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CHLOROPHYLL FLUORESCENCE AND PHOTOSYSTEM II PHOTOCHEMISTRY OF DESICCATION TOLERANT CYANOBACTERIUM *SCYTONEMA GEITLERI* ON RE-HYDRATION AND DE-HYDRATION

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ABSTRACT - Chlorophyll fluorescence analysis provides a sensitive and near instantaneous measurement of the behavior of photosynthetic system during and after stress. The photosystem II (PS II) activity during gradual re-hydration and de-hydration of desiccation tolerant cyanobacterium *Scytonema geitleri* was studied in intact cells/mats. Three types dry, GSDR (grown but slowly dried) and GQDR (grown but quickly dried) mats of the cyanobacterium on re-hydration at 0 MPa osmotic water potential for different time periods in light, showed enhancement in the values of chlorophyll fluorescence parameters Fo (minimal fluorescence level with all PSII reaction centers open), Fv (variable fluorescence in dark adapted state), Fv/Fm (maximum efficiency of PSII photochemistry in dark adapted state), ΔF/Fm' (quantum yield of PSII electron transport), Fv'/Fm'(efficiency of excitation capture by open reaction centers of PSII) and qP (photochemical fluorescence quenching coefficient; oxidation state of PSII) with increasing incubation periods in intact cells/mats. Contrary to this, qN (non-photochemical fluorescence quenching coefficient) showed first gradual increase and then decreasing pattern in all the mats A decreasing pattern in the values of Fm, Fv, Fv/Fm, Fv'/Fm' ΔF/Fm' and qP in cells/mats on lowering of the water potentials (dehydration) was observed. Different pattern of first increase then decrease on lowering of matric water potentials was observed in qN. Decrease in the values of fluorescence parameters on de-hydration of wetted mats may be accompanied by an alteration of the inner structure and composition of the thylakoid membranes. Increase in the values of fluorescence parameters on re-hydration of dry mats favor the possibilities of 'repair' by means of re-synthesis and / or re-arrangement of the damaged / changed composition of thylakoid membranes of the desiccation tolerant cyanobacterium *Scytonema geitleri,* on re-wetting.

Key words : Chlorophyll fluorescence, de-hydration, re-hydration, maximum quantum yield of PS II photochemistry, photochemical fluorescence quenching, non-photochemical fluorescence quenching, efficiency of excitation capture by open PS II reaction centers, quantum yield of PS II electron transport.

INTRODUCTION

Many cyanobacteria are well known which withstand drying to extremely low water content of 5-10 % of their dry weight. No liquid phase remains in the cells at this point, however the cyanobacteria recovered their normal metabolism within minutes or hours on re-hydration. This may be due to recovery from dryness or re-organization, of all the essential metabolic processes or systems including photosynthetic apparatus of the cyanobacteria. Several cellular processes such as photosynthesis, respiration, nitrogen fixation, carbon balance, protein synthesis and maintenance of membrane integrity have been suggested to explain the ability of such desiccation tolerant organisms to survive extreme changes in water content (Brock, 1975; Stewart *el al,* 1978; Potts and Friedmann, 1981; Potts and Morrison, 1986). Photosynthetic electron transports related to PS I, PS II and whole chain as well as water splitting, light absorption and transfer of excitation energy by light harvesting pigments are known to be affected by water content variation (Wiltens *et al,* 1978; Vertucci *et al,* 1985). Photosystem II photochemistry has been shown to be more sensitive to water stress (Masojidek *et al,* 1991; van Rensburg and Kruger 1993; Masojidek *et al,* 2000). Several *in vivo* studies demonstrated that water stress resulted in damage to the oxygen-evolving complex of PS II (Canaani *et al,* 1986; Toivonen and Vidaver, 1988) and to the PS II reaction centres (Satoh *et al,* 1983; Havaux *et al,* 1986;).

Abbreviations: Chl *a* – chlorophyll *a*; car- carotenoids PS I- photosystem I; PS II - photosystem II; DCMU - 3 (3,4 - dichlorophenyl)-1, 1-dimethylurea; chl *a* – chlorophyll *a*; car – carotenoids; Fo, Fv, Fm – minimum, variable and maximum fluorescence in dark - adapted state after DCMU application; Fo', Fv', Fm' – minimum, variable and maximum fluorescence in light – adapted state; Fv/Fm – maximum photoochemical quantum yield of PS II (maximum efficiency of PS II photochemistry); Fv'/Fm', - efficiency of exciton capture by PS II reaction centers; ΔF/Fm, - quantum yield of PS II electron transport; qP – photochemical fluorescence quenching; qN non – photochemical fluorescence quenching. GQDR – grown but quickly dried; GSDR – grown but slowly dried.

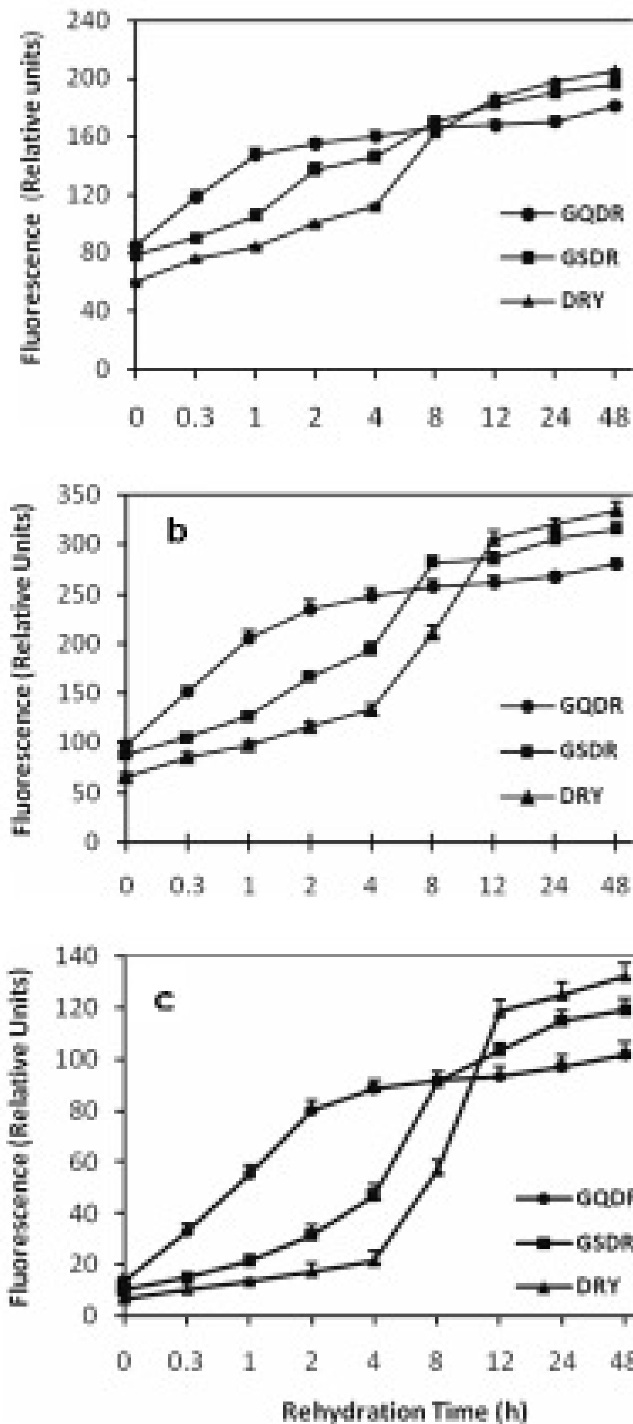

Fig. 1 : Changes in (*a*) Fo -minimum fluorescence, (b) Fm - maximum fluorescence and (c) Fv - variable fluorescence in GQDR (grown and quickly dried mats), GSDR (grown and slowly dried mats) and Dry mats of *S. geitleri* re-hydrated at 0 MPa osmotic water potential for different time periods in light. Values are means ± SE (n= 3).

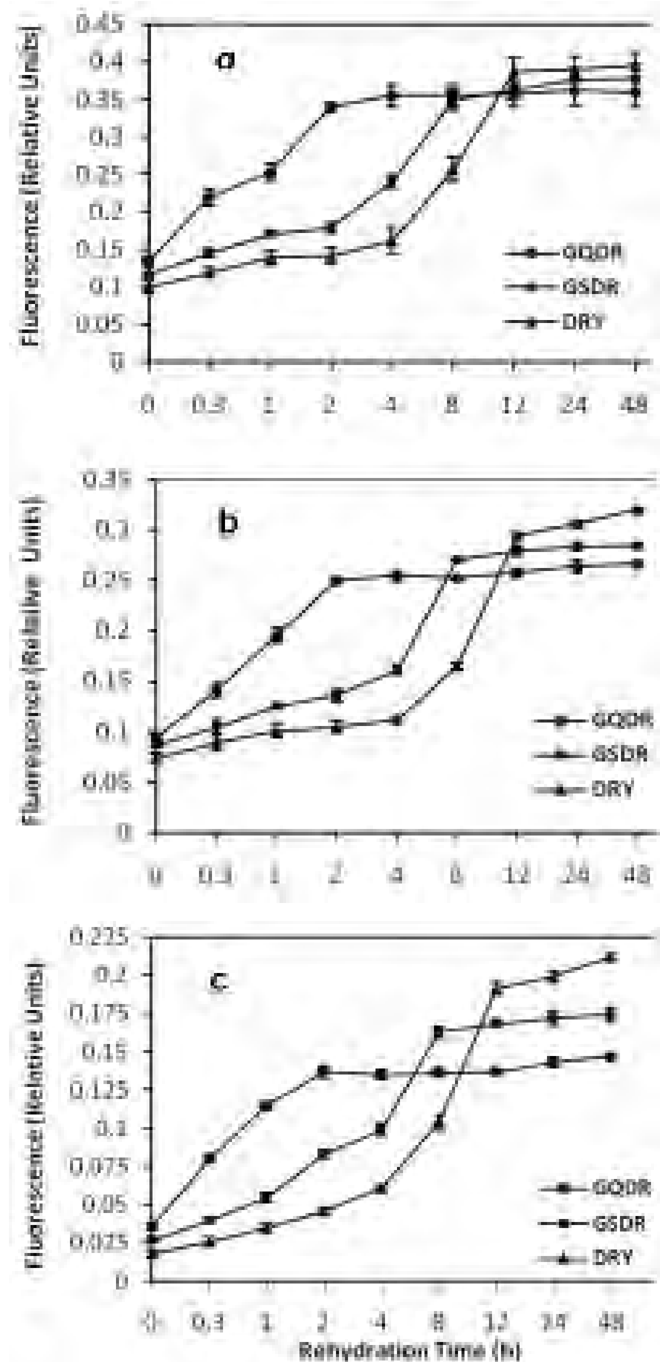

Fig. 2 : Changes in (*a*) Fv/Fm -maximum photochemical efficiency of PS II, (b) Fv'/Fm' – efficiency of excitation capture by open PS II reaction centers, and (c) ΔF/Fm' (quantum yield of PS II electron transport) in GQDR (grown and quickly dried mats), GSDR (grown and slowly dried mats) and Dry mats of *S. geitleri* re-hydrated at 0 MPa osmotic water potential for different time periods in light. Values are means ± SE (n= 3).

The function of photosynthetic pigments has been well established as light harvesting and water oxidation in photosystem II (PSII) which is identified by chlorophyll a fluorescence. The light energy absorbed by the chlorophyll (Chl) of photosynthetic organisms drives photosynthesis and is also dissipated as heat and fluorescence. The energy distribution between photochemical activity and thermal dissipation can be estimated from Chlorophyll fluorescence parameters. Although it is generally the case that more than half of the Chl *a* within oxygenic organisms is associated with PS I, changes in the yield of Chl *a*

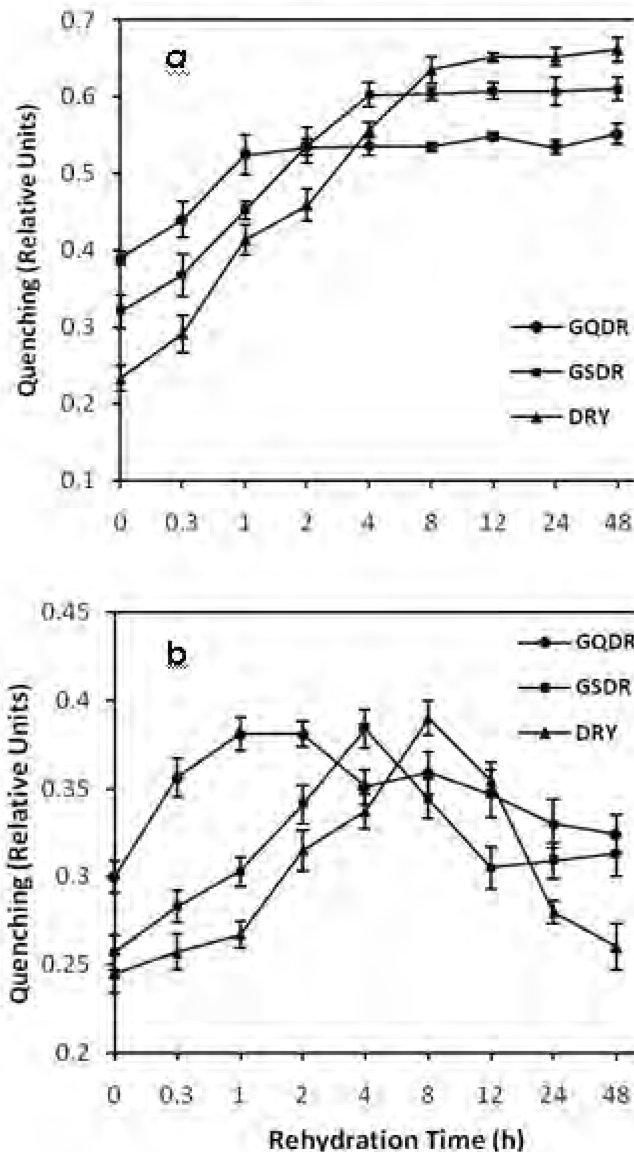

Fig. 3 : Changes in (*a*) qP - photochemical fluorescence quenching, and (b) qN – non-photochemical fluorescence quenching in GQDR (grown and quickly dried mats), GSDR (grown and slowly dried mats) and Dry mats of *S. geitleri* re-hydrated at 0MPa osmotic water potential for different time periods in light. Values are means ± SE (n= 3).

fluorescence are usually interpreted exclusively in the context of PS II. Therefore chlorophyll fluorescence measurement is one of the most widely used techniques for the measurement of the behavior of photosynthetic system especially PS II during and following stresses. Steel *et al* (1992) using a modulated fluorometer studied the changes in the PS II activity (reflecting in Fv/Fm and ΔF/Fm') during drying and gradual re-hydration of the desiccation tolerant moss *Torula ruralis* ssp. *ruraliformis* and the desiccation-sensitive moss *Dicranella palustris*. A strong depression of the photochemical quantum yield of PS II (Fv/Fm) in

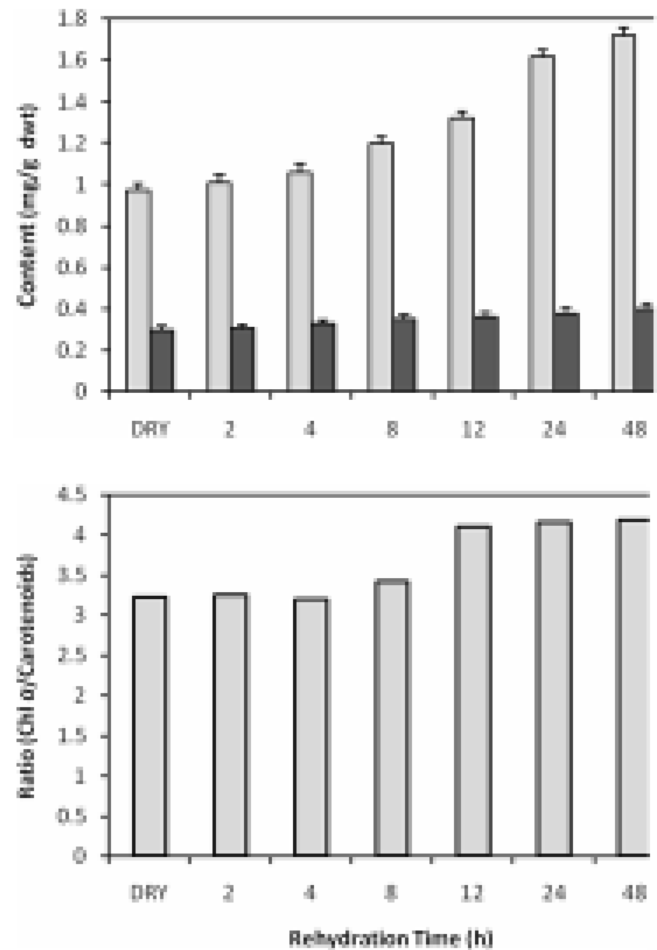

Fig. 4 : Amount (a) and ratio (b) of Chl *a* ( ) and carotenoids ( ) of thylakoids isolated from *S. geitleri* dry mats and dry mats re-hydrated at 0 MPa osmotic water potential for different time periods under growing conditions. Values are means ± SE (n=3).

Chlorococcum on exposure to 0.2 M NaCl was reported by Masojidek *et al* 2000. Kongake *et al* (2009) have reported that Fv/Fm, $Ö_{PS II}$, qP and NPQ in salt-stressed seedlings in rice decreased significantly. Masojidek *et al* (2000) have also reported that the decrease in Fv/Fm was accompanied by an increase in the non-photochemical quenching coefficient (qN). Osmotic stress in *Dunaliella tertiolecta*, inhibits non-cyclic electron transport and stimulates cyclic electron transport and fluorescence emission arising from PS I at 77 K, suggesting that the inhibition of PS II activity may b*e* due to pH dependent down-regulation and state- 2 transition (Gilmour *et al,* 1985). Endo *et al* (1995) have suggested that, the inhibition of quantum yield of PS II photochemistry by osmotic stress in *Chlamydomonas renihardtii,* is due to an increase in non-photochemical quenching which is attributable to a state 2 transition. Satoh *et al* (1983), in a red alga *Porphyra perforata,* shown that the decrease in excitation energy reaching PS II reaction centers and the inhibition of the oxidizing side of the PS II by salt stress

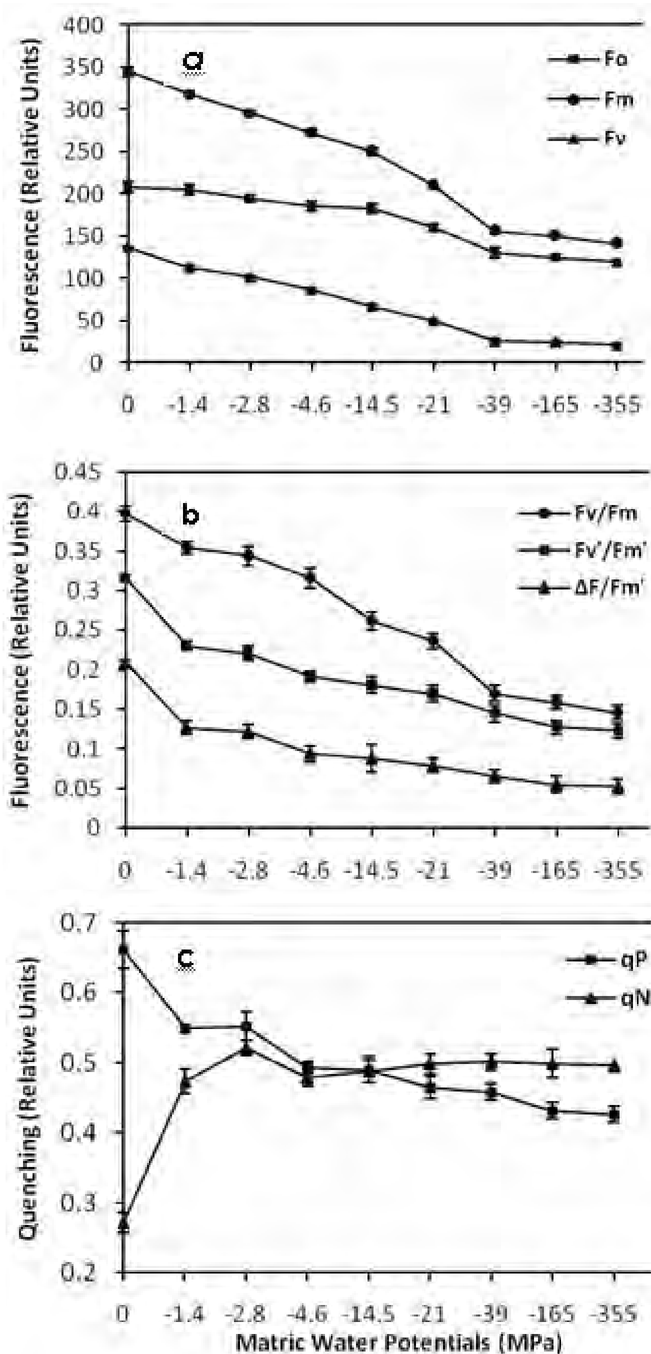

Fig. 5 : Changes in (*a*) Fo- minimum fluorescence, Fm- maximum fluorescence, Fv- variable fluorescence, (b) Fv/Fm- maximum photochemical efficiency of PS II, Fv'/Fm'- efficiency of excitation capture by open PS II reaction centers, ΔF/Fm' (quantum yield of PS II electrontransport) and (c) qP- photochemical fluorescence quenching & qN- non-photochemical fluorescence quenching in growing mats of *S. geitleri* de-hydrated at different matric water potentials for 48 h in dark. Values are means ± SE (n=3).

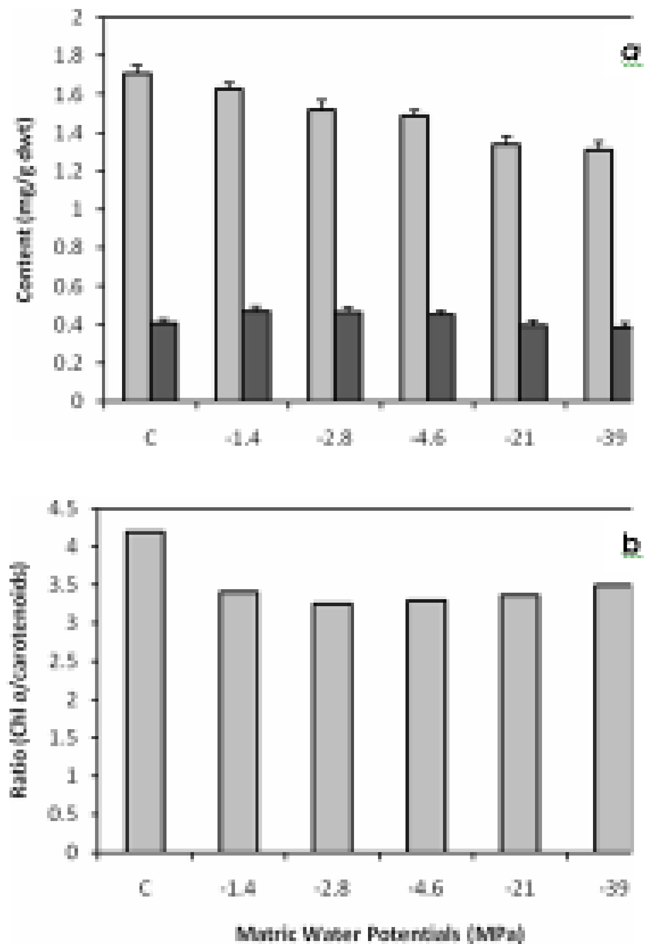

Fig. 6 : Amount (*a*) and ratio (b) of Chl *a* ( ) and carotenoids ( ) of thylakoids isolated from *S. geitleri* growing mats (C) and growing mats de-hydrated at different matric water potential (MPa) for 48 h in dark. Values are means ± SE (n=3).

resulted in a decrease in PS II activity. Wiltens *et al* (1978) by drying several marine algae demonstrated that the inhibition of PS II activity by desiccation was probably due to the loss of the water-splitting system of PS II. In this connection, Lu *et al* (1998) have suggested that inhibition of quantum yield of PS II electron transport in *Spirulina platensis* by osmotic stress is due to an increase in the proportion of the non-reducing PSII reaction centers.

Noticeably, looking on the observation of Baker (1991) that it is PS II that plays a significant role in response of photosynthesis to environmental perturbations, and few studies on PS II photochemistry under water stress, in the recent years, attempts are made to understand the photochemistry of photosystems under the conditions of water stresses by applying fluorescence techniques (Schreiber *et al.* 1995; Campbell and Oquist. 1996; Masojidek *et al.* 2000). However, most of the studies made under water stress conditions are mainly confined to osmotic variations (Endo *et al,* 1995, Lu *et al,* 1998, Papageorgiou *et al,* 1998; Masojidek *et al,* 2000). Osmotic stress has been found ineffective on Fo and qN in *Spirulina plantensis* (Lu *et al,* 1998), but lowered the values of state 1 of *Synechococcus* sp. PCC7942

(Papageorgiou *et al,* 1998). The values of Fm have been lowered on lowering of water level in almost all the cases (*Lu et al,* 1998; Papageorgiou *et al,* 1998), resulting in lowering down of maximum quantum yield of PS II photochemistry (Fv/Fm). Besides, the efficiency of the PS II in light acclimated conditions declined in *Synechococcus* sp. PCC7942 on application of increasing concentrations of sorbitol and manitol (Papageorgiou *et al,* 1998). However, only a few detailed studies on the photochemical changes, induced in desiccation tolerant organisms, subjected first to water stress and then allowed to recover, have been made.

Scytonema geitleri, on its terrestrial habitats, roof-tops in particular, has been observed to face extremes of environmental stresses mainly desiccation. The characteristic features of the cyanobacterium showing recovery of metabolic activities after extremes of desiccation and higher temperature (up to 70°C) under dry state (Talpasayi and Tripathi, 1987) provide a model to understand the process of recovery and stability of the photosynthesis and its apparatus during the course of wetting and drying at its habitats. Hence, the work presented in this paper has been mainly aimed to understand PS II photochemistry during re-hydration and de-hydration of air dried and grown mats of the cyanobacterium respectively.

MATERIALS AND METHODS

Organism and culture conditions

Dry mats *of Scytonema geitleri,* collected by scraping from surfaces of roof- top *o*f buildings during late rainy season, were gently washed with water to remove maximum possible soil particles. The mats free from soil particles were stored in a desiccator containing concentrated sulphuric acid in dark at 25±1°C. To obtain the grown mats of the cyanobacterium, the stored mats were treated osmotically at 0 MPa water potential solutions and incubated for different time periods, as required, at 25±1°C under 35 $\mu Em^{-2} s^{-1}$ light intensity of fluorescent tube light. Fully grown mats dried matrically in the atmosphere of concentrated sulphuric acid were treated as grown but quickly dried mats (GQDR), whereas mats dried over concentrated sulphuric acid after 24 h pre-drying in the atmosphere of -21 MPa were treated as grown but slowly dried mats (GSDR).

Isolation of thylakoid membrane

Thylakoid membranes were isolated by following the method described by Samuelsson and Prezelin (1986) and Leegood and Malkin (1986) with some modifications.

Measurement of chlorophyll fluorescence

Chlorophyll fluorescence measurements were performed using the pulse-amplitude-modulated *(PAM)* fluorometer (Hansatech, Great Britain, Model MK II) indry and grown mats of *Scytonema geitleri* according to method described by Campbell *et al* (1998) and Lu *et al* (1998). Samples were dark-adapted for 15 minutes prior to measurement of fluorescence signals. Measurements were made at room temperature (25± 1°C).

The chlorophyll fluorescence was excited with a modulated yellow light (peak wavelength, 585 nm; modulation frequency 4.8 KHz approximately) provided by an array of yellow light emitting diodes. Fluorescence emission was detected with a photodiode through 700nm interference filter. The minimal fluorescence level (Fo) with all PS II reaction centers open, was measured using the modulated light, which was sufficiently low (1.6 $\mu Em^{-2} s^{-1}$) and do not induce any significant variable fluorescence (essentially no photosynthesis). The maximal fluorescence level (Fm^d) with PS II reaction centers closed was determined by using 1s high intensity saturating light pulse (8000 $\mu Em^{-2} s^{-1}$, blue light excitation) on dark-adapted samples. The saturating light pulse source was a 12V, 24W halogen lamp. The light was passed to the samples using Hansatech A8 fiber optic cable. Then, the samples were continuously illuminated with a white actinic light at an irradiance of 200 $\mu Em^{-2} s^{-1}$, which was equivalent to the actual growth light for the cyanobacterium. The steady-state value of fluorescence (Fs) reaching within about 10 minutes was thereafter recorded and a second saturating pulse at 8000 $\mu Em^{-2} s^{-1}$ was imposed to determine the maximal fluorescence level in light-adapted state (Fm’). The minimal fluorescence level in light-adapted state (Fo’) was determined by switching-off actinic light. The true maximal fluorescence (Fm) was measured after addition of DCMU (10 µM) to samples under irradiance of 8000 $\mu Em^{-2} s^{-1}$

Using original fluorescence parameters Fo, Fm, Fo’, Fm’, and Fs as indicated in Fig. 1, the derived variables were calculated:

i. Fv =Fm -Fo

ii. Fv’ = Fm’ - Fo’

iii. Fv/Fm = (Fm -Fo)/Fm

iv. qP = (Fm’ -Fs) / (Fm’ -Fo’) = ?F/Fv’

vi. qN =1- (Fm’ - Fo’)/(Fm - Fo) = 1- Fv’/Fv

vii. Fv’/ Fm’ = (Fm’ -Fo’) /Fm’

viii. ΔF/ Fm’= (Fm’ -Fs)/ Fm’

Fluorescence nomenclature and calculation was according to van Kooten and Snel (1990) and Genty *et al*

(1989).

Re-hydration

Dry, grown but quickly dried (GQDR) and grown but slowly dried (GSDR) mats *of Scytonema geitleri* rehydrated at 0 MPa for different time periods, 0.30, 1, 2, 4, 8, 12, 24 and 48 h in growing light. After re-hydration mats were pre-incubated in dark for 15 min and chlorophyll fluorescence was measured as described above.

De-hydration

Dry mats *of* the *Scytonema geitleri* grown at 0 MPa osmotic water potential for 48 h under growing condition were de-hydrated matrically at –1.4, -2.8, -14.5, -21, -39, -165, and –355 MPa for 48 h. Water potentials corresponding to –1.4, -2.8, -14.5, -21, -39, -165, and –355 MPa, were obtained by using salt solutions of NaCl, NaoH and $CaCl_2$ as mentioned by Vertucci and Roos (1993) and Lang (1967).

Extraction and estimation of Chl a and carotenoids

Extraction of Chl *a,* and carotenoids in the cyanobacterial samples were made according to the methods described by Tripathi (1963). For quantitative estimation of Chl *a*, the formula proposed by Talling and Driver (1963) and for carotenoids formula of Myers and Kratz (1955) was used.

RESULTS

All the three types of dry mats of the cyanobacterium when subjected to re-hydration at 0 MPa (osmotic water potential) for different time periods in light (Figs.1, 2 & 3) showed enhancement in the values of Fo (Fig. 1a), Fm (Fig. 1b), Fv (Fig. 1c), Fv/Fm (Fig. 2a), Fv'/Fm' (Fig.2b) and ΔF/Fm' (Fig. 2c) with increasing the incubation period. The GQDR mats showed comparatively faster recovery. Almost within 1-2 h, all the parameters of the chlorophyll fluorescence recovered to their maximum values. Among these parameters, the recovery in the values of Fv/Fm was quite high (Fig. 1b). Followed by the GQDR mats, GSDR mats reflected the quickness in recovery in the parameters of the chlorophyll fluorescence. They recovered to their maximum in the time periods of 4-8 h. However, values were considerably higher than the GQDR mats. Though, dry mats took 8-12 h in acquiring maximum recovery in chlorophyll fluorescence parameters, the recovery in the values was maximum among all the re-hydration treatments.

Photochemical quenching of fluorescence (qP) followed almost similar pattern as other parameters of the chlorophyll fluorescence. The GQDR mats showed enhancement in the value of qP just after 2 h incubation (Fig. 3a), which became almost stationary on increasing the re-hydration period at 0 MPa osmotic water potential in light; whereas GSDR mats as well as dry mats took nearly 8 and 12 h, respectively, for attaining the maximum qP values. Of course, in GSDR and dry mats qP value was initially very low which started increasing only after 2 and 4 h of incubation respectively (Fig. 3a) Contrary to these, non-photochemical fluorescence quenching (qN) showed a gradual increase in its value in the first 1, 2 and 4 h of re-hydration in the GQDR, GSDR and dry mats respectively, and thereafter, qN showed decreasing pattern in these three types of mats. Noticeably, the enhancement and decrease in the values of qN were very distinct in GSDR as well as in dry mats (Fig. 3b).

An increase in the amount of Chl *a* and carotenoids of thylakoid membranes of the cyanobacterium on re-hydration of dry mats of the cyanobacterium at 0 MPa osmotic water potential was observed (Fig. 4a). There was a gradual increase (4.13, 9.27, 23.71, and 36.08%) in Chl *a* content with increasing incubation periods from 2 to 12 h, and thereafter, a sharp increase by 67.0% and 77.3% was observed in thylakoid membranes of mats grown for 24 and 48 h, respectively (Fig.4a). Increase in carotenoids' content was comparatively low, as it showed only 35 % enhancement even after 48 h incubation (Fig. 4a). The Ratio Chl *a* / carotenoids also indicated a greater increase in the Chl *a* amount compared to carotenoids (Fig. 4b). The ratio between Chl *a* and carotenoids increased from 3.23:1 to 4.3:1 after 48 h incubation of dry mats of the cyanobacterium (Fig.4b).

Impact of dehydration on chlorophyll fluorescence of the cyanobacterium was studied in details emphasizing the PS II photochemisry by monitoring the different fluorescence parameters in the dry mats grown for 48 h and then dehydrated at different matric water potentials (Fig. 5). 48 h grown mats, when matrically de-hydrated at -1.4, -2.8, -14.5, -21.0, -39, -165, and -355 MPa water potentials for 48 h in dark, showed a decrease in the values of Fo, Fm, Fv (Fig. 5a), Fv/Fm, Fv'/Fm', ΔF/Fm' (Fig. 5b) and qP (Fig.5c) on lowering of water potentials. The pattern of the decrease in the values of the fluorescence parameters was almost same in all. However, decrease was distinct in Fm, Fv, and qP parameters after -2.8 MPa. Noticeably, all the parameters showed considerable values even at -165 and -355 MPa water potentials. Different patterns of first increase then decrease on lowering of water potentials were observed in qN values (Fig. 5c). It reached its maximum at -2.8 MPa and then gradually decreased. However, the qN was higher than the photochemical fluorescence quenching (qP) at water potentials lower than -14.5 MPa.

To assess the impact of dehydration on photosynthetic

pigments of the cyanobacterium, Chl *a* and carotenoids were estimated in thylakoid membranes isolated from osmotically grown at 0 MPa for 48h and also from 48 h osmotically grown mats dehydrated at matric water potentials of -1.4, -2.8, -4.6 -21, and -39 for 48 h in dark. The content of Chl *a,* compared to its control i.e. grown mats, was found to be decreased gradually by 4.68, 11.11, 13.00, 21.64 and 22.3% at -1.4, -2.8, -4.6, -21, and -39 MPa, respectively (Fig. 6*a*). Contrary to the Chl *a*, carotenoids showed initial enhancement in its amount, compared to its control, by 13.25, 12.69 and 9.77 at -1.4, -2.8 and -4.6 MPa, respectively. On further lowering of the water potentials less or almost no change in carotenoids' content with respect to grown mats' thylakoid menbranes was recorded. Noticeably, on further lowering of water potentials, Chl *a* /carotenoids ratio decreased sharply in thylakoids isolated from mats treated at -1.4 and -2.8 MPa and then increased but not up to the level of 48h grown mats' thylakoid membranes i.e. control (Fig. 6b).

DISCUSSION

A wide variability in time span (ranging from few minutes to several hours) for recovery/loss of metabolic activities such as respiration, photosynthesis, nitrogen fixation, protein synthesis, etc. in desiccated/growing cyanobacterial forms on re-hydration/de-hydration has been reported (Stewart *et al,* 1978; Potts and Morrison, 1986; Chen and Lai, 1996; Yoshinobu *et al,* 2000). In oxygenic phototrophs, chlorophyll fluorescence provides valuable information on photochemistry i.e. the energetic of primary photosynthetic reactions, the extent and nature of photoprotective processes and electron transport rates. In view of their potentiality, the chlorophyll fluorescence techniques have been employed in investigating the photochemistry of *S. geitleri* on re-hydration and de-hydration.

Incubation of the three types of the mats of the cyanobacterium - dry, GQDR and GSDR mats at different periods at 0 MPa osmotic water potential demonstrated quick recovery of almost all the fluorescence parameters in grown but quickly dried mats in comparison to the other two types of the mats. The dry mats took maximum time in the recovery of the fluorescence parameters (Figs. 1, 2, & 3). Such observations suggest about certain changes in the structure of the cellular components during the course of drying and wetting. These changes are more prominent in the cells dried for longer duration, in particular. It is clear from the above that the rate of rise of fluorescence is more affected by the amount and time of hydration. More the time and amount of hydration, there was more rise fluorescence. It has been proposed by several workers that electron donation to PS II is more sensitive to water level (Wiltens *et al,* 1978; Eastman *et al,* 1997). It has also been suggested that the reason for such type of observations may be due to impact of water content on light absorption, transfer of excitation energy by light harvesting pigments, electron transports and content and composition of pigments (Vertucci *et al,* 1985; Masojidek *et al,* 2000).

On re-hydration of dry mats, the values of Fo gradually increased with initial low values up to 2 h, and after 4 h, there was a sharp increase, which slowed down after 12 h of incubation (Fig. 1a). Fo fluorescence has been found varying depending on the cellular phycobiliprotein concentration. Fo fluorescence increases, particularly once the phycocyanin content is increased above a threshold level (Campbell *et al,* 1998). It is not expected that within such a short span of incubation of the mat at 0 MPa osmotic water potential, the content of phycocyanin will cross its threshold level. Second, the GQDR and GSDR mats, which are expected to have more phycobiliproteins, showed initial high amount of Fo fluorescence in comparison to dry mats, but latter (after 12 h) the values of Fo were found lesser than dry mats. Hence, the reason for enhancement in the Fo values on re-hydration of the dry mats on increasing the incubation periods may be attributed to a factor other than phycocyanin.

The values of Fv and Fm fluorescence are also followed almost similar pattern as Fo fluorescence in the dry, GQDR and GSDR mats on incubation at 0 MPa for different time periods (Figs. 1b,c), which suggest that the total conversion of all the centers from PS II° (open) to PS II°⁻ (closed) state. The GQDR mats showed faster recovery in their activities pertaining to Fv and Fm than GSDR mats and dry mats showed slower than the GSDR mats. Nonetheless, compared to Fo values, the Fm values are slightly higher from the initial to final periods of incubation under growing conditions; which resulted in low values of Fv at initial period and considerably higher values of Fv at the final periods of incubation. These observations reflect that light absorbing components, phycobilisomes in particular, were not so changed under dry states compared to that of photochemical reaction centers, as Fo values are more dependent on content of phycocyanin (Campbell *et al.* 1998). Since Fv/Fm, the maximum quantum yield of PS II photochemistry is a ratio of Fv and Fm, it also follow almost similar pattern to Fv and Fm on re-hydration for different time periods in all the three types of mats (Fig. 2a)

Similar to Fo, Fv, Fm, and Fv/Fm, effective quantum yield (ΔF/Fm') and efficiency of excitation capture by

open PS II (Fv'/Fm') of the three types of the mats increased on increasing the re-hydration period (Figs. 2b &c). Thus, it seems that there is not any major difference in the recovery of dark/DCMU (closed PS II) and light (open PS II) adapted chlorophyll fluorescence parameters of the mats during re-hydartion. The values of fluorescence parameters recorded for the *S. geitleri* mats in present study are much nearer to the values reported for natural habitats (Luttge *et a1,* 1995). Luttge *et al.* (1995) working on natural cyanobacterial materials reported up to 0.37, 0.33 and 0.93 as values of Fv/Fm, ?F/Fm' and qP, respectively, at 240 µmoles photons $m^{-2}s^{-1}$ whereas, 0.34, 0.21 and 0.662 as values for Fv/Fm, ?F/Fm' and qP, respectively, were observed in the present study, at the almost similar amount of irradiance. On the other hands, recovery of all these fluorescence parameters on rewetting of the three types of the mats of *S. geitleri* (Figs.1 & 2) has been more comparable with the short (4 days) and long term (21 days) desiccated mosses, where it has been shown that the recovery of the fluorescence parameters in short term desiccated mosses was very fast (within 10-15 min), whereas the long term desiccated mosses showed gradual recovery in the parameters and took more time for complete recovery on re-hydration (Csintalan *et al,* 1999). Such a quick recovery in the fluorescence parameters was not observed in the present case. However, GQDR mats showed values much comparable to the bryophytes, whereas, dry mats of the cyanobacterium took nearly 12h for full recovery of the fluorescence parameters.

It has been observed that the protection mechanisms such as production of osmolytes, antioxidants production, and sub-cellular re-organisation, etc. involved against damages due to desiccation are generally introduced during drying and it has been suggested that the time taken for their induction and establishment precludes survival of rapid drying (Oliver *et al,* 1998, Tuba *et al,* 1998, Farrant *et al,* 1999). It is likely that on increasing the duration of re-hydration the values of all the fluorescence parameters increased mainly due to availability of water molecules which resulted in reorientation in the light harvesting components of the PS II allowing more absorption of light, but probably, and subsequently due to occurrence of either Q_A under reduced state or any other means favoring non-transference of light energy to the PS II reaction centre resulting in dissipation of irradiation in the form of fluorescence. The possibility of reorientation of the pigments in the light harvesting or core antennae complexes is further strengthened by the observation of lesser values of all the studied fluorescence parameters in the GSDR and GQDR mats compared to the dry mats incubated for more than 12 h; which suggest that compared to the dry mats, light absorbing units of PS II are in better position for light absorption in the grown mats or/and lesser possibility of occurrence of PS II reaction centers, such as Q_A under reduced state. Further, the gradual increase in the value of fluorescence parameters till the end of the experiment i.e. 48 h suggest that the reorientation of the light absorbing systems goes on during the process of re-hydration particularly in the dry mats of the cyanobacterium. Potts *et al,* (1983) have shown that in desiccated cells of *Chroococcus* 524 and *Chroococcus* N41, the cell wall, cell membrane, thylakoid membranes and cyanophycin granules and carboxysomes are intact, which further favors the hypothesis of reorientations or conformational changes in the photosynthetic apparatus rather than any major damage in the structure requiring 'repair' on re-hydration. However, CO_2 diffusion, 'repair' of systems/functions is also suggested for differential recovery of such parameters on re-hydration (Csintalan *et al,* 1999; Calatayud *et al,* 1997). After longer desiccation or in more sensitive species, where course of recovery is more prolonged, it is assumed that repair processes are to play a greater role (Bewley and Oliver, 1992; Oliver, 1996). Hence, there a possibility of repair/synthesis of damaged cellular organisations also persists, mainly, in the dry than GSDR and GQDR cyanobacterium during the later period of recovery on re-hydration.

Besides phycobiliproteins, Chl *a* is the major photosynthetic pigment present in the thylakoids of cyanobacteria (Lee, 1989). Among the accessories pigments, carotenoids hold important position particularly in transferring energy to chlorophyll, releasing excess excited levels of chlorophyll molecules and scavenging of free radicals of oxygen molecules (Sarry *et al,* 1994; Demmig-Adams *et al*, 1995; Alscher *et al,* 1997). However, the impact of duration of re-hydration and dehydration of the dry and growing mats of the cyanobacterium *S. geiltleri* has clearly generated information about greater synthesis of Chl *a* and low synthesis of carotenoids during the process of re-hydration (Figs. 4) and loss of Chl *a* and more availability of carotenoids during dehydration of the cyanobacterium (Figs. 6). It may be noted that the chlorophyll of sub-aerial cyanobacteria has been found more stable to de-hydration compared to other aquatic cyanobacteria (Tripathi 1983). However, the observations of loss and synthesis of Chl *a*, and synthesis and loss of carotenoids during dehydration and re-hydration, respectively, is much in accordance with other observations made in different lower as well as higher plants subjected to the abiotic stresses, specifically desiccation (water deficit; Chen and Lai, 1996), salinity (Singh and Dubey, 1995), chilling (Haldimann, 1997) and high light intensity (Merzlyak *et*

al, 1998). Likely, the enhancement and decrease in the level of Chl *a* on re-hydration and dehydration, respectively, might be responsible for revival and enhancement, and diminishing and lowering in the activity of photosynthesis of the cyanobacterium. Chlorophyll is the main light-absorbing pigment in the light harvesting complex, the inner antennae and also in the reaction centres. The rate of the fluorescence rise provides information on efficiency of light harvesting by the photosynthetic units. Rapid rise signals large and efficient light harvesting complexes whereas slow rise means small or inefficient antennae (see Koblizek *et al,* 2005). Further, there is quasi-linear relationship between óPSII (effective absorption cross section) and Fo (see Koblizek *et al,* 1999). These signals that increase in fluorescence on re-hydration may parallels the synthesis of chlorophylls.

On the other hand, the low increasing trend in the values of carotenoids on re-hydration may be suggested due to its function as accessory pigments in entrapment of photon and transfer of energy to the chlorophyll molecules, which is probably, not so required during re-hydration at 0 MPa osmotic water potential at growing conditions. However, the enhancement in the amount of carotenoids on dehydration of the growing mats may be to protect the chlorophyll molecules from photo-damage, as it is well documented that at lower water potentials generation of free radicals of oxygen, singlet oxygen and excessive excitation of chlorophyll molecules, which is responsible for photo-damage of photosynthetic pigments, are inevitable (Merzlyak and Hendry, 1994, Sgherri *et al,* 1996,). Further, Dreuw *et al* (2005) have proposed that carotenoids (eg. Zeaxanthin form complex with excited chlorophylls and quenched them and decrease the chlorophyll fluorescence. Extremely less recovery in the content of Chl *a* and carotenoids at lower water potentials is mainly due to extremely low availability of water for synthesis of the pigments, because at these water levels, no metabolic activities are known in the cyanobacterium (Potts and Bowman, 1985, Potts ,1994).

Lowering of photosynthetic activities by introduction of water stress has been reported in various photosynthetic algae, also (Endo *el al,* 1995, Gilmour *et al,* 1985). In *Dunaliella tertiolecta,* osmotic stress inhibits non-cyclic electron transport and stimulates cyclic electron transport and fluorescence emission arising from PS I at 77° K, suggesting that inhibition of PS II activity may be involved in the ΔpH-dependent down regulation and state-2 transition (Gilmour *et al,* 1985). Endo *et al,* (1995) have recently shown that, in *Chlamydomonas reihardtii,* the inhibition of quantum yield of PS II photochemistry by osmotic stress is due to an increase in non-photochemical quenching which is attributable to a state-2 transition. In red alga *Porphyra perforata,* Satoh e*t al* (1983) have demonstrated that the decrease in excitation energy reaching PS II reaction centres and the inhibition of the oxidizing side of PS II by salt stress resulted in a decrease in PS II activity. Notondo *et al* (2005) reported decrease in Fv/Fm, qP and ETR, and increase in qN under salt stress. Additionally, as stated above, drying may enhance loss of PS II activity by damaging water-splitting system (Wiltens *et al,* 1978). Whereas, studying on *Spirulina pIatensis,* it has been suggested by Lu *et al* (1998) that inhibition of quantum yield of PS II electron transport by water stress may be due to an increase in the proportion of the QB-non-reducing PS II centres.

Contrary to re-hydration, the cyanobacterium S *geitIeri* on de-hydration (matric lowering of water potentials) of mats reflected a decrease in almost all the fluorescence parameters, excepting qN. Values of qN increased on initial state of de-hydration and then showed decrease in its values below -2.8 MPa hydration level in the intact cells (Fig. 5). The values of Fo have been proposed to be affected by activity of PS I and the content of phycocyanin. As stated above, the content of phycocyanin is not so high in these terrestrial organisms. PS I electron transport has been observed to be lowered down on lowering of the state of hydration either matrically or osmotically (data not shown). However, Fo and qN have been shown unaffected by lowering in the water level by application of osmotic stress in *Spirulina pIantensis* (Lu *et al* 1998). Introduction of osmotic stress has shown lowering in the values of state 1 of *Synechococcus* sp. PCC7942 (Papageorgiou *et al,* 1998). However, the values of Fm have been lowered on lowering of water level in almost all the cases (Lu *et al,* 1998, Papageorgiou *et al,* 1998), because of which maximum quantum yield of PS II photochemistry (Fv/Fm) has also been found affected. Besides, the efficiency of the PS II in light acclimated conditions declined in *Synechococcus* sp. PCC7942 on application increasing concentrations of sorbitol and manitol (Papageorgiou *et al,* 1998). Our findings of decrease in the values of almost all the parameters have been in accordance with the reports also. Similarly decrease in the values of Fv/Fm, ØPS II, qP and qN had been reported in mosses (Csintalan *et al,* 1999) and rice seedlings (Kongake *et al,* 2009). In the mosses as well as in the present study, both, fluorescence parameters were not so affected at certain level of lowering of water level. The lowering in the parameters is considerable only when water level goes down drastically below the level required for inhibition of O_2 evolution and CO_2 fixation (data not shown). It is evident

from above observations that osmotic and matric water stresses behave differently. Probably, osmotic water stress created by 0.8 M mannitol, and 0.24 and 0.72 M sorbitol has not been so effective in modifing fluorescence parameters than matric water stress.

As it has been discussed for recovery of photosynthetic apparatus after re-hydration of dried mats and thylakoids, it may be said for the impact of drying (desiccation) that matric water stress is able to make certain structural changes in the photosynthetic apparatus that require 'repair' during re-hydration. This hypothesis is further supported by the observations on protein profile of thylakoid membranes of the *S. geitleri* which showed increase in amount of D1, D2 and CP43 proteins on re-hydration (data not shown).

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