




Study of biochemical and biophysical adjustments during transition from desiccation-to-fully-hydrated states in *Riccia gangetica* and *Semibarbula orientalis*

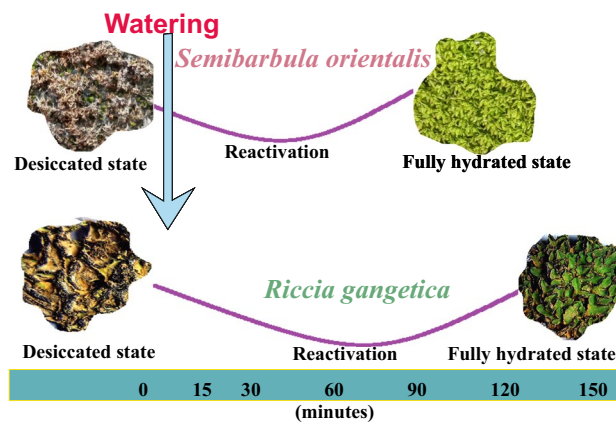
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Abstract

Liverworts are considered as the earliest-divergent clade of land plants, while in phylogenetics mosses are the more advanced sister group to a clade that existed between hornworts and tracheophytes. In the present studies, a comparative analysis was done to understand the biochemical and physiological changes during transition from desiccated to hydrated phases of *Semibarbula orientalis* (moss) and *Riccia gangetica* (Liverwort). In dehydrated state, resurrection plants enter in quiescent phase and stop all molecular, biochemical and physiological processes until the following rehydration. Biochemical (chl content, proline accumulation, expression of antioxidant enzymes) and biophysical (fluorescence values, density of active reaction centers, specific and phenomenological fluxes, and performance of PSII) studies were done to unravel the mechanism of reactivation during the transition from desiccation-to-hydration. In both bryophytes, SOD, CAT and POD remained in inactive form in the fully desiccated state. In contrast, upon the availability of water, rapid upregulation in the activity of antioxidant enzymes was observed in *S. orientalis* as compared with *R. gangetica*. Even upto 15 min of rehydration, significant accumulation of proline reflects its role to prevent hypo-osmotic shock in *S. orientalis*. Rehydration quickly activated photosynthetic machinery in *S. orientalis* than *R. gangetica*. The results clearly reflect that *S. orientalis* have more efficient survival strategies at biochemical and physiological levels as compared with *R. gangetica*.

Graphical abstract



Keywords Abiotic stress · Desiccation · Bryophytes · *Semibarbula orientalis* · *Riccia gangetica* · Antioxidants · Photosynthesis · Chlorophyll *a* fluorescence · Reactive oxygen species · Hypo-osmotic shock

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Introduction

Water scarcity is one of the greatest dilemmas which greatly affect the distribution of plants across the globe. The frequent and longer drought episodes due to global climate change negatively influence the ecosystem and its components. Bryophytes, the first land plants, are believed to be the richest group with resurrection abilities (de León and Montesano 2013). They grow at diverse environmental places such as tree trunks, walls, rock faces, hills, and even at extreme hot and cold regions (Dey and De 2012). The absence of well define vascular system, epidermis, thick cuticle and small size make the plants suitable for experimentation to unravel the mechanism of various abiotic stresses (Onele et al. 2018). During the entire life cycle, these plants encounter numerous dehydration and rehydration cycles, which are regulated by several molecular, biochemical and physiological pathways (Proctor et al. 2007).

Due to the severity of drought, plants deal with many complications such as overproduction of reactive oxygen species (ROS), ion toxicity, osmotic stress, protein degradation, enzyme inactivation, nutrition imbalance, membrane disintegration, bleaching in pigments, DNA damage, and ultimately cell death (Todaka et al. 2015; Cruz de Carvalho et al. 2017; Forni et al. 2017; Zhanassova et al. 2021). Oxidative damage is one of the most deleterious results of desiccation (Mundree et al. 2000; Mihailova et al. 2022). To ameliorate these impediments, resurrection plants are well equipped with enzymatic and non-enzymatic antioxidants such as SOD (superoxide dismutase), POD (peroxidase), CAT (catalase), proline etc. SOD is a metalloenzyme and a vital component of the ROS scavenging system. It converts superoxides into molecular oxygen and H_2O_2 (Scandalios 1993). To abate the toxic effect of H_2O_2 produced, dismutation is also a major step to prevent enzymes of the Calvin cycle (Kaiser 1979). At this time POD and CAT play a major role to control H_2O_2 level in cells. The activity of antioxidants is also associated with increased concentration of proline (Ashraf and Foolad, 2007), which significantly act as osmoregulatory and ROS scavengers (Szabados and Saviouré 2010; Ghosh et al. 2022). Accumulation of proline is a positive sign of peak desiccation tolerance among plants (Verslues et al. 2014). The relative water content (RWC) represents the level of cellular water which indicates the physiological status of water during dehydration and rehydration (Sánchez-Rodríguez et al. 2010).

The chlorophyll *a* fluorescence (chlF) is an impactful technique to study various physiological responses of plants during water reactivation of the desiccated thalli (Schlensog and Schroeter 2001; Kalaji et al. 2014). The quick rehydration induces a metabolic imbalance in the

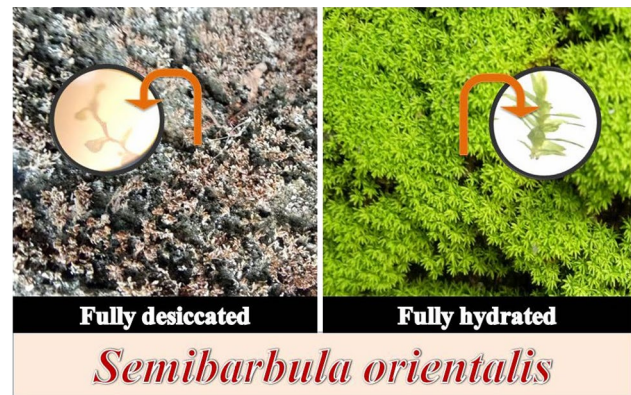


Fig. 1 *S. orientalis* in fully desiccated with 0% RWC (1) and fully hydrated states (2)

resurrection plant which activates the antioxidant system to protect cells from oxidative damage (Rakić et al. 2014). Desiccation alters the primary photochemistry of PS-II, Fv/Fm and performance indexes (Bhatt et al. 2019; Soni 2020). In contrary to this, the bryophytes shows quick physiological activation after rewatering. To unravel these protective strategies in bryophytes, two different clades were taken for the experiment. Liverworts are considered as an earliest-divergent clade of land plants, which are characterized by the presence of oil bodies, polar organizers, elaters and lacking stomata, (Brown and Lemmon 2011; Crandall-Stotler and Stotler 2012; Duckett and Ligrone 1995; Flores et al. 2018, 2020; Ligrone et al. 2012). Vascular and mechanical tissues are also absent in the seta of sporophytes of liverworts. On the other hand in phylogenetics, mosses are the more advanced sister group to a clade that existed between hornworts and tracheophytes, having septate and multicellular rhizoids, filamentous protonemata, a spiral leafy mature gametophyte and the presence of stomata (Chang and Graham 2011; Harris et al. 2020; Ligrone et al. 2012; Merced and Renzaglia 2017; Qiu, 2008; Qiu et al. 2007). In the present studies, comparative analyses were done to understand the biochemical and physiological changes during watering of desiccated thalli of *S. orientalis* (Fig. 1) and *R. gangetica* (Fig. 2).

Material and method

Plants material and culture conditions

Well hydrated rosettes of moss *S. orientalis* and Liverwort *R. gangetica* were collected from the 3 locations of forest area near Udaipur city, India (24.6367° N, 73.6801° E) during July 2019. The 5 large size patches of each plants were



Fig. 2 *R. gangetica* (b) in fully desiccated with 0% RWC (1) and fully hydrated states (2)

collected with the upper crusts of soil by cluster sampling method and maintained in the laboratory for 2 weeks under 12/12 light and dark conditions at 65–70% relative humidity. After 2 weeks, plants were subjected to desiccation by ceasing water supply for 1 month. Thereafter, biochemical and physiological analyses were done to understand the changes during transition from desiccation to rehydration phases in both thalli. Desiccated thalli were rehydrated by spraying water and measurements were conducted during 0 (fully desiccated), 05, 15, 30, 60, 90, 120, 150 min.

Relative water content (RWC)

RWC is measured at an assortment of times to perceive plant water status. For this purpose, 1 g of both plant samples i.e. *R. gangetica* and *S. orientalis* were collected to determine RWC as per the following formula (Turner 1981)

$$\% \text{ RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100,$$

where FW is fresh weight at the time of sample collection, DW is dry weight and TW is the turgid weight of plant samples.

Chlorophyll contents

Arnon (1949) method was used to determine the total chlorophyll content in *R. gangetica* and moss *S. orientalis* by crushing fresh leaves in 80% acetone. The extract was collected in a 3ml vial and cold centrifuged (4 °C) at 5000 rpm for 5 min. The supernatant was analyzed through spectrophotometer at 663 and 645nm against blank acetone for measurements of chl. *a* and *b* respectively. The total

chlorophyll content was calculated by using absorbance taken with the help of the following formula (Arnon 1949)

$$\text{Total chl.} = 20.2 \times (A_{645}) + 8.02 \times (A_{663}),$$

where A is the absorbance at 645 and 663 nm.

H₂O₂ quantification

H₂O₂ content was determined by method described by Velikova et al. (2000). 1 g of samples was homogenized in 0.1% TCA and cold centrifuged (4 °C) at 10,000 rpm for 10 min. 75 µl TCA, 0.5 M KI and 5 mM K₂HPO₄ were added to form reaction mixture. Samples were kept at 25 °C till 20 min. and the optical density was noted at 390 nm. Reaction mixture without KI is used as a blank. The peroxide content was calculated with help of standard curve of pure H₂O₂ standards, whose absorbance was measured at the same wavelength.

SOD assay

The SOD (EC 1.15.1.1) was assayed by Kono (1978) method. The experimental plant samples of *R. gangetica* and *S. orientalis* were homogenized with 50 mM phosphate buffer (pH 7.0) and cold centrifuged at 10,000 rpm for 10 min. The supernatant was mixed with 50 mM potassium buffer (pH 10), 500 µl *p*-nitrobluetetrazolium chloride (NBT), 100 µl Triton and 100 µl hydroxylamine-HCl to form a reaction mixture. The absorbance was recorded at 540 nm by spectrophotometer and the specific activity was expressed in terms of unit ml⁻¹g⁻¹ protein.

POD assay

POD (EC 1.11.1.7) activities were measured by assessing the mixture containing 2 ml of 0.05 mM phosphate buffer (pH 7.0) and 1.0 ml guaiacol (1%) and enzyme extract prepared by samples of both plants *R. gangetica* and moss *S. orientalis* at various time intervals. 0.2 ml of 0.3% H₂O₂ was instantly mixed to initiate the reaction. The absorbance was taken at 470 nm for every 15 s up to 3 min and the data were expressed in terms of absorbance s⁻¹ mg⁻¹ protein according to Racusen and Foote (1965) method.

CAT assay

CAT (EC 1.11.1.6) activity was estimated by Teranishi et al. (1974) method during rewatering of desiccated plants of *R. gangetica* and *S. orientalis* from each variety and treatment. The reaction mixture was prepared of 50 mM potassium buffer (pH 7.0), 20 mM peroxide, and 0.1 ml enzyme solutions. At last, addition of 2 ml of Titanium reagent blocked

the reaction and further, it was centrifuged at 10,000 rpm for 10 min. The optical density was observed at 410 nm and the Catalase activity was expressed as unit g^{-1} FW

Proline estimation

For determination of proline, fresh thallus of both plants were harvested and homogenated in 10 ml of 3% sulpholylic acid and then filtered. The filtered solution was mixed with an acidic nin-hydrin solution, and treated with 100 °C for 60 min in a water bath. The reaction was ended by incubating the mixture in ice for 5 min. Absorbance was taken at 520 nm after the addition of toluene. The proline content in tissues of thallus was calculated from a standard curve of proline by using Bates et al. (1973) method.

Chlorophyll fluorescence analysis

The O–J–I–P transients were measured in the dark chamber by using the Plant Efficiency Analyzer, PEA (Hansatech Instruments, Kings Lynn, UK). Plant materials were fixed in leaf clip of 4mm diameter and exposed to the red light of $3000 \mu\text{mol m}^{-2} \text{s}^{-1}$ by using three high-intensity light-emitting diodes. The fluorescence was measured after 0, 05, 15, 30, 60, 90, 120, 150 minutes of re-watering and analyzed according to the JIP-test measurements as per the formula (Strasser and Strasser, 1995; Tsimilli-Michael and Strasser, 2001)

F_m = Maximum fluorescence,

$RC/CS = (ABS/CS)(RC/ABS)$,

$ABS/CS = \text{approximately proportional to } F_o$,

$ET_o/CS = (ET_o/RC) \times (RC/CS)$,

$PIcs = RC/CS \times [\phi_{po}/(1 - \phi_{po}) \times [\Psi_o/(1 - \Psi_o)]]$.

Statistical analysis

All experiments were conducted in triplicates for both plants *R. gangetica* and *S. orientalis*. The three mean values of all experiments were compared at $p < 0.05$ by using One-way analysis of variance (ANOVA) and after obtaining significance differences the two groups were compared by bonferoni correction method at $p < 0.05$ by using M.S. Excel.

Results and discussion

Water is one of the most abundant and vital compounds on earth, which is required for the survival of all living organisms. However, there are few organisms which belongs to

bryophytes, lichens and angiosperms that have the potential to stay alive after complete loss of water content. The process of anhydrobiosis is evolved when plants were migrated from aquatic to land regions during the evolution. During dehydrated state, resurrection organisms enter a quiescent phase, and stop all molecular, biochemical and physiological processes until they follow rehydration. Bryophytes, commonly known as the amphibians of the plant kingdom, developed the remarkable ability to grow at terrestrial habitats through developing various survival strategies from molecular to physiological levels. In the present work, comparative studies were performed between desiccated thalli of *R. gangetica* (liverwort) and *S. orientalis* (moss) to analyze the biochemical and biophysical strategies required for reactivation upon watering.

The effect of water stress on relative water content (RWC) has been investigated by several researchers. Under severe drought stress, some specific plants encountered irreversible damage when RWC drops below 20%. In the present study, fully desiccated thalli of resurrection bryophytes *S. orientalis* and *R. gangetica* showed 0% RWC which rapidly increased after watering (Fig. 3a). The RWC reached upto 82.3% and 79.12% in 150 min

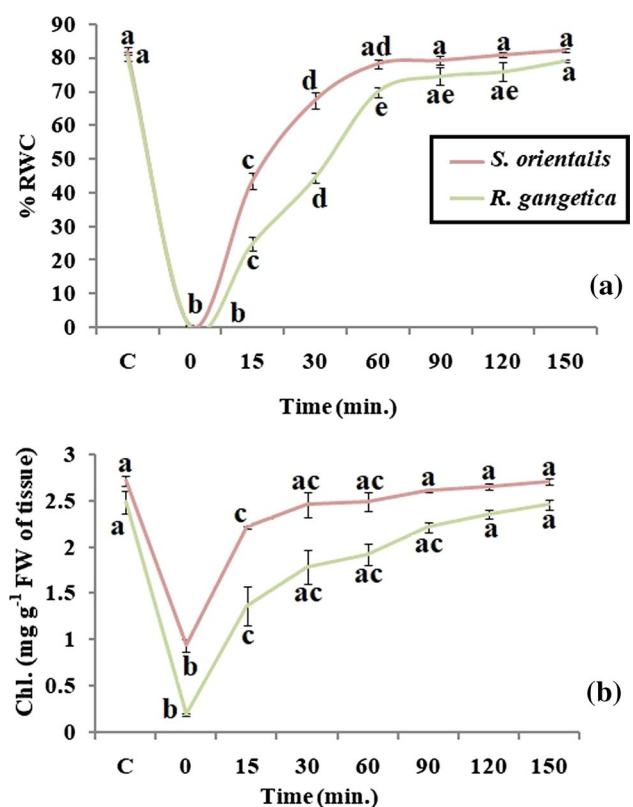


Fig. 3 Changes in RWC (a), chl. content (b) in *S. orientalis* and *R. gangetica* during desiccation to fully hydrated transition

after rewatering *S. orientalis* and *R. gangetica*, respectively (Fig. 3a). The RWC reached up to the control level in both the plants within 150 min. Low RWC of plants allied with falling in water potential and accumulation of some organic solutes such as glucose, sucrose, glucosinolates, proline and some other osmoprotectants which play a major role to retain the metabolic activities of plants (Mattson and Haack 1987; Verslues et al. 2006; Wanjiku and Bohne 2017). During desiccation, slow growth and photosynthetic shutdown reduce their metabolic requirements to adjust the cellular, metabolic and physiological status of vegetative tissues in resurrection plants (Challabathula et al. 2016). Coupling of the photosynthetic shutdown with other metabolic adjustments minimize damage and also protect photosynthetic machinery in resurrection plants (Farrant et al. 2015; Urano et al. 2017), but due to this premature photosynthesis inhibition plants faces energy deficiency which is maintained by using alternate reserve energy sources or by reducing energy expenditure (Asami et al. 2018) which may protect plants from irreversible damage. The speedy recovery in RWC is the indication of fast water retention capacity which helps *S. orientalis* and *R. gangetica* both plants to survive under desiccation.

In bryophytes with daily cycles of dehydration/rehydration, neoxanthin stabilizes the structure of chls by binding with light-harvesting chlorophyll protein (LHCP). The present study showed, rewatering markedly enhanced the chl contents in both bryophytes. After 150 min of rewatering, the chlorophyll concentration reached up to 2.70 mg/g FW and 2.45 mg/g FW in *S. orientalis* and *R. gangetica*, respectively (Fig. 3b). The chl content was only 1.28% and 0.68% lower to the control level after 150 min of rehydration in moss and *R. gangetica*, respectively. Although, the chl reformation was much faster in moss (Fig. 3b), this water-induced rapid increment in chl contents shows the poikilochlorophyllous activation by the rapid re-synthesis of inactive chl molecules into active forms (Bewley 1979; Csintalan et al. 1996).

Formation of ROS during respiration and photosynthesis in mitochondria, peroxisomes and chloroplasts results in 'oxidative burst' (You and Chan 2015). A light mediated overexpression of chl. molecules are the main cause of excess ROS production during desiccated state (Apel and Hirt 2004; Blokhina et al. 2003; Fryer et al. 2003; Wang et al. 2010). The H_2O_2 concentration was highest in fully desiccated thalii (Fig. 4) of both the plants. The peroxide concentration was decreased with increasing time after re-watering, however, a wide variation in ROS scavenging enzymes was observed during the transition of desiccation to a fully hydrated state in both bryophytes. It was observed that SOD, CAT and POD remained inactive during the fully desiccated phase and became active upon the availability of water. A progressive reduction was observed in the H_2O_2 content just after 15 min of

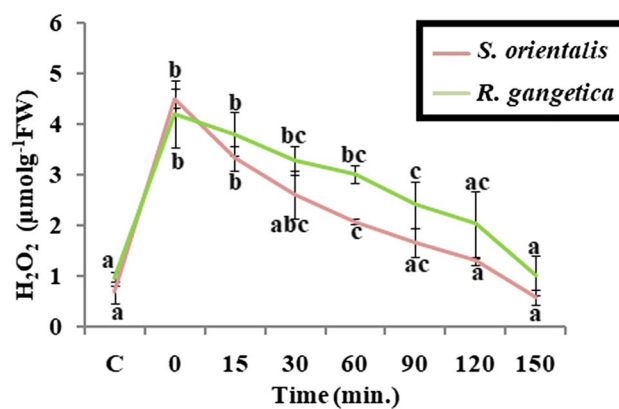
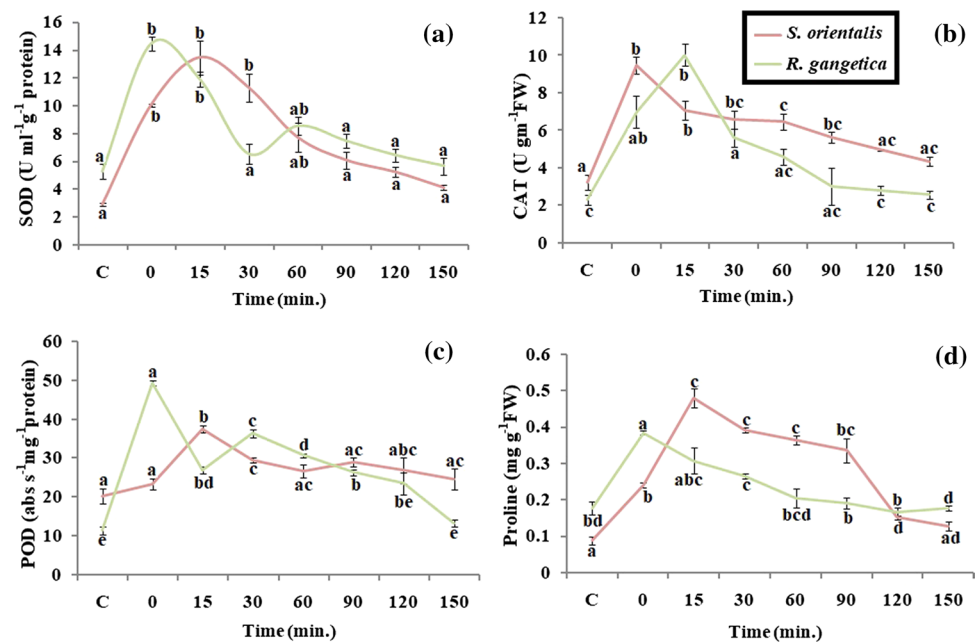


Fig. 4 Change in peroxide concentration with the time in *S. orientalis* and *R. gangetica* during desiccation to fully hydrated transition

rewatering in *S. orientalis* and *R. gangetica* due to the higher activity of ROS-scavenging enzymes (Fig. 4).

The mechanism of desiccation tolerance and reactivation of resurrection plants is a typical phenomenon, dominantly dependent on rehydration-based cellular repairing (Oliver et al. 2004; Oliver and Derek Bewley 2010). At the time of rehydration, plants show oscillatory, fast, vulnerable and unstable recovery which induced maximum antioxidant action for defending cells from ROS, these antioxidant activities are the clear indication of metabolic imbalance (Rakić et al. 2014; Mansoor et al. 2022) in both plants during reactivations. Upon watering, the activity of SOD and CAT initially increased and thereafter slightly declined in *S. orientalis* (Fig. 5a, b). The highest SOD and CAT activity was observed after 15 min of watering (Fig. 5a, b), the higher concentration of the H_2O_2 enhanced the CAT activity in *R. gangetica*. Initial increment in the activities of SOD and CAT during moderate rehydration is a consequence of shifting in redox potential by increased production of ROS (Sgherri et al. 2004; Jovanović et al. 2011). With the course of time, the SOD and CAT activities decreased up to the control level in *S. orientalis* (Fig. 5a, b), and due to the significant drop in peroxide concentration similar results were obtained in *Syzygium maire* (van der Walt et al. 2022). But the SOD activity slight enhanced at 60 min, therefore the amount of H_2O_2 was higher in *R. gangetica* as compare to the moss. Peroxidases are capable of eliminating the access level of H_2O_2 formed during non-enzymatic or enzymatic dismutation which also plays a vital role in cell wall remodeling (Rakić et al. 2014). Therefore, changes in peroxidase activity have been frequently correlated to the response of resistance or susceptibility of organisms to environmental stresses. A temporary electrolytic leakage through the plasma membrane is coordinated with high activities of SOD, CAT and POD during the initial state of rehydration (Sgherri et al. 2004; Jovanović et al. 2011). In the present

Fig. 5 SOD (a), CAT (b), POD (c) and proline accumulation (d) during desiccation to fully hydrated transition in *S. orientalis* and *R. gangetica*



study, the availability of water continuously declined the activity of POD in *S. orientalis* (Fig. 5c). SOD and CAT activity continuously declined after the watering process in *R. gangetica* (Fig. 5a, b). On the other hand, expression of POD initially increased up to 15 min and thereafter declined with increasing watering period in *R. gangetica* (Fig. 5c). The lowering POD activities may be due to the higher oxidation within ascorbate–glutathione cycle (Veljovic-Jovanovic et al. 2006; Rakić et al. 2014).

Proline is the most important osmolyte which allocates cells to hold more water and also act as a metal chelator, antioxidant and signaling molecule which contributes to osmoregulation in plants under various types of biotic and abiotic stresses to stabilize subcellular complexes and redox buffering (Anwar Hossain et al. 2014; Ashraf and Foolad 2007; De Ronde et al. 2004; Gururani et al. 2015; Parvaiz and Satyawati, 2008; Wang et al. 2015). Therefore, proline accumulation is the positive sign of desiccation tolerance. In the present investigation, control plants contain very low amount of proline in both the plants which was evidently increased in desiccated thalii of *S. orientalis* and *R. gangetica*. Proline concentration was higher in desiccated thalii of *R. gangetica* as compare to *S. orientalis*. But it was slightly increased after 15 min of watering and then continuously decreased in *S. orientalis* (Fig. 5d). After a long term, desiccating plants quickly rehydrated, this initial phase generate metabolic imbalance and plants faces hypo-osmotic shock during hydration (Zhou et al. 2022). To prevent plant from this shock, proline concentration enhanced after 15 min of watering in the moss, which is great sign of adaptation to cope the stress conditions. In contrast, proline content rapidly dropped after watering in *R. gangetica* (Fig. 5d) and

finally reached up to the control level. These data collaborated with previous research works, that the proline accumulation is directly proportional to the ability of drought tolerance of plants (Efeoğlu et al. 2009). Our results showed that the *S. orientalis* has advanced strategies to deals with the osmotic stress by a slow drop of proline during rewatering.

Water-induced quick reactivation of photosynthetic apparatus has been observed in many plant species (Heber et al. 2011; Soni 2020). Upon watering, maximal fluorescence (F_m) increased several folds in both the thalli and mimic to the control level, however it was higher in *S. orientalis* than *R. gangetica* (Fig. 6a). The comparative rapid increase of F_m in *S. orientalis* indicates that the watering process quickly converts inactive chl into active chl molecules (Fig. 6b). Similarly the concentration of active PSII reaction centers (RC/CS) markedly enhanced in *S. orientalis* as compared to *R. gangetica* (Fig. 6b). In hydrated thalii of *R. gangetica*, the numbers of active RC/CS were unable to touch the control level even after 150 min. Results indicate that PSII reaction centers remained in their inactive form during the desiccation state of both bryophytes. Traces of water transformed inactive forms of reactions centers into an active one.

Upon watering, phenomenological fluxes [ABS/CS (absorbance per cross section) and ET/CS (electron transfer per cross section)] remarkably increased in *S. orientalis* (Table 1). The rapid increase of ABS/CS and ET/CS in *S. orientalis* is due to the increased amount of chl molecules and functional light-harvesting complexes (LHCs). Despite the rapid increase in chl molecules, no significant variations in ABS/CS and ET/CS were observed in *R. gangetica* (Table 1), which may be due to the lack of functional LHCs. At the beginning of rehydration-fast recovery, zeaxanthin

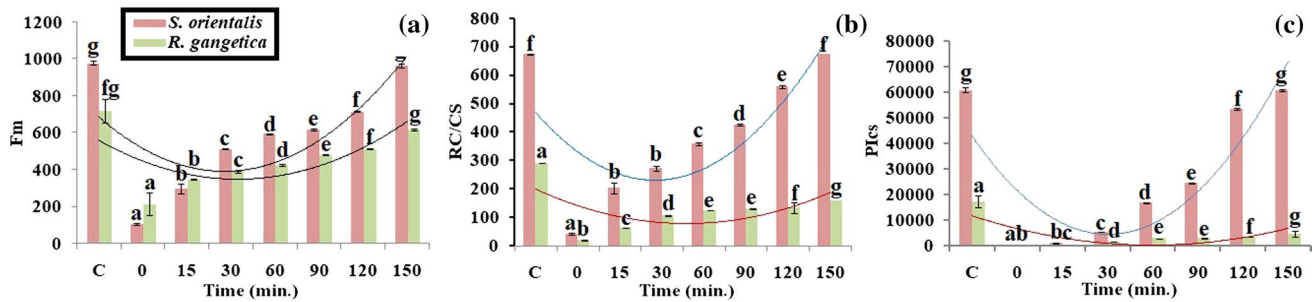


Fig. 6 Maximum fluorescence-Fm (a), density of active PS II-RC/CS (b) and photosynthetic performance index-PIcs (c) during desiccation to fully hydrated transition in *S. orientalis* and *R. gangetica*

Table 1 Variations in absorption and electron transport efficiency of PS-II in *S. orientalis* and *R. gangetica* during desiccation to fully hydrated transition

Time (min)	ABS/CS (absorption per cross section)		ET/CS (electron transfer per cross section)	
	<i>S. orientalis</i>	<i>R. gangetica</i>	<i>S. orientalis</i>	<i>R. gangetica</i>
C	726.54 ± 6.47 ^e	284.91 ± 2.83 ^d	547.90 ± 3.01 ^g	182.46 ± 1.72 ^g
0	111.00 ± 1.00 ^a	161.00 ± 1.00 ^a	005.02 ± 0.16 ^a	15.44 ± 0.67 ^a
15	306.33 ± 4.50 ^b	215.66 ± 0.57 ^b	133.87 ± 0.80 ^b	44.40 ± 0.70 ^b
30	316.33 ± 1.52 ^b	157.66 ± 1.52 ^a	256.96 ± 0.08 ^c	77.40 ± 1.93 ^c
60	517.33 ± 4.50 ^c	166.66 ± 2.08 ^a	282.00 ± 0.03 ^d	87.33 ± 0.57 ^d
90	595.66 ± 1.57 ^d	173.66 ± 3.78 ^a	303.42 ± 0.66 ^e	91.00 ± 1.41 ^{de}
120	620.00 ± 8.71 ^d	192.33 ± 1.15 ^c	409.00 ± 0.10 ^f	91.79 ± 0.70 ^e
150	722.00 ± 6.08 ^e	223.00 ± 2.00 ^b	541.07 ± 0.41 ^g	99.74 ± 0.65 ^f

plays a crucial role in non-radiative energy dissipation when ET/CS lower in *R. gangetica* energy dissipated in the form of heat which must be recognized by the de-epoxidation state of the xanthophyll cycle and non-photochemical quenching (Augusti et al. 2001).

Performance indexes (PI) introduced by Strasser et al. (2004), provides in-depth information about the overall photosynthetic potential of PSI and PSII. The watering process significantly increased the PI per cross section (PIcs) in *S. orientalis* (Fig. 6c). In contrary to this, PIcs slightly increased after 15 min of watering and thereafter remained constant due to the ceased ET/CS till 150 min and unable to recover in *R. gangetica* (Fig. 6c) which shows higher reactivation potential of moss.

Conclusion

Our study focused on the biochemical and physiological reactivation strategies of desiccated bryophytes. The increased ROS concentration in fully desiccated plants shows the higher intensity of stress. Meanwhile, chlorophyll and RWC enhanced after re-watering both the plants. The retardation in H₂O₂ concentration was supported by the activity of antioxidants (SOD, CAT and POD). The

activity of antioxidants enhanced when H₂O₂ was higher and it reshuffled up to the control level after rehydration. All reaction centers become active after 150 min of hydration in *S. orientalis* therefore, the Fm reached up to the control level while the *R. gangetica* takes more time to be active physiologically. It has also been shown by comparative analyses of PIcs of both the plants. The biochemical (chl content, proline accumulation, expression of antioxidant enzymes) and biophysical (fluorescence values, density of active reaction centers, ABS/CS, ET/CS, performance of PSII and PSI) studies during the desiccation-to-hydrated state demonstrate that *S. orientalis* has developed high potential to grow at land habitats during the evolution as compared with *R. gangetica*.

Author contributions UB and VS designed the whole work, UB perform the experiment and wrote the article. VS supervised the whole work and corrected the manuscript.

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Declarations

Conflict of interest No competing interest.


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