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Summary of the PhD thesis

Macro-organization of Photosynthetic Complexes During Salt Stress Acclimation of Green Microalgae and in Plant Chloroplast Acetyltransferase and Ser/Thr Kinase Mutants

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Background

Salinity stress is an important environmental abiotic stress that poses a serious threat to photosynthetic organisms. They have evolved efficient mechanisms to adjust their photosynthetic apparatus to maintain photosynthetic efficiency and adequate photoprotection.

The photosynthetic membranes, so called thylakoid membranes (TM), are assembled into highly organized multilamellar systems. This implies the ability of thylakoid membranes to remodel and suggests structural flexibility, they can actively participate in different photosynthetic regulatory mechanisms. Accumulating evidences indicate that the photosynthetic supercomplexes, embedded into the TM, undergo supramolecular reorganization during acclimation to abiotic stresses [1]. Our knowledge of the mechanisms responsible for the fine-tuning of photosynthesis in a wide range of environmental conditions in various organisms and in relation to the structural flexibility of thylakoid membranes, specifically, is far from complete.

The main advantage of the TM organization into grana is to separate both photosystems to prevent energy spillover, to enable controlled distribution of the excitation energy between the photosystems through state transitions [2]. Dynamic organization of the TMs is a prerequisite for the regulatory processes of photosynthetic light reactions. Protein phosphorylation is a key regulatory mechanism mediated by STN7 kinase in plants and STT7 kinase in algae causing state transitions [3]. During state transitions the PSII is associated with LHCII in state 1, upon LHCII phosphorylation LHCII leaves the PSII-enriched stacked region and migrates to PSI-containing unstacked stromal region, then it interacts with PSI forming PSI-LHCII supercomplexes in state 2 [4]. Role of membrane reorganization during salt stress acclimation has not been explored in detail, yet.

In plants *stn7* mutant is defective in state transition due to lack of the

phosphorylation of LHCII and interaction between PSI and LHCII [5]. Recent study demonstrated that the chloroplast acetyl transferase GNAT2 plays a key role in the regulation of photosynthetic light reactions [6]. The *gnat2* mutant is unable to form the PSI-LHCII complex specific to the state transition and balance the excitation energy between the two photosystems although there are no defects in the LHCII phosphorylation [6]. The role of STN7 and GNAT2 in the macro-organization of thylakoid membranes needs further examinations.

References

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Aims

The major aim of the presented project was to better understand the dynamics of photosynthetic complexes and membrane organization during salt stress in biotechnologically important microalgal strains revealing the relation to their stress tolerance and in *Arabidopsis thaliana* chloroplast mutants.

To gain a better insight into salt stress response of microalgae we studied:

1. The acclimation responses of *E. gracilis* to moderate salt stress by investigating the morphological and pigment composition changes in addition to the macro-organization of photosynthetic apparatus.
2. The changes in the structure and function of photosynthetic apparatus under salinity stress in *C. reinhardtii*.

Understanding the acclimation responses of algae to salinity stress is essential to advance basic research in algal biology and biotechnology.

3. In addition, we studied the macro-organization of the photosynthetic complexes in thylakoid membrane of *A. thaliana* chloroplasts in the cases of chloroplast acetyltransferase *gnat2* and Ser/Thr kinase *stn7* mutants. The *gnat2* mutant exhibiting reduced lysine acetylation of specific thylakoid proteins. The STN7 kinase regulates the organization and dynamics of thylakoid structure through phosphorylation.

A thorough characterization of the responses of photosynthetic apparatus to salt stress could be important for increasing agricultural productivity and the plant mutants help understanding the dynamics of thylakoid membrane.

Applied methods and techniques

- Scanning electron microscopy (SEM)
- Transmission electron microscopy (TEM)
- Small-angle neutron scattering (SANS)
- Absorption and Circular dichroism (CD) spectroscopy
- Steady-state low temperature fluorescence spectroscopy
- Time-resolved fluorescence spectroscopy
- Thylakoid isolation
- Blue native – PAGE
- High performance liquid chromatography (HPLC)
- Chl *a* fluorescence measurements

Summary of findings

In this thesis, we demonstrated that *Chlamydomonas reinhardtii* and *Euglena gracilis* use specific strategies to overcome the effects of salinity.

We characterized the physiological effects of moderate salt stress in *E. gracilis* by using various biophysical and biochemical techniques like SEM, TEM, SANS, HPLC, BN-PAGE, Chl a fluorescence, CD and 77 K fluorescence spectroscopy and techniques.

- **In *E. gracilis* cells, salt stress had a direct effect on the growth rate and chlorophyll content**. Growth rate and chlorophyll content of the cells decreased with salt treatment in a concentration dependent manner.
- **TEM and SANS results revealed that repeat distances of the thylakoid membranes decreased significantly** with increasing salt treatment.
- The intensity of major psi-type bands was decreased suggesting significant changes in the macro-organization of thylakoid membranes. **The amplitude of psi-type CD showed significant decrease with time in concentration dependent manner**.
- No significant changes were observed in the photosynthetic energy transfer, functional state of PSII, PSI activity and the composition of pigment-protein complexes of the salt-treated cells.
- The content of each carotenoid increased individually and thus the ratio of total carotenoid content to Chl increased significantly.
- **Accumulation of paramylon increased** with increasing salt treatment and the highest levels of paramylon was observed in the cells grown in the presence of 150 mM NaCl. The increase was 2.5, 3.5 and 5-fold in cells treated with 50, 100 and 150 mM NaCl respectively.

These results were published in Kanna et al. (2021) *Frontiers in Plant Science* 12: 725699; doi: 10.3389/fpls.2021.725699.

Further, the physiological effects of salt treatment in *C. reinhardtii* were characterized using TEM, CD, 77 K and time-resolved fluorescence spectroscopy. By using the *stt7* and *pgrl1* mutants, we investigated the role of state transitions and cyclic electron flow during salt stress acclimation.

- **In *C. reinhardtii* cells, chlorophyll content of the cells decreased** with increasing salt treatment in WT, *stt7* and *pgrl1* strains.
- **Repeat distances of the thylakoid membranes** of 100 mM NaCl treated cells of WT **increased** by about 2 nm when compared to the control. However, this effect was not observed in *stt7* mutant.
- **The major psi-type bands are decreased** in all strains, even at moderate salt concentrations, suggesting significant changes in the macro-organization of thylakoid membranes.
- In all *C. reinhardtii* strains grown in high-salinity media, a significant reduction in the relative intensity of PSII fluorescence emission bands was observed. Moreover, our results suggested increase in aggregated LHCII and presence of uncoupled LHCs and free chlorophylls under salt stress. These effects were more evident in *stt7* cells grown under 150 mM NaCl conditions.
- Average fluorescence lifetimes increased in the salt-treated cells indicating a decreased photochemical quantum yield of PSII. We **observed an increase in the relative amplitudes and fluorescence intensities of fluorescence lifetime component of free LHCs in the salt-treated cells of WT** which is more prominent in *stt7*. Uncoupling of LHCs occurs also in the absence of phosphorylation (in the *stt7* mutant) – even more so than in WT at high salt concentration.
- The more severe salt induced effects hint that LHCII phosphorylation in this case is an acclimatory stress response stabilizing the photosynthetic apparatus.

Based on these results, a manuscript is in preparation for publication.

Moreover, **the role of chloroplast proteins STN7 kinase and GNAT2 in the thylakoid membrane macro-organization of *A. thaliana* was also demonstrated**.

- **The intensity of red positive psi-type CD band** i.e., (+)690 nm band of the leaves and thylakoid membranes of ***gnat2* and *stn7* mutants is significantly less** when compared to the WT.
- **The amplitude of other psi-type CD bands** i.e., the (−)674 nm and (+)506 nm bands **of the thylakoid membranes of both the mutants were significantly reduced** suggesting that the macro-organization is different compared to the WT.

This set of results was published in Rantala et al. (2022) *Plant and Cell Physiology*, https://doi.org/10.1093/pcp/pcac096

Conclusions

The following list summarizes the main novel scientific results of this thesis work:

1. *E. gracilis* cells can maintain the photosynthetic activity under moderate salt stress with modification of the pigment composition and the reorganization of the thylakoid membranes. Moreover, the increase in paramylon content prepares the cells for a higher osmotic shock. It appears that *E. gracilis* cells can compensate for moderate salt stress, by adjusting metabolism, without extreme effects on other cell functions.

2. It seems that *E. gracilis* possesses inherent capabilities including the incredible metabolic and physiological versatility which enables acclimation to the hostile saline environment making it an excellent target for commercial use.

3. In *C. reinhardtii*, moderate salt stress disturbed macro-organization of PSII:LHCII protein complexes and induced significant alteration in the distribution of excitation energy between the two photosystems, favoring PSI and an energetic detachment of LHCII from PSII.

4. In *C. reinhardtii*, while diminished efficiency of the cyclic electron flow exerts minor additional effect on salt stress acclimation, the incapability of state transitions causes severe damages in the (macro-)organization of the pigment-protein complexes – suggesting that state transitions play an important role in the acclimatory response of the photosynthetic apparatus of *C. reinhardtii* cells to salinity.

5. Changes in the structure or arrangement of LHCII and PSII-LHCII supercomplex across the thylakoid membrane were suggested in *stn7* and *gnat2* mutants of *A. thaliana* by their CD spectra.

6. It seems that in *A. thaliana*, both phosphorylation and acetylation play key role in the macro-organization of thylakoid membranes. Since GNAT2 protein is involved in state transitions, it would be interesting to study its role in the stress adaptation.

ÖSSZEFOGLALÓ

Doktori munkám során célom volt tanulmányozni sóstressz hatására bekövetkezett változásokat i) a *Chlamydomonas reinhardtii* (*C. reinhardtii*) fotoszintetikus apparátus szerkezetében és működésében ii) az *Euglena gracilis* (*E. gracilis*) akklimatizációs reakcióiban, továbbá iii) feltárni a proteinek acetilezésének/ foszforilálásának szerepét az *Arabidopsis thaliana* (*A. thaliana*) fotoszintetikus apparátusának membrándinamikájában.

A sóstressz negatív hatással volt az *E. gracilis* sejtek növekedésére és klorofill tartalmára, valamint kihatott a tilakod membránok (TM) makro-szerveződésére, de nem volt hatása a fotoszintetikus folyamatokra és a fotoszintetikus komplexek összetételére. Ugyanakkor a karotinoid-klorofill arány és a biotechnológiai szempontból jelentős paramilon tartalom megnőtt. A raktározott poliszacharid felhalmozódása, a pigment összetételben és a TM szerveződésében bekövetkező változások segíthetik az *E. gracilis* sejtek sóstresszhez történő alkalmazkodását.

Vizsgáltuk a sóstressz hatását a *C. reinhardtii* vad típusú és két *stt7* és a *pgrl1* mutáns törzsére. Kimutattuk, hogy a sóstressz kihatott a TM ismétlődési távolságára; a pigment-fehérje komplexek szerveződésére és működésére. Az *stt7* mutáns fokozott sóstressz érzékenysége, arra utal, hogy a fotoszintetikus állapotátmenetek kulcsszerepet játszanak a sóstresszhez való alkalmazkodásban.

Vizsgáltuk a kloroplaszt acetil-transzferáz (GNAT2) és az STN7 kináz szerepét *A. thaliana* TM szerveződésében. A CD spektroszkópiai vizsgálatok a vad típushoz képest eltérést mutattak a PSII-LHCII szuperkomplex és/vagy az LHCII szerkezetében és elrendezésében mind a *gnat2*, és az *stn7* mutánsokban. Ezek az eredmények megerősítették, hogy a GNAT2 az STN7 kinázhoz hasonlóan részt vesz a TM makroszervezésének alakításában.

Általánosságban elmondható, hogy a membrán dinamikus szerveződése

kulcsszerepet játszik a fotoszintetikus organizmusok stressz-adaptációjában, melyben a fehérjék poszttranszlációs módosításának is szerepe lehet.

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List of publications

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Publications related to the PhD thesis

1. **Kanna, S.D.,** Domonkos, I., Kóbori T. O., Dergez, Á., Böde K., Nagyapáti S., Zsiros O., Ünnep R., Nagy G., Garab G., Szilák L., Solymosi K., Kovács, L., Ughy B. (2021) Salt stress induces paramylon accumulation and fine-tuning of the macro-organization of thylakoid membranes in *Euglena gracilis* cells. ***Frontiers in Plant Science***, 12 (IF 6.627)

2. Marjaana, R**.,** Ivanauskaite, A., Laihonen, L., **Kanna, S.D.,** Ughy, B., Mulo, P. (2022) Chloroplast acetyltransferase GNAT2 is involved in the organization and dynamics of thylakoid structure. ***Plant and Cell Physiology*** (IF 4.927)

3. Devadasu, E., **Kanna, S.D.,** Neelam, S., Nama, S., Akhtar, P., Polgar, T.F., Ughy, B., Garab, G., Lambrev, P.H., Subramanyam, R. Long- and short-term acclimation of the photosynthetic apparatus to salinity in *Chlamydomonas reinhardtii*: The role of Stt7 protein kinase. (MS under preparation).

Other Publications

Dlouhy, O., Karlický, V., Javornik. U., Kurasová, I., Zsiros, O., Šket, P., **Kanna, S.D**., Böde, K., Večeřová, K., Urban. O., Gasanoff, E.S., Plavec, J., Špunda, V., Ughy B., Garab, G. (2022) Structural entities associated with different lipid phases of plant thylakoid membranes – selective susceptibilities to different lipases and proteases. ***Cells MDPI,*** (IF 7.66)

Communications at international conferences

1. **Kanna SD**, Akhtar P, Neelam S, Nama S, Lambrev PH, Garab G, Subramanyam R, Ughy B. Salt-stress induced changes in the supramolecular organization of the photosynthetic membranes of *Chlamydomonas reinhardtii*. 8th international symposium on "Microalgae and seaweed products in plant/soil-systems", 26th – 27th June 2017, Mosonmagyaróvár (Hungary) [Poster presentation]

2. **Kanna SD**, Akhtar P, Neelam S, Nama S, Lambrev PH, Garab G, Subramanyam R, Ughy B. Salt-stress induced changes in the supramolecular organization of the photosynthetic membranes of *Chlamydomonas reinhardtii*. 8th International Conference on "Photosynthesis and Hydrogen Energy Research for Sustainability", 30th October - 4th November 2017, Hyderabad (India) [Poster presentation]

3. **Kanna SD**, Akhtar P, Lambrev PH, Garab G, Subramanyam R, Ughy B. Effect of salt stress on biotechnologically important microalgal strains. Young Researchers in Biosciences International Symposium, 23rd – 29th July 2018, Cluj-Napoca (Romania) [Oral presentation]

4. **Kanna SD**, Domonkos I, Akhtar P, Lambrev PH, Garab G, Subramanyam R, Ughy B. Effect of salt stress on biotechnologically important microalgal strains. 9th Symposium on "Microalgae and seaweed products in plant/soil-systems", 25th – 26th June 2019, Mosonmagyaróvár (Hungary) [Oral presentation]

5. **Kanna SD**, Kóbori TO, Dergez Á, Domonkos I, Zsiros O, Ünnep R, Nagy G, Böde K, Garab G, Ughy B. Effects of salt stress on a biotechnologically important microalgal strain. International Congress on Biophysics of Photosynthesis from molecules to the field, 2nd – 4th October 2019, Rome (Italy) [Poster presentation]

6. **Kanna SD**, Akhtar P, Neelam S, Devadasu E, Nama S, Lambrev PH, Kóbori TO, Dergez Á, Domonkos I, Garab G, Subramanyam R, Ughy B. Salt stress induced changes in the supramolecular organization of the photosynthetic membranes of *Chlamydomonas reinhardtii* and *Euglena gracilis*. 19th International Conference on the Cell and Molecular Biology of *Chlamydomonas*, 28th August - 2nd September 2021, Iles des Embiez (France) [Poster presentation]

7. Nagyapáti S, Böde K, **Kanna SD**, Pettko-Szandtner A, Szilák L, Ughy B. Bacterial growth phases in *Synechococcus elongatus* PCC7942. ProSynfest 2020, 16th - 19th March 2022, Córdoba (Spain) [Poster presentation]

Fellowships and Prizes

1. Biological Research Centre of the Hungarian Academy of Sciences *PhD scholarship* (2016)

2. 8th International Conference on "Photosynthesis and Hydrogen Energy Research for Sustainability" (2017) *Poster prize*

Declaration from the corresponding author

on the contribution of Sai Divya Kanna to the following scientific article:

Sai Divya Kanna, Ildikó Domonkos, Tímea Ottília Kóbori, Ágnes Dergez, Kinga Böde, Sarolta Nagyapáti, Ottó Zsiros, Renáta Ünnep, Gergely Nagy, Győző Garab, László Szilák, Katalin Solymosi, László Kovács, Bettina Ughy

Salt stress induces paramylon accumulation and fine-tuning of the macro-organization of thylakoid membranes in *Euglena gracilis* cells.

2021, Frontiers in Plant Science, 12:725699., doi: 10.3389/fpls.2021.725699. eCollection 2021.

I hereby declare that Sai Divya Kanna has had the major contribution to the preparation and implementation of the experiments described in the article, the analysis of the experimental results and in part of the writing of the article. The published article is part of her doctoral dissertation entitled "Macro-organization of photosynthetic complexes during salt stress acclimation of green microalgae and in plant chloroplast acetyltransferase and ser/thr ptrotein kinase mutants" in chapter 4.2 "salt stress acclimation of *Euglena gracilis*."

I declare we do not intend to use for other PhD dissertation the results obtained by Sai Divya Kanna.

Dr. Bettina Ughy

Szeged, 2022.08.24

Declaration from the co-author

on the contribution of Sai Divya Kanna to the following scientific article:

Sai Divya Kanna, Ildikó Domonkos, Tímea Ottília Kóbori, Ágnes Dergez, Kinga Böde, Sarolta Nagyapáti, Ottó Zsiros, Renáta Ünnep, Gergely Nagy, Győző Garab, László Szilák, Katalin Solymosi, László Kovács, Bettina Ughy

Salt stress induces paramylon accumulation and fine-tuning of the macro-organization of thylakoid membranes in *Euglena gracilis* cells.

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As a PhD student I do not intend to use for my PhD dissertation the results obtained by Sai Divya Kanna.

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Szeged, 2022.08.24

Declaration from the co-author

on the contribution of Sai Divya Kanna to the following scientific article:

Sai Divya Kanna, Ildikó Domonkos, Tímea Ottília Kóbori, Ágnes Dergez, Kinga Böde, Sarolta Nagyapáti, Ottó Zsiros, Renáta Ünnep, Gergely Nagy, Győző Garab, László Szilák, Katalin Solymosi, László Kovács, Bettina Ughy

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As a PhD student I do not intend to use for my PhD dissertation the results related to the article and represented in the above mentioned thesis of Sai Divya Kanna.

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Declaration from the corresponding author

on the contribution of Sai Divya Kanna to the following scientific article:

Marjaana Rantala, Aiste Ivanauskaite, Laura Laihonen, **Sai Divya Kanna**, Bettina Ughy, Paula Mulo

Chloroplast acetyltransferase GNAT2 is involved in the organization and dynamics of the thylakoid structure.

Plant and Cell Physiology. 2022 Jul 6:pcac096. doi: 10.1093/pcp/pcac096.

I hereby declare that Sai Divya Kanna has contributed to the preparation and implementation of the CD experiments described in the article, to the analysis of the CD results and the writing of the article. She is allowed to use the above mentioned parts of the published article as part of her doctoral dissertation entitled "Macro-organization of photosynthetic complexes during salt stress acclimation of green microalgae and in plant chloroplast acetyltransferase and ser/thr ptrotein kinase mutants" in the chapter 4.3 "Role of GNAT2 in the organization of the thylakoid membrane of *Arabidopsis thaliana*."

The CD experiments and results represented in the above article and represented in the thesis by Sai Divya Kanna is not intended to use for other PhD titles.

Prof. Paula Mulo

Turku, 12th August 2022.

Declaration from the corresponding author

on the contribution of Sai Divya Kanna to the following scientific article (going to be submitted):

Elsinraju Devadasu[¶], Sai Divya Kanna[¶], Satyabala Neelam[¶], Srilatha Nama, Parveen Akhtar, Tamás F. Polgár, Bettina Ughy, Győző Garab, Petar H. Lambrev and Rajagopal Subramanyam (2022) Long- and short-term acclimation of the photosynthetic apparatus to salinity in *Chlamydomonas reinhardtii*. The role of Stt7 protein kinase

I declare that Sai Divya Kanna has had the lead contribution (sharing first author) to the preparation and implementation of some of the experiments described in the article, the analysis of the experimental results and the writing of the article. The article and the results to be published therein have not been referred to for the purpose of acquiring any other academic degrees or titles.

Dr. Subramanyam Rajagopal

Hyderabad, 12th July 2022.