BIO Web of Conferences **17**, 00231 (2020) https://doi.org/10.1051/bioconf/20201700231
FIES 2019

Effect of calcium applications on ion accumulation in different organs of pepper plant under salt stress

F. Yasar* and *O.* Uzal

Van Yuzuncu Yıl University, Van, Turkey

Abstract. The present study was carried out in a climate chamber and water culture of physiology laboratory of Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Horticulture. Demre pepper varieties are used in the study of different levels of calcium (Ca) morphological and biochemical effects of pepper plants under salt stress were investigated. The study was carried out in 16/8 hour light / dark photoperiod, 25 ºC and % 70 humid climate chamber in controlled conditions. In order to determine the biochemical changes occurring in stressed plants, the amount of ions Na, K, Ca, Cl in root, stem and leaves of plants and Ca/Na ratio were determined. The accumulation of Na and Cl ions was found to decrease in root, stem and leaves as the dose of Ca increased. It can be said that increasing doses of Ca applications under salt stress are partially effective in reducing the negative effects of salt.

1 Introduction

NaCl reduces water potential and also adversely affects plant growth by disrupting the ion balance in the cell. High NaCl intake leads to an increase in the accumulation of Na + and Cl- ions in the cell and a decrease in Ca + 2, K + and Mg + 2 concentrations [1]. Na + entering the plant cell disrupts membrane potential and facilitates passive entry of extracellular Cl into the cell via anion channels [2, 3]. High salt concentrations decrease the calcium uptake and transport of the plant and cause calcium deficiency and ion imbalance in the plant [4, 5]. In plants under salt stress, it has been reported that potassium is a cofactor for many enzymes and may reduce the harmful effect of NaCl by external application of Ca [6].

Calcium is an element that has a positive effect on salt stress. High doses of exogenous calcium reduce the permeability of the cell membrane to Na + ion. In this way, the accumulation of sodium in the cell and plant is prevented by passive uptake [7, 8]. The common ideas of researchers trying to explain the role of calcium in the protection of salt stress through various mechanisms; calcium to strengthen the cell membrane and ion selectivity in the uptake and transport. Ca2 + ion, cross-linking the cell membrane with negatively charged basic groups and thus preserving the structural integrity of the cell membrane is also made in the description [4, 9]. Rengel [10] (1992) suggested that the calcium element binds to the cell membrane to control the permeability and prevents the release of calcium present in the cell.

In this study, different doses of Ca were applied to pepper plant exposed to salt stress and effects of applications on ion intake were determined.

2 Materials and methods

Demre sharp pepper variety was used in the study. The experiment was carried out in a split air-conditioned climate chamber with a humidity of 70 % and a temperature of 25±2 ºC in a normal atmosphere and in a water culture. In the study, first of all, pepper seeds were sown in 150x25x5 cm plastic seedlings (9 holes with a diameter of 0.5 cm) filled with pumice sieved, and watered with fountain water.

For the better development of the seedlings, the cotyledon leaves coming horizontally and the first true leaves began to be seen, irrigation was started with Hoagland nutrient solution [11]. Seedlings, which also formed the 2nd true leaves in the pumice environment, were taken to water culture in 25x25x18 cm size plastic cuvettes filled with Hoagland nutrient solution. Specially prepared and perforated for each seedling in plastic trays pepper seedlings were placed by wrapping with small sponge pieces. The trays were placed on the trays with the plant roots in the nutrient solution. The aeration was done by immersing the thin plastic hoses connected to the aquarium pump into the nutrient solution.

Seedlings were grown in water culture for two weeks and salt application was started on seedlings having 4–5 real leaves. The experiment was designed according to the exact chance design with three replications and 15 replicates per repetition.

NaCl was added to the nutrient solution (1/2 Hoagland) with a salt concentration of 75 mM. During the regeneration of the repeating solutions every week, the same concentration of salt applications was maintained. Pepper seedlings with sal*t (NaCl) 5 different doses (150*, 200, 250, 300, 350 pp*m) Ca was added. As a result, 6* different applications were performed as

* Corresponding author: fyasar@yyu.edu.tr

control, salt + Ca (150 ppm, 200 ppm, 250 ppm, 300 ppm, 350 ppm). The nutrient solution used was prepared according to [11]. The ppm values of all nutrients are given in Table 1.

Table 1. Contents of the nutrient solution used (ppm).

Elements	App. 1 Control (ppm)	App. 2 Ca1+Salt (ppm)	App. 3 Ca2+ Salt (ppm)	App. 4 Ca3+Salt (ppm)	App. 5 Ca4+Salt (ppm)	App. 6 Ca5 + Salt (ppm)
Nitrogen (N)	186	186	186	186	186	186
Phosphorus (P)	31	31	31	31	31	31
Potassium (K)	167	167	167	167	167	167
Magnesium (Mg)	49,28	49,28	49,28	49,28	49,28	49,28
Calcium (Ca)	**200**	**150**	**200**	**250**	**300**	**350**
Sulfur (S)	66	66	66	66	66	66
Iron (Fe)	3.3	3.3	3.3	3.3	3.3	3.3
Manganese (Mn)	0.031	0.031	0.031	0.031	0.031	0.031
Boron (B)	0.205	0.205	0.205	0.205	0.205	0.205
Copper(Cu)	0.015	0.015	0.015	0.015	0.015	0.015
Zinc (Zn)	0.023	0.023	0.023	0.023	0.023	0.023

Sampling for analyzes was performed on the 20th day of salt application. Na^+, K^+, Ca^{+2}, and Cl^- contents were determined in these samples.

3 Mineral element analysis

For the ion concentration determinations, fresh root, stem, and leaf samples were extracted in 0.1 N of nitric acid. The sodium, potassium (K+) and calcium (Ca+2) concentrations were measured by atomic absorption spectrometry [12], Cl ion was measured by an automatic chloridometer (Buchler – Cotlove chloridometer) which was analyzed by colorimetric amperometric titration with silver ions. Ion amounts in the fresh plant samples were determined as fresh weight (g/mg Fresh Weight) [13].

In order to evaluate the data obtained as a result of the study, Statgraphics was subjected to variance analysis in statistical analysis package program. Duncan multiple comparison test (P <0.05).

4 Results

At the end of 20 days of 75 mM NaCl stress application, it was observed that there were differences between the plant organs (root, stem and leaf) in terms of Na +, K, Ca, Ca / Na and Cl accumulation (Tables 2–5 and 6).

When the Na ion in the root was examined, the lowest value in terms of Na ion was obtained from Ca5 + Salt (2.474) application compared to the control group (1.125), while the highest value was observed in Ca3 + Salt (3,369) application. When the Na ion in the stem was examined, the lowest value in terms of Na ion was obtained from Ca2 + Salt (2.105) application compared to the control group (1.226), while the highest value was observed in Ca1 + Salt (7.169) application. When the Na ion in the leaf was examined, the lowest value in terms of Na ion was obtained from Ca3 + Salt (2.697) application compared to the control group (0.764), while the highest value was measured in Ca1 + Salt (4.839) application. Na deposition in the roots, stem and leaves of all applications increased compared to the control group. In general, the amount of Na ion was found to accumulate more in the stem.

Table 2. Na ion accumulations determined in plant organs (μ g/mg T.A.)

Application	Root Na	Stem Na	Leaf Na	P Value
Control	1.125 C a	1.226 E a	0.764 B a	0.1170*
Ca 1+ NaCl	2.886 AB b	7.169 A a	4.839 A ab	0.0381*
Ca 2+ NaCl	2.786 AB a	2.105 DE a	4.113 A a	0.1546*
Ca 3+ NaCl	3.369 A a	3.448 CD a	2.697 AB a	0.3875*
Ca 4+ NaCl	3.074 AB b	5. 516 B ab	4.429 A b	0.0226*
Ca 5+ NaCl	2.474 B b	3.698 C a	2.963 AB ab	0.0668*
P Value	0.0005*	0.0000*	0.0175*	

Differences between means with the same superscript capital letter in the same column are not significant at P ≤ 0.05. Differences between means with the same superscript small letter in the same row are not significant at P ≤ 0.05.

Table 3. K ion accumulations determined in plant organs (μ g/mg T.A.)

Application	Root K	Stem K	Leaf K	P Value
Control	5.493 A a	10.457 A b	9.388 AB b	0.0010*
Ca 1+ NaCl	2.374 C a	3.157 D b	7.423 B b	0.0009*
Ca 2+ NaCl	2.996 BC b	4.721 CD b	9.905 AB a	0.0207*
Ca 3+ NaCl	3.884 B b	6.734 BC b	12.505 A a	0.0048*
Ca 4+ NaCl	3.617 B c	7.132 B b	9.781 AB a	0.0010*
Ca 5+ NaCl	3.514 BC c	5.069 CD b	7.124 B a	0.0027*
P Value	0.0019*	0.0001*	0.0724*	

Differences between means with the same superscript capital letter in the same column are not significant at P ≤ 0.05. Differences between means with the same superscript small letter in the same row are not significant at P ≤ 0.05.

When the K ions in the root were examined, the lowest value in terms of K ions was observed in Ca1 Salt (2,374) application and the highest value was observed in Ca 3 Salt (3,884) application compared to the control (5,493) group. When looking at the amount of K in the stem (10.457) compared to the highest value of Ca 4 Salt (7.132) application, the lowest value was seen in Ca 1 Salt (3.157) application. When the K ion in the leaf was examined, the lowest amount of K ion was observed from Ca 5 Salt (7.124) application and the highest value was observed in Ca 3 Salt (12.505) application. Among the organs, K ion was found to accumulate mostly in the leaves. In all other applications, it was determined that the accumulation of K ions in the root and stem decreased compared to the control group.

When the Ca ion in the root was examined, the highest value was found in Ca 5+ Salt (3.642) application and the lowest value was found in Ca 1 + Salt (0.798) application compared to control (4.245) group. Compared to the control group, the accumulation of calcium ions in the root was reduced in all other applications. When the amount of Ca in the stem was examined, the highest value was seen in Ca 1 + Salt (2.343) application and the lowest value was found in Ca 2 + Salt (0.813) application compared to control (1.693) group. When the amount of Ca in the leaves was examined, the highest value was found in Ca 4 + Salt (3,445) application and the lowest value was found in Ca 3 + Salt (1,867) application compared to control (2.105) group. Compared to the control group, it was observed that the calcium ion accumulation in the leaf increased in all applications except Ca 3+ Salt application.

Table 4. Ca Ion accumulations determined in plant organs (µ g/mg T.A.)

Application	Root Ca	Stem Ca	Leaf Ca	P Value
Control	4.245 A a	1.693 BC b	2.105 BC b	0.0067*
Ca 1+ NaCl	0.798 C b	2.343 A a	2.807 AB a	0.0006*
Ca 2+ NaCl	2.934 B a	0.813 D b	2.772 AB a	0.0009*
Ca 3+ NaCl	2.553 B a	1.319 C b	1.867 C ab	0.0580*
Ca 4+ NaCl	3.561 AB a	2.010 AB b	3.445 A ab	0.0689*
Ca 5+ NaCl	3.642 AB a	1.665 BC c	2.447 BC b	0.0000*
P Value	0.0007*	0.0001*	0.0167*	

Differences between means with the same superscript capital letter in the same column are not significant at $P \leq 0.05$. Differences between means with the same superscript small letter in the same row are not significant at $P \leq 0.05$.

Table 5. Ca^+ / Na^+ ratio determined in plant organs (µ g/mg T.A.)

Application	Root Ca/ Na	Stem Ca/Na	Leaf Ca/Na	P Value
Control	4.386 A a	1.384 A c	2.751 A b	0.0002*
Ca 1+ NaCl	0.279 D b	0.337 B b	0.651 B a	0.0016*
Ca 2+ NaCl	1.063 BC a	0.388 B c	0.744 B b	0.0061*
Ca 3+ NaCl	0.754 CD a	0.397 B b	0.708 B a	0.0310*
Ca 4+ NaCl	1.257 BC a	0.362 B c	0.821 B b	0.0029*
Ca 5+ NaCl	1.527 B a	0.452 B b	0.837 B b	0.0033*
P Value	0.0000*	0.0000*	0.0000*	

Differences between means with the same superscript capital letter in the same column are not significant at $P \leq 0.05$. Differences between means with the same superscript small letter in the same row are not significant at $P \leq 0.05$.

It was observed that Ca + / Na + ratio in root, stem and leaves decreased in all applications compared to control group. The highest Ca + / Na + ratio was observed in Ca 5 + Salt application after control application.

Table 6. Cl ion accumulations determined in plant organs (µ g/ mg T.A.)

Application	Root Cl	Stem Cl	Leaf Cl	P Value
Control	0.238 C a	0.302 C a	0.369 D a	0.3486*
Ca 1+ NaCl	1.41 AB b	2.759 A a	2.584 A a	0.0025*
Ca 2+ NaCl	1.311 AB b	2.643 A a	1.745 B b	0.0054*
Ca 3+ NaCl	1.113 B b	1.515 B a	1.244 C b	0.0270*
Ca 4+ NaCl	1.605 A b	2.566 A a	2.220 A a	0.0121*
Ca 5+ NaCl	1.602 A a	1.893 B a	1.523 BC a	0.1232*
P Value	0.0000*	0.0000*	0.0000*	

Differences between means with the same superscript capital letter in the same column are not significant at $P \leq 0.05$. Differences between means with the same superscript small letter in the same row are not significant at $P \leq 0.05$.

When the Cl ions in the root were examined, the lowest value in terms of Cl ions was obtained from Ca 3 + Salt (1,113) application and the highest value was observed in Ca 4 + Salt (1.605) application compared to the control (0.238) group. When the Cl ions in the stem were examined, the highest value was obtained from Ca 1 + Salt (2.759) application and the lowest value was found in Ca 3 + Salt (1.515) application compared to the control (0.302) group. When the Cl ion accumulation in the leaf was examined, the lowest value of this ion was observed in Ca 3 + Salt (1.244) application and the highest value was observed in Ca 1 + Salt (2.584) application compared to the control (0.369) group. In general, the amount of Cl ion was found to accumulate more in the stem part.

5 Conclusion

The results show that calcium uptake and transport in plants with high concentrations of salt is reduced. Thus leading to calcium deficiency and ion imbalance in the plant [4, 5]. Since calcium is an element having a positive effect on salt stress, high dose exogenous calcium decreases the permeability of the cell membrane against Na + ion, prevents the accumulation of sodium in the cell and in the plant by passive uptake and provides ion balance [7, 8], therefore, calcium protects plants against salt stress, and therefore plants are less affected by salt stress.

It is one of the most important reasons for the decrease and damage in the growth of pepper plants grown under the stress of salt, which is the amount of Na that they take from the environment by means of roots and accumulate in their bodies in excess and toxic level. Especially in low dose Ca applications, pepper plants accumulated higher Na in their stems. When the organs of the plants were examined in terms of Na accumulation, there was a balanced distribution of Na between all three organs and decreased intake. In the 4th application, it is seen that the ion uptake is reduced and there is a balanced distribution. Ion regulation is one of the most important features seen in plants with high tolerance to salt.

The results obtained in our study showed that the appropriate Ca dose (4th application) adjusted the ion uptake and distribution well. Plants with high NaCl salt concentration receive excessive Na ions. The uptake of Ca and K ions, which is very similar to sodium ion due to their ionic diameters and electrical charges, is prevented.

On the other hand, if the appropriate Ca application is performed, the uptake of low Na and Cl ions under salt conditions as well as higher K and Ca uptake increases are the key mechanisms of tolerance. Tissues of plants with better tolerance to salt stress are generally capable of producing a higher Ca / Na and K / Na ratio. This means that plants can be protected from the negative effects of salt stress if Ca is applied at the appropriate rate. Many studies to determine tolerance to salt stress in plants (eggplant, beans, melons, tomatoes and peppers), K / Na, Ca / Na ratios in different plant organs and the determination of Na concentrations in plant tissues is an important parameter [14–17].

One of the most striking results in our study; As the Ca ion dose increases, there is an increase in the amount of K ions in the roots, stems and leaves of pepper plants. At the same time, it was observed that there was an increase as it went up from the roots to the leaves. Especially in the 3rd dose of Ca, there was a significant increase in K accumulation in the leaves compared to the control.

In plants grown in soil conditions where salt is high, a high proportion of Na ions are taken by the roots and the amount of this ion is increased by transporting this ion to the plant organs. Due to the decrease in K and Ca uptake and transport, their proportion in plant organs decreases. While Ca and K ions play a key role in physiological events, Na has no effect as a nutrient. In addition, the increase of Na ion against K and Ca ions causes ion imbalance [18].

The application of Ca in different doses of nutrient solution given to pepper plants grown in salt stress environment revealed differences in Ca intake of plants naturally. The main objective, however, is to determine which dose of Ca reduces the salt stress effect and which dose will achieve ion balance in Ca uptake. In our study, different results were obtained at different doses. There was no systematic increase in doses in all three organs. In particular, a reduction in intake of Ca 4 was observed. Calcium is less accumulated in the stem, while it is higher in the roots and leaves. Ca / Na ratio is very important in the process uptake of calcium from the roots.

The presence of high amounts of sodium in the nutrient medium reduces calcium uptake. In such saline conditions, when extra Ca is given to plants, it may be mentioned that sodium in the plasma of the root hairs can be replaced by calcium, and it is stated that there may be decreases in Na uptake and therefore stress-reducing effect [19]. Yaşar et al. [20] under salt stress, two sensitive and two tolerant eggplant varieties used in the study, Na and Cl ion accumulation was found to be higher in the genotype sensitive. They reported a decrease in the K and Ca amounts of these genotypes. Tolerant genotypes showed a decrease in Na and Cl uptake and an increase in K and Ca uptake. Yaşar et al., [21, 22], Üzal, [23], Üzal and Yıldız, [24] showed that K and Ca uptake had a significant effect on salt stress tolerance in plants.

It was observed that there are differences in Cl accumulation in root, stem and leaves of pepper plants according to calcium doses. Cl increased in all organs compared to control. For Cl, we cannot see a systematic decrease or increase due to the increase in Ca doses. However, a more important result is that the 4th dose of Ca also proved to be the most appropriate dose in Cl uptake. The 4th dose had a significant positive effect on both Cl intake and distribution. The closest control value in terms of Cl uptake was obtained from plants grown at the 4th dose of Ca. It has been reported that Cl accumulation increases in plants under salt stress, but in studies conducted in different species, the increase in tolerant ones is less [14–16, 23].

The results obtained from these studies and our studies showed that the 4th dose of Ca decreased Cl intake and thus provided a tolerance to salt stress in plants. As a result, proper doses of Ca can protect the plant from the toxic effect of salt by maintaining ion balance in the cell, even under salt stress.

References

1. A.K. Parida, A.B. Das, *Salt Tolerance and Salinity Effects on Plants, a Review*, Ecotoxicol. and Environm. Safety, **60**, 324–349 (2005)
2. X. Niu, R.A. Bressan, P.M. Hasegawa, J.M. Pardo, *Ion Homeostasis in Nacl Stress Environments*, Plant Physiol., **109(3)**, 735 (1995)
3. N. Tuteja, *Mechanisms of High Salinity Tolerance in Plants*, Meth. in Enzymol., **428**, 419-438 (Academic Press, 2007)
4. G.R. Cramer, A. Läuchli, E. Epstein, *Effects of Nacl and Cacl2 on İon Activities in Complex Nutrient Solutions and Root Growth of Cotton*, Plant Physiol., **81(3)**, 792–797 (1986)
5. J. Huang, R.E. Redmann, *Solute Adjustment to Salinity and Calcium Supply in Cultivated and Wild Barley*, J. of Plant Nutrit., **18(7)**, 1371–1389 (1995)
6. P.M. Hasegawa, R.A. Bressan, J.K. Zhu, H.J. Bohnert, *Plant Cellular and Molecular Responses to High Salinity*, Annual Review of Plant Biol., **51(1)**, 463–499 (2000)
7. R. Hoffmann, J. Tufarıello, M.A. Bısson, *Effect of Divalent Cations on Na+ Permeability of Chara Corallina and Freshwater Grown Chara Buckellii*, J. of Experim. Botany, **40(8)**, 875–881 (1989)
8. J. Whittington, F.A. Smith, *Calcium-Salinity İnteractions Affect İon Transport in Chara Corallina*, Plant, Cell & Environment, **15(6)**, 727–733 (1992)
9. A. Lauchil, *Calcium, Salinity and the Plasma Membrane*, Calc. in Plant Growth and Developm., 26–35 (1990)
10. Z. Rengel, *The role of calcium in salt toxicity*, Plant, Cell and Environm., **15**, 625–632 (1992)
11. D.R. Hoagland, D.I. Arnon, *The Water Culture Method for Growing Plants Without Soil*, Circular California Agricult. Experim. Station., **1**, 347–461 (1938)
12. B. Kacar, *Bitki ve Toprağın Kimyasal Analizleri. III Toprak Analizleri* (Ankara Üniversitesi Ziraat Fakültesi Eğitim, Araştırma ve Geliştirme Vakfı Yayınları: 3, Ankara, 1994)
13. E. Taleisnik, G. Peyran, C. Arias, *Respose of Chlorisgayana Cultivars to Salinity. 1. Germination and Early Vegetatif Growth*, Tropical Grassland, **31**, 232–240 (1997)
14. F. Yaşar, *Invitro And In Vivo Investigation Of Some Antioxidant Enzyme Activities In Atiplant Genotics Under Salt Stress*, PhD Thesis Not Published (Van Yuzuncu Yil University, Institute of Science and Technology, Van, 2003)
15. H. Aktas, K. Abak, I. Cakmak, *Genotypic Variation in The Response of Pepper to Salinity*, Sciantia Hort, **110**, 260–266 (2006)
16. Ş. Kuşvuran, Ş. Ellialtıoğlu, K. Abak, F. Yaşar, *Responses of Some Melon (Cucumis Sp.) Genotypes to Salt Stress*, J. of Agricult. Sci. (Turkey, 2007)

17. H.Y. Dasgan, S. Koc, *Evaluation of Salt Tolerance in Common Bean Genotypes by İon Regulation and Searching for Screening Parameters,* J. of Food, Agricult. Environm., **7(2)**, 363–372 (2009)
18. G.N. Al-Karaki, *Growth, Water Use Efficiency, and Sodium and Potassium Acquisition by Tomato Cultivars Grown Under Salt Stress,* J. of Plant Nutrit., **23(1)**, 1–8 (2000)
19. C. Kemmler, A. Krauss, *K and stress tolerance Büntheof Agriculture,* Research station of Kali und Salz A.C. Bünteweg 8, D-3000 (Hannover, 1971)
20. F. Yaşar, S. Ellialtıoglu, S. Kusvuran, *Ion and Lipid Peroxide Content in Sensitive and Tolerant Eggplant Callus Cultured Under Salt Stress,* Europ. J. of Hortıcult. Scı., **71(4)**, 169–172 (2006a)
21. F. Yasar, O. Uzal, S. Tufenkci, K. Yildiz, *Ion Accumulation in Different Organs of Green Bean Genotypes Grown Under Salt Stres,* Europ. J. of Hortıcult. Scı., **71**, 169–172 (2006b)
22. F. Yasar, O. Uzal, O. Yasar, *Identification of Ion Accumulation and Distribution Mechanisms in Watermelon Seedlings (Citrullus lanatus (Thunb.) Mansf.) Grown under Salt Stress,* Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, **23(3)**, 209–214 (2013)
23. Ö. Üzal, *Effects of Jasmonic Acid on Plant Growth and Antioxidant Enzyme Activities in Some Strawberry Cultivars Grown Under Salt Stress,* PhD dissertation thesis (Graduate School of Natural and Applied Sciences, Department of Horticulture, Van, 2009)
24. Ö. Üzal, K. Yıldız, Bazı Çilek *(Fragaria X Ananassa L.) Çeşitlerinin Tuz Stresine Tepkileri. Reactions to Salt Stress of Some Strawberry (Fragaria X Ananassa L.),* Cultivars Yüzüncü Yıl Univer. J. of Agricult. Sci., **24(2)**, 159–167 (2014)