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 plant growth and development as a result of a disruption of the homeostasis. Therefore, understanding the mechanism of plants for tolerating salinity stress is essential in order to develop new techniques that may improve the tolerance for optimizing crop yields. In this paper, we compare the response of cucumbers (*Cucumis sativus* L.) and tomatoes (*Solanum lycopersicum* L.), grown by hydroponic cultures, to a moderate salinity of NaCl 60 mM. For that, root hydraulic conductance, the relative water content of leaves (RWC), stomatal conductance, fresh weight and dry weight ratios, and Na concentrations in the 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 the Na content after the treatment. In addition, the stomatal conductance in cucumbers was reduced, accompanied by a decrease of the fresh/dry weight ratio in the roots. Conversely, neither of those parameters changed in tomatoes. These experiments confirm the evidence that cucumbers and tomatoes follow different strategies in their adaptation to salinity, tomatoes being 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; cucumbers; tomatoes

Citation: Martinez-Alonso, A.; Carvajal, M.; Barzana, G. Different Strategies to Tolerate Salinity Involving Water Relations. *Biol. Life Sci. Forum* **2022**, *11*, 41. https://doi.org/10.3390/IECPS2021-12035

Academic Editor: 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 plant areas [1]. Among all the abiotic factors, salinity is one of the most harmful for crop yields. Salinity stress causes various effects on plant physiology, such as a reduction of seed germination and plant growth as a result of osmotic stress [2]. In the early stages, plants under salinity conditions could experiment some disturbances, such as the inhibition of cell expansion or stomata closure [3]. If this exposure is prolonged in the long term, more serious phenomena could take place, including the reduction of physiological and metabolic activity, alteration of primary and secondary metabolites synthesis [2], early senescence, an increase of cytotoxic ions [3], and last, cell death [4]. Osmotic stress also affects the water balance, causing water deficit, the alteration of ion fluxes, or water potential reduction, which 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 tissues. At the physiological level, osmotic adjustment plays a fundamental role in maintaining the 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 ion 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 cucumbers (*Cucumis sativus* L.) and tomatoes (*Solanum lycopersicum* L.) in a controlled environment. Cucumber plants are considered salt-sensitive, while tomatoes have been described as a highly resistant crop [9,10]. Therefore, the objective of this study is to determine the effects of salinity in relation to water in cucumbers and tomatoes and to determine the possible mechanisms involved in stress tolerance. For that, we measure some physiological parameters like root hydraulic conductance, the relative water content, and fresh and dry weight ratios. In addition, the 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 cucumbers (*Cucumis sativus*) and tomatoes (*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: a 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 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 of: 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. The L_0 was calculated as

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

where J_v is the exuded sap flow rate and $\Delta\Psi$ 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 g^{-1} root DW MPa^{-1}.

2.3. Relative Water Content (RWC)

The 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

The 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 roots 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 into a fine powder and were digested in a microwave oven (CEM Mars Xpress, Holtsville, New York, NY, USA), by HNO_3:$HClO_4$ (2:1) digestion. The ion concentration (mmol/g DW) was detected by inductively coupled plasma (ICP) analysis (Optima 3000, PerkinElmer, Waltham, MA, USA).

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 cucumbers and tomatoes 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 roots to shoot in plants under salinity stress regarding the control ones [12].

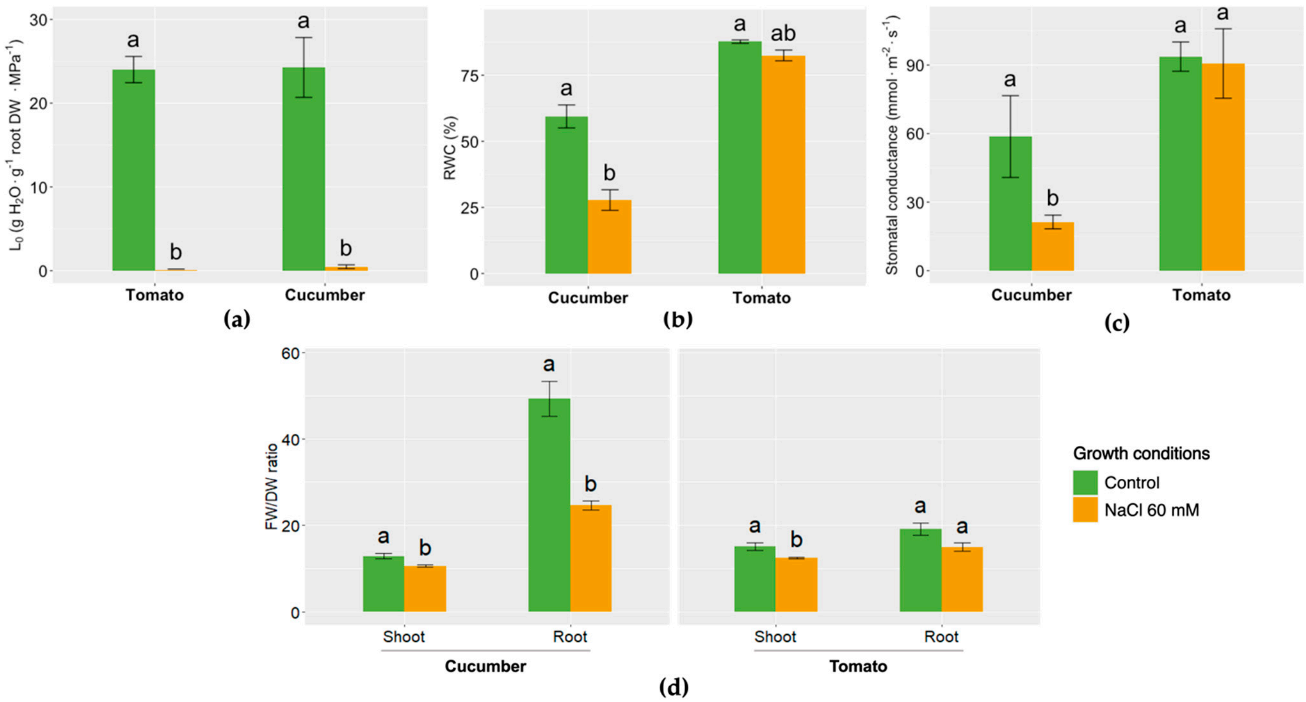

Figure 1. Physiological parameters: (**a**) root hydraulic conductance (L_0), (**b**) relative water content (RWC), (**c**) stomatal conductance, and (**d**) FW/DW ratio in shoots and roots in the control conditions and salinity conditions (NaCl 60 mM). Each bar represents the mean of 4 biological replicates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

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 cucumbers, this value decreased by almost 50%

in salinity conditions. Similar results appeared with stomatal conductance, which only decreased significantly in plants of cucumbers subjected to salinity, as appears in Figure 1c. In the case of the FW/DW ratio, it declined both in shoots and roots in cucumber plants grown with salinity. In plants of tomatoes, this was only reduced in shoots; however, no significant differences were found in the roots (Figure 1d). Finally, the concentration of Na in the shoots and roots of both species was significantly higher in plants stressed by NaCl than in the controls.

A remarkable fact of the tomato plants grown under salinity is that a decrease of L_0 appeared, but the stomatal conductance did not significantly change. This could indicate that the water movement inside the plant was maintained. In addition, in the same plants, RWC did not change in an opposite way compared to cucumbers (they drop by almost half), indicating that, in tomatoes, the water state balance of a plant was maintained [13]. The analysis of the Na concentration was related to those results (Figure 2). Comparing these results by species, for tomato plants treated with NaCl, both in roots and shoots, 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 tomatoes have a greater resistance to salinity.

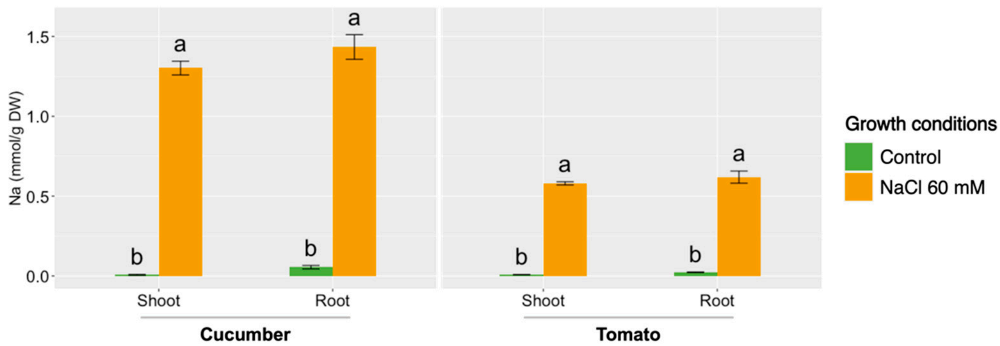

Figure 2. Na concentrations in shoots and roots in the control conditions and salinity conditions (NaCl 60 mM). Each bar represents the mean of 4 biological replicates ± SEM. Columns with different letters differ significantly according to Duncan's test ($p \leq 0.05$).

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 in 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 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 plants 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 plants has a considerable influence on their adaptation to salinity stress.
2. Tomatoes are able to resist salinity better than cucumbers, as most of the water relations in the plant are not 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.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/IECPS2021-12035/s1.

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.; and 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 through the Strategic Programme "MISIONES" of CDTI (Ref.: MIP-20201045).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability 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. [CrossRef] [PubMed]
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*, 1626. [CrossRef] [PubMed]
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*, 1579. [CrossRef]
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. [CrossRef]
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. [CrossRef]
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. [CrossRef]
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. [CrossRef]
10. Chen, T.W.; Pineda, I.M.G.; Brand, A.M.; Stutzel, H. Determining Ion Toxicity in Cucumber under Salinity Stress. *Agronomy* **2020**, *10*, 677. [CrossRef]
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. [CrossRef] [PubMed]
12. Steudle, E.; Peterson, C. How does water get through roots? *J. Exp. Bot.* **1998**, *49*, 775–788. [CrossRef]
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*; Springer: Cham, Switzerland, 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. [CrossRef] [PubMed]
17. Afzal, Z.; Howton, T.; Sun, Y.; Mukhtar, M. The Roles of Aquaporins in Plant Stress Responses. *J. Dev. Biol.* **2016**, *4*, 9. [CrossRef]