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Research article

Relation between water status and desiccation-affected genes in the lichen photobiont *Trebouxia gelatinosa*

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ABSTRACT

The relation between water status and expression profiles of desiccation -related genes has been studied in the desiccation tolerant (DT) aeroterrestrial green microalga *Trebouxia gelatinosa*, a common lichen photobiont. Algal colonies were desiccated in controlled conditions and during desiccation water content (WC) and water potential (Ψ) were measured to find the turgor loss point (Ψ_{tlp}). Quantitative real-time PCR was performed to measure the expression of ten genes related to photosynthesis, antioxidant defense, expansins, heat shock proteins (HSPs), and desiccation related proteins in algal colonies collected during desiccation when still at full turgor (WC $>$ 6 g H_2O g^{-1} dry weight), immediately before and after Ψ_{tlp} (-4 MPa; WC $\sim$ 1 g H_2O g^{-1} dry weight) and before and after complete desiccation (WC $<$ 0.01 g H_2O g^{-1} dry weight), quantifying the HSP70 protein levels by immunodetection. Our analysis showed that the expression of eight out of ten genes changed immediately before and after Ψ_{tlp}. Interestingly, the expression of five out of ten genes changed also before complete desiccation, i.e. between 0.2 and 0.01 g H_2O g^{-1} dry weight. However, the HSP70 protein levels were not affected by changes in water status. The study provides new evidences of the link between the loss of turgor and the expression of genes related to the desiccation tolerance of *T. gelatinosa*, suggesting the former as a signal triggering inducible mechanisms.

1. Introduction

Desiccation tolerance is the ability to survive and recover metabolism after drying to 0.1 g H_2O g^{-1} of dry mass (Kranner et al., 2008; Farrant et al., 2012). This value corresponds to a water potential (Ψ) of ~ -100 MPa or even lower (Fernàndez-Marin et al., 2016). Desiccation tolerant (DT) organisms survive in this state for ecologically relevant periods of time and resume a normal metabolic activity in minutes to days, as soon as water becomes available again. Desiccation tolerance can be found in phylogenetically distant taxa, from fungi to nematodes, rotifers, and tardigrades (Alpert, 2006; Gaff and Oliver, 2013) and is typical of organisms which colonize substrates or environments with little and unpredictable water availability (Nardini et al., 2013). DT photoautotrophs include cyanobacteria, micro-algae, lichens, bryophytes, several clubmosses and ferns and a few hundred adult angiosperms plus most of the angiosperms at the embryo stage (as seeds) (Alpert, 2006). Aero-terrestrial microalgae, in particular, have a global distribution and typically occur in biofilms on soil, rocks, leaves, tree barks, and man-made substrata (Lüttge and Büdel, 2010), being very important for primary production and nutrient cycling (Holzinger and Karsten, 2013). Furthermore, some taxa form long-living, stable symbiosis with DT fungi, *i.e.* lichens, and *Trebouxia* (Chlorophyta) is the most common and widespread genus of lichen photobionts (Hawksworth et al., 1995). All Trebouxias (c. 30 species; (Friedl, 1989)) are DT, and the mechanisms of their desiccation tolerance are not yet understood.

To cope with the effects derived by water loss, DT photoautotrophs apply multiple strategies. In most DT vascular plants, in which desiccation usually occurs in terms of days, the protection/repair mechanisms are activated by desiccation itself (Kranner et al., 2002; Gasulla et al., 2013). DT non-vascular plants, on the other side, are subjected to frequent cycles of desiccation/rehydration lasting even a few minutes and thus they mostly rely on constitutive protection mechanisms (Fernàndez-Marin et al., 2016; Alpert, 2006; Candotto Carniel

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et al., 2016).

When desiccation is moderate, DT photoautotrophs accumulate compatible osmolytes that allow osmotic adjustments (Fernàndez-Marin et al., 2016). An increased expression and accumulation of dehydrins, late embryogenesis abundant proteins, and heat shock proteins (HSPs) is commonly observed (Gechev et al., 2012). Desiccation causes impairment of redox equilibrium, and both vascular and non-vascular DT photoautotrophs must avoid oxidative damage caused by reactive oxygen species (ROS) (Dinakar et al., 2012). An effective antioxidant system based on protective enzymes and non-enzymatic molecules (Kranner et al., 2005) is one of the most important prerequisites for desiccation tolerance (Holzinger et al., 2011).

During desiccation, one of the major threats is the loss of cell turgor, because the cell starts to shrink (Honegger et al., 1996) and is subjected to mechanical and biochemical modifications that could lead to irreversible damages (Fernàndez-Marin et al., 2016; Dinakar et al., 2016). For these reasons, cell turgor loss is considered one of the best indicators of water stress in plant science (Dinakar et al., 2016).

At physiological level, turgor loss can be monitored by measuring the water potential (Ψ), that assesses the water status in terms of potential energy per unit volume. At the same time, morphological modifications caused by desiccation, including turgor loss, can be monitored at microscopic level with the use of special chambers that allow observations of samples equilibrated at various relative humidity (RH) (Lajos et al., 2016). Ψ measurements are not regularly applied in lichenological studies (Lange et al., 2007; Hartard et al., 2009) as they are more time consuming than weighing to quantify the water content. However, Ψ is definitely more informative, because it allows a description of the water status derived on irreversible thermodynamics, and the discrimination between extra- and intracellular water loss (Nardini et al., 2013). Ψ of distilled water is 0 MPa, and the potential of cells in equilibrium is slightly negative due to the solutes (González and Reigosa Roger, 2001). When the cells lose water during desiccation, the decrease of turgor pressure (P_t, MPa) and/or the increase of solutes concentration cause a drop in the water potential (González and Reigosa Roger, 2001). When P_t reaches 0, cell turgor is lost, Ψ is mostly determined by the osmotic potential (*i.e.* cell solutes concentration) and known as Ψ at turgor loss point (Ψ_{tlp}). This parameter describes the ability of an organism to maintain cell turgor in the face of fluctuating water availability (Lenz et al., 2006). Below Ψ_{tlp}, important metabolic processes start to slow down or decrease their efficiency, such as photosynthesis (Petruzzellis et al., 2017). It is reasonable to think that this point might be perceived by the cells which then trigger some countermeasures, starting from changes in gene expression and followed by changes of protein and metabolite levels to cope with the modifications induced by water loss.

To date, few studies have been conducted on the turgor loss response in DT non-vascular plants (Holzinger and Karsten, 2013), and an estimation of Ψ in relation to water status would be important to understand if and how this process is involved in desiccation tolerance of these organisms.

Up to date, a single work was performed considering both Ψ and RWC in the lichen photobiont *Trebouxia sp.* TR9 (Centeno et al., 2016), showing that, at metabolomic level, water status affects mainly cell wall, extracellular polysaccharides, polyols and antioxidant protection. Candotto Carniel et al. (2016) showed that desiccation tolerance of *Trebouxia gelatinosa* Archibald mostly relies on constitutive mechanisms, but desiccation and rehydration affect also the gene expression of components of the photosynthetic apparatus, the ROS-scavenging system, HSPs, expansins, and desiccation related proteins (DRPs). The latter, firstly described in the resurrection plant *Craterostigma plantagineum,* have been linked to desiccation tolerance (Bartels et al., 1990). One of these DRPs, predicted to exist as small gene family (pcC13-62, (Piatkowski et al., 1990)), share similarities with *T. gelatinosa* DRPs (Candotto Carniel et al., 2016).

Here, we aimed at understanding how the water status of *T. gelatinosa* causes changes in the expression of stress- and desiccation-related genes. We monitored the expression of selected genes related to desiccation tolerance in colonies of *T. gelatinosa* during desiccation in order to verify whether the loss of turgor can trigger inducible tolerance mechanisms.

2. Materials and methods

2.1. Cultures of Trebouxia photobiont

Trebouxia gelatinosa was isolated following Yamamoto et al. (2002) from thalli of *Flavoparmelia caperata* (L.) Hale collected in the Classical Karst (NW Italy). The algal cultures were subcultured on solid Trebouxia Medium (TM; 1.5% agar) (Ahmadjian et al., 1973) every 30–45 days and kept in a thermostatic chamber at 18 ± 1 °C and 20 ± 2 μmol photons $m^{-2} s^{-1}$ with a light/dark regime of 14/10 h and 53 ± 2% RH.

Axenic cultures of *T. gelatinosa* were inoculated using 100 μl of a water suspension of approximately 3.5×10^6 cells mL^{-1} on cellulose acetate membranes (25 mm diameter, pore size 0.45 μm, Sartorius Lab Holding GmbH), which were laid on 25 mL of solid TM (1.5% agar) (Ahmadjian et al., 1973) at the bottom of Microbox Junior 40 vessels (Duchefa Biochemie), equipped with a micro-filter strip on the cover which allows gas exchange while keeping the internal volume in sterile conditions. The vessels, each containing six membranes, were kept in the thermostatic chamber at the same conditions reported above. On the 30th day of growth, three groups of colonies (weight: 0.22 ± 0.01 g) were used for Ψ_{tlp} assessment, desiccation treatment and chlorophyll *a* fluorescence measurements.

2.2. Desiccation treatment

Sixty-three colonies were randomly (Lehmer Pseudo random number generator) selected from the vessels and placed in groups of seven along the border of single filter paper discs (Whatman, 60 ± 5 g m^{-2}, 25 mm diameter) wetted with 100 μl of distilled water inside plate lids, and left to desiccate at the air of the thermostatic chamber, at the same conditions described above.

During desiccation, 9 sampling points were selected as defined in a preliminary experiment performed at the same conditions, in which the decreasing weight of fully-hydrated *T. gelatinosa* colonies was followed over time with a precision balance. In the final experiment, at each sampling point (T0-T8), one plate lid with seven colonies was randomly selected and one of its colonies was placed in a dew point water potential meter (WP4, Decagon Devices, Inc.) to measure the water potential (Ψ). Then this colony was gently transferred from the membrane to a pre-weighed labeled 1.5 mL tube, soaked in liquid nitrogen and freeze-dried for 48 h. After freeze-drying, the tube was weighed on a precision balance to obtain the dry weight (DW) of the colony.

Just before finishing the Ψ measurement, the other six colonies were transferred on the plate lid from the membrane to pre-weighed labeled 1.5 mL tubes, weighed on a precision balance for water content (WC) estimation, soaked in liquid nitrogen and stored at −80 °C. For the last time point (T8), colonies were kept in silica-gel for 24 h. The six colonies of each plate lid represent six replicates of the same sampling point (sample), which were referred to the specific Ψ measured on the seventh replicate.

The water content (WC) was expressed as g H_2O g^{-1} dry weight and calculated as WC = [(FW – DW)/DW], where the fresh weight (FW) was the weight at the sampling point, and the dry weight (DW) was the average weight of the freeze-dried colonies. The relative water content was calculated as RWC = [(FW – DW)/(IFW - DW)] × 100 following Nardini et al. (2013), where the water lost until the decline of Ψ was interpreted as extracellular, and subtracted from the T0 fresh weight to get the initial fresh weight (IFW), which was considered the weight at full turgor (Nardini et al., 2013).