Metabolic strategy underlying resurrection in carpet moss, Hypnum cupressiforme Hedw. subjected to a desiccation-rehydration cycle

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Metabolic strategy underlying resurrection in carpet moss, *Hypnum cupressiforme* Hedw. subjected to a desiccation-rehydration cycle

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Abstract

In the present study, we investigated comprehensive metabolic divergence, highlighting potential desiccation- and resurrection-related traits in the carpet moss Hypnum cupressiforme Hedw. subjected to a desiccation-rehydration cycle. The high-resolution liquid chromatography-mass spectrometry (HRLC-MS) analysis revealed significant metabolic differences between desiccated and rehydrated conditions, reflected in differences in the relative abundances of metabolites, including amino acids, carbohydrates, organic acids, and secondary metabolites. The metabolic pathways linked to amino acid biosynthesis and metabolism, sugar biosynthesis, energy metabolism, and secondary metabolite biosynthesis were significantly enriched in moss upon rehydration. Besides causing a metabolic shift, considerable differences in stress markers, including hydrogen peroxide (H_2O_2), the superoxide radical ($O_2^{\bullet-}$), and malondialdehyde (MDA), were also observed between the desiccated and rehydrated moss samples. This could be attributed to the enhanced activity of antioxidant enzymes, such as catalase (CAT) and superoxide dismutase (SOD), which effectively scavenged and controlled the overproduction of reactive oxygen species (ROS) in cells. However, no significant difference in photosynthetic pigment concentration was observed in the moss under the treatment conditions. Rehydration triggered a significant surge of metabolites, reflecting the rapid upregulation and reactivation of metabolism, highlighting remarkable resurrection traits in the moss.

Keywords: Desiccation, *Hypnum cupressiforme,* metabolomics, resurrection traits, stress markers

Introduction

Water availability is the foremost requirement for a plant to survive. Due to the increasing impact of climate change, precipitation patterns worldwide have changed significantly, leading to droughts and water scarcity. Studies on climate models have highlighted the urgent need to focus primarily on the emerging problem of global water scarcity and recurrent droughts, while also underscoring the importance of assessing tolerance and sensitivity to such stresses and their effects on plants (Farrant and Oliver 2004). Plants respond to water deficit or drought conditions in various ways, primarily through distinct traits that regulate complex signaling and response systems. Thus, it is essential to

identify plant functional traits, which are crucial for understanding stress response, adaptation, and tolerance. Throughout evolution, plants have developed strategies to survive harsh environmental conditions, including prolonged periods of drought and water scarcity; thus, plant adaptation to desiccation is one of the most significant aspects of present-day scientific research. It is well established that desiccation stress is generally unfavorable for plants; however, a few species, known as resurrection plants, can survive extremely dry conditions and rapidly recover upon rehydration. Such plants reactivate their physiological and metabolic function to recover quickly from such conditions (Yobi et al. 2012; Gaff and Oliver 2013; Coe et al. 2021). Among plants, mosses are considered suitable models for studying desiccation tolerance (Alpert 2000). Numerous studies have demonstrated that mosses possess a unique mechanism to balance cellular water potential during prolonged dry periods and return to their normal physiological state upon rehydration (Alpert 2000; Liang et al. 2021; Morales-Sánchez et al. 2022). Additionally, mosses can withstand stress-induced oxidative damage by modulating their metabolic and cellular functions (Petkova et al. 2023).

Plants respond to stress in multifarious ways. It is well established that under stress, plants undergo a multitude of cellular changes, including the overproduction of reactive oxygen species (ROS), which leads to oxidative stress (Choudhury et al. 2013; Noctor et al. 2018; Nadarajah 2020). The onset of oxidative stress affects both structural and functional traits, thereby impacting its growth and survival (Foyer 2018; Foyer and Hanke 2022). However, plants have well-defined mechanisms to counterbalance ROS overproduction by the antioxidant system, which comprises enzymes and non-enzymatic metabolites (Apel and Hirt 2004; Considine and Foyer 2021; Zhang et al. 2022). Studies on moss species, such as *Macrocoma orthotrichoides*, have shown that ROS overproduction is largely associated with oxidative stress and differential responses in antioxidant metabolism (Neto et al. 2025). The increase in ROS production and consecutive oxidative stress during desiccation has also been previously reported in various species of bryophytes, such as *Syntrichia ruralis* (Ruchika et al. 2021; Salih et al. 2024), *Selaginella brachystachya* (Shivaraj et al. 2021), *Hyophila propagulifera* (Ramyashree et al. 2021), *Bryum argentum* (Yuqing et al. 2021), and *Campylopus flexuosus* (Usha et al. 2021). In recent years, the use of high-throughput screening (HTS) approaches, combined with various analytical tools, has significantly enhanced our understanding of plant responses to stresses

(Choudhury et al. 2021). One such approach is metabolomics, which aims to assess changes in an organism's metabolic profile in response to environmental perturbations. Any change in the organism's metabolome will be reflected in its phenotype, and such changes could be effectively correlated with stress adaptation and tolerance (Yobi et al. 2012; Fabregas and Fernie 2019; Oliver et al. 2020). Thus, the identification of metabolic traits remains one of the most important perspectives for comprehending plant responses and adaptation to desiccation.

The genus *Hypnum* is largely cosmopolitan in distribution and found in almost all ecosystems, but is less common in tropical and subtropical regions (Si et al. 2021). In India, the genus is represented by six (06) species, among which *H. cupressiforme* is mainly found in the Indian states of Assam, Sikkim, and Jammu and Kashmir (Dandotiya et al. 2011; Barukiyal 2011). *Hypnum cupressiforme* Hedw. is also known as plaitmoss or carpet moss, a remarkable species with high desiccation tolerance, enabling it to survive severe water stress and to recover rapidly upon rehydration (Cruz de Carvalho et al. 2020). Chlorophyll fluorescence and membrane permeability studies have also revealed significant traits, which complement *H. cupressiforme* as a desiccation-tolerant moss (Deltoro et al. 1998). Additionally, as a desiccation-tolerant moss species, it is also a probable source of phytochemicals and metabolites with potential antioxidant, anti-inflammatory, immunomodulatory, and DNA-protective activities (Lunic et al. 2020; Petkova et al. 2025). Studies have also suggested that *H. cupressiforme* contains active metabolites, which makes it an interesting moss species from a pharmaceutical standpoint (Bdrize et al. 2020). The presence of diverse secondary metabolites can also be linked to the moss species' tolerance to environmental stresses (Izquieta-Rojano et al., 2018). Since metabolite levels vary substantially under stress, fluctuations in metabolite levels may reflect the moss's metabolic response to environmental water availability.

Given the multifaceted potential of a metabolomics-based approach to illustrate variations in the metabolome and prospective desiccation traits in *H. cupressiforme*, the present study was conceptualized. It is based on the proposition that rehydration after a period of desiccation could substantially reveal important metabolic markers, which would be vital towards a comprehensive understanding of resurrection traits and their adaptability to desiccation.

Materials and methods

Collection and identification of moss samples

The moss samples were collected from concrete walls on a bio-colonized layer of organic matter in Silchar town, Assam, India (24.82° N latitude and 92°80 E longitude) in October 2024. The moss grows mainly in a wide range of habitats due to its high ecological adaptability (Dollery et al., 2022; Delimann et al., 2025). Samples were carefully removed from the substrate with a scalpel and immediately transferred into a plastic box for transportation to the laboratory. The specimens were divided into two sets: one for taxonomic identification and the other for desiccation stress studies. For taxonomic identification, the moss samples were dried and preserved in herbarium packets. The dried specimen, complete with data on the label, was deposited in the Assam University Silchar Central Herbarium (AUSCH) bearing specimen no. 001 and Voucher Accession no. 8651. The specimen was identified based on morphological and anatomical observations. For the morpho-anatomical study, the dried specimen was first soaked in water and allowed to regain its usual shape. The external morphological features were studied under a stereo zoom dissecting microscope (Leitz-Leica, Germany) and identified based on observed features and relevant literature (Box 1).

Box 1. Taxonomic description and identification of the moss sample

Hypnum cupressiforme **Hedw. Sp. Musc. Frond.: 291. 1801**

Plants procumbent, forming dense mats. Gametophytes are small to medium-sized, green to yellowish green. Stem 2 – 5 cm, creeping, nearly unbranched to irregularly branched or rarely pinnate. Leaves falcate, nearly secund, ovate–oblong, 1.2 – 1.8 x 0.5 – 0.8 mm, apices acute to acuminate, base non-decurrent to weakly decurrent, margin almost entire, sometimes proximally recurved. Costa double, median laminal cells thin walled, smooth, linear – flexuose 50 – 70 x 5 – 10 µm; basal cells thick-walled, wider than median cells; alar cells prominent, rectangular – subquadrate – hexagonal, hyaline. Branch leaves are similar in shape to stem leaves, smaller.

Habitat: Terrestrial, growing on shady, damp soil over rock along the bank of the stream. *Distribution*: Africa; Asia: China, India (Assam, Jammu & Kashmir, Sikkim, Tamil Nadu), Japan, Korea; Europe; North America. *Specimens examined:* India, Assam University, Campus, Silchar; Assam, Borail Wildlife Sanctuary, Maruachera, 26.11.2024, *D. Bhattacharyya* and *S. Choudhury* 001 [Assam University Silchar Central Herbarium, (AUSCH); Accession No.8651].

Experimental setup

The collected moss samples were thoroughly cleaned to remove any attached foreign material. The moss samples were placed inside a glass desiccator containing coarse, self-indicating silica gel until the moisture content of the samples reached less than 15% (<15%). At this stage, the samples were considered markedly desiccated. The desiccated samples were used for biochemical and metabolomic

analysis. For rehydration, the desiccated moss gametophytes were transferred to a beaker containing 100 mL of distilled water (dH_2O) and placed inside the growth chamber, which received 400 Lux illumination on a 12-hour light and 12-hour dark cycle at 30°C with 40% relative humidity (RH). The relative water content (RWC) was checked at a gap of every 6h, until it reached a value exceeding fifty percent (>50%). At this point, when the RWC was greater than 50% (> 50%), the samples were harvested to assess the resurrection traits through a comparative analysis of stress markers and metabolic profiles of *H. cupressiforme*, compared to the desiccated condition. The RWC was calculated following the formula RWC (%) = $(F_{wt} - D_{wt}) / (T_{wt} - D_{wt}) \times 100$, where F_{wt} is the fresh weight of the moss samples, T_{wt} is the turgid weight of the moss sample, and D_{wt} is the dry weight of the samples (Yobi et. al. 2012). The experimental setup for the present study is illustrated in Fig. 1.

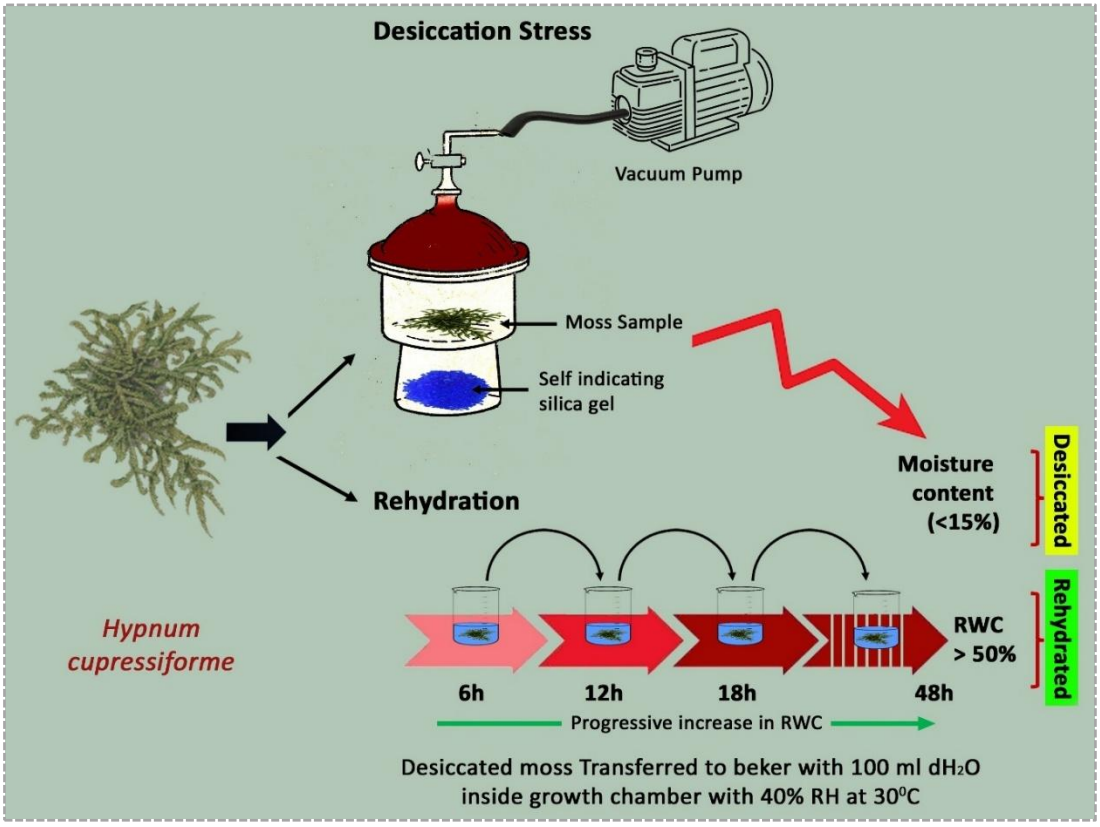


Fig. 1. Illustrative representation of experimental setup for induction of desiccation stress and subsequent rehydration

Determination of photosynthetic pigments and stress markers

The photosynthetic pigment content was determined as per the method of Strickland and Parsons (1972). 0.2g of tissue samples were frozen with liquid nitrogen and ground to a fine powder. The grounded samples were transferred to 50 ml tubes containing 90% (v/v) acetone and rested overnight at 4°C. The homogenate was centrifuged at 5,000 rpm for 15min and the supernatant was collected to

estimate the concentration of photosynthetic pigments. The absorbance of the supernatant was recorded at 665, 630, 645, 750, and 480 nm.

The stress markers were measured as ROS content, including hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\bullet-}$), as per the methods of Sagisaka (1976) and Elstner and Heupel (1976), respectively. For the determination of H_2O_2, 0.2g of tissue samples was ground into a fine powder with liquid nitrogen and homogenized with 10% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 rpm for 15 min at 4°C, and the supernatant extract was collected. The final reaction mixture comprised 1.6 ml supernatant tissue extraction and 0.4 ml each of 50% (w/v) TCA, ferrous ammonium sulphate ($FeNHSO_4$), and 2.5 mM potassium ferrocyanide (KSCN). The absorbance of the mixture was recorded at 480 nm. For the determination of $O_2^{\bullet-}$, 0.2g of tissue sample was ground into a fine powder with liquid nitrogen and homogenized with ice-cold 65mM Na-phosphate buffer (pH 7.8). The homogenate was allowed to rest on an ice bath for 5 min, then centrifuged at 5,000 g for 10 min at 4°C to collect the supernatant. The assay mixture contained 0.9 ml of Na-phosphate buffer, 0.1ml of 10mM hydroxylamine hydrochloride, and 1ml of supernatant extract. The mixture was allowed to rest at room temperature for 20 min, followed by the addition of 1ml each of sulphanilamide, α-napthyl, and diethyl ether. The content was centrifuged at 3,000 rpm for 5 min at 4°C, and the absorbance was recorded at 530 nm.

Lipid peroxidation was determined as malondialdehyde (MDA) content using the method of Heath and Packer (1968). 0.2g of tissue sample was ground to a fine powder with liquid nitrogen and homogenized with 0.1% (w/v) TCA. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C, and the supernatant extract was collected. The final reaction mixture for the determination of MDA comprised 1 ml of 20% (w/v) TCA containing 0.5% (w/v) thiobarbituric acid (TBA) and 100 µl of butylated hydroxytoluene (BHT, a 4% solution in ethanol). The assay mixture was heated in a water bath at 95°C, cooled, and the absorbance was recorded at 532 and 600 nm.

Antioxidant enzyme activity

For the determination of antioxidant enzyme activity, 0.2 g of tissue sample was ground in liquid nitrogen and homogenized in 0.1 M ice-cold Na-phosphate buffer (pH 7.2) using a pre-chilled mortar and pestle. The homogenate was centrifuged at 16,000 g at 4°C for 20 min, and the supernatant was

collected. Antioxidant enzyme activities were determined as catalase (CAT) [EC 1.11.1.6] and superoxide dismutase (SOD) [EC 1.15.1.1] using the methods of Aebi (1984) and Beauchamp and Fridovich (1971), respectively. The assay mixture for CAT activity comprised 1 ml each of 0.1 M Na-phosphate buffer, enzyme extract, and 30 mM H_2O_2. The mixture was incubated for 5 min in an ice bath, and the absorbance was recorded at 240 nm. The assay mixture for the determination of SOD activity comprised 100 mM Na-phosphate buffer (pH 7.8), 13 mM methionine, 2.25 mM nitroblue tetrazolium salt (NBT), 600 µM riboflavin, and enzyme extract. The reaction mixture was incubated at room temperature for 5 min under white fluorescent illumination, after which the absorbance was recorded at 560 nm.

Extraction of metabolites

For the extraction of metabolites, 50 mg of the tissue sample was ground in liquid nitrogen, then extracted with 600 µl of ice-cold methanol and ultrasonicated for 15 min, with a 30-second pause after 1 min of sonication. The homogenate was vortexed 5-6 times for 30 seconds each and centrifuged at 15,000 g for 15 min at 4°C. The supernatant was collected, and the pellets were resuspended in methanol and extracted as described above. Both supernatant extracts were pooled and dried under vacuum. The samples were redissolved in LC-grade methanol, filtered through a syringe filter, and analyzed by LC-MS. The data acquisition parameters were set with a minimum mass (m/z) of 85 and a maximum mass (m/z) of 1100 with a scan rate of 1.00 spectra sec^{-1}. 3 µL of the sample was injected at a flow rate of 0.3 mL min^{-1}, with a stop time of 30 min at 1200.00 bar. The solvent is composed of ultrapure water containing 0.1% (v/v) formic acid (solvent A) and 100% LC-grade acetonitrile (solvent B). The solvent flow was initially set to 95% solvent A and 5% solvent B at 1 min, and the gradient was set to 100% solvent B for 5 min starting at 25 min. The metabolites were identified using the Compound Discoverer 3.2 platform (Thermo Scientific, USA).

Metabolic data analysis

The metabolic datasets were analyzed using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca) to evaluate metabolic disparities in the moss under desiccated conditions and upon rehydration. To compare metabolic disparities in moss under desiccated and rehydrated conditions, the metabolic

datasets were analyzed using principal component analysis (PCA). PCA is an unsupervised statistical method used in metabolomics to analyze sample clustering and thus separate samples by treatment condition. PCA also indicates the dataset's suitability for deriving meaningful conclusions from the results. The metabolic datasets were also subjected to comparative analyses of percent distributions to highlight the contributions of different metabolites to the overall metabolic composition of the sample under experimental conditions. The heat map analysis was performed to identify stress-specific metabolic shifts and co-regulation of metabolites in the moss samples. Heat maps visualize the relative patterns of metabolite abundance and their correlations in a sample under study, and identify treatment-specific changes. Further, the volcano plots were generated from the metabolic datasets using fold change (log2FC) and p-values to identify the specific contributions of metabolites to separating the treatments. The pathway enrichment analysis was done to identify significantly affected biological pathways in moss under desiccated conditions and upon rehydration. This is based on mapping metabolic datasets to identify prospective, biologically relevant pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/).

Statistical analysis

The datasets presented are means of three replicates. The datasets for analysis of photosynthetic pigments, stress markers, and antioxidant activity were statistically evaluated to determine significant differences among the treatment groups at $p < 0.001$, 0.01, and 0.05. The datasets were further subjected to a two-sample paired test to identify the relative differences among the tested parameters under the treatment conditions and to determine whether the differences are statistically significant. The datasets present mean and median values for the treatment conditions, with 95% confidence intervals and the Wilcoxon signed-rank test. All statistical analyses were performed using INSAT (ver. 3.0), Past 4.0, and MS Excel (Microsoft Inc.).

Results

Changes stress responses to desiccation and rehydration

In the present study, we report significant upregulation of metabolic function and oxidative stress responses in the moss *H. cupressiforme* following rehydration. These results indicated that, upon rehydration, the moss rejuvenates its physiological and metabolic processes, which are vital to recover

from desiccation. The concentration of photosynthetic pigments, such as chlorophyll a, b, c, total chlorophyll, and carotenoid, showed a moderate increase after rehydration, which indicated potential recovery from desiccated conditions (Fig. 2A). Although chlorophyll a showed the maximum variation ($p < 0.05$), followed by a moderate difference in total chlorophyll content in the moss under the treatment conditions, no significant ($p > 0.05$) differences were observed in case of chlorophyll b, c, and carotenoid content. The ROS, such as H_2O_2 and $O_2^{\bullet-}$ showed a significant decline ($p < 0.001$) upon rehydration, which clearly indicated restoration of cellular redox homeostasis, concomitant with which a decline in MDA level was also observed after rehydration (Fig. 2B). Thus, the desiccation-induced oxidative stress was significantly regulated upon rehydration. The activities of antioxidant enzymes such as CAT and SOD were significantly ($p < 0.001$) raised in the moss upon rehydration, which could be correlated to substantial mitigation of ROS-induced oxidative stress (Fig. 2C). To study the similarity in response of the tested parameters, hierarchical cluster analysis (HCA) was performed, where the y-axis represents the Euclidean distance, with higher and lower similarity represented by smaller and greater distances, respectively (Fig. 2D). Both chlorophyll c and carotenoid showed the strongest similarity, representing an identical response during the desiccated condition and after rehydration. The MDA and chlorophyll b were linked to the chlorophyll c and carotenoid cluster, indicating that pigment loss and oxidative stress are concurrent responses in the moss during desiccation stress. ROS (H_2O_2 and $O_2^{\bullet-}$) and chlorophyll a also join the above cluster of pigments, indicating that changes in their concentrations are closely linked. Antioxidant enzymes like CAT and SOD, along with total chlorophyll, form a separate cluster, indicating a close relationship between the upregulation of antioxidant metabolism and the recovery of photosynthesis. The HCA represents that the moss *H. cupressiforme* responds to rehydration by upregulating photosynthesis and limiting the ROS-induced oxidative stress through enhanced antioxidant activity. A bivariate scatter plot with regression analysis was used to validate changes in the response of each tested parameter upon rehydration relative to the desiccated condition (Fig. 2E). The linear regression line showed a positive slope, indicating a positive relationship between the two conditions. Parameters such as photosynthetic pigments (total chlorophyll), CAT, and SOD increased after rehydration. In contrast, no substantial differences were observed in chlorophyll a,

b, and c, or in carotenoid content across the treatment regimes. ROS (H_2O_2 and $O_2^{\bullet-}$) and oxidative stress marker (MDA) showed a drastic decline after rehydration, reflecting restoration of cellular redox homeostasis. The results of the biochemical parameters tested reflected a remarkable resurrection trait in the moss, with oxidative stress regulation as the fundamental underlying mechanism.

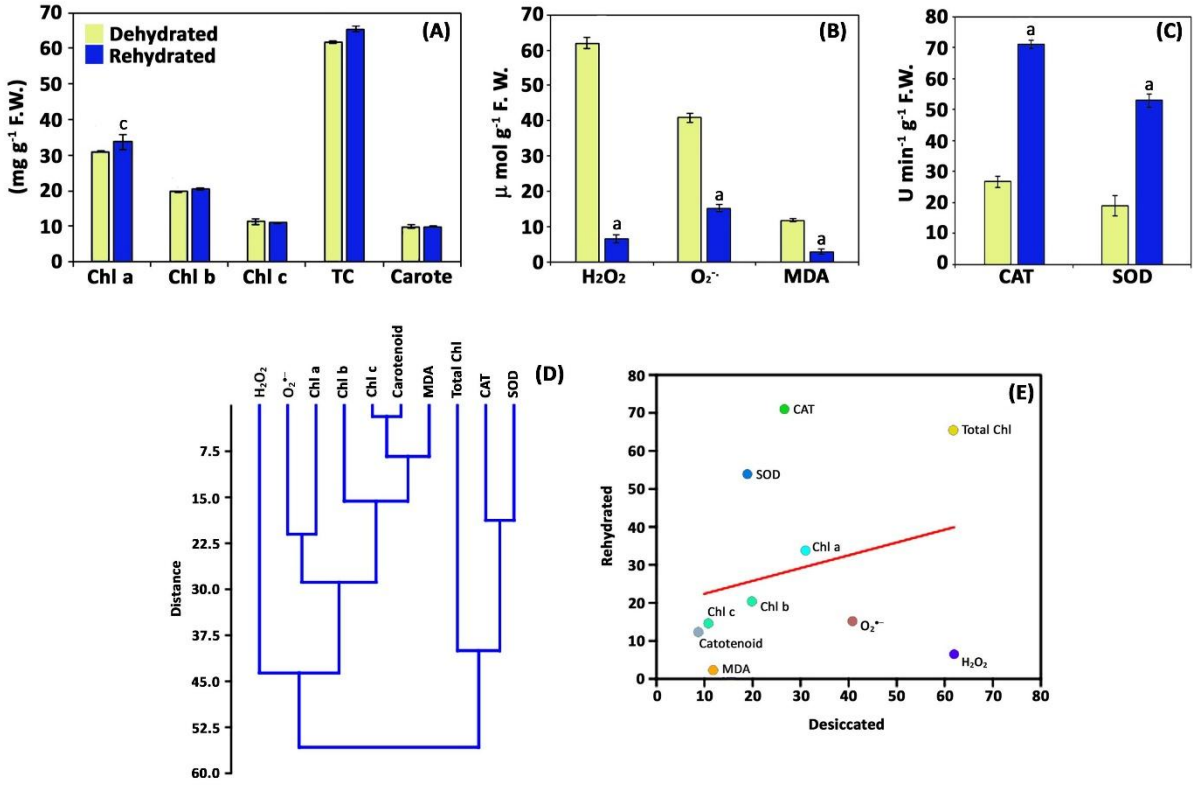


Fig. 2. Changes in photosynthetic pigments (A), ROS and MDA levels (B), activities of CAT and SOD (C) in moss under desiccated conditions and upon rehydration. The data presented are the means of three replicates (n = 3). The letters a, b, and c represented the significant changes observed after rehydration relative to desiccated conditions. (D) Hierarchical cluster analysis (HCA) plot showing the similarity in response of the tested parameters. (E) Bivariate scatter plot with regression analysis showing changes in parameters upon rehydration relative to the desiccated condition.

HRLC-MS-based identification of metabolites

Metabolite profiling of aqueous methanolic extracts by HRLC-MS revealed significant metabolic differences in the moss under desiccated conditions and after rehydration (Fig. 3 and Tables 1, 2, and 3). The amino acid and its derivatives showed a substantial disparity in the moss under both desiccated and rehydrated conditions. In the desiccated condition, the abundance of the amino acids and their derivatives, such as GABA, D-Proline, valine, tyrosine, L-aspartic acid, phenylalanine, D-(+)-pyroglutamic acid, fructosyl glycine, pyroleucine, 2-aminoadipic acid, D-homoproline, and L-

glutamyl-L-leucine was observed, compared to those under desiccated conditions. These metabolites are likely unique to the moss upon rehydration and clearly indicate that, upon rehydration, the moss regains metabolic stability, thereby ensuring flexibility in maintaining physiological and cellular homeostasis.

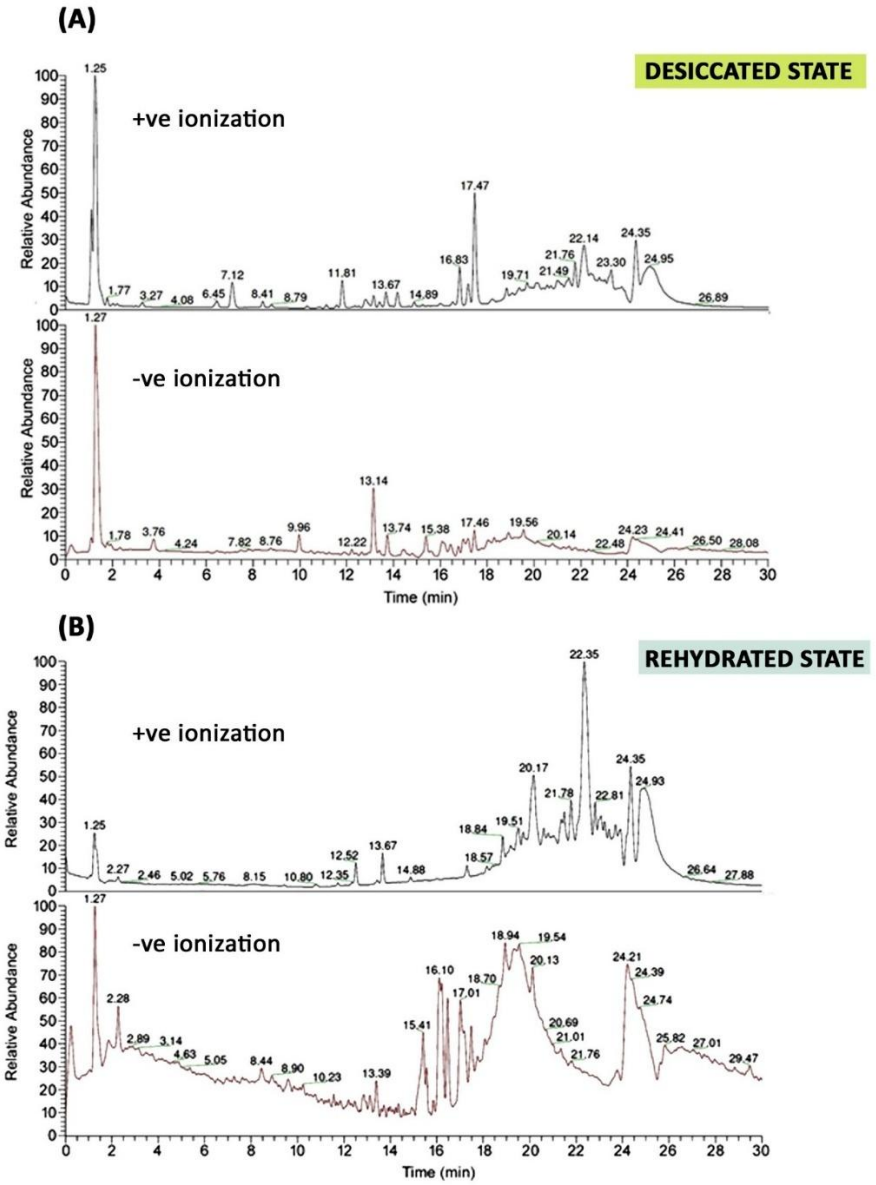


Fig. 3. Liquid chromatogram (LC) obtained under positive (+ve) and negative (-ve) ionization modes showing different metabolites observed in the moss under desiccated conditions (A) and upon rehydration (B).

Table 1. Amino acids, their derivatives, and peptides identified in the moss *Hypnum cupressiforme* Hedw. using HRLC-MS during desiccation and upon rehydration.

S. No.	Name of the compound	Formula	Molecular weight	Experimental Conditions	
				Rehydrated	Desiccated
Amino Acids and Derivatives					
1	Betaine	$C_5H_{11}NO_s$	117.07	+	+
2	Sitagliptin	$C_{16}H_{15}F_6N_5O$	407.31	-	+
3	Asparagine	$C_4H_8N_2O_3$	132.05	+	+
4	L-Histidinal	$C_6H_9N_3O_2$	155.15	-	+
5	L(-)-Pipecolinic acid	$C_6H_{11}NO_2$	129.07	+	+
6	Ectoine	$C_6H_{10}N_2O_2$	142.16	+	+
7	γ-amino butyric acid (GABA)	$C_4H_9NO_2$	103.12	-	+
8	Isoleucine	$C_6H_{13}NO_2$	131.09	+	+
9	L-Serine	$C_3H_7NO_3$	105.04	-	+
10	Tyrosine	$C_9H_{11}NO_3$	181.19	+	-
11	L-Aspartic acid	$C_4H_7NO_4$	133.1	+	-
12	D-Proline	$C_5H_9O_2$	115.06	+	-
13	Valine	$C_5H_{11}NO_2$	117.07	+	-
14	Phenylalanine	$C_9H_{11}NO_2$	165.07	+	-
15	D-(+)-Pyroglutamic Acid	$C_5H_7NO_3$	129.04	+	-
16	Fructosyl glycine	$C_8H_{15}NO_7$	237.21	+	-
17	Prolylleucine	$C_{19}H_{26}N_2O_5$	362.42	+	-
18	2-Aminoadipic acid	$C_6H_{11}NO_4$	161.06	+	-
19	D-Homoproline	$C_6H_{11}NO_2$	126.16	+	-
20	L-Glutamyl-L-leucine	$C_{11}H_{20}N_2O_5$	260.29	+	-
21	gln-ile-lys	$C_{17}H_{33}N_5O_5$	387.5	+	-
22	phe-asp-arg	$C_{15}H_{23}N_5O_3$	321.18	+	-

Among carbohydrates, sugars, and fatty acids, the metabolites that were abundant in the moss after rehydration include sucrose, D-glucose, lactose, 16-hydroxyhexadeconic acid, 13(S)-HOTrE, coriolic acid, palmitic acid, lysolecithin, styrene, and linoleic acid (Table 2). The accumulation of sucrose and D-glucose in the moss after rehydration could be linked to efficient energy metabolism or other specific responses, particularly osmotic adjustment. The higher levels of fatty acid derivatives in the moss upon rehydration indicated a strong response in terms of membrane stability and lipid signalling in response to rehydration.

Table 2. Carbohydrates, sugars, and fatty acids identified in the moss *Hypnum cupressiforme* Hedw. using HRLC-MS during desiccation and upon rehydration

S. No.	Name of the compound	Formula	Molecular weight	Experimental Conditions	
				Rehydrated	Desiccated
Sugar, Carbohydrates, Fatty Acids and derivatives					
1	Trehalose	$C_{12}H_{22}O_{11}$	342.11	+	+
2	D-Raffinose	$C_{18}H_{32}O_{16}$	504.16	+	+
3	D-Mannitol	$C_6H_{14}O_6$	182.07	+	+
4	Sucrose	$C_{12}H_{22}O_{11}$	342.11	+	-
5	D-Glucose	$C_6H_{12}O_6$	180.06	+	-
6	Lactose	$C_{12}H_{22}O_{11}$	342.11	+	-
7	Phloionolic acid	$C_{18}H_{36}O_5$	332.48	-	+
8	Oleamide	$C_{18}H_{35}NO$	281.27	+	+
9	Erucamide	$C_{22}H_{43}NO$	337.59	+	+
10	Palmitylethanolamide	$C_{18}H_{37}NO_2$	299.49	-	+
11	2-Amino-1,3,4-octadecanetriol	$C_{18}H_{39}NO_3$	317.29	-	+
12	2-Aminopalmitic acid	$C_{16}H_{33}NO_2$	271.44	-	+
13	11-Aminoundecanoic acid	$C_{11}H_{23}NO_2$	201.31	-	+
14	16-hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$	272.23	+	-
15	13(S)-HOTrE	$C_{18}H_{30}O_3$	294.21	+	-
16	Coriolic acid [(±) 13-HODE]	$C_{18}H_{32}O_3$	296.35	+	-
17	Palmitic acid	$C_{16}H_{32}O_2$	256.24	+	-
18	Lysolecithin	$C_{24}H_{50}NO_7P$	495.63	+	-
19	Styrene	C_8H_8	104.15	+	-
20	α-Linoleic acid	$C_{18}H_{32}O_2$	280.44	+	-

A substantial variation in the composition of organic acids and secondary metabolites was also observed in the moss after rehydration, compared to the desiccated condition (Table 3). This includes the metabolites such as citric acid, azelaic acid, L-malic acid, D-gluconic acid, 4-methoxycinnamic acid, 2-furoic acid, quinic acid, nicotinic acid, coenzyme Q2, ubiquinone, adenosine, and cytosine. Metabolites that were abundantly present in moss under desiccated conditions include chelidonic acid, stearyldiethanolaime, luraylbetaine, berberine, trigonelline, 3-methylhistamine, and 3,5-dimethylpyrazole. Additionally, a few metabolites were abundantly present in moss samples under both desiccated and rehydrated conditions, including urocanic acid, quinic acid, nicotinamide, choline, guanine, and uridine. The metabolic variation reveals multifaceted functions of the metabolites that include energy metabolism, stress-specific responses, osmoprotection, ROS detoxification, and antioxidant defense.

Table 3. Organic acids and secondary metabolites identified in the moss *Hypnum cupressiforme* Hedw. using HRLC-MS during desiccation and upon rehydration.

S. No.	Name of the compound	Formula	Molecular weight	Experimental Conditions	
				Rehydrated	Desiccated
Organic Compounds (Organic acids, vitamins, co-enzymes, etc.)					
1	Chelidonic acid	C7H4O6	184.10	-	+
2	Urocanic acid	$C_6H_6N_2O_2$	138.04	+	+
3	Citric acid	$C_6H_8O_7$	192.02	+	-
4	Azelaic acid	$C_9H_{16}O_4$	188.22	+	-
5	L-(-)-Malic acid	$C_4H_6O_5$	137.08	+	-
6	D-Gluconic acid	$C_6H_{12}O_7$	196.16	+	-
7	4-methoxycinnamic acid	$C_{10}H_{10}O_3$	178.06	+	-
8	2-Furoic acid	$C_5H_4O_3$	112.08	+	-
9	Quinic acid	$C_7H_{12}O_6$	192.06	+	+
10	Nicotinamide	$C_5H_5N_5O$	151.04	+	+
11	Stearyldiethanolamine	$C_{22}H_{47}NO_2$	357.61	-	+
12	Choline	$C_5H_{13}NO$	103.09	+	+
13	Luraylbetaine	$C_{16}H_{33}NO_2$	271.45	-	+
14	Berberine	$C_{20}H_{18}NO_4$	336.36	-	+
15	Trigonelline	$C_7H_7NO_2$	137.04	-	+
16	3-Methylhistamine	$C_6H_{11}N_3$	124.17	-	+
17	3,5-Dimethylpyrazole	$C_5H_8N_2$	96.13	-	+
18	Guanine	$C_5H_5N_5O$	151.04	+	+
19	Uridine	$C_9H_{12}N_2O_6$	244.2	+	+
20	D-Stachydrine	$C_7H_{13}NO_2$	143.09	+	-
21	Adenosine	$C_{10}H_{13}N_5O_4$	267.09	+	-
22	Nicotinic acid	C_6H_5NO	123.03	+	-
23	coenzyme Q2	$C_{19}H_{26}O_4$	318.41	+	-
24	α-Tocopherol	$C_{29}H_{50}O_2$	430.71	+	-
25	Ubiquinone Q4	$C_{29}H_{42}O_4$	454.65	+	-
26	Cytosine	$C_4H_5N_3O$	111.1	+	-

Principal component analysis (PCA) and PCA-Biplot

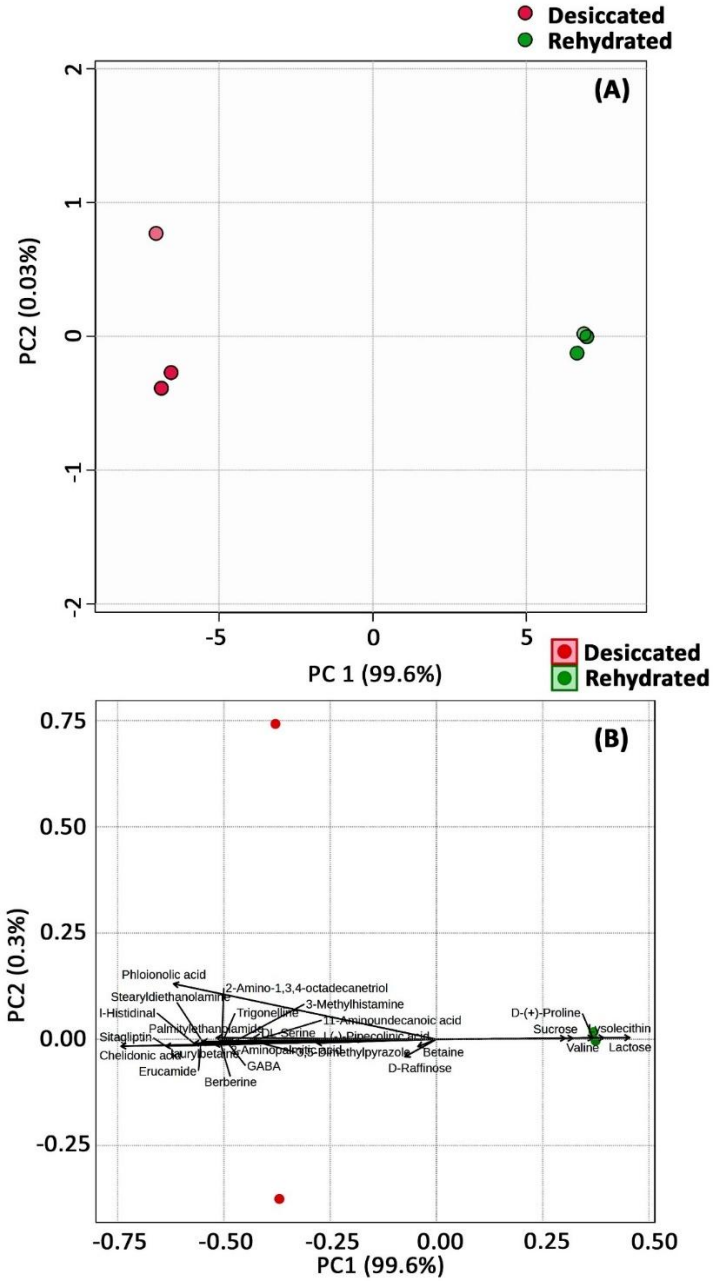
To study the disparity in metabolite abundance in the moss under desiccated conditions and upon rehydration, we performed principal component analysis (PCA) (Fig. 4A). The PCA explains a clear separation between the desiccated and rehydrated conditions in the moss, where PC1 and PC2, showed 99.6% and 0.3% variance, respectively. PC1 accounts for the greatest variance among the metabolites, indicating a clear separation between desiccated and rehydrated conditions. To identify the contribution of the metabolites in separating the desiccated and rehydrated conditions in the moss, we performed the

PCA biplot analysis considering the top twenty-five (25) metabolites (Fig. 4B). The metabolites that are enriched in the moss under desiccated conditions include phoionolic acid, I-histidinal, sterayldiethanolamine, sitagliptin, laurylbetaine, and L-pipecholic acid. This indicates that during desiccation, these metabolites function to provide substantial protection, enabling the moss to survive harsh conditions. The metabolites that clustered to the right-hand portion of the biplot include sucrose, D-(+)-proline, valine, lactose, and lysolecithin, indicating higher accumulation in the moss after rehydration. A few of the metabolites that contributed partly to both conditions, including GABA, raffinose, betaine, palmitoylethanolamide, and DL-serine, were clustered at the centre of the biplot. The PCA and biplot analyses revealed a distinct metabolic profile in the moss, indicating a substantial metabolic overhaul after rehydration, enabling it to recover from a desiccated state.

Heat map and fold-change (FC) analysis

To identify the differences in metabolite abundance in the moss under desiccated conditions and upon rehydration, we performed heat map analysis (Fig. 5). The heat map analysis clearly revealed a separation between the desiccated and rehydrated conditions. Several metabolites, largely related to stress-protective function, were strongly accumulated in the moss in its desiccated state. In the rehydrated state, amino acids, TCA intermediates, sugars, and fatty acid derivatives were abundant. Further, to classify the biologically as well as statistically significant metabolites in the moss upon rehydration compared to the desiccated conditions, we used volcano plots by considering the fold change (log2FC) and *p-value* (0.001 to 1.0) (Fig. 6). Metabolites that were significantly upregulated in the moss upon rehydration include D-(+)-proline, valine, citric acid, L-phenylalanine, sucrose, choline, L-malic acid, D-stachydrine, and 16-hydroxyhexadecanoic acid. Compared with desiccated conditions, metabolites such as stearidyl ethanolamine, erucamide, palmitylethanolamide, laurylbetaine, chelidonic acid, sitagliptin, L(-)-pipecolinic acid, I-histidinal, and phloionic acid were significantly downregulated in the moss after rehydration. The upregulated metabolites in the moss in response to rehydration function as antioxidants and osmoprotectants. The upregulation of these metabolites also indicated a significant enhancement of metabolic processes, including amino acid, lipid, citrate (TCA) cycle, and carbohydrate metabolism. A strong metabolic reprogramming was observed in the moss upon rehydration, thus improving the cellular metabolism. Higher levels of TCA cycle intermediates in the

359 moss upon rehydration clearly reflected a highly functioning TCA cycle, fuelling energy-rich
360 compounds and precursors for amino acid metabolism. Other metabolites, such as D-proline, choline,
361 and ectoine, serve as compatible solutes, providing osmoprotection in the moss. Sucrose and lactose
362 fuelled the carbon pool, while higher levels of choline and lysolecithin were possibly associated with
363 membrane stability in the moss during rehydration. The metabolites downregulated in the moss are
364 specifically induced by desiccation. This may be because, after rehydration, the pool of these
365 metabolites returns to its normal level.



366

367 **Fig. 4.** Principal component analysis (PCA) (A) and biplot analysis (B), showing disparity in metabolite
368 abundance in the moss *H. cupressiforme* under desiccated conditions and after rehydration.

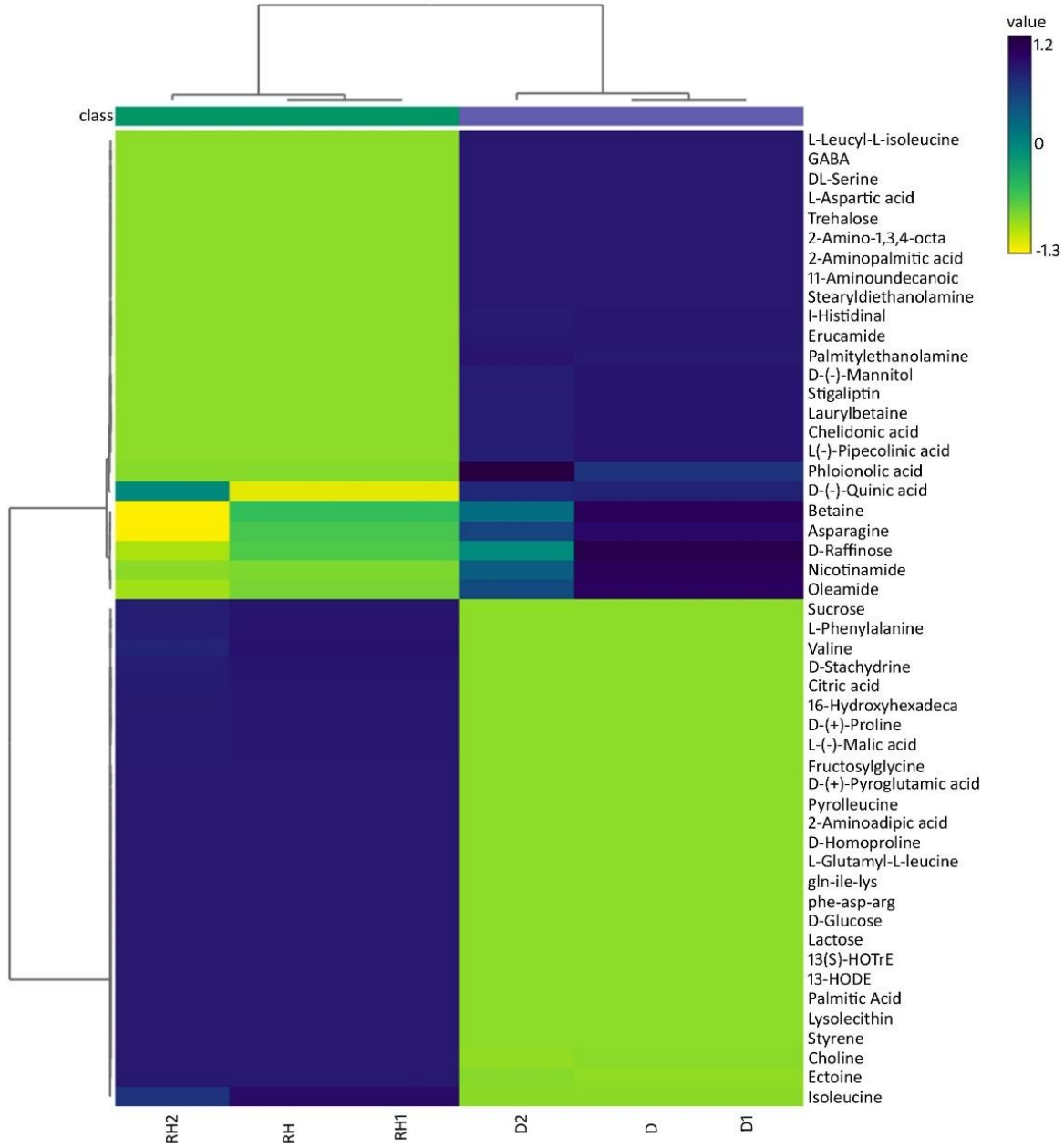


Fig. 5. Heat map analysis representing the difference in metabolite abundance in the moss *H. cupressiforme* under desiccated conditions and after rehydration.

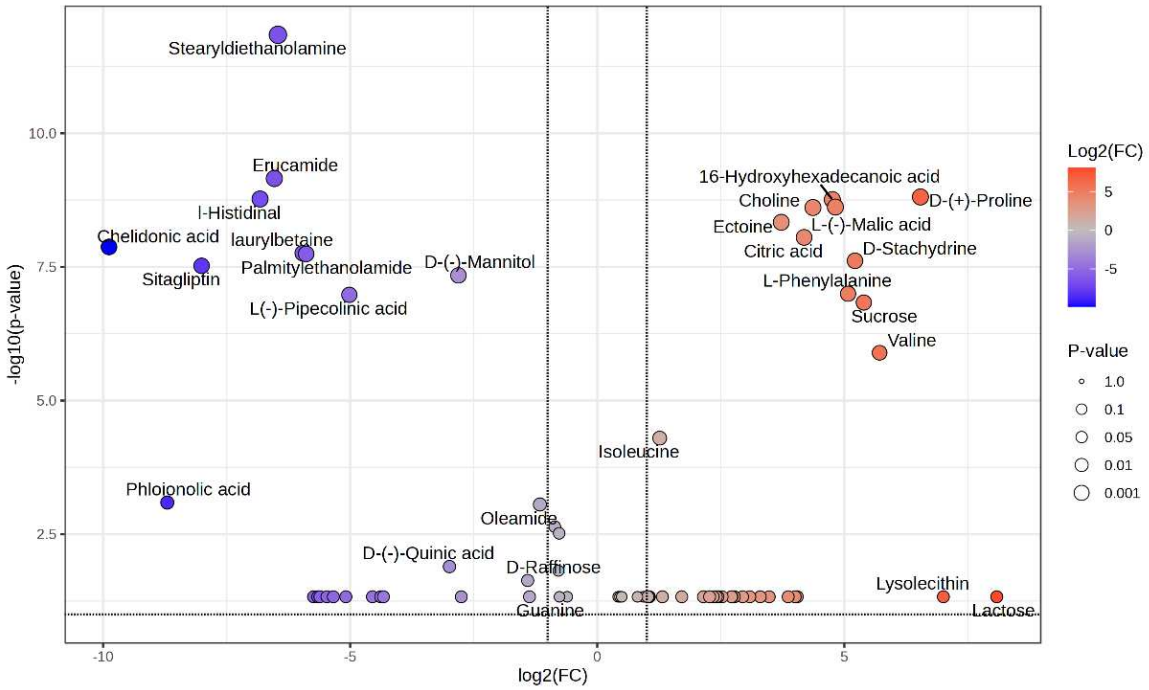


Fig. 6. Volcano plots showing upregulated (red) and downregulated (blue) metabolites in the moss *H. cupressiforme* upon rehydration compared to the desiccated condition.

Pathway analysis

The significantly upregulated metabolic pathway in the moss after rehydration was explained using KEGG pathway enrichment analysis based on enrichment ratios and *p-values* ($p = 0.2 – 0.001$) (Fig. 4A-C). The pathway enrichment analysis revealed that amino acid biosynthesis and degradation pathways are significantly enriched in the moss upon rehydration (Fig. 8A). These pathways include those for phenylalanine, tyrosine, and tryptophan biosynthesis, indicating distinct roles in stress signaling and antioxidant function. In addition, pathways related to alanine, aspartate, and glutamate metabolism, the biosynthesis of valine, leucine, and isoleucine, phenylalanine metabolism, the biosynthesis of pantothenate and CoA, glycine, serine, and threonine metabolism, and arginine and proline metabolism showed the greatest enrichment. This indicates that the pathways involved in amino acid metabolism are significantly upregulated in the moss after rehydration, facilitating the normalization of essential cellular metabolic processes. As shown in Fig. 4B, rehydration also enriched pathways related to sugar and lipid metabolism. These enriched pathways mainly include galactose metabolism, starch and sucrose metabolism, unsaturated fatty acid biosynthesis, fatty acid elongation and degradation pathways, and linoleic acid metabolism. The enrichment of these metabolic pathways suggests that rehydration after prolonged desiccation induces significant changes in energy metabolism,

membrane stability, and lipid signaling. The rehydration after prolonged desiccation also affected major metabolic pathways in the moss, showing statistically significant enrichment (Fig. 4C). These pathways include metabolites associated with nicotinate and nicotinamide metabolism, the citrate (TCA) cycle, glyoxylate and dicarboxylate metabolism, purine metabolism, the pentose phosphate pathway, and pyruvate metabolism. Rehydration may have led to the possible refurbishment of major metabolic pathways involved in energy metabolism. The enrichment of these metabolic pathways indicates a robust recovery mechanism in the moss upon rehydration.

Based on the pathway enrichment analysis, a metabolic network model was developed to explain the metabolic shift in the moss upon rehydration (Fig. 5). This model illustrates a clear metabolic reprogramming, leading to substantial stress amelioration and restoration of cellular homeostasis. Rehydration affects amino acid metabolism, providing the intermediates necessary for energy metabolism, such as the citric acid (TCA) cycle. Galactose metabolism may be interconnected with trehalose synthesis, a significant metabolite associated with osmoprotection and the amelioration of oxidative stress. Additionally, it can also synthesize sugar moieties, such as D-glucose, raffinose, and sucrose, as well as sugar alcohols, by fuelling specific metabolic pathways with intermediates. Nicotinate and nicotinamide biosynthesis are linked to the synthesis of high-energy compounds, such as NADH, which is required in various metabolic steps. Fructose metabolism is presumed to be associated with an increase in the pyruvate pool, which is converted to acetyl-CoA and enters the citrate or the TCA cycle. Alternatively, acetyl-CoA may initiate fatty acid biosynthesis, which is essential for biological membranes and is also involved in stress signaling responses. Thus, the moss *H. cupressiforme* exhibits a strong recovery upon rehydration, enriching its carbohydrate and sugar reserves, and enhancing amino acid biosynthesis and metabolism. This affects metabolic pathways, leading to specific stress signaling responses, thus restoring cellular function and redox homeostasis

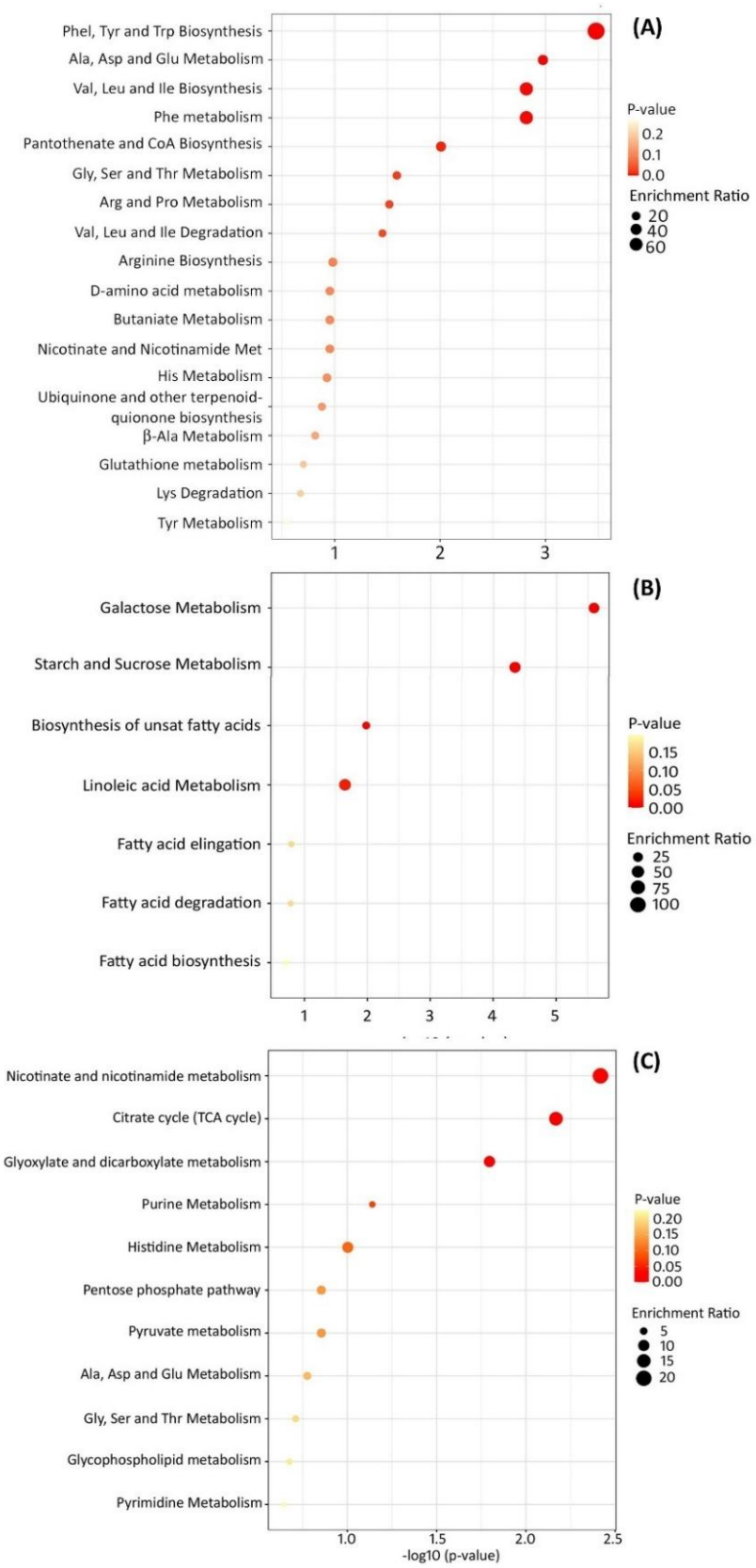


Fig. 7. KEGG pathway enrichment analysis showing enriched metabolic pathways in the moss *H. cupressiforme* after rehydration. The panels represent the enrichment of pathways associated with amino acid metabolism (A), sugar and lipid metabolism (B), and energy metabolism (C). The colour gradients indicate the *p-value,* and the bubble size represents the enrichment ratio.

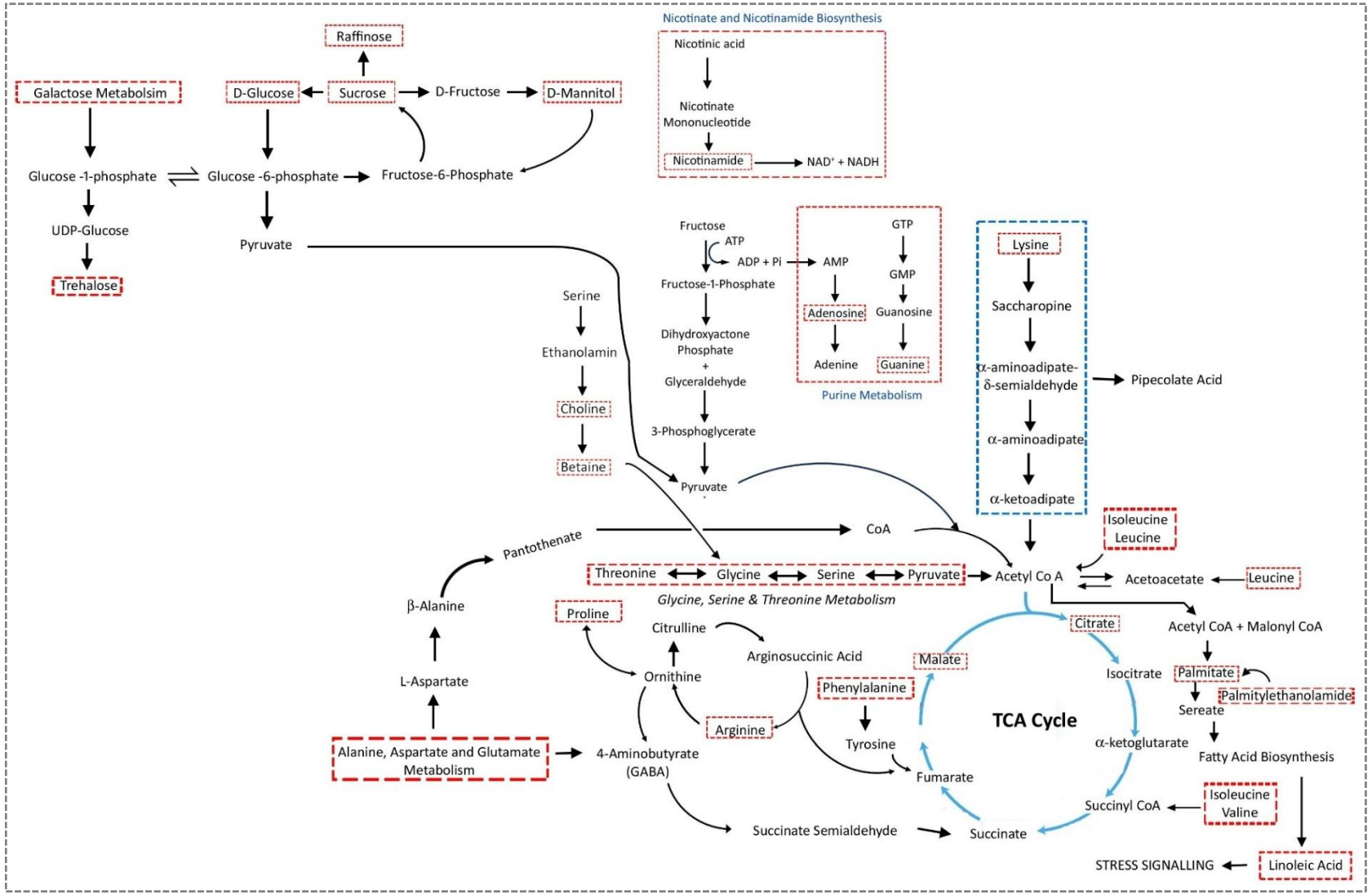


Fig. 8. Metabolic network model representing the metabolic upregulation in the moss *H. cupressiforme* upon rehydration after a prolonged period of desiccation

Discussion

Mosses are widely considered among the most suitable organisms for studying mechanisms of desiccation stress responses and tolerance due to their remarkable capacity to survive under harsh conditions (Stark et al. 2013; Oliver et al. 2020). In the present study, we report significant upregulation of diverse metabolites and alleviation of oxidative stress responses in the moss *H. cupressiforme* following rehydration. These results indicated that the moss rejuvenates its physiological and metabolic processes upon rehydration, which appears vital for tolerating desiccation or dehydration. Using a metabolomics approach, we dissected the metabolic reprogramming in moss following rehydration after desiccation. These two conditions in the moss yielded a unique metabolite profile: a diverse set of metabolites remained exclusively abundant under either desiccated or rehydrated conditions, and a few showed variable abundance in both conditions. Upon rehydration, the metabolites that were significantly upregulated include D-(+)-proline, valine, citric acid, choline, L-phenylalanine, sucrose, choline, L-malic acid, D-stachydrine, and 16-hydroxyhexadecanoic acid. The accumulation of proline has been widely reported in plants subjected to various environmental stresses, particularly during drought (Szabados and Savoure, 2010). This amino acid is abundantly synthesized in plants under drought stress, where it acts as an osmoprotectant (Szabados and Savoure 2010; Wang et al. 2022). High accumulation of proline has been previously reported in plants such as watermelon, peanuts, and maize under drought or water-deficit conditions (Furlan et. al. 2020; Wang et. al. 2022; Khan et. al. 2025). Higher proline levels in *H. cupressiforme* upon rehydration indicated its substantial role as an osmoprotectant that may be crucial for its survival. The accumulation of valine, a branched-chain amino acid, facilitates essential stress signaling activities, such as fuelling TCA intermediates, regulating nitrogen metabolism, and mitigating ROS-induced oxidative damage (Bowne et al., 2012; Zivanovic et al., 2020; Zhang et al., 2025).

Citrate is an intermediate of the TCA cycle. Higher levels of citrate may be associated with the condensation of acetyl-CoA. Although several studies have clearly demonstrated the significance of citrate in stress amelioration and tolerance, most have focused on exogenous citrate supplementation during stress (Mallhi et al. 2019; Tahhib-ul-Arif et al. 2021; Ali et al. 2024). The accumulation of L-malic acid was also concomitant with an active TCA cycle, a key process in energy metabolism. In

460 rehydrated moss samples, significantly higher sucrose levels were observed, serving as an energy
461 source, whereas sucrose metabolism was affected under desiccated conditions. A similar pattern was
462 previously reported in soybean under drought stress (Du et. al. 2020). In the moss *Physcomitrella*
463 *patens*, higher sucrose levels have been reported to be associated with osmoprotection and
464 cryoprotection during desiccation and freezing stress (Oldenhof et al. 2006). Furthermore, an increase
465 in sucrose levels can also be correlated with protection against desiccation, as reported in mosses
466 (Crowe 2014; Gao et al. 2017). Sucrose buildup in moss upon rehydration can be attributed to enhanced
467 carbon fixation, which fuels the production of other sugar metabolites, including D-glucose, raffinose,
468 D-fructose, and D-mannitol. Plants subjected to various abiotic stresses, including salinity and drought
469 (Hoffmann 2010; Thomas et al. 2022; Li et al. 2022).

470 The D-mannitol levels in moss were higher upon rehydration than in the desiccated moss
471 samples. D-mannitol acts as an osmoprotectant in plants, thereby improving cellular osmolarity and
472 reducing water loss (Le and McQueen Manson 2006). Upon rehydration, a significant increase in
473 raffinose level was also observed in the moss. Raffinose is an essential metabolite of the raffinose family
474 oligosaccharides (RFOs), which have been previously reported to play a crucial role in desiccation
475 tolerance (Li et al. 2017; Jing et al. 2018; Yan et al. 2022). The increase in levels of RFOs has been well
476 documented in plants subjected to a wide array of stresses, such as drought, high temperature, cold, and
477 salinity stresses, where it improves plant tolerance and facilitates normal physiological functions (Peters
478 et al. 2007; Amtmann 2009; Sun et al. 2013; Gu et al. 2019; Li et al. 2020). After rehydration, the high-
479 level monosaccharide D-glucose was observed in moss, along with higher levels of disaccharides such
480 as lactose and trehalose. Besides being the primary energy source, D-glucose has multifaceted
481 functions, which include modulation of osmoregulation, opening and closing of stomata, enhancing
482 antioxidant efficiency, promoting pigment biosynthesis, and influencing plant growth by interacting
483 with key growth regulators (Sidiqui et al. 2020; Perdomo et al. 2023). Though higher levels of lactose
484 were observed in moss post-rehydration, its putative role in stress tolerance or amelioration is not yet
485 clearly known in plants (Sami et al. 2016). Trehalose acts as a compatible solute and induces
486 vitrification under water-deficit conditions (Jain and Roy 2009; Ramirez et al. 2024; Dietsch et al.
487 2025).

Additionally, a few of the amino acids and their derivatives exhibited higher accumulation in the moss after rehydration. Higher levels of betaine reflect its osmoregulatory role in improving cell volume and restoring cellular homeostasis upon rehydration (Dobrijevic et. al. 2023). In plants, aspartic acid has a multifarious role in regulating stress responses. Studies in wheat seedlings have shown that aspartic acid regulates antioxidant defense metabolism and reduces ROS overproduction during salinity stress (Sadak et al. 2022). It is an important component in the aspartate metabolic pathway and also associated with various pathways such as TCA cycle, nucleotide metabolism, energy metabolism, and biosynthesis of plant hormones (Lei et. al. 2022). Aminoadipic acid is an intermediate of lysine catabolism in plants and animals (Azevedo and Lea 2001; Gulyas et. al. 2023). In a previous study on rice plants subjected to drought stress, the levels of aminoadipic acid were observed to be significantly high; thus, it was considered a biomarker for grain yield stability (Melandri et. al. 2020). Higher levels of aminoadipic acid in *H. cupressiforme* after rehydration, as compared to the desiccated conditions, may have an essential role in desiccation tolerance. The branched-chain amino acids, such as isoleucine, were high after rehydration in the moss. In previous studies on wheat seedlings subjected to drought stress, the level of isoleucine was found to be exceedingly high, which reflects its potential role in regulating drought stress (Bowne et. al. 2012). Furthermore, studies have shown that isoleucine is crucial in maintaining turgor pressure, with a concentration substantially higher than that of proline (Huang and Jander 2017). In the case of *H. cupressiforme*, levels of isoleucine were higher than those of proline, which indicated its potential role in drought or dehydration tolerance. Several studies have also clearly shown that amino acids such as asparagine (Oddy et. al. 2022), valine (Bowne et. al. 2012), phenylalanine (Ramzan et. al. 2023), fructosylglycine (Gonzalez-Villagra et. al. 2022), ectoine (Czech et. al. 2018), and tyrosine (Wang et.al. 2022) have a possible role in tolerance to drought. Our result in *H. cupressiforme* upon rehydration showed a substantial increase in the levels of these amino acids, indicating its potential role in conferring desiccation tolerance.

In desiccation-tolerant plant species, trehalose is also known as a substitute for water, thus being vital for tolerance to desiccation stress (Dietsch et al. 2025). Studies in yeast have demonstrated that an increase in intracellular trehalose levels can prevent protein degradation and enhance the functionality of cellular molecules (Tapia et al. 2015). In moss *H. cupressiforme*, higher trehalose levels after

rehydration indicated a strong stress tolerance response. The 13-HODE levels were significantly high in moss after rehydration, along with other metabolites such as 13(s)-HOTrE, Oleamide, Lysolecithin, 16-hydroxyhexadeconic acid, and linoleic acid. 13-HODE is an oxidized fatty acid that belongs to the oxylipin sub-pathway (Yang et al. 2018). Though its function in stress tolerance or amelioration is mainly unknown, studies have also reported its 13-HODE potential role in disease resistance, ABA signalling, and ROS signalling pathways (He and Ding 2020). 13(s)-HOTrE is an oxylipin, whose role in desiccation stress is largely unknown. Phospholipids, such as Lysolecitin or lyso-phosphatidylcholine, regulate diverse physiological functions in plants during biotic stress (Volz et al. 2021). In *Arabidopsis*, lysolecithin was found to increase the levels of salicylic acid, thereby enhancing plant immunity (Volz et al. 2021). However, its role in desiccation or other abiotic stress remains largely unknown. In the moss, we observed 05 a fivefold rise in linolenic acid levels after rehydration compared to the dehydrated ones. It is also linked to jasmonic acid synthesis and antioxidant metabolism, thereby imparting a strong function in stress tolerance and amelioration (Zi et al. 2022).

In case of *H. cupressiforme,* rehydration up to >50% RWC resulted in a significant increase in levels of carbohydrates, lipids, and fatty acids, conferring tolerance to desiccation. In plants, choline serves as the precursor for glycine betaine (GB), a compatible solute that may regulate desiccation tolerance (Dace et al. 2023). In moss, an increase in choline levels upon rehydration indicates its role in GB biosynthesis, conferring protection against desiccation-induced physiological alterations. Nicotinamide also regulates stress signalling in plants, protecting against DNA damage, increasing aconitase activity, and improving GSH content in plants (Berglund et al. 2017). Thus, higher nicotinamide levels conferred a significant antioxidative effect upon rehydration of moss. Although the role of urocanic acid in human health is primarily known (Hart and Norval, 2021), its potential function in regulating plant stress responses remains to be assessed. The effect of rehydration in the moss after a prolonged period of desiccation. Our results showed that the pigment content in moss did not considerably change either in dehydrated conditions or upon rehydration. In previous studies on desiccation recovery in the aquatic moss *Fontinalis antipyretica*, it was reported that chlorophyll levels are mostly preserved during desiccated conditions and their concentration increases substantially upon rehydration (de Carvalho et al., 2011, 2012, 2019). Our study revealed that *H. cupressiforme* possesses

a robust pigment system that is consistently conserved, regardless of whether it is hydrated or dehydrated. In previous studies, similar responses were observed in terms of ROS production and oxidative stress, as reported in mosses *Fontinalis antipyretica* and *Hyophila propagulifera* upon rehydration (de Carvalho et al., 2012; Ramyashree et al., 2021). The results revealed a robust mechanism in moss that upregulates antioxidant metabolism, enabling efficient ROS detoxification and allowing restoration of physiological and cellular homeostasis upon rehydration.

Conclusion

Understanding desiccation tolerance traits in plants highlights the impact of changing climatic conditions on biodiversity. Desiccation tolerance is a primitive trait that is well-exhibited by several plant groups, including cyanobacteria, green algae, lichens, bryophytes, pteridophytes, and seed plants (Oliver et al. 2022). It is an evolutionary adaptation that enables the plants to thrive under extreme water scarcity and rejuvenates upon rehydration. The results of our study indicated that *H. cupressiforme* is a desiccation-tolerant moss that maintains its physiological and metabolic features upon rehydration. In addition to this aspect, the moss possesses a wide range of diverse metabolites that can offer potential benefits in numerous ways. Furthermore, the moss possesses a unique ability to hold water. This unique attribute can be productively utilized to retain soil moisture and nutrients in drought-prone areas, aiming towards sustainable soil and nutrient management.

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Authors' contribution

SN and PD**:** Experimentation, data acquisition, methodology; SD: Taxonomic identification, data analysis, review and editing; DB**:** Taxonomic identification, resources, methodology, data analysis, visualization, review and editing; DM**:** Data analysis; visualization, editing, reviewing; SC**:** Conceptualization, design, data curation, data analysis, resources, methodology, writing original draft, review, editing, supervision.

571 **Date availability statement**

572 All the datasets are available in the manuscript and supplementary materials.

573 **Disclosure statement**

574 We declare that there is no conflict of interest

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