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Surviving Abiotic Stress: The Relationship between High Light and High Salt Tolerance

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***Abstract*—**The mechanism of abiotic stress tolerance is crucial for plants to survive in harsh condition and the knowledge of this mechanism can be use to solve the problem of declining productivity of plants or crops around the world. However in-depth description is still unclear and it is argued, in particular that there is a relationship between high salinity tolerance and the ability to tolerate high light condition. In this study, *Dunaliella salina*, which can withstand high salt was used as a model. Chlorophyll fluorometer for non-photochemical quenching (NPQ) measurement and high-performance liquid chromatography for pigment determination was used. The results show that NPQ value and the amount of pigment were increased along with the levels of salinity. However, it establish a clear relationship between high salt and high light but the further study to optimized the solutions mentioned above is still required.

***Keywords*—**Abiotic stress tolerance, *Dunaliella salina*, Non-photochemical quenching, Zeaxanthin.

I. Introduction

ABIOTIC stress, the negative effect of non-living factors on the living organisms is a very important factor that affects the growth and yield of crops around the world. High salinity is one of the factors which should be considered today. The response of plants to salt stress has been studied previously in Arabidopsis [1] and rice [2]. However, these species are not well adapted to the presence of very high salt concentration. There are some plants that are adapted to grow normally under such conditions. One of them, the unicellular green algae *Dunaliella salina* is well known for its ability to grow in high salinity and can even survive in a salt evaporation pond, which is considered a very high salt concentration. Knowledge of the mechanisms of abiotic stress tolerance is crucial for resolving problems with the growth and yield of crops. In order to survive, *D. salina* responds and adapts to high salinity by complex mechanisms, including enhancement of photosynthetic CO_2 assimiliation, and synthesis of glycerol to provide protection against osmotic pressure. Enhanced photosynthetic activity of *D. salina* at high salinity is interesting because in most plants and cyanobacteria, photosynthesis is inhibited or not affected by salt stress [3], [4], [5], [6], [7], [8], [9], [10]. In addition, high light stress responding mechanisms, including non-photochemical quenching (NPQ), adjustment of the light-harvesting antenna size, and zeaxanthin accumulation, are also found in this alga [11], [12]. In this research, *Dunaliella salina*, was used as model organisms to determine the effect of salt concentration on induction kinetic of NPQ and to find out the actual factor that regulates induction of NPQ and accumulation of zeaxanthin apart from the level of light intensity. Since it has been reported recently that avalibility of CO_2 affects induction of NPQ but not zeaxanthin accumulation [13].

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II. Materials and Method

A. Plant Material and Growth Conditions

Unicellular green alga *Dunaliella salina* Teod. Strain 1644 was obtained from the UTEX culture collection. It was grown photoautotrophically in 125 ml Erlenmeyer flask with gentle shaking twice a day. The growth medium was an artificial hyper saline medium containing 40 mM Tris-HCl (pH7.5), 5 mM KNO_3, 5 mM $MgSO_4$, 0.3 mM $CaCl_2$, 0.1 mM KH_2PO_4, 2 µM $FeCl_3$, 20 µM EDTA, 150 µM H_3BO_3, 10 µM $MnCl_2$, 2 µM Na_2MoO_4, 2 µM $NaVO_3$, 0.8 µM $ZnCl_2$, 0.2 µM $CoCl_2$ and 0.3 µM $CuCl_2$. An inorganic carbon source was supplied in the form of $NaHCO_3$ at a final concentration of 25 mM. Growth temperature was maintained at 28-30˚C. To adjust cell number for starter point, hemocytometer was used to count under microscope. Cell movements were inhibited by 5% iodine solution. Growth of the algae cultures were measured by OD measurement at 678 nm by spectrophotometer.

B. Chlorophyll Fluorescence Measurement and NPQ Analysis

Standard modulated chlorophyll fluorescence analyses similar to those previously described [14], [15] were performed using an FMS2 fluorometer. Briefly, cuvettes containing 1 ml *D. salina* cells were subjected to Chl fluorescence measurements. The algal cultures were subjected to a 1 s flash of saturated white light (10,000 µmol photons $m^{-2}s^{-1}$) to measure Fm and then illuminated with white actinic light (2,000 µmol photons $m^{-2}s^{-1}$) for 10 min. During the 10 min actinic illumination, the same flashes of saturated white light previously used to measure Fm were given once in a while to the cells for determination of Fm'. NPQ was calculated as (Fm-Fm')/Fm'.

C. Determination of Photosynthetic Pigments

Pigment compositions in the algal cell were determined by HPLC analysis. *D. salina* cells aliquots were taken and centrifuged for 3 min at 3,000 g. Cell pellets were resuspended in 90% acetone and debris were removed by centrifugation at

16,000 g for 3 min and subjected to HPLC analysis as described previously [16]. Briefly, a Waters Nova-Pak® C18 3.9x150 mm HPLC column was used. 10 µl of filtered supernatant (0.2 µm nylon filter) from pigment extracts were subjected to HPLC analysis. The pigments were separated using a solvent mixture of ddH_2O, acetonitrile, methanol and ethyl acetate. During the run, the solvent concentration was 14% ddH_2O, 84% acetonitrile and 2% methanol from 0 min to 9 min. From 9 min to 11 min, the solvent mixture consisted of 68% methanol and 32% ethyl acetate. From 11 min to 12 min, the solvent mixture consisted of the initial solvent mixture. A post-run followed for 3 min with the initial solvent mixture. The flow rate was constant with 1.2 ml/min. Pigments were detected at λ = 445 nm. Identification of individual pigments were determined from the HPLC profiles compared with data from previous work [17] with standard samples of chlorophyll and carotenoid. The amount of individual pigment was determined from the individual peak area from HPLC profiles.

III. Results and Discussion

To determine the effect of low NaCl concentration and cultivation time on the induction kinetics of NPQ in *D.salina*, cells were cultivated under 60 µmol photons $m^{-2}s^{-1}$ in growth medium that does not contain NaCl (0 M). Cell aliquots were taken after 10 day or 20 day and subjected to NPQ analysis with or without DCMU treatment. When grown for 10 days, the NPQ value, in the absence of DCMU, was gradually increased along with the period of time under actinic light reaching the value of ~0.6 at the end of the analysis (Fig. 1A; solid symbols). In the presence of DCMU the overall induction was similar to that without DCMU treatment except that the NPQ reached to a value ~0.5 at the end of the experiment (Fig. 1A; open symbols). There is no discernible transient NPQ in this growth condition. The 20-day-old culture, regardless of the DCMU treatment, exhibited a similar NPQ induction kinetics to that of the 10-day-old (Fig. 1B). Again, there was no detectable transient NPQ even when the alga was grown for 20 days. When *D. salina* was grown for 10 and 20 days in normal growth medium (1.5 M NaCl), the transient NPQ was observed (Fig. 2; solid symbols). Such transient NPQ was almost completely relaxed in the presence of DCMU (Fig. 2; open symbols). When *D. salina* was grown in high salinity growth medium (3 M NaCl), strong transient NPQ rapidly induced reaching the value of more than 2 (Fig. 3; solid symbols). This transient NPQ was sustained in such a way that it could not be completely relaxed within a few min like that observed in the earlier results. The results of NPQ induction in response to different of salinity from low to high (Fig. 1, 2, 3), can demonstrate that, under the lowest salinity (0 M NaCl), the alga does not need glycerol to balance the osmotic pressure, no need to enhance carbon assimilation, so the leftover availability of carbon source was high. With plenty of the carbon source left in the medium, Calvin cycle is not inhibited, photosynthetic electron transport is normal, leading to normal level of the proton gradient across the thylakoid membrane. Under such condition, the transient energy dependent NPQ does not occurs and the NPQ that was induced under high light illumination was energy- independent NPQ. This finding has never been reported before in the literature.

In higher salinity (1.5 & 3 M NaCl), the alga needs more glycerol to counteract the osmotic pressure. In order to synthesize more glycerol, it needs to enhance the rate of carbon assimilation. Thus, when grown for the same period of time, the alga grown in medium with higher salinity level depletes more carbon source, so the leftover in the medium was lower. Less available carbon source leads to inactivation of the Calvin cycle, reduced redox components, proton accumulation in the lumen, leading to the formation of rapid transiently induced energy-dependent NPQ upon illumination with HL.

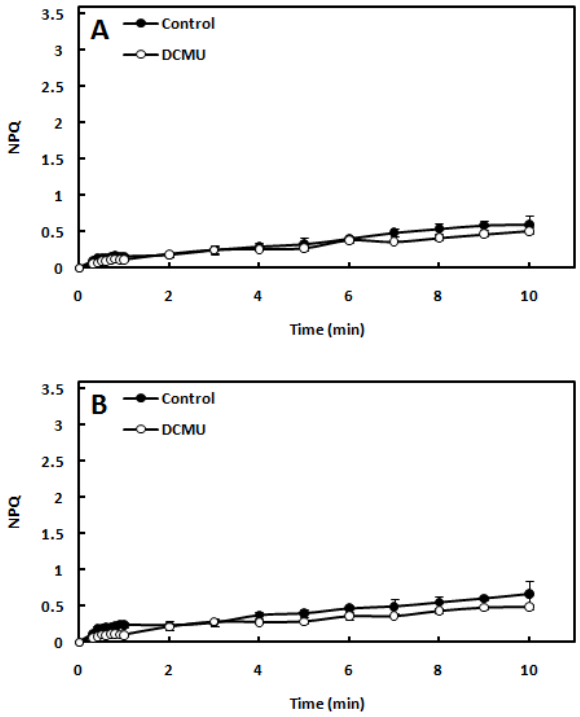

Fig. 1 Induction kinetics of NPQ in *D.salina* cultivated under 60 µmol photons $m^{-2}s^{-1}$ in 0 M NaCl media. Cells were cultivated under 60 µmol photons $m^{-2}s^{-1}$ in 0 M NaCl media. Cell aliquots were taken from 10 day (A) and 20 day (B) cultures and subjected the NPQ measurement as described in Materials and methods. Analyses were performed in the presence (open circle) or absence (solid circle) of 10 µM DCMU. Data points shown are averages of three measurement±SE

The pattern of NPQ in response to different salinity was similar to the NPQ in shade-grown leaves of avocado [13]. Takayama and coworkers showed that even in the same leaf, NPQ was different in different regions on the leaf due to different carbon assimilation in each region. In response of *D. salina* to high salinity, the overall NPQ were increased along with the salinity from low to high, similar to that reported in *Arabidopsis* [9]. Yet, in some other plant species, salt stress

has no effect on NPQ induction [10], [18].

To correlate the degree of transient NPQ observed along the increasing salinity in *D.salina* with pigment composition. *D. salina* was cultivated under 60 µmol photons $m^{-2}s^{-1}$ in 0 M, 1.5 M and 3 M NaCl media. Cell aliquots were taken after 10 and 20 days and subjected to pigment analysis by HPLC. I observed that DES of *D. salina* was increase proportionally to the cultivation time and salinity (Fig. 4A). The Ch *a* to Ch *b* ratio did not seem to differ in different salinity concentration (Fig. 4B). However, as the cultivation prolonged, the ratio was higher (Fig. 4B).

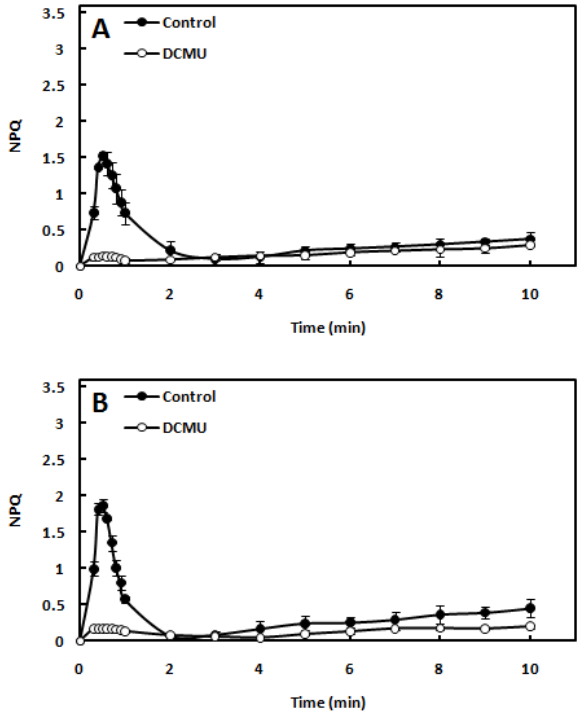

Fig. 2 Induction kinetics of NPQ in *D.salina* cultivated under 60 µmol photons $m^{-2}s^{-1}$ in 1.5 M NaCl media. Cells were cultivated under 60 µmol photons $m^{-2}s^{-1}$ in 1.5 M NaCl media. Cell aliquots were taken from 10 day (A) and 20 day (B) cultures and subjected the NPQ measurement as described in Materials and methods. Analyses were performed in the presence (open circle) or absence (solid circle) of 10 µM DCMU. Data points shown are averages of three measurement±SE

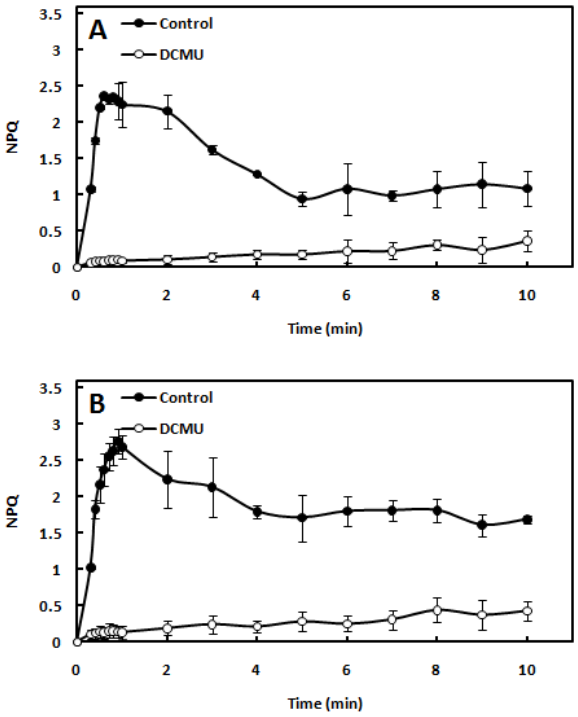

Fig. 3 Induction kinetics of NPQ in *D.salina* cultivated under 60 µmol photons $m^{-2}s^{-1}$ in 3 M NaCl media. Cells were cultivated under 60 µmol photons $m^{-2}s^{-1}$ in 3 M NaCl media. Cell aliquots were taken from 10 day (A) and 20 day (B) cultures and subjected the NPQ measurement as described in Materials and methods. Analyses were performed in the presence (open circle) or absence (solid circle) of 10 µM DCMU. Data points shown are averages of three measurement±SE

IV. Conclusion

To determine the effect of carbon source availability on induction kinetic of NPQ and accumulation of zeaxanthin, induction kinetics of NPQ and accumulation of zeaxanthin were followed and compared between cultures from different culture period, different sodium chloride concentration in growth media as the indirect way to change carbon source availability in this alga.

The result showed that, the development of NPQ in *D. salina* is actually depend on carbon source availability and can be divided into three apparent stages along with the level of carbon source availability from high to low, first stage is the energy-independent NPQ follow by transient energy-dependent NPQ and the last stage is almost dominated by energy-dependent NPQ.

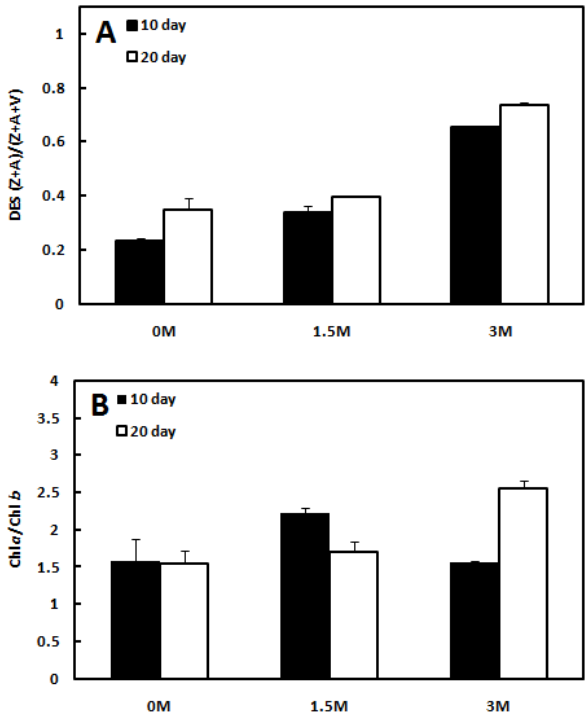

Fig. 4 Pigment compositions of *D.salina* cultivated under 60 µmol photons $m^{-2}s^{-1}$ in 0 M NaCl media, 1.5 M NaCl media and 3 M NaCl media. Cells were cultivated under 60 µmol photons $m^{-2}s^{-1}$ in 0 M NaCl media, 1.5 M NaCl media and 3 M NaCl media respectively. Cell aliquots were taken from 10 day (solid bar) and 20 day (open bar) cultures and subjected the pigment determination as described in Materials and methods. A De- epoxidation state (DES) of the xanthophylls cycle. B Chl *a* to Chl *b* ratio. Data points shown are averages of three measurement±SE

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