Final report of the project[1]
The effect of salt and drought stress on plastid structure and function
FK_17 124748, PI: Katalin Solymosi
2017-10-01 – 2023-09-30

Background and aims of the project

Global climate change leading to extreme weather conditions poses a significant threat to food security. It increases the frequency and severity of floods, waterlogging and drought. Decreased precipitation leads to the expansion of semi-arid and desert areas and often results in improper irrigation practices using groundwater and no drainage management. This causes salinization, i.e. the accumulation of water-soluble salts in the soil. Several alarming estimations suggest that soil salinity is constantly increasing and already affects approximately 833 million hectares of arable land (Munns and Tester 2008, Qadir et al. 2014) in more than half of the world's countries (Corbishley and Pearce 2007). According to the United Nations, the global population reached 8 billion by mid-November 2022. Around 1.5 billion inhabitants are affected by soil salinity and half of the human population is prone to high water scarcity for at least one month a year.

The water condition of crops and their resilience to drought are crucial for agricultural output. Both drought stress and saline soils present significant economic challenges, leading to stunted growth, delayed development, and reduced yields of cultivated plants. Estimates indicate that the annual global cost of land degradation caused by salinity could exceed $12 billion (Volkov, 2015) or $27.3 billion (Qadir et al., 2014) due to decreased crop production. Yearly global agricultural losses associated with drought stress are also thought to be in the range of billions of dollars. Hungary's agriculture is particularly vulnerable to extreme precipitation and aridity as well as soil salinization, jeopardizing the cultivation of major crops like wheat, maize and barley. Moreover, urban areas and lands located close to highways are also exposed to soil salinity problems due to excessive use of salts for de-icing roads during winter.

Thus, breeding of drought- and salt-tolerant crops and crop varieties is essential to mitigate those challenges. Therefore, it is important to improve our understanding of the complex mechanisms of plant tolerance to salt and drought stress. These involve multiple genes and parameters. Ion transport mechanisms play a crucial role in salt tolerance, suggesting potential targets for improving crop performance under stress conditions as we have reviewed in detail (**Wani et al. 2020**)[2]. High salt stress is commonly viewed as comprising two main components: hyperosmotic stress, which reduces water potential, thereby restricting water uptake and impeding cell expansion and growth; and hyperionic stress, which disrupts the uptake and balance of essential

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[1] We highly appreciate the possibility to submit the final report of FK124748 now. The reason for this extension is that due to COVID pandemic, some measurements and the publication process of several interesting results had taken much more time than originally planned (in some cases it took years), and in the final report we aimed to show our results as published after peer review, to illustrate the success of the project not only by the results/data gained.

[2] Papers published in the frame of this project and provided at the end of this document in the list of publications will be indicated in bold.

metals, and may lead to specific toxicity from ions such as Na^+, and, to a lesser extent, Cl^- (Miyake et al., 2006; Munns and Tester, 2008; Maathuis et al., 2014).

Due to photosynthesis and other important metabolic processes, plastids and their adaptability to stressors significantly impact plant growth and yield. Literature data about the response of plastid structure and function to drought and salt stress is still limited and somewhat controversial due to, among others, the lack of *in vivo* noninvasive methods to investigate plastid ultrastructure. Some authors report swelling of the thylakoid lumen of chloroplasts under drought (osmotic) or salt stress, while others observed no alterations. However, the observed changes depend of course on the crop species or variety used, the length or strength of the applied treatment, as well as the developmental stage at which the stress occurred. The main research questions formulated in the project were the following:

1. **How is it possible to distinguish the osmotic and specific ionic components of salt stress on plastid structure and function?**
2. **Which plastid types or developmental stages are most affected by salt and osmotic (and drought) stress?**
3. **Which stress treatments can be considered as good models of naturally or agriculturally relevant stress processes?**

Below we'll present the major outputs of the above OTKA FK project. This project enabled the PI to involve several BSc, MSc, and PhD students in the work (resulting in several theses and TDK participation), and to build the ELTE Plastid Biology research group. There were changes mostly in the administrative staff associated with the project, and Renáta Ünnep went on maternity leave and was therefore replaced by Márton Markó and Gergely Nagy in some small-angle neutron scattering (SANS) measurements.

Results

During the project, first, we carefully selected and optimized the plant cultivars and species, as well as, the applied treatments. As planned in our proposal, we have also performed literature reviews related to the project and plastid biology (**Wani et al. 2020, Solymosi and Mysliwa-Kurdziel, 2021**, **Aronsson and Solymosi, 2024**), and worked on optimizing methods for thylakoid isolation and proteomic analyses related to this work (**Sárvári et al. 2022**).

According to current agricultural protocols, wheat seedlings are sown deep in the soil. This also means that during the first few days of their germination, these seedlings develop in the absence of sunlight, and their leaves containing etioplasts may be directly prone to high soil salinity. This may even affect their greening when they reach the soil surface. In spite of their agricultural relevance, not many studies have investigated the effect of salt stress on etioplasts.

As a continuation of our earlier works with a salt-sensitive wheat (*Triticum aestivum*) cultivar (Giza 168) from Egypt (Abdelkader et al. 2007), we started to test one salt-tolerant Egyptian cultivar (Sids1) (Figure 1, **Solymosi et al.** - manuscript). In addition to normal fixation, a specific transmission electron microscopic (TEM) fixation was also performed with the conventional glutaraldehyde solution (2.5% glutaraldehyde in 70 mM phosphate buffer) supplemented with the same salt concentration (600 mM KCl:NaCl) as the previous salt-stress treatment of etiolated leaves of Giza 168 and Sids1. The inner membrane system of plastids (i.e. prolamellar bodies, PLBs) was often hardly visible when compared with samples obtained using conventional fixation (Figure 1). In the sensitive cultivar the swelling of the intrathylakoidal space was observed with both fixation protocols, while in the tolerant cultivar only small vesicles were observed in the conventional fixative, and large swollen intrathylakoidal spaces appeared during salt-supplemented fixation (i.e. prolonged salt exposure) (Figure 1). This indicates that the swelling is probably induced by salt and is not related to reverse plasmolysis or osmolarity changes during fixation; thus, we decided to use conventional fixation protocols in our later analyses.

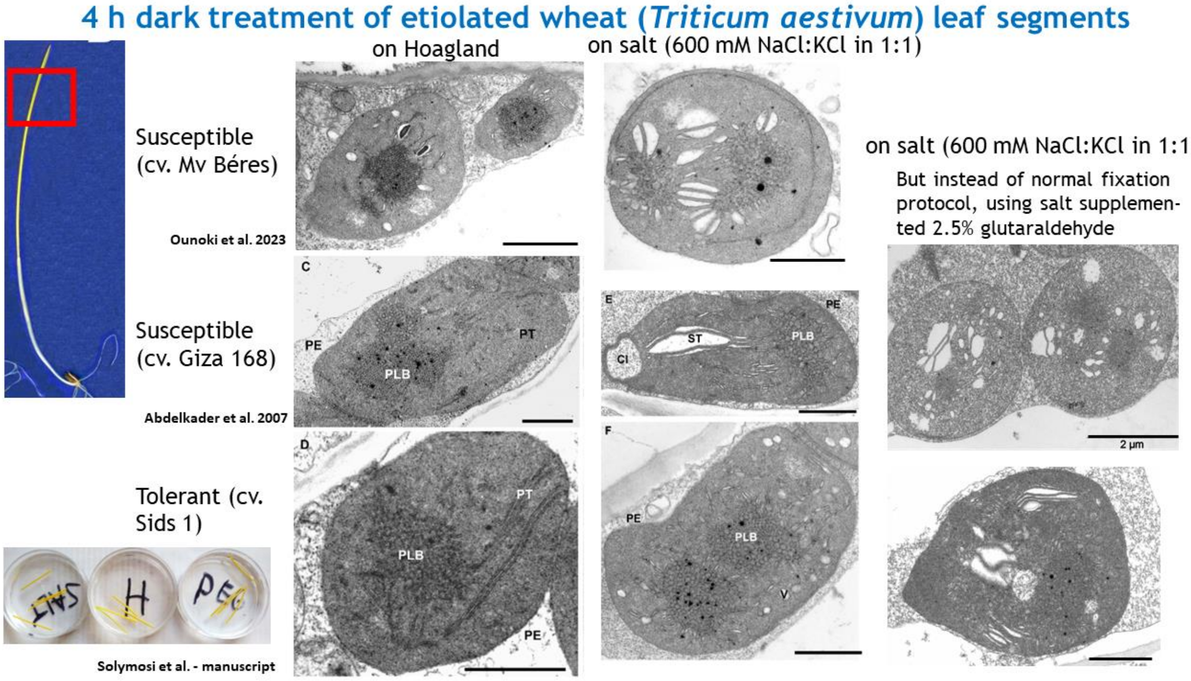


*Figure 1. Comparison of etioplast ultrastructure in the studied wheat cultivars under salt stress and specific fixation protocol (Abdelkader et al. 2007, **Ounoki et al. 2023, Solymosi et al. – manuscript**).*

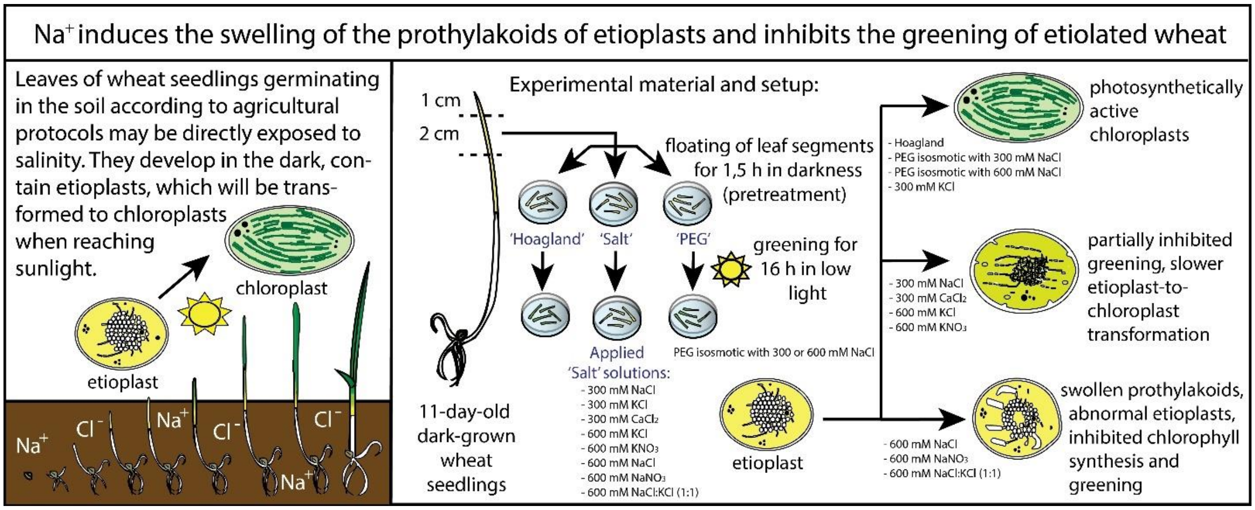
Comparison of chlorophyll (Chl) biosynthesis during the greening of the excised etiolated leaves of the two cultivars showed slower greening capacity and membrane rearrangement in the tolerant cultivar under normal conditions (after 3 h greening with 50 µmol photons m^{-2} s^{-1} white light on Hoagland solution the Chl fluorescence band at 740 nm did not appear in 77K fluorescence emission spectra, and PLBs were still observed on electron micrographs). Salt stress inhibited the greening in both cultivars both in terms of thylakoid/granum development (PLBs were retained after 8 h greening) and in the formation of the Chl-protein complexes of the photosynthetic apparatus (with only a band at 681 nm being present in them), but the plants recovered after transfer to a salt depleted nutrient solution, this recovery being faster in the sensitive cultivar.

Similarly to control samples illuminated on Hoagland for 8 h, 250 nmol g^{-1} fresh mass (FM) Chl was produced in salt stressed samples after their transfer to Hoagland solution and further 12 h illumination, while this amount was 140 nmol g^{-1} FM in the tolerant one during the recovery, and approx. 40 nmol g^{-1} FM was produced under salt stress in both cultivars. HPLC analyses of carotenoid pigments using methanol/acetonitrile/ethylacetate three phase system, according to Wright and Jeffrey (1997), indicated that salt stress induced an increase in zeaxanthin and a decrease in violaxanthin and antheraxanthin relative contents, irrespective of light regime or cultivar, indicating that the violaxanthin cycle pigments are mostly affected by salt stress. We observed similar changes in the carotenoid contents in green and greening wheat seedlings.

During dark recovery of salt-stressed and 3-h-illuminated leaves (recovered on Hoagland solution for 12 h in the dark) the regeneration of photoactive protochlorophyllide with fluorescence emission at 655 nm was remarkably delayed in the tolerant cultivar. The delayed structural conversion of plastids, and slower Chl biosynthesis in the tolerant cultivar might be a consequence of resource relocation towards salt-protection mechanisms (**Solymosi et al.** - manuscript).

Later, for further analyses, we also tested the Hungarian wheat cultivar Mv Béres that was previously investigated by Janda et al. (2016) and Darkó et al. (2019). In terms of salt sensitivity, this cultivar was similar, or even more sensitive to salinity than Giza 168 (Figure 1) (**Ounoki et al. 2023**). Interestingly, the salt stress induced swelling of the prothylakoid lumen was absent from epidermal cells (e.g. etioplasts of stomatal guard cells or epidermal plastids) and was only observed in the etioplasts of mesophyll cells of the sensitive cultivars. Our systematic analyses performed on the salt-sensitive Mv Béres wheat cultivar revealed that osmotic stress alone (e.g. the use of isosmotic polyethylene glycol 6000 – PEG) did not induce the swelling of the prothylakoid lumen of etioplasts, but swelling was always associated with the presence of both the osmotic and ionic components of the salt stress (**Ounoki et al. 2023**). Our detailed analyses revealed that among the used ions (Na^+, K^+ and Cl^- all present in the original 600 mM NaCl:KCl, 1:1 solution), the swelling of the intrathylakoidal space and hindered Chl biosynthesis, greening and inhibited transformation of etioplasts to chloroplasts (Figure 2, **Sóti et al. 2023**) could be clearly associated with solutions containing high concentrations of Na^+ ions. In other solutions, the greening either proceeded almost normally (e.g. on PEG solutions or 300 mM KCl) or was only partially slowed down or inhibited (Figure 2).

In the same wheat cultivar (Mv Béres) and experimental system, we have systematically compared the effect of salt and osmotic (drought) stress on different plastid types, in order to elucidate which plastid developmental stage or which stages of the greening process are more prone to salt or osmotic (drought) stress induced ultrastructural alterations (Figure 3, **Ounoki et al. 2023**). We observed no visible alterations, thus no swelling of the intrathylakoidal space in case of the same salt stress or isosmotic stress treatment of young and fully developed chloroplasts of light-grown seedlings. We calculated the granum repeat distance (RD) values of the chloroplasts of control and salt-stressed light-grown leaves based on the analysis of TEM images of previously chemically fixed and embedded samples as well as the SANS profiles of *in vivo* leaf segments (for graphical representation of the granum RD values see Figure 4). Our data obtained with both methods have revealed a shrinkage of the granum RD value upon salt stress when compared with the control (Hoagland treated) sample.


Na^+ induces the swelling of the prothylakoids of etioplasts and inhibits the greening of etiolated wheat

Leaves of wheat seedlings germinating in the soil according to agricultural protocols may be directly exposed to salinity. They develop in the dark, contain etioplasts, which will be transformed to chloroplasts when reaching sunlight.

Experimental material and setup:

floating of leaf segments for 1,5 h in darkness (pretreatment)

greening for 16 h in low light

Applied 'Salt' solutions:
- 300 mM NaCl
- 300 mM KCl
- 300 mM $CaCl_2$
- 600 mM KCl
- 600 mM KNO_3
- 600 mM NaCl
- 600 mM $NaNO_3$
- 600 mM NaCl:KCl (1:1)

PEG isosmotic with 300 or 600 mM NaCl

- Hoagland
- PEG isosmotic with 300 mM NaCl
- PEG isosmotic with 600 mM NaCl
- 300 mM KCl

photosynthetically active chloroplasts

- 300 mM NaCl
- 300 mM $CaCl_2$
- 600 mM KCl
- 600 mM KNO_3

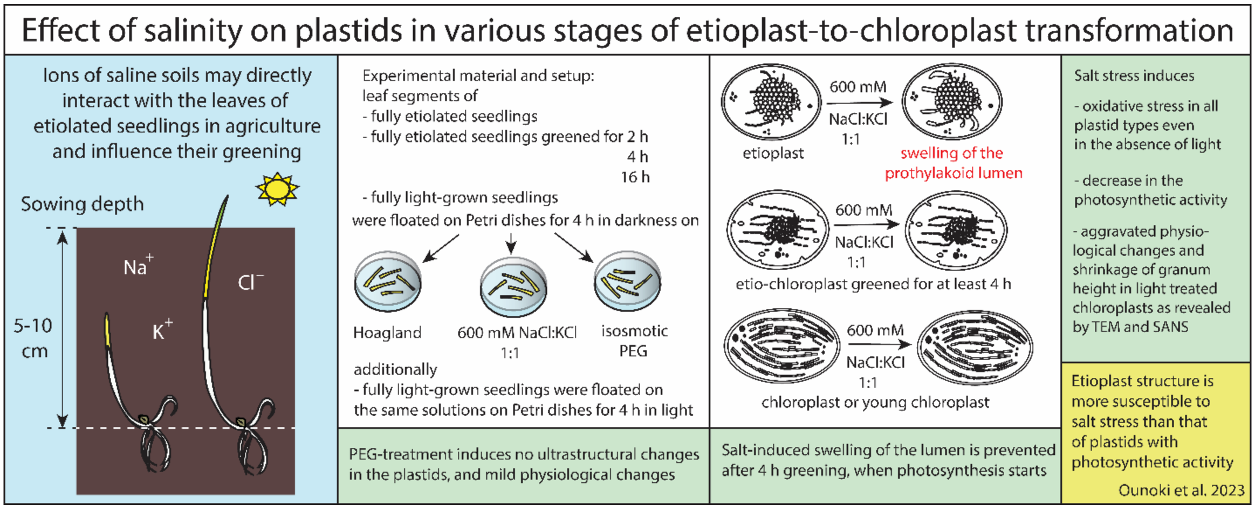
partially inhibited greening, slower etioplast-to-chloroplast transformation

- 600 mM NaCl
- 600 mM $NaNO_3$
- 600 mM NaCl:KCl (1:1)

swollen prothylakoids, abnormal etioplasts, inhibited chlorophyll synthesis and greening

Figure 2. Summarizing the effect of various salt and osmotic stress treatments on the greening of leaf segments of etiolated wheat (cv. Mv Béres) seedlings (Graphical abstract of ***Sóti et al. 2023****).*

The swelling of the intrathylakoidal space of the (pro)thylakoid lumen was observed by TEM only in etioplasts, and in the youngest greening stages, i.e. in the etio-chloroplasts of leaf segments of plants illuminated only for 2 h, and then treated in the dark for 4 h on the salt solution. Swelling was absent from plants greened for 4 h and having functionally active photosynthetic apparatus. Our physiological, biophysical and biochemical data showed that isosmotic PEG treatment had only mild effect on plastid structure and function.


Effect of salinity on plastids in various stages of etioplast-to-chloroplast transformation

Ions of saline soils may directly interact with the leaves of etiolated seedlings in agriculture and influence their greening

Experimental material and setup:
leaf segments of
- fully etiolated seedlings
- fully etiolated seedlings greened for 2 h
4 h
16 h
- fully light-grown seedlings

were floated on Petri dishes for 4 h in darkness on

additionally
- fully light-grown seedlings were floated on the same solutions on Petri dishes for 4 h in light

PEG-treatment induces no ultrastructural changes in the plastids, and mild physiological changes

Salt-induced swelling of the lumen is prevented after 4 h greening, when photosynthesis starts

Salt stress induces
- oxidative stress in all plastid types even in the absence of light
- decrease in the photosynthetic activity
- aggravated physiological changes and shrinkage of granum height in light treated chloroplasts as revealed by TEM and SANS

Etioplast structure is more susceptible to salt stress than that of plastids with photosynthetic activity

Ounoki et al. 2023

Figure 3. Graphical abstract summarizing the observations of ***Ounoki et al. (2023)*** *about etioplasts, and about non-photosynthetic etio-chloroplasts being most prone to the salt stress induced swelling of the (pro)thylakoids, while chloroplasts resisting it.*

Our data indicated that salinity causes lipid peroxidation and is inhibiting the greening of etiolated plants and Chl biosynthesis around the last steps of the process, e.g. after the reaction step of protochlorophyllide-chlorophyllide photoreduction catalyzed by NADPH:protochlorophyllide oxidoreductase (LPOR) enzyme. We have also observed that salinity strongly influenced the structure of the etioplast inner membrane system. Therefore, we summarized knowledge about the last steps of Chl biosynthesis and the role of membrane lipids and membranes in them (see **Solymosi and Mysliwa-Kurdziel, 2021**). A detailed overview of this particular reaction step, and a detailed insight into the molecular structure, dimerization or oligomerization, interactions and functioning of the LPOR enzyme are also important to understand how and at which points Chl biosynthesis may be inhibited by salinity or drought stress. Therefore, in collaboration with Swedish researchers, we performed detailed computational biochemistry studies on the enzyme structure (see **Hassan et al. 2021**). This is particularly interesting, as both under drought and salt stress, the biosynthesis of Chl precursors and Chl-protein complexes is stopped at an intermediary Chl(ide) spectral form with emission maximum around 680-682 nm, which may be attributed to disaggregated LPOR complexes after the so-called Shibata shift. The latter spectral shift reflects major reorganizations that are still not fully understood and characterized in the oligomeric structure of LPOR linked to the stromal surface of the PLB membranes.

As the swelling of the intrathylakoidal space of etioplasts was associated with treatment with high concentrations of NaCl solution (**Sóti et al. 2023**), but was not observed in fully developed chloroplasts (**Ounoki et al. 2023**), we decided to investigate the effect of various well-known chloroplast located ion channels on plastid ultrastructure. In collaboration with Prof. Cornelia Spetea's research group (University of Gothenburg, Sweden), as a continuation of our earlier works on single mutants (the K^+/H^+ antiporter KEA3, the Cl^- channel/transporter CLCe and the voltage-dependent Cl^- channel VCCN1) (Figure 4), we also generated and investigated double (*clcekea3*, *clcevccn1* and *kea3vccn1*, abbreviated later as *CK*, *CV* and *KV*, respectively) and triple (*clcekea3vccn1*, abbreviated as *KVC*) mutants of *Arabidopsis thaliana* lacking different thylakoid ion transporters/channels.

First, we characterized the chloroplasts of the above mutants under normal, unstressed conditions, and determined that the different transport components function independently in the adjustment of photosynthetic energy conversion to the light environment by maintaining the ionic balance within the chloroplasts during photosynthesis (**Dukic et al., 2019**). Surprisingly, our data indicated that the photosynthetic phenotype (a lower fluorescence level at the "I" step of the OJIP transient in the single, double, and triple mutants lacking CLCe) was strongly affected by the chloride concentrations present in tap water used for watering the plants. Tap water contained 7-135 mg L^{-1} Cl^- (up to 3.8 mM) and 13-57 mg L^{-1} Na^+ (up to 2.5 mM) as determined from IC or ICP-MS measurements, and the above phenotype was only reproduced in plants watered with distilled water. Our TEM analyses showed that the *vccn1* mutants had significantly increased granum diameter (490 nm) when compared with wild-type (WT) plants and other genotypes (460-470 nm) in the dark, and also in the light (440 nm vs. 400-420 nm).

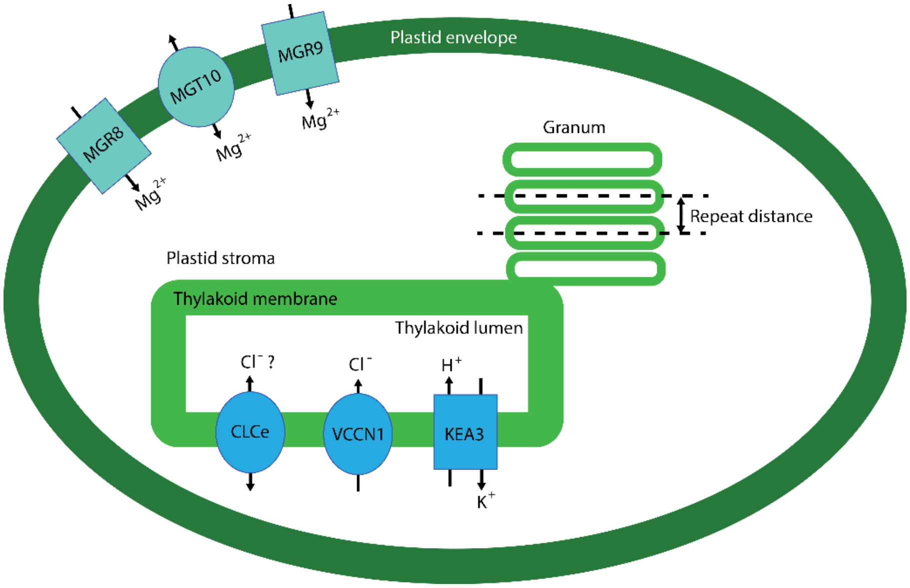


Figure 4. Scheme summarizing the chloroplast transporter mutants studied in the project, and indicating the granum repeat distance value (comprising two thylakoid membranes, one interthylakoidal space, and two halves of the thylakoid lumen).

We have shown that detached arabidopsis leaves of the *clce* mutant lines preincubated with 150 mM KCl for 30 min before measurements had restored the OJIP fluorescence phenotype (similar to WT characteristics) (**Dukic et al. 2019**). This proves that the thylakoids of plants lacking the CLCe channel have disturbed chloride homeostasis under low Cl^- availability.

Given our previous data, we decided to focus on optimizing the salt stress treatments of WT arabidopsis plants, as well as, single, double, and triple mutants of *kea3*, *vccn1* and *clce* (**Schubert et al. – manuscript**). Altogether this represented 8 different genotypes. As the expression of the various ion channels is altered during plant development, we analyzed how salt stress affected chloroplast structure and function at various developmental stages of the mutants (i.e. in the leaves of 1-, 4- and 8-week-old plants). Due to the high salt sensitivity of arabidopsis, we first applied 200 mM NaCl salt stress. However, we observed only slight changes, thus later we also performed experiments with higher concentration (300 mM NaCl) treatments. For comparison with our data obtained in wheat (see above – **Ounoki et al. 2023**), on selected genotypes and developmental stages we have also applied 600 mM NaCl stress for 4 h in the dark. With all biological repetitions and respective control measurements this represented a huge amount of data, which we are briefly summarizing below.

First, we checked the effect of a 30-min treatment with 200 mM NaCl (dissolved in distilled water) on detached leaves. We treated the leaves under white light (100 µmol photons m^{-2} s^{-1}), because light is thought to facilitate ion uptake of chloroplasts.

The Qy light and Qy dark values (i.e. actual and maximal quantum efficiencies of PSII, respectively) of the cotyledons of 1-week-old plants showed no significant difference among the WT plants and of the mutant plants, and the 30-min treatment with 200 mM NaCl also did not result in significant change in any of the studied genotypes. However, Qy and OJIP data on cotyledons were somewhat unconclusive because of big data scattering due to high biological variability. For instance, mutation also affected germination rates with *K*, *KV* and *KVC* plants having slower (delayed) germination, resulting in slightly smaller plants at week 1 and even at week 4.

In the case of the leaves of untreated, 4-week-old control plants, the Qy light values of the WT plants were significantly different (higher) only from those single, double or triple mutants that were lacking the CLCe channel (i.e. *C*, *CK*, *CV,* and *KVC*). The Qy dark value of the WT plants was significantly higher than that of *CV* and *KVC* mutants, but was similar in all other mutants. Application of 200 mM NaCl treatment to the 4-week-old detached leaves only induced a significant decline in the Qy light values of the WT and the *KVC* mutants, and the Qy dark values were significantly altered (i.e. decreased) by salt stress only in WT plants. This indicates complex compensation mechanisms and interrelation between the various channels, and indeed their involvement in salt stress induced alterations in photosynthetic activity. The CLCe channel plays an important role in influencing photosynthetic activity in control plants, but its loss may be at least partially compensated by excess salinity.

The Qy light and Qy dark values of the leaves of untreated 8-week-old WT plants differed significantly only from *CK*, *CV* and *KVC* plants (were higher in the WT) (Figure 5). The 200 mM NaCl treatment significantly decreased Qy light in *K*, *V*, *KV,* while increased it significantly in *KVC*. Qy dark was only negatively affected by the same salt stress treatment in the *KV* mutant (Figure 5). This complex pattern of photosynthetic alterations also points to the role of CLCe in the regulation of photosynthetic activity.

As described above and in **Dukic et al. (2019)**, due to the absence of CLCe, the OJIP transients of the *C*, *CK*, *CV* and *KVC* plants differed from WT (and other genotypes). The application of a 200 mM NaCl treatment restored the OJIP transient in these mutants, while the OJIP transients of other mutants were not affected by salt stress. The same tendency was observed in 1-week- and 4-week-old plants. In the case of 8-week-old plants the differences were less pronounced. Similar trends were observed when 300 mM NaCl was applied to the leaves. 300 mM NaCl treatment resulted in most cases in stronger effect on photosynthetic activity (significant decrease in the Qy dark and Qy light values in more genotypes).

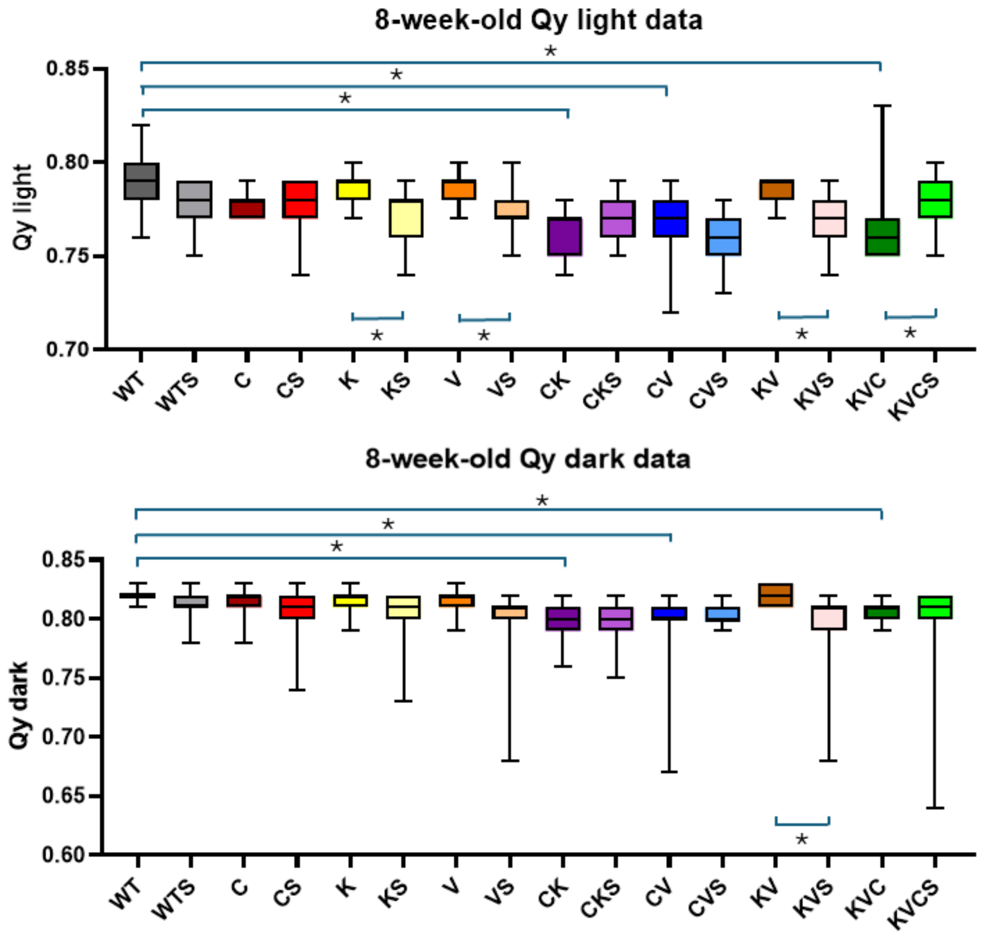


*Figure 5. Qy light and Qy dark values of 8-week-old Arabidopsis thaliana leaves of the studied genotypes with or without salt stress treatment ('S' after the genotype's name indicates 30-min-long 200 mM NaCl treatment in light.) Significant differences are indicated with *.*

We performed electron microscopic investigations only on selected genotypes and treatments. We observed no difference in the chloroplasts of the cotyledons of 1-week-old WT and *KVC* plants, however, 200 mM treatment induced swelling in the *KVC* plants (Figure 6). 300 mM NaCl treatment induced swelling of the intrathylakoidal space in all studied genotypes (WT, *C*, *K*, *V*, *KVC*). For comparison with our data in wheat (**Ounoki et al. 2023**), we also treated the cotyledons of WT and KVC plants with 600 mM NaCl:KCl (1:1) for 4 h in the dark. Starch disappeared from the chloroplasts, and concentric membranes and swelling of the thylakoids appeared in them as an effect of the high-concentration salt stress treatment.

On the other hand, 200 mM NaCl treatment did not induce any changes in the fully differentiated chloroplasts of 4-week-old WT and *KVC* plants (Figure 6) and of the chloroplasts of the 8-week-old leaves of all the 8 genotypes (not shown). This indicates that young chloroplasts of cotyledons are more sensitive to salt stress and the ion transport components may play a role in stabilising thylakoid ultrastructure.

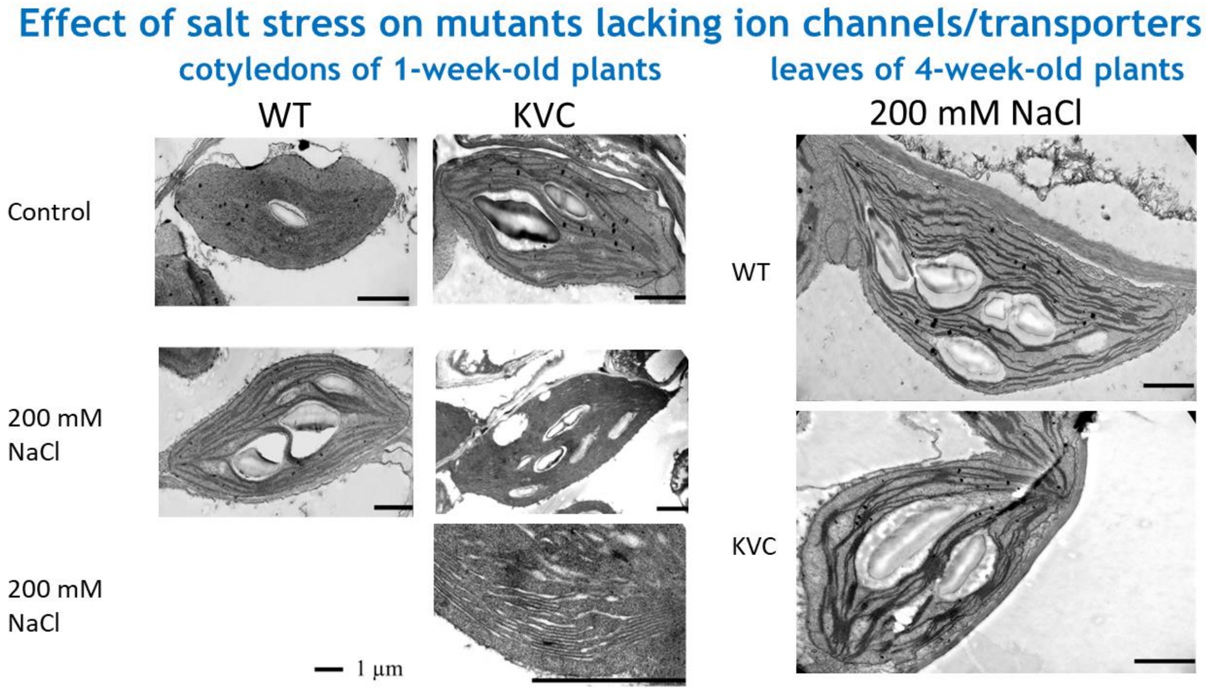


Figure 6. Effect of salt stress on chloroplast structure in mutants lacking ion channels/transporters studied in ***Dukic et al. (2019)*** *and* ***Schubert et al. - manuscript****.*

We also performed ultrastructural analyses on 1-week-old cotyledons of dark-germinated (etiolated) arabidopsis plants (Figure 7). Prominent nucleoid regions were observed in the mutants, and the mutants lacking KEA3 (and having delayed germination) had the less prothylakoid membranes. Interestingly, 300 mM NaCl treatment of the entire seedlings for 30 min in the dark resulted in the formation of vesicles at the PLB periphery in the *KVC* plants but not in WT (Figure 7). Treatment of the etiolated seedlings with 600 mM NaCl:KCl (1:1) for 4 h in the dark resulted in the loss of cellular integrity both in WT and *KVC* plants.

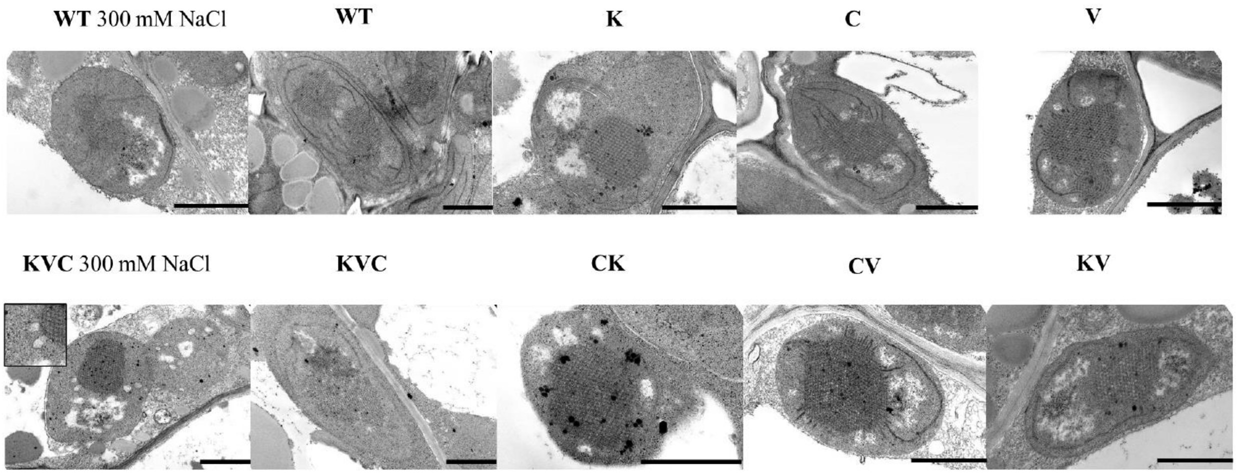
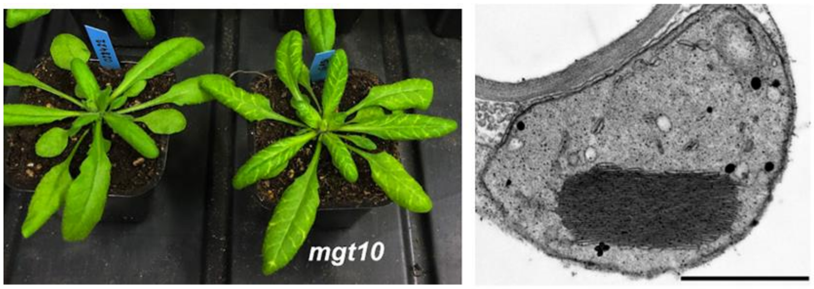


*Figure 7. Etioplasts of cotyledons of 1-week-old dark-germinated Arabidopsis thaliana seedlings. Salt stress was applied only to WT and KVC (****Schubert et al. - manuscript****).*

Magnesium plays an important role in photosynthesis (it is the central ion of the Chl molecule, but is also involved in the regulation of Chl biosynthesis, RuBisCO's activity, and the H$^+$ gradient across the thylakoid membrane). It is also thought to have an important role in thylakoid stacking. In this respect, it was interesting to investigate – in collaboration with Cornelia Spetea's research group – the role of envelope located MGR8 and MGR9 Mg^{2+} transporters and MGT10 Mg^{2+} channel in chloroplast structure and function in arabidopsis (Figure 4) (**Dukic et al. 2023**). We have shown that these Mg^{2+} transporting components have an important role in photosynthesis and in activating photoprotective mechanisms under strong light conditions. The *mgt10* knock-down plants had a chlorotic phenotype, i.e. yellow leaf venation (Figure 8). This channel is thought to be preferentially expressed in the bundle sheath cells and veins. In these and the surrounding mesophyll cells, we observed peculiar plastid structure in the *mgt10* plants: chloroplasts with macrograna and several small vesicles, but lacking stroma thylakoids were present (Figure 8). The non-chlorotic intervenal leaf regions contained regular chloroplasts.



Dukic et al. 2023

*Figure 8. Phenotype and plastid ultrastructure in mgt10 knock-down plants (**Dukic et al. 2023**).*

Sometimes these peculiar plastids could be located in the vicinity of normally developed chloroplasts within the same mesophyll cell next to the vein region. No such visible phenotype and ultrastructural difference was present in the *mgr8-2* and *mgr9-1* mutants. The Na$^+$ and K$^+$ contents of the chloroplasts of the single mutant lines were similar to those of WT plants, however, the Na$^+$ and K$^+$ contents of the *mgt10mgr8-2* mutant were significantly higher than those of WT plants (**Dukic et al. 2023**). Further experiments might be needed to elucidate whether MGR8 and MGR9 are Na$^+$-dependent transporters, and why the double mutants had altered Na$^+$ and K$^+$ homeostasis. Elemental analyses require relatively large amounts of plant material, and do not provide information about the potential intracellular or intrachloroplastic localization of the given ions. During plastid isolation, ion exchange processes may also occur. Therefore, in our other studies (e.g. dealing with small wheat leaf segments) we did not to perform them.

After our observations obtained in excised or detached leaves, we continued our studies in agriculturally more relevant experimental settings: we investigated the effect of salt and osmotic (drought) stress in a dicot medicinal crop, spearmint (*Mentha spicata* L. var. *crispa* „Moroccan") (**Ounoki et al. 2021**) to check if it may be cultivated in slightly salt contaminated areas. When compared to control plants treated with distilled water, low concentrations of NaCl (5 mM, 25 mM) did not affect vegetative reproduction, i.e. the formation of adventitious roots on freshly cut shoots immersed into the given solutions for 2 weeks. However, when a 50 mM NaCl treatment was applied, no root formation was observed, and leaves started to exhibit slight chlorotic and senescence symptoms, along with significant decline in the relative water and relative Chl contents (SPAD index) and photosynthetic activity (Qy light and Qy dark). Chloroplasts were electron-dense but grana were still distinguishable in the brownish regions of the 50 mM NaCl treated leaves, while swelling of the thylakoid lumen was only observed in the chloroplasts of wilted green regions of the same leaves (Figure 9D). The essential oil composition was not affected by any of these treatments, which indicates that spearmint may be cultivated in agricultural fields with less than 50 mM salt concentration in the soil without significant effect on its medicinal and culinary uses.

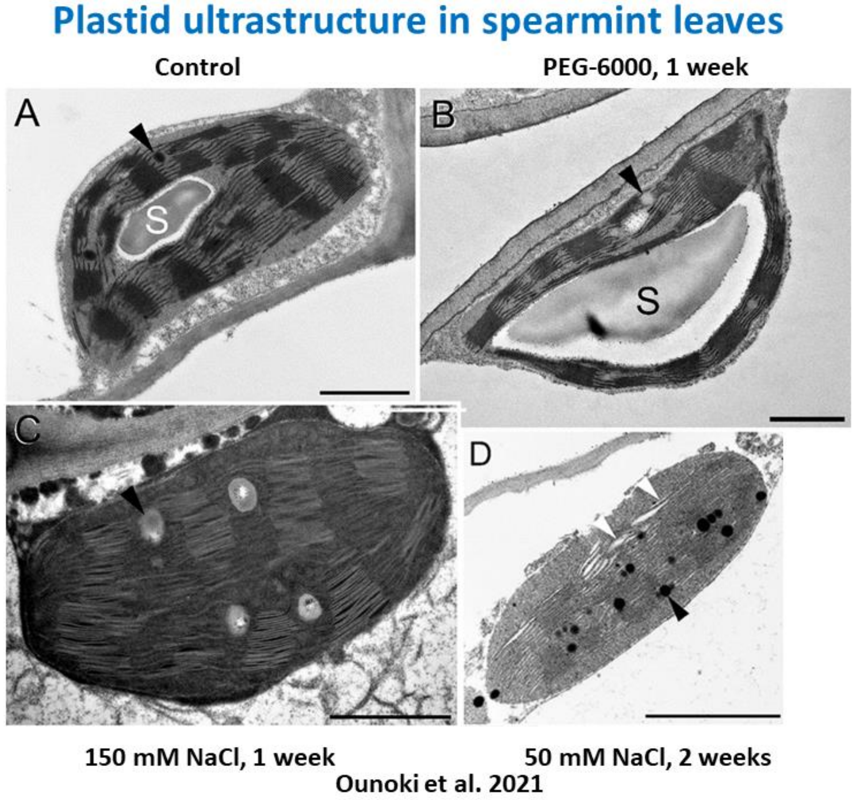


*Figure 9. Chloroplast ultrastructure in spearmint leaves (**Ounoki et al. 2023**).*

In another set of experiments, we investigated the effect of 1-week-long treatment with 150 mM NaCl solution and isosmotic PEG solution on rootless and previously rooted plants. Through these experiments we wanted to optimize a new potential model for SANS experiments and to demonstrate the role(s) of (adventitious) roots in stress tolerance. The leaves of rootless PEG- or salt treated plants became fully brownish and dry already after 1 week of treatment, with loss of photosynthetic activity and disorganization of the cells, but surprisingly the chloroplasts and their grana could be still distinguished in them. Rooted plants treated with isosmotic PEG solution had similar characteristics to control plants at the end of the 1-week-long period (Figure 9A and B), while rooted plants treated with 150 mM NaCl showed chlorotic symptoms, lower relative water and Chl contents (SPAD indices) and photosynthetic activity, disorganization of the Chl-protein complexes (as monitored by 77K fluorescence spectroscopy) and loss of the regularity of the chloroplast inner membranes. The stroma of these plastids became highly electron-dense, and electron-transparent spotted bodies (inclusions) appeared in them, while the size of plastoglobuli increased (Figure 9C, black arrowhead). SANS of intact rooted spearmint control plants showed typical scattering curve with a Bragg peak indicating granum periodicity. However, this peak disappeared in salt stressed plants, and RD values could not be calculated on the TEM micrographs either, in line with our observation that grana lost their regular structure. The essential oil composition of the rooted plants was not affected by salt or osmotic stress. Our data show that under natural conditions, intact spearmint plants are much better adapted to osmotic (drought) stress (i.e. PEG treatment), than to high soil salinity, and the observed chlorotic symptoms and observed ultrastructural and functional changes can be directly associated with salt stress.

While optimizing our SANS measurement protocols, we had several preliminary measurements with other plant species including pea (*Pisum sativum* L. „Rajnai törpe”) and *Aspidistra elatior* Blume plants. Some of these seemed to be inconclusive (pea), while in the case of *Aspidistra* we observed interesting SANS diffraction patterns in control plants. Therefore, we started to develop salt (600 mM NaCl) and isosmotic PEG treatments, coupled with physiological investigations. However, due to the limited availability of SANS infrastructure and beamtime, we could not perform those SANS measurements, and decided to focus on the above-mentioned studies dealing with salt stress in intact dicot plants (spearmint – **Ounoki et al. 2021**), monocot leaf segments (wheat – **Ounoki et al. 2023**) and a unicellular model organism, and on studies deciphering the effect of drought stress and subsequent recovery on the photosynthetic apparatus in two other crops (see below).

The changes induced by salt stress in the chloroplast thylakoid structure may be put into a larger, evolutionary context using different photosynthetic model organisms. Therefore, we wanted to extend our observations to unicellular organisms, in which the effect of salt stress may be more direct and less complex. *Euglena gracilis* Klebs cells contain a secondary endosymbiosis-derived chloroplast of green algal origin, and have different, lamellar organization of the thylakoid membranes. In collaboration with Bettina Ughy and her research group (HUN-REN Biological Research Center) we investigated the effect of salinity on the chloroplast structure (granum repeat distance – RD – as determined by TEM and also by SANS) and photosynthesis of *Euglena* cells (**Kanna et al. 2021**). In the context of this grant, it is relevant that we found significant shrinkage of the thylakoid lamellar structures upon salt stress with both methods. This is in line with our data obtained for the grana of salt stressed wheat plants, and proves that SANS and TEM data are in good correlation, and that SANS is suitable for *in vivo* measurements of thylakoid RD values.

Our comparative data using isosmotic PEG solutions as osmotic (or drought) stress revealed that it had a milder effect on the structure and metabolism of plastids than salt stress (**Ounoki et al. 2021, 2023, Sóti et al. 2023**). In order to study the effect of drought stress in an agriculturally more relevant setting, we decided to perform analyses in soil-grown plants with various drought and desiccation tolerance.

First, in collaboration with Katya Georgieva’s lab (Bulgarian Academy of Sciences, Bulgaria) we chose *Haberlea rhodopensis* Friv., a homoiochlorophyllous vegetative desiccation tolerant plant which can retain the structural integrity of its chloroplasts even under almost full desiccation (e.g. leaf relative water content decreased to 5-10%), and then regenerate cell structure and photosynthetic activity relatively quickly. In our comparative structural investigations we have shown that the formation and then the disappearance of small secondary vacuoles are involved in the dehydration and rehydration processes, respectively (Figure 10), and have also described minor differences during the regeneration of the plants after freezing (frost)- or drought-induced desiccation (**Mihailova et al. 2022**). Chloroplast ultrastructure was retained intact in both cases and no swelling of the intrathylakoidal space was observed. The granum RD values (as monitored by TEM) were significantly lower only under drought-induced desiccation and increased upon rehydration (Figure 10). This indicates shrinkage, i.e. the loss of water from the intrathylakoidal (lumen) and interthylakoidal aqueous phases of grana upon dehydration. Unfortunately, we could not get SANS signals from intact *Haberlea rhodopensis* leaves during our preliminary experiments at the Paul Scherrer Institute (Switzerland).

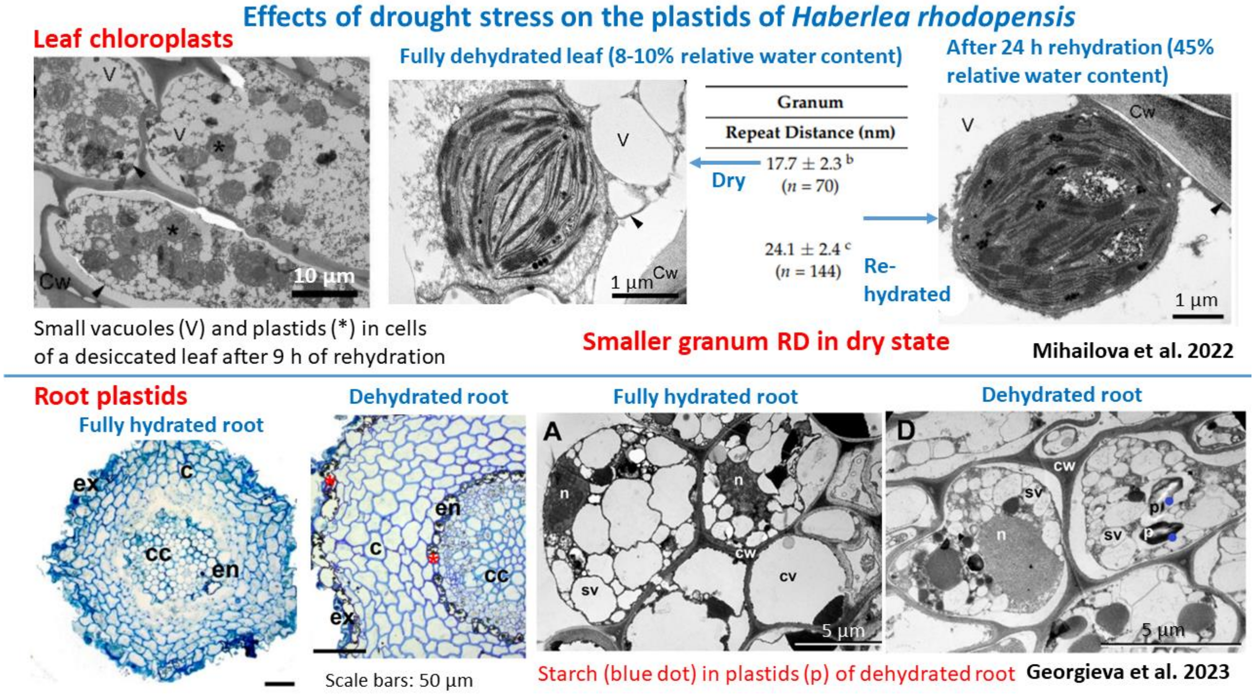
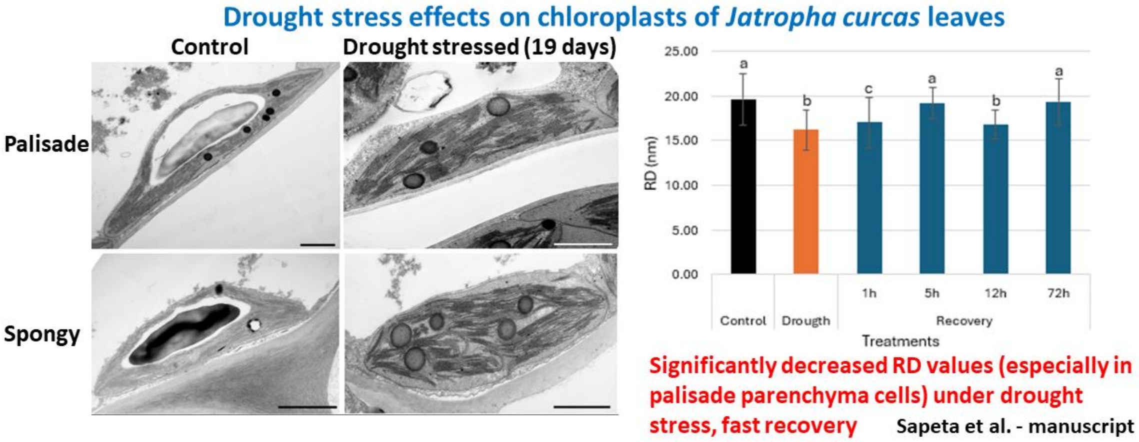


*Figure 10. Effects of desiccation (dehydration) and subsequent rehydration on plastids structure in Haberlea rhodopensis leaves and roots (**Mihailova et al. 2022, Georgieva et al. 2023**).*

Most data – especially (ultra)structural analyses – studying the effect of desiccation and rehydration in vegetative desiccation tolerant plants are dealing with leaf chloroplasts. Therefore, in the following study we focused on metabolic and structural changes observed during dehydration of the roots (**Georgieva et al. 2023**). During dehydration, extensive buildup of dense intracellular accretions was observed in the exodermis (ex) and endodermis (en) cell layers of the root cortex (Figure 10). Heterochromatinous nuclei and several small vacuoles were present in both cortex (c) and central cylinder (cc) cells of the fully hydrated roots, but more vacuoles appeared upon dehydration, especially in the cortex. Starch accumulation, observed in amyloplasts, was evident in the central cylinder cells of desiccated roots (Figure 10D).

In order to better understand the effect of drought stress on chloroplast structure, we also investigated a stem succulent crop (*Jatropha curcas* L.) and a particularly drought-tolerant ornamental crop showing leaf succulence (*Ctenanthe setosa* /Roscoe/ Eichler). In both cases, we could monitor the changes of the granum RD values using TEM and also SANS *in vivo*.

The effect of drought stress and subsequent recovery on the growth, physiology (e.g. photosynthetic activity) and transcriptome of *Jatropha curcas* leaves is already well described (Sapeta et al. 2013, 2016). For drought stress treatment, 46-day-old *Jatropha curcas* plants were either normally watered (control) or not watered for 19 days (**Sapeta et al. – manuscript**). On day 19, freshly excised leaf pieces of control, drought-stressed, drought-stressed but subsequently rewatered plants were measured by SANS (without any D_2O infiltration) and were also simultaneously sampled for TEM. With both methods we found that RD values in drought-stressed plants were smaller than in the control (indicating potential shrinkage of the grana), however, after rewatering of the plants in their pots, the RD values were recovered within a few hours (Figure 11). TEM analyses showed increased number and size of plastoglobuli, and disappearance of starch in the drought-stressed plants, but no visible alterations in the thylakoid membranes. We found significant differences in the RD values of grana of plastids located in palisade and spongy parenchyma cells under drought stress and recovery, with the latter always having larger RD values. Important differences were also observed in the Chl content (20% decrease), Chl a/b ratio, in the activity (PSII quantum yield, excitation energy allocation) and organization of the photosynthetic apparatus (decrease in PSII supercomplexes and increase in PSI complexes containing LHCII due to state transition). However, these changes were restored during recovery.



Significantly decreased RD values (especially in palisade parenchyma cells) under drought stress, fast recovery

Sapeta et al. - manuscript

*Figure 11. The effect of drought stress on chloroplast structure of Jatropha curcas leaves (**Sapeta et al. - manuscript**).*

In the case of *Ctenanthe setosa* leaves, our conventional biochemical, biophysical, physiological and (ultra)structural analyses indicated no significant changes in the Chl content, 77K fluorescence emission spectra, Qy dark values (maximal quantum efficiency of PSII), chloroplast ultrastructure, whilst Qy light values (the actual quantum efficiency of PSII) decreased, the amounts of PSI-LHCII complexes and PSII monomers declined and that of PSII supercomplexes increased in the thylakoids upon 40-49 days of drought stress (water withdrawal) (Figure 12) (**Hembrom et al. – manuscript**). The adaxial hypodermis layer and thus the leaf thickness, as well as chloroplast length and granum repeat distance values decreased upon drought stress as shown by light microscopy, TEM, and SANS, respectively. SANS provided a unique insight into biological variability of granum RD values among the different leaves of the same plant. Furthermore, placing one leaf of the drought-stressed intact plant in the neutron beam and rewatering its pot there, enabled us for the first time to monitor the *in vivo* fast structural recovery of the granum structure of drought stressed leaves which happened already 18 h after rewatering, while functional and biochemical recovery only occurred on a longer time scale (Figure 12).

Ctenanthe setosa plants were unique in retaining their chloroplast structure and function even under long-term drought stress, therefore, we performed transcriptomic analyses of control and drought stressed leaves in order to understand the molecular mechanisms involved in drought tolerance. Unfortunately, we encountered technical problems with RNA isolation from the samples and also with the bioinformatics analyses, so further experiments would be necessary to get reliable information on changes in the transcriptome of *Ctenanthe setosa* upon drought stress.

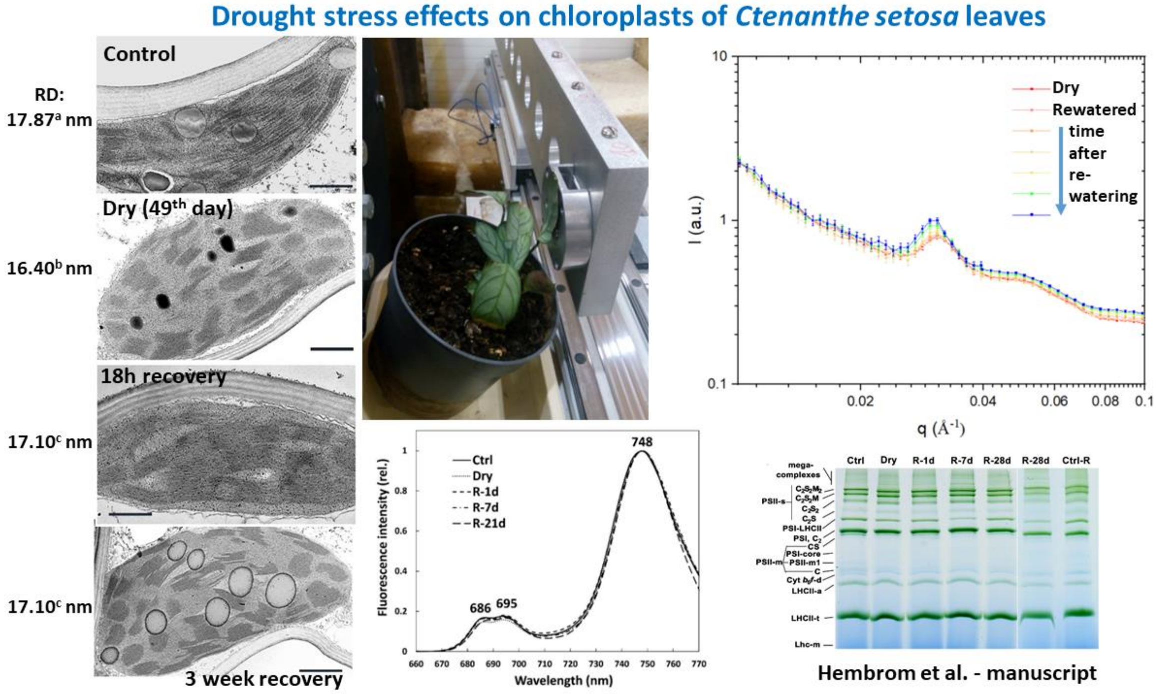


*Figure 12. Effects of drought stress on chloroplasts of Ctenanthe setosa leaves (**Hembrom et al. - manuscript**).*

To get information about the effect of drought stress on important drought-sensitive crops like wheat (*Triticum aestivum* L. cv. Szecsuán) and barley (*Hordeum vulgare* L. cv. Morex), we exposed 3-week-old wheat and barley plants to water withdrawal for 2 weeks, in collaboration with László Sági (HUN-REN ATK/Martonvásár) (**Horváth et al. – manuscript**). We observed significant changes upon drought stress: decline in the leaf relative water content (to approx. 30 and 20% RWC), number of leaves, photosynthetic parameters (Qy dark and F_v/F_m values, alterations in the OJIP curves) after 2 weeks of drought stress. Light and electron microscopic investigations revealed strong damage to leaf cell and chloroplast integrity of the drought stressed plants (Figure 13). In these cells we observed chloroplasts with swollen thylakoid membranes. In other studied cultivars and genotypes with less water loss and physiological alterations, the swelling was absent.

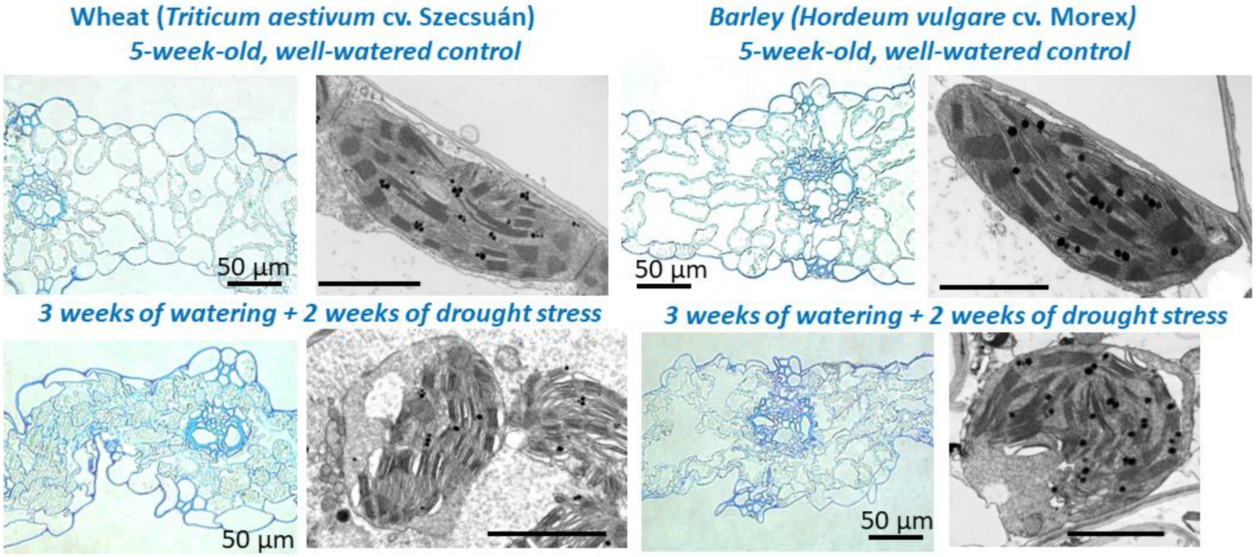


*Figure 13. Effect of drought stress on wheat and barley leaves (**Horváth et al.** - manuscript).*

Conclusions

Using isosmotic PEG treatment we could model the osmotic component of salt stress. In our experimental settings, osmotic stress alone did not induce important physiological or structural alterations. Even severe drought stress when applied under agriculturally relevant settings (using water withdrawal in case of potted plants) did not result in the swelling of the thylakoids in vegetative desiccation tolerant and succulent plants, but resulted in a reversible shrinkage of the grana as monitored both by TEM and *in vivo* SANS measurements. The swelling of the thylakoid lumen was observed only after extreme drought stress inducing irreversible changes and loss of cellular integrity in drought sensitive crops. In case of the desiccation tolerant *Haberlea rhodopensis*, the structure of leaf chloroplasts was less affected by desiccation than that of root plastids.

The ionic component of salt stress, especially high concentrations of Na^+ had the most detrimental effect on etioplast structure (inducing peculiar swelling of the intrathylakoidal space), inhibiting the greening, Chl biosynthesis, the development of the photosynthetic apparatus and thus the etioplast-to-chloroplast transformation in wheat. Potassium salts and $CaCl_2$ had milder effect on the plants, and thus may represent more environment-friendly ice-melting compounds in winter, as they can be expected to do less harm to the leaves developing from seeds germinating in the soil under natural or agricultural conditions. Etioplasts, young etio-chloroplasts with no photosynthetic activity and chloroplasts of young developmental stages (e.g. cotyledons) were more sensitive to salt stress than fully differentiated chloroplasts. The various studied chloroplast ion transport components influenced the photosynthetic activity and plastid ultrastructure in a very complex manner. Depending on the applied treatment and on the species or cultivar used, salt stress induced either shrinkage of grana or the loss of their regularity in chloroplasts, but no swelling, except for long-term severe salt stress resulting in the irreversible wilting of the leaves**.** However, granum structure was even well retained in fully dry, brown leaf regions, indicating the importance of preserving this structure of the photosynthetic apparatus under stressful conditions. To the best of our knowledge, this was the first time when SANS was used to study the biological variability of granum ultrastructure and its membrane dynamics *in vivo* in intact plants and under agriculturally relevant stress conditions.

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Publications related to the project[3]

12 published papers, 1 book chapter, 5 manuscripts, 27 conference abstracts, 2 BSc theses, 3 MSc theses, participation of 4 students at various TDK competitions, 4 PhD topics related to the project, 13 science outreach publications (including podcast, TV interview and press releases)

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[3] Names of participants of the OTKA FK124748 project are underlined (including also students supervised by the PI and involved in research related to the grant)

Published peer-reviewed papers (12, cumulative impact factor: 62.465):

1. Ounoki R, Sóti A, Ünnep R, Sipka G, Sárvári É, Garab G, Solymosi K# (2023) **Etioplasts are more susceptible to salinity stress than chloroplasts and photosynthetically active etio-chloroplasts of wheat (*Triticum aestivum* L.).** *Physiologia Plantarum* **175** (6): e14100. doi: 10.1111/ppl.14100. #Corresponding author (IF2022: 6.4, Scimago Rank: D1)
2. Sóti A, Ounoki R, Kósa A, Mysliwa-Kurdziel B, Sárvári É, Solymosi K# (2023) **Ionic, not the osmotic component, is responsible for the salinity induced inhibition of greening in etiolated wheat (Triticum aestivum L. cv. Mv Béres) leaves – a comparative study.** *Planta* **258**: 102. doi:.10.1007/s00425-023-04255-4 #Corresponding author (IF2022: 4.3, Scimago Rank: Q1)
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14. Solymosi K, Abdelkader AF, Böddi B, Aronsson H: **Differences in plastid development of salt tolerant and susceptible wheat cultivars under unstressed conditions and under salt stress.** *Manuscript to be submitted in May*
15. Sapeta H*, Hembrom R*, Markó M, Nagy G, Sárvári É, Oliveira M, Solymosi K: **The reversibility of drought stress induced ultrastructural and physiological alterations in *Jatropha curcas* leaves.** *Manuscript in preparation, * equal contribution*
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19. Schubert HF, Sóti A, Hembrom R, Ounoki R, Enkhbileg R, Dukic R, Spetea C, Solymosi K: **Effect of salt stress on etioplast and chloroplast membranes of thylakoid transporter mutants of Arabidopsis thaliana.** (2 posters) Magyar Biofizikai Társaság XXIX. Kongresszusa, Budapest, 2023. 08. 28-31., Program és absztrakt könyv, Derényi I, Grama L, Solymosi K (eds.), ISBN 978-615-01-8688-7, pp. 120.
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31. Ágh F, Ounoki R, Hembrom R, Szögi-Tatár B, Böszörményi A, Solymosi K: **Effect of salt and osmotic stress on spearmint (*Mentha spicata* var. *crispa* 'Moroccan') plants.** (presentation)

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32. Ounoki R, Sóti A, Kósa A, Mysliwa-Kurdziel B, Solymosi K: **Comparative analysis of the effects of salt and osmotic stress on dark and light grown wheat**. (presentation) In: Magyar Biofizikai Társaság Fotobiológiai Miniszimpóziuma, Budapest, 2020. 12. 18. online symposium.
33. Horváth S, Hembrom R, Sági L, Solymosi K: **Szárazságstressz hatásának összehasonlítása újonnan előállított búza alloplazmás árpa növényekben és szülői vonalaikban**. (presentation) In: Magyar Biofizikai Társaság Fotobiológiai Miniszimpóziuma, Budapest, 2020. 12. 18. online symposium.
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40. Ounoki R, Hembrom R, Ágh F, Szögi-Tatár B, Böszörményi A, Solymosi K: **Comparison of the ionic and osmotic effects of salt stress on spearmint (Mentha spicata L. var. crispa).** (poster) In: 11th Scandinavian Plant Physiology Society PhD Student Conference, Turku, Finland, 2020. 09. 02-04.
41. Ounoki R, Hembrom R, Solymosi K: **Studies on the effect of salt and drought stress on chloroplast ultrastuctures in various crops.** (presentation - 581.132:631.52) Agriculture-2020, Online Conference of the Nikolaev State Agricultural Experimental Station of IIA of NAAS of Ukraine, Nikolaev, Ukraine, 2020. 04. 10.

42. Mysliwa-Kurdziel B, Sóti A, Kósa A, Solymosi K: **Comparative analyses of plastid structure and greening under drought and salt stress** (p.31, poster and oral presentation) In: 3rd Agriculture and Climate Change Conference, Budapest, 2019. 03. 24-26.
43. Schmidthoffer I, Solymosi K, Skribanek A: **Drought tolerance of spring barley varieties** (poster, p.40) In: 3rd Agriculture and Climate Change Conference, Budapest, 2019. 03. 24-26.
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52. Nagy G, Zsiros O, Solymosi K, Ünnep R, Porcar L, Peters J, Finazzi G, Garab G: **Adaptation mechanisms of photosynthetic machineries as revealed by neutron scattering**. (presentation) In: Synergies with Hungary – ILL workshop on recent progress in the Hungarian neutron user community, Budapest, 2018. 11. 07.
53. Solymosi K, Ünnep R, Almásy L, Markó M, Nagy G, Zsíros O, Garab G: **Invasive and noninvasive structural analysis possibilities of plastids under various stress conditions**. (presentation, p.

14.) In: Program and Abstracts of the Meeting of Hungarian Photosynthesis Researchers, Mátrafüred, 2018. 11. 07-09.Garab G, Janda T, Solymosi K, Darkó É, Pál M (eds), ISBN 978-615-00-3750-9.

54. Sóti A, Horváth S, Solymosi K: **Structural alterations of plastids in ion channel mutants and under salt stress.** (poster – p. 129) In: *XXIV. Növénynemesítési Tudományos Nap,* Abstract Book, MTA, Hungary, Karsai I, Polgár Z (eds.) ISBN 978-615-00-1469-2.
55. Brillouet J-M, Aronsson H, Solymosi K**: The ins and outs of plastid membrane trafficking under normal development and stress conditions.** (poster, p. 95) In: 8th Regional Biophysics Conference: Book of Abstracts, Slovenian Biophysical Society, Zrece. Majaron H, Cotar P, Koren M, Zima NG (eds.).

BSc theses related to the project (2):

56. Sóti A (2018) **The effect of salt stress on plastid structure and function** (in Hungarian with English abstract). BSc thesis, Eötvös Loránd University. (Supervisor: Solymosi K)
57. Horváth S (2019) **The effect of drought stress on chloroplast structure** (in Hungarian with English abstract). BSc thesis, Eötvös Loránd University. (Supervisor: Solymosi K)

MSc theses related to the project (3):

58. Sóti A (2020) **Comparative analyses about the effect of salt and osmotic stress during the greening of wheat plants**. MSc thesis, Eötvös Loránd University. (Supervisor: Solymosi K)
59. Horváth S (2021) **Effect of drought stress on wheat alloplasmic barley plants and their parental lines.** MSc thesis, Eötvös Loránd University. (Supervisor: Solymosi K)
60. Schubert HF (2022) **The effect of salt stress on the structure of etioplasts and chloroplasts in various thylakoid ion transporter mutants of arabidopsis (Arabidopsis thaliana).** MSc thesis, Eötvös Loránd University. (Supervisor: Solymosi K)

PhD theses related to the topic (4):

61. Ounoki R (2024) **Structural and functional changes induced by salt stress in crop plastids of various developmental stages**. Eötvös Loránd University, Doctoral School of Biology, Experimental Plant Biology Doctoral Program, Stipendium Hungaricum Scholarship. Thesis submitted in January 2024. (Supervisor: Solymosi K)
62. Hembrom R: ***The effect of drought stress on the photosynthesis of crops***. Eötvös Loránd University, Doctoral School of Biology, Experimental Plant Biology Doctoral Program, Stipendium Hungaricum Scholarship, 2019-2023 – ongoing (COVID-related extension of the scholarship until April 2024). (Supervisor: Solymosi K)
63. Sóti A: ***The effect of salt stress on chlorophyll synthesis and greening***. Eötvös Loránd University, Doctoral School of Biology, Experimental Plant Biology Doctoral Program, 2020-2024 – ongoing. (Supervisor: Solymosi K)
64. Schubert HF**: *The role of thylakoid transport components in stress tolerance.*** *E*ötvös Loránd University, Doctoral School of Biology, Experimental Plant Biology Doctoral Program, 2022-2026 – ongoing. Since 2024 in the frame of KDP. (Supervisor: Solymosi K)

TDK (Scientific Student Circle) participation (4 students, presenting at 8 TDK and OFKD competitions):

65. Sóti A (2019) **Comparative analyses about the effect of salt and osmotic stress during the greening of wheat plants**. Student’s Scientific Circle Competition (TDK), Eötvös Loránd University. (Supervisor: Solymosi K)
66. Ágh F (2020) **Studies on the effect of salt and osmotic stress in spearmint (Mentha crispa ‘Moroccan’) plants.** Student’s Scientific Circle Competition (TDK), Semmelweis University, 3rd Place in Pharmaceutical Sciences A. (Supervisors: Böszörményi A and Solymosi K)
67. Horváth S (2020) **Effect of drought stress on wheat alloplasmic barley plants and their parental lines.** Student’s Scientific Circle Competition in Biology (BTDK), Eötvös Loránd University, 2020, 1st Prize in the “Cell Biology and Immunology” Session; and National Student’s Scientific Circle Competition in Biology (OTDK in Biology), Kolozsvár, 2021, 1st Prize in the “Plant Physiology 2” Session. (Supervisor: Solymosi K)
68. Horváth S (2020) **Comparison of the effects of drought stress in newly bred wheat alloplasmic barley plants and their parental lines**. Student’s Scientific Circle Competition in Environmental Sciences (KTDK), Eötvös Loránd University, 2020, 1st Prize in the KTDK Session. (Supervisor: Solymosi K)
69. Schubert HF (2021) **The effect of salt stress on the structure of etioplasts and chloroplasts in various thylakoid ion transporter mutants of arabidopsis (Arabidopsis thaliana).** Student’s Scientific Circle Competition in Biology (BTDK), Eötvös Loránd University, 4th Prize, 2021; Student’s Scientific Circle Competition in Environmental Sciences (KTDK), Eötvös Loránd University, 2021; and National Higher Education Environmental Sciences Student Conference (OFKD), 2022. (Supervisor: Solymosi K)

Science outreach (publications, press releases, podcasts) (13)**:**

70. https://www.youtube.com/watch?v=Uj5tbXLisf4&t=976s
71. http://novumtv.hu/adasaink/2024-02-05
72. https://www.eurekalert.org/news-releases/1007220
73. https://biologia.elte.hu/en/content/for-the-benefit-of-our-plants-it-makes-a-difference-what-kind-of-salt-we-use-on-the-roads-in-winter.t.37094
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75. https://www.eurekalert.org/news-releases/999970
76. https://biologia.elte.hu/en/content/magnesium-deficiency-is-detrimental-to-plants.t.35675
77. https://biologia.elte.hu/content/a-magnezium-hianya-a-novenyeknek-sem-jo.t.35675
78. Élet és Tudomány, LXXVIII. évfolyam, 2023/47. lapszám, **’Só show**’ címlapfotó (tibeti konyhasó kristályok polarizációs mikroszkópos képe, 1473. o.), és ‘**Nem mindegy, mit használunk jégtelenítésre! Sóból is megárt a sok**’, 1478-1480. o., 2023.11.24.
79. Élet és Tudomány, LXXVIII. évfolyam, 2023/37. lapszám, **’Különleges színtestek – a magnézium hiánya a növényeknek sem jó’**, 1174-1175. o., 2023.09.15.
80. Élet és Tudomány, LXXVIII. évfolyam, 2023/33. lapszám, **’Amit a gyökerek és a levelek mesélnek – Így él túl az Orfeusz-virág’**, 1043-1045. o., 2023.08.18.
81. Élet és Tudomány, LXXV. évfolyam, 2020/19. lapszám, **’Gyógynövények hatóanyagösszetételét befolyásoló tényezők – A rozmaring aromája’**, 596-597. o., 2020.05.1.
82. Élet és Tudomány, LXXIII. évfolyam, 2018/25. lapszám, **’Különleges membránszerveződések – Csőhálózatok a sejtben’**, 774-776. o., 2018.07.22.