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Influence of dehydration rate on cell sucrose and water relations parameters in an inducible desiccation tolerant aquatic bryophyte

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ABSTRACT

During desiccation, plant cells are subjected to very low water potentials. Osmoregulation through increase of soluble materials (*e.g.* soluble sugars, compatible inorganic ions) is a response to the decreasing turgor pressure in the cells. In bryophytes, sucrose acts as an osmolyte and also stabilizing membranes and proteins through vitrification. We used psychrometric measurements in the aquatic bryophyte *Fontinalis antipyretica* Hedw. to construct pressure–volume isotherms and determine the water relations parameters under fast and slow dehydration rates. Sucrose was also quantified. The starting hypothesis was that a slow dehydration rate would increase sucrose concentration, thereby decreasing the osmotic potential at turgor loss point, and would also increase cell wall elasticity, postponing turgor loss and allowing time for induction of molecular and structural acclimation mechanisms. In fact, we found that slowly dehydrated samples presented more elastic cell walls, allowing cells to shrink and maintain turgor, helping to better preserve their metabolic functions and therefore to induce desiccation tolerance (DT). On the other hand, in fast dehydrated samples the osmotic potential at turgor loss point decreased, indicating the activity of osmoregulation processes, possibly connected to the increase observed in sucrose content. Upon rehydration, fast dried samples lost 50% of the sucrose through leakage due to cell membrane rupture, while slow dehydrated leaves maintained their sucrose content constant. DT appears to be achieved through slow dehydration, meaning that a high sucrose content alone does not contribute to DT establishment. Moreover, in natural conditions external water can be maintained at very high values due to the life form of *F. antipyretica*, which grows in long and compact floating stems in streams, allowing a slow dehydration rate required for induction of other DT mechanisms.

1. Introduction

Bryophytes are typically poikilohydric plants, with water content depending on the humidity of the surrounding atmosphere. Unlike vascular plants, these organisms lack conducting tissues, absorbing external water directly. In addition to the symplast water fraction (within the protoplasts) and the apoplast water fraction (cell walls and spaces between cells) also present in vascular plants, a third fraction, the external capillary water, must be taken into account (Dilks and Proctor, 1979; Beckett, 1997; Proctor et al., 1998; Proctor and Tuba, 2002). Bryophytes also exhibit different mechanisms to delay water loss and are often able to tolerate extreme desiccation (Proctor and Pence, 2002).

The water loss that takes place when tissues are exposed to a drying atmosphere can be counteracted by a decrease of the cellular osmotic potential achieved by increasing soluble molecules concentration, thereby maintain homeostasis and preserving cell structure and function. The construction of pressure–volume (*PV*) curves allows the determination of several water relations parameters that describe this process (Santarius, 1994; Beckett, 1997; Proctor et al., 1998; Hájek and Beckett, 2008), allowing the assessment of osmoregulation.

Osmotic adjustment enables turgor to be maintained during mild dehydration, involving compatible solutes, namely potassium and sugars such as sucrose (Ingram and Bartels, 1996). This sugar has an important role in desiccation tolerance (DT), acting as an osmoregulator and preventing denaturation of important

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macromolecules and membranes, contributing to biological vitrification of the cytoplasm of dried cells, slowing down damaging reactions with reactive oxygen species and maintaining cell structure intact (Crowe et al., 1992; Smirnoff, 1992). High concentrations of sucrose were found in dry tissues of DT plants (Buitink et al., 2002). In vascular DT plants, such as *Craterostigma plantagineum* Hochst., the sugar 2-octulose is present at high contents in well-watered conditions. During dehydration it is converted to sucrose which can increase up to 40% of dry weight (DW) (Ingram and Bartels, 1996). In the bryophyte *Syntrichia ruralis* (Hedw.) F. Weber & D. Mohr, sucrose makes up approximately 10% of the DW and its concentration is maintained during desiccation and rehydration (Bewley et al., 1978), while for the bryophyte *Physcomitrella patens* (Hedw.) Bruch & Schimp, it remains at 4% of DW (Oldenhof et al., 2006). Nevertheless, the impact of the drying rate in tissue sucrose concentration has been scarcely studied in bryophytes (Cruz de Carvalho et al., 2014).

Drying rate is crucial for DT induction in bryophytes (Stark et al., 2013; Greenwood and Stark, 2014), namely in the aquatic bryophyte *Fontinalis antipyretica* Hedw., where slow drying was previously shown to be essential (Cruz de Carvalho et al., 2011, 2012, 2014). The aim of the present work was to investigate how contrasting (fast and slow) dehydration rates change cell water relations in an aquatic bryophyte and how those changes explain the need for slow dehydration to induce DT. Sucrose content was evaluated as well as the role of this sugar on cell osmoregulation. The initial hypothesis was that sucrose content is essential to DT induction and is related with dehydration rate, leading to differences in water relation parameters, especially in osmotic potential. Thus, a higher sucrose content would be expected in a slow dehydration rate in order to osmoregulate, compensating the water loss and postponing the shutdown of the metabolism, allowing time for acclimation and to attain the high cell survival previously observed (Cruz de Carvalho et al., 2011). For the same reasons, a higher cell wall elasticity was expected in the slow dehydrated samples.

2. Materials and methods

2.1. Plant material and culture conditions

Bryophyte samples of *F. antipyretica* were collected in a shaded stream bordered by *Quercus pyrenaica* Willd., *Castanea sativa* Mill., and *Cistus* sp. woodlands at the *Serra de S. Mamede Natural Park*, central Portugal in a clean stream, away from important human impact. Samples were transported to the laboratory under cooling conditions (about 5 °C) and cleaned of debris and sediments in distilled water. Bryophyte were grown in a modified Knop culture medium (Traubenberg and Ah-Peng, 2004) under controlled conditions (17 °C day/13 °C night, photosynthetic photon flux density [PPFD] of 20–30 $\mu mol\ m^{-2}\ s^{-1}$ and photoperiod of 16 h). Samples were left in the lab for no more than 60 days before analysis. In the water relations assay, each sample consisted of a single 1 cm shoot tip, while in the sucrose assay samples consisted of ten shoot tips bundled together. Relative water content (RWC) was calculated according to Deltoro et al. (1998). After blotting any external water from the tips and before drying, samples where weighted to determine full turgor weight (FTW). After the dehydration treatment, fresh weight (stress weight) was determined. At the end of the assays, dry weight (DW) was determined by placing the samples at 80 °C for 48 h.

2.2. Dehydration induction

Different dehydration rates were attained by placing shoots in small containers over saturated salt solutions of K_2SO_4 (slow dehydration, 95% relative humidity [RH], −6 MPa, 0.08 ± 0.01 g $H_2O\ g^{-1}$ dry weight h^{-1}, *circa* 24 h) and $Ca(NO_3)_2{\cdot}4H_2O$ (fast dehydration, 50% RH, −100 MPa, 0.74 ± 0.12 g $H_2O\ g^{-1}$ dry weight h^{-1}, *circa* 3 h). During this period, conditions where maintained under controlled temperature (*circa* 20–23 °C) and at low PAR (2–5 $\mu mol\ m^{-2}\ s^{-1}$). In the water relations assay, slow dehydration was attained by air drying bryophytes at lab conditions (*circa* 80% RH) a value lower than the one used in previous works (Cruz de Carvalho et al., 2011, 2012, 2014), but still high enough to allow slow dehydration (0.25 ± 0.02 g $H_2O\ g^{-1}$ dry weight h^{-1}, *circa* 8 h). Rehydration was made through immersion in culture medium for 72 h under similar growth conditions.

2.3. Water potential determination and water relation components calculation

Slow dehydrated (blotted and non-blotted) and fast dehydrated (blotted) samples were placed in a small steel cup (319 mm^3) and introduced in a C-52 chamber (Wescor Electronics, Logan, USA) linked to a PsyPro microvoltmeter (Water Potential System, Wescor Electronics, Logan, USA) for an equilibration period of 30 min before water potential (ψ) measurement. Between measurements, samples were allowed to lose water inside small containers containing a saturated salt solution (see previous description of dehydration induction). Five standard solutions of NaCl (0, 0.05, 0.25, 0.5 and 1 molal) where used to establish a calibration curve. After RWC determination, *PV* curves where plotted as $-1/\psi$ *versus* 1 − RWC. All water relations components were calculated according to Hájek and Beckett (2008). At low ψ, turgor does not contribute to ψ and the *PV* curve is linear. Apoplastic water was determined as the *x*-intercept of the linear portion of the *PV* curve. Osmotic potential (ψ_π) at full turgor ($\psi_{\pi s}$) was calculated as the *y*-intercept of the same linear portion of the *PV* curve. Turgor potential (ψ_p) was determined as the difference of the extrapolated linear portion and the real curve. For simplification of visualization, it was not plotted on the graph. Turgor loss point (TLP) was determined at the point where ψ_p reached zero, where it was also determined the osmotic potential at turgor loss point ($\psi_{\pi TLP}$). Above TLP, ψ_p increased linearly until full turgor was achieved and, after plotted against relative water content (RWC), cell walls elasticity modulus (ε) was determined as the slope of the linear function in this correlation (for more details see Supplementary information). Blotting was an efficient method to remove all capillary water, not affecting the course of water potential below RWC = 100% (see Supplement data, Fig. 2)

2.4. Sucrose quantification

Soluble sugars extraction was performed as described by Arrabaça (1981) on blotted shoots submitted to the small container drying method described in Section 2.2. Shoots with approximately 0.1 g (control: $n = 17$; treatments: $n = 4$) were quickly grinded with 500 µl of 80% (v/v) ethanol in a mortar with pestle at room temperature. The homogenate was quickly transferred to tubes with 1 ml of 80% (v/v) ethanol and placed for 5 min at 80 °C. Extracts were cooled on ice, and then centrifuged at 16,000 × *g*, for 5 min in a microcentrifuge (Centrifuge 5415C, Eppendorf). The supernatant was transferred to another tube, evaporated in nitrogen (N_2) flow, resuspended in 1 ml of ultra-pure water and filtered through 0.45 µm filters (Dinistrat Filter RC 25, Sartorius). Sucrose quantification was determined following the resorcinol (1,3-dihidroxybenzene) method (Roe, 1934). Briefly, 100 µl of the extract or the recovery solution were added to 0.25 ml of resorcinol solution (1% diluted in absolute ethanol (w/v)), and to 0.75 ml of a solution of 30% HCl (w/v), mixed and incubated at 80 °C for 8 min. Samples were cooled on ice and absorbance at 405 nm