CHARACTERIZATION OF PHOTOSYSTEM II
HETEROGENIETY
IN RESPONSE TO SALINITY STRESS

SYNOPSIS FOR
Ph.D.
REGISTRATION

SUBMITTED BY
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INTRODUCTION

Life on Earth ultimately depends on the energy derived from the Sun. Photosynthesis is the only process of biological importance that can harvest solar energy. In photosynthesis, the plants use solar energy to oxidize water, thereby releasing oxygen and reduce carbon dioxide forming large carbon compounds primarily sugars. The Photosynthetic complex comprises of two photosystems: Photosystem II and I. Photosystem II (PSII) is a multisubunit pigment protein complex embedded in the thylakoid membrane of the chloroplast. It utilizes light for photochemical energy conversion and is also involved in the regulation of energy flow. PS II consist of at least 25 different types of protein subunits many of which are bound to the thylakoid membrane. Some subunits are involved in the capturing of solar energy and the regulation of energy flow; while others are directly or indirectly responsible for photochemistry, including the oxidation of water to molecular oxygen. The central part of complete PS II unit is formed by the so called core complex, a well defined structure of which the reaction centre protein D1, D2 and core antenna protein CP 47 and CP 43 are largest and functionally the most important components (Boekema et al. 1999) and The PS II complex also consist of three extrinsic proteins, a cluster of four manganese atom and two specific tyrosine residue, one on D1 called TyrZ (Yz) and other on D2 called TyrD (YD) (Bacon Ke 2000)

PS II complex is not homogenous in nature and differs in its structure and function both. This diverse nature of PS II is known as heterogeneity. Heterogeneity is mainly classified in to two classes i. e. static heterogeneity and dynamic heterogeneity. Static heterogeneity is correlated with the grana or stroma structure and heterogeneity of antenna size heterogeneity with respect to the ability of sustaining electron transfer. Dynamic heterogeneity is implied when the functional changes of the photosynthetic apparatus occur under different circumstances (Lavergne and Braintais 1996). Extent and nature of PS II heterogeneity may vary under different physiological states i.e. salinity stress, temperature stress etc. Photosynthetic performance is limited due to the biotic and abiotic stress factors.
Photosystem II has been found to be more heterogeneous than other components such as PS I and Cyt b$_6$f in various aspects. Two major fractions i.e. PS II $\alpha$ and PS II $\beta$ were distinguished with respect to PS II antenna heterogeneity and heterogeneity related with energy connectivity. Determination of antenna heterogeneity of PS II from fluorescence rise curve method, measured with 3-(3’ 4’dichlorophenyl)-1, 1 dimethyl urea (DCMU) for the first time was introduced by (Melis and Homann 1975, 1976, Strasser 1981). By using this method three types of heterogeneities were determined i.e. PS II$\alpha$, PS II$\beta$ and PS II$\gamma$ (Sinclair and Spence 1988, Hsu and Lee 1991). PS II $\alpha$ is mainly located in grana region of thylakoid membrane and is characterized by a large light harvesting antenna while PS II $\beta$ is mainly located in the stroma region of the thylakoid membrane and is characterized by a small light harvesting antenna. When only PS II $\alpha$ and PS II $\beta$ were considered, analyses of the DCMU fluorescence rise curve revealed about 65-80% PS II $\alpha$ and rest PS II $\beta$ centres (Lazar et.al. 2001). PS II $\beta$ differ from PS II $\alpha$ among other thing, in the fact that the former has an exponential fluorescence rise in presence of DCMU in comparison with PS II $\alpha$ which has sigmoidal one. It is generally accepted that the exponential increase in the fluorescence intensity reflects energetic separation of PS II$\beta$ (Melis 1979, Melis and Anderson 1983). The determination of PS II antenna heterogeneity was also done without DCMU. In the flash fluorescence induction measurement, a very strong 100 $\mu$s flash is applied which causes transient closure of PS II (Nedbal et al. 1999). On the basis of biochemical and fluorescence method it was found that about 20 -40% PS II can not reduce Q$_B$ and PQ pool. These PS II centres were called the Q$_B$ non-reducing centres or inactive PS II centres as different from the Q$_B$ reducing or active PS II centres that can reduce Q$_B$ and PQ pool. It was suggested that Q$_B$ reducing type PS II centres can be of $\alpha$ type (Ghirardi and Melis 1988). Another heterogeneity related to PS II is in the formation of domains with different rates of the PQ pool reduction; 50-70% of PQ molecules from the PQ pool are reduced fast and suggested that fast reducing PQ pool represents the pool from grana region of thylakoid membrane. (Joliot et al.1992, Kirchoff et al. 2000)
Increasing salinity in soil affects the plants. Effects of NaCl stress are mainly of two types. Primary stress i.e. direct membrane damage leading to metabolic alteration and secondary stress i.e. osmotic stress. The increase in NaCl concentration above 100 mM inhibited the photoreduction of ferricyanide in uncoupled isolated spinach thylakoids and consequently decrease the rate of the primary electron flow to ferricyanide. Exposing leaf cell to high salinity can causes ionic imbalance and toxicity effect as well as drought effect in plants (Robert and Nott 1982). The high salinity of surrounding solution causes changes in water and ionic status in cell. High salt stress in wheat leaves causes retardation of chlorophyll accumulation, partly by reducing the rate of porphyrin formation (Abdelkader et al. 2007) High salinity markedly reduced oxygen evolution in isolated thylakoids from NaCl treated plants. Salt stress inhibited the apparent quantum efficiency of photosynthesis and PS II actively while stimulating PS I activity and dark respiration significantly. Salt stress also resulted in decrease in overall electron transport rates which could not be restored by DPC. Salt stress resulted in decrease in growth and relative water content as well as net photosynthesis in maize. The effect of salinity on in vitro growth morphogenesis, photosynthetic rate, PS II efficiency (Fv/Fm) and chlorophyll content were investigated in *Centaurium erythraea* (Siler et al. 2007). Chlorophyll a, b and total chlorophyll content had a decreasing trend with increasing supply of NaCl in Growth medium. The accumulations of intracellular sodium ion during salt stress change the ratio of K: Na, which probably affects the bioenergetic process of photosynthesis.

**RATIONALE OF THE WORK**

Increase in salinity in soil and an effect on plants has become a vast problem for agriculture. Increase in salt concentration in soil reduces the productivity of the plants, so it necessary to know the cause of reduction of productivity.

In this project we explore the alteration of photosystem II heterogeneity under salinity stress. The result obtained from this study may help in designing plant resistant or tolerant to salt stress in future. This is a basic study to establish if stress affects the structural and functional heterogeneity of PS II. The effects on PS II heterogeneity may
affect photosynthesis and eventually crop yield. Assortment of measurements using both preparative biochemistry and biophysical techniques are likely to provide the markers for monitoring the PS II heterogeneity during early stages of salinity stress as well as for screening the tolerance in varieties and transgenics.

OBJECTIVES

1. To study the effect of salt treatment on the rate of electron transport mediated by PS II and PS I.

2. To measure heterogeneity in antenna size by measuring PS II α and PS II β centres as a function of salt stress.

3. To determine functional heterogeneity by monitoring Q$_B$ reducing and Q$_B$ non-reducing centres as a function of salt stress.

4. To study heterogeneities in the arrangement of PS II in granal and Stromal lamellae in response to salt stress.

METHODOLOGY

HYDROPONICS: For the salinity stress we adopt the hydroponics and grow the plant in soil less culture. The term hydroponics originally meant nutrient solution culture. Hydroponics or soil less culture is a technology for growing the plant in nutrient solution that supply all nutrient elements needed for optimum growth with and without use of inert medium such as gravel, vermiculite, rock wool, saw dust, coir dust etc. to provide mechanical support. Hydroponics or soil less culture reduces the possibilities of soil born disease and pests.

PREPARATION OF THYLAKOID MEMBRANES: The present work involves working with young plants, intact leaves and isolated thylakoid membranes under salt treatment. The
thylakoid membrane fragments prepared by following the method described in Kuwabara and Murata (1982). Various biophysical and biochemical tools will be used to analyze PS II heterogeneity as a function of salt stress.

**Electron Transfer Measurement:** Different acceptor and donor will be used to monitor the partial electron transport.

(i). **The Hill Reaction:** Water to DCPIP reaction will be used for studying electron transfer of PS II. In the Hill reaction measures PS II rates by measuring amount of dye reduced mg chl$^{-1}$ h$^{-1}$.

(ii). **Oxygen Evolution:** To study the effect of salinity stress on oxygen evolving complex, oxygen evolution rates will be measured by using oxygraph system.

(iii). **Oxygen Consumption:** The electron transfer rates of PS I (DCPIP to MV or DAD/TMPD to MV) will be studied by measuring oxygen consumption rates on the YSI oxygen monitor.

**Steady State Fluorescence:** The fluorescence measurement will be done in order to understand the change in energy spill over due to the effect of salinity stress. Change in fluorescence intensity with salinity treatment in PS II is monitored at room temperature.

**Low Temperature Fluorescence:** Any change in the energy distribution between two photosystem by salinity stress monitored at 77K by low temperature fluorescence emission spectra.

**Fluorescence Induction Kinetics:** In photosynthetic system the processes by which the energy is dissipated are either photochemical or non photochemical. Photochemical processes utilize absorbed energy for photochemistry during which electron donation from pigment to an acceptor molecule occurs and such processes direct energy for the chemical work involved in photosynthesis. Non-photochemical processes dissipate energy from the photosynthetic apparatus in a manner that does not result in photochemistry. Energy is usually reemitted from the sample in form of heat and red far-
red radiation, which is known as chl a fluorescence. The fluorescence induction curves use to measure quantum yield of chl a fluorescence in leaves as well as in isolated thylakoid membranes. Fluorescence induction curves are useful for the differentiation of Q_B reducing and Q_B non reducing PS II centres as well as PS IIα and PS IIβ centres. The fluorescence induction curves will be measured by photosynthesis efficiency analyzer (PEA Hansatech instruments).

GEL ELECTROPHRESIS: Protein profile will be monitored during different steps of the experiments by SDS-PAGE electrophoresis.

EPR STUDIES: Experiments will be done to explore measurement of PS II heterogeneity using EPR

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