

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

Rutanachai Thaipratum

Abstract—The mechanism of abiotic stress tolerance is crucial for plants to survive in harsh condition and the knowledge of this mechanism can be used 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 establishes a clear relationship between high salt and high light but the further study to optimize 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 CO₂ assimilation, 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 availability of CO₂ affects induction of NPQ but not zeaxanthin accumulation [13].

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 KNO₃, 5 mM MgSO₄, 0.3 mM CaCl₂, 0.1 mM KH₂PO₄, 2 μM FeCl₃, 20 μM EDTA, 150 μM H₃BO₃, 10 μM MnCl₂, 2 μM Na₂MoO₄, 2 μM NaVO₃, 0.8 μM ZnCl₂, 0.2 μM CoCl₂ and 0.3 μM CuCl₂. An inorganic carbon source was supplied in the form of NaHCO₃ 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⁻²s⁻¹) to measure F_m and then illuminated with white actinic light (2,000 μmol photons m⁻²s⁻¹) for 10 min. During the 10 min actinic illumination, the same flashes of saturated white light previously used to measure F_m were given once in a while to the cells for determination of F_m'. NPQ was calculated as (F_m-F_m')/F_m'.

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 ddH₂O, acetonitrile, methanol and ethyl acetate. During the run, the solvent concentration was 14% ddH₂O, 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⁻²s⁻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.

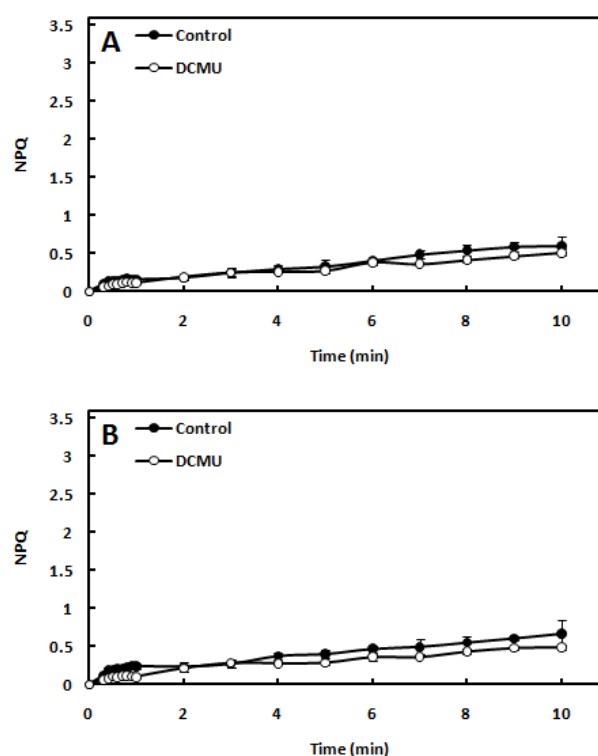


Fig. 1 Induction kinetics of NPQ in *D.salina* cultivated under 60 µmol photons m⁻²s⁻¹ in 0 M NaCl media. Cells were cultivated under 60 µmol photons m⁻²s⁻¹ in 0 M NaCl media. Cell aliquots were taken from 10 day (A) and 20 day (B) cultures and subjected the NPQ measurement as described in Materials 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 $60 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ 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).

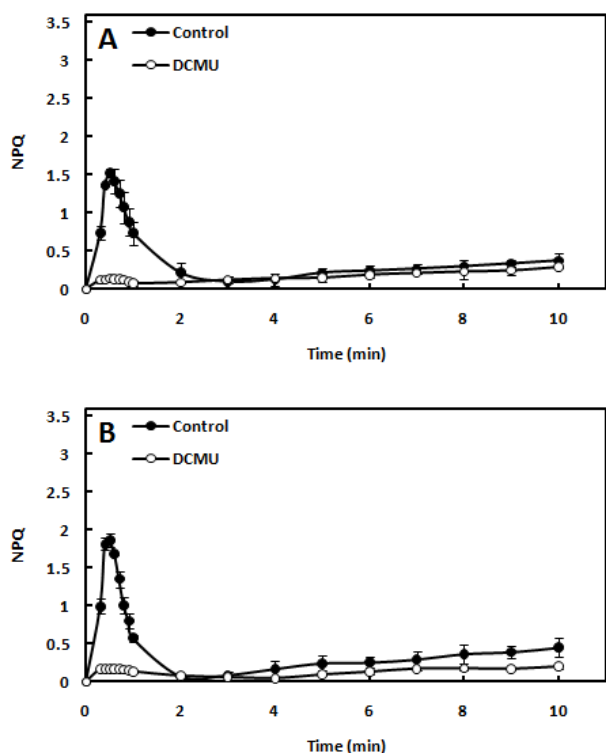


Fig. 2 Induction kinetics of NPQ in *D. salina* cultivated under $60 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ in 1.5 M NaCl media. Cells were cultivated under $60 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ in 1.5 M NaCl media. Cell aliquots were taken from 10 day (A) and 20 day (B) cultures and subjected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

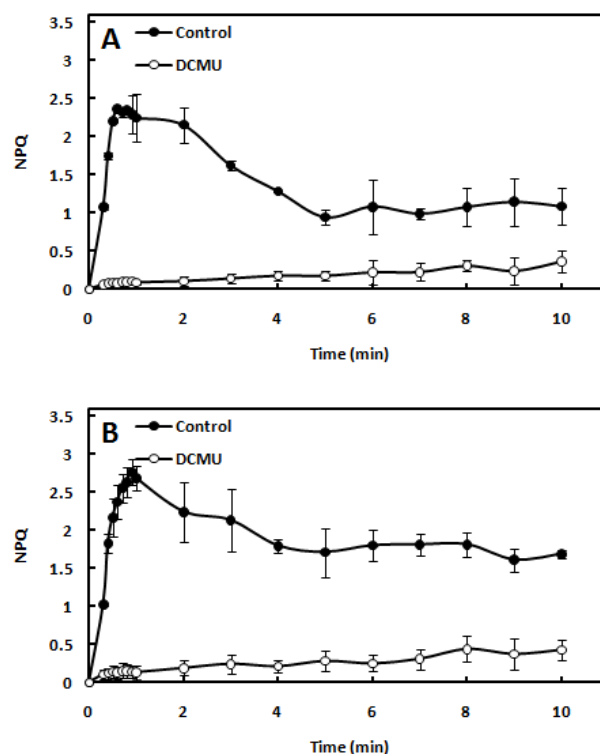


Fig. 3 Induction kinetics of NPQ in *D. salina* cultivated under $60 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ in 3 M NaCl media. Cells were cultivated under $60 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ in 3 M NaCl media. Cell aliquots were taken from 10 day (A) and 20 day (B) cultures and subjected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

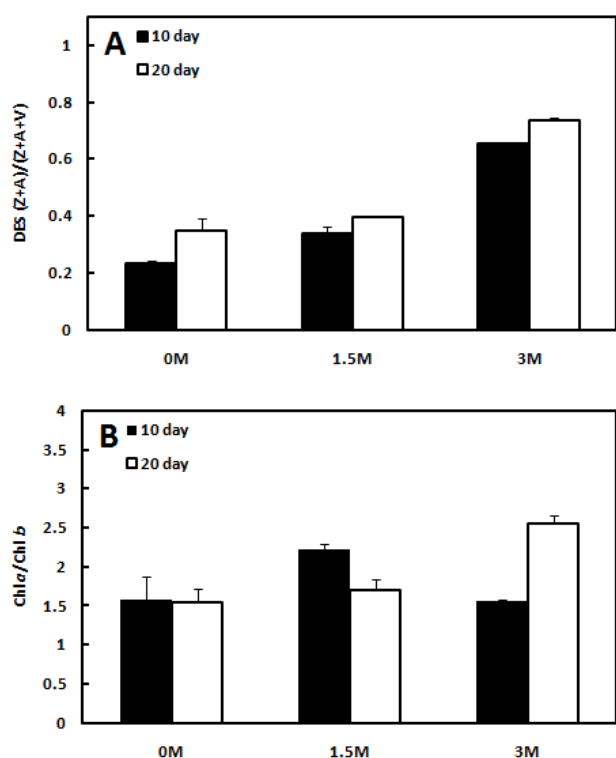


Fig. 4 Pigment compositions of *D.salina* cultivated under 60 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ in 0 M NaCl media, 1.5 M NaCl media and 3 M NaCl media. Cells were cultivated under 60 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ in 0 M NaCl media, 1.5 M NaCl media and 3 M NaCl media respectively. 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 \pm SE

ACKNOWLEDGMENT

This work was financially supported by Research and Development of Suan Sunandha Rajabhat University, Bangkok, Thailand.

REFERENCES

[1] S. Consortium, "Analysis of genome sequence of the flowering plant *Arabidopsis thaliana*", *Nature*, 2000, pp. 796-815.
 [2] SA. Goff, D. Ricke, TH. Lan, G. Presting, R. Wang, M. Dunn, J. Grazebrook, A. Sessions, P. Oeller, H. Varma, et al, "A draft sequence of the rice genome (*Oryza sativa* L. ssp. japonica)", *Science*, 2002, pp. 92-100.
 [3] HX. Chen, WJ. Li, SZ. An, HY. Gao, "Characterization of PSII photochemistry and thermostability in salt-treated *Rumex leaves*", *J. Plant Physiol.*, 2004, pp. 257-264.
 [4] S. Chen, N. Gollop, B. Heuer, "Proteomic analysis of salt-stressed tomato (*Solanum lycopersicum*) seedlings effect of genotype and exogenous application of glycinebetaine", *J Exp Bot.*, 2009, pp. 2005-2019.
 [5] CH. Huang, WL. He, JK. Guo, XX. Chang, PX. Su, LX. Zhang, "Increased sensitivity to salt stress in an ascorbate-deficient *Arabidopsis* mutant", *J Exp Bot.*, 2005, pp. 3041-3049.
 [6] AJ. Liska, A. Shevchenko, U. Pick, A. Katz, "Enhanced photosynthesis and redox energy production contribute to salinity tolerance in *Dunaliella*, as revealed by homology-based proteomics", *Plant Physiol.*, 2004, pp. 2806-2817.

[7] A. Oren, "A hundred years of *Dunaliella* research: 1905-2005", 2005, *Saline Syst* 1: 2.
 [8] AK. Parida, AB. Das, "Salt tolerance and salinity effects on plants: a review", *Ecotoxicology and Environmental Safety*, 2005, pp. 324-349.
 [9] P. Stepien, GN. Johnson, "Contrasting Responses of Photosynthesis to Salt Stress in the Glycophyte *Arabidopsis* and the Halophyte *Thellungiella* Role of the Plastid Terminal Oxidase as an Alternative Electron Sink", *Plant Physiol.*, 2009, pp. 1154-1165.
 [10] L. Zribi, F. Gharbi, F. Rezgui, S. Rejeb, H. Nahdi, MN. Rejeb, "Application of chlorophyll fluorescence for the diagnosis of salt stress in tomato (*Solanum lycopersicum* (variety Rio Grande)", *Scientia Horticulturae*, 2009, pp. 367-372.
 [11] T. Masuda, JEW. Polle, A. Melis, "Biosynthesis and distribution of chlorophyll among the photosystems during recovery of the green alga *Dunaliella salina* from irradiance stress", *Plant Physiol.*, 2002, pp. 603-614.
 [12] K. Yokthongwattana, T. Savchenko, JE. Polle, A. Melis, "Isolation and characterization of a xanthophyll-rich fraction from the thylakoid membrane of *Dunaliella salina* (green algae)", *Photochem Photobiol Sci*, 2005, pp. 1028-1034.
 [13] K. Takayama, B. Osmond, K. Omasa, "Imaging Heterogeneity of Xanthophyll-Independent Non-photochemical Quenching During Photosynthetic Induction in Shade-Grown Leaves of Avocado (*Persea americana* L.)", In: Allen JF, Gantt E, Golbeck JH, and Osmond B (eds) *Photosynthesis. Energy from the Sun: 14th International Congress on Photosynthesis*. Springer, 2008, pp. 681-685.
 [14] XP. Li, P. Müller-Moulé, AM. Gilmore, KK. Niyogi, "PsbS-dependent enhancement of feedback de-excitation protects photosystem II from photoinhibition", *Proc Natl Acad Sci*, 2002, pp. 15222-15227.
 [15] I. Baroli, BL. Gutman, HK. Ledford, JW. Shin, BL. Chin, M. Havaux, KK. Niyogi, "Photo-oxidative stress in a xanthophylls-deficient mutant of *Chlamydomonas*", *J Biol Chem*, 2004, pp. 6337-6344.
 [16] E. Jin, JEW. Polle, A. Melis, "Involvement of zeaxanthin and of the Cbr protein in the repair of photosystem-II from photoinhibition in the green alga *Dunaliella salina*", *Biochim Biophys Acta*, 2001, pp. 244-259.
 [17] E. Jin, B. Feth, A. Melis, "A mutant of the green alga *Dunaliella salina* constitutively accumulates zeaxanthin under all growth conditions", *Biotechnol Bioeng*, 2003, pp. 115-124.
 [18] CH. Huang, WL. He, JK. Guo, XX. Chang, PX. Su, LX. Zhang, "Increased sensitivity to salt stress in an ascorbate-deficient *Arabidopsis* mutant", *J Exp Bot*, 2005, pp. 3041-3049.

Rutanachai Thaipratum was born in Nakhon Ratchasima City (the city in the northeast of Thailand) on March 20, 1982. He received his B.Sc. degree in Biochemistry from Khon Kaen University (KKU), Khon Kaen, Thailand in 2002 and Ph.D. degree in Biochemistry from Mahidol University (MU), Bangkok, Thailand in 2009. Since June 2010, he has been a lecturer of the Department of Science at Suan Sunandha Rajabhat University (SSRU), in Bangkok. His main research interests include plant biochemistry and physiology.