Rehydration Quickly Assembles Photosynthetic Complexes in Desiccation Tolerant *Riccia gangetica*

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ABSTRACT

Resurrection plants such as lichens and some bryophytes have remarkable ability to survive under desiccation. At this stage no water content remains in the plant cellular system, but upon rehydration these plants subsequently regain their normal metabolism within seconds or minutes. To understand the physiological strategies of desiccation tolerance and hydration-induced photosynthetic recovery, we studied the polyphasic chlorophyll *a* fluorescence OJIP kinetics in *Riccia gangetica* during desiccation and well-hydrated states. Fv/Fm (maximal quantum efficiency of PSII) of desiccated thallus was found 0.047, which increased upto 0.79 within 24h of rehydration. Similarly, rehydration remarkably induced the light harvesting efficiency and rate of electron transport in *R. gangetica*. Results of present studies reveal that *R. gangetica* has high ability to survive under desiccation phase and quickly recover the photosynthetic process upon rehydration through the activation of light harvesting complexes, and conversion of inactive PS II into functional state.

Introduction

Figure 1:

- Riccia gangetica* in desiccated state, and
- After 24 h of rehydration

Desiccation tolerance is a unique potential of lichens and some bryophytes [1]. The idea of a repair-based mechanism of desiccation tolerance in bryophytes was introduced by Bewley [2]. *Riccia gangetica* (Ricciaceae) has physiological and biochemical adaption to equilibrate their cellular water levels with atmospheric water levels, a trait known as poikilohydry. At desiccated stage, the thalli of *R. gangetica* appear to be dead-brown, dried and lifeless (Figure 1a). Traces of water rapidly activate the photosynthetic

process (Figure 1b). Hydration induced quick activation of photosynthesis indicates that all components of light reaction and enzymes are preserved throughout the events of desiccation. JIP test based on polyphasic chlorophyll *a* fluorescence analysis has become one of the powerful tools to study survival strategies in lichens and bryophytes [3,4]. In present investigations, efforts were done to answer the following questions

- How the photosynthetic machinery is preserved in desiccated *R. gangetica*?, and
- How rehydration reactivates the photosynthetic process in desiccated thallus within seconds or minutes? To answer these questions, polyphasic chlorophyll fluorescence OJIP kinetics were studied during desiccation and rehydration process in *R. gangetica*.

Materials and Methods

Plant Materials & Measurement of Polyphasic Chlorophyll Fluorescence Kinetics: Dehydrated thalli of *R. gangetica* were collected from botanical garden, University College of Science, Udaipur (India) during rainy season (August, 2019). The Chlorophyll *a* fluorescence parameter (F_0 -minimum fluorescence, F_m - minimal

fluorescence) were measured by HandyPEA (HansaTech, UK) according to the JIP- test [3,4]. The leaf clip (HansaTech, UK) was modified so as to facilitate the addition of water from the back side even during multiple light pulse measurements. Prior to hydration, F_0 -minimum fluorescence, and F_M - minimal fluorescence of desiccated thalli were recorded (Tables 1 & 2). Upon hydration, fluorescence transient O-J-I-P were recorded at 5Sec, 1h and 24h. The phenomenological energy fluxes (Absorption flux per cross section-ABS/CS, trapped energy flux per cross section-TR/CS, and electron transport flux per cross section -ET/CS), the density of active PS II reaction center (RI/CS), quantum yield of primary photochemistry ($F_v/F_M - \phi_{po}$), quantum yield of electron transport ET/ABS (ϕ_{Eo}), quantum yield of energy dissipation (ϕ_{Do}) were calculated as per the equations of JIP- test [3]

Table 1: Data extracted from the recorded fluorescence transient O-J-I-P.

Data extracted from the Recorded Fluorescence Transient O-J-I-P	
	$F_0 \cong F_{50\mu s}$
	$F_m = F_p$

Table 2: Data extracted from the recorded fluorescence transient O-J-I-P.

Phenomenological Energy Fluxes (Per Excited Cross Section – CS)	
ABS /CS- Absorption flux per cross section	F_0
TR /CS- trapped energy flux per cross section	$j_{po} \times (ABS / CS)$
ET / CS- electron transport flux per cross section	$j_{po} \times y_0 \times (ABS / CS)$

Performance Index on Cross Section Basis $PI_{CS} = \frac{ABS}{CS} \times \frac{1 - (F_0 / F_M)}{M_0 / V_j} \times \frac{F_M - F_0}{F_0} \times \frac{1 - V_j}{V_j}$

Results and Discussion

Desiccation-tolerance, the skill to survive without water for longer period and then recover normal metabolic activity upon rehydration, is one of the most remarkable features of bryophytes. This ability to survive under desiccation has also been observed in lichens and bryophytes [3,4]. In this study, we chose the *R. gangetica*, one of the dominant bryophytes of Rajasthan (India), to investigate the mechanism of conservation of photosynthetic potential under desiccation and rapid recovery of photosynthesis upon rehydration. Desiccated thalli did not show any variation between F_0 and F_M , which shows complete inactivation of photosynthetic activity (Figure 2). A quick induction in chlorophyll fluorescence was noticed when desiccated thalli were hydrated. Chlorophyll a fluorescence increased continuously from initial (F_0) to maximal (F_M) intensity in after 5 seconds of watering (Tables 3 & 4). Two intermediate peaks F_j (chlorophyll fluorescence at 2ms) and F_i (chlorophyll fluorescence at 300ms) were formed between F_0 and F_M , forming a typical OJIP curve. Rapid increase in fluorescence indicates quick recovery of photosynthetic machinery in *R. gangetica*.

Table 3: Density of reaction centers

Density of Reaction Centers	
$RC/CS = \phi_{po} \cdot (V_j / M_0) \cdot \frac{ABS}{CS}$	Density of reaction centers (Q_A -reducing PSII reaction centers)

Table 4: Quantum efficiencies or flux ratios.

Quantum Efficiencies or Flux Ratios	
j_{po} or TR / ABS or F_v / F_M	Quantum yield of primary photochemistry
ϕ_{Eo} or ET / ABS	Quantum yield of electron transport
ϕ_{Do} or DI / ABS	Quantum yield of energy dissipation

Figure 2: Induction of chlorophyll a fluorescence during desiccation and rehydration stage in *R. gangetica*.

F_v/F_m (j_{p0} or TR / ABS) is a sensitive indicator of plant photosynthetic performance. Lower values of F_v/F_m indicates down-regulation of photosynthesis in plants (Maxwell & Johnson 2000). Upon rehydration, F_v/F_m apparently increased from 0.047 (desiccated state) to 0.79 (24h after rehydration). Increase of F_v/F_m indicates that the thalli have remarkable ability to restore photosynthetic performance by the activation of light harvesting

complexes and other proteins/enzymes involved in photosynthesis process. Similarly, quantum yield of electron transport (ϕ_{E0} or ET / ABS) increased with increasing the duration of hydration upto 24h. A drastic decline in quantum yield of energy dissipation (ϕ_{D0} or DI / ABS) indicates the utilization of light energy into electron transport process (Figure 3a).

Figure 3:

a. Radar plot and

b. leaf model showing the variations in photosynthetic parameters during desiccation and various rehydration stages in *R. gangetica* (black dots are representing inactive PSII RCs and intensity of leaf color is indicating chlorophyll concentration).

Density of active reaction (RC/CS) centers increased upon rehydration (inactive RCs are denoted by black dots in leaf model of desiccated thallus in Figure 3b). Increase in RC/CS is due to the conversion of inactive PSII into functional state. Absorbance per cross section (ABS/CS) rapidly increased during rehydration process. It may be due to the quick assembly of light harvesting complexes (LHCs). Trapping (TR/CS) and electron transport per cross section (ET/CS) also enhanced when desiccated thalli were rehydrated (Figure 3b). Increment in TR/CS and ET/CS suggests that rehydration converts inactive PSII reaction centers into active forms. Performance indices provide information about the overall photosynthetic performance of PSI and PSII. In present studies,

rehydration rapidly increased the performance index (PIcs) in *R. gangetica*. On the basis of all photosynthetic parameters, it is concluded that *R. gangetica* has evolved a remarkable physiological adaption to protect their photosynthetic machinery under desiccation stage. The results also indicate that during desiccation PS II reaction centers become inactive and act as 'energy dissipating units', while they convert into 'energy utilizing units' upon rehydration.

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