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Photochemical efficacy analysis using chlorophyll fluorescence of *Dicranopteris linearis* in response to desiccation and rehydration stress

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ABSTRACT

Exploring the mechanism of desiccation tolerance is critical in order to unravel the position of ferns in tropical region of the earth. Desiccation in plants induces morphological deformities, ROSs formation, oxidation of protein, nucleic acids, peroxidation of cell membranes, antioxidants machinery and photosynthetic efficacy. The present study is planned to analyze the pigment and fluorescence responses of the forked fern - *Dicranopteris Linearis* (Burm.F.) Underw. against desiccation and rehydration stress with a view to select drought tolerant marker species. Fronds of the fern were subjected to various regimes of desiccation rehydration stress ((a) 2 (b) 4 (c) 6 (d) 8 and (e) 10 days). Initially chlorophyll a and b pigments were decreased (2nd day) followed by an increase indicating the physiological resurrection of the stressed plants during subsequent days of desiccation phase. Meanwhile, carotenoids showed a steady increase till 6th day followed by a decrease. The quantum yield potential of photosystem II (F_v/F_m) was 0.61, 0.77, 0.79, 0.80 and 0.76 respectively, when subjected to 2, 4, 6, 8 and 10 days of desiccation. The F_v/F_m ratio, Fm, F_v, Fo the potential parameters of chlorophyll fluorescence can be used in the early detection of desiccation stress in the fern. Further, the quantum yields (F_v/F_m), photosynthetic quenching and ERT and non-photochemical quenching were maintained remarkably in the fronds till the 8 d of desiccation stress. Further studies are warranted at molecular levels to unravel the mechanism of desiccation tolerant ability in the fern.

KEY WORDS: CHLOROPHYLL FLUORESCENCE; CHLOROPHYLL; CAROTENOIDS; PHOTOSYNTHETIC EFFICACY

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INTRODUCTION

Desiccation is the most alarming stress faced by plants. The loss of water in the tissues leads to the denaturation of essential biomolecules and subsequently, degeneration of cell organelles (Alpert, 2006). Animals actively avoid desiccation by movements, while plants are static and therefore subjected to water loss and recover back slowly during rehydration (Alpert, 2006). Resurrection plants such as *Myrothamnus flabellifolius*, *Xerophyta viscose*, and *Sporobolus stapfianus* were proven drought tolerant species (even up to 90% water loss) (Alpert, 2006). Adaptation to desiccation is based on the ability of the organism to equilibrate its internal water potential with the desiccating environment, and also their drastic ability to regain normal activities after rehydration (Alpert, 2000).

Compared to vascular plants (Vicre *et al.,* 2004), the mechanisms involved in desiccation among lower plants like ferns is poorly understood. Most of the drought stress initiates the activation of antioxidant enzymes such as catalase, superoxide dismutase, ascorbate peroxidase and glutathione reductase (GR) etc. (Burritt *et al.,* 2002) to counter balance desiccation-mediated oxidative stress. Similarly, *many macro algae* resist desiccation tolerance through the photosynthetic system including chloroplast (Zou and Gao, 2002a,b). *Fucus vesiculosus* was evaluated by molecular approaches to unravel the responses to desiccation via the genes encoding photosynthetic and ribosomal proteins (Pearson *et al.,* 2010 Maghsoudi *et al.*, 2015).

The photosystem II reaction center was critical in photosynthetic pathway against drought stress (Wen *et al.*, 2005) i.e., desiccation stress reduces the photosynthetic electron transport activity and also the fluorescence of photosystem II (PSII). Inactivation of PSII leads to derailment of the water-splitting complex, disturbance of pigment protein complexes in thylakoids, which further influences regulation of energy transfer and finally, the photochemical reaction center of PSII was deactivated (Wise *et al.*, 2004). Impact of plants exposed to desiccation stress was drastic but, its recovery was gradual or ceased due to the injury to PSII components (Sinsawat *et al.*, 2004 and Kifah and Jaroslav, 2015).

The use of fluorescence parameters permit to analyze the reduction in electron transport disorder via the emission of heat in the form of IR radiation or by fluorescence. This technique is based on the light kinetics absorbed by antenna pigments and the excitation energy transferred to the reaction centers of photosystem I and II (Zhani *et al.*, 2012).

Contreras-Porcia *et al.*, (2011) analyzed the interrelationship between F_m and F_o in *Porphyra columbina* collected from the different intertidal regions. Generally, in the optimal conditions the proportion of radiant energy emitted as fluorescence is decreased. Meanwhile, during stressed conditions, the chlorophyll fluorescence will be altered (Kadir and Von Weihe 2007). So, *in vivo* fluorescence of chlorophyll provides an early sign of photosynthetic malfunction and can be used as marker to localize the possible sites of damage induced by stress within the cells. In this juncture, the present study is aimed to analyze the photosynthetic pigments and their efficiency in the fern against different duration of desiccation and rehydration.

MATERIAL AND METHODS

QUANTIFICATION OF PHOTOSYNTHETIC PIGMENTS

Photosynthetic pigments were estimated in 80% acetone extract. 1 g tissue was homogenized with 1.5 ml of 80% chilled acetone. The homogenate was centrifuged at 3000 rpm for 5 min. The aliquots were made up to 3 ml by using 80% acetone and the absorbance was read at 470, 648 and 664 nm spectrophotometrically against 80% acetone as blank. Total chlorophyll as well as chlorophyll *a* and *b* concentrations and carotenoids were calculated according to the protocol of Arnon (1949).

Chlorophyll fluorescence emission from the upper and lower surface of the leaves of the fern was measured by a modulated fluorometer (OS 500;Opti Sciences;Inc;Tyngsboro;Mass). Maximum fluorescence yield (F_m) was determined during saturating flash (3000 µmol $m^{-2}s\text{-}^{2}$). The actual fluorescence level (F) was monitored to ensure that it was stable. To obtain the maximal fluorescence yield under illumination (F_m), the leaf was exposed to a saturating flash during exposure to actinic light (210 µmol $m^{-2}s^{-1}$). To determine the minimal level of fluorescence during illumination (F_0), the leaf was continuously illuminated with far-red light (730 nm) to rapidly reoxidize the PSII centers. All measurements were conducted at 25°C (Demmig-Adams *et al.*, 1996).

The minimal fluorescence level (F_0) with all PSII reaction centers open and the maximal fluorescence level (F_m) with all PSII reaction centers closed were determined on dark-adapted leaves. Then the leaves were continuously illuminated with a white actinic light at an irradiance of 180 µmol m^{-2} s^{-1} to measure the steady-state value of fluorescence (F_s), which occurred at about 6 min after the initiation of white actinic light. The maximal fluorescence level in the light-adapted state (F_m') was recorded after subjecting the leaf to a second saturating pulse at 8000 µmol m^{-2} s^{-1}.

The minimal fluorescence level in the light-adapted state (F_0) was determined by exposing the leaf to far-red light for 3s.Using both light and dark fluorescence data, the following parameters were calculated:

- F_v (maximum variable chlorophyll fluorescence yield in a dark-adapted state) was calculated following Maxwell and Johnson (2000): $F_v = F_m - F_0$
- F_v/F_m (the maximal efficiency of PSII photochemistry in the dark-adapted state) was calculated as: $F_v/F_m = (F_m - F_0)/F_m$
- **qP** (the photochemical quenching coefficient): $qP = (F_m' - F_s) / (F_m' - F_0')$
- **qN** (non-photochemical quenching coefficient): $qN = 1 - (F_m' - F_0') / (Fm - F0)$.
- **φPSII** (the actual quantum yield of PSII electron transport in the light-adapted state):
- $\varphi PSII = (F_m' - F_s)/ F_m'$, which was equal to the product of qP and F_v'/F_m'. Thus, ϕ PSII depends on the degree of closure of PSII reaction centers and the efficiency of excitation energy capture in PSII.
- **ETR** (Apparent photosynthetic electron transport rate): Apparent electron transport rates (ETR) are derived from effective quantum yields of photosystem II ($\Delta F/F_m'$ or Y(II)) according to ETR = Y(II) x PAR x 0.42. In this equation, the PAR corresponds to the quantum flux density of photosynthetically active radiation, and the 0.42 is the product of light absorptance by an average green leaf (0.84) times the fraction of absorbed quanta available for photosystem II (0.5).

Data were statistically analyzed using ANOVA followed by Tukey's test (SPSS 14.0; SPSS Chicago, IL, USA). Significant differences were analyzed based on $P < 0.05$ and $P < 0.01$. Percentage data were subjected to arc sine transformation prior to statistical analysis.

RESULTS AND DISCUSSION

Generally, desiccation tolerance was evaluated as the capacity of the plants to mitigate the excess ROS formed and thereby attenuating the oxidative stress within the plant. Photosynthetic components such as enzymes, chlorophylls, and carotenoids levels depend on the severity and duration of stress. In the present analysis, the total chlorophyll content was maintained by the fern during the different periods of desiccation stress (4 to 10th day) i.e., showed an increase from 0.352 (in control) to 0.353 mg/g at 10th day of desiccation treatment in the fern (Table-1). The optimal maintenance of chlorophyll content directly reflects the functional status of the fern against the desiccation stress management. Chlorophyll a/b ratio showed an initial increase (2nd day) followed by an decrease with the degree of desiccation till the day 10 of treatment. Carotenoids, the major accessory pigments showed steady increase up to 6th day and then decreased marginally (10th day) (Table-1). The pigments are effective antioxidants and therefore, protect the cells from oxidative stress by mitigating ROSs formed during photo-oxidative stress.

Chlorophyll fluorescence emission is a versatile tool for quick and non-intrusive estimation of the photosynthetic activity and photoinhibition in the leaves against environmental stresses. Initially, after 2 d desiccation the F_0 level increased whereas, the F_m value decreased in the frond when compared to control. This leads to a decline in the maximum quantum yield of PSII (F_v/F_m) to 0.61. From 4th to 8th day of desiccation F_0, F_m, F_v and F_v/F_m values were maintained. The effective quantum yield of PSII (ϕPSII) and photochemical quenching (qP) also showed a similar trend. In contrast, the non-photochemical quenching (qN) was increased at 2 d desiccated fronds with improvement in apparent photosynthetic electron transport rate (ETR) also. F_o/F_m ratio, known as the basal quantum yield displayed a range from 0.198 to 0.38 (Table – 2). Rehydration application regained these parameters of desiccation stressed ferns compared to the stressed plants. A significant correlation was observed in 2 d desiccated ferns compared with in terms of marginal necrosis in the fronds.

Generally, the chlorophyll fluorescence parameters are ideal markers of PSII and photosynthetic activity in stressed plants (Kifah and Jaroslav, 2015). Maximum quantum efficacy of PSII (F_v/F_m) refers the photosynthetic efficiency of the leaf (Maghsoudi et al., 2015). Thus, F_v/F_m is widely used to evaluate stress-induced impairment in

Table 1: Pigment content in the fern treated with desiccation and rehydration stress (D - desiccated; R- rehydrated)

	Control	2D	2R	4D	4R	6D	6R	8D	8R	10D	10R
Chl a(mg/g)	0.226	0.197	0.261	0.240	0.285	0.237	0.309	0.284	0.325	0.21	0.226
Chl b(mg/g)	0.126	0.109	0.123	0.136	0.142	0.166	0.217	0.191	0.224	0.128	0.127
Chl a/chl b	1.79	1.8	2.12	1.76	2.00	1.42	1.42	1.48	1.45	1.64	1.78
Total chl	0.352	0.306	0.384	0.376	0.427	0.403	0.526	0.475	0.549	0.338	0.353
Carotenoids(mg/g)	0.051	0.057	0.066	0.062	0.059	0.083	0.113	0.070	0.088	0.075	0.089
Tot chl/tot car	6.9	5.37	5.81	6.06	7.23	4.85	4.65	6.78	6.23	4.50	3.97

Table 2: Chlorophyll fluorescence parameters

	control	2 D	4D	6D	8D	10D
F_0	121	158	133	127	125	123
F_m	612	410	584	610	629	512
F_v	491	252	451	483	504	389
F_v/F_m	0.80	0.61	0.77	0.79	0.80	0.76
ϕ	0.67	0.50	0.62	0.67	0.68	0.60
qP	0.82	0.71	0.8	0.83	0.84	0.78
NPQ	0.68	0.76	0.65	0.66	0.65	0.70
ERT	78	66	75	78	79	70
F_0/F_m	0.198	0.38	0.22	0.208	0.199	0.24

the chloroplast. The present results revealed that desiccation stress resulted an initial reduced F_v/F_m (Table – 2) which may be due to the decreased efficiency of energy transfer from the antennae to the reaction centers and / or inhibition of the activity circumscribed around PSII reaction centers (Abdeshahian *et al*., 2010). The decline in F_v/F_m suggests the possible damage occurred to PSII (Yamane *et al*., 2008). Amirjani (2010) reported that the decline in F_v/F_m might retard the rate of photosynthesis, thereby inhibiting plant growth and development.

Lepedu *et al*., (2012) reported a reduction in photochemistry among drought-stressed cowpea plants but, its overall photosynthetic efficiency remained unaffected. The desiccation induced suppression noticed initially (2d after desiccation) with the apparent photosynthetic electron transport rate (ETR) revealing the initial imbalance of the fern against drought stress with an increase of qN. This may be to counter balance the excessive light energy with reduction in photosynthetic rate. Thus, the photochemical down-regulation related with the stress payway to reduction in ETR (Arabzadeh, 2013).

In this study, 2 day desiccation increased the non-photochemical quenching (qN) but later qN was maintained at optimal level. Yamane *et al*., (2008) reported chloroplast damage in rice due to photo-inhibition that was induced by high salinity condition. Photo-inhibition may also retard and reverse the reduction in photosynthetic efficacy that partially impairs transformation of radiation energy into net assimilatory products. Hazem *et al*., (2011) reported the effect of salt stress on photosystem II efficiency and CO_2 assimilation of two Syrian barley landraces. Further, the excessive light energy could be dissipated as heat through qN. Cha-um (2013) suggested that adequate supply of CO_2 for carbon reactions may prevent photoinhibition, which has been reflected as significantly higher F_v/F_m value in the cowpea plants and others against salinity stress. In the present study, desiccation initially reduced the F_v/F_m values, but was subsequently maintained significantly in the fern during 4, 6, 8th days of desiccation.There were different interpretations regarding the variation in the level of F_0 such as an estimation of the relative size of the antenna pigment complexes of the PSII (Kadir and Von Weihe, 2007).

Contreras-Porcia *et al*., (2011) also suggested that in *Porphyra columbina* an increase in F_0 leads to symptom of damage to the PSII reaction center, resulting in a reduction in absorbed light and a subsequent increase in unused emitted light. The results of present study showed that desiccation stress reduced F_m initially, but maintained the photosynthetic quenching. Maghsoudi *et al*., (2015) also showed a reduction in F_m but an increase in F_o/F_m in wheat seedlings under water deficit stress. Additionally, De Lucena *et al*., (2012) reported in mango that a reduction in F_v/F_m ratio, under stress conditions, is often an indicator of photoinhibition or injury to PSII complex. Therefore, the increase in non-photochemical quenching can be expected under desiccation stress as a result of decrease in the utilization of light energy due to a drought-induced reduction in PSII activity (F_v/F_m). This might explain the increase in the value of F_0/F_m in silicon treated wheat under water-deficit conditions (Maghsoudi *et al*., 2015).

Several studies have reported stress-induced increases in the values of F_0/F_m and qN and decreases in F_v/F_m, qP, F_0, and cpPSII (Pellegrini *et al*., 2011; De Lucena *et al*., 2012; Contreras-Porcia *et al*., 2011). In the fern, *D. linearis*, under desiccation stress at 4, 6, 8 days significantly increased the value of F_v/F_m as well as that of qP (Table – 2). Reductions in the values of F_0/F_m and qN and increases in F_v/F_m, qP, *F0,* and ϕ PSII have been reported in many plants under abiotic stress conditions (Pellegrini *et al*., 2011; De Lucena *et al*., 2012; Contreras-Porcia *et al*., 2011). Similarly, Al-aghabary *et al*., (2004) have reported in tomato that addition of silicon to the root growing medium of salt-stressed plants enhanced F_v/F_m as well as improved the photochemical efficiency of PSII through antioxidant machineries.

CONCLUSION

The present results revealed that the ferns can withstand desiccation via maximum quantum yield of PSII and photochemical quenching. The chlorophyll fluorescence and photosynthetic pigments suggest enhanced drought tolerance of the ferns. The fern alleviate the adverse effects of desiccation through the pigments and also effective ROSs scavenging mechanism. Further studies are warranted at molecular level to analyze the antioxidant enzymes and stress protein up regulation in the fern.

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