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Evaluation of Physiological and Biochemical Responses against to Salinity in Local Rice (*Oryza sativa* L.) under *in vitro* Conditions

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ABSTRACT: In a local rice cultivar (Karacadağ), physiological and biochemical changes caused by different salt types (NaCI, $CaCI_2$, $MgCI_2$) and their concentrations (25, 50, 75, 150, 300 mM) were investigated under stress conditions. The germination percentage was not affected at low concentrations in each of 3 types salt tested but it decreased significantly as the concentration increased. In development stages of seedling, it was determined that as the concentration increased in all salt types, plant growth and relative water content (RWC) decreased. Salinity was mostly affected the photosynthetic pigment contents of the plants and there was a difference significantly between the results according to the salt concentration. In general, it was determined that malondialdehyde (MDA) content increased depending on the concentration in all salt types that tested. The highest cell membrane damage was found in the 75 mM application of $CaCI_2$ with 4.1820 µmol /g MDA. As a result, it was determined that germination and seedling development stages were negatively affected in the salt types tested, especially at high concentrations.

Keywords; Rice; Karacadağ; salt stress; *in vitro*

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INTRODUCTION

Salinity, negatively affects the growth of plants by causing changes in their structural, physiological, biochemical and molecular mechanisms. Generally, Ca^{+2}, Na^{+}, Mg^{+2} cations and SO_4^{-2}, CI^- anions lead to the salinity stress. The most harmful effects of salt stress are composed by NaCI, $CaCI_2$ and Na_2SO_4 salts (Koyuncu, 2008).

Tissue culture studies are used in researches to reveal the physiological and biochemical basis of salinity mechanisms and salt tolerance in plants. The tissue culture applications have provided many advantages when used completely controlled and homogen material at physiological studies (Ellialtıoğlu and Tıpırdamaz, 1998; Yokaş et al., 2008). In recent years, there have been many studies using different *in vitro* culture techniques in different plant species to determine the effects of salt stress (Koyuncu, 2012; Benderradji et al., 2012).

The rice is estimated to have more than 140.000 species around the World (Sürek, 2002). The presence of so many varieties of rice can be explained by the sense of quality and taste that varies according to countries, regions and even different districts (Akay, 2010). Rice is an important food source and the most important cultivation plant after wheat. It is also one of the plants most affected by salinity, so the decrease in yield due to salt stress factor is an important problem (Siahpoosh et al., 2012; Rajakumar, 2013). Karacadağ local rice variety is known as one of the most grown rice varieties in Southeastern Anatolian region of Turkey (Kaya, 2013). There are restricted studies about effecting of salt stress in the literatüre (Orcan et al., 2019). In this study it was intended that in a Karacadağ rice cultivar, physiological and biochemical changes under stress conditions caused by different salt types were investigated.

MATERIALS AND METHODS

Sterilization

In this study, Karacadağ rice variety obtained from local producers around Diyarbakır was used as plant material. After the rice seeds was waited in 70% ethanol for 30 seconds, optimum surface sterilization was performed by being waited in 5% NaOCl for 60 minutes.

Germination

Aseptic rice seeds were incubated in Magenda GA-7 culture dishes containing 1/4 MS medium supplemented with 0, 25, 50, 75, 150, 300 mM of NaCI, $CaCI_2$ or $MgCI_2$, and was left to developed in the growth chamber. All nutrient media was supported with 30 g sucrose and 5.458 g agar. After period of 3-week, germination datas were taken.

Growth parameters

Seeds were first germinated in hormone-free MS medium. After 1 week, the germinated rice seedlings were transferred to MS medium containing 25, 50, 75, 150, 300 mM concentrations of NaCI, $CaCI_2$ or $MgCI_2$. At the end of the 3-week culture period, the aerial parts /roots of the rice seedlings were harvested separately and then kept in deep freeze (-42 °C) until analysis was performed. Measurement of shoot/root lenght and fresh/dry weight were taken immediately after harvesting to determine salinity effects on growth of seedlings.

Relative water contents (RWC%)

Every samples' RWC were calculated according to formula below as % (Barr and Weatherley, 1962).

RWC% = [(FW – DW) / (TW – DW)]x100
TW=Turgor weight, DW=Dry weight, FW=Fresh weight

Photosynthetical pigments contents

Photosynthetical pigments content was measured according to Arnon (1949). 0.25 gr leaves were homogenised in 80% acetone and the samples were centrifuged at 5000xg for 5 min at 4°C. The extracts were measured to the absorbans values as spectrophotometric for *chlorophyll a* at 480 nm, for *chlorophyll b at 645 nm* and for *carotenoid at 480 nm* after santrifuged at 5000 rpm for 5 minutes.

Malondialdehyde content (MDA)

MDA amount were determined according to Ohkawa et al. (1979). MDA quantity was calculated by being drawn graphic of MDA standart curves with 1,1,3,3-Tetramethoxypropane in the tissues.

Statistical Analyzes

Statistical analysis was done with the Duncan multiple range test (one-way ANOVA) using SPSS 20.0. The significance refers to the statistical level at $p \leq 0.05$.

RESULTS AND DISCUSSION

Germination

As the NaCI increased, germination percentage decreased in all three tested salt types (Figures 1a-c). When compared to the control group, germination percentage was decreased 15% at 150 mM NaCI, 27% for $CaCI_2$ and 28% for $MgCI_2$. While seeds were not germinated at 300 mM $MgCI_2$ and $CaCI_2$, 54% of the seeds were germinated with NaCI. Among the tested salt types, it was determined that $MgCl_2$ was more negatively affected the germination. Parallel to this result, it has been reported that the germination rate of rice varieties decreases with increasing salinity in the studies by Asch and Wopereis (2001), and Tun et al. (2003). Similarly, Tatar (2006) declared that IR31785, Kral, Demir, IR4630 and Yavuz rice cultivars had an inverse relationship between salinity levels and germination rate germination percentage decreased with increasing salinity.

Growth parameters

As the NaCI concentration increased, the growth of the plants slowed down and decreased the root and shoot length (Table 1). It was determined that the decrease in shoot length of seedlings at low NaCI concentrations (25 mM and 75 mM) were about 5% low rate and this ratio was found not to be statistically significant.

Table 1. Effects of salinity stress on shoot and root length.

		Salt Concentration (mM)					
	Salt Types	**Control**	**25**	**50**	**75**	**150**	**300**
Shoot length (cm)	NaCI	21.38 ± 2.74^{a}	20.33 ± 3.44^{a}	14.17 ± 5.07^{b}	19.45 ± 1.96^{a}	10.24 ± 2.31^{c}	1.12 ± 0.26^{d}
	$CaCl_2$	21.38 ± 2.74^{ab}	22.24 ± 2.64^{a}	19.99 ± 2.75^{b}	15.34 ± 3.12^{c}	3.24 ± 0.40^{d}	0.00 ± 0.00^{e}
	$MgCl_2$	21.38 ± 2.74^{a}	17.91 ± 3.83^{b}	7.46 ± 1.43^{c}	22.39 ± 3.11^{a}	1.71 ± 0.68^{d}	0.00 ± 0.00^{e}
Root length (cm)	NaCI	3.13 ± 0.35^{ab}	3.00 ± 0.50^{ab}	2.54 ± 0.63^{c}	3.07 ± 1.02^{ab}	4.40 ± 1.20^{a}	0.07 ± 0.00^{d}
	$CaCl_2$	3.13 ± 0.35^{a}	2.64 ± 0.53^{a}	1.95 ± 0.50^{b}	1.08 ± 0.17^{c}	0.21 ± 0.01^{d}	0.00 ± 0.00^{e}
	$MgCl_2$	3.13 ± 0.35^{a}	4.07 ± 1.05^{a}	2.02 ± 0.95^{b}	4.53 ± 1.07^{a}	0.01 ± 0.00^{c}	0.00 ± 0.00^{e}

Values are mean ± sd (n: 15). Distinct lettering in each line indicate important difference in $p \leq 0.05$ level.

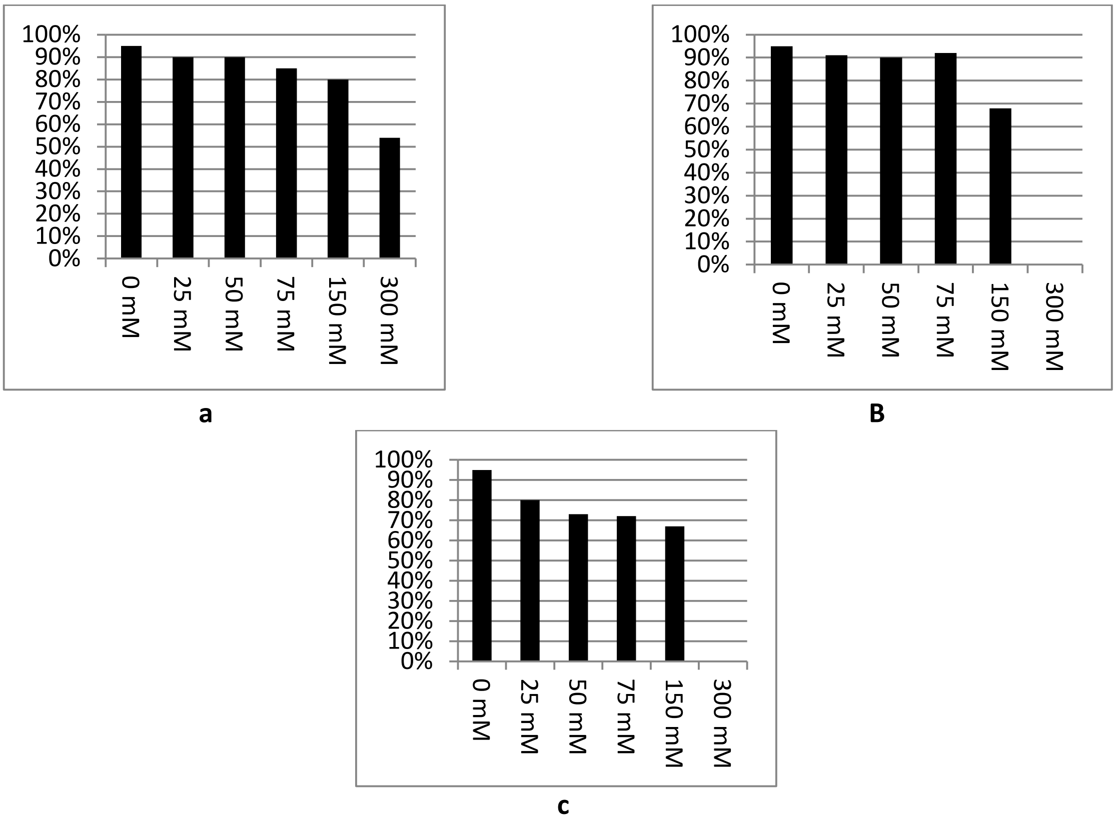

Figure 1 The germination ratio of each 3 salt varieties. a**:** NaCI, b**:** $CaCI_2$, c**:** $MgCI_2$

At high NaCI applications it was a significant decrease in shoot length and this reduction reached 50% at 150 mM NaCl and 95% at 300 mM NaCl application (Figure 2a-b). When we examined the effects of NaCl salt applications on the root length; it was established that root length decreased significantly (98% rate) for 300 mM NaCl and this decrease was found to be statistically significant. In the $CaCl_2$ salt application, when the concentrations tested in terms of shoot length were compared with the control group (Figure 2c), a significant decrease was observed as statistically at 75 and 150 mM concentrations (25% and 85%, respectively). Similarly, as the NaCI content increased, there was a decrease in root length. Statistically, this decrease was significant at concentrations of 50, 75 and 150 mM (Table 1). At high concentrations of $CaCl_2$ (150 and 300 mM), root growth was found to be very weak, whereas at 300 mM concentration plants were not shown development. As the same of other types of salts, it was determined that there was no elongation in shoot length as the $MgCI_2$ concentration increases (Figure 2d). The shoot length was also found significant when compared with the control group as statistical (except for the 75 mM $MgCI_2$ application). When the effect of $MgCI_2$ on root length was examined, the root length of 25 and 75 mM salt applied was increased, but it was seen that this increase was not statistically significant (Table 1).

In our study the salt applications were affected the shoot/root length and caused generally decrease as salt concentration increase at all salt varieties. Similar to our findings, Demiral and Türkan (2005) notified in their study on rice varieties that the salinity affected negatively the root length.

Effect of salinity stress on biomass

In this study we investigated the effect on biomass (fresh weight-dry weight -FW/DW) of aerial part during seedling development, it was determined that all tested salt types had a negative effect on the plant development and generally causing weight loss. When compared with control group, it was determined that FW/DW of the rice seedlings was decrease at 25 mM NaCI applied about 20%, at 50 and 150 mM applied about 50%, at 75 mM applied about 35%. Low $CaCI_2$ concentrations (25 and 50 mM) were not statistically significant, but a significant decrease was observed at high concentrations (75 mM) of $CaCl_2$ (Table 2). At the application of 50 mM $MgCl_2$, in terms of both FW and DW was decrease compared to the control and this decrease was important as statistical. When the DW of the seedlings examined it was seen that generally significant decrease in parallel with the FW when investigated to DW of aerial part of the seedlings.

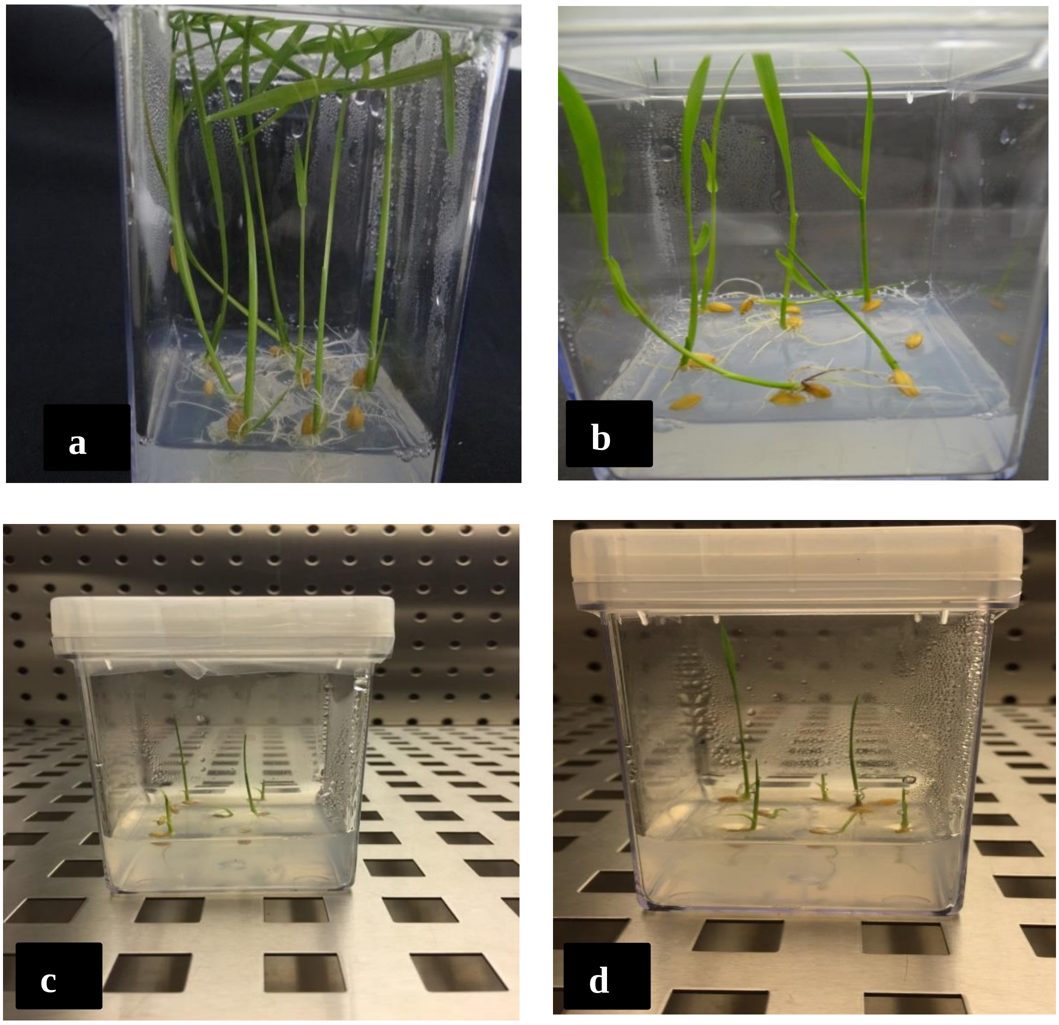

Figure 2 General view of the rice seedlings which developing (3-weeks) in a**)** salt-free culture medium b**)** 150 mM NaCI c) 150 mM $CaCI_2$ d**)** 150 mM $MgCI_2$

Table 2. Effects of salinity stress on aerial part dry/fresh weigth of rice

		Salt Concentration (mM)					
	Salt Types	**Control**	**25**	**50**	**75**	**150**	**300**
Fresh weigth (g)	NaCI	0.5800±0.13^{a}	0.4600±0.03^{b}	0.2900±0.02^{c}	0.3733±0.02^{bc}	0.2933±0.02^{c}	0.00±0.00
	$CaCI_2$	0.5800±0.13^{a}	0.5467±0.02^{ab}	0.4867±0.08^{ab}	0.4000±0.06^{b}	0.00±0.00	0.00±0.00
	$MgCI_2$	0.5816±0.13^{a}	0.6230±0.05^{a}	0.2957±0.01^{b}	0.4538±0.15^{ab}	0.00±0.00	0.00±0.00
Dry weigth (g)	NaCI	0.0900±0.02^{a}	0.0700±0.00^{b}	0.0500±0.01^{bc}	0.0533±0.00^{bc}	0.0400±0.00^{c}	0.00±0.00
	$CaCI_2$	0.0900±0.02^{a}	0.1100±0.00^{a}	0.1027±0.01^{a}	0.0800±0.00^{a}	0.00±0.00	0.00±0.00
	$MgCI_2$	0.0932±0.02^{ab}	0.0969±0.00^{a}	0.0475±0.00^{c}	0.0635±0.02^{bc}	0.00±0.00	0.00±0.00

Differ letters in each line indicate significant difference at 0.05 level.

Root development was negatively affected and there was general weight loss at all salt applications tested when compared to the control. As the NaCI concentration increased, there was a decrease in root FW (except for 150 mM NaCI) according to control (Table 3). Also, at 150 mM salt application of root FW was increase according to the control different from the other applications. The root FW was decrease and this decrease was significant as statistically at the all concentration (25, 50, 75 mM) of $CaCI_2$. When $MgCl_2$ salt applications (except for 25 mM) compared with control group, it was determined that occurring of weight loss was significant as statistically. As for root DW, it was determined that weight loss compared to control group in all salt applications similar with FW in generally (except for 50 mM $MgCI_2$). Besides, the data could not be obtained at high concentrations (150 and 300 mM) of $CaCI_2$, $MgCI_2$ and 300 mM NaCI applications because the plants were not show development.

Table 3. Effects of salinity stress on root dry/fresh weigth.

		Salt Concentration (mM)					
	Salt Types	**Control**	**25**	**50**	**75**	**150**	**300**
Fresh weigth (g)	NaCI	0.2667 ± 0.05^{b}	0.2567 ± 0.03^{b}	0.1300 ± 0.02^{c}	0.0833 ± 0.00^{c}	0.4367 ± 0.00^{a}	0.00 ± 0.00
	$CaCI_2$	0.3327 ± 0.03^{a}	0.2752 ± 0.02^{b}	0.0903 ± 0.00^{c}	0.0583 ± 0.00^{c}	0.00 ± 0.00	0.00 ± 0.00
	$MgCI_2$	0.2667 ± 0.05^{a}	0.2133 ± 0.05^{ab}	0.0733 ± 0.057^{c}	0.1733 ± 0.05^{b}	0.00 ± 0.00	0.00 ± 0.00
Dry weigth (g)	NaCI	0.0203 ± 0.00^{a}	0.0200 ± 0.00^{a}	0.0133 ± 0.00^{b}	0.0070 ± 0.00^{c}	0.0100 ± 0.00^{bc}	0.00 ± 0.00
	$CaCI_2$	0.0245 ± 0.00^{b}	0.0455 ± 0.00^{a}	0.0206 ± 0.00^{c}	0.0175 ± 0.00^{c}	0.00 ± 0.00	0.00 ± 0.00
	$MgCI_2$	0.0200 ± 0.00^{b}	0.0200 ± 0.00^{b}	0.0933 ± 0.01^{a}	0.0167 ± 0.002^{b}	0.00 ± 0.00	0.00 ± 0.00

Differ letters in each line indicate significant difference at 0.05 level.

In our study, it was determined that all tested salt varieties effects negatively the plant growth and caused generally weight losing. Similarly, Demiral and Türkan (2005) and Vaidyanathan et al. (2003) notified that the weigth losing occurred in plants with salinity stress at the studies of the rice.

RWC

Generally, the leaf RWC was decreased in all test groups according to control, and these data were statistically significant (Table 4). It was detected that decreases of RWC was inversely correlated with applied salt concentration, so the RWC was decreased as salt concentration increased. The lowest RWC was determined to be 61.33% at 150 mM application for NaCl, 78.30% at 75 mM application for $CaCl_2$ and 75.25% at 75 mM application for $MgCl_2$. Roots RWC was decreased as the NaCl concentration increased, similar with the aerial parts. As seen in Table 4 this decrease was also important according to the control as statistically. The lowest RWC was shown in 150 mM NaCl application which is the highest salt application (67.66%).

Table 4. Effects of salinity stress on relative water content (RWC% ± Sd).

		Salt Concentration (mM)					
	Salt Types	**Control**	**25**	**50**	**75**	**150**	**300**
Green Fraction	NaCI	88.53 ± 1.70^{a}	85.30 ± 1.11^{ab}	79.10 ± 4.05^{bc}	71.96 ± 2.20^{c}	61.33 ± 9.09^{d}	0.00 ± 0.00
	$CaCI_2$	79.10 ± 4.05^{a}	79.53 ± 2.03^{a}	78.36 ± 1.87^{a}	78.30 ± 0.60^{a}	0.00 ± 0.00	0.00 ± 0.00
	$MgCl_2$	86.10 ± 2.23^{a}	81.15 ± 1.91^{ab}	79.13 ± 4.04^{b}	75.25 ± 4.59^{b}	0.00 ± 0.00	0.00 ± 0.00
Root	NaCI	97.86 ± 0.47^{a}	90.86 ± 4.21^{b}	84.00 ± 6.00^{c}	78.00 ± 1.00^{c}	67.66 ± 3.51^{d}	0.00 ± 0.00
	$CaCI_2$	98.01 ± 0.38^{a}	95.33 ± 0.14^{a}	79.50 ± 8.63^{b}	81.12 ± 2.46^{b}	0.00 ± 0.00	0.00 ± 0.00
	$MgCl_2$	97.93 ± 0.45^{a}	97.46 ± 0.45^{a}	83.80 ± 2.45^{b}	72.80 ± 0.26^{c}	0.00 ± 0.00	0.00 ± 0.00

Differ letters in each line indicate significant difference at 0.05 level.

As a result, it was found that $MgCl_2$ and $CaCl_2$ showed negative affect on seedling growth more than NaCl among the tested salt varieties. The increase in salt level in the culture medium cause a decrease in water potential, resulting in physiological drought in the plant. Khan and Panda (2008) studied different NaCI concentrations on the roots of two rice cultivars. It is recorded that the RWC% value is decreased in both varieties. The results of the researchers were consistent with our study that both the aerial parts and root RWC decreased with increasing salt concentration.

Photosynthetic pigment contents

The effect of salinity on photsynthetic pigment content showed differences depending on the concentration of salt rather than salt varieties. In NaCI application, the pigment content was negatively affected, and it was observed that a general decrease in contents of pigment with increasing salt concentration (Table 5). $CaCI_2$ applications led to an overall decrease in pigment contents compared to the control group. However, *chlorophyll b* and total *chlorophyll* contents increased depending on control at 25 mM, which is the lowest concentration, and it was found to be statistically significant. All photosynthetic pigment contents decreased with increasing concentration of $MgCI_2$. However, the content of *chlorophyll a* was found to be higher at the 75 mM application than the control group, and this increase was statistically significant. It was determined that the highest *carotenoid* content among $MgCI_2$ applications were 75 mM.

Table 5. Effect of salinity stress on photosynthetic pigment contents

	Salt Concentration (mM)	Chlorophyll-a content (µg/g) ± Sd	Chlorophyll-b content (µg/g) ± Sd	Total Chlorophyll content (µg/g) ± Sd	Carotenoid content (µg/g) ± Sd
NaCl	Control	618.33 ± 0.57^{b}	484.37 ± 1.00^{a}	1100.33 ± 0.57^{a}	0.36 ± 0.00^{a}
	25	614.75 ± 1.00^{c}	318.33 ± 1.52^{d}	930.30 ± 0.10^{c}	0.28 ± 0.00^{c}
	50	625.33 ± 0.57^{a}	384.33 ± 1.52^{c}	1000.66 ± 0.57^{bc}	0.33 ± 0.00^{b}
	75	625.42 ± 1.00^{a}	403.66 ± 1.52^{b}	1020.33 ± 0.57^{bc}	0.31 ± 0.00^{b}
	150	452.66 ± 0.57^{d}	153.00 ± 1.00^{e}	600.60 ± 0.20^{d}	0.20 ± 0.00^{d}
Differ letters in each column indicate significant difference at 0.05 level.					
	Control	618.33 ± 0.57^{a}	484.00 ± 1.00^{b}	1102.33 ± 1.52^{b}	0.36 ± 0.00^{a}
	25	617.80 ± 1.00^{a}	518.54 ± 2.00^{a}	1135.66 ± 0.57^{a}	0.32 ± 0.00^{b}
	50	613.53 ± 1.00^{b}	312.33 ± 1.52^{d}	925.66 ± 0.57^{d}	0.29 ± 0.00^{d}
$CaCl_2$	75	611.66 ± 0.57^{c}	317.33 ± 1.52^{c}	929.33 ± 1.52^{c}	0.30 ± 0.00^{c}
	150	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Differ letters in each column indicate significant difference at 0.05 level.					
	Control	618.33 ± 0.57^{b}	484.00 ± 1.00^{a}	1102.33 ± 1.52^{a}	0.36 ± 0.00^{a}
	25	618.10 ± 1.00^{b}	339.66 ± 1.52^{c}	957.33 ± 0.57^{c}	0.29 ± 0.00^{c}
$MgCl_2$	50	472.81 ± 1.00^{c}	173.36 ± 1.00^{d}	646.33 ± 1.52^{d}	0.17 ± 0.00^{d}
	75	621.33 ± 0.57^{a}	446.33 ± 1.52^{b}	1068.00 ± 2.00^{b}	0.33 ± 0.00^{b}
	150	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Differ letters in each column indicate significant difference at 0.05 level.					

Many researchs reveal that the salinity stress caused decreases of chlorophyll pigment amount in rice as most of plants (Garcia et al., 1997; Mitsuya et al., 2003; Ali et al., 2004; Turan et al., 2007). In our study, photosynthetic pigment contents generally showed decreases depending on salt concentration in the rice seedlings. However, the *chlorophyll a* content for 75 mM $MgCI_2$ application and *chlorophyll b*/*total chlorophyll* contents for 25 mM $CaCI_2$ application increased according to the control group. Similar with our study, in the previous studies photosynthetic pigment contents shown increases with increasing salt concentration (Hossain et al., 2006).

MDA content

The salinity caused an increase in the MDA amount with increasing salt concentration. The highest MDA value was obtained from application of 150 mM NaCI with 3.5067 µmol/g, at 75 mM $CaCl_2$ with 4.1820 µmol/g and at 75 mM $MgCl_2$ with 3.6 µmol/g (Table 6). The MDA content was higher in the $CaCl_2$ than the other two salts (NaCI, $MgCI_2$). Among the tested applications, it was found that the largest cell membrane damage occurred in the 75 mM application of $CaCI_2$ with the highest MDA content.

Table 6. Effect of salinity stress on MDA content (lipid peroxydaiton) (µmol/g ± Sd).

	Salt Concentration (mM)					
Salt Types	**Control**	**25**	**50**	**75**	**150**	**300**
NaCI	2.69±0.04^d	2.89±0.04^c	2.76±0.04^d	3.01±0.04^b	3.50±0.053^a	0.00±0.00
$CaCl_2$	2.69±0.04^c	2.64±0.04^c	3.72±0.02^b	4.18±0.05^a	0.00±0.00	0.00±0.00
$MgCl_2$	2.69± 0.04^c	2.51±0.04^d	3.25±0.05^b	3.36±0.04^a	0.00±0.00	0.00±0.00

Differ letters in each line indicate significant difference at 0.05 level.

The salinity stress causes free radical formation in the plants. These radicals cause irreversible damage to lipids and proteins by leading to peroxydaiton of cell membrane lipid (Kuşvuran, 2010). Result of lipid peroxydation of cell membrane, MDA reveals as the last product (Ohkawa et al., 1979). The MDA content showed differences according to applied salt types and proportionally increased as the salt concentration increase in all salt types generally in our study. Rice seedlings reached higher value in $CaCI_2$ than other 2 salt types (NaCI, $MgCI_2$) hence cell damage was more at $CaCI_2$. Demiral and Türkan (2004) reported that the MDA amount of the rice plants, which is exposed to increased salt concentration, occured increases. Orcan et al. (2019) reported that different concentrations of NaCI stress increased MDA content with different rice varieties.

CONCLUSIONS

As a result, it was determined that Karacadağ local rice, which exposed salt stress under *in vitro* condition, was effected negatively in both germination and seedling growth stages. In order to minimize yield losses as a result of these adverse, future researches on the development of salinity tolerance mechanisms should be supported by physiological, genetic and biochemical studies.

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