rhizomes (Fig. 22a, compare with Fig. 21). Numerous vesicles and vacuoles varying in size and shape rather than structure accumulate in some phloem and xylem parenchyma cells (Fig. 22b).



Figs. 21–23. Comparison of ultrastructure of vascular parenchyma cells in hydrated and dehydrated states of *P. vulgare* rhizomes. Visible are dense chromatin in large clusters in nucleus (Fig. 22a), and the formation of small vacuoles and a number of small vesicles with electron-dense precipitates in ABA-untreated/dehydrated xylem parenchyma cell (Fig. 22b). **Figs. 24–26.** Comparison of amyloplast ultrastructure in vascular parenchyma cells from hydrated and dehydrated rhizome. Note that amyloplasts of ABA-untreated rhizomes contain smaller starch grains (Fig. 25). N – nucleus; V – vacuole; S – starch grain. Bar = 2 μm .

Such cells also occur in the control (compare to Figs. 10 or 21). These globular structures resemble those found in the other cells of the stele. Morphological criteria could not distinguish the origin of individual vesicles, but most of them probably originated from the Golgi apparatus. Some may have been formed *de novo* as a result of mitigation of water stress in cells. There are no structural changes in the mitochondria, endoplasmic reticulum or Golgi apparatus. Mannitol dehydration treatment does not disturb the continuity of membranes in the analyzed cells. In rhizomes pretreated with abscisic acid, no major structural changes in the vascular parenchyma cells are noted (Fig. 23). No nuclei with dense chromatin or vesicle formations are observed. As in other types of cells, in dehydrated vascular parenchyma cells the starch grains are smaller than in hydrated cells.

These changes involve mainly rhizomes not pretreated with ABA (Fig. 25 vs. Fig. 24). Amyloplasts of ABA-treated rhizomes (Fig. 26) contain starch grains comparable in size to the control.

DISCUSSION

Our results suggest that some elements of *Polypodium vulgare* rhizome anatomy protect against harm from water deficit stress. In this species, endodermis with lignified and suberized Casparian strips prevents water loss from the cells of the vascular bundle. The thicker cell walls of cortical cells in the close vicinity of the endodermis could also play a part in maintaining water inside the stele. This might be critical to rhizome survival during dehydration; regeneration in fern tissue cul-

ture begins from pericycle cells (secondary meristem) (Zenkteler, 2000). These adaptations could play an important part in fern survival during water stress in the wild.

In the analyzed material we observed only a few changes in cell ultrastructure after mannitol dehydration treatment. In the vast majority of observations the changes involved rhizomes without ABA pretreatment. The character of the observed alterations was true to type for dehydrated cells: nuclear chromatin condensation was visible as areas with dense chromatin, changes in amyloplast structure, and microvacuolation, but membrane alterations were not observed. Nonlethal stress did not cause visible macroscopic damage.

In different stress conditions, changes in ultrastructure are usually uniform. The structure of the interphase nuclei is usually changed, and chromatin condensation during water deficit stress is associated with arrest of metabolism in various plant species (Čiamporová, 1997). According to Čiamporová and Mistrik (1993), very early responses to water deficit include rearrangement of ER elements and the appearance of concentric cisternae of ER. Endoplasmic reticulum and mitochondria are the most sensitive cell components; their structure changes very quickly after water stress. In stelar elements of *P. vulgare* rhizomes, ER were rarely visible, usually in the form of short cisternae in cross section, and this did not change during dehydration, unlike ER in cortex parenchyma cells, which formed concentric arrangements of their cisternae (Zenkteler and Bagniewska-Zadworna, 2005).

Generally, increasing vacuolation and vesicle formation was observed in the endodermis and vascular parenchymatous cells in this work. In some cases we could not be sure whether there were more vacuoles in the cell or rather one vacuole crossed by cytoplasmic strands, particularly since it was also observed in some hydrated cells. We confirmed earlier observations in various organs and different species by Barrieu et al. (1999), Farrant (2000), Cooper and Farrant (2002) and Brighigna et al. (2002). These authors suggested that microvacuoles might stabilize the cytoplasm, since increased vacuole volume is important for accumulation of osmotically active substances and osmotic adjustment under water stress (Čiamporová and Mistrik, 1993).

In our experiment the Golgi apparatus in the analyzed cells produced many small vesicles, which apparently contained compact material impregnated by electron-dense substances, but disintegration of dictyosomes was not observed. We concluded that most of the vesicles likely originated from Golgi bodies (as we described earlier for cortex parenchyma cells; Zenkteler and Bagniewska-Zadworna, 2005). Moreover, the appearance of small vesicles, especially those associated with the plasma membrane,

may indicate the occurrence of exocytotic processes that could remove and/or replace damage in membranes (Corbinneau et al., 2004). However, the dehydration technique applied here and used earlier for in vitro storage of dehydrated explants (Bagniewska-Zadworna and Zenkteler, 2002) resulted in nonlethal stress which did not disturb the continuity of membranes in stele cells of *P. vulgare* rhizomes. In ABA-untreated rhizomes we also found that the starch grains were smaller. Starch hydrolysis might be a source of carbon for sucrose synthesis, which is an important osmolyte promoting osmotic adjustment during plant drying (Scott, 2000). That could be very important in mitigating water deficit.

The response of *Polypodium vulgare* to rhizome dehydration was presented in this work and in a previous one (Zenkteler and Bagniewska-Zadworna, 2005). The extent of ultrastructural changes in the presently investigated stelar elements was lower than in cortex parenchyma cells. We indicated the elements of the *P. vulgaris* stele that probably constitute structural adaptations against drying. The ultrastructural changes during water deficit resembled those in other species. ABA pretreatment prevented visible dehydration-induced ultrastructural changes; this phytohormone helped maintain membrane continuity in stelar cells. Maintenance of selective membrane permeability is a crucial element of dehydration tolerance in plants.

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