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The effect of dehydration with or without abscisic acid pretreatment on buds regeneration from *Polypodium vulgare* L. rhizomes

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Abstract *Polypodium vulgare* L., a widely distributed fern, is water-stress tolerant. Under controlled dehydration conditions (20% mannitol, 9 h) without and with abscisic acid (ABA) pretreatment (2 mg l^{-1}, 24 h), dehydration tolerance and regenerative, potential of common polypody rhizomes was investigated. We demonstrated the positive effect of ABA on changes in dehydrated rhizome metabolism. ABA pretreatment reduced electrolyte leakage from cells: it has also a role in regulating sucrose accumulation and thus, osmotic adjustment. Our findings confirm that *P. vulgare* rhizomes are well adapted to stress conditions through maintaining of ability to bud formation by dehydrated and rehydrated rhizomes.

Keywords Abscisic acid (ABA) · Dehydration tolerance · Electrolyte leakage · *Polypodium vulgare* · Solute accumulation

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Abbreviations

ABA	Abscisic acid
(+ABA)	ABA-treated rhizomes
(−ABA)	ABA-untreated rhizomes
DEH	Dehydration
REH	Rehydration
RWC	Relative water content
Ψ_{osm}	Osmotic potential

Introduction

Water deficit stress in plants can be caused not only by soil drought but also by inaccessibility of water. Major strategies of plant resistance to desiccation rely on avoidance or tolerance of dehydration. Mechanisms at both the whole plant level and the cellular level can contribute to the avoidance of desiccation during the plant life cycle. Most vascular plants are not tolerant to desiccation. Only a small group of plants, called resurrection or poikilohydric plants, are desiccation tolerant (DT) and have the ability to survive repeated water loss from their vegetative tissues (Bewley 1979). Approximately 60–70% of pteridophytes belong to resurrection plants (Oliver et al. 2000). Proctor and Tuba (2002) mention 21 genera of DT pteridophytes, including *Polypodium*. *Polypodium vulgare* L. is an example of DT ferns that can withstand successive dry periods in their life cycles (Kappen 1964). This species is well adapted to withstand salt, heat stress and frost also.

According to Proctor and Tuba (2002), there are two kinds of plant adaptations to desiccation, independently of taxonomic position. The first, includes factors

preserving integrity of membranes and macromolecules and the second, includes structural adaptations and protection against oxidative stress. Moreover exposure of vegetative tissues to exogenous abscisic acid (ABA) also enhances dehydration tolerance in plants (Wang et al. 2002). An increasing body of evidence indicates that both desiccation and endogenous or exogenously applied ABA are able to induce changes in content, usually synthesis of stress proteins (Reynolds and Bewley 1993a, b; Ditzer et al. 2001); some compatible cellular solutes, like proline (Bandurska 2000); sugar alcohols, like mannitol (Adebe et al. 2003); soluble carbohydrates (Pukacka and Pukacki 1997; Whittaker et al. 2004); quaternary ammonium compounds, like betaine; and other low-molecular-weight metabolites (Morgan 1984). In higher plants these osmolytes guarantee osmotic adjustment and play an important role in cell function, as they participate in prevention of changes in membrane permeability and enzyme deactivation (Crowe et al. 1987; Kermode 1990). Cell membrane stability is one of the best physiological indicators of water deficit tolerance, which prevents leakage of solutes and electrolytes (Blum 1988).

Abscisic acid content increases in plants exposed to environmental stresses (Bianco-Trinchant and Le Page-Degivry 1998; Bandurska and Stroiński 2003). An increase in endogenous ABA in response to desiccation may arise from an increase in ABA biosynthesis enzymes and/or a decrease in ABA breakdown (Bray 2002). ABA is a root-to-shoot chemical long-distance signal of stress conditions, being a regulatory link between stress factors and plant responses (Jackson 2002; Wilkinson and Davies 2002). How ABA signal and signal transduction events are traduced into a physiological and biochemical response is a very interesting subject of many research projects in recent years. It is proposed that ABA is transported via xylem from roots to leaves, is responsible for stomatal closure (Comstock 2002; Holbrook et al. 2002), and is involved in the regulation of expression of a number of genes in plant tissues (Bartels et al. 1990; Bray 2002). The genes that are not associated with ABA-dependent pathways and are intensively expressed during desiccation in plants, are called ABA-independent genes (Bray 1997). Desiccation, which induces ABA accumulation, also leads to the up-regulation of the antioxidant defence system through reduction of toxin accumulation and free radical damage (Jiang and Zhang 2004; Yoshida et al. 2004). In addition, the vast majority of vegetative desiccation-tolerant plants can survive dehydration only if water loss is slow, extended over several hours to days (Reynolds and Bewley 1993a, b). However,

ABA treatment can stimulate desiccation-tolerance in rapidly dried tissues—incubation for at least 18 h is necessary for water stress survival (Reynolds and Bewley 1993a).

Thus ABA-dependent dehydration tolerance is connected with minimisation of mechanical changes associated with turgor loss, maintenance of the functional integrity of macromolecules and cell membranes, and initiation of repair mechanisms upon rehydration, because not only successful survival during desiccation but also the recovery after stress are crucial. For these reasons, *P. vulgare* rhizomes were exposed to three successive mannitol dehydrations alternating with rehydrations in water, with or without a preceding ABA treatment.

The aim of this work was to evaluate the ability of *P. vulgare* rhizome to bud formation after dehydrations and the relationship between dehydration and application of exogenous ABA.

Materials and methods

Rhizome dehydration

Adult sporophytes of *P. vulgare* L. were collected from the wild in Mosina near Poznań, Poland. Leaves and roots were cut off, and the rhizomes were watered to full turgor before treatment. The whole rhizomes were incubated for 24 h in 2 mg l^{-1} abscisic acid (+ABA) solutions or in water—20 ml per 1 g of tissue (–ABA; as was described earlier, Zenkteler and Bagniewska-Zadworna 2005). Plant material was dehydrated in mannitol solution (200 g l^{-1}, 10 ml per 1 g of tissue) for 9 h. Rehydration was performed in water (20 ml per 1 g of tissue) for 15 h at 21 ± 2°C.

Estimation of tissue water deficit in rhizomes was determined on the basis of relative water content: RWC = $[(W - DW_1)/(TW - DW_2)] \times 100\%$, where W = sample fresh weight after treatment, TW = sample turgid weight, and DW = sample dry weight.

Determination of electrolyte leakage

After each dehydration treatment, the pieces of rhizomes (0.5 g) were immersed in 5 ml of bidistilled water for 24 h at room temperature. The impedance technique was used to estimate the specific conductivity of exudates. The impedance spectrum was measured by modified method of Lewandowski and Świderska (2003), applying the Atlas Sollich System, Poland. Then, the same rhizomes were killed by boiling for 30 min in caped glass tubes, and after 24 h,

the electrical conductivity of the solution was measured for the second time. The percentage of electrolyte leakage (EL) was expressed as described by Pukacka and Czubak ([1998](#)), following the formula: $EL = [C_0(C_0 + C_1)^{-1}] \times 100\%$, where C_0 is the conductance after 24-h incubation, C_1 is the conductance after boiling rhizomes at 100°C. The EL was treated as an indicator of the membrane permeability in rhizome tissues.

Ion content

Determination of the content of potassium, sodium and calcium ions was performed using inductively coupled plasma optical emission spectrometry method (Fernandes et al. [2005](#)). Dry weight of rhizomes was done by incubation at 120°C for 48 h, and then the material was milled. Samples (0.5 g) were flooded for 24 h with nitric acid and perchloric acid solution and then mineralised in this solution first at 130–140°C, next at 200–210°C. Samples were fed into the plasma using a peristaltic pump. Identification and quantification of each ion was measured with an optical emission spectrometer coupled with a computer, which controlled the analysis.

Determination of cellular osmotic potential

Osmotic potential (Ψ_{osm}) of rhizome cell sap was estimated by using freeze-point osmometer (Fiska, Uxbridge, MA, USA) in mOsm. The osmometer was calibrated with standard NaCl solutions; according to Sun ([2002](#)), and osmotic potential of the cell sap (in MPa) was calculated according to the published standard curve from a series of solutions. As mentioned earlier, all rhizomes were watered to full turgor before the experiment. According to Alves and Setter ([2004](#)), to investigate the possible osmotic adjustment during successive treatments, the amount of water lost after dehydrations was recorded and the values were corrected and normalised to the common water content, and the observed osmotic adjustment was calculated using the formula: $\Delta\Psi_{osm} = \Psi_{osm(control)} - \Psi_{osm(stress)}$.

Sucrose analysis

High-pressure liquid chromatography was used to perform sucrose determination (Bonn and Bobleter [1984](#)). Pieces of rhizomes (3 g of fresh weight) were homogenised in water. Determination of sucrose was carried out on MERCK-HITACHI system consisted of autosampler (model L-7250), pump (model L-7100)

and refractive index detector (model L-7490). Analyses were performed isocratically at flow rate 0.8 ml min^{-1} at 30°C, on column Aminex HPX-87H, 300 mm × 7.8 mm (BIO-RAD). Sulphuric acid (0.005 M) as a mobile phase was used. Samples were filtered (0.45 μm, Millex-GS, Millipore) and injected to column. Standard was used to identify peaks in chromatograms, and peak area was used to determine the samples' concentrations. It was done by computer integration (Chromatography Data Station Software) operated in the mode of external standard.

Starch determination

Starch content was measured using the modified Ewers' polarimetric method (Radley [1976](#)). Rhizomes were dried at 120°C for 48 h and milled. Twenty-five millilitres of 2.56% HCl was added to 2.5 g of samples and solutions were shaken. Erlenmeyer' flasks with materials were placed in a beaker of boiling water and stirred for 15 min, then solutions were immediately cooled to room temperature (+20°C). Samples were treated with 5 ml of Carrez' solutions [10.6% of potassium hexacyanoferrate (II) trihydrate and 21.9% of zinc acetate dihydrate with 3 g of glacial acetic acid]. Each sample was filtered and starch content was measured at $\lambda = 589.3$ nm. The data were expressed in % of dry weight and counted over to μM of starch (triacetate) per 1 g of dry weight.

ABA content

High-performance liquid chromatography-modified method according to Bandurska and Stroiński ([2003](#)) was used for the estimation of ABA content. After each dehydration, ABA-untreated organs were frozen and stored at –20°C until analysis. Pieces of rhizomes (3 g of fresh weight) were homogenised in 80% methanol. For estimating extraction efficiency, ABA was added during homogenisation. Extractions were centrifuged at 2,800g for 10 min, microfiltered through 0.45 μm filters and injected on column. Determinations of ABA were carried out on MERCK-HITACHI system with DAD (model L-7455) set at 260 nm. Analyses were performed isocratically at flow rate 1 ml min^{-1}, at 30°C, on column Lichrospher®RP-18, 250 mm × 4 mm. Methanol/water/acetic acid (40:60:1) as a mobile phase was used. Quantity inject of samples—40 μl. Standard (±-cis,trans-ABA) was used to identify peaks in chromatograms, and peak area was used to determine the samples' concentrations. The identity of each peak was confirmed as described earlier.

Transmission electron microscopy analysis

Pieces of rhizomes (about 2 mm³) were fixed with 4% glutaraldehyde and 4% paraformaldehyde mixture (1:1; pH 6.8) for 2 h and post-fixed with 1% osmium tetroxide (OsO₄) for 2 h, as was described by Zenkteler and Bagniewska-Zadworna (2005). Samples were dehydrated in a graded acetone series (from 10 to 100%) and embedded in the low-viscosity Spurr's resin (Spurr 1969). Ultrathin sections (0.1 μm), obtained using an ultramicrotome, were stained for 10 min with uranyl acetate and lead citrate (Reynolds 1963) and examined with a JEM 1200 EX II (Jeol, Japan) transmission electron microscope, operating at 80 kV. On average, five samples from three different rhizomes were investigated.

Test of rhizome ability to regeneration

For estimation of rhizome survival rate, the rehydrated rhizomes were placed on Petri dishes on humid blotting paper for 48 h in the dark and then for a month of preculture in the growth chamber (continuous light: 40 μM m⁻² s⁻¹ of photon fluxes at 21 ± 2°C). During the preculture the number of developed meristems and shoot tips were calculated and the time, which was indispensable for the beginning of bud development was estimated.

Reagents and statistical analysis

The reagents were obtained from the following companies: Sigma (St Louis, MO, USA), Polysciences (Warrington, PA, USA) and Polish Chemical Reagents (Gliwice, Poland).

Statistical analysis was conducted using STATISTICA PL 6.0 software (StatSoft Poland Inc., Tulsa, OH, USA)

Results

The analysis of the rhizomes after dehydration in a hypertonic solution of mannitol showed that in ABA-untreated tissues the RWC was significantly reduced. After three successive dehydrations, the RWC values decreased from 100 to 68.9, 56.3 and 55.8%, respectively (Fig. 1—values inside the bars). By contrast, in ABA-treated rhizomes, the reduction of RWC values was not so high and reached 77.7% after the third dehydration. After rehydrations of ABA-treated organs, they recovered fresh weight and their RWC was similar to the control. The RWC of ABA-untreated

organs after 15 h of rehydrations was slightly lower than in the control (on average 97.7%). ABA pretreatment facilitated water recovery also at the end of experiments.

Measurements of electrical conductivity of the exudates indicated that under water-deficit conditions, EL from the examined rhizomes increased during the successive dehydrations (Fig. 1). Ion leakage was significantly more strongly reduced ($P < 0.001$) in ABA-treated rhizomes (solute leakage after the third dehydration from the ABA-untreated was almost one-third higher than from the ABA-treated). The increasing ion leakage was correlated with lower RWC values of ABA-untreated rhizomes (Fig. 1). Dehydration, imposed on ABA-untreated rhizomes induced an increase in membrane permeability.

As demonstrated in Table 1, the content of K⁺, Na⁺, and Ca²⁺ in ABA-untreated rhizomes declined after successive dehydrations and rehydrations. This confirmed that some changes in membrane's permeability and EL occurred in this variant of the experiment (during 9 h of mannitol dehydration followed by rehydration in water). On average, the highest decline in ion content of ABA-untreated organs was recorded for sodium ions (36%), while the lowest, for potassium ions (17%) after the third treatment cycle (Table 1). In ABA-treated rhizomes, sodium ion content did not change significantly after the first dehydration/rehydration in comparison to the control. Just after the successive treatments, the Na⁺ content declined. ABA application seems to promote also the lack of changes in Ca²⁺ content even after the second dehydration and in K⁺ content after the first dehydration (Table 1). Accumulation of these three ions in ABA-treated
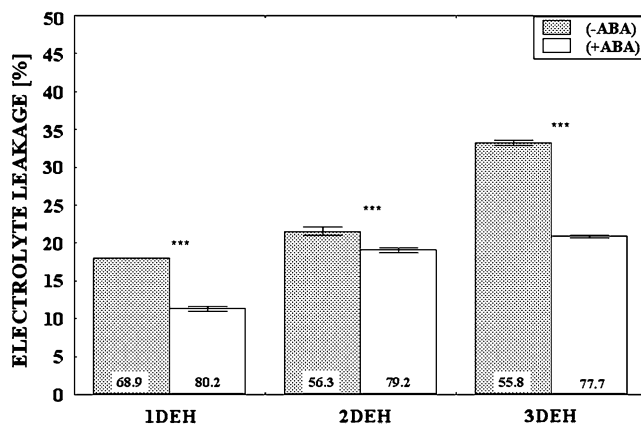

Fig. 1 EL from ABA-untreated and -treated rhizomes of *P. vulgare* after successive dehydrations. *Values* inside the *bars* mean the RWC rhizomes (%); ±means SE; ***$P < 0.001$ according to Student's *t*-test

Table 1 Inorganic ions content after successive dehydrations/rehydrations in *P. vulgare* rhizomes without (–ABA) and with (+ABA) abscisic acid pretreatment

Treatment	K^+ (mg g^{-1} d.w.)		Na^+ (mg g^{-1} d.w.)		Ca^{2+} (mg g^{-1} d.w.)	
	(–ABA)	(+ABA)	(–ABA)	(+ABA)	(–ABA)	(+ABA)
Control	11.92 ± 0.12 a	11.92 ± 0.12 a	0.28 ± 0.00 a	0.28 ± 0.00 a	5.75 ± 0.02 a	5.75 ± 0.02 a
1DEH	10.81 ± 0.16 b	11.81 ± 0.16 ab***	0.24 ± 0.01 b	0.28 ± 0.01 a**	5.01 ± 0.01 b	5.73 ± 0.05 a**
1REH	10.45 ± 0.42 bc	11.39 ± 0.03 bc***	0.23 ± 0.01 bc	0.26 ± 0.01 ab*	5.02 ± 0.01 b	5.58 ± 0.03 b***
2DEH	10.45 ± 0.04 bc	11.04 ± 0.06 c**	0.22 ± 0.00 cde	0.24 ± 0.01 bc**	4.91 ± 0.02 c	5.66 ± 0.02 ab***
2REH	10.00 ± 0.01 cd	10.97 ± 0.06 c**	0.21 ± 0.01 cd	0.23 ± 0.00 cd*	4.84 ± 0.02 cd	5.19 ± 0.05 c**
3DEH	9.96 ± 0.05 cd	10.25 ± 0.30 d	0.19 ± 0.00 de	0.22 ± 0.00 d**	4.80 ± 0.04 d	5.17 ± 0.05 c***
3REH	9.86 ± 0.24 d	10.20 ± 0.25 d	0.18 ± 0.00 e	0.20 ± 0.00 d**	4.42 ± 0.04 e	4.98 ± 0.02 d***

Means designated by the same letter do not differ significantly, at the level according to Tukey's HSD test; * represents differences between ABA-treated rhizomes and untreated ones

$*P < 0.05$, $**P < 0.01$, $***P < 0.001$ according to Student's *t*-test

organs is certainly associated with a possibility of osmotic adjustment in rhizome tissues.

After rhizome incubation in the hypertonic solution of mannitol, osmotic adjustment (the reduction of osmotic potential in relation to the control) was estimated. The osmotic adjustment of rhizome cells was the highest after the first dehydration and amounted to 0.2 MPa (Fig. 2). The extent of Ψ_{osm} reduction of ABA-treated rhizome cells was even more negative, causing osmotic adjustment by 0.25 MPa after the first dehydration (Fig. 2). After successive treatments, osmotic adjustment also occurred. Possibly, concentrations of the compounds protecting the rhizome against dehydration increased. All measurements of osmotic adjustment showed significant differences between dehydrated or rehydrated rhizomes with and without ABA incubation (Fig. 2).
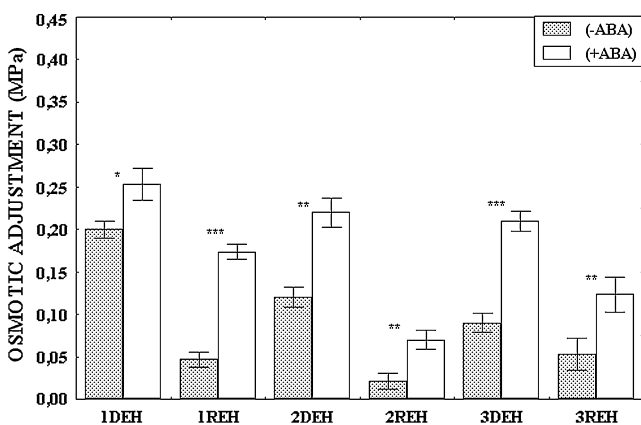

Fig. 2 Osmotic adjustment in *P. vulgare* rhizomes after successive dehydrations and rehydrations without and with ABA pretreatment (reduction of osmotic potential in relation to the control; values of Ψ_{osm} were corrected for water loss during dehydrations); ±means SE; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ according to Student's *t*-test

Additionally, the observed osmotic adjustment was associated with an increase in sucrose content in the dehydrated rhizome cells (Fig. 3a). All rhizomes accumulated large amounts of sucrose in response to only 9 h of water deficit. ABA pretreatment also promoted synthesis of this carbohydrate. Sucrose content increased after successive dehydrations. In this case, the most dynamic changes were observed after the first dehydration; on average, sucrose increased most strongly in ABA-treated organs (by 23%). In ABA-untreated rhizomes, the sucrose content increased only by 8%. After successive rehydrations, the content of sucrose decreased and after the second and third rehydrations, it was similar to the control. The sucrose content of ABA-treated rhizomes was statistically higher than that of the untreated ones after successive dehydrations and rehydrations (except for the second rehydration, Fig. 3a). On the contrary, ABA treatment lowered starch content after successive dehydrations, in comparison to non-stressed, control rhizomes (Fig. 3b). The starch content declined strongly after the first dehydration by about 24% (–ABA) and 31% (+ABA), as compared to the control. After rehydrations, when sucrose content decreased, starch content increased (Fig. 3a, b). It is possible that starch hydrolysis products could be a substrate for sucrose synthesis. The differences in starch content between ABA-treated and -untreated rhizomes were statistically significant ($P < 0.05$, except for the third dehydration and rehydration).

Interestingly, our results proved that the endogenous ABA content in the control amounted to 22 nM g^{-1} d.w. (±0.15 SE) and did not change significantly after the mannitol dehydration treatment of examined rhizomes (Table 2).

Under a transmission electron microscope, we observed that amyloplasts in rhizome storage parenchyma
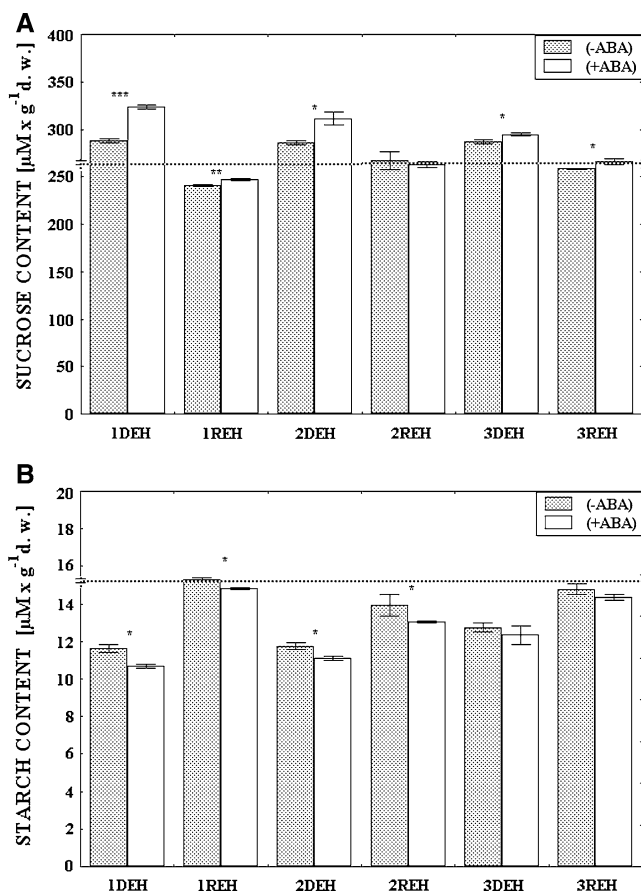
Fig. 3 Effects of successive dehydrations and rehydrations *P. vulgare* rhizomes and the ABA pretreatment on sucrose (**a**) and starch (**b**) content. Interrupted line shows control values; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ according to Student's *t*-test

cells included usually one big starch grain, which almost completely filled the amyloplast (Fig. 4a). After dehydration, we observed a reduction in the size and an increasing number of starch grains and sometimes, light areas in the stroma of amyloplasts in rhizome storage parenchyma cells. Amyloplast contained structures resembling thylakoids and few plastoglobuli (Fig. 4b, c). In the vast majority of amyloplasts, membranes were not degraded.

Table 2 Abscisic acid content after successive dehydrations in *P. vulgare* rhizomes

Treatment	ABA content (nM g^{-1} d.w.)
Control	22.00 ± 0.15 a
1DEH	22.02 ± 0.14 a
2DEH	21.86 ± 0.56 a
3DEH	22.43 ± 0.76 a

Means designated by the same letter do not differ significantly, at the level according to Tukey's HSD test; ±means SE

Dehydrated *P. vulgare* rhizomes lost their turgor and became flaccid. The colour of rhizome tissues changed from orange or light brown (in the control) to dark brown (after dehydrations). ABA-untreated organs were more browned than treated ones. The rhizomes maintained their ability to bud formation. Five to seven days after the beginning of preculture in the light, the initiation of bud development was observed through the shoot tips becoming green. The lower decline in RWC in ABA-treated rhizomes was not correlated with a higher survival rate of shoot tips after dehydration. ABA-untreated organs showed a delay in shoot tip development only for a few days (Fig. 5a). The survival rate of ABA-treated and -untreated rhizomes was also comparable to the control (after 22 days of preculture, Fig. 5). The number of developed shoot tips after dehydration amounted to 7 (±0.5 SE) for every 10 cm of rhizome and showed no significant differences in comparison to the control. These results confirmed that mannitol dehydration was not a sublethal stress for rhizomes of *P. vulgare*, because a sublethal stress would exert a stronger influence on their potential for meristem development.

All these results suggest that ABA application might enhance the dehydration tolerance of rhizome tissues of *P. vulgare*.

Discussion

The physiological and biochemical processes that occurred during dehydration of *P. vulgare* rhizomes determined the responses of tissues to stress. In the current study, water-stress was applied by three repeated dehydration/rehydration cycles. During the exposure to mannitol dehydration, the rate of water loss in rhizomes was not rapid.

Water-stress causes changes in membrane structure and composition, and causes significant cell membrane injuries and ion leakage from plant tissues (Blum 1988). The increasing leakage from ABA-untreated rhizomes indicates that some changes in membrane permeability occurred, but EL reached only a maximum of 32.7%. It has been estimated that if EL exceeded 50%, disorders in membrane permeability would be serious. That would lead to death, especially after long-term or rapid desiccation (Bewley 1979). Dehydration resulting from water-deficit could directly lead to the generation of reactive oxygen species, which react with membrane phospholipids, causing their de-esterification and finally membrane degradation (Huang 2001). Although oxidative stress is a primary component of desiccation-induced membrane
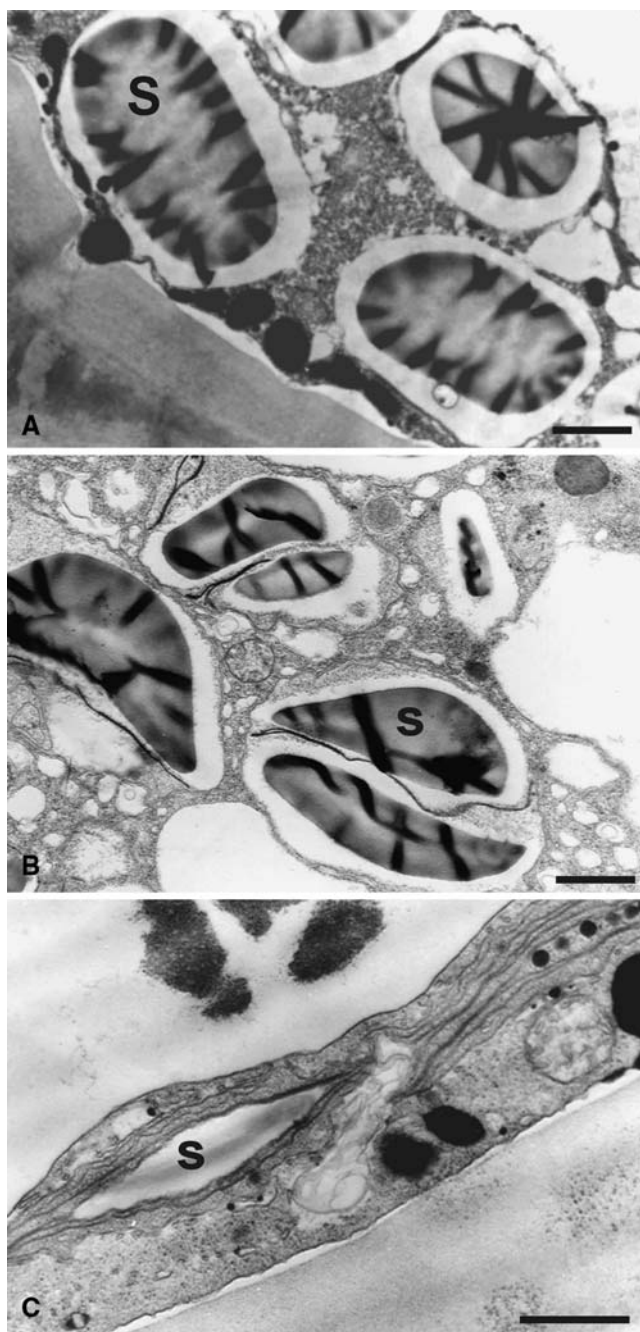
Fig. 4 Starch grains (*s*) in amyloplasts of control (**a**) and dehydrated (**b**, **c**) rhizome parenchyma cells. Note the smaller size of starch grains after successive treatments, the increased number of plastoglobuli and structure of remaining thylakoids; bars = 1 μm

Fig. 5 Development of buds from dehydrated ABA-untreated (**a**) and -treated (**b**) *P. vulgare* dehydrated and rehydrated rhizomes, after preculture

injuries, there exist antioxidative systems, which protect against membrane degradation (Mittler 2002). EL from plant tissues concerns mainly potassium ion. Symptom of potassium deficiency as the light areas in amyloplast stroma after dehydration of *Zea mays* (Heght-Buchholtz and Marschner 1970) was also ob-
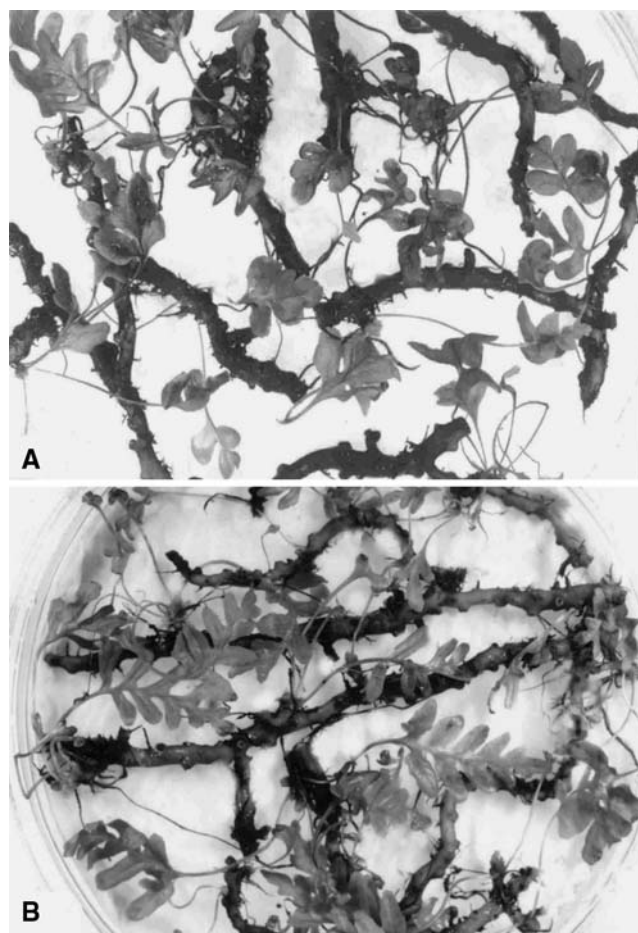
served in the amyloplasts of *P. vulgare* rhizome parenchymatic cells. More interesting is our finding that exogenously applied ABA limited EL from dehydrated rhizomes also, irrespective of the analysed ion. Ion content in ABA-untreated rhizomes declined after successive dehydrations and rehydrations, which confirmed that some changes in membrane's permeability occurred even during the experiment. Furthermore, the induction of ion accumulation by ABA could also provide a greater opportunity for osmotic adjustment, so the ABA-induced accumulation of inorganic ions (K^+, Na^+ and Ca^{2+}) correlated with a lower osmotic potential. Similarly, Wang et al. (2002) demonstrated that concentrations of inorganic ions were higher if orchid protocorms were treated with ABA.

In ABA-treated rhizomes the reduction of water loss was lower, while osmotic adjustment was higher. This means that ABA-treated tissues contained more water than untreated ones and they accumulated or synthesised more osmolytes in cells to increase the

extent of osmotic adjustment. Our results are in agreement with earlier observations showing that ABA pretreatment promotes a decline in osmotic potential in fresh and dried cells (Wang et al. 2002, 2003). Alves and Setter (2004) claimed that potassium salts and proline (but not soluble sugars) were the major contributors to osmotic adjustment in cassava leaves (from 0.14 to 0.51 MPa).

In this study, we demonstrated that sucrose was accumulated after dehydration of *P. vulgare* rhizomes. A major part of osmotic adjustment after treatments (both dehydrations and rehydrations) might originate from the accumulation of soluble sugars, especially sucrose. However, a decrease in sucrose content after rehydration proved that this adjustment was due also to the accumulation of other metabolites, which were not determined in this work (also of mannitol, in which rhizomes were incubated).

It is well known that inducible protection mechanisms are necessary to survive during desiccation. There are many reports on solutes being synthesised and accumulated in response to dehydration, especially soluble sugars (Crowe et al. 1987). The increase in sugar content concerned: sucrose or other disaccharides and oligosaccharides, such as raffinose and stachyose. An important role in induction of dehydration tolerance in plants is played by sucrose to raffinose ratio (Black et al. 1999). Furthermore, basing on the presented data, we propose that ABA application induced sucrose synthesis in dehydrated rhizomes. Earlier, Scott (2000) also reported on an increase in sucrose accumulation induced by endogenous ABA in *Craterostigma* leaves. This could be associated with the ability of resurrection plants to survive dehydration.

Soluble sugars are a major factor in vitrification—the formation of a glassy matrix in the cytoplasm of dehydrated cells (Hoekstra et al. 2001; Bruni and Leopold 1991). Moreover, sugars play a role in the maintenance of the stability of plasma membranes through water substitution in membranes (Crowe et al. 1987). Our results, as well as those presented by Reynolds and Bewley (1993a) for *Polypodium virginianum* leaves, indicated also that during the desiccation sucrose content increased and starch content declined. Possibly, the products from starch hydrolysis could be the substrate for sucrose synthesis. Whittaker et al. (2001) and Veramendi et al. (1999) proposed that carbon, required for sucrose synthesis, might originate from starch breakdown. Another possible source of carbon is 2-octulose, which is accumulated in hydrated leaves of *Craterostigma plantagineum*. During desiccation, as sucrose was accumulated, 2-octulose was metabolised (Bartels and Salamini 2001).

For many years it has been generally accepted that ABA content increases when water content declines. ABA increases even six- to sevenfold in the dehydrated leaves of *Craterostigma* (Bartels et al. 1990). Bandurska and Stroiński (2003) proved that during only 30 min of polyethylene glycol treatment, ABA content in leaves increased. There are many results confirming it, like those in the literature cited by Alves and Setter (2004). Interestingly, in the more primitive, non-vascular plant *Tortula ruralis*, which is one of the best-known resurrection mosses, ABA did not occur (Bewley et al. 1993). Dehydrations of maize roots and leaves in ethylene glycol had no effect on ABA accumulation, in contrast to dehydration in polyethylene glycol. Both tissues accumulated ABA during air-drying (Jia et al. 2001). Reynolds and Bewley (1993b) proved that in cut-off leaves of the desiccation-tolerant fern *P. virginianum*, ABA did not increase in dried tissues after 12 h of slow dehydration; it even decreased after 10 days of drying (when their fresh weight declined to 20%). At the same time, application of exogenous ABA enabled survival of detached leaves even after a rapid desiccation. Our results are compatible with their observations. In the polystelic rhizomes of *P. vulgare*, this ABA level is probably mostly due to the ABA normally circulated and transported in xylem sap and phloem sap. Most likely, this phytohormone might be rather synthesised in roots under water-deficit and then transported to shoots and leaves. The mannitol dehydration treatment is a slow-rate water stress; ABA content might increase after a rapid water loss. Dehydration tolerance in rhizomes of *P. vulgare* appears to be mediated by both ABA-dependent and ABA-independent signalling pathways. Perhaps, a further analysis concerning free and bound (ABA-GE—glucose ester) ABA content could be helpful to determine how ABA is involved in dehydration tolerance of *P. vulgare* rhizomes, because Sauter et al. (2002) postulated that in many plants under stress conditions, ABA-GE concentration was higher than the concentration of free ABA. This could explain the lack of changes in ABA content in dehydrated rhizomes. It is still unclear how the endogenous and exogenously applied ABA are involved in induction of fern dehydration tolerance.

The proposed technique of mannitol-dehydration was useful to investigate if dehydrated and rehydrated rhizomes maintained the capability to bud formation. Both ABA-treated and -untreated ones maintained this ability that confirmed that dehydration does not derange rhizome morphogenic activity. Similar effects were observed in desiccation tolerance and successful rewatering of model DT vascular plant *C. plantagineum*,

which is fully functional within 24 h and its reproductivity had been activated within 14 days upon watering (Scott 2000). Bryophytes and some pteridophytes recover during few hours (Proctor and Tuba 2002) but the slowest documented recovery of DT plant is for *Myrothamnus flabellifolia*—48 h (Scott 2000). In the case of ABA-treated *P. virginianum* fronds, recovery is possible after 24 h of rehydration following only slow dehydration (Reynolds and Bewley 1993a).

In summary, our results prove the positive effect of ABA on changes in *P. vulgare* rhizome metabolism during dehydration. ABA pretreatment reduced EL from cells and induced synthesis of soluble sugars. Our results and earlier evidence clearly show that ABA has a role in regulating solute accumulation and thus osmotic adjustment and adaptation to stress conditions through maintaining of ability to bud formation. Our findings confirm that the fern tissues did not synthesise ABA during dehydration of detached organs if water loss was not severe.

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