Final Thesis

Analysis of salt tolerance in three widely used accessions of *Arabidopsis thaliana*: a photosynthetic approach

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Salt stress is one of the major problems in the present world’s agriculture. Plants encounter drought stress even in the availability of water because of osmotic imbalance in the cell due to excess salts. Plants avoid water uptake, which in turn decreases the photosynthetic activity. In this work, we measured the effect of salt stress on three accessions of Arabidopsis thaliana (Columbia (Col-0), Landsberg erecta (Ler-0), Wassilewskija (Ws-4)) by subjecting the plants to stress with 0-150 mM NaCl followed by recovery. The impact of the stress was clearly observed in all three accessions during stress and recovery period. Chlorophyll content in leaves decreased with increasing salt concentration. Proline levels increased during salt stress conditions. Non-photochemical quenching and PSII activity slightly decreased under stress conditions. Salt treated plants showed slow acidification of lumen with delayed Non-photochemical quenching in recovery phase. Ler-0 was the most sensitive ecotype to salt stress followed by Ws-4 and Col-0.

Keyword: Salt tolerance, photosynthetic activity, chlorophyll fluorescence, Arabidopsis ecotypes.
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1. ABSTRACT

Salt stress is one of the major problems in the present world’s agriculture. Plants encounter drought stress even in the availability of water because of osmotic imbalance in the cell due to excess salts. Plants avoid water uptake, which in turn decreases the photosynthetic activity. In this work, we measured the effect of salt stress on three accessions of *Arabidopsis thaliana* (Columbia (Col-0), Landsberg erecta (Ler-0), Wassilewskija (Ws-4)) by subjecting the plants to stress with 0-150 mM NaCl followed by recovery. The impact of the stress was clearly observed in all three accessions during stress and recovery period. Chlorophyll content in leaves decreased with increasing salt concentration. Proline levels increased during salt stress conditions. Non-photochemical quenching and PSII activity slightly decreased under stress conditions. Salt treated plants showed slow acidification of lumen with delayed Non-photochemical quenching in recovery phase. Ler-0 was the most sensitive ecotype to salt stress followed by Ws-4 and Col-0.

**Key Words:** salt tolerance, photosynthetic activity, chlorophyll fluorescence, *Arabidopsis* ecotypes.

2. Abbreviations:
   ATP - Adenosine triphosphate
   Chl - Chlorophyll
   ETR - Electron Transport Rate
   $F_0$ - Minimal fluorescence from dark adapted leaf
   $F_m$ - Maximal fluorescence from dark adapted
   PAR - Photosynthetic Active Radiation
   PSI - Photosystem I
   PSII - Photosystem II
   NPQ - Non-Photochemical Quenching
   $Q_A$ - Primary electron acceptor of PSII.
   VDE - Violaxanthin De-Epoxidase.
3. Introduction
Some environmental stresses adversely affect plant growth, crop yield. Salinity is one of major environmental stresses encountered by terrestrial plants. High levels of salts are located in the regions near to sea shore and estuaries. In contrast, salinity is also caused by wash away of natural salt deposits into adjoining areas by irrigation waters. Irrigation water contains salt concentrations lower than 2.5 mM (Zeiger and Taiz, 2004). Plants growing in semi-arid and arid regions often encounter poor irrigation with higher salt concentrations. Electrical conductivity rises with increasing salt concentrations and lowers the osmotic potential in cells (Zeiger and Taiz, 2004).

3.1 Arabidopsis thaliana as a model plant
Arabidopsis thaliana is a small flowering plant of Brassicaceae family, having genome with well-studied genetic resources for modern plant biology. Despite of fact that Arabidopsis being true glycophyte, many salt tolerance studies are done to understand the adaptive responses of the plant. Variation among the physiological traits in all the natural accessions has favoured the research in identifying the novel salt tolerance mechanisms in commonly used Arabidopsis accessions like Columbia (Col-0), Landsberg erecta (Ler-0), Wassilewskija (Ws-4) and to compare the mechanisms with halophytes such as Thellungiella halophila (Stepien and Johnson., 2009).

Three Ecotype in Focus

![Three Arabidopsis accessions under focus for the current study.](image_url)

3.2 Photosynthesis in plants
The chloroplast thylakoid membrane is the site for the light-dependent reactions in photosynthesis in plants. This photosynthetic membrane of thylakoids consists of four major protein complexes (LHCII-PSII, cytochrome b6f, PSI-LHCI and ATP synthase), capable of absorbing sunlight, exciting NADP+ by transferring...
the electron obtained from water breakdown and ATP synthesis. Plants can acclimatize to variations in light environment, composition, function and arrangement of photosynthetic apparatus as a response to long term changes. But, the intermediate components of the photochemistry can kill the plants as the energy generate in the thylakoid membrane by the LHC during the water breakdown is the source for these intermediates. Energy transfer within the proteins of the membrane was solution for the problems caused by products (Merchant and Sawaya, 2005).

Generally energy from sunlight is first taken by active photosynthetic pigments of leaf. This energy is not completely utilized in driving photochemistry. The light energy absorbed by the exited chlorophyll in leaf undergoes four mechanisms:

1) Chl fluorescence (re-emission of photon to return to ground state),
2) Non-photochemical quenching (Converting absorbed energy and emitting in the form of Heat),
3) Energy transfer (Chl transfers its energy to other chl molecule)

3.3 Soil salinity and photosynthesis

In the current world wide agriculture salinity is the major factor impairing the productivity. According to Martinez-beltran and Manzur (2005) total global salt-affected soils make up to 831 million hectares. This has increased after the intrusion of salt by natural disasters like tsunami. It is very important to note that salt stress in field crops is getting energized with irrigation process and increasing human activities to spring up the nutrient content (addition of inorganic fertilizers). Salt stressed plants show a decreased photosynthetic efficiency, but the underlying mechanism is not completely known. There are grounds for the increasing salinity and changes occurring in plants that have undergone different mechanisms to tolerate the increasing salt concentration. Multiple factors are implicated as the reason for decrease in photosynthetic performance during increasing salinity. Stomatal closure with decreased partial pressure of CO2 and non-stomatal factors such as decreased protein and pigment concentrations due to ionic changes are also included as one of the reasons. (Bethke and Drew, 1992; Nagy and Galiba, 1995; Sibole et al. 1998; Kolcheskii et al.1995). Generally salt stress causes the activation of Salt Overly Sensitive pathway (SOS pathway) which involves in removal of excessive salts present in plant cell. As photosynthetic performance of plants under salt stress we did not concern about the plants response in removing the excessive salts during and after stress.

We all did learn from our very childhood that photosynthesis requires water uptake to undergo light reactions in chloroplasts. As the breakdown of water molecule takes place at the PSII (Pushkar et al., 2007). The salinity activity is likely to be associated with PSII and protection these pigments form ion toxicity is important as chlorophyll pigments are the only source of carbon fixation and biomass production in plants.
3.4 How do plants protect themselves from this stress?
Osmoprotectants like amino acids (proline, glycine-betaine), disaccharides (trihalose) and sugar alcohols mannitol and sorbitol are produced and accumulated in salt treated cells, as salt-stress response strategy. These are expected to reduce the osmotic potential in stressed cells protecting various cell structures and proteins during stress (Sahi et al., 2006). Hyperosmotic stress is observed in many conditions in plants such as dehydration caused during salinity, drought and cold conditions initiates the biosynthesis of Abscisic acid (ABA) and ABA activates set of genes induced by drought condition. Increased concentration of ABA inhibits the seed germination (Kornneef et al., 1984).

In general proline synthesis is related with rapidly dividing tissues and embryo development (Lehmann et al., 2010). Proline is highly found in pollen and seeds. Accumulation of proline in organs like leaf is one of the wide physiological responses reported after various stress conditions Heat and Cold Temperatures, heavy metal toxicity and atmospheric pollution are few abiotic stresses (Bohnert et al., 1995; Sleator and Hill., 2002).

3.5 Chlorophyll fluorescence and Electron Transport Rate
Chlorophyll fluorescence is the light re-emitted by the plant chlorophyll after the photosynthetic process. Chl fluorescence is a rapid and non-intrusive tool used to screen the amount of tolerance. Previous studies on salt stress using chl fluorescence was done in crop plants Barley, wheat, sorghum (Belkhodja et al., 94; Zair et al., 2003; Netendo et al.,2004) and Model plants like Arabidopsis and Thellungiella (Stepien and Johnson, 2009).

3.6 Previous studies in salt stress
Salt stress and salinity are the main area of concern in past decade which resulted in discovery and understanding plants response to stress. Discovery of mechanisms involving SOS pathway by Exchange of Salts, Na+/H+ transporters (AtNHX1) (Qiu et al., 2003; Apse et al.,2003; Verslues et al.,2007; Gong et al., 2004).

4 Aims of the project and experimental hypothesis
4.1 Aim
Specific aim was to investigate salt tolerance in three selected accessions of Arabidopsis. This was performed by comparing the photosynthetic performance during and after stress treatment

4.2 Hypothesis
Distinct response in relation to salt stress of these three accessions was expected, and to be reflected in distinct photosynthetic performance.
5.0 Materials and methods
5.1 Plant material and growth conditions
Seeds of Arabidopsis ecotypes (Col-0, Ler-0 and Ws-4) were obtained from Arabidopsis Biological Resource Centre (ABRC, Ohio (USA)). The seeds were disinfected using 2% sodium dodecyl sulphate (SDS), 70% ethanol (v/v) and washed with distilled water. Disinfected seeds were planted in boxes containing agar tips with nutrient medium, and incubated at 4°C for 48 hours followed by 22°C under growth light for germination. Germinated seedlings were transplanted into trays containing nutrient solution and aeration system. Plants were grown hydroponically at 21°C – 25°C at an irradiance of 150 µmol m⁻² s⁻¹ under 8 h-light/16 h-dark cycles and relative humidity of 70% for 3-4 weeks (Yin et al., 2010).

5.2 Salt stress treatment
For stress experiments 4 week old plants were transferred to hydroponic system containing 0 mM (Control), 50 mM, 75 mM, 100 mM, and 150 mM sodium chloride (NaCl). Plants were grown for one week, and then transferred for recovery to a system lacking NaCl for one additional week (Stress and recovery). 150 mM NaCl concentration was only used for the screening of NaCl concentrations in hydroponic system.

5.3 Visual inspection of Plants
During the development of Arabidopsis seedlings, visual inspection was performed for the survival rate at increasing concentrations of NaCl, and recovery rate after salt treatment.

5.4 Effect of salinity on Chlorophyll fluorescence
Chl fluorescence kinetics measurements were recorded on detached leaves. Measurements of chlorophyll fluorescence were done using a pulse amplitude modulator (PAM-210, Walz, Germany) a modulated fluorescence recorder. Various photosynthetic parameters such as minimal and maximal fluorescence (Fₐ & Fₘ) for dark adapted leaves and Fₐ’ and Fₘ’ were measured for light adapted leaves respectively. The maximum quantum efficiency of PSII photochemistry (Fᵥ/Fₘ) was recorded as a ratio of variable fluorescence (when QA is maximally oxidized) from the dark adapted leaf and maximal fluorescence (when QA is maximally reduced) from dark adapted leaf.

For determination of non-photochemical quenching (NPQ) slow kinetics of Chl fluorescence induction were recorded in leaves detached from the 16 h dark adapted plants, exposed for 15 min to PAR 1250 µmol m⁻² s⁻¹. The determination of ETR was also performed on the dark and light adapted leaves (Yin et al., 2010).

5.5 Effect of salinity on total Chl content
In this experiment we quantified the amount of Chl in intact leaves. Leaves from control, salt treated and stress recovered plants were collected, washed in distilled
water, 50 mg leaf tissue is weighed and extracted in 1 ml of 95% (v/v) ethanol by boiling at 90°C. Chl content was measured according to reference (Lichtenthaler., 1983). The Chl content was expressed as mg Chl per gram leaf. The chlorophyll a/b ratio, amount of Chl a, Chl b and carotenoids were also determined.

5.6 Proline content analysis
Proline measurements were performed using the modified protocol of Bates et al (1973) (Suryan and Kirdmane 2009). Totally 100 mg of leaf tissue was collected from each plant group. The tissue was ground in mortar along with liquid nitrogen. 3 % 5-sulfosalicylic acid was added to homogenate (5 µl/mg tissue). Samples are centrifuged for 5 min at maximum speed. Reaction mixture is prepared in separate tube with 100 µl of 3 % 5-Sulfosalicylic acid, 200 µl Glacial acetic acid(GAA), 200 µl of acid ninhydrin (reagent mixture of 1.25 mg Ninhydrin in 30 ml GAA and 20 ml of 6 M H₃PO₄), add 100 µl of supernatant and incubated at 96 °C for 60 min. reaction was terminated by placing reaction tubes on ice for 5 min and 1 ml Toluene was added to reaction samples and vortexed for 20 s. Chromophore containing Toluene(pink) was extracted into fresh tubes after separation of organic and water phases. L-proline (MERK, Germany) was used as standard. 10, 20, 30, 40, 50 µg/ml concentrations were prepared. Absorbance was measured at 520 nm using Toluene as reference.

5.7. Statistical analysis
The statistical analysis and Analysis of variance (ANOVA) was performed using GraphPad prism (V5.0) software.

6.0 Results
6.1 Stress reduces plant growth
The plants were subjected to one week stress with nutrient solution containing NaCl followed by one week recovery in solution without NaCl. Initially the growth rate was the same for all ecotypes, but the NaCl treatment has changed the growth levels. We have found that the salt treated plants showed stunted root growth with brown root tips under stress as compared to control (0mM). The plants subjected to salt stress (Ws & Ler 100 mM) showed a loss in chlorophyll as shown in Fig 2B, chlorophyll changes were not found in Col accession. The Salinity effect was high at Conc of 150mM (see fig 2C) all the accessions showed colour change Green-yellow and wilt on day 3 of stress which led to plant death
Figure 2 A) Before applying salt stress. B) 7 days after applying salt stress (100mM) C) 150mM on day 5 of salt stress.

6.2 Chlorophyll content analysis
Pigment analysis was performed in the detached leaves from plants at Day 0, 3, 5 and 7 of stress followed by 7 days of recovery. The chlorophyll content in the
control and salt treated plants is shown in the graph (Fig. 2). During the salt treated conditions the amount of Chl a decreased with increasing concentrations. There is no difference in terms of sensitivity between the accessions. The graph below (Fig. 3) showing the amount of Chl a, Chl b & Carotenoids (C(x+c)). The Chl a/b ratio is shown in table 1. Decrease in the pigment levels were found on day 7 of stress. The difference was not significant between 0 mM and 100 mM col (P=0.069(P>0.05)) and Ws (P=0.179(P>0.05)). Significant difference in the chlorophyll content was observed only in Ler between 0 mM & 100 mM (P=0.0471 (P<0.05)).

![Chlorophyll (mg/g leaf)](image)

**Figure 3.** Impact of salt stress on the Chl content of the three accessions after one of week of stress. Chl measured on day 7 of salt treatment. X-axis represents the Concentration of NaCl used for treatment with each accession; Y-axis represents mg Chl per gram leaf. Green bars represent Chl a, Blue bars Chl b, and Yellow bars represent carotenoids. Data represents ±SD, N=5-7.

**Table 1.** Chl a/b ratio in salt treated plants are determined from above graph using chlorophyll extraction method of Lichtenthaler et al (1983).

<table>
<thead>
<tr>
<th>Accessions</th>
<th>0 mM (NaCl)</th>
<th>50 mM (NaCl)</th>
<th>75 mM (NaCl)</th>
<th>100 mM (NaCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Col-0</td>
<td>3.49±0.32</td>
<td>3.602±0.42</td>
<td>2.974±0.103</td>
<td>2.047±0.0327</td>
</tr>
<tr>
<td>Ws-4</td>
<td>4.051±0.35</td>
<td>3.693±0.21</td>
<td>3.691±0.65</td>
<td>3.402±0.119</td>
</tr>
<tr>
<td>Ler-0</td>
<td>4.23±0.53</td>
<td>3.861±0.17</td>
<td>3.891±0.216</td>
<td>3.843±0.151</td>
</tr>
</tbody>
</table>

**6.3 Proline analysis**

Proline analysis was performed on the day 0 (0 hours), day 2 (48 hours), and day 7 of salt treatment. Leaf tissue was collected randomly from all the plants in each group and measured according to Bates et al (1973). Proline levels present on day
7 of stress are shown in graph below which has a significant increase in Col and Ws.

![Proline levels on day 7 in NaCl treated Plants. Salt treated plants showed an increase of twice when compared to control. The Col-100mM and Ws-100mM showed a significant increase in proline. Col (P=0.023), Ler(P=0.09), Ws(P=0.0014). Difference means ±SD, N=5](image)

The significant difference was not observed between the three accessions. The levels of proline decreased in leaf tissue on day 7 of recovery.

6.4 Salt stress effect  PSII Activity

In order to investigate the photosynthetic mechanisms during stress conditions fluorescence parameters such as $F_o$, $F_m$, $F_v/F_m$, were recorded in Col, Ler and Ws for all the used concentrations from 16 h dark-adapted plants during stress and recovery condition. Minimum and maximum fluorescence ($F_o$ & $F_m$) slightly and the differences were compared to readings from stress and recovery, neither of them have any impact on the $F_v/F_m$ showed in table 2. To investigate the Non photochemical quenching (NPQ) activity in these plants during stress and recovery the induction kinetics for NPQ were recorded for 15 min at PAR 1250 µmol /m$^2$/s$^{-1}$ in dark adapted plants and graphs were plotted Time Vs. NPQ for every one minute readings. Though there was no significant difference in the NPQ during stress conditions, but we found a shift change in all three accessions during the recovery in the 75 mM & 100 mM.
Table 2. $F_v/F_m$ taken on the day 6 of stress treatment $n=8$-10 measurements were made during light conditions (3 hours after the light activation of photosystems).

<table>
<thead>
<tr>
<th>Accession</th>
<th>Conc (NaCl)</th>
<th>0 mM</th>
<th>50 mM</th>
<th>75 mM</th>
<th>100 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Col-0</td>
<td></td>
<td>0.79±0.023</td>
<td>0.815±0.027</td>
<td>0.797±0.017</td>
<td>0.801±0.030</td>
</tr>
<tr>
<td>Ler-0</td>
<td></td>
<td>0.813±0.061</td>
<td>0.824±0.007</td>
<td>0.79±0.043</td>
<td>0.8±0.007</td>
</tr>
<tr>
<td>Ws-4</td>
<td></td>
<td>0.821±0.013</td>
<td>0.80±0.045</td>
<td>0.816±0.017</td>
<td>0.82±0.06</td>
</tr>
</tbody>
</table>

Figure 5 Slow induction kinetics of Non-photochemical Quenching on day 0 of stress and day 7 of stress treatment. The plants were dark incubated and measured by exposing to 1240 µmolm$^{-2}$s$^{-1}$ for 15 minutes. The measurements were made for every 20 sec intervals for all 3 accessions of Arabidopsis for four different concentrations. Labels: A, B and C represent the NPQ measurement in individual accessions. Green curve represents 0mM, Blue 50mM, Orange 75mM, Red 100mM. Data represent the means ±SD, $n=4$-6.

The Induction kinetics in control and salt treated plants showed no significant difference during stress condition. All the data points coincided with control when the standard deviation is considered.
Figure 6 Slow induction kinetics of Non-photochemical Quenching on day 0 of stress and day 7 of recovery treatment. The plants were dark incubated and measured by exposing to 1240µmol m⁻² s⁻¹ for 15 minutes. The measurements were made for every 20 sec intervals for all 3 accessions of Arabidopsis for four different concentrations. Labels: D, E and F represent the NPQ measurement in individual accession. Green curve represents 0mM, Blue 50mM, Orange 75mM, Red 100mM. Data represent the means ±SD, n= 4-6.

When examined very closely salt treated plants showed a slower NPQ at first 40 seconds. In dark adapted salt treated plants, faster phase of NPQ showed stable delay, Col and Ws 100 mM showed a faster and higher steady state than control group. Ler-100 mM showed a slow and lower Steady state.
Figure 7, Electron transport rate (ETR) for 3 hour light adapted plants under stress conditions. The ETR is slightly higher in salt treated plants when compared to control but the difference was not significant. G,H& I represent for individual accessions Col-0, Ler-0 & Ws-4 respectively. Data represent ± SD. N=5-7.

The light adapted plants showed a higher ETR (%) when compared to dark adapted plants (data not shown). Dark-adapted plants control group showed 60-75% of ETR. The Light adapted plants showed ETR values ranging from 90-120% in control group plants (Fig 7). The ETR measurements conducted on the light adapted salt treated plants showed variable results. Neither increase nor decrease of ETR could be assessed during stress condition.
Figure 8, Electron transport rate (ETR) for 3 hour light adapted plants under RECOVERY conditions. The ETR was slightly higher in salt treated plants when compared to control but the difference was not significant. J, K & L represent for individual accessions Col-0, Ler-0 & Ws-4 respectively. Data represent ± SD. N=5-7.

The ETR has slightly increased during the Recovery phase (Fig 8). The graphs with labels K and L show the difference in ETR with change in salt treatment. The Max ETR was at 840 PAR in control whereas it shifted to 1240 PAR in stressed plants.

7. Discussion
The effect of salt tolerance in the plants has been subject of research for decades (Flowers et al, 1977). The majority of the studies performed involved rice, maize wheat, and Arabidopsis. So far the research was done using molecular analysis to understand the genetic mechanisms involved in the selective activation of stress signalling pathways. Understanding photosynthesis under salt treatment has been the present area of interest since 1990s’ where the photosynthetic response to salt stress has been studied by comparing a glycophyte to halophyte (Stepien and Johnson, 2009).
7.1 Salt tolerance and sensitivity

Arabidopsis plants are grown in a hydroponic system for the four week and then transferred into salt stress system. Plants showed gradual response to lower salt concentrations. Many studies revealed that water and osmotic potential decreases with increasing concentrations of NaCl whereas, turgor pressure rises under such condition. The chlorophyll loss was observed in Ler and Ws 75mM, 100 mM during 6th and 7th day stress treatment, leaves slowly tend turn yellow, The change of colour green-yellow was not observed in the Columbia accession. At 150mM concentration all the plants started to loose chlorophyll by 3rd day of stress treatments. The studies conducted on crop plants showed decrease in photosynthetic pigments of leaves under salt stress (Hernandez et al., 1999; Gadallah, 1999). From the previous studies (Apse et al, 1999) plants tolerated the highest salt concentration of 200mM (by increasing the NaCl concentration) showed decreased growth having small stature. By this we can implicate that the Hydroponic system used for the project helped us to identify the tolerance level in one week of stress provided to Arabidopsis accessions.

7.2 Chlorophyll fluorescence and salt stress

Abiotic or biotic stress factors affect the Fv/Fm in C4 plants (Pfundell, 1998). The impact of stress was not heavily observed in plants during the stress treatment as the Fv/Fm was affected by exposure to salt. Previous stress studies on Arabidopsis showed decrease in Fv/Fm for 100 mM concentration upon 14 day prolonged stress treatment and increased NPQ under stress conditions (Stepien and Johnsson 2009). No increase in NPQ might be because of short stress duration. No decrease in the ETR during the stress describes the active photosystems and electron transport from PS II to PS I under stress condition. There is a shift in the Electron transport rate during recovery phase in the stressed plants. The Maximum ETR in the light adapted plants was observed at 1240µmol m⁻² s⁻¹ (PAR) whereas control plants showed the Max ETR at 840µmol m⁻² s⁻¹ PAR. The Slower study state (NPQ) in the Ler-100mM and change in Max ETR (1240 PAR) might lead to speculation both the parameters are related in-terms of excitation of Chl molecule. But the shift of path at 50, 75 and 100 mM concentrations in all the accessions in the first 40 seconds and a fast and higher NPQ explains slow acidification of lumen followed by rapid conversion of Vx to Zx.

7.3 Salt stress decreases chlorophyll content

We found decrease in the chlorophyll pigment content with increasing Salt concentrations. This condition may be speculated as the loss of photoprotective ability and synthesis of chlorophyll. Many salinity studies reported the decrease of Chlorophyll pigments is due to production of ROS in the photosynthetic membrane and stress induced oxidative damage (Taylor et al.,2009; Hernandez et
NaCl stress increases synthesis of chlorophyllase, this degrades the chlorophyll in sunflower plants (Santos., 2004). The decrease in the Chl a explains the failure of Photoprotective mechanisms operated by plants.

### 7.4 Proline role under stress

An increased concentration of Proline was observed in the plant leaves after 48 h of stress. This could be protective mechanism, as higher levels of proline were recorded on day 7 of stress. These levels dropped to control levels in the week recovery treatment. Rise and drop of proline in salt treated plants supports previous studies indicating protective role of proline in the cell system. Unclear and too long sentence previously many reports were about the increase in proline levels in plants under salt stress as an osmotic adjustment in other plant varieties like rice, wheat, sorghum and Arabidopsis. (Hasegawa et al., 2000; Mordi and Ismail, 2007), Studies regarding decrease in proline levels were mostly based on drought stress and decrease of proline after re-watering plants (Voetberg and Stewart, 1984). Decrease of proline levels under recovery after salt treatment explains the role as the Osmoprotectants.

### Conclusion:

From our results it is evident that salinity tolerance limit is 100mM for a hydroponic system. Even though the stress duration was not enough to observe the clear difference in photosynthetic performance of plants, from the decrease in chlorophyll content and plant growth I speculate that salinity decreases photoinhibitive and photoprotective ability in Arabidopsis accessions. I speculate the delayed response in NPQ in the first 40 seconds might be the effect of pH change in the cell system causing slow excitation of the Chl molecules. Slow steady state in Ler-100 mM can be implicated to delayed acidification of lumen. Ler-0 was the most sensitive ecotype to salt stress followed by Ws-4 and Col-0.

### Acknowledgments:

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