Ultrastructural changes in rhizome parenchyma of *Polypodium vulgare* during dehydration with or without abscisic acid pretreatment

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Abstract

Common polypody (*Polypodium vulgare* L.) belongs to desiccation-tolerant ferns. The structure of storage parenchyma of their rhizome was examined by transmission electron microscopy after dehydration and subsequent rewetting. Analysis revealed that treatment with supplemental abscisic acid resulted in protection of cells against ultrastructural damage compared to untreated ones. Dehydration rate appears to modify the ability of rhizome parenchyma to stand water stress.

Additional key words: common polypody, desiccation tolerance, fern, TEM analysis.

Introduction

Polypodium vulgare, as an herb with medicinal properties (*Rhizoma Polypodii*) is often subjected to chemical analysis (Grzybek 1983, Hegnauer 1986, Duke 2001). Its rhizomes contain 52 active compounds (Duke 2001), from which three represent high diuretic activity, two have antibiotic properties and another two have cytostatic features (Grzybek 1983). Moreover, the abundance of mono-, di- and oligosaccharides in rhizome cells has been correlated with its desiccation tolerance (Reynolds and Bewley 1993, Bagniewska-Zadworna and Zenkteler 2003). The species belongs to poikilohydric plants and can withstand the loss of 45 % of its water content (Bagniewska-Zadworna and Zenkteler 2002). For this reason the species has been targeted as a model plant for long term *in vitro* storage.

The damage to cell structure in tissues, subjected to different levels of dehydration has been recently reviewed in some species (Farrant 2000, Brighina *et al.* 2002, Cooper and Farrant 2002). The diversity of drought injury symptoms in the diverse tissues makes it difficult to find an explanation of ultracellular events associated with tolerance. However, the ultrastructure of storage parenchyma in *P. vulgare* rhizome is not yet known, especially in circumstances of slow dehydration.

The objective of the present study was to establish whether abscisic acid (ABA) pretreatment protect rhizome parenchyma cells against ultrastructural injury.

Materials and methods

Plants and tratments: Rhizomes of *P. vulgare* were collected from natural sites near the Wielkopolska National Park. They were cleaned of leaves and roots and washed before being used in experiments.

Dehydration of *P. vulgare* rhizomes was conducted in a hypertonic solution of mannitol (20 %, -3.14 MPa; *Sigma,* St. Louis, USA) for 9 h at the temperature of 19 - 20 °C. Prior to dehydration half of the rhizomes were incubated in a 2 mg dm^{-3} ABA (*Sigma*) for 24 h. ABA-treated rhizomes lost 19.8 % of water and their relative water content (RWC) was 80.2 %; untreated ones lost 31.1 % of water and reach 68.9 % RWC.

Received 28 April 2004, *accepted* 27 August 2004.

Abreviations: ABA - abscisic acid; DT - desiccation-tolerant; ER - endoplasmic reticulum; TEM - transmission electron microscopy.

Acknowledgements: This work has been supported by grant PU-II/49 shared between A. Mickiewicz University and Agriculture Academy in Poznań.

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The control rhizomes were soaked in water. Before the subsequent mannitol-dehydration the rhizomes were immersed in sterile distilled water and maintained at room temperature for 15 h.

Electron microscopy analysis: Pieces of rhizomes (2 mm^3) were fixed with 4 % glutaraldehyde and 4 % paraformaldehyde (1:1; pH 6.8; *Polysciences,* Warrington, USA) for 2 h and post-fixed with 1 % osmium tetraoxide for 2 h at room temperature. The fixed material was counterstained for 1h with 2 % uranyl acetate (*Polysciences*). Samples were dehydrated in a graded acetone series (from 10 to 100 %) and embedded in low-viscosity Spurr's resin (Spurr 1969). Ultrathin sections (0.1 μm), obtained using ultramicrotom *Ultracut S* (*Leica-Reichert*, Bensheim, Germany) were collected on *Formvar*-coated nickel grids and stained with uranyl acetate and lead citrate (Reynolds 1963) and examined with a *JEM 1200 EX II* (*Jeol*, Tokyo, Japan) transmission electron microscope, operating at 80 kV. An average of 5 samples from three different rhizomes per sampling were investigated. For each sample, 10 ultrathin sections (dehydrated/rehydrated, with/without ABA treatment and control) were examined under electron microscopy.

Results

Parenchyma ultrastructure in *P. vulgare* rhizomes before dehydration: Parenchyma cortex cells were delimited by hypodermis from outside and by endodermis from every meristele element inside (Ogura 1972). A parenchyma cell contains a nucleus with two nucleoli, numerous amyloplasts, mitochondria, rough endoplasmic reticulum (ER), Golgi apparatus, a few small vacuoles, plasma membranes and has a relatively thick primary walls.

The amyloplasts in the rhizome storage parenchyma are uniformly distributed throughout the cell (Fig. 1). The number of amyloplasts per cell cross section was variable and ranged from 5 to 25. The amyloplasts contain a rudimentary thylakoid system which consists of only a few thylakoids extending along the main axis of the organelle. Starch granules occupy the central area of every plastid (Fig. 2). Structure of the mitochondria was true to type, they were cylindrical in shape, and ranged from round to elongate (Fig. 3). The mitochondria size was highly variable.

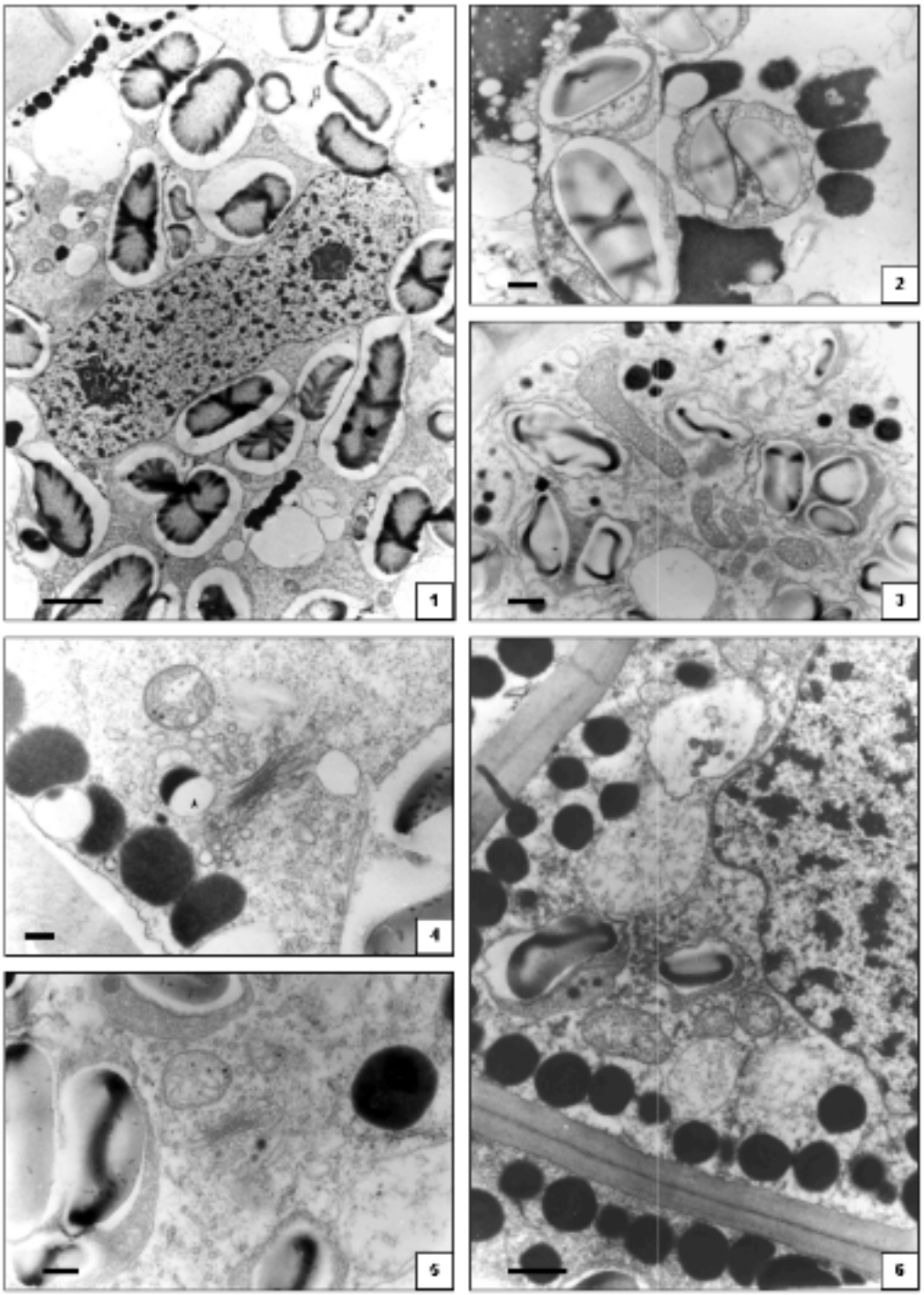
Tangential section through dictyosomes (Figs. 4, 5) showed several electron transparent, smooth surfaced vesicles released from cisternae close to the trans region that have migrated towards the plasma membrane. The endoplasmic reticulum (ER) has high plasticity to become also a repository of resin granules that may be transported to a wall or undergo storage deposition.

Most of the parenchyma cells have one distinctive structural characteristics: the presence of numerous secretory Golgi-derived vesicles filled with a dark content and distributed in the cytoplasm close to the plasma membrane (Fig. 6). These vesicles appear to be a ubiquitous feature of the storage parenchyma of *P. vulgare* rhizomes.

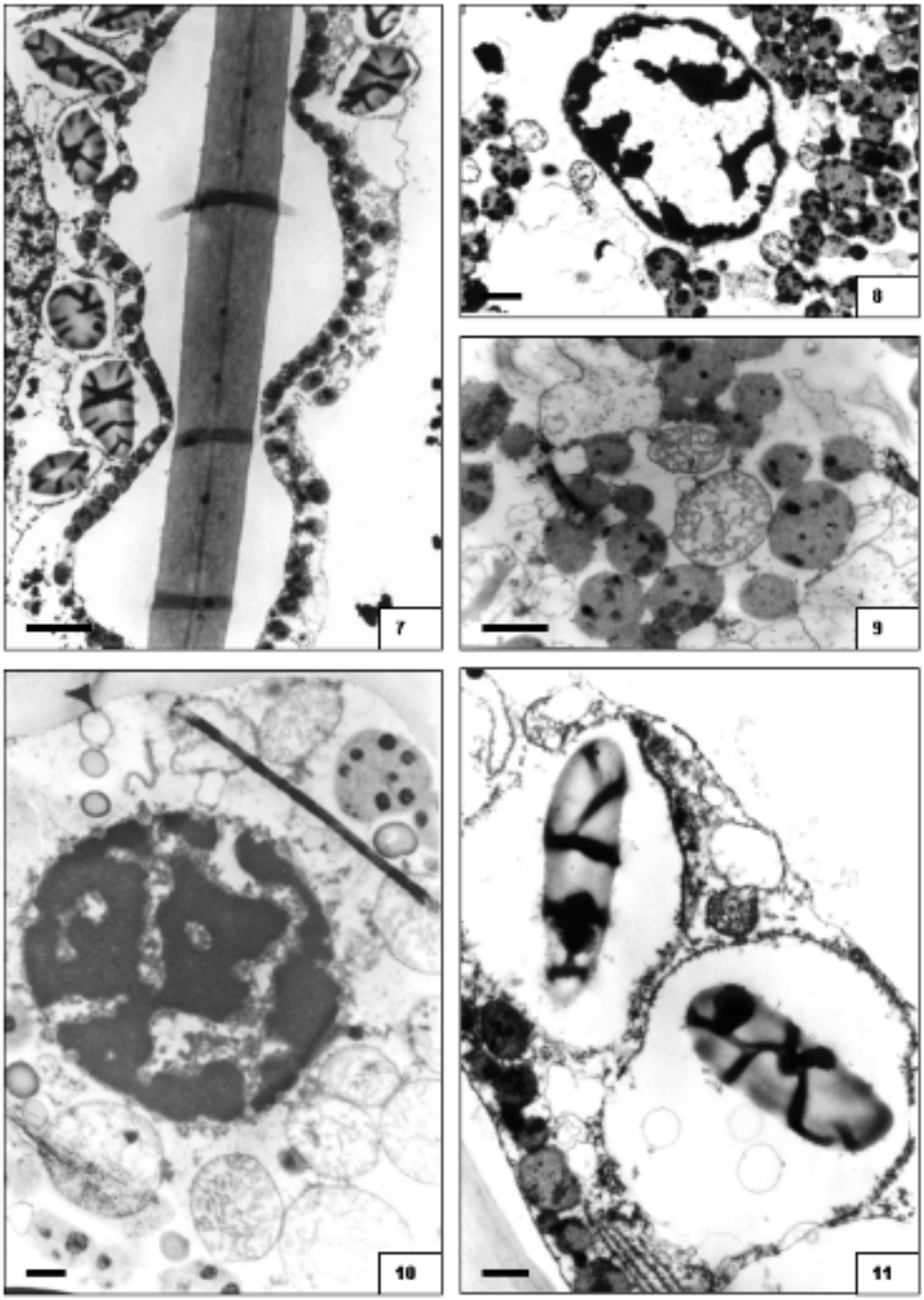
Parenchyma ultrastructure in *P. vulgare* rhizomes after dehydration: The two dehydration protocols (with or without an ABA pretreatment) resulted in markedly different ultrastructural changes. Parenchyma pretreated with ABA before the first dehydration show some plasma membrane withdrawal from the cell walls, but there was no breakage of the plasma membrane and cell walls did fold (Fig. 7). The nuclear chromatin becomes much condensed at the periphery of the nucleus (Fig. 8). Amyloplasts had intact envelopes and vacuoles did not show subdivision. Mitochondria have accumulated near the nucleus (Fig. 8) and their cisternae became swollen (Fig. 9). Peripheral cytoplasm of *P. vulgare* parenchyma cells was packed with secretory vesicles containing phenols responsible for the dark colour of precipitates (Fig. 7). The osmiophilic vesicles increase in size with diminished volume of starch grains inside amyloplasts. Some fused to form larger bodies (Fig. 9).

After the second dehydration (in trial with ABA pretreatment) vacuoles had a more crenate border and less dense contents. The nuclei were contracted, filled by a condensed mass of chromatin and surrounded by swollen mitochondria (Fig. 10). Amyloplasts membranes become discontinuous (Fig. 11). In the lumen of rough ER cisternae we observed osmiophilic aggregates. The most striking feature of ER after dehydration was the numerous parallel, concentric membrane systems (Fig. 12). These cisternae had a constant luminal width. The mitochondria appear to be more resistant to water stress than the amyloplasts (Fig. 13 *versus* Fig. 11).

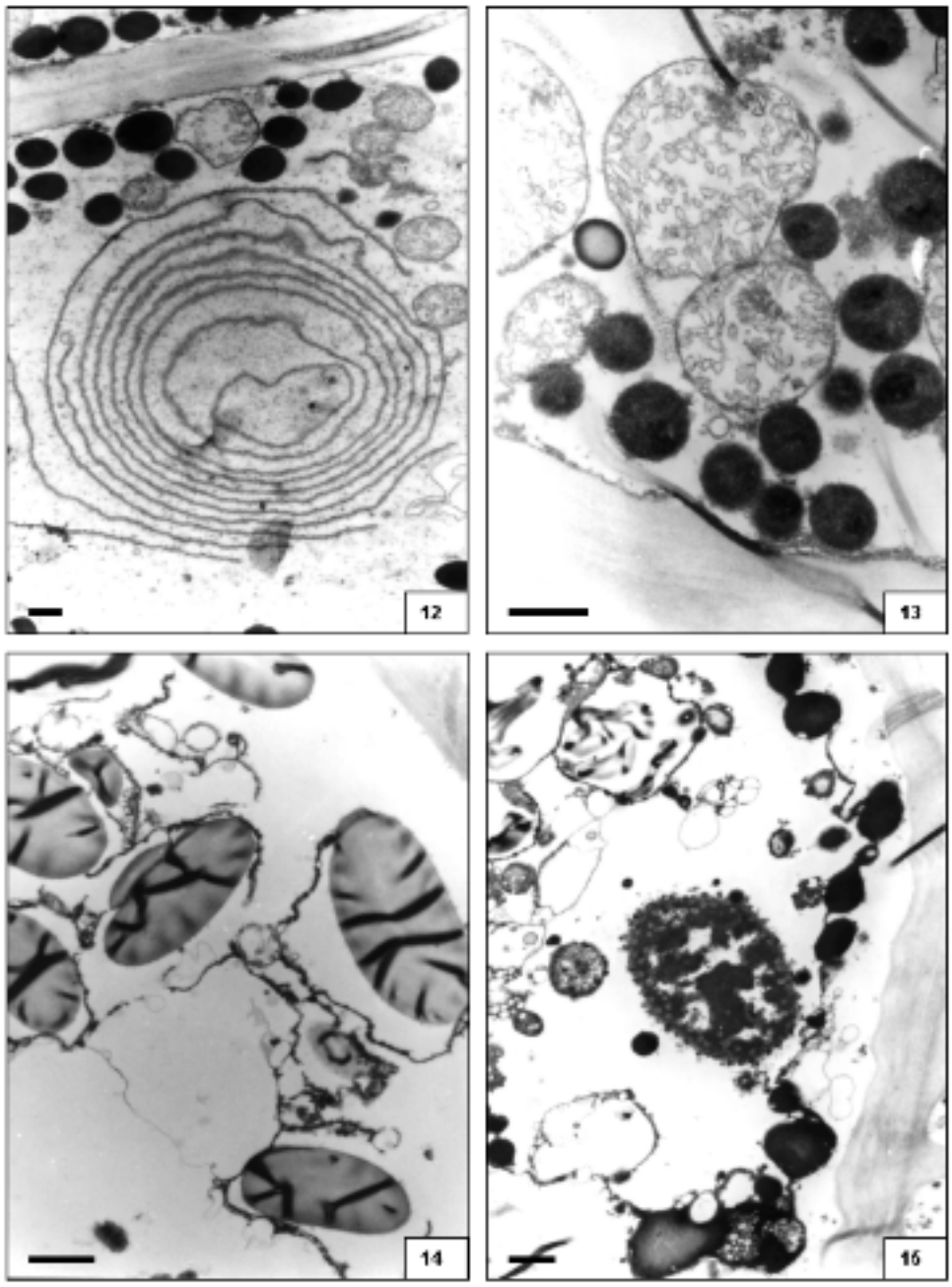
A feature peculiar to dehydration without ABA pretreatment was the progressive dispersal and breakdown of the outer and inner amyloplasts membrane after the first dehydration (Fig. 14). All other organelles become progressively less structured and their protoplasts strongly disintegrated (Fig. 15). In contrast to cell walls of leaf mesophyll cells in some desiccation-tolerant plants, which folded during dehydration, cell walls in *P. vulgare* rhizome parenchyma did not fold after dehydration with ABA pretreatment. Only cells, which had not been pretreated with ABA, showed slightly folding walls (Fig. 15).



Figs. 1 - 6. Subcellular organization of *P. vulgare* rhizome parenchyma cells in hydrated state. Fig. 1. Section through part of a typical storage parenchyma cell containing a dense protoplast with numerous mitochondria and amyloplasts surrounding a large nucleus with two nucleoli (*bar* = 2 μm). Fig. 2. Amyloplasts containing one or two starch grains and only a few thylakoids (*bar* = 0.5 μm). Fig. 3. Spherical and lenghtened mitochondria with numerous cristae and well discernible boundary membranes (*bar* = 0.5 μm). Fig. 4. Golgi apparatus in a cell producing many osmiophilic vesicles restricted to the trans-face of the dictyosome. *Asterisk* indicates vesicles that may have originated from Golgi apparatus (*bar* = 0.2 μm). Fig. 5. The Golgi network composed of a trans-cisternae and a reticulum next to the dictyosome. Note the presence of mitochondria in the close vicinity of dictyosome (*bar* = 0.2 μm). Fig. 6. Numerous secretory vesicles along plasma membrane the two neighbour cells. Inner and outer membranes of nucleus, amyloplasts and mitochondria show no damage (*bar* = 2 μm).



Figs. 7 - 11. Subcellular organization of *P. vulgare* rhizome parenchyma cells after dehydration proceeded by an ABA treatment. (Figs. 7 - 9 after the first dehydration, Figs. 10 - 11 after the second dehydration). Fig. 7. Reversible plasmolysis as an early response of parenchymatic cells to dehydration. Note numerous secretory vesicles in close proximity to the cell membrane (*bar* = 2 μm). Fig. 8. Condensation of nuclear chromatin near the nuclear envelope. Note numerous secretory droplets with dark, phenolic precipitates (*bar* = 1 μm). Fig. 9. Swollen mitochondria with distorted inner membranes (*bar* = 0.5 μm). Fig. 10. Contraction of the nucleus and assemblage of condensed chromatin masses with the envelope. Note numerous swollen mitochondria surrounding the nucleus (*bar* = 0.5 μm). Fig. 11. Swollen amyloplasts with disintegrated boundary membranes, their stromal contents mixing with the cytoplasm. Starch grains reduced in size (*bar* = 0.5 μm).



Figs. 12 - 15. Subcellular organization of *P. vulgare* rhizome parenchyma cells (Figs. 12 - 13 after dehydration with ABA pretreatment). Fig. 12. Concentric, parallel system of ER membrane (*bar* = 0.5 μm). Fig. 13. Highly swollen mitochondria with dilation of cristae. Their membranes are still integral (*bar* = 0.5 μm). Severe ultrastructural cell injury after only the first dehydration without ABA pretreatment (Figs. 14 - 15). Fig. 14. Cell plasmolysis and a total disruption of amyloplast envelope (*bar* = 1 μm). Fig. 15. Disruption and subsequent malformation of all organelles. Accumulation of condensed secretory droplets in the cell periphery. Note the slight wall folding (*bar* = 1 μm).

Discussion

Analysis of *P. vulgare* ultrastructure revealed that storage parenchyma cells contained numerous amyloplasts, were rich in ER and had well-defined mitochondria. The ER has high plasticity to become also a repository of resine granules that may be transported to a wall or undergo storage deposition. Majority of the cells lacked a large

central vacuole, however they did contain numerous smaller vacuoles. We documented the presence of a vesicle-mediated system for transport and storage of secondary metabolites.

An earlier phytochemical analysis showed that rhizome parenchyma of *Polypodium* contains numerous metabolites, such as phenolic compounds, resin and specific substances such as filicin, floroglucine and cautchouc (Duke 2001). In *Polypodium vulgare* resin was produced and accumulated in the form of vesicles in the majority of the rhizome parenchyma cells (Zenkteler and Grzybek 1995). Our previous studies showed that the resin (a mixture of polyphenols, cinnamyl derivatives, terpenoids, and fatty acids) plays an important role in restriction of numerous soil borne microorganisms from *P. vulgare* rhizoplana (Zenkteler 1995). In other desiccation tolerant species *Parthenium argentatum* resin biosynthesis is regulated by environmental conditions, especially by water stress. It has been shown that, when under water stress RWC fell to values < 30 %, water and osmotic potentials fall to -3.5 MPa. These data suggested a significant osmotic adjustment in the stressed plants (Mills 1994).

Analysis of the cell ultrastructure revealed that the plasma membranes of storage parenchyma were intact after the first dehydration of rhizomes pretreated with ABA, while those without the ABA treatment had ruptured. Continuity of the plasma membrane was essential to the survival of the parenchyma cells. The presence of di- and oligosaccharides and antioxidants has been correlated with desiccation tolerance in *Polypodium* because these compounds can stabilize cell internal membranes during water stress (Bagniewska-Zadworna and Zenkteler 2002). It was hypothesized that resurrection ferns that are adapted to a severe water stress may maintain a greater capacity for accumulation of disaccharides supported by starch hydrolysis. This has been shown in an experiment conducted to assess the dehydration tolerance of *Polypodium vulgare* under controlled conditions (Bagniewska-Zadworna and Zenkteler 2003).

This work has demonstrated a highly protective function of abscisic acid in *Polypodium* cells experiencing a severe dehydration. ABA appears to help in maintenance of membrane integrity in those cells.

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