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ANTIOXIDANT CAPACITY AND MEMBRANE PERMEABILITY UNDER REWETTING RECOVERY OF *PORELLA PLATYPHYLLA* (L.) PFEIFF., A DESICCATION-TOLERANT LIVERWORT

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Abstract: Relatively constant photosynthetic pigment content (chlorophyll *a*, chlorophyll *b*, and carotenoids) in *Porella platyphylla* (L.) Pfeiff. when experiencing frequent desiccation/rehydration cycles support photosynthetic efficiency, directly influencing its growth and survival and essential for resilience against oxidative stress and its ability to maintain cellular integrity. When actual pigment composition reports healthy physiological condition and functionality the homoiochlorophyllous desiccation tolerance 'machinery' plays a crucial role in the antioxidant defense mechanisms of the liverwort. After a short period of desiccation (1 week), an approximately 200% increase in DPPH inhibition was shown during the first hour of rehydration, indicating a substantial enhancement in antioxidant activity. Folin-Ciocalteu (F-C) assay revealed exceptionally high polyphenol content for *P. platyphylla*, whose values were significantly higher than those typically reported for a wide range of plants. There is a need for further research to explore the specific types of polyphenols present in *P. platyphylla* and their bioactive properties or determine the potentially overestimated polyphenol content by F-C assay. The antioxidant capacity of *P. platyphylla* in mg $FeSO_4$ equivalent appears to be relatively low. However, it shows a remarkable increase in the antioxidant capacity after 48 hours of rehydration, with an increase of almost 900% compared to the initial 24 hours. The difference in the results from the two antioxidant capacity assays (DPPH, FRAP) for the same sample can be attributed to the specific types of antioxidant molecules present in *P. platyphylla*. GC-MS analysis has revealed the presence of several metabolic compounds, those amounts decreased during rehydration. D-turanose was identified as a substantial component. Membrane permeability after a short period of desiccation in *P. platyphylla* recovered at 90% during the 48 hours of rewetting, while after a medium-long period of desiccation, the recovery was 50%, and only 5-10% after a very prolonged desiccation.

Keywords: *Porella platyphylla*, antioxidant capacity, desiccation tolerance, rehydration, membrane permeability, photosynthetic pigments

INTRODUCTION

Bryophytes represent a very diverse and non-homogeneous group of plants (Shaw *et al.* 2011). Most species tested inhabit shady and moist environments, such as damp trees and rocks, or alongside streams and pools. However, the bryophytes from other ecological groups, and also from open habitats (Wolski *et al.* 2021), are often overlooked. Bryophytes are recognized for their high content of biologically active compounds (Asakawa and Ludwiczuk 2018), extensively used in ethnopharmacology for treating wounds and burns. Specifically, bryophytes exhibit antibacterial, antifungal, antiviral, antioxidant, antiplatelet, antithrombin, insecticidal, neuroprotective properties, and antiproliferative activity against cancer cells (Cheng *et al.* 2012; Vollár *et al.* 2018). These cryptogamic plants are utilized for various purposes across many cultures because of the diverse compounds they contain, such as terpenoids, simple benzoic, cinnamic, and phthalic acid derivatives, coumarins, and nitrogen-containing aromatic compounds like benzonaphthol-xanthenones. The chemical composition of bryophytes can vary significantly depending on their taxonomic group, leading to a wide range of properties. Factors contributing to this diversity include habitat, seasonal changes, water and moisture exposure levels, and materials absorbed from the environment (Heinrichs 2000).

Porella platyphylla, a leafy liverwort (Özenoğlu Kiremit and Keçeli 2009), thrives in shady and moist environments like many bryophytes. A key feature of *Porella platyphylla*, as with many bryophytes, is its desiccation tolerance (DT) the ability to withstand extreme drying and resume normal metabolic activity upon rehydration (Marschall and Proctor 1999). Desiccation tolerance is considered an ancient evolutionary trait, present across all kingdoms of life, from cyanobacteria to angiosperms. Even desiccation-intolerant species may harbor the genes necessary for this trait, though they may not always express them. Desiccation tolerance (DT) is common in poikilohydric bryophytes, but not universal. Among vascular plants (homoiohydric), true desiccation-tolerant (abbreviated also as DT) plants are rare, occurring in only 0.15%. As DT bryophytes dry out, they pass through the water stress levels at which DT vascular plants succeed but only briefly face these water conditions, i.e. they only temporarily face the problem of continuing their metabolism under water stress (Proctor *et al.*

2007). DT bryophyte cells have 2 stable states: one is the full turgor state and the other is the desiccated state. *P. platyphylla*, alternate between active photosynthesis and metabolic dormancy, drying out when water is unavailable and resuming activity upon rehydration. DT bryophyte's tolerance to desiccation is greatly influenced by the relative length of time spent in the two states, the intensity of desiccation, and the associated temperature and light conditions. Vascular DTs do not survive desiccation if it is too rapid (less than 12 h), as 'time-consuming' inductive cell defense mechanisms (e.g. protein synthesis) are required for the development of their desiccation tolerance. DT bryophytes can tolerate dehydration for short periods (up to 1 h, but typically 3 h) and fully recover their metabolism after rewetting. A fundamental difference in their strategy compared to vascular DTs is the existence of an active rehydration-induced repair and regeneration mechanism, which is not based on the transcriptional activity of well-defined tolerance genes, but on a much faster translational control. It is an ABA-independent constitutive defense system that incorporates some key elements of the inducible system characterized in vascular plants. The meaning of this constitutive system is that it pre-expresses protective proteins, pre-forms mRNA-protein complexes, and maintains their levels during desiccation. Furthermore, a constitutive trait is that the amount of osmotically active carbohydrates (mainly sucrose) is significant and their levels are virtually unchanged during desiccation and rewetting.

Upon rehydration, recovery of respiration, photosynthesis, and protein synthesis takes place within minutes or an hour or two; recovery of the cell cycle, food transport, and the cytoskeleton may take 20 hours or more. Sustaining a positive carbon balance is essential for enduring recurrent cycles of desiccation and rewetting. Significant growth requires continuously wet periods of a few days or more. The mechanisms of DT in bryophytes, including expression of LEA proteins, high content of non-reducing sugars, and effective antioxidant and photo-protection, are at least partly constitutive, allowing survival of rapid drying, and employing an active rehydration-induced repair and recovery mechanism (Proctor *et al.* 2007; Marschall 2010).

Studies on *Porella platyphylla* have demonstrated its ability to recover rapidly from desiccation. Modulated chlorophyll fluorometry has been used in research to investigate *P. platyphylla*'s

photosynthetic response to desiccation and subsequent rehydration, providing insights into the plant's physiological systems under stress. According to a study (Marschall and Proctor 1999), *P. platyphylla* quickly regains its ability to photosynthesize after being desiccated for seven days. Whether in low light or complete darkness, the maximal quantum yield (F_v/F_m) and effective quantum yield (ΦPSII) both revert to normal after two hours of rehydration. Relative water content (RWC) decreased dramatically during drying, with both parameters falling below 0.5; however, photochemical quenching was substantially unaffected. Under stress, non-photochemical quenching (*NPQ*) rose and peaked after recovery in light. However, within 24 hours, *NPQ* reverted to normal levels, suggesting that photosynthetic performance was temporarily impacted.

Its carbohydrate metabolism is another crucial factor contributing to *P. platyphylla*'s desiccation tolerance. Starch and reducing sugars are present at relatively low concentrations in *P. platyphylla*. Sucrose and fructan are the main soluble carbohydrates. Sucrose and fructans have shapes suitable for association with the polar head groups of phospholipids in place of water and for preventing damaging phase transitions in membranes during desiccation. Additionally, these sugars maintain a vitreous phase in the cytoplasm of desiccated cells, which minimizes protein denaturation. Fructans can be inserted between the head groups of different kinds of phospholipids with some preference for phosphatidylethanolamine. They are the key regulators of adaptation to various environmental stresses, act as antioxidants, scavenging ROS, and prevent cell damage under abiotic stress conditions. Fructan-accumulating species contain only traces (~1%) of starch, which means that fructan is a real alternative to starch. Fructans accumulate in the vacuole, where they play an important role in turgor regulation. More molecules mean these cells are more resistant to osmotic pressure or cold. The size of fructan polymers can be altered quickly; this could be an explanation for their role in osmotic adjustment. Fructans likely protect plants from various environmental stresses such as frost and drought by stabilizing membranes. Starch synthesis drops dramatically when the temperature decreases below 10°C, but photosynthesis and fructan production are much less sensitive to low temperatures, suggesting that fructan production benefits those plants, which actively

photosynthesize during the winter and early spring. The soluble carbohydrate pool is well-balanced in *P. platyphylla* (sugar feeding, dark starvation, desiccation, and low temperature have little effect), and fructans are conserved at the expense of a substantial sucrose stock. In higher plants, a negative correlation between sucrose concentration and soluble acid invertase activity was observed. The high sucrose concentration coupled with high acid invertase activity in *Porella platyphylla.* Dark starvation significantly increased the activity of acid invertase, i.e. acid invertase is active in the dark. Glucose and fructose treatment in the dark significantly decreased the activity of the enzyme. When applied in the light, it increased, a role associated with fructan accumulation, consistent with an increase in the concentration of low molecular weight carbohydrates. Desiccation reduced the glucose, fructose, and sucrose content of the leaves. One hour after rehydration, glucose, and fructose contents were 5 times higher than before dehydration. Sucrose and fructan contents were at pre-desiccation levels 1 hour after rehydration. During desiccation, the amount of low molecular weight fructans decreased while that of high molecular weight fructans increased, suggesting that fructan polymerization occurred during desiccation. The concentration of fructans increased under low water potential, doubling at Ψ=-0.62 MPa, while specific acid invertase showed only a small increase at Ψ= -4.5 MPa. According to this regulation, invertase is crucial for maintaining a balance between energy consumption and sucrose preservation throughout the plant's desiccation recovery. These adaptation strategies highlight *P. platyphylla*'s resilience to severe dehydration (Marschall *et al.* 1998).

In addition to its carbohydrate metabolism, *P. platyphylla* produces a wide range of secondary metabolites, including sesquiterpenoids and diterpenoids. Along with three known pinguisanes and the sacculatane perrottetianal B, studies have identified a new sesquiterpenoid derivative of pinguisanoic acid, methyl 2α-hydroxy-6-oxo-11-pinguisanoate, and a new hemiacetal sacculatane diterpenoid, (13S)-15ξ-hydroxysacculaporellin, from the liverwort. These findings further highlight the chemical diversity of *P. platyphylla* and contribute to our understanding of its secondary metabolism (Buchanan *et al.* 1996).

While the desiccation tolerance of. *P. platyphylla* has been well studied (Marschall *et al.* 1995; Marschall 1998; Marschall *et al.* 1998; Marschall and Proctor 1999; Marschall 2010; Marschall and Sütő 2022) but its antioxidant capacity remains relatively unexplored compared to other bryophytes. In a recent study (Aydin 2020), the objective was to determine the free radical scavenging activities, fatty acid, and vitamin contents of *Dicranum scoparium* and *Porella platyphylla*. *Dicranum scoparium* exhibited a significantly higher DPPH radical scavenging effect compared to *P. platyphylla*. A strong correlation exists between the phenolic compound content in methanol extracts of the plants and their DPPH radical scavenging efficiency. *Dicranum scoparium* had higher amounts of D-3 α-tocopherol, stigmasterol, and betasterol. *P. platyphylla* contained higher levels of all unsaturated fatty acids except for α-linolenic acid when compared to *Dicranum scoparium* (Aydin 2020). Although, *P. platyphylla* showed lower DPPH scavenging activity in this study, its high unsaturated fatty acid content indicates it could still be a potent antioxidant.

Additionally, *P. platyphylla* has demonstrated significant anti-oxidant capacity (AC) and high total phenolic compound (TPC) content, particularly in the n-butanol fraction. A prominent phenolic acid, p-hydroxybenzoic acid (p-HBA), is found in free form and likely contributes to the strong antioxidant activity observed in the species (Yildirim Akatin *et al.* 2024). These findings suggest that *P. platyphylla* could serve as a valuable source of bioactive compounds with potential applications in biotechnology and ecological adaptation.

In light of its demonstrated desiccation tolerance and its potential for antioxidant activity, this study aims to further investigate the antioxidant properties, and membrane integrity of *P. platyphylla*, focusing on its unique chemical composition, and its capacity to withstand desiccation and recover quickly upon rehydration. Understanding how desiccation tolerance and antioxidant capacity are interrelated could provide valuable insights into the plant's resilience mechanisms and its potential biotechnological applications.

MATERIALS AND METHODS

Plant material

Porella platyphylla was collected from an area of limestone woodland in the Bükk Mountains, north-east Hungary, near Felsőtárkány village in the late spring season. The field history of the collected material was unknown; therefore, field-collected plants were allowed a state of uninterrupted hydration under nonstressful conditions, to lose any physiological hardening to DT that has been gained by experiencing, under field conditions, wetting and drying events (Marschall and Sütő 2022). The plants were acclimatized at 100% relative humidity (r.h.) in glass desiccators, at 20°C, at a photosynthetic photon flux density (PPFD) of 100 μmol m^{-2} s^{-1}, and a photoperiod of 12/12 h light/dark for 3 days before desiccation treatment. So-called 'natural drying' meant drying in laboratory air (at 20°C, ~35% RH, in natural light, infiltrating through the laboratory windows) was applied for 1 week. When testing membrane permeability during rewetting recovery three periods of desiccation were applied before rehydration: a short period (1 week), a medium-long period (1 month), and a very prolonged period (6 months) of desiccation. The air-dried weight of only the green parts of the liverwort samples was measured with analytical accuracy (0.02 g). Samples with known air-dried weight are used for extraction or set up for rehydration. Rewetting was carried out with distilled water. Rehydrated samples were monitored for various physiological parameters after 1, 24, and 48 hours, and kept in a desiccator with 100% RH between measurements, in natural light (outdoor, natural light infiltrating through the laboratory window) (n=3).

Determination of Pigment Content

Pigment analyses followed Lichtenthaler and Wellburn (1983). The air-dry bryophyte samples were extracted in 96% (v/v) ethanol and absorbance at 470, 649, and 665 nm read on a spectrophotometer (Varian Cary 3E).

Determination of Antioxidant Capacity – DPPH Test

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay is a well-established method for evaluating the antioxidant activity in plant extracts, leveraging the ability of antioxidants to neutralize DPPH radicals. DPPH, a stable free radical with a distinct purple color, undergoes a color change from purple to yellow upon reduction, a shift that can be quantified spectrophotometrically (λ = 517 nm) (Blois 1958; Frankel and Meyer 2000). DPPH was added to the different volumes (100, 200, 300, 400, and 500 µl) of 96% (v/v) ethanolic extracts of *P. platyphylla* to test inhibition.

Determination of Antioxidant Capacity – Folin-Ciocalteu Assay

The determination of phenol content was based on the reduction of phenolic compounds by the Folin-Ciocalteu reagent under alkaline conditions resulting in a blue-colored complex whose intensity is proportional to the total phenol content in the sample (Pérez *et al.* 2023). Ethanolic extracts (96% (v/v)) of *P. platyphylla* were used in the Folin-Ciocalteu assay. Absorbance was measured at 765 nm. The polyphenol content of the samples was calculated in terms of gallic acid equivalents.

Determination of Antioxidant Capacity – FRAP Assay

The ferric-reducing antioxidant power (FRAP) assay is based on the reducing ability of antioxidants, which involves the reduction in Fe^{3+}-2,4,6-tripyridyl-s-triazine (TPTZ) complex while taking absorbance at 593 nm (Benzie and Strain 1996). Ethanolic extracts (96% (v/v)) of *P. platyphylla* were used in the FRAP assay.

Analytical Measurements – GC-MS Analysis

For the GC-MS measurement (Perkin Elmer Autosystem XL GC and Turbomass MS), the 96% (v/v) ethanolic extracts of *P. platyphylla* underwent a derivatization procedure as follows: the extracts were first dried under a stream of N_2 (5.0) gas at room temperature. The dried samples were then redissolved in 50 µL of pyridine to prepare them for derivatization. Following this, 50 µL of BSTFA + TMCS (N, O-Bis(trimethylsilyl)trifluoroacetamide with 1% Trimethylchloro-

silane) was added, and the mixture was heated at 70°C for 1 hour to complete the derivatization reaction. After cooling, 1 µL of the derivatized samples was injected into the GC-MS for analysis, enabling precise measurement of the target compounds.

The Perkin Elmer Autosystem XL GC was equipped with a Turbomass MS series mass spectrometer. The GC-MS was installed with an HP-5 ms capillary column ((5%-Phenyl)-methylpolysiloxane phase, 30 m × 0.25 mm × 0.5 µm) (Agilent). Helium 6.0 was the carrier gas with a constant flow rate of 1.0 ml min^{-1}. The injector temperature was 270°C. Injected volume: 1 µl. Chromatographic conditions were as follows: the oven was held for 2 min at 70°C, and heated to 150°C at 25°C min^{-1}, heated to 200°C at 6°C min^{-1}, heated to 280°C at 10°C min^{-1} and held 5.2 min at this temperature. The mass spectrometer operated in the EI+ mode with a source temperature of 200°C, an ionizing voltage of 70 eV, and a transfer line temperature of 270°C. The mass spectrometer scanned masses from 30 to 650 m/z at a rate of 0.1 scan s^{-1} the detection time windows: 3 to 25.27 min. Peak identification was carried out by comparison of the derivatization sample mass spectra with spectra in the NIST/EPA/NIH Mass Spectral Database.

Measurement of ion leakage / Membrane permeability

A modified Szalai's method (Marschall and Sütő 2022) was used to determine the changes in membrane permeability. 0.1 g (fresh weight) of plant material was put in centrifuge tubes containing 20 ml ultrapure distilled water (Milli-Q 50). The samples were shaken for an hour and the electrolytes leaked from the plant cells in the water were measured using an electrical conductivity meter (C1) (Hach, HQ40D conductometer). Then the samples were boiled (at 100 ˚C) in the same tube containing the distilled water to complete membrane disruption. After cooling down to room temperature samples were shaken for an hour again, and then the membrane permeability of the samples was measured by a conductivity meter as well (C2). The final conductivity was calculated as percent ion leakage, (C1 / C2) x 100.

Statistical analysis

Statistical analysis was performed with the SigmaPlot 11.0 software (Systat Software Inc., San Jose, CA, U.S.A.) using one-way analysis of variance. The presence or absence of significant differences between each treatment was detected by the Holm-Sidak test. Where probabilities are quoted the data have been subject to analysis of variance. Errors, where indicated, are standard deviations. Measurements were performed in three separate replicates (n=3) and also 3 replicates were treated in each condition. Replicates for analysis were from three separate samples; they were not homogenized. Replicates represent a single branch. There were not necessary any transformations during the statistical analysis.

RESULTS AND DISCUSSION

Photosynthetic pigment composition

Studying photosynthetic pigments, including chlorophyll *a*, chlorophyll *b*, and carotenoids, in *Porella platyphylla* is essential for understanding its physiological condition and functionality. These pigments are integral to the liverwort's photosynthetic efficiency, directly influencing its growth and survival. In terms of antioxidant activity, chlorophylls and carotenoids can indicate the plant's capacity to mitigate oxidative stress. Both pigment types are recognized for their antioxidant properties, which protect plant cells from oxidative damage caused by reactive oxygen species (ROS). Therefore, quantifying the photosynthetic pigment content can provide valuable insights into the plant's resilience against oxidative stress and its ability to maintain cellular integrity. This assessment aligns with our DPPH inhibition findings, suggesting a link between pigment concentration and antioxidant activity. Based on preserving photosynthetic pigments during desiccation P*. platyphylla* belongs to the homoiochlorophyllous type. Our study's pigment results align with those reported by Marschall and Proctor (2004), confirming that *P. platyphylla* exhibits typical pigment concentrations associated with healthy plant samples (*Figure 1*). This accordance with the literature data not only confirms our results but also supports the hypothesis that photosynthetic pigments and the homoio-chlorophyllous desiccation tolerance 'machinery' play a crucial role in the antioxidant defense mechanisms of *P. platyphylla*.

Bryophytes can achieve photoprotection during dehydration through non-photochemical quenching processes in which xanthophylls harmlessly dissipate the excess absorbed energy as heat (Deltoro *et al.* 1998; Csintalan *et al.* 1999). Such protection seems particularly beneficial during the rehydration phase when the gametophytes experience considerable photooxidative damage.

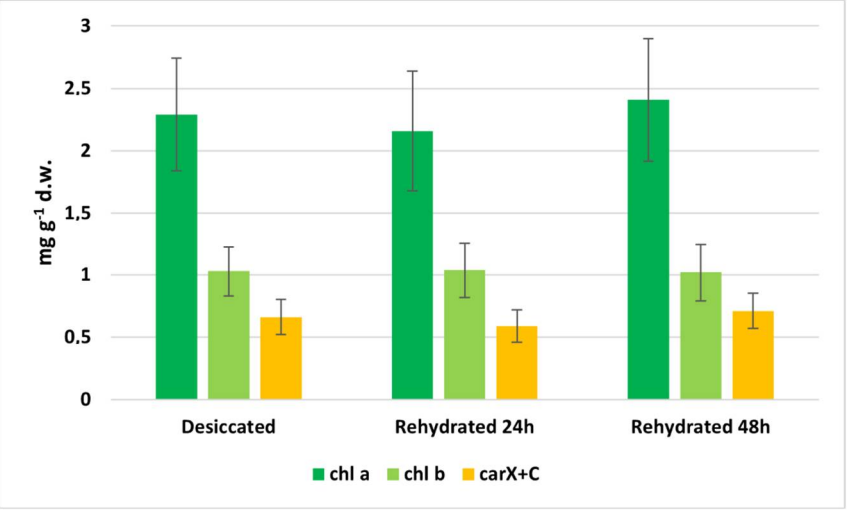

Figure 1. Photosynthetic pigment content (chlorophyll *a*, *b,* and total carotenoids in mg g^{-1} d.w.) of *Porella platyphylla* after a short period of desiccation, 24 hours and 48 hours following rehydration. Error bars are STDs, where n=3.

Antioxidant capacity

The rehydration of *P. platyphylla* resulted in an approximate 200% increase in **DPPH inhibition**, indicating a substantial enhancement in antioxidant activity over time. Initial DPPH inhibition values demonstrated significant increases upon rehydration, with the most pronounced effect observed after 1 hour following rehydration (*Figure 2*). These results suggest that rehydration may activate or amplify the liverwort's inherent antioxidant mechanisms. This increase in DPPH inhibition could be attributed to multiple factors. Rehydration may activate specific metabolic pathways that promote the synthesis of antioxidant compounds, or it may facilitate the release of antioxidants that were previously bound within the cellular structure, making them more available for free radical

scavenging. The levels of oxidative damage increase directly upon rehydration, especially in light conditions. Since electrons keep reaching the photosystems but photosynthesis is not fully recovered, ROS are highly produced upon rehydration (Minibayeva and Beckett 2001; Beckett *et al*. 2004), increasing damage during the initial phase, especially at the chloroplast level. Additionally, this ROS burst can also act, to a certain level, as a defense against pathogenic fungi and bacteria that can attack cells upon rehydration (Minibayeva and Beckett 2001). Detailed metabolic profiling and molecular studies are necessary to elucidate the precise mechanisms driving this marked increase in antioxidant activity. Additionally, environmental factors during rehydration, such as temperature and light exposure, deserve attention, as they may influence the synthesis and release of antioxidant compounds. The greatest value recorded for *P. platyphylla* in the literature for the DPPH test was 62.39% (Aydin 2020), and the results here (*Figure 2*) show that during the first hour of rehydration, it can go higher than that. Comparative studies with other bryophyte species could further illuminate the unique properties of *P. platyphylla* that contribute to its high antioxidant capacity when rehydrated.

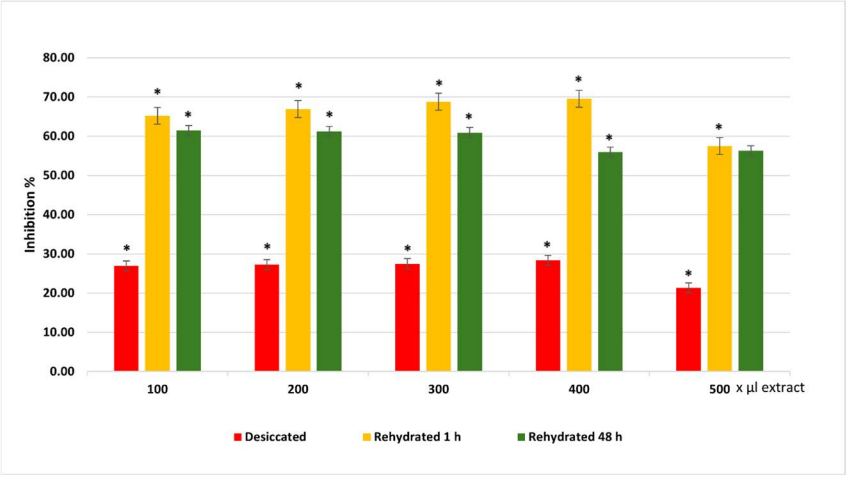

Figure 2. The antioxidant capacity of *Porella platyphylla,* as inhibition %, was determined by the DPPH test after a short period of desiccation, 24 hours and 48 hours following rehydration. DPPH was added to the different volumes (100, 200, 300, 400, and 500 µl) of 96% (v/v) ethanolic extracts of *P. platyphylla* to test inhibition. * = Statistically significant ($p<0.05$). Error bars are STDs, where n=3.

Our pilot **Folin-Ciocalteu assay** results for *P. platyphylla* reveal exceptionally high polyphenol content, with values of 44960 mg, 31700 mg, and 45130 mg of gallic acid equivalents (GAE) per gram of dry matter. These values are significantly higher than those typically reported for a wide range of plants. For context, many fruits and vegetables exhibit polyphenol content in the range of 10-100 mg GAE/g dry weight (Basu and Maier 2016), while even plants known for their high polyphenol content, such as certain berries or tea leaves, rarely exceed 300-400 mg GAE/g dry weight (Ankolekar *et al.* 2011). However, it is important to consider the Folin-Ciocalteu test is not specifically designed for phenolic compounds, as the reagent could be reduced by other nonphenolic compounds also present in the sample, with the risk of content overestimation. Our results support the need for further research to explore the specific types of polyphenols present in *P. platyphylla* and their bioactive properties. Additionally, comparative studies with other high-polyphenol plants could provide further insights into the unique attributes of *P. platyphylla* that contribute to its elevated polyphenol levels.

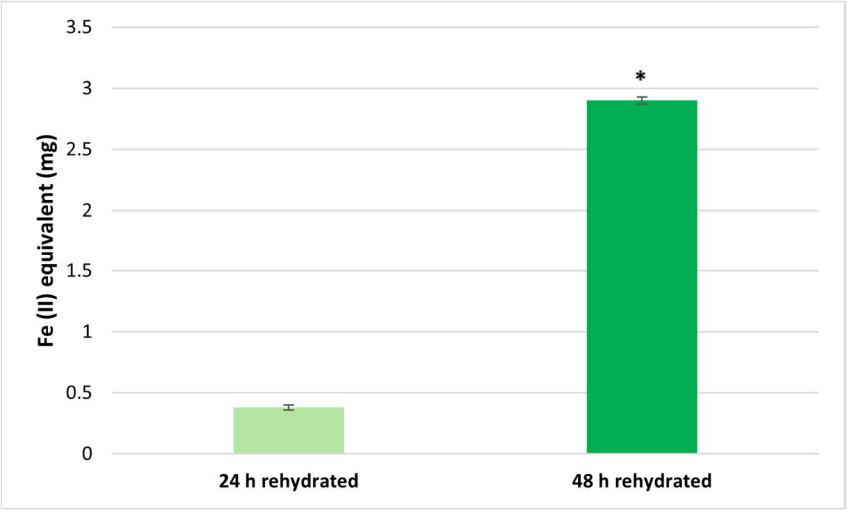

Figure 3. The antioxidant capacity of *Porella platyphylla,* as the ferric-reducing antioxidant power (FRAP) is based on the reducing ability of antioxidants, which involves the reduction in Fe^{3+}-2,4,6-tripyridyl-s-triazine (TPTZ) complex after a short period of desiccation, 24 hours and 48 hours following rehydration. Ethanolic extracts (96% (v/v)) of *P. platyphylla* were used in the FRAP assay. * = Statistically significant ($p<0.05$). Error bars are STDs, where n=3.

Our **FRAP assay** findings (*Figure 3*) indicate a remarkable increase in the antioxidant capacity of *P. platyphylla* after 48 hours of rehydration, with an increase of almost 900% compared to the initial 24 hours. When compared to the literature values for mosses such as *Ceratodon purpureus* and *Tortula muralis* (Wolski *et al.* 2021), where antioxidant activities range between 60-120 mg $FeSO_4$ equivalent, the antioxidant capacity of *P. platyphylla* appears to be relatively low. However, this significant enhancement in antioxidant activity during 48 hours of rehydration has not been previously observed in the literature, marking a novel discovery in the study of this liverwort.

The difference in the results from these two assays (DPPH, FRAP) for the same sample can be attributed to the specific types of antioxidant molecules present in *P. platyphylla*. Some antioxidants may have stronger reducing power, thus showing higher activity in the FRAP assay, while others may be more effective in scavenging free radicals, showing higher activity in the DPPH assay (*Figure 2*, *3*).

Result of the GC-MS analysis

GC-MS analysis has revealed the presence of several metabolic compounds, those amounts decreased during rehydration (*Figure* 4). The most prominent peak identified was D-turanose, a compound typically found in bryophytes. The identification of D-turanose in *P. platyphylla* is noteworthy as it provides insights into the metabolic profile of the liverwort. This sugar, along with other detected compounds, could play a role in the liverwort's response to desiccation and the subsequent rehydration and its recovery capacity. The detailed analysis of these metabolites can further our understanding of the biochemical pathways active in *P. platyphylla*, potentially linking specific metabolites to enhanced antioxidant activity observed after rehydration.

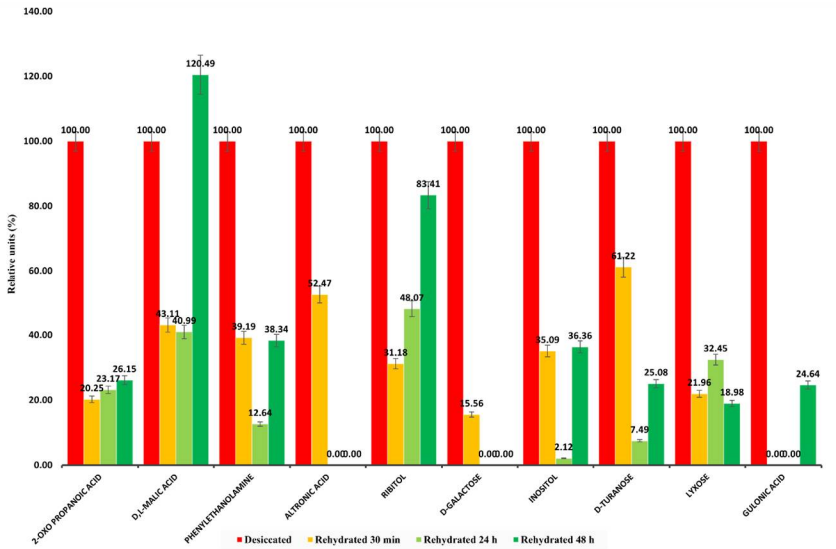

Figure 4. Metabolic components of *Porella platyphylla,* determined by GC-MS after a short period of desiccation, 30 minutes, 24 hours, and 48 hours following rehydration. Ethanolic extracts (96% (v/v)) of *P. platyphylla* underwent a derivatization procedure as written in Materials and Methods. Each component is potted in the graph in relative units (%). Error bars are STDs, where n=3.

Membrane permeability measurements

Desiccation disrupts cellular membranes in bryophytes due to mechanical stress, oxidative damage, and loss of structural integrity. This results in the leakage of ions such as K^+, and Ca^{2+} from the cell interior, and other solutes upon rehydration. Electrolyte leakage, measured as ion conductivity or solute release into rehydration water, is a critical physiological parameter used to assess cell membrane integrity and damage during these stress events. Studies report significant electrolyte leakage immediately after rehydration, indicative of membrane damage. However, many bryophytes, especially desiccation-tolerant species, show rapid repair of membranes, minimizing long-term leakage. Desiccation-tolerant bryophytes, such as *Syntrichia ruralis* and *Syntrichia caninervis*, exhibit lower initial leakage and faster recovery compared to desiccation-sensitive species. Electrolyte leakage peaks during the first few minutes to hours post-rehydration and stabilizes over time as membranes repair. Cells recover their form very rapidly after

rehydration (from 30 seconds to one minute) (Glime 2017). Desiccation-tolerant bryophytes employ protective mechanisms, including the accumulation of late embryogenesis abundant (LEA) proteins, sugars (e.g., trehalose), and antioxidant systems, which mitigate damage and leakage (Glime 2017). In the more DT species, this leakage is transient, probably due to lipid-phase transitions occurring in the plasma membrane (Crowe *et al.* 1992). In the more sensitive species, this leakage is more substantial (Crowe *et al.* 1992), eventually leading to complete loss of all intracellular content and cell death. Membrane permeability after a short period of desiccation in *P. platyphylla* recovered at 90% during the 48 hours of rewetting (*Figure 5A*). While after a medium-long period of desiccation, the recovery was 50%, and only 5-10% after a very prolonged desiccation (*Figure 5B, C*). Oliver *et al.* (1993) found that electrolyte leakage alone was not a reliable measure of desiccation tolerance in *Syntrichia ruralis*. Factors such as the rate of desiccation, exposure to light during drying, and temperature influence the extent of membrane damage and leakage. Repeated cycles of desiccation and rehydration may lead to cumulative damage, increasing electrolyte leakage in sensitive species. Bryophytes from arid environments show lower electrolyte leakage compared to species from mesic habitats, underscoring their evolutionary adaptations. Electrolyte leakage can be a more reliable measure of the effect of desiccation if the field physiological history of the collected bryophytes is known. Electrolyte leakage studies provide insights into the resilience and stress physiology of bryophytes. These findings are pivotal in understanding desiccation tolerance mechanisms, which have implications for ecosystem resilience and biotechnological applications. Further research should focus on the molecular mechanisms underpinning membrane repair in desiccation-tolerant bryophytes, and the long-term effects of repeated desiccation/rehydration cycles on membrane integrity. Comparative studies should provide data across broader bryophyte taxa to identify universal and species-specific tolerance strategies.

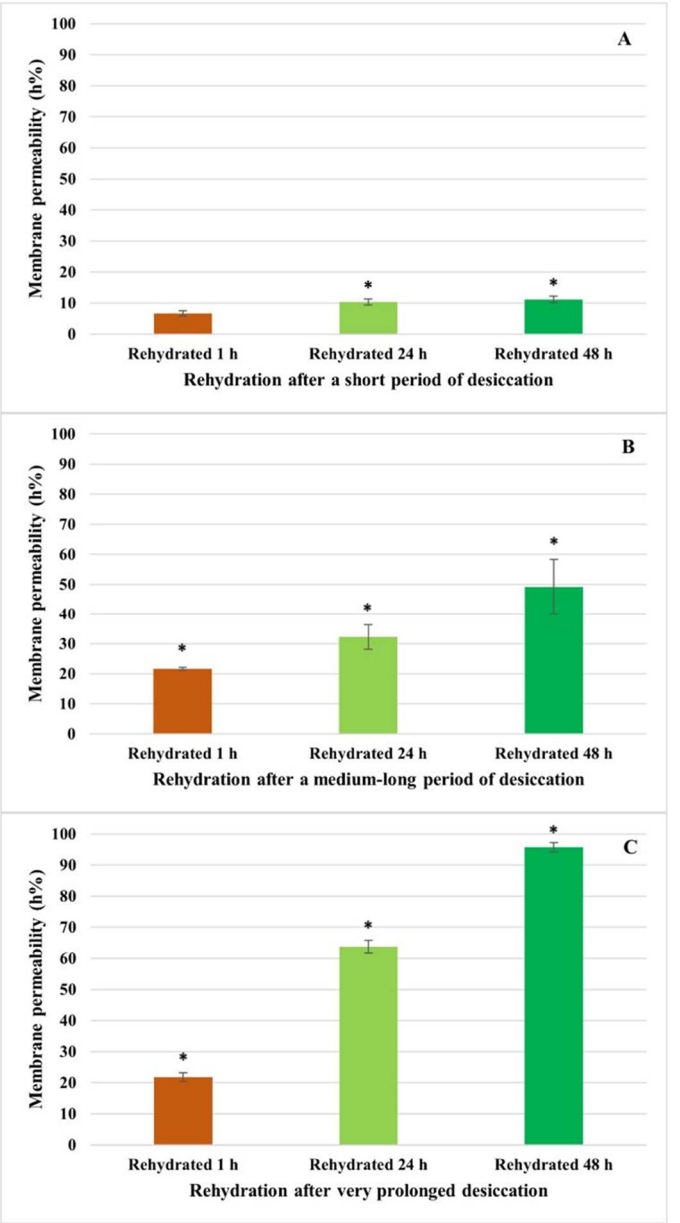

Figure 5. Membrane permeability during rewetting recovery in *Porella platyphylla*. Ion leakage (%) was measured in *P. platyphylla* **A**) after a short period (1 week) of desiccation and after 1, 24, and 48 hours of subsequent rehydration; **B**) after a medium-long period (1 month) of desiccation and after 1, 24 and 48 hours of subsequent rehydration; **C**) after a very prolonged period (6 months) of desiccation and after 1, 24 and 48 hours of subsequent rehydration. * = Statistically significant ($p<0.05$). Error bars are STDs, where n=3.

CONCLUSIONS

Porella platyphylla a desiccation-tolerant liverwort can achieve photoprotection during the frequent desiccation/ rehydration cycles through non-photochemical quenching processes in which xanthophylls harmlessly dissipate the excess absorbed energy as heat and with a help of effective antioxidant capacity especially under the rehydration phase when the gametophytes exposed to considerable photooxidative stress. *P. platyphylla* has a significant antioxidant capacity and a high content of total phenolic compounds. Further studies should focus on quantifying these metabolites and exploring their biological roles, which may reveal new aspects of how rehydration enhances the liverwort's antioxidant properties. To further understand the mechanisms behind this significant increase in antioxidant capacity, additional studies are needed. Conducting detailed metabolic profiling before and after rehydration could identify specific compounds responsible for the increased antioxidant activity. Extending the rehydration period beyond 48 hours and monitoring antioxidant capacity at various intervals could provide a clearer picture of the dynamics of antioxidant synthesis and release. Investigating the impact of other environmental stress factors, such as light, temperature, and nutrient availability, in combination with rehydration could offer insights into optimal conditions for maximizing antioxidant production. Exploring the genetic and molecular basis of the observed increase in antioxidant activity could help identify key genes and regulatory mechanisms involved in this process. Further research is needed to understand in more detail the rehydration process and to fully characterize the liverwort's antioxidant potential.

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