*biology and life sciences forum*

MDPI

*Proceeding Paper*

# Different Strategies to Tolerate Salinity Involving Water Relations †

**Alberto Martinez-Alonso *, Micaela Carvajal and Gloria Barzana**

Aquaporins Group, Centro de Edafología y Biología Aplicada del Segura, CEBAS-CSIC, Campus Universitario de Espinardo-25, E-30100 Murcia, Spain; mcarvaja@cebas.csic.es (M.C.); gbarzana@cebas.csic.es (G.B.)

* Correspondence: amalonso@cebas.csic.es; Tel.: +34-968-296-200 (ext. 6245)

† Presented at the 2nd International Electronic Conference on Plant Sciences—10th Anniversary of Journal Plants, 1–15 December 2021; Available online: https://iecps2021.sciforum.net/.

**Abstract:** Salinity is one of the main limiting factors in agriculture, which can affect plants growth and development, as a result of a disruption of homeostasis. Therefore, the understanding of the mechanism of the plants for tolerate salinity stress is essential in order to develop new techniques that may improve tolerance for optimizing crop yields. In this paper, we compare the response of Cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* L.), grown by hydroponic culture, to a moderate salinity of NaCl 60 mM. For that, root hydraulic conductance, relative water content of leaves (RWC), stomatal conductance, fresh weight and dry weight ratio, and Na concentration in shoot and root were measured. The results showed a significant decrease of root hydraulic conductance in both species treated with NaCl, revealing a higher resistance to water passage from root to shoot, probably influenced by the increase of Na content after the treatment. In addition, stomatal conductance in cucumber was reduced, accompanied by a decrease of fresh/dry weight ratio in the root. Conversely, neither of those parameters changed in tomato. These experiments confirm the evidence that cucumber and tomato follow different strategies in the adaptation to salinity, being tomato more resistant probably due to the role of membrane water transporters. Despite that, more specific studies would be needed in order to support this conclusion.

**Keywords:** salinity resistance; water relations; water transport; aquaporins; cucumber; tomato

**Citation:** Martinez-Alonso, A.; Carvajal, M.; Barzana, G. Different Strategies to Tolerate Salinity Involving Water Relations. *Biol. Life Sci. Forum* **2021**, *1*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Fulai Liu

Published: 2 December 2021



## 1. Introduction

Plants are sensitive to the effects of abiotic stresses. The severity of those effects has been increased as a consequence of climate change, which is endangering the agricultural productivity in several planet areas [1]. Among all the abiotic factors, salinity is one of the most harmful for crops yield. Salinity stress causes various effects on plant physiology, such as reduction of seed germination and plant growth as a result of an osmotic stress [2]. In the early stages, plants under salinity conditions could experiment some disturbances, such as inhibition of cell expansion or stomata closure [3]. If this exposure is prolonged in a long term, more serious phenomena could take place, including reduction of physiological and metabolic activity, alteration of primary and secondary metabolites synthesis [2], early senescence, an increase of cytotoxic ions [3], and, as a last, cell death [4]. Osmotic stress also affects water balance, causing water deficit, alteration of ion fluxes, or water potential reduction, what leads to a loss of cell turgor or plant dehydration, among other effects [5,6]. In addition, salinity can alter the uptake of some essential mineral nutrients, like N, P, and K [7].

To deal with these problems, plants, throughout evolution, have developed several resistance mechanisms to avoid the harmful effects of salinity and, therefore, to allow them to grow in hostile environments. Salt avoidance and salt exclusion are the two main strategies followed by plants to alleviate the damaging effects of NaCl in the tissues. At the physiological level, osmotic adjustment plays a fundamental role in maintaining water balance. This is achieved by accumulating a large amount of osmolytes like organic solutes, which can stabilize the cell osmotic potential, or by controlling ions transport pathways. Furthermore, in recent studies, it has been demonstrated that some membrane transporters perform a significant role in improving plant adaptation to salinity by maintaining water flow in the tissues [4,8].

In this paper, we compare the adaptability to salinity of cucumber (*Cucumis sativus* L.) and tomato (*Solanum lycopersicum* L.) in a controlled environment. Cucumber plants are considered salt-sensitive while tomato has been described as high resistant crop [9,10]. Therefore, the objective of this study is to determine the effects of salinity in the water relations in cucumber and tomato and to determine the possible mechanisms involved in stress tolerance. For that, some physiological parameters like root hydraulic conductance, relative water content, and fresh and dry weight ratio. In addition, sodium (Na) concentration in the tissues was determined.

## 2. Materials and Methods

### *2.1. Plant Material and Growth Conditions*

The experiments were carried out with plants of cucumber (*Cucumis sativus*) and tomato (*Solanum lycopersicum*). Seeds were pre-hydrated with deionized water with continuous aeration for 24 h. Then, the seeds were germinated in vermiculite under dark conditions in a 28 °C chamber for 2 days. After that, small plants were grown in hydroponic culture in a growth chamber under controlled conditions: 16-h light and 8-h dark cycle with temperatures of 25 and 20 °C and relative humidity of 80% and 60%, respectively. The photosynthetically-active radiation (PAR) was of 400 μmol $m^{-2}$ $s^{-1}$, provided by LEDs.

For each species, the experimental design consisted of 16 plants placed in 4 12 L containers of $n$ = 4 each one with Hoagland's nutrient solution aerated continuously, composed by: 6 $KNO_3$, 4 $Ca(NO_3)_2$, 1 $KH_2PO_4$ and 1 $MgSO_4$ (mM), and 25 $H_3BO_3$, 2 $MnSO_4$, 2 $ZnSO_4$, 0.5 $CuSO_4$, 0.5 $(NH_4)_6Mo_7O_{24}$ and 20 Fe-EDDHA (μM). The solution was replaced every week. After 2 weeks, NaCl was added to 2 of the containers until reaching a 60 mM concentration. The other 2 served as controls. The plants continued growing under these conditions for 12 days until sampling.

### *2.2. Root Hydraulic Conductance ($L_0$)*

Root hydraulic conductance ($L_0$) was measured on roots detached from the shoot, which were exuding under atmospheric pressure [11] for 10 min for control plants and 120 min for the NaCl treated ones. L0 was calculated as

$$L_0 = J_v/\Delta\Psi \tag{1}$$

where $J_v$ is the exuded sap flow rate and ΔΨ the osmotic potential difference between the exuded sap and the nutrient solution into which the plants were placed. The measurements were carried out 3 h after the onset of light. The $L_0$ value was expressed in $gH_2O \cdot g^{-1}$ root $DW \cdot MPa^{-1}$.

### *2.3. Relative Water Content (RWC)*

Relative water content (RWC) was calculated using a 1 $cm^2$ fragment from 4 fully developed leaves, in which fresh weight, full-turgor weight, and dry weight were measured. For the turgor weight, the fragments were kept in darkness and humidity in a 4 °C chamber for 24 h. For the dry weight, the fragments were placed in a 60 °C oven for 2 days.

### *2.4. Stomatal Conductance*

Stomatal conductance (mmol·$m^{-2}$·$s^{-1}$) was measured using the TPS-2 Portable Photosynthesis System (PP Systems, Inc., Amesbury, MA, USA). Each measure was taken in the second, third, and fourth fully expanded leaves.

### *2.5. Fresh Weight and Dry Weight Ratio*

Fresh weight (FW) and dry weight (DW) were measured from the shoot and root of each plant. For DW, each sample was placed in a 60 °C oven for 3 days. After this, the ratio between FW and DW was calculated.

### *2.6. Ions Concentration*

Dry shoots and roots were ground to a fine powder and were digested in a microwave oven (CEM Mars Xpress, NC, USA), by $HNO_3$: $HClO_4$ (2:1) digestion. The ions concentration (mmol/g DW) was detected by inductively coupled plasma (ICP) analysis (Optima 3000, PerkinElmer).

### *2.7. Statistical Analysis*

The statistical analysis of the previous parameters was carried out with 32 variables (2 species × 8 plants × 2 conditions). This analysis was performed using RStudio (RStudio PBC, Boston, MA, USA) with R version 4.1.0. All the parameters were analyzed using one-way ANOVA, followed by Duncan's multiple comparison test, determining significant differences between both treatments at $p \leq 0.05$.

## 3. Results and Discussion

The results showed that cucumber and tomato were affected differently by salinity stress. As an exception, $L_0$ under controlled conditions was considerably lower in plants treated with NaCl in both species, as can be seen in Figure 1a. This result indicated a high increase in water passage resistance from root to shoot in plants under salinity stress regarding the control ones [12].

However, the rest of the measured parameters gave different results for each species. RWC, which expresses the water balance in the tissues [13], did not significantly change in tomato plants (Figure 1b). However, in cucumber, this value decreased by almost 50% in salinity conditions. Similar results appeared with stomatal conductance, which only significative decreased in plants of cucumber subjected to salinity, as it appears in Figure 1c. In the case of FW/DW ratio, it declined both in shoots and roots in cucumber plants grown with salinity. In plants of tomato, this was only reduced in shoots, however, no significant differences were found in roots (Figure 1d). Finally, the concentration of Na in shoots and roots of both species was significantly higher in plants stressed by NaCl than in controls.

A remarkable fact in tomato plants grown under salinity is that a decrease of $L_0$ appeared but stomatal conductance did not significantly change. This could indicate that water movement inside the plant was maintained. In addition, in the same plants, RWC did not change in an opposite way than cucumber (they drop by almost half), indicating that in tomato the water state balance of a plant was maintained [13]. The analysis of the Na concentration was related to those results. Comparing these results by species, tomato plants treated with NaCl, both in root and shoot, the increment of concentration was nearly 50% lower than in cucumber plants. This factor reveals the possible existence of some mechanisms in the roots that avoid Na uptake. All these results lead us to confirm that tomato has a greater resistance to salinity.

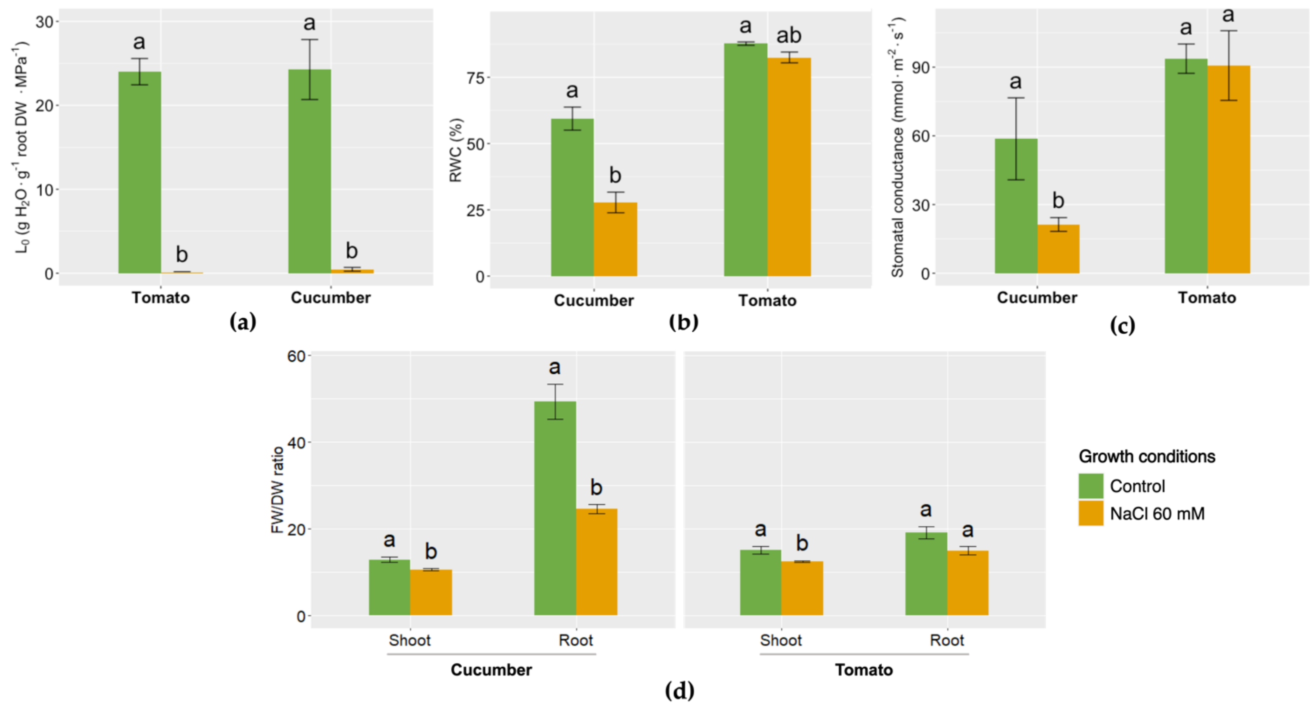


**Figure 1.** Physiological parameters: (**a**) Root hydraulic conductance ($L_0$), (**b**) relative water content (RWC), (**c**) stomatal conductance, and (**d**) FW/DW ratio in shoots and roots in control conditions and salinity conditions (NaCl 60 mM). Each bar represents the mean of 4 biological replicates ± SEM. Columns with different letters differ significantly according to Duncan's test ($p \leq 0.05$).

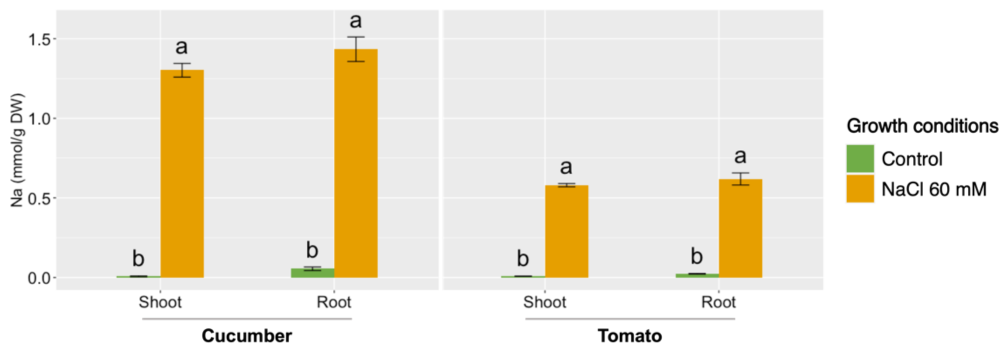


**Figure 2.** Na concentration in shoots and roots in control conditions and salinity conditions (NaCl 60 mM). Each bar represents the mean of 4 biological replicates ± SEM. Columns with different letters differ significantly according to Duncan's test ($p \leq 0.05$).

Membrane water transporters could have a significant influence on this better adaptation since may allow water passage through the cells even when osmotic imbalance blocks this movement by other pathways [6]. One of these transporters could be aquaporins. These are transmembrane proteins presented in most organisms, including higher plants, belonging to the MIP superfamily (major intrinsic proteins), that intervene in the water selective transport and other solutes [14–16]. Moreover, it has been shown that some tonoplast aquaporins can transport some ions into the vacuole, which could alleviate the osmotic imbalance [17]. However, it will be necessary to carry out more studies in order to confirm the possible implications of aquaporins in the water balance maintenance in the plant under salinity stress.

## 4. Conclusions

In light of all these results, the main conclusions of this study are:

1. The maintenance of the water balance in the plant has a considerable influence on the adaptation to salinity stress.
2. Tomato is able to resist salinity better than cucumber, as most of the water relations in the plant have not been altered.
3. Membrane water transporters, like aquaporins, could have a key role in relieving the harmful effects of salinity in the plant, although more in-depth studies will be needed in order to confirm this fact.

**Author Contributions:** Conceptualization, A.M.-A., M.C. and G.B.; methodology, A.M.-A., M.C. and G.B.; software, A.M.-A.; validation, M.C.; formal analysis, A.M.-A.; investigation, A.M.-A. and G.B.; resources, M.C.; data curation, A.M.-A.; writing—original draft preparation, A.M.-A.; writing—review and editing, A.M.-A. and M.C.; visualization, A.M.-A. and M.C.; supervision, M.C.; project administration, M.C.; funding acquisition, M.C. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was funded by the Spanish Ministry of Science by through the Strategic Programme "MISIONES" of CDTI Ref. MIP-20201045.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Acknowledgments:** The authors thank Ramiro Arnedo, S.A. (Spain). for providing the seeds.

**Conflicts of Interest:** The authors declare no conflict of interest

## References

1. Grene, R.; Provart, N.J.; Pardo, J.M. Editorial: Resistance to Salinity and Water Scarcity in Higher Plants. Insights From Extremophiles and Stress-Adapted Plants: Tools, Discoveries and Future Prospects. *Front. Plant Sci.* **2019**, *10*, 3. https://doi.org/10.3389/fpls.2019.00373.
2. Abdel-Farid, I.B.; Marghany, M.R.; Rowezek, M.M.; Sheded, M.G. Effect of Salinity Stress on Growth and MetabolomicProfiling of Cucumis sativus and Solanum lycopersicum. *Plants* **2020**, *9*, 19. https://doi.org/10.3390/plants9111626.
3. Imran, Q.M.; Falak, N.; Hussain, A.; Mun, B.G.; Yun, B.W. Abiotic Stress in Plants; Stress Perception to Molecular Response and Role of Biotechnological Tools in Stress Resistance. *Agronomy* **2021**, *11*, 20. https://doi.org/10.3390/agronomy11081579.
4. Chen, H.; Jiang, J.G. Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. *Environ. Rev.* **2010**, *18*, 309–319. https://doi.org/10.1139/a10-014.
5. Fernandez-Garcia, N.; Martinez, V.; Carvajal, M. Effect of salinity on growth, mineral composition, and water relations of grafted tomato plants. *J. Plant Nutr. Soil Sci.* **2004**, *167*, 616–622. https://doi.org/10.1002/jpln.200420416.
6. Djanaguiraman, M.; Prasad, P.V.V. Effects of Salinity on Ion Transport, Water Relations and Oxidative Damage. In *Ecophysiology and Responses of Plants under Salt Stress*; Ahmad, P., Azooz, M.M., Prasad, M.N.V., Eds.; Springer: New York, NY, USA, 2013; pp. 89–114.
7. Bidalia, A.; Vikram, K.; Yamal, G.; Rao, K.S. *Effect of Salinity on Soil Nutrients and Plant Health*; Springer-Verlag Singapore Pte Ltd.: Singapore, 2019; pp. 273–297.
8. Munir, N.; Hasnain, M.; Roessner, U.; Abideen, Z. Strategies in improving plant salinity resistance and use of salinity resistant plants for economic sustainability. *Crit. Rev. Environ. Sci.Technol.* **2021**, 47. https://doi.org/10.1080/10643389.2021.1877033.
9. Alpaslan, M.; Gunes, A. Interactive effects of boron and salinity stress on the growth, membrane permeability and mineral composition of tomato and cucumber plants. *Plant Soil* **2001**, *236*, 123–128. https://doi.org/10.1023/a:1011931831273.
10. Chen, T.W.; Pineda, I.M.G.; Brand, A.M.; Stutzel, H. Determining Ion Toxicity in Cucumber under Salinity Stress. *Agronomy* **2020**, *10*, 15. https://doi.org/10.3390/agronomy10050677.
11. Aroca, R.; Amodeo, G.; Fernandez-Illescas, S.; Herman, E.M.; Chaumont, F.; Chrispeels, M.J. The role of Aquaporins and membrane damage in chilling and hydrogen peroxide induced changes in the hydraulic conductance of maize roots. *Plant Physiol.* **2005**, *137*, 341–353. https://doi.org/10.1104/pp.104.051045.
12. Steudle, E.; Peterson, C. How does water get through roots? *J. Exp. Bot.* **1998**, *49*, 775–788. https://doi.org/10.1093/jxb/49.322.775.
13. González, L.; González-Vilar, M. Determination of Relative Water Content. In *Handbook of Plant Ecophysiology Techniques*; Reigosa Roger, M.J., Ed.; Springer: Dordrecht, The Netherlands, 2001; pp. 207–212.
14. Chaumont, F.; Tyerman, S. *Plant Aquaporins: From Transport to Signaling*; 2017; pp. 1–353.
15. Buchanan, B.; Gruissem, W.; Jones, R. *Biochemistry & Molecular Biology of Plants*, 2nd ed.; Wiley Blackwell: Chichester, UK, 2015; p. 1264.

16. Chaumont, F.; Tyerman, S. Aquaporins: Highly Regulated Channels Controlling Plant Water Relations. *Plant Physiol.* **2014**, *164*, 1600–1618. https://doi.org/10.1104/pp.113.233791.
17. Afzal, Z.; Howton, T.; Sun, Y.; Mukhtar, M. The Roles of Aquaporins in Plant Stress Responses. *J. Dev. Biol.* **2016**, *4*. https://doi.org/10.3390/jdb4010009.