International Journal of Secondary Metabolite
2024, Vol. 11, No. 1, 144–156

https://doi.org/10.21448/ijsm.1335099

journal homepage: https://dergipark.org.tr/en/pub/ijsm

Research Article

Effect of Na, Mg, Ca chloride salts on mineral element, proline and total protein contents in rice (*Oryza sativa* L.) grown *in vitro*

Mehmet Yusuf Orcan [1*], **Pınar Orcan** [2]

[1]Batman University, Food Analyzes Application and Research Center, Batman, Türkiye
[2]Batman University, Technical Science Vocational School, Department of Food Process, Batman, Türkiye

ARTICLE HISTORY

Received: Aug. 01, 2023
Accepted: Jan. 03, 2024

KEYWORDS

Mineral element,
Rice,
Prolin,
Salinity stress,
Total protein.

Abstract: In this study, the effects of different types and concentrations of salts on local Siverek rice plant (*Oryza sativa* L.) grown *in vitro* were investigated in terms of mineral elements (K, Ca, P, Mg, Na, Fe, Cu, Zn, Mn, Mo, Co), proline, and total protein content. Sterilized seeds were planted in hormone-free and salt-free MS medium. After one week, the seedlings were subjected to different concentrations of NaCl, $CaCl_2$, and $MgCl_2$ salts (0, 30 mM, 90 mM) in order to evaluate the effect of salinity on plant growth and development. In response to salt stress, a decrease in nutrient elements was observed for all three types of salt compared to the control group, which can be attributed to disruptions in ion balance. Changes in element levels generally showed varying levels of increase or decrease depending on both the type and concentration of the salt and these changes were statistically significant. The increase in proline level was found to be directly proportional to the changes in the amounts of Ca, Mg, K, and Na elements. Both total protein and proline content showed the lowest values for all salt concentrations with $CaCl_2$, while the highest values were obtained with NaCl. In conclusion, the changes in the level of mineral elements, total protein, and proline content levels, which decrease or increase in different ratios, depending on the type and concentration rising of the salt, are associated with the varying tolerance of the plant to different types of salts.

1. INTRODUCTION

Soil salinity is one of the environmental stresses worldwide adversely affecting plant growth and productivity. Approximately one billion hectares of land are affected by salt stress globally (Fageria *et al*., 2012), and this number is increasing every year. The significant impact of high salinity on crop growth and development is of great concern, as it can affect agricultural productivity on more than 20% of the global cultivated land (Botella *et al*., 2005; Fahad *et al*., 2019; Dramalis *et al*., 2021).

*CONTACT: Mehmet Yusuf ORCAN ✉ mehmetyusuf.orcan@batman.edu.tr Batman University, Food Analyzes Application and Research Center, Batman, Türkiye

e-ISSN: 2148-6905 / © IJSM 2024

Nutrient depletion, disruption of soil aggregates, and impairment of plant growth and development are among the main effects of soil salinization (Kordrostami *et al*., 2017). Salinity impedes plant growth through three main principles; namely, 1) water deficiency (water stress), 2) ion toxicity, and 3) intracellular fluid due to imbalances in ion transport disruption of the mineral balance (Marschner, 1995; Akyol *et al*., 2020). Osmotic stress results from reduced water availability due to salt stress, which leads to impaired nutrient transport efficiency, including macro elements such as nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca). This, in turn, leads to nutrient deficiency and ion toxicity (Razzaq *et al*., 2020). Salinity exerts diverse stresses on crops, leading to imbalances in nutrient uptake, toxic ion accumulation, and exposure to oxidative and osmotic stress (Wang *et al*., 2012; Singh *et al*., 2018). When ions expand in the soil, plants are exposed to salt stress. These ions accumulate at higher levels, leading to a decrease in essential ions within the plants. Consequently, this disrupts the relationship between the plant and water by inducing conditions resembling drought, causing osmotic stress. This stress factor is accountable for the reduction in stomatal conductance and the activities of photosynthetic enzymes, ultimately triggering the generation of reactive oxygen species (ROS) in plants (Hasanuzzman *et al*, 2021). ROS have the potential to harm cellular components such as cell membranes, proteins, lipids, and genetic materials (DNA and RNA). Additionally, they may trigger programmed cell death (Kumar *et al*, 2020). While the primary response mechanism to osmotic degradation is recognized, the exact molecules involved and their specific roles are yet to be fully elucidated. The cytosolic Ca++ level increases in response to osmotic imbalance by unknown sensors, which is sensed by SOS2 SOS3 (salt overly sensitive) protein complex. SOS2-SOS3 complex activates the SOS1 protein by phosphorylate. SOS1 functions as a Na^+/H^+ antiporter protein located in the cell membrane, facilitating the expulsion of Na ions from the cell. SOS2 can also regulate the activity of NHX1 (Na/H antiporter) and V-ATPase (vacuoler type ATPase) antiport independently of SOS3. It can be regulated by SCaBP (SOS liked Ca^{++} bindings protein), which targets the tonoplast. Salinity can lead to an increase in ABA (abscisic acid) accumulation, which is activated by ABI1 and ABI2, negatively affecting the SOS system and NHX1 (Shanker, 2011).

Rice (*Oryza sativa* L.) is one of the most important food sources, and there are still countries that cover a considerable part of their energy requirements from this plant or its derivatives. Salinity stress affects rice in several ways, causing changes in the physiological and morphological structures of cells, as well as affecting the synthesis of certain biochemicals (Kumar *et al*., 2012; Liu *et al*., 2010; Nam *et al*., 2012; Pani *et al*., 2012; Rajendran *et al*., 2009; Wang *et al*., 2013; Orcan *et al*., 2017; Mondal *et al*., 2018). Saline soils lead to ion imbalances, which negatively affect both the growth and yield of rice plants and their nutrient content, thus affecting the overall quality of the crop (Rao *et al*., 2013).

The studies on salt stress in rice generally focus on the effects of NaCl, and there is a lack of research on the effects of $CaCl_2$ and $MgCl_2$ salts. In addition, there are no studies on the content of mineral elements in local Karacadağ rice (Siverek population) under the influence of NaCl and various salts. Therefore, the aim of this study was to investigate the effects of different salt types (NaCl, $CaCl_2$, and $MgCl_2$) and concentrations (0, 30, 90 mM) on the content of important micro- and macro-mineral nutrients such as potassium (K), calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), molybdenum (Mo), and cobalt (Co), as well as on the total protein and proline amounts in the local Karacadağ rice (Siverek population) under salt stress.

2. MATERIAL and METHODS

2.1. Seed Sterilization

The Karacadağ rice variety, which was obtained from local growers near Diyarbakır, Türkiye, served as the plant material in this study. Prior to the experiments, the rice seeds underwent a 30-second soak in 70% ethanol, followed by optimal surface sterilization through a 60-minute soak in 5% NaOCl.

2.2. *In vitro* Conditions

MS medium was prepared with MS main solution, MS-1, MS-2, complex chelator, vitamin mixture, and B1 vitamin containing thiamine. The nutrient media were formulated by combining 30 g of sucrose and 5,458 g of agar with 1liter of distilled water. The medium was adjusted to pH 5,7 by using acid and base. The prepared medium was autoclaved at 121^0C and 1 atm pressure for 25 minutes. The final solution was transferred to a Magenda GA-7 (purchased from Merck KGaA, Darmstadt, Germany) culture vessel in portions of approximately 50-60 mL.

2.3. Plant Growth

The sterilized seeds were planted in magendas containing hormone-free and salt-free MS medium to germinate. After 1 week of germination, in order to evaluate the effect of salinity stress on plant growth and development, the seedlings were transferred to a culture vessel containing separately NaCl, $CaCl_2$ and $MgCl_2$ salts at different concentrations (control, 30 mM, 90 mM). Rice seedlings were grown in salt-free medium for 3 weeks as a control and under salt stress conditions. At the end of the 3-week growth period, the green part and the roots of the *in vitro* grown plants were harvested.

2.4. Determination of Mineral Element Contents

Harvested and dried samples were weighed. To determine elemental contents, weighed samples were digested in 7mL nitric acid (65% HNO_3) and 1mL hydrochloric acid (37% HCl) using a microwave digester. Digested samples were adjusted to 15mL by adding extra pure water. The samples were then analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) to quantify the elements.

2.5. Determination of Proline Content

In accordance with the technique outlined by Bates *et al*. (1975), proline determination was conducted. The samples were subjected to protein precipitation by sulphosalicylic acid treatment, followed by centrifugation, and the supernatant was then transferred to a fresh tube. After reacting the supernatant with glacial acetic acid and ninhydrin reagent, the reaction was stopped by cooling the tubes on ice. The proline products were subsequently extracted with toluene by vortex mixing, and the absorbance of the toluene phase was recorded at 518 nm. Proline concentrations were computed using a set of proline standards (0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 mM).

2.6. Determination of Total Protein Content

For protein extraction, leaves were treated with an extraction buffer comprising 0.01 M Tris-HCl, 10% glycerol, 5% PVP, and 1% Triton X 100 at pH 6.8. Subsequently, the total protein content of the extracts was quantified according to the Bradford method (Bradford, 1976).

2.7. Statistical Analysis

Triplicate experiments were conducted for all measurements, and the data were reported as mean ± standard deviation (SD). Statistical analysis was carried out using SPSS 20.0 for Windows (SPSS Inc., Chicago, USA), with the significance of differences tested using one-way analysis of variance (ANOVA) and Duncan test.

3. RESULTS

3.1. Mineral Element Contents

Under saline conditions, the reduction in the osmotic potential of the soil solution leads to a reduction in the water potential and thus a reduction in water uptake by plants. It is known that this situation is caused by direct toxic effects of Na and similar cations present in the environment and by the reduced uptake of K, Ca and N, leading to disturbances in the ionic balance (İnal *et al*., 1995; Yakıt & Tuna, 2006). The changes in mineral contents in the Siverek rice population exposed to NaCl, $CaCl_2$, and $MgCl_2$ salts at concentrations of 0, 30, and 90 mM are shown in Table 1. High Na levels in the soil solution can reduce the uptake and mobility of K by inhibiting its transport to the growth zone, leading to a decrease in growth quality of both vegetative and reproductive organs (Dionisio & Tobita, 2000). Ghoulam *et al*. (2002) on sugar beet, Lacerda *et al*. (2002) on sorghum and Essa (2002) on soybean reported an increase in Na content in leaves and roots due to increased Na concentration, while the content of cations such as Ca and K decreased under NaCl salt stress. In our study, the Siverek rice treated with NaCl at all concentrations showed a decrease in the elements Ca, K and Mg. In varieties of *Medicago sativa* (alfaalfa), an increase in the concentration of NaCl led to a decrease in the contents of Ca and Mg. (Bhattarai *et al*., 2022). A decrease in Ca content was observed in rice varieties with varying levels of salt tolerance in response to salt stress, while the change in K content in the leaves of the treated plants was not statistically significant (Thu *et al*., 2017). Mokabel *et al.* (2022) reported that using the microbiome to alleviate the effects of salt stress in Solanum melongena (eggplant) resulted in a significant reduction in Ca and K levels compared to the control group. In addition, they observed a decrease in Mg content in plants treated with 200 mM NaCl, but this decrease was not statistically significant. These studies emphasize the importance of understanding how individual plant species respond to salt stress and mineral nutrient content. Bernstein *et al*. (2017) reported that 30 mM NaCl stress in basil (*Ocimum basilicum*) slowed plant growth, decreased Ca and K concentrations, and increased Na concentration. Similarly, in lentil (*Lens esculenta* Moench) treated with different chlorine salts ($CaCl_2$, $MgCl_2$, and NaCl), increasing concentrations led to a decrease in germination rate, N, Ca, Mg, and K content, while increasing Na content (Karaman & Kaya, 2015). Çalışkan *et al*. (2017) subjected basil (*Ocimum basilicum* L.) to salt stress using different concentrations of NaCl, $CaCl_2$ and $MgCl_2$ (between 1 and 8 ds/m). The lowest root weight was obtained at the highest concentration for each salt type, and the highest root and leaf weight were observed in the control group. The importance of salt stress levels for basil plant development was emphasized by the researchers. In our study, there was a decrease in the amounts of K and Mg elements under $CaCl_2$ salt stress and a decrease in Ca and K elements under $MgCl_2$ stress compared to the control group. The decrease in element quantities under both types of salt stress can be attributed to a disturbance of the ionic balance.

Due to the competition between Na and especially cationic elements such as K and Ca, the balance of Na^+/K^+ and Na^+/Ca^{++} is quickly disturbed. This situation leads to K and/or Ca deficiency, which hinders osmoregulation and enzyme activation and has a negative effect on plant metabolism. Studies have found a positive correlation between the amount of K and Ca in leaves and the increase in plant resistance under saline conditions, as well as a direct relationship between high K^+/Na^+ and Ca^{++}/Na^+ ratios and salt tolerance (Vicente *et al*., 2004; Martinez-Atienza *et al*., 2007; Deinlein *et al*., 2014). In our study, the highest K^+/Na^+ ratio was observed in the treatment with $CaCl_2$, $MgCl_2$ and NaCl, respectively, at low salt concentrations compared to the control. At high salt concentrations, the group treated with $MgCl_2$ exhibited the highest K^+/Na^+ ratio. Therefore, it can be concluded from our study that Ca and Mg containing salt solutions, although not as strong as NaCl, still had some negative effects on the macro-element content in rice.

Table 1. Mineral content of elements in Siverek rice exposed to different salt types and concentrations (μg/g)*

Mineral Element	Control	NaCl		$CaCl_2$		$MgCl_2$	
	0 mM	30 mM	90 mM	30 mM	90 mM	30 mM	90 mM
Ca	831.64 ± 16.99^{c}	472.84 ± 9.40^{f}	509.49 ± 10.2^{e}	2928.65 ± 59^{b}	5687.50± 113.68^{a}	194.93± 3.90^{g}	647.48± 13.01^{d}
K	3529.06 ± 63.52^{a}	1678.15 ± 30.2^{f}	2324.59 ± 41.84^{b}	1793.84 ± 32.27^{d}	1134.37± 20.41^{g}	1691.71± 30.43^{e}	2011.57± 36.21^{c}
Mg	54.42 ± 2.16^{e}	18.99 ± 0.74^{g}	44.89 ± 1.79^{f}	131.28 ± 5.24^{c}	60.48± 2.48^{d}	441.20± 17.64^{b}	575.65 ± 22.92^{a}
Na	143.27 ± 3.57^{c}	195.55 ± 4.87^{b}	670.11 ± 16.74^{a}	89.35 ± 2.22^{f}	110.96± 2.75^{d}	103.52± 2.57^{e}	147.82± 3.67^{c}
P	489.32 ± 22.98^{b}	319.80 ± 14.95^{g}	553.88 ± 26.12^{a}	340.97 ± 15.98^{f}	433.97± 20.35^{c}	375.46± 17.62^{e}	389.51± 18.28^{d}
Fe	96.91 ± 3.84^{c}	80.04 ± 3.28^{d}	112.61 ± 4.44^{b}	24.07 ± 0.96^{g}	57.38± 2.28^{f}	67.79 ± 2.68^{e}	139.95± 5.56^{a}
Zn	27.85 ± 0.13^{b}	29.34 ± 0.14^{a}	24.32 ± 0.12^{c}	21.58 ± 0.01^{d}	27.04± 0.13^{b}	27.24± 0.13^{b}	23.89± 0.11^{c}
Co	0.293 ± 0.01^{e}	0.296 ± 0.01^{e}	0.31 ± 0.02^{d}	0.44 ± 0.02^{c}	0.51± 0.02^{a}	0.30± 0.01^{d}	0.46± 0.02^{b}
Cu	8.48 ± 0.12^{g}	10.21 ± 0.14^{e}	19.34 ± 0.20^{a}	9.36 ± 0.12^{f}	13.12± 0.15^{c}	12.67± 0.13^{d}	15.25± 0.18^{b}
Mn	4.36 ± 0.13^{g}	36.77 ± 1.28^{a}	9.04 ± 0.30^{f}	16.67 ± 0.57^{d}	11.59± 0.42^{e}	30.52± 1.06^{b}	17.20± 0.60^{c}
Mo	3.74 ± 0.18^{a}	1.43 ± 0.07^{e}	0.99 ± 0.04^{g}	1.38 ± 0.06^{f}	1.59± 0.07^{d}	2.24± 0.11^{b}	1.79± 0.08^{c}

Note: Different letters (a-g) in each line indicate significant differences by ANOVA and Duncan's test compared to the control group.

Manganese (Mn) acts primarily as a cofactor in important metabolic processes related to photosynthesis, lipid biosynthesis, and oxidative stress in plants. When Mn is deficient, plant growth and yield decrease, and sensitivity to various stress factors such as pathogens, salinity, and frost damage increases (Socha & Guerinot, 2014; Sevilmiş *et al*., 2020). On the other hand, Mn ions have been found to play a role in ageing-related processes, regulation of protein synthesis and chlorophyll synthesis, although these aspects have not been extensively studied (Leidi *et al*., 1991). During transport, Mn competes with other cations as many carriers are used to transport the different cations. This competition can have a negative effect on Mn uptake (Schimansky, 1981; Kacar & Katkat, 2010). Scagel *et al*. (2017) studied the growth and development of basil plants under stress induced by $CaCl_2$ and NaCl. The researchers reported that while Mn uptake is increased by NaCl treatment, it is reduced by $CaCl_2$ treatment. In their study Bhattarai *et al*. (2022) applied NaCl salt stress to different varieties of *Medicago sativa* plants and found an increase in Mn content in all tested cultivars with increasing NaCl concentration. In the present study, an increase was observed in Mn content in all salt types and concentrations applied compared to the control group, but this increase varied according to salt type and concentration. In the control group, the Mn content was measured at 4.36 µg/g, while in the 30 mM salt treatments it was 36.77 µg/g for NaCl, 30.52 µg/g for $MgCl_2$ and 16.67 µg/g for $CaCl_2$. For the 90 mM salt treatments, it was found to be 17.20 µg/g for $MgCl_2$, 11.59 µg/g for $CaCl_2$ and 9.04 µg/g for NaCl.

The importance of molybdenum (Mo) arises from its presence in the structure of nitrogenase and reductase enzymes and the role of the nitrogenase enzyme in the vital activity of microorganisms that fix elemental N from the atmosphere and convert it to N in the soil. Considering this role of Mo, a study was conducted to determine the contribution of Mo fertilization to the N content of chickpea plants using different doses of Mo (0, 0.05, 0.10, 0.15, and 0.20 ppm). Considering the total N gain, the highest value was reached at 0.15 ppm Mo, and the further the dose deviated from this value, the lower the N gain became. Mo deficiency had a negative effect on root formation, resulting in underdeveloped green parts of the plant, and consequently low N gain from the soil (Vural & Müftüoğlu, 2012). In our study, Mo content was found to be lower in all treatments than in the control, with the highest decrease in NaCl and the lowest in $MgCl_2$.

In a study by Zhang *et al*. (2022), the content of certain elements was measured in different rice varieties exposed to different NaCl concentrations as salt stress. The P content increased with increasing salt concentration, although this increase was statistically different depending on the rice variety. Similarly, stress studies conducted on different plants have also recorded varying levels of increase and decrease in P content depending on the species and concentration (Bhattarai *et al*., 2022; Mokabel *et al*., 2022). In our study, as the salt concentration of NaCl and CaCl2 increased, the P content increased and was found to be higher than the control at 90 mM NaCl concentration. However, for $MgCl_2$, there was a varying increase or decrease in P content depending on the concentration, and overall, there was a decrease compared with the control at all concentrations. The P content in all treatments was statistically different.

Furthermore, Malkoç and Aydın (2003) reported that an increase in salt dose generally resulted in a decrease in the content of N, P, K, Ca, Mg, Fe, Mn, Zn, and Cu depending on the variety of maize (*Zea mays* L.) and bean plants. Zeiner *et al*. (2022) applied 200 mmol/L NaCl to different varieties of brassica plants grown in a hydroponic system. The researchers reported a decrease in Fe content in the white cabbage variety compared to the control, while there was no change in the Kale and Chinese cabbage varieties. In our study, the Fe content increased with the rising concentration in all three types of salts, and it was higher than the control at 90 mM $MgCl_2$.

The Cu content was higher than the control in all salt treatments, with a more pronounced increase observed in NaCl and $MgCl_2$ compared to $CaCl_2$. In terms of Zn, the highest increase was observed at 30 mM NaCl compared to the control (27.858 µg/g). For Co, particularly in $MgCl_2$, the Co content increased with an increase in concentration and it was higher than in the control. In the present study, these elements showed varying degrees of increase and decrease depending on the concentration and type of salts.

3.2. Proline Content

Under stressful conditions, plants synthesize and accumulate organic substances like proline within the cell, which plays a significant role in maintaining membrane integrity and osmotic balance (Tuna & Eroğlu, 2017). Proline amino acid not only contributes to osmotic regulation but also plays a role in adjusting cytosolic pH, preserving the integrity of cellular structures and proteins and activating enzymatic processes under stress conditions (Büyük *et al*., 2012). Therefore, in the present study, the proline content of the samples was examined in comparison with the control in response to different concentrations and types of salt stress (Figure 1).

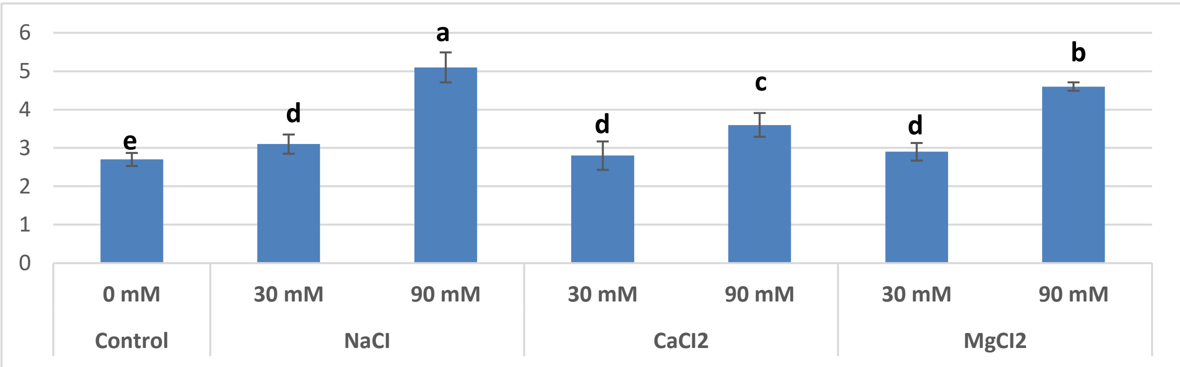

Figure 1. Effect of different salinity stress on proline content (mM/g).

Vertical bars indicate ± SE. Letters indicate significant differences compared with control group.

The study conducted by Özden *et al*. (2011) reported an increase in proline levels in grape (*Vitis vinifera*) plants exposed to salt stress. Similarly, in our study, when compared to the control group, the proline content showed a consistent increase with the rising concentration of each salt type.

The study by Özcan *et al*. (2000) also reported an increase in proline and Na concentrations, while K content decreased in plants under salt stress. Abdelhamid *et al.* (2013) reported that proline treatment through foliar spraying increased P and K concentrations while decreasing Na concentration under salt stress to bean (*Phaseolus vulgaris* L.) plants. Suleiman *et al.* (2023) studied native desert plant species under saline conditions and found that proline accumulation increased with increasing salinity level. In our study, the lowest proline level was obtained from the 30 mM $CaCl_2$ treatment, while the highest values were obtained from the 90 mM NaCl treatment, when compared to the control group. Considering the literature and our study, it can be concluded that the decrease in proline levels is directly related to changes in Ca, Mg, Na, and K element levels, which vary depending on the type of salts used. This could be evidence that the plant has developed a tolerance or self-protective mechanism against stress.

3.3. Total Protein Content

Secondary effects of salinity include impaired protein functionality and reduced chlorophyll content due to limited photosynthesis (Zhu, 2001; Elhindi *et al*., 2017; Kaya & İnan, 2017). Data on the total protein content of extracts from the Siverek rice population exposed to three types of salts (NaCl, $CaCl_2$, and $MgCl_2$) at different concentrations (30 and 90 mM) compared to the control (0 mM) are presented in Figure 2.

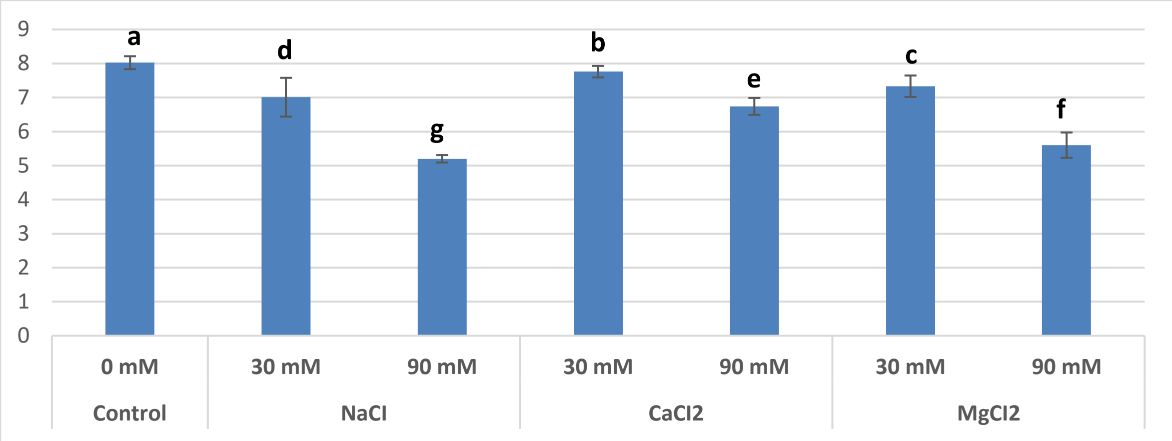

Figure 2. Effect of different salinity stress on total protein content (mg/g).
Vertical bars indicate ± SE. Letters indicate significant differences compared with control group.

Rajakumar (2013) investigated the effect of salinity (NaCl) on seed germination and some biochemical cursors of rice (*Oryza sativa* L.) under *in vitro* conditions. Higher NaCl concentrations resulted in increased proline accumulation and reduced total protein content in the study and it was found that NaCl had a negative effect on seed germination and total biomass. In our study increasing salt concentration resulted in rising proline content and reducing total protein content when compared with the control group. Among all treatments, the lowest decrease in total protein content was observed in $CaCl_2$ treatment, while the highest was observed in NaCl treatments. Demir and Kocaçalışkan (2001) found that soluble protein content decreased in salt-sensitive bean (*Phaseolus vulgaris* L.), and Karaman and Kaya (2015) reported a similar decrease in lentil under NaCl treatment, which is consistent with our findings.

4. DISCUSSION and CONCLUSION

In the present study, the mineral element contents, total protein, and proline levels were investigated for the first time in the Siverek rice population under different types/concentrations of salt. It is concluded that the reason for changes in cations may be relevant with their concentration and competition with each other. A decrease in Ca, Mg, and K levels during NaCl treatment or a decrease in Na, Mg, and K levels during $CaCl_2$ treatment may suggest evidence for it.

The changes in mineral elements involved in biochemical and enzymatic activities are remarkable. Mn plays a crucial role as a micronutrient in supporting the growth and development of plants. The subcellular Mn amount to maintain Mn-dependent metabolic processes such as ROS scavenging, and photosynthesis is mediated by a multitude of transport proteins from diverse gene families (Alejandro *et al*., 2020). Mn level increased in all treatments, but total protein content decreased in the current study. This change in manganese may be linked to other metabolic events related to the stress response other than protein content. Cu acts as a cofactor in proteins engaged in electron transfer reactions and serves as a vital micronutrient for plant growth (Burkhead *et al*., 2009). It can be concluded that similar changes in both Cu and Mn levels may considerably affect the generation of ROS due to their role in the electron transport system.

Salt stress studies focusing on NaCl have usually led to insufficient information on other chloride compounds, sulphates, nitrates, etc. and to confusion of information. Therefore, understanding the effects of different types of salts on plants and the complex responses of plants to these effects remains an area of interest. Further studies on salt stress, supported by molecular investigations, could identify genes that are activated in response to stress caused by different types of salts. Therefore, this study is essential for understanding the stress caused by

NaCl and other chloride compounds in rice and may shed light on the complex responses of plants to different types of salts.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

All authors carried out the experiments, conducted the data analysis, and wrote and approved the final version of the manuscript.

Orcid

Mehmet Yusuf Orcan https://orcid.org/0000-0002-5953-1178
Pınar Orcan https://orcid.org/0000-0001-8666-4542

REFERENCES

Abdelhamid, M.T., Rady, M.M., Osman, A.S., & Abdalla, M.A. (2013). Exogenous application of proline alleviates salt-induced oxidative stress in *Phaseolus vulgaris* L. *The Journal of Horticultural Science and Biotechnology*, *88*(4), 439-446. https://doi.org/10.1080/14620316.2013.11512989

Akyol, T.Y., Yılmaz, O., Uzilday, B., Uzilday, R.Ö., & Türkan, İ. (2020). Plant response to salinity: an analysis of ROS formation, signaling, and antioxidant defense. *Turkish Journal of Botany*, *44*(1), Article 1. https://doi.org/10.3906/bot-1911-15

Alejandro, S., Holler, S., Meier, B., & Peiter, E. (2020). Manganese in plants: from acquisition to subcellular allocation. *Frontiers in Plant Science*, *11*, 300. https://doi.org/10.3389/fpls.2020.00300

Bates, L.S., Waldren, R.P., & Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, *39*, 205-207. https://doi.org/10.1007/BF00018060

Bernstein, N., Sela, S., Dudai, N., & Gorbatsevich, E. (2017). Salinity stress does not affect root uptake, dissemination and persistence of salmonella in sweet-basil (*Ocimum basilicum*). *Frontiers in Plant Science*, *8*, 675. https://doi.org/10.3389/fpls.2017.00675

Bhattarai, S., Lundell, S., & Biligetu, B. (2022). Effect of sodium chloride salt on germination, growth, and elemental composition of alfalfa cultivars with different tolerances to salinity. *Agronomy*, *12*, 2516. https://doi.org/10.3390/agronomy12102516l

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Botella, M.A., Rosado, A., Bressan, R.A., & Hasegawa, P.M. (2005). Plant adaptive responses to salinity stress. *Plant Abiotic Stress*. Wiley-Blackwell. https://doi.org/10.1002/9780470988503

Burkhead, J.L., Gogolin Reynolds, K.A., Abdel-Ghany, S.E., Cohu, C.M., & Pilon, M. (2009). Copper homeostasis. *New Phytologist*, *182*, 799-816. https://doi.org/10.1111/j.1469-8137.2009.02846.x

Büyük, İ., Aydın, S.S., & Aras, S. (2012). Bitkilerin stres koşullarında verdiği moleküler cevaplar [Molecular responses of plants to stress conditions]. *Türk Hijyen ve Deneysel Biyoloji Dergisi*, *69*(2), 97-110.

Çalışkan, O., Kurt, D., Temizel, K.E., & Odabas, M.S. (2017). Effect of salt stress and irrigation water on growth and development of sweet basil (*Ocimum basilicum L.*). *Open Agriculture*, *2*(1), 589-594. https://doi.org/10.1515/opag-2017-0062

Deinlein, U., Stephan, A.B., Horie, T., Luo, W., Xu, G., & Schroeder, J.I. (2014). Plant salt-tolerance mechanisms. *Trends in Plant Science*, *19*, 371-379. https://doi.org/10.1016/j.tplants.2014.02.001

Demir, Y., & Kocaçalışkan, I. (2001). Effects of NaCl and proline on polyphenol oxidase activity in bean seedlings. *Biologia Plantarum*, *44*(4), 607-609. https://doi.org/10.1023/A:1013715425310

Dionisio, M.L.S., & Tobita, S. (2000). Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance. *Journal of Plant Physiology*, *157*(1), 54-58. https://doi.org/10.1016/S0176-1617(00)80135-2

Dramalis, C., Katsantonis, D., & Koutroubas, S.D. (2021). Rice growth, assimilate translocation, and grain quality in response to salinity under Mediterranean conditions. *AIMS Agriculture and Food*, *6*, 255–272. https://doi.org/10.3934/agrfood.2021017

Elhindi, K.M., Al-Amri, S.M., Abdel-Salam, E.M., & Al-Suhaibani, N.A. (2017). Effectiveness of salicylic acid in mitigating salt-induced adverse effects on different physio-biochemical attributes in sweet basil (*Ocimum basilicum* L.). *Journal of Plant Nutrition, 40*(6), 908-919. https://doi.org/10.1080/01904167.2016.1270311

Essa, T.A. (2002). Effect of salinity stress on growth and nutrient composition of three soybean (*Glycine max* L. Merrill) cultivars. *Journal of Agronomy and Crop Science, 188*(2), 86-93. https://doi.org/10.1046/j.1439-037X.2002.00537.x

Fageria, N.K., Stone, L.F., & Santos, A.B.D. (2012). Breeding for salinity tolerance. *Plant Breeding for Abiotic Stress Tolerance*. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-30553-5_7

Fahad, S., Noor, M., Adnan, M., Khan, M.A., Rahman, I.U., Alam, M., & Nasim, W. (2019). Abiotic Stress and Rice Grain Quality, 571-583. Cambridge, UK: *Elsevier*. https://doi.org/10.1016/B978-0-12-814332-2.00028-9

Ghoulam, C., Foursy, A., & Fores, K. (2002). Effects of salt stress on growth, inorganic ions, and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environmental and Experimental Botany, 47*, 39-50. https://doi.org/10.1016/S0098-8472(01)00109-5

Hasanuzzaman, M., Raihan, M.R.H., Masud, A.A.C., Rahman, K., Nowroz, F., Rahman, M., Nahar, K., & Fujita, M. (2021). Regulation of reactive oxygen species and antioxidant defense in plants under salinity. *International Journal of Moleculer Science*, *22*, 9326. https://doi.org/10.3390/ijms22179326

İnal, A., Güneş, A., & Aktaş, M. (1995). Effects of chloride and partial substitution of reduced forms of nitrogen for nitrate in the nutrient solution on the nitrate, total nitrogen, and chlorine contents of onion. *Journal of Plant Nutrition*, *18*, 2219-2227. https://doi.org/10.1080/01904169509365058

Kacar, B., & Katkat, V. (2010). *Bitki Besleme*. 5. Baskı, Nobel Yayın Dağıtım Tic. Ltd.

Karaman, R., & Kaya, M. (2017). Mercimeğe (*Lens esculanta* Moench) uygulanan farklı klor tuzu ve dozlarının kimi ilk gelişme özelliklerine etkisi [Effect of Different Chloride Salts and Doses on Early Growth Characters in Lentil]. *Journal of Agricultural Sciences*, *23*, 10-21.

Kaya, A., & İnan, M. (2017). Effect of salicylic acid on some morphological, physiological and biochemical parameters of basil plant (*Ocimum basilicum* L.) which was subjected to salt (NaCl) stress. *Harran Tarım ve Gıda Bilimleri Dergisi/Harran Journal of Agricultural and Food Science, 21*(3), 332-342. https://doi.org/10.29050/harranziraat.339489

Kordrostami, M., Rabiei, B., & Hassani Kumleh, H. (2017). Biochemical, physiological, and molecular evaluation of rice cultivars differing in salt tolerance at the seedling stage. *Physiology and Molecular Biology of Plants*, *23*, 529–544. https://doi.org/10.1007/s12298-017-0440-0

Kumar, G., Kushwaha, H.R., Panjabi-Sabharwal, V., Kumari, S., Joshi, R., Karan, R., Mittal, S., Pareek, S.L.S., & Pareek, A. (2012). Clustered metallothionein genes are co-regulated in rice and ectopic expression of OsMT1e-P confers multiple abiotic stress tolerance in tobacco via ROS scavenging. *BMC Plant Biology, 12,* 107. https://doi.org/10.1186/1471-2229-12-107

Kumar, A., Singh, S., Gaurav, A.K., Srivastava, S., & Verma. J.P. (2020). Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants. *Frontiers in Microbiology*, *11*, 1216. https://doi.org/10.3389/fmicb.2020.01216

Lacerda, C.F., Cambraia, J., Oliva, M.A., & Ruiz, H.A. (2002). Changes in growth and in solute concentrations in sorghum leaves and roots during salt stress recovery. *Environmental and Experimental Botany*, *54*, 69–76. https://doi.org/10.1016/j.envexpbot.2004.06.004

Leidi, E. O., Nogales, R., & Lips, S. H. (1991). Effect of salinity on cotton plants grown under nitrate or ammonium nutrition at different calcium levels. *Field Crops Research*, *26*(1), 35-44. https://doi.org/10.1016/0378-4290(91)90055-Z

Liu, S., Zheng, L., Xue, Y., Zhang, Q., Wang, L., & Shou, H. (2010). Overexpression of OsVP1 and OsNHX1 increases tolerance to drought and salinity in rice. *Journal of Plant Biology, 53*, 444–452. https://doi.org/10.1007/s12374-010-9135-6

Malkoç, M., & Aydın, A. (2003). Mısır (*Zea mays* L.) ve fasulye (*Phaseolus vulgaris* L.)'nin gelişimi ve bitki besin maddeleri içeriğine farklı tuz uygulamalarının etkisi [Effects of Different Salt Sources on *Zea Mays* and *Phaseolus* Growth and Mineral Content]. *Turkish Journal of Agriculture and Forestry*, *34*(3), 211–216.

Marschner, H. (1995). *Mineral Nutrition of Higher Plants*, 2nd ed. Academic Press, The Netherlands.

Martínez-Atienza, J., Jiang, X., Garciadeblas, B., Mendoza, I., Zhu, J.K., Pardo, J.M., & Quintero, F.J. (2007). Conservation of the salt overly sensitive pathway in rice. https://doi.org/10.1104/pp.106.092635

Mokabel, S., Olama, Z., Ali, S., & El-Dakak, R. (2022). The role of plant growth promoting rhizosphere microbiome as alternative biofertilizer in boosting *Solanum melongena* L. adaptation to salinity stress. *Plants, 11*(5), 659. https://doi.org/10.3390/plants11050659

Mondal, T.K., Rawal, H.C., Chowrasia, S., Varshney, D., Panda, A.K., Mazumdar, A., Kaur, H., Gaikwad, K., Sharma, T.R., & Singh, N.K. (2018). Draft genome sequence of the first monocot-halophytic species *Oryza coarctata* reveals stress-specific genes. *Scientific Reports, 8,* 13698. https://doi.org/10.1038/s41598-018-31518-y

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Plant Physiology, 15,* 473-497.

Nam, M.H., Huh, S.M., Kim, K.M., Park, W.J., Seo, J.B., Cho, K., & Kim, D.Y. (2012). Comparative proteomic analysis of early salt stress-responsive proteins in roots of SnRK2 transgenic rice. *Proteomics Science*, *10*(25), 15. https://doi.org/10.1186/1477-5956-10-25

Orcan, P., Işıkalan, Ç., & Akbaş, F. (2019). Evaluation of salinity tolerance in rice (*Oryza sativa* L.) using water potential, biomass, membrane damage, and osmoprotective compounds. *Fresenius Environmental Bulletin, 28*(4a), 3313-3323.

Özcan, H., Turan, M.A., Koç, Ö., Çıkılı, Y., & Taban, S. (2000). Tuz stresinde bazı nohut (*Cicer arietinum* L.) çeşitlerinin gelişimi ve prolin, sodyum, klor, fosfor ve potasyum konsantrasyonlarındaki değişimler [Growth and variations in proline, sodium, chloride, phosphorus and potassiumconcentrations of chickpea (*Cicer arietinum*L. cvs.) Varieties Under Salinity Stress]. *Turkish Journal of Agriculture and Forestry, 24,* 649-654.

Özden, M., Dikilitaş, M., Gürsöz, S., & Ak, B.E. (2011). 110r anacı üzerine aşılı şiraz üzüm (*Vitis Vinifera* L.) çeşidinin NaCl ve prolin uygulamalarına karşı fizyolojik ve biyokimyasal tepkileri [Physiological and biochemical responses of syrah vines (*Vitis vinifera* L.) grafted

on 110 r rootstock to NaCl and proline applications]. *Harran Üniversitesi Ziraat Fakültesi Dergisi*, *15*(1), 1-9.
Pani, D.R., Sarangi, S.K., Subudhi, H.N., Misra, R.C., & Bhandari, D.C. (2012). Exploration, evaluation, and conservation of salt-tolerant rice genetic resources from the Sundarbans region of West Bengal. *Journal of the Indian Society of Coastal Agricultural Research, 30*(1), 45-53.
Rajakumar, R. (2013). A study on the effect of salt stress on seed germination and biochemical parameters of rice (*Oryza sativa* L.) under in vitro conditions. *Asian Journal of Plant Science and Research*, *3*(6), 20-25.
Rajendran, K., Tester, M., & Roy, S.T. (2009). Quantifying the three main components of salinity tolerance in cereals. *Plant, Cell and Environment*, *32*, 237-249. https://doi.org/10.1111/j.1365-3040.2008.01916.x
Rao, P.S., Mishra, B., & Gupta, S. (2013). Effects of soil salinity and alkalinity on grain quality of tolerant, semi-tolerant, and sensitive rice genotypes. *Rice Science*, *20*(4), 284–291. https://doi.org/10.1016/S1672-6308(13)60136-5
Razzaq, A., Ali, A., Safdar, L.B., Zafar, M.M., Rui, Y., Shakeel, A., Shaukat, A., Ashraf, M., Gong, W., & Yuan, Y. (2020). Salt stress induces physiochemical alterations in rice grain composition and quality. *Journal of Food Science*, *85*(1). https://doi.org/10.1111/1750-3841.14983
Scagel, C.F., & Bryla, D.R. (2017). Salt exclusion and mycorrhizal symbiosis increase tolerance to NaCl and $CaCl_2$ salinity in 'Siam Queen' Basil. *HortScience*, *52*(2), 278-287. https://doi.org/10.21273/HORTSCI11256-16
Schimansky, C. (1981). The influence of certain experimental parameters on the flux characteristics of Mg-28 in the case of barley seedlings in hydroculture experiments. *Landwirtschaftliche Forschung, 34*(3), 154-163.
Shanker, A. (2011). Abiotic Stress in Plants - Mechanisms and Adaptations. *InTech*.
Sevilmiş, U., Sevilmiş, D., Ölmez, Y.A., Aykanat, S., & Özcan, O.B. (2020). Soyada bitki besleme ve hastalıklarla mücadele yöntemi olarak yapraktan mangan uygulamaları [Effect of foliar manganese applications on yield quality and diseases progresses of soybean]. *Ziraat Mühendisliği, 369*, 4-21.https://doi.org/10.33724/zm.675983
Singh, A.S., Ramteke, P.W., Paul, A., David, A.A., Shukla, P.K., & Lal, E.P. (2018). Influence of jeevamrutha on seed germination of *Ocimum basilicum* L. under NaCl salinity stress. *Journal of Pharmacognosy and Phytochemistry, 7*(2), 705-707.
Socha, A.L., & Guerinot, M.L. (2014). Mn-euvering manganese: The role of transporter gene family members in manganese uptake and mobilization in plants. *Frontiers in Plant Science, 5*, 106. https://doi.org/10.3389/fpls.2014.00106
Suleiman, M.K., Bhatt, A., Madouh, T.A., Islam, M.A., Jacob, S., Thomas, R.R.,&Sivadasan, M.T. (2023). Effects of salt stress on growth, proline and mineral content in native desert species. *Sustainability*, *15*, 6232. https://doi.org/10.3390/su15076232
Thu, T.T.P., Yasui, H., & Yamakawa, T. (2017). Effects of salt stress on plant growth characteristics and mineral content in diverse rice genotypes. *Soil Science and Plant Nutrition*, *63*(3), 264-273. https://doi.org/10.1080/00380768.2017.1323672
Tilman, D., Balzer, C., Hill, J., & Befort, B.L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences*, *108*(50), 20260–20264. https://doi.org/10.1073/pnas.11164371
Tuna, A.L., & Eroğlu, B. (2017). Tuz stresi altındaki biber (*Capsicum annuum* L.) bitkisinde bazı organik ve inorganik bileşiklerin antioksidatif sisteme etkileri [Effects of some organic and inorganic compounds on antioxidative system in pepper (*Capsicum annuum* L.) plant under salt stress]. *Anadolu Journal of Agricultural Sciences, 32*, 121-131. https://doi.org/10.7161/omuanajas.289038

Vicente, O., Boscaiu, M., Naranjo, M.A., Estrelles, E., Belles, J.M., & Soriano, P. (2004). Responses to salt stress in the halophyte *Plantago crassifolia* (Plantaginaceae). *Journal of Arid Environments, 58*(4), 463-48. https://doi.org/10.1016/j.jaridenv.2003.12.003

Vuralın, A., & Müftüoğlu, N.M. (2012). Farklı Dozlarda Uygulanan Molibdenin Bakla (*Vicia faba* L.) Bitkisinin Azot İçeriğine Etkisi[The effect of different doses Molybdenum applied to nitrogen content of broad bean (*Vicia faba* L.)]. *Ege Üniversitesi Ziraat Fakültesi Dergisi, 49*(1), 53-62.

Wang, H., Zhang, M., Guo, R., Shi, D., Liu, B., Lin, X., & Yang, C. (2012). Effects of salt stress on ion balance and nitrogen metabolism of old and young leaves in rice (*Oryza sativa* L.). *BMC Plant Biology*, *12*, 194. https://doi.org/10.1186/1471-2229-12-194

Wang, P., Sun, X., Li, C., Wei, Z., Liang, D., & Ma, F. (2013). Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple. *Journal of Pineal Research, 54*(3), 292-302. https://doi.org/10.1111/jpi.12017

Yakıt, S., & Tuna, A.L. (2006). Tuz stresi altındaki mısır bitkisinde (*Zea mays* L.) stres parametreleri üzerine Ca, Mg Ve K'nın etkileri [The Effects of Ca, K and Mg on the stress parameters of the maize (*Zea mays* L.) plant under salinity stress]. *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 19*(1), 59-67.

Zhang, R., Wang, Y., Hussain, S., Yang, S., Li, R., Liu, S., Chen, Y., Wei, H., Dai, Q., & Hou, H. (2022). Study on the effect of salt stress on yield and grain quality among different rice varieties. *Frontiers in Plant Science, 13*, 918460. https://doi.org/10.3389/fpls.2022.918460

Zhu, J.K. (2001). Plant salt tolerance. *Trends in Plant Science, 6*(2), 66-71. https://doi.org/10.1016/S1360-1385(00)01838-0

Zeiner, M., Cindrić, I.J., Nemet, I., Franjković, K., & Sondi, B.S. (2022). Influence of soil salinity on selected element contents in different brassica species. *Molecules, 27*(6), 1878. https://doi.org/10.3390/molecules27061878