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Original Article

Effect of Salinity Stress on Plant Growth Parameters and Antioxidant Activity In Some Cucumber Cultivars (*Cucumis sativus* L.) Grown Under *In Vitro* Conditions

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ABSTRACT

In this study, effects of salinity (NaCl) at different concentrations (0, 50, 100, 150, 200, 250, 300 mM) on the growth of cucumber plants were determined in *in vitro* conditions. The study was carried out in the plant tissue culture laboratory of Agricultural Sciences and Technology Faculty of Sivas Science and Technology University (Sivas, Türkiye) . Experiments were conducted with 3 different cucumber varieties. Mureshige and Skoog (MS) was used as the basic nutrient medium. During the study, some parameters were evaluated such as germination rate (%), salt tolerance index (%), stem fresh and dry weight (g), root fresh and dry weight (g), actual water content (%), stem and root lengths and visual scale values. Also, antioxidant capacity were determined by DPPH and ABTS. When the effect of different NaCl doses on the varieties was evaluated, the highest antioxidant capacity was obtained from HD medium (173.21 µmol TE g^{-1} dw) in the DPPH analysis and from HC medium (251.06 µmol TE g^{-1} dw) in the ABTS analysis. In the study, the highest germination rate was obtained in the variety 1 (94.36%) and in the HA medium (96.05%). In the actual water content, the variety average was the highest in the variety 2 (91.16%) and the medium average was the highest in the HA (91.71%). The highest stem length was determined in the variety 1 (26.40 mm) and in the HA medium (24.48 mm). Considering the results of the study, although there were differences among the varieties, the increase in NaCl concentration negatively affected germination and plant growth in cucumber.

Key words: *In vitro*, NaCl, Cucumber, Antioxidant

In Vitro Koşullarda Yetiştirilen Bazı Hıyar Çeşitlerinde (*Cucumis sativus* L.) Tuzluluk Stresinin Bitki Büyüme Parametreleri ve Antioksidan Aktivite Üzerine Etkisi

ÖZ

Bu çalışmada, farklı konsantrasyonlarda (0, 50, 100, 150, 200, 250, 300 mM) tuzun (NaCl) *in vitro* koşullarda hıyar bitkilerinin büyümesi üzerine etkileri tespit edilmiştir. Çalışma, Sivas Bilim ve Teknoloji Üniversitesi Tarım Bilimleri ve Teknoloji Fakültesi bitki doku kültürü laboratuvarında gerçekleştirilmiştir. Denemeler, 3 farklı hıyar çeşidi ile yürütülmüştür. Çalışmada temel besin ortamı olarak Murashige ve Skoog (MS) kullanılmıştır. Çalışma süresince, çimlenme oranı (%), tuz tolerans indeksi (%), gövde yaş ve kuru ağırlığı (g), kök yaş ve kuru ağırlığı (g), gerçek su içeriği (%), gövde ve kök uzunlukları ve görsel skala değerleri değerlendirilmiştir. Ayrıca, antioksidan kapasite DPPH ve ABTS ile belirlenmiştir. Farklı tuz dozlarının çeşitler üzerindeki etkisi değerlendirildiğinde, DPPH analizinde en yüksek antioksidan kapasite değeri HD ortamından (173,21 µmol TE g^{-1} kuru madde), ABTS analizinde ise HC ortamından (251,06 µmol TE g^{-1} kuru madde) elde edilmiştir. Çalışmada, en yüksek çimlenme oranı çeşit 1'de (%94,36), ve HA ortamından (%96,05) elde edilmiştir.

Gerçek su içeriğinde çeşit ortalaması en yüksek 2 numaralı çeşitte (%91,16), orta ortalaması ise en yüksek HA ortamında (%91.71) tespit edilmiştir. En yüksek gövde uzunluğu 1 numaralı çeşitte (26.40 mm) ve HA ortamında (24.48 mm) belirlenmiştir. Çalışma sonuçları dikkate alındığında, çeşitler arasında farklılıklar olmasına rağmen, NaCl konsantrasyonundaki artış hıyarda çimlenmeyi ve bitki büyümesini olumsuz etkilemiştir.

Anahtar kelimeler: *In vitro*, NaCl, Hıyar, Antioksidan

INTRODUCTION

Cucumber (*Cucumis sativus* L.), belongs to the *Cucurbitaceae* family, is native to Asia and has been consumed for more than 3000 years (Pandey and Kujur, 2022; Gelaye, 2023). It has very high water content and commonly consumed in salads and as pickle, and desserts. It is also rich in phosphorus, potassium, and oxalic acid, about 80% of the edible part of the cucumber fruit consists of 95% water, 0.7% protein, 0.1% fat, 3.4% carbohydrate, 0.4% fiber, and 0.4% ash (Onimisi and Ovansa, 2015; Abbey et al., 2017; Gupta et al., 2021). Cucumber is also useful in preventing jaundice and similar diseases and constipation. Since cucumber is a low-calorie vegetable, it helps weight loss by regulating blood sugar (Abbey et al., 2017). Cucumber cultivation is carried out both in open field and greenhouse as table and pickled cucumbers, and the production amount is 94,718,396 tons in the world (FAO, 2022). In Türkiye, cucumber cultivation is mostly carried out in the Mediterranean and Aegean and Marmara Regions with a production of 1,871,712 tons (TÜİK, 2023).

Salinity stress is one of the most common abiotic stresses that cause significant losses in agricultural production, especially in arid and semi-arid regions. According to FAO, approximately 800 million hectares of land worldwide are affected by salinity. Therefore, it is vital to know the mechanisms of salinity tolerance in order to obtain plants that respond better to abiotic stress. At the same time, it is necessary to achieve these goals through sustainable agricultural practices that will ensure more productive crops in the future climate change scenario (Hernández, 2019). The first symptoms of salt stress are manifested through exposure to osmotic stress, which is associated with the accumulation of phytotoxic ions in the roots, occurring from the early hours to several days later (Acosta-Motos et al., 2017). All these responses to salinity contribute to detrimental effects on plants, but there are plants tolerant to NaCl that can implement a series of adaptations to acclimatize to salinity, helping them survive. These adaptation mechanisms include morphological, physiological, biochemical, and molecular changes (Acosta-Motos et al., 2017). High salt levels affect 20% of cultivated land and half of the irrigated area (Chinnusamy et al., 2005; Sarwar et al., 2021).

Antioxidants neutralize free radicals and reactive oxygen species, preventing oxidative damage to cell membranes, proteins, DNA, and lipids, while also playing a protective role against environmental stress factors (temperature fluctuations, salt stress, drought, etc.), allowing plants to grow and develop healthily. Antioxidants protect plant health by enhancing their resistance to environmental stresses and supporting their productivity (Tabassum et al., 2024). In plants under salt stress, the activity of certain enzymes (glutathione peroxidase, superoxide dismutase, peroxidase, catalase, etc.) may change. These enzymes help neutralize free radicals and reactive oxygen species (ROS), preserving the plant's cellular structures (Zia-ur-Rehman et al., 2023). During salt stress, the regulation of potassium and sodium balance can affect the effectiveness of the plant's antioxidant systems. Maintaining this balance can enable better adaptation of the plants (Kanwal et al., 2024). As a result, salt stress can alter the antioxidant capacity in plants. However, this effect may vary depending on the plant species, the severity of the stress, and its duration. Some plants may tolerate this stress better, while others may experience more oxidative damage and lower productivity (Zia-ur-Rehman et al., 2023; Gill et al., 2024).

Plant tissue culture is the process of transferring parts of a plant to artificial nutrient media under sterile conditions. The goal of plant tissue culture were to obtain disease especially virus-free materials, to propagate species that are difficult to reproduce, to protect species at risk of extinction, and to produce a large number of plants in a short period. In this context, the aim of this study was to determine the effects of NaCl at different concentrations (0, 50, 100, 150, 200, 250, 300 mM) on the growth parameters of cucumber under *in vitro* conditions. The study also aimed to determine the antioxidant capacity of cucumber varieties in response to salt stress applied at different doses.

MATERIALS AND METHODS

This study was carried out in the plant tissue culture and also quality and soil analysis laboratories of Agricultural Sciences and Technology Faculty of Sivas University of Science and Technology (Sivas, Türkiye). Besta F1 (1), MRC 1724 F1 (2) and Tulga F1 (3) varieties obtained from Manier Seed Company (Adana, Türkiye) were used in the study.

Preparation of Nutrient Media and Seed Sowing

Murashige and Skoog (MS) (1962) was used as the basal nutrient medium. Sucrose and agar were used as the carbon source and solidification, respectively. Different doses of NaCl (0, 50, 100, 150, 200, 250, 300 mM) were tested (Table 1). After adjusting pH of the nutrient media to 5.8, they were sterilized in an autoclave for 15 minutes at 121°C and a pressure of 1.2 atm. After sterilization, the media were poured into petri dishes n a sterile bench. The cucumber seeds used in the experiments were kept in a 25% sodium hypochlorite solution for 20 minutes, then they were rinsed 4-5 times with sterile distilled water. The seeds sterilized were sown into the previously prepared nutrient media.in the sterile benchAfter sowing, the cucumber seeds were kept in the plant growth room at a temperature of 25 ± 2°C with light intensity of 3000 lux for an 8-hours dark and 16-hours light conditions. During the study, germination rate (%) (Kaya et al., 2006), salt tolerance index (%) (Rahman et al., 2008; Khayatnezhad et al., 2011; Güldüren, 2012; Aydın and Atıcı, 2015), real water content (RWC, %), stem fresh weight (g), stem dry weight (g), root fresh weight (g), root dry weight (g), stem length (mm), root length (mm) (Keleş, 2019) and visual scale evaluations in plants after salt stress (Daşgan et al., 2002) were evaluated.

Table 1. Cucumber varieties and NaCl doses used in the study

NaCl Dose (mM)	Variety		
	1	2	3
0 (Control)	HA	HA	HA
50	HB	HB	HB
100	HC	HC	HC
150	HD	HD	HD
200	HE	HE	HE
250	HF	HF	HF
300	HG	HG	HG

Determination of Antioxidant Capacity by DPPH

For DPPH, 1 gram of dried samples was weighed, 25 ml of 80% methanol was added, and the mixture was shaken in a shaking water bath for 2.5 hours at room temperature. The homogenized samples were transferred to a falcon tube and centrifuged at 7,000 rpm for 15 minutes. The supernatant of the centrifuged samples was filtered through blue band filter paper, and the resulting liquid extract was stored at +4°C. The radical DPPH scavenging activity of the extract was determined by modifying the method described by Masuda et al. (1999). A 1900 µl DPPH solution was added to 100 µl of the sample and kept in the dark for 30 minutes. The samples were analyzed in a microplate reader at 517 nm, and absorbance values were recorded. Trolox was used as a reference antioxidant for the calculation of antioxidant capacity. Solutions of different concentrations were prepared and analyzed under the same conditions to obtain a calibration curve. The antioxidant capacity of the samples was determined as µmol TE g^{-1} dry weight (dw) in terms of trolox equivalent using this curve.

Determination of Antioxidant Capacity by ABTS

A modified spectrophotometric method developed by Re et al. (1999) was used. 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (7 mM) and potassium peroxydisulfate solution (2.45 mM) were dissolved in 50 ml and 50 ml of distilled water, respectively. To prepare the stock solution, 10 ml of ABTS solution was taken, and 10 ml of $K_2O_8S_2$ (potassium persulfate) solution was added. The mixture was kept in the dark for 16 hours at room temperature to prepare the ABTS radical solution. The working solution was prepared by diluting the stock solution to give an absorbance value of 0.700 ± 0.02 at 734 nm, and the initial absorbance value was determined. Then, 3 ml of the working solution (ABTS + peroxydisulfate) or standard/sample was added to 100 µl of the extract, and after mixing, the reaction was allowed to occur for 10 minutes in a dark environment at room temperature. Absorbance values were then read at 734 nm. Trolox was used as a reference antioxidant for the calculation of antioxidant capacity. Solutions of different concentrations were prepared and analyzed under the same conditions, and a calibration curve was obtained. The antioxidant capacity of the samples was determined as µmol TE g^{-1} dry weight (dw) in terms of trolox equivalent using this curve.

Statistical Analysis

This study was carried out according to the Factorial Experimental Design with 4 replications and 5 petri dishes in each replication. Statistical analyses were performed using the JMP program.

RESULTS AND DISCUSSION

The average germination rate of different cucumber varieties and nutrient media including different doses of NaCl were given in Table 2. Variety mean, medium mean and variety x medium interaction were found to be statistically significant. Variety average was the highest in variety 1 (94.36%) and the lowest in variety 2 (84.99%). In terms of medium average, the highest germination rate was observed in HA (96.05%) and the lowest in HG (84.80%). Singh and Gopal (2019) determined the effects of water and salt stress on germination rate, germination time and seedling growth of onion seeds. They found that increasing stress level caused a gradual and significant decrease in germination rate and seedling growth. Abdel-Farid et al. (2020) determined the effects of different doses of NaCl (25, 50, 100 and 200 mM) on seed germination and seedling growth of *Cucumis sativus* and *Solanum lycopersicum* in *in vitro* and *in vivo* studies. Seed germination rate and time was slightly decreased and delayed and shoot length was significantly reduced under the highest salt concentration (200 mM) in cucumber, while seed germination rate, germination time and seedling growth were significantly affected under all NaCl concentrations in tomato. Öztürk Gökçe et al. (2022) determined the effect of different salt concentrations on onion breeding lines and obtained the highest germination rate in the control medium without salt. Our study results were similar.

Table 2. Average germination percentage (%) of different cucumber varieties on different NaCl concentrations

	Variety			
Medium	1	2	3	Medium mean
HA	98.00ab	92.00f	98.16a	96.05A
HB	96.36abc	90.91f	96.12bc	94.46B
HC	95.36cd	85.00hı	96.00cd	92.12C
HD	94.54cd	84.91hı	94.16de	91.20C
HE	92.54ef	83.63ıj	92.10f	89.42D
HF	92.03f	81.81j	88.36g	87.40E
HG	91.72f	76.67k	86.00h	84.80F
Variety mean	94.36*A*	84.99*C*	92.99*B*	
$LSD_{variety}$***:0.74	LSD_{medium}***:1.13	$LSD_{varietyxmedium}$***:1.95		

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. (2) *** $p \leq 0.001$.

The differences in salt tolerance index between different cucumber varieties and nutrient media prepared at different concentrations of NaCl were found statistically significant (Table 3). In terms of salt tolerance index, variety mean was the highest in the variety 1 (96.29%) and the lowest in the variety 2 (92.38%). The medium average was the highest in HA (100.00%) and lowest in HG (88.18%). When the variety x medium interaction was analyzed, the highest salt tolerance index was obtained from HA (100.00%) medium of varieties 1, 2 and 3, and the lowest was found in HG (83.34%) medium of variety 2, which were statistically in the same group. Kara et al. (2024) determined the growth of tomato plants in MS medium including sodium chloride at different concentrations (0, 50, 100, 150 and 200 mM) under *in vitro* conditions. In their study, the lowest salt tolerance index was observed in DE (200 mM) (81.25%) medium.

Table 3. Salt tolerance index of cucumber varieties at the different concentartions of NaCl (%)

	Variety			
Medium	1	2	3	Medium mean
HA	100.00a	100.00a	100.00a	100.00A
HB	98.33abc	98.82ab	97.92bc	98.36B
HC	97.31bcd	92.39gh	97.80bcd	95.83C
HD	96.47cd	92.29gh	95.93de	94.90C
HE	94.43ef	90.90hı	93.83fg	93.05D
HF	93.91fg	88.92ıj	90.02ı	90.95E
HG	93.59fg	83.34k	87.61j	88.18F
Variety mean	96.29*A*	92.38*C*	94.73*B*	
$LSD_{variety}$***:0.75	LSD_{medium}***:1.15	$LSD_{varietyxmedium}$***:1.99		

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. (2) *** $p \leq 0.001$.

Table 4 shows the actual water contents of different cucumber varieties and nutrient media including NaCl at various concentrations. The variety mean and medium mean were found to be statistically significant in the experiments. The variety mean was the highest in the variety 2 (91.16%) and the lowest in the variety 1 (89.07%). In terms of medium mean, the highest actual water content was found in HA (91.71%), while the lowest was detected in HG (89.44%) and HF (88.74%), which were in the same group.

Table 4. Actual water contents of cucumber varieties at the different concentartions of NaCl (%)

	Variety			
Medium	1	2	3	Medium mean
HA	90.82	92.24	92.07	91.71A
HB	90.17	91.01	90.56	90.58B
HC	89.75	90.68	90.24	90.22BC
HD	88.17	91.90	90.34	90.13BC
HE	89.56	91.19	89.91	90.22BC
HF	87.92	90.76	89.65	89.44CD
HG	87.09	90.33	88.80	88.74D
Variety mean	89.07*C*	91.16*A*	90.22*B*	
$LSD_{variety}$***:0.69	LSD_{medium} ***:1.06	$LSD_{varietyxmedium}$: N.S.		

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. N.S. – non significant (2) *** $p \leq 0.001$.

The stem fresh weight of different cucumber varieties and nutrient media including NaCl at different concentrations was given in Table 5. As a result of the analyses, both variety mean and medium mean were found to be statistically significant. According to Table 5, the highest the variety average was obtained from variety 2 (0.401 g), while the lowest was found in the varieties 3 (0.306 g) and 1 (0.302 g), which were statistically in the same group. The medium mean was the highest in HA (0.408 g) and the lowest in HG (0.251 g). Abu-Romman et al. (2012) reported that the shoot fresh and dry weights of cucumber plants decreased with increasing doses of NaCl (0.0, 25, 50, 75, and 100 mM). Kıran et al. (2015) examined the response of some eggplant rootstocks to salinity stress. When the plants reached to 4-5 true leaves stage, a decrease in the fresh weight of the green parts was observed at a concentration of 100 mM. The results obtained from our study have also showed a similar trend.

Table 5. Stem fresh weight of different cucumber varieties at the different concentartions of NaCl (g)

	Variety			
Medium	1	2	3	Medium mean
HA	0.396	0.456	0.372	0.408A
HB	0.356	0.455	0.330	0.380AB
HC	0.339	0.450	0.329	0.372AB
HD	0.274	0.447	0.305	0.342BC
HE	0.264	0.383	0.300	0.316CD
HF	0.251	0.310	0.290	0.284DE
HG	0.213	0.304		0.251E
Variety mean	0.302*B*	0.401*A*	0.306*B*	
$LSD_{variety}$***:0.03	LSD_{medium}***:0.04	$LSD_{varietyxmedium}$: N.S.		

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. N.S. – non significant (2) *** $p \leq 0.001$.

Table 6 shows the stem dry weights of different cucumber varieties and nutrient media including NaCl at various concentrations. Both variety mean and medium mean were found to be statistically significant in the analyses. According to the Table 6, the highest variety mean was observed in the variety 2 (0.034 g), while the lowest was found in the varieties 1 (0.031 g) and 3 (0.031 g), which were statistically in the same group. The medium mean was the highest in HA (0.035 g) and the lowest in HG (0.027 g).

Table 6. Stem dry weight of different cucumber varieties at the different concentartions of NaCl (g)

	Variety			
Medium	1	2	3	Medium mean
HA	0.0341	0.0379	0.0341	0.035A
HB	0.0317	0.0382	0.0333	0.034AB
HC	0.0305	0.0383	0.0326	0.034AB
HD	0.0306	0.0341	0.0318	0.032BC
HE	0.0306	0.0332	0.0307	0.032BC
HF	0.0314	0.0304	0.0285	0.030CD
HG	0.0275	0.0289	0.0247	0.027D
Variety mean	0.031*B*	0.034*A*	0.031*B*	
$LSD_{variety}$***:0.74	LSD_{medium}***:1.13	$LSD_{varietyxmedium}$: N.S.		

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. N.S. – non significant (2) *** $p \leq 0.001$.

The root fresh weight of different cucumber cultivars and nutrient media including NaCl at various concentrations was shown in Table 7. Both variety mean and medium mean were found to be statistically significant in the experiments. According to Table 7, the highest variety mean was found in the variety 2 (0.289 g), while the lowest was found in the variety 1 (0.088 g). The medium mean was the highest in HA (0.220 g) and the lowest in HG (0.100 g). In a study carried out by Afsar et al. (2020), the response of arugula to salt stress was investigated and shoot length, plant height, number of leaves, plant fresh weight, and plant dry weight were significantly reduced in plants under salt stress compared to the control. Baktemur (2023) investigated the growth of zucchini plants in nutrient media containing different concentrations of sodium chloride under *in vitro* conditions. The highest root fresh weight was observed in KD medium (3.78 g), while the lowest root fresh weight was found in the 250 mM in KF medium (2.03 g) including 250 mM NaCl.

Table 7. Root fresh weight of different cucumber varieties at the different concentartions of NaCl (g)

	Variety			
Medium	1	2	3	Medium mean
HA	0.130	0.384	0.147	0.220A
HB	0.117	0.341	0.135	0.198AB
HC	0.094	0.295	0.125	0.172BC
HD	0.077	0.294	0.120	0.164CD
HE	0.077	0.266	0.119	0.154CD
HF	0.068	0.247	0.080	0.132DE
HG	0.055	0.195	0.051	0.100E
Variety mean	0.088*C*	0.289*A*	0.111	
			B	
$LSD_{variety}$***:0.02		LSD_{medium}***:0.13	$LSD_{varietyxmedium}$: N.S.	

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. N.S. – non significant (2) *** $p \leq 0.001$.

Table 8 shows root dry weights of different cucumber varieties and nutrient media including NaCl at various concentrations. The differences in the analyses were not statistically significant.

Table 8. Root dry weight of different cucumber varieties at the different concentartions of NaCl (g)

	Variety			
Medium	1	2	3	Medium mean
HA	0.011	0.024	0.011	0.015
HB	0.007	0.019	0.008	0.011
HC	0.007	0.018	0.008	0.011
HD	0.006	0.016	0.007	0.010
HE	0.006	0.014	0.007	0.009
HF	0.005	0.001	0.006	0.008
HG	0.004	0.016	0.004	0.007
Variety mean	0.006	0.016	0.007	
$LSD_{variety}$***: 0.002		LSD_{medium}***: 0.005	$LSD_{varietyxmedium}$***: 0.003	

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. (2) *** $p \leq 0.001$.

The average stem length of different cucumber varieties and nutrient media including NaCl at various concentrations was presented in Table 9. Both variety mean and medium mean were found to be statistically significant in the study. The highest variety mean was observed in the variety 1 (26.40 mm), while the lowest was found in the variety 3 (21.55 mm). In terms of medium mean, the highest stem length was observed in HA (24.48 mm), and the lowest was in HG (20.23 mm). In a study carried out by Alrahman et al. (2005), growth and physiological characteristics of cucumber under *in vitro* salt stress (0, 50, 75, and 100 mM NaCl) determined and shoot length, fresh shoot weight, dry shoot weight, root length, and number of roots generally decreased in response to increasing salt concentrations. Kara et al. (2024) found that stem length was the highest in the DA medium (57.04 mm) and shortest in the DE medium (39.37 mm) in tomato plant growth subjected to salt stress.

Table 9. Stem lenght of different cucumber varieties at the different concentartions of NaCl (mm)

	Variety			
Medium	1	2	3	Medium mean
HA	30.50	27.08	24.86	24.48A
HB	29.18	27.02	22.61	26.27AB
HC	26.97	26.06	22.43	25.15BC
HD	25.71	24.95	22.44	24.37CD
HE	24.71	24.81	21.21	23.58DE
HF	24.08	24.37	19.09	22.51E
HG	23.66	18.87	18.18	20.23F
Variety mean	26.40*A*	24.74*B*	21.55*C*	
$LSD_{variety}$***:0.06	LSD_{medium}***:0.13	$LSD_{varietyxmedium}$: N.S.		

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. N.S. – non significant (2) *** $p \leq 0.001$.

Table 10 shows the average root length of different cucumber varieties and nutrient media including NaCl at various concentrations. Variety mean, medium mean, and variety x medium interaction were found to be statistically significant in the experiments. The highest variety mean was observed in the variety 2 (32.91 mm), while the lowest was found in the variety 1 (22.62 mm). In terms of medium mean, the highest root length was obtained from HA (36.11 mm), and the lowest was in HG (17.36 mm). When the variety x medium interaction was analyzed, the maximum root length was determined in the variety 2-HA medium (41.83 mm), and the minimum root length was found in the variety 1HF medium(14.16 mm), variety 3-HG medium (11.95 mm), and variety 1-HF medium(11.83 mm), which were statistically in the same group. Öztekin and Tüzel (2011) determined that root length decreased with the increase in salt levels in tomato. Kurum et al. (2013) investigated the effects of different salt doses on root length on rootstock squash varieties; the longest root was obtained in the control group (54.3 cm), while the shortest root was recorded at 16 dS m- (30.1 cm). Mahmood and Pırlak (2023) investigated the responses of aronia plants to salt stress in both *in vitro* and *in vivo* conditions. In the experiments, it was observed that plant height, plant dry weight, root length, chlorophyll content, and protein content decreased with increasing salt concentrations. Similar results were obtained in our study.

Table 10. Root lenght of different cucumber varieties at the different concentartions of NaCl (mm)

	Variety			
Medium	1	2	3	Medium mean
HA	35.08b	41.83a	31.42cd	36.11A
HB	27.26efg	35.23b	30.87cd	31.11B
HC	24.36gh	34.29bc	30.66de	29.77BC
HD	24.13gh	31.57cd	30.03def	28.58C
HE	21.51h	29.73def	28.14def	26.46D
HF	14.16ı	29.44def	26.91fg	23.50E
HG	11.83ı	28.31def	11.95ı	17.36F
Variety mean	22.62*C*	32.91*A*	27.14*B*	
$LSD_{variety}$***:1.32	LSD_{medium}***:2.02	$LSD_{varietyxmedium}$***:3.49		

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. (2) *** $p \leq 0.001$.

The visual scale evaluations for different cucumber varieties and nutrient media including NaCl at various concentrations was presented in Table 11. Both variety average and medium average were found to be statistically significant in the experiments. The highest variety average was obtainedfrom variety 2 (2.97), while the lowest was found in the variety1 (1.69). In terms of medium average, the highest damage was observed in HG (3.40) and HF (3.07), while the least damage was observed in HA (1.33) (HA was in the same group with HG

and HF. The importance of visual scale evaluation was reportes as important paameter by Aktaş (2002) in pepper, Daşgan et al. (2002) in tomato, and Kuşvuran (2010) in melon for selection of genotypes. Kuşvuran (2011) examined the responses of genotypes to salt stress in determining salt-tolerant genotypes in okra. It was reported that the genotypes Okr-49, Okr-6, Okr-92, and Okr-36 were the least affected by salt stress with scale values ranging from 2 to 2.50, while the genotypes Okr-93 and Okr-112 were the most damaged by salt stress with scale values of 4. Baktemur (2023) reported increased damage in zucchini plants with increased NaCl concentrations. Similar trends were observed in our study.

Table 11. Visual scala values (1-5) of different cucumber varieties at the different concentartions of NaCl

	Variety			
Medium	1	2	3	Medium mean
HA	1.00	1.80	1.20	1.33D
HB	1.60	2.60	2.20	2.13C
HC	1.60	2.60	2.40	2.20BC
HD	1.60	2.80	2.60	2.33BC
HE	1.60	3.40	2.60	2.53B
HF	1.80	3.80	3.60	3.07A
HG	2.60	3.80	3.80	3.40A
Variety mean	1.69*C*	2.97*A*	2.63 *B*	
	$LSD_{variety}$***:0.08	LSD_{medium}***:0.12	$LSD_{varietyxmedium}$: N.S.	

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. N.S. – non significant (2) *** $p \leq 0.001$.

The antioxidant capacity of different cucumber varieties and nutrient media including NaCl at various concentrations was presented according to DPPH method (Table 12). Variety mean, medium mean, and variety x medium interaction were found to be statistically significant. The highest variety mean was obtained from variety 1 (163.57 µmol TE g^{-1} dw), while the lowest was found in variety 2 (147.68 µmol TE g^{-1} dw). In terms of medium mean, the highest average was observed in HD (173.21 µmol TE g^{-1} dw), and the lowest was in HF (138.36 µmol TE g^{-1} dw). Nasrin et al. (2015) reported that the scavenging percentage (%) of DPPH free radicals was concentration-dependent. Specifically, the concentration of the extract between 5-100 µg/ml significantly increased the inhibitory activity. The IC50 value for MCS was 13.06 µg/ml, while it was 13.17 µg/ml for standard ascorbic acid. In a study conducted Shariff et al (2021) by polyphenol content and antioxidant capacity of different parts of cucumber were determined. Results showed that the ethanol peel extract contained the highest levels of phenolics (23.08 mg GAE/g) and flavonoids (14.02 mg QE/g). Furthermore, the ethanol bark extract showed a significantly ($p<0.05$) higher FRAP value. Pearson correlation analysis revealed positive correlations ($p<0.01$) between FRAP analysis and TPC (Total Phenolic Content) and TFC (Total Flavonoid Content).

Table 12. Antioxidant activity (µmol TE g^{-1} dw) recorded by DPPH analysis in the nutrient media including different concentrations of NaCl

	Variety			
Medium	1	2	3	Medium mean
HA	148.97ef	139.59h	147.22fg	145.26D
HB	162.86d	141.41h	161.27d	155.18C
HC	183.14a	161.71d	168.27c	171.05AB
HD	181.08a	163.77cd	174.78b	173.21A
HE	182.87a	152.36e	175.75b	170.33B
HF	139.56h	131.14ı	144.37fgh	138.36E
HG	146.50fg	143.81gh	153.19e	147.83D
Variety mean	163.57*A*	147.68*C*	160.69*B*	
	$LSD_{variety}$ ***: 2.83	LSD_{medium}***: 1.85	$LSD_{varietyxmedium}$***: 4.90	

In Table 13, the antioxidant capacity of different cucumber varieties and nutrient media including NaCl at different concentrations was presented according to ABTS method. In the study, the variety average, medium average, and the variety × medium interaction were found to be statistically significant. The variety average was the highest in the variety 3 (242.17 µmol TE g^{-1} dw) and the lowest in the variety 2 (210.56 µmol TE g^{-1} dw). Regarding the medium average, the highest values were found in HC (251.06 µmol TE g^{-1} dw) and HE (249.02 µmol TE g^{-1} dw) media, while the lowest value was observed in the HF (208.15 µmol TE g^{-1} dw) medium. Kanwal et al. (2024) conducted a study to alleviate the negative effects of sodium chloride (NaCl) on pea through foliar application of ascorbic acid (AsA). They found that enzymatic antioxidants, such as SOD (22.3%), POD (34.1%), and CAT (39%),were increased in both varieties under stress,. In another study, a salt stress was simulated by irrigating with a 100 mM NaCl solution, and then the leaves were sprayed with 1.0 mM salicylic acid (SA) to determine resistance of cherry rootstocks to salt stress (Xu et al., 2024). The salt treatment increased the antioxidant enzyme (peroxidase, catalase, and superoxide dismutase) activities in cherry rootstocks under salt stress, and this increase was more pronounced under salt stress with SA application (Xu et al., 2024). In plants under salt stress, the activity of certain enzymes (glutathione peroxidase, superoxide dismutase, peroxidase, catalase, etc.) may change. These enzymes help neutralize free radicals and reactive oxygen species (ROS), preserving the plant's cellular structures (Zia-ur-Rehman et al., 2023).

Table 13. Antioxidant activity (µmol TE g^{-1} dw) of different cucumber varieties determined by ABTS analysis in the nutrient media including different concentrations of NaCl

	Variety			
Medium	1	2	3	Medium mean
HA	226.46f	198.54j	234.39e	219.79D
HB	235.08e	201.51ıj	251.27c	229.29C
HC	263.42a	233.58e	256.18bc	251.06A
HD	257.49b	210.65h	245.10d	237.75B
HE	255.38bc	226.99f	264.69a	249.02A
HF	218.51g	196.54j	209.40h	208.15E
HG	218.34g	206.13hı	234.19e	219.56D
Variety mean	239.24*B*	210.56*C*	242.*17A*	
$LSD_{variety}$***: 3.41	LSD_{medium}***: 4.90		$LSD_{varietyxmedium}$***: 5.91	

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. (2) *** $p \leq 0.001$.

CONCLUSION

This study aimed to determine the effects of of salt applications at different concentrations on the growth of cucumber plants under *in vitro* conditions. The negative effects of salinity, one of the most important abiotic stresses, are increasing globally. As usable agricultural areas have continued to decrease, it is crucial to develop salinity-resistant plant varieties. As seen from the results of this study, plant damage increases with higher salinity concentrations.

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Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Author Contributions

M.D. : Setting up experiments and performing all analyses, statistical analyses & writing; Y.Ç: Antioxidant analyses, E. K. : Data analysis-review, editing & writing, G. B.: Designing of the study, controling of all analysis and measurements, supervising & writing.

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