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Research Article

PROBING THE EFFECTS OF SALINITY STRESS ON PHOTOSYNTHESIS IN *SPIRODELA POLYRHIZA* L. - A POTENTIAL FEEDSTOCK FOR BIOFUEL PRODUCTION

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ABSTRACT
Photosynthetic performance, through polyphasic chlorophyll fluorescence OJIP analysis was evaluated in great duckweed *Spirodela polyrhiza* under salt stress condition. Chlorophyll fluorescence induction showed physiologically adaption in *S. polyrhiza* against salt stress. Due the activation of inactive reaction centers, the rate of photosynthesis was increased slightly when *S. polyrhiza* were subjected to mild and moderate salt stress. Activation of inactive reaction centers under mild and moderate saline condition clearly shows the physiological adaptation in giant duckweed. High salt concentration decreased the rate of electron transportation both at reaction center (ET/RC) and cross section (ET/CS) levels. Light trapping efficiency of reaction center (TR/RC) slightly decreased with increasing concentration of NaCl. Absorbance per cross section (ABS/CS) was also declined drastically with increasing salt stress condition. The study clearly indicates the higher potential of salt tolerance in *S. polyrhiza.*

Keywords: *Salt Stress, Spirodela polyrhiza, Photosynthesis, Chlorophyll Fluorescence, JIP Test*

INTRODUCTION
The over salinity of the soil/water is one of the main factors that limits the growth and development of plants in their natural habitats. It is an ever-increasing problem in arid and semi-arid regions *i.e.* Rajasthan (India). Anthropogenic activities such as industrial discharge and decomposition of biological waste result in increased salt levels in aquatic ecosystems (Wang *et al.,* 2008). Depending on the ability of plants to grow in saline environments, they are classified as either glycophytes or euhalophytes, and their response to salt stress differs in terms of toxic ion uptake, ion compartmentation and/or exclusion, osmotic regulation, CO_2 assimilation, photosynthetic electron transport, chlorophyll content and fluorescence, reactive oxygen species (ROS) generation, and antioxidant defense mechanism (Tang *et al.,* 2015). Salt stress affects photosynthesis both in the short and long term. In the short term, salinity can affect photosynthetic process by stomatal limitations, leading to a decrease in carbon assimilation (Parida, and Das, 2005). This effect can produce rapid growth inhibition, even after just a few hours of salt exposure (Hernández and Almansa, 2002). In the long term, salinity stress can also affect the photosynthetic process due to salt accumulation in young leaves (Munns and Tester, 2008) and decreases in chlorophyll concentration even in halophytes (Duarte *et al.,* 2013). The photosynthesis rate drops due to stomatal closure, inhibition of the electron transfer chain and CO_2 fixing enzymes (Chaves *et al.,* 2009).
Duckweed is a group of small and simple floating aquatic angiosperms that belongs to family Lemnaceae, comprised of 38 species (Les *et al.,* 2002). Vegetative mode of reproduction is a characteristic feature of Lemnaceae (Hillman, 1961). Duckweeds are traditionally used as animal feeds because they are excellent sources of protein and starch (Goopy and Murray, 2003). Duckweeds have high biomass yield and short life spans and can be easily harvested by surface skimming; thus, they have been concurrently considered as a potential feedstock for the production of biofuel and biogas (Cui and Cheng, 2015). Moreover, duckweeds are capable of heavy metal accumulation (Bergmann *et al.,* 2000) and phytoremediation (Bocuk *et al.,* 2013).
Great duckweed *Spirodela polyrhiza* L. is one of the most common duckweed species, found on the water surface of ponds, lakesides, rice fields, pools, ditches, and slow-flowing streams (Figure 1 A).

The plant body or frond contains one or two metristematic pockets at its proximal ends from which new daughter fronds develop. It is distributed almost all over the world, suggesting adaptation to a wide variety of environmental conditions. In the present study, greater duckweed was used as the test organism because it is easy to grow, has a short life cycle and is very sensitive to various pollutants (Appenroth *et al.*, 2010). Efforts were made to understand the physiological defense mechanism in *S. polyrhiza* to various concentrations of NaCl.

MATERIALS AND METHODS

Collection of S. polyrhiza: Plants were collected from a local natural Ayad River of Udaipur district of Rajasthan (India). After collection, macrophytes were thoroughly cleaned under running water in order to eliminate any remains of sediment and particles. The plants were then subjected to various salt treatments (Figure 1 B).

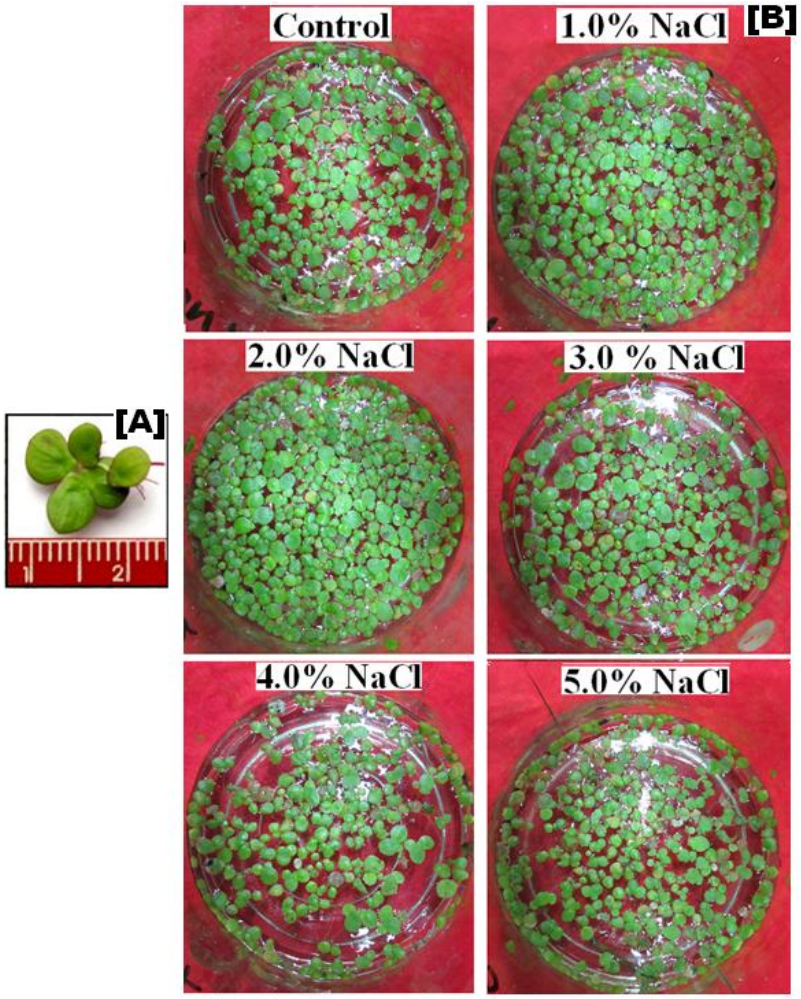

Figure 1: (A) Giant Duckweed (*S. polyrhiza*), and (B) Its Growth in Various Concentration of NaCl

Measurement of Polyphasic Chlorophyll Fluorescence Kinetics: Chlorophyll *a* fluorescence O-J-I-P transients were recorded after 24 hours of salt treatment at 20°C under dim green light with a Plant Efficiency Analyzer, PEA (Hansatech Instruments, Kings Lynn, Norfolk, U.K.). Fluorescence transients were induced over a leaf area of 4 mm diameter by a red light (peak at 650 nm) of 3000 $\mu molm^{-2}s^{-1}$ (sufficient excitation intensity to ensure closure of all PSII RCs to obtain a true fluorescence intensity of

F_M) provided by a high intensity LED array of three light emitting diodes. A total measuring time of one second was used thought out the experiments.

JIP Test: The Chlorophyll *a* fluorescence transient O-J-I-P was analyzed according to the JIP- test (Strasser and Strasser, 1995; Strasser and Tsimilli-Michael, 2001). The extracted and technical parameters, specific energy fluxes (per reaction center), phenomenological energy fluxes (per cross section), quantum efficiencies or flux ratios, density of reaction centers and performance indexes were calculated by using the equations of JIP- test (Table 1).

Table 1: Formulae and Glossary of Terms Used by the JIP-Test for the Analysis of Chlorophyll *a* Fluorescence Transient OJIP Emitted by Dark-Adapted Photosynthetic Samples

Extracted and Technical Fluorescence Parameters	
$F_o = F_{50\ \mu s}$	fluorescence intensity at 50 μs
$F_{100\ \mu s}$	fluorescence intensity at 100 μs
$F_{300\ \mu sp}$	fluorescence intensity at 300 μs
F_J	fluorescence intensity at the J step (at 2 ms)
F_I	fluorescence intensity at the I step (at 30 ms)
F_M	maximal fluorescence intensity
Specific Fluxes or Specific Activities	
$ABS/RC = M_0 \cdot (1/V_J) \cdot (1/\phi_{Po})$	Absorption flux per reaction center
$TR_0/RC = M_0 \cdot (1/V_J)$	Trapped energy flux per reaction center
$ET_0/RC = M_0 \cdot (1/V_J) \cdot \psi_0$	Electron transport flux per reaction center
$DI_0/RC = (ABS/RC) - (TR_0/RC)$	Dissipated energy flux per reaction center
Phenomenological Fluxes or Phenomenological Activities	
ABS/CS	Absorption flux per cross section
$TR_0/CS = \phi_{Po} \cdot (ABS/CS)$	Trapped energy flux per cross section
$ET_0/CS = \phi_{Po} \cdot \psi_0 \cdot (ABS/CS)$	Electron transport flux/ cross section
$DI_0/CS = (ABS/CS) - (TR_0/CS)$	Dissipated energy flux per cross section
Quantum Efficiencies or Flux Ratios	
$\phi_{Po} = TR_0/ABS = [1 - (F_o/F_m)] = F_v/F_m$	Maximum quantum yield for primary photochemistry
Density of RCs	
$RC/CS = \phi_{Po} \cdot (V_J/M_0) \cdot ABS/CS$	Density of reaction centers (Q_A-reducing PSII reaction centers)

RESULTS AND DISCUSSION

Both biotic and abiotic stresses are the natural part of all types of ecosystem and are, in many respects, a positive force in shaping adaptive capacities. Plants adapt to abiotic stresses by altering the biochemical and physiological pathways that reprogram the whole plant to attain a new metabolic and cellular homeostasis. The effects of short-term salt stress on photosynthetic performance of the aquatic angiosperm *S. polyrhiza* were examined in present studies. Chlorophyll fluorescence induction showed that *S. polyrhiza* are physiologically adapted against salt stress (Figure 2). Mild and moderate salt concentration enhanced the photosynthetic rate of *S. polyrhiza*.

The plants were found sensitive for severe salt stress. High salt concentration caused inhibition of electron transportation per reaction center (ET/RC) and per cross section (ET/CS). Light trapping efficiency of reaction center (TR/RC) slightly decreased with increasing concentration of NaCl into the water. Absorbance per cross section (ABS/CS) was also declined drastically with increasing salt stress condition. On the other hand, a remarkable enhancement in absorbance per reaction center (ABS/RC) was noted in plants growing in salt stress condition. Mind and moderate exposure of salt stress enhanced performance index per reaction center (PI_{abs}) and per cross section (PI_{cs}). Severe salt stress drastically declined the performance index per reaction center (PI_{abs}) and per cross section (PI_{cs}) in *S. polyrhiza* (Figure 3 A).

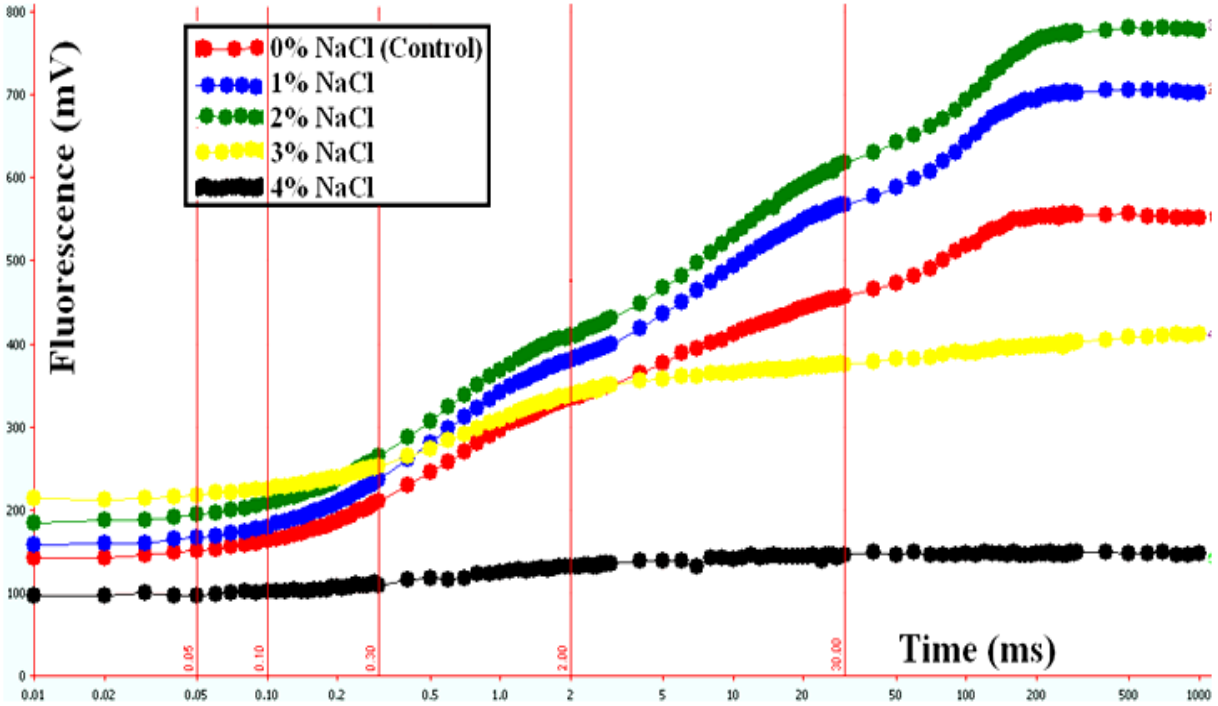

Figure 2: Chlorophyll Fluorescence OJIP Curve of *S. polyrhiza* Growing in Various Concentration of NaCl

Reduction of photosynthetic pigment content is a physiological marker of abiotic stress in plants. Giant duckweed fronds started to show signs of chlorosis after a 2 d exposure to high NaCl treatments. Due the activation of inactive reaction centers, the rate of photosynthesis was increased slightly when mild and moderate NaCl treatments were applied on *S. polyrhiza* for short term (Figure 3 B). Activation of reaction center under mild and moderate salt condition shows the physiological adaptation in giant duckweed. High concentration of NaCl adversely affected the rate of photosynthesis in *S. polyrhiza.*

However, concentration of active reaction centers declined in severe salt stress condition, but plants were found capable to perform photosynthesis. It may be due to the enhancement of functional potential of remaining active reaction centers. Similar results were also observed in various plant species such as *S. polyrhiza* (Appenroth *et al.*, 2001) and *Anabaena* (Bueno *et al.*, 2004). Decline in ABS/CS might be due to the degradation of total chlorophyll contents.

It has been shown that the decline in the rate of photosynthesis in drought stress is primarily due to CO_2 deficiency, as the photochemical efficiency could be brought back to normal after a fast transition of leaves to an environment enriched in CO_2 (Meyer and Genty, 1998). Decrease in PI_{abs} and PI_{cs} indicates that photosynthesis was negatively affected by salt stress in *S. polyrhiza*. According to the theory suggested by Farquhar and Sharkey (1982), declined PI_{abs} and PI_{cs} resulted from stomatal limitation, as stomatal conductance and CO_2 concentration concomitantly decreased in the *S. polyrhiza* under severe salt treatments. The most common response to salinity is a decrease in stomatal conductance in plant leaves, and it may be caused by salt-induced alteration in water status and local synthesis of abscisic acid in stomatal guard cells (Munns and Tester, 2008). The study indicates adaptive nature of *S. polyrhiza* to salt stress.

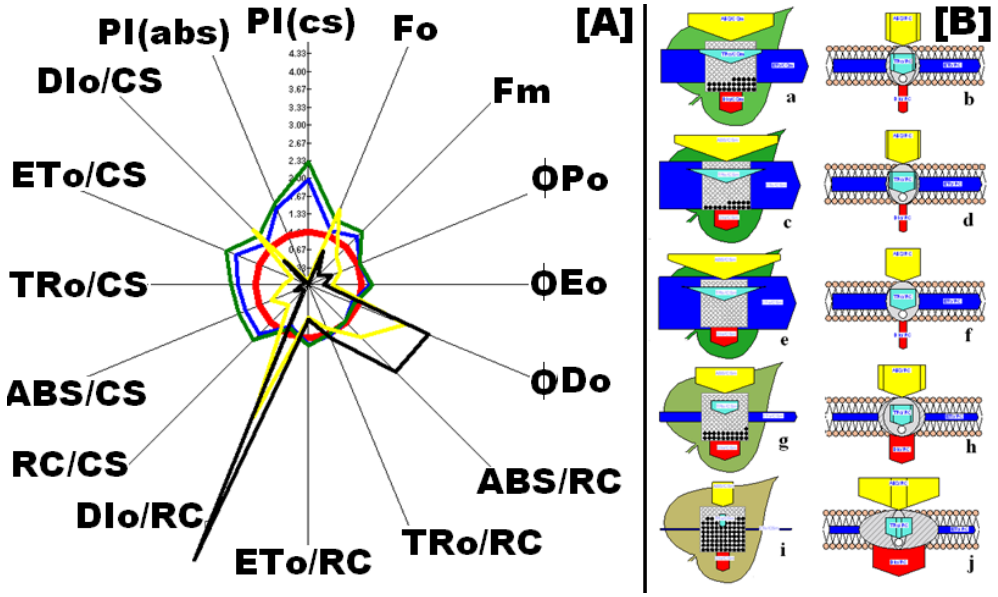

Figure 3: (A) Radar Plot Showing Effect of Salt Stress on Various Photosynthetic Parameters, and (B) Leaf and Reaction Center Models are Showing the Effect of Various NaCl Concentrations (a,b- 0%; c,d-1%; e,f-2%; g,h- 3%; i,j-4%) on Photosynthetic Performance of *S. polyrhiza* (Black Dots in Leaf Models Representing Inactive PS II Reaction Centers)

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