Journal of Genetics, Genomics & Plant Breeding 7(3) 52-61 (July, 2023)
ISSN (Online): 2581-3293

RESEARCH ARTICLE

Measuring canopy temperature to determine the level of salt stress tolerance of sus1 mutant in *Arabidopsis thaliana*

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Manuscript received: June1, 2023; Decision on manuscript, June 30, 2023; Accepted: July 2, 2023

Abstract

Increasing salinity in irrigation water and soil represents a crucial problem against plant growth and development especially the agricultural crops. A number of plants own the ability to adapt to salt by recruiting a set of genes that are expressed and regulated under salt stress conditions. Using the model plant; Arabidopsis in studying gene function and regulation has a great importance in plant biotechnology. This research mainly aims to estimate the level of salt stress resistance of Arabidopsis plants by measuring plant canopy temperature. After adaptation to salt stress, Columbia 0 'wild type' and sus1 mutant of the gene sucrose synthase 'SUS1' plants were grown to the appropriate stage and then treated with NaCl solution at 0.0, 100 & 200 mM. Plant canopy temperature, plant morphological changes, plant survival, and leave chlorophyll content were estimated. The results showed that there were no significant differences between the wild type and the mutant in plant temperature and plant survival. The morphology of sus1 mutant plants was severely affected by salinity at 100 mM NaCl after 7 days comparing to wild type which may indicate a function of the SUS1 gene in salt stress response. Leave content of chlorophyll a, b, and total chlorophyll was significantly increased in both Columbia 0 and sus1 plants when treated with 200 mM NaCl comparing to 0.0 and 100 mM but Columbia 0 plants preserved higher amount of chlorophyll comparing to sus1. Having found a less amount of chlorophyll in leaves in the mutant might be refereed to presences of a T-DNA within the SUS1 gene sequence which knocked out the gene, and thus may indicate a role of SUS1 gene in chlorophyll synthesis. More investigations are needed to confirm the possible leaf structural changes that allow plants to preserve more chlorophyll under higher salt levels and also, the possible function of the SUS1 gene in chlorophyll synthesis.

Keywords: T-DNA, salt stress, thermal imaging, chlorophyll, Arabidopsis

Introduction

Salinity stress is one of the most effecting abiotic stresses on the distribution and growth of plants and agricultural crop worldwide. Plants show different physiological, biochemical, and molecular responses to different abiotic stress conditions. Among these stressful conditions, soil salinity which is considered to be the most important abiotic stress that negatively affects plants in a number of ways, depending on the extent and duration of the stress. Salinization occurs as a result of the accumulation of high levels of different minerals used in field fertilization and irrigation leading to receding wide spaces of crop cultivation and production. In the last two decades, scientist focused on how to overcome the effects of the salinity on the agricultural crops after the amazing progress in plant biotechnology research seeking to producing plant with high capability of salt adaptation and tolerance. To study the gene function and regulation, a huge number of Arabidopsis mutants using transferred DNA (T-DNA) strategy were produced. The focus was mainly on the abiotic stresses involved genes and transcription factors; especially salt stress. It is well known that the site of the inserted DNA significantly effects on the level of expression of the targeted gene; the knock-out mutant usually results no protein from the gene in which T-DNA is inserted in (Krysan, Young, and Sussman, 1999a). Studying the salt stress related genes, a set of sucrose synthesis (SUS) genes were found to be expressed in different plant tissues under stress conditions such as drought (Xiao *et al.,* 2014). Also, the relationship of the sucrose synthase1 gene to salinity stress throughout triggering the expression of this gene was confirmed by Lu *et al.,* (Lu, Li, and Jiang, 2010). On the other hand, it had been proofed that sucrose synthase gene was found to be expressed under drought, cold, and high osmosis (Baud, Vaultier, and Rochat, 2004).

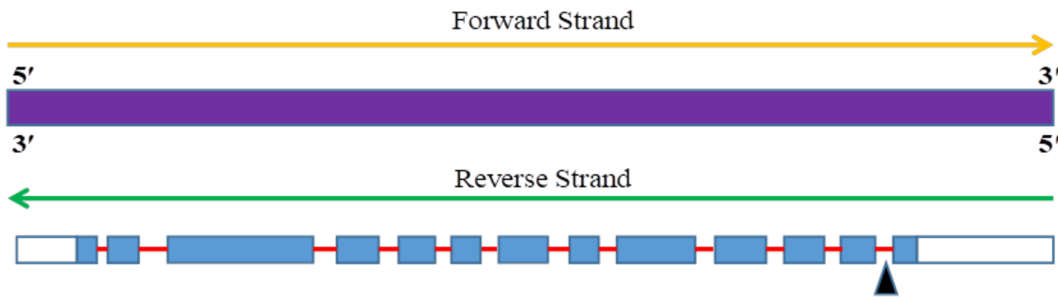
In recent, scientists tend to using remote sensing to diagnose the abiotic stress levels plants suffer. The thermal and inflorescence imaging are being applied and suggested to be an alternative non-destructive and fast technique to diagnose and determine the plant response and tolerance to different abiotic stresses such as salinity and drought (Esmaeili *et al.,* 2017) (Stutsel *et al.,* 2021), (Yao *et al.,* 2018) and (Pineda, Barón, and Pérez-Bueno, 2021) (Stoll and Jones, 2007) (Urrestarazu, 2013; Menegassi *et al.,* 2022). It has been found that the thermal imaging was very active in scanning a wide range of the temperature of wheat vegetative group under slat stress (Bayoumi *et al.,* 2014). In soybean, measuring the temperature of the vegetation was proved as a fast and active mean to evaluate the level of stress plants exposed to comparing to the classical physiological methods (Kim *et al.,* 2014). The research shows that the salinity stress creates a wide range in canopy temperature; so the mean can distinguish between stressed and non-stressed vegetation (Bayoumi et al., 2014). In general, the salinity affects the morphological properties of plants including the changes in leave color resulting from the accumulation of anthocyanin which indicates the plant response to stress condition. Studies show that the anthocyanin accumulated in significant amounts in corn leaves upon exposing plants to 150 mM NaCl (Donghyun, 2020). On the other hand, many investigations showed that the abiotic stresses such as salinity may cause increase in leave chlorophyll content. In Columbia 0, treated plants with 75 mM NaCl accumulated more chlorophyll and showed dark green color of leaves (Ruiz Carrasco *et al.,* 2007).

Materials and methods

This experiment was done in a controlled growth chamber to diagnose the salt stress level of Arabidopsis thaliana using a thermo-camera (Testo 875). A T-DNA mutant line “sus1” (SALK_112854), N676548 of Sucrose Synthase 1 “SUS1” gene (At5G20830) containing 13 exons and 12 introns, was used for this study comparing to Columbia 0 plants. This gene is located on the reverse strand on the chromosome 5 consisting of 5179 bp located between 7055398…..7050217 base pairs. The T-DNA is inserted in the intron 1 of the gene (Fig.1).

Small pots were filled in with the same amount of peat moss according to the weight and then were watered with the same amount of water according to the volume. Seeds were then surface sown using a small sterilized (70% ethanol) tweezer. Directly after sowing, post were covered by kling film and kept under 4c° temperature and continuous light for 4 days. After 4 days, room temperature was modified to 20 ±2 and 10 hrs light; with light intensity 115µmol/m2/s, and 14 hrs dark. The relative humidity in the growth room was kept between 50-65%. Fife days after germination, small holes were made in the kling film and was left for a week. The kling film was then completely removed and regular irrigation was done using 50 ml of tap water. After 7 days, only one seedling per pot was selected according to the size and regular watering was kept until salt treatment was done. The experiment included two factors; NaCl concentration (0.0, 100 and 200mM) and Arabidopsis genotypes (Columbia 0 'wild type' and sus1 mutant line). The SALK_112854 mutant line 'stock no. N676548' of the gene SUS1 was used for this study. Upon reaching a specific growth stage according to (Boyes et al., 2001), plants from both lines specified for salt treatment were watered with low salt concentrated solution (20mM NaC) for 4 days (Elhaj, 2009) using 50 ml of the salty solution and the control plants from both lines were watered with tap water. All plants were then left for 4 days without any treatment. Two days after treating plants with high salt concentrations, plant leave samples for chlorophyll determination were collected. higher salt treatments using 100 and 200mM NaCl continued for 5 times "day by day" using 50 ml volume of the solution, and the 0.0mM plants of both lines were watered with distilled water (EC=2.5ppm) using the same volume. During the period of the salt treatment, plants were watched for morphological changes; wilting, yellowing, and dryness. After 10 days of starting salt treatment, plants were extensively washed with tap water. Then, plants were accounted for survival after one day later. All period long of the experiment, thermal and normal images were taken and the temperature degrees were analyzed using the version 3.3 Testo IRSOFT software.

Fig. 1: The Sucrose Synthase 1 gene structure in Arabidopsis showing the T-DNA insertion site; exons (in blue), introns (red lines), UTRs (empty triangles), site of T-DNA (black arrow), redesigned from TAIR (TAIR, 1999)



For chlorophyll a, b and total chlorophyll determination, samples after collection were kept refrigerated for later use. Next day, samples were ground in 5 ml acetone 80%. Mixtures were filtered and the supernatants were collected in small flasks. Solutions were completed to the final volume (25 ml), and a small amount (5ml) of each sample was taken to estimate the chlorophyll contents and a sample of acetone 80% was used as a blank. A spectrophotometer apparatus; JENWAY 6300 was used for the purpose and then the amount of the chlorophyll was calculated in mg/g fresh weight according to (Arnon, 1949), (Porra, Thompson, and Kriedemann, 1989) (Gogoi and Basumatary, 2018) and (Ozrecberoglu and Kahramanoglu, 2020).

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Chlorophyll $a = \frac{(12.7\ x\ \text{A663} - 2.69\ x\ \text{A645})}{\text{sample wight}\ x\ 1000} x\ volume$ **Chlorophyll** $b = \frac{(22.9\ x\ \text{A645} - 4.68\ x\ \text{A663})}{\text{sample wight}\ x\ 1000} x\ volume$

Total chlorophyll $= \frac{(8.02\ x\ \text{A663} + 20.2\ x\ \text{A645})}{\text{sample wight}\ x\ 1000} x\ volume$

Statistical analysis was done using Minitab 5; ANOVA, General Linear Model. References were sited using EndNote 6 program and APA style was used for listing references. This experiment aims to the study of response of Arabidopsis sus1 mutant of Sucrose Synthase 1 (SUS1) gene under salt stress using thermal imaging including some morphological and physiological investigations.

Results and discussion

Measuring plant canopy temperature

Results show that there were not significant differences in canopy temperature between Columbia 0 and sus1 mutant plants (Fig. 2), but the salt concentration affected significantly the temperature of the vegetation which was higher in plants treated with 200mM than 100mM NaCl comparing to control. Thermal images show the normal water content (blue color) in the control plants of both genotypes but the normal color started to disappear by disappeared after 9 days at 200 mM NaCl (Fig. 3). These results indicate the effect of the salt on the absorption of the water which is reflected by increasing the temperature of the plant canopy, but at the same time did not clarify whether the sus1 mutant was more sensitive to water deprivation than the control plants. Applying higher salt concentrations might be needed to get more clarification about the effect of salt on sus1 mutant water uptake in relation to our expectation considering the involvement of the SUS1 gene in salt and water deficiency response.

Fig. 2: Effect of salt stress on Arabidopsis Columbia 0 and sus1 mutant canopy temperature at day 0 (after salt adaptation), 7 and 9 days after high salt concentration (0.0, 100 and 200mM) treatment

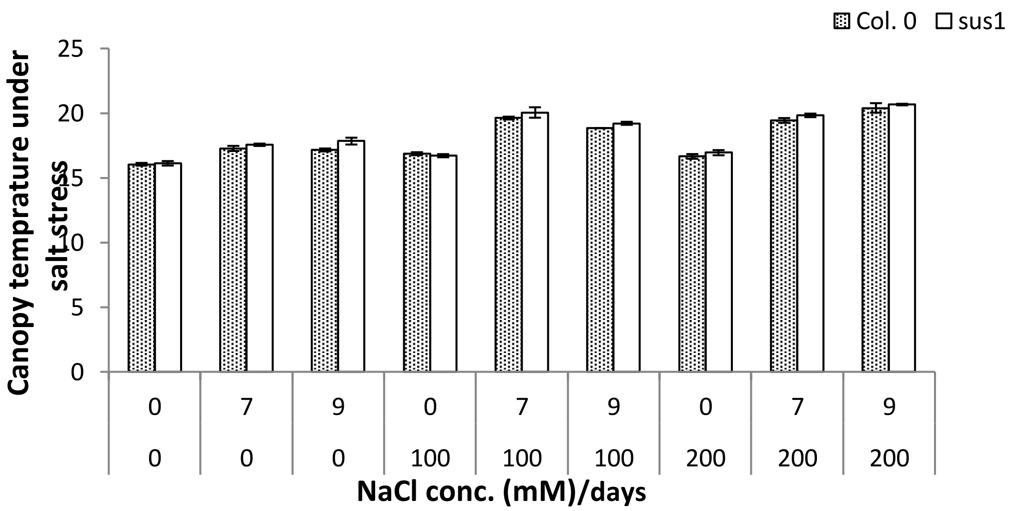
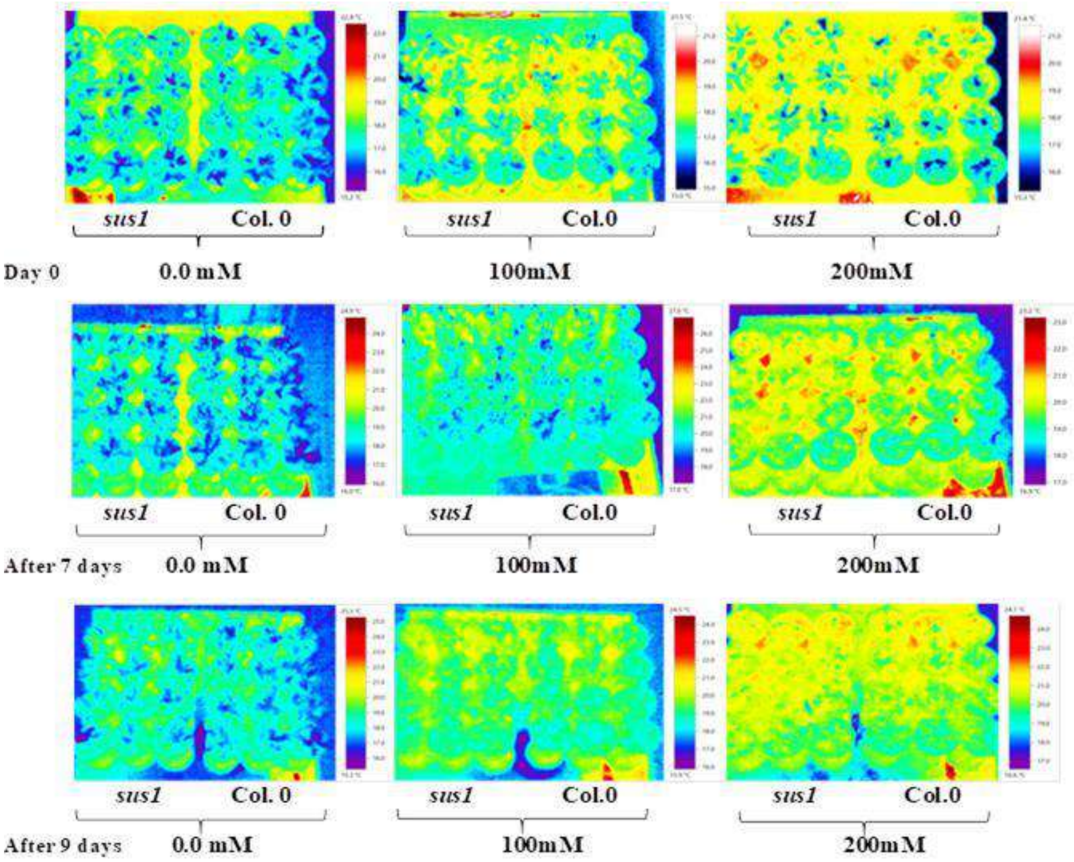


Fig. 3: Effect of salinity on the plant canopy temperature of Columbia 0 and sus1 mutant at day 0 (the last day of salt adaptation at 20 mM NaCl) and, after 7 and 9 days from salt treatment (NaCl) at 0.0, 100 and 200 mM



Considering the morphological changes that take place upon the exposure to salinity stress, wilting, discoloration, and desiccation were visually estimated and counted in both genotypes. A significant difference was found between sus1 mutant and Columbia 0 'wild type' when treated with 100 mM after 7 days exposure period; the mutant was less affected by salt treatment than the wild type as estimated by less wilting, yellowing and dryness (Fig. 4 and 5). Apart from time of exposure, both genotypes were affected similarly by salt when treated with higher salt concentrations (200mM). These results indicates that the mutant was more tolerant to salt comparing to wild type in keeping the morphology of plants but only at certain salt concentration and for certain period of time. These results may indicate a function of the sucrose synthase 1 in the salt stress response.

The SUS1 gene holds a T-DNA within the gene sequence; in the intron 1 (Fig. 1), and according to Krysan *et al.*, Krysan, Young, and Sussman, 1999a and Wang, 2008, this gene was knocked out and will not produce a functional protein as its first part of the sequence is interrupted by the insert. Thus, there is a possibility that another neighboring gene was activated instead (Tamura et al., 2016) under the salinity conditions, or the inserted T-DNA might have increased the gene expression in this null mutant under salt as it located in the first initial part of the gene (Missihoun, Kirch, and Bartels, 2012).

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This can be referred to presence of some specific salt response cis-acting elements in the left border of the insert that increase the level of expression of the targeted gene. Similar results were obtained by Elhaj (Elhaj, 2009) upon exposing some salt stress responsive mutants; kup1 and chx17. This explanation was based on the fact that inserting a T-DNA may lead to gain of function of a gene (Krysan, Young, and Sussman, 1999b) depending on the cis-acting elements present at the left border, flanking the promoter or at the beginning of the gene. The results show that there were no significant differences between Columbia 0 'wild type' and sus1 mutant plants in survival average after 10 days of imposing salt stress (Fig. 6 and 7). Being similarly affected by salt, this might be referred to the long exposure time (10 days) of salt which negatively smashed the growth of both phenotypes.

Fig. 4: Effect of salinity on the plant morphology of *Arabidopsis Columbia* 0 and sus1 mutant at day 0, 4 and 7 days salt treatment (0.0, 100 and 200 mM NaCl). 'Day 0'= the last day of salt adaptation (20 mM NaCl)

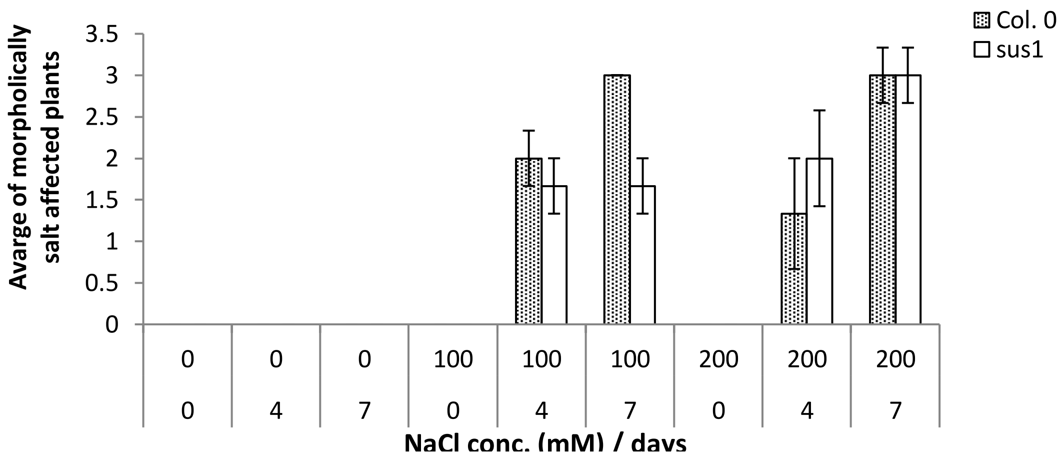


Fig. 5: Effect of salinity on the plant morphology of *Arabidopsis Columbia* 0 and sus1 mutant at day 0, 4 and7 days salt treatment (0.0, 100 and 200 mM NaCl). 'Day 0'= the last day of salt adaptation (20 mM NaCl)

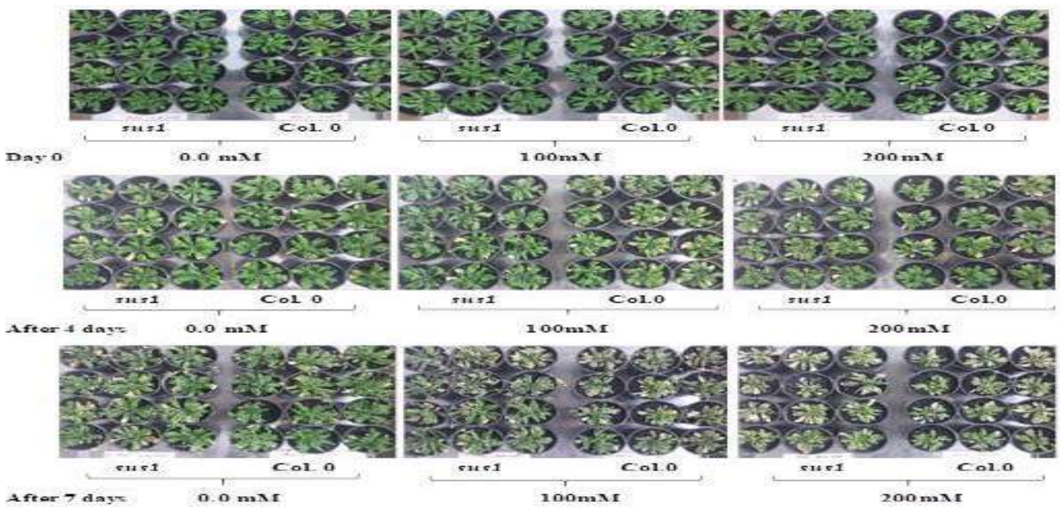


Fig. 6: Effect of salinity on the survival average of *Arabidopsis Columbia* 0 and sus1 mutant after 10 days of salt treatment (NaCl) at 0.0, 100 and 200 mM.

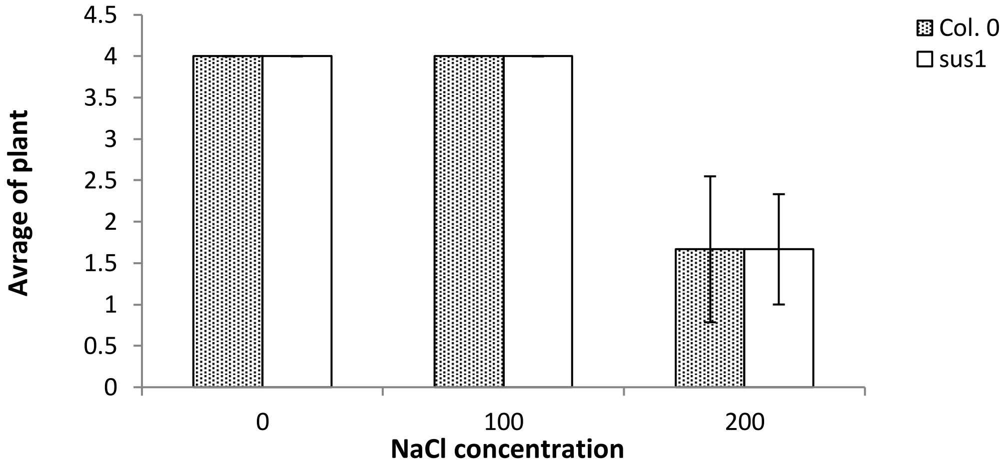
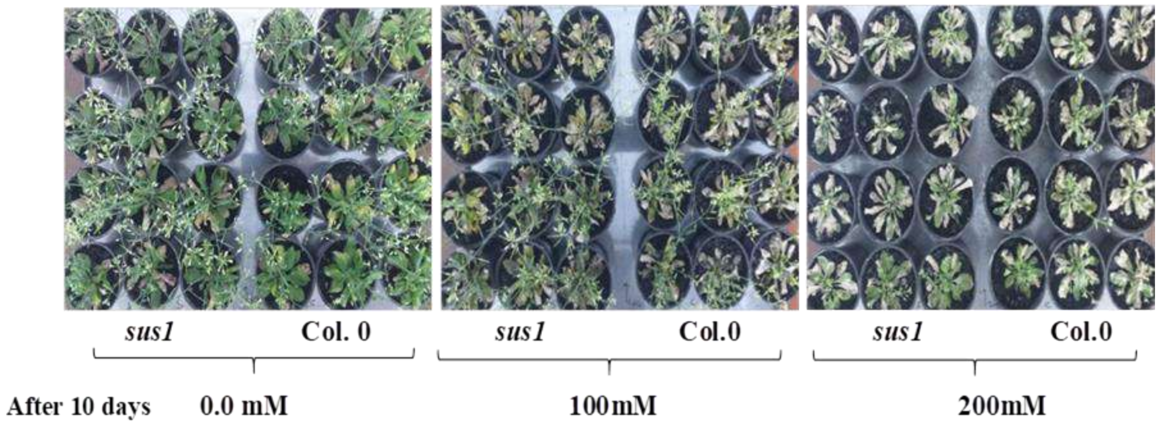


Fig. 7: Effect of salinity on the survival average of Columbia 0 and sus1 mutant after 10 days of salt treatment (NaCl) at 0.0, 100 and 200 mM.



Effect of salinity on leaf chlorophyll content

The chlorophyll content was determined in leave samples two days after of imposing salt stress after plants been adapted to salt at 20 mM. The results show that there was an increase in chlorophyll a, b and total chlorophyll after treating plant with 200 mM NaCl especially in Columbia 0 'wild type' plants (Fig. 8). Increasing leave chlorophyll content has been reported in many plants such as tomato (Ruiz Carrasco *et al.*, 2007) and rice (Misra *et al.*, 1997). In sunflower, it was found that the salinity has led to a decrease in the chlorophyll content only on the long term, but on the short term the level of chlorophyll in leaves was increased under salt stress (Heidari, Bandehagh, and Toorchi, 2014). Similar results were obtained when Arabidopsis plants exposed to a lower salt concentration of NaCl (Rolly *et al.*, 2020).

The explanation of such results was interpreted as the salt stressed plants had some structural changes like leave thickness or the increase of the number of chloroplast in the stressed leaves which have led to more tolerance to salt stress by keeping higher level of chlorophyll content (Ruiz Carrasco *et al.*, 2007). In our experiment, even though there was an increase in the chlorophyll content with the increase in salt concentration, the mutant sus1 had significant less chlorophyll a, b and total chlorophyll than the Columbia 0 'wild type' treated with 200 mM NaCl. These results might indicate a specific function of the SUS1 gene in the chlorophyll synthesis that was not reported before (Fig. 8 and 9). Disruption of the SUS1 gene by the T-DNA insert as the gene was knocked out (Krysan, Young, and Sussman, 1999b) may explain why the chlorophyll levels were significantly reduced in the mutant comparing to the wild type plants.

Fig. 8: Effect of salt stress on chlorophyll a, b and total chlorophyll content of *Arabidopsis Columbia* 0 and sus1 mutant after 4-days salt adaptation (20mM NaCl) followed by 2-days high salt concentration treatment (0.0, 100 and 200mM NaCl)

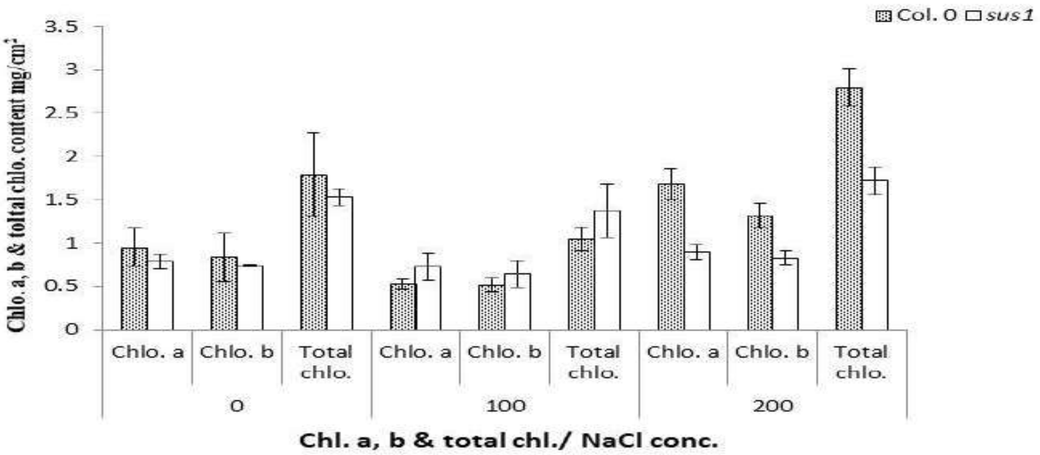
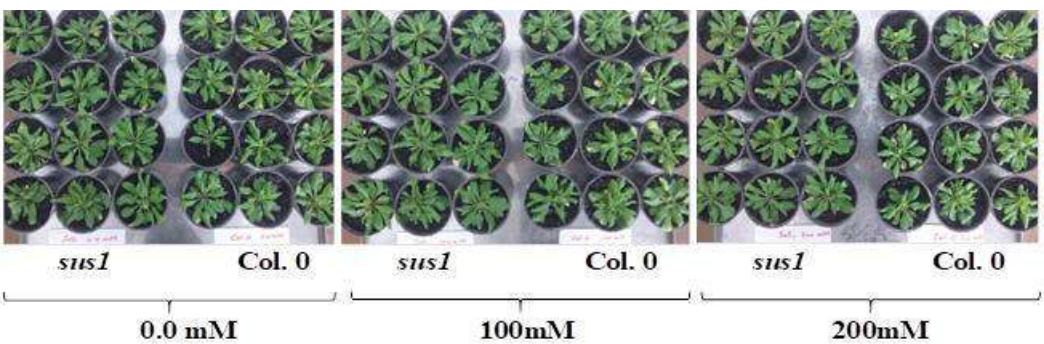


Fig. 9: Effect of salt stress on chlorophyll a, b and total chlorophyll content of *Arabidopsis Columbia* 0 and sus1 mutant after 4-days salt adaptation (20mM NaCl) followed by 2-days high salt concentration treatment (0.0, 100 and 200mM NaCl)



References

1. Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. polyphenoloxidase in beta vulgaris. Plant Physi., 24 (1): 1-15.
2. Baud, S., M. N. Vaultier, and Rochat, C. 2004. Structure and expression profile of the sucrose synthase multigene family in Arabidopsis. J. Exp. Bot., 55 (396): 397-409.
3. Bayoumi, T., S. El-Hendawy, M. Yousef, M. Emam, and Okasha, S. 2014. Application of infrared thermal imagery for monitoring salt tolerant of wheat genotypes. American J. Sci., 10 (12): 227-234.
4. Boyes, D. C., A. M. Zayed, R. Ascenzi, A. J. Mccaskill, N. E. Hoffman, K. R. Davis, and Görlach, J. 2001. Growth stage-based phenotypic analysis of Arabidopsis: a model for high throughput functional genomics in plants. Plant Cell 13 (7): 1499-1510.
5. Donghyun, J. S., Choi Kim, C. 2020. Effect of salt stress on the anthocyanin content and associated genes in *Sorghum bicolor* L. Korean J. Agril. Sci., 47 (1): 105 - 117.
6. Elhaj, A. 2009. Molecular and physiological investigations of Arabidopsis mutant lines under abiotic stresses. Newcastle University, Newcastle upon Tyne, Ph.D. Thesis.
7. Esmaeili, A., K. Poustini, H. Ahmadi, and Abbasi, A. 2017. Use of IR thermography in screening wheat (*Triticum aestivum* L.) cultivars for salt tolerance. Arch. Agron. Soil Sci. 63(2): 161-170.
8. Gogoi, M., and Basumatary, M. 2018. Estimation of the chlorophyll concentration in seven Citrus species of Kokrajhar district, BTAD, Assam, India. Trop.Plant Res., 5(1): 83-87.
9. Heidari, A., A. Bandehagh, and Toorchi, M. 2014. Effects of NaCl Stress on chlorophyll content and chlorophyll fluorescence in sunflower (*Helianthus annuus* L.) Lines. YYU. J. Agr. Sci. 24(2): 111-120.
10. Kim, J.W., T.Y. Lee, G. Nah, and Kim, D.S. 2014. Potential of thermal image analysis for screening salt stress-tolerant soybean (*Glycine max*). Plant Genet. Resour. 12: S134-S136.
11. Krysan, P. J., J. C. Young, and Sussman, M. R. 1999a. T-DNA as an insertional mutagen in Arabidopsis. The Plant Cell., 11(12): 2283-2290.
12. Lu, S., T. Li, and Jiang, J. 2010. Effects of salinity on sucrose metabolism during tomato fruit development. African J. Biotech., 9 (6): 842-849.
13. Menegassi, L. C., V. C. Benassi, L. R. Trevisan, F. Rossi, and Gomes, T. M. 2022. Thermal imaging for stress assessment in rice cultivation drip-irrigated with saline water. Eng. agríc. 42 (5).
14. Misra, A. N., S. M. Sahu, M. Misra, P. Singh, I. Meera, N. Das, M. Kar, et al. 1997. Sodium chloride induced changes in leaf growth, and pigment and protein contents in two rice cultivars. Bio. Plantarum., 39: 257-262.
15. Missihoun, T. D., H. H. Kirch, and Bartels, D. 2012. T-DNA insertion mutants reveal complex expression patterns of the aldehyde dehydrogenase 3H1 locus in *Arabidopsis thaliana*. J. Exp. Bot., 13(63): 3887-3898.
16. Ozrecberoglu, N., and Kahramanoglu, I. 2020. Mathematical models for the estimation of leaf chlorophyll content based on RGB colours of contact imaging with smartphones: A pomegranate example. Folia Horticul., 32 (1):57-67
17. Pineda, M., M. Barón, and Pérez-Bueno, M.-L. 2021. Thermal imaging for plant stress detection and phenotyping. Remote Sens.,13(1):68.

18. Porra, R. J., W. A. Thompson, and Kriedemann, P. E. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimi. et Biophysi. Acta (BBA) – Bioenergetics.,975(3): 384-394
19. Rolly, N. K., Q. M. Imran, I. J. Lee, and Yun, B. W. 2020. Salinity stress-mediated suppression of expression of salt overly sensitive signaling pathway genes suggests negative regulation by AtbZIP62 transcription factor in Arabidopsis thaliana. Int. J. Mol. Sci., 21 (5):1726
20. Ruiz Carrasco, K., F. R., A. Tassoni, and Bagni, N. 2007. Identification of two phenotypes of Arabidopsis thaliana under in vitro salt stress conditions. Biol. Plantarum., 51: 436-442.
21. Stoll, M., and Jones, H. 2007. Thermal imaging as a viable tool for monitoring plant stress. J. Int. des Sci. de la Vigne et du Vin., 41(2): 77-84.
22. Stutsel, B., K. Johansen, Y. M. Malbéteau, and Mccabe, M. F. 2021. Detecting plant stress using thermal and optical imagery from an unoccupied aerial vehicle. Front. Plant Sci., 12: 734944.
23. TAIR. 2003. The Arabidopsis information resources. Nucleic Acids. Res.,31(1):224-8.
24. Tamura, K., T. Kawabayashi, T. Shikanai, and Hara-Nishimura, I. 2016. Decreased expression of a gene caused by a T-DNA insertion in an adjacent gene in Arabidopsis. PLoS One 11: e0147911.
25. Urrestarazu, M. 2013. Infrared thermography used to diagnose the effects of salinity in a soilless culture. Quant Infrared Thermogr J., 10 (1): 1-8.
26. WANG, Y. 2008. How effective is T-DNA insertional mutagenesis in *Arabidopsis*? J Biochem Tech., 1(1):11-20.
27. Xiao, X., C. Tang, Y. Fang, M. Yang, B. Zhou, J. Qi, and Zhang, Y. 2014. Structure and expression profile of the sucrose synthase gene family in the rubber tree: indicative of roles in stress response and sucrose utilization in the laticifers. FEBS J., 281 (1): 291-305.
28. Yao, J., D. Sun, H. Cen, H. Xu, H. Weng, F. Yuan, and He, Y. 2018. Phenotyping of Arabidopsis drought stress response using kinetic chlorophyll fluorescence and multicolor fluorescence imaging. Front. Plant Sci., 9: 603-603.