International Journal of
Agriculture, Environment and Food Sciences
e-ISSN : 2618-5946
www.jaefs.com

Research Article
Int J Agric Environ Food Sci 6 (1): 91-99 (2022)

Leaf and root-growth characteristics contributing to salt tolerance of backcrossed pepper (*Capsicum annuum* L.) progenies under hydroponic conditions

Firdes Ulas*

Erciyes University, Faculty of Agriculture, Department of Horticulture, Kayseri, Türkiye

*Corresponding Author: fulas@erciyes.edu.tr

Citation
Ulas, F. (2022). Leaf and root-growth characteristics contributing to salt tolerance of backcrossed pepper (*Capsicum annuum* L.) progenies under hydroponic conditions. International Journal of Agriculture, Environment and Food Sciences, 6 (1), 91-99.
Doi
https://doi.org/10.31015/jaefs.2022.1.13

Received: 01 January 2022
Accepted: 08 March 2022
Published Online: 15 March 2022
Revised: 02 April 2022

Year: 2022
Volume: 6
Issue: 1 (March)
Pages: 91-99

Available online
http://www.jaefs.com
https://dergipark.org.tr/jaefs

Abstract
The aim of this study was to determine the genotypic differences in salt tolerance of third back-crossed peeper progenies and their respective parents through examining the changes in the shoot growth at agronomical, root growth at morphological and leaf development at physiological levels under salt stress. A hydroponic experiment was conducted by using an aerated Deep-Water Culture (DWC) technique in a controlled growth chamber of Erciyes University, Agricultural Faculty in Kayseri, Turkey. Five pepper plants (BC3-1, BC3-2, BC3-3, BC3-4, BC3-5) were selected from the third backcrossed (BC3) progenies of Sena and Kopan. Plants were grown in 8 L pots filled continuously aerated nutrient solution under at two electrical conductivity (EC) levels (control at 1.0 dS m^{-1} and salt at 8.0 dS m^{-1}) in RBD design with four replications for six weeks. Significant reductions in leaf, shoot and root fresh and dry biomass productions, total leaf area, total root length, and total root volume of pepper plants were recorded under hydroponic salt stress. On the other hand, significant differences in salt tolerance among backcrossed peeper progenies and their respective parents existed. Particularly the progeny of BC3-3 was more tolerant characterized to salinity than the other progenies of third backcrossed and their respective parents. This was highly associated with vigorous root growth (root fresh and dry weight, total root length and volume) and photosynthetically active leaves (total leaf area, leaf chlorophyll index, chloride exclusion) under hydroponic salt stress. These traits could be useful characters to select and breed salt-tolerant pepper varieties for sustainable agriculture in the future.

Keywords
Back crossing, *Chile penguin*, Hydroponic system, Salinity, SPAD, Total root length

Introduction
Salinity is the one of the main abiotic stress factors that adversely affects crop growth and productivity by decreasing yield and quality, particularly in arid and semi-arid regions (Huez-López et al., 2011). Although, some advanced management practices were developed in recent decades, currently, more than one billion hectares of land, accounting for approximately 25% of the global land area is being affected by salinity. Due to natural salinization or unsuitable irrigation practices, this area is increasing by up to 10 million hectares of land every year. In Turkey, 4 million hectares of land was affected by salinization (Abidalrazzaq Musluh Al Rubaye et al., 2021). Salinity stress is rising in agricultural areas owing to numerous factors including rising in sea levels, low-level rainfall, high-level evaporation, climate change, excessive irrigation without proper drainage in lands, and underlying rocks rich in harmful salts etc. (Kumar et al., 2013). In addition, if the current scenario of salinity stress would continue, 50% of the land currently cultivated for agriculture may be lost by 2050 [Wang et al 2003]. Most of the crop plants are sensitive to salinity and thus under excessively saline conditions the crops usually exhibit shorter life cycles or limited plant growth and diminished biomass production (Abidalrazzaq Musluh Al Rubaye et al., 2021). It has been demonstrated that salinity stress causes various major problems in crop plants such as intercellular damage and metabolic disturbance (Chartzoulakis and Klapaki, 2000),

reduction in stomatal conductance, transpiration rate and net photosynthesis (Lycoskoufis et al., 2005), water stress in source leaves and carbohydrate shortage in sink leaves (Kurtar et al., 2016), ion toxicity by excessive Na^+ and Cl^- accumulation and reduction of K^+ uptake (Wu et al., 2013), and inhibition in root growth, nutrient uptake (Colla et al., 2013).

Pepper (*Capsicum annuum* L.) is an important horticultural crop in the world due to its economic significance and the nutritional value of fruits. Global pepper production has increased over the last 20 years from 17 to 36 million tons (Mt), and the cultivated area has expanded by about 35% particularly in arid and semiarid regions (Tripodi and Kumar, 2019). Pepper is a warm-season crop possessing vulnerability features to abiotic and biotic factors. Pepper plants are considered moderately sensitive, sensitive, or highly susceptible to salinity stress (Penella et al., 2017). Dry pepper cultivation in Turkey is generally carried out in arid and semi-arid regions in the southeastern part of the country. As mentioned above, one of the important problems in arid and semi-arid regions is continuing salinization in the soil. For this reason, the breeding and releasing of salt stress-tolerant varieties are important for the sustainability of dry pepper production in these areas. To avoid or reduce salt stress impacts and hinder yield losses for sustainable pepper production, integrated salt management strategies that take into consideration improved soil and crop management practices are necessary. Moreover, another way is to improve the salinity tolerance of high-yielding salt susceptible pepper cultivars would be applying an interspecific hybridization breeding technique.

In the past decades to enhance salinity tolerance in crops, several strategies such as conventional selection and breeding techniques have been recommended and applied (Shannon, 1998). Interspecific hybridization has been used to introgress useful traits from wild and related species into cultivated varieties in many Solanaceous crops, particularly in terms of pest and disease resistance. The Capsicum genus involve 27 species, 5 domesticated and 22 wild-type species. As genetic resources for breeding enhanced *Capsicum annuum*, wild and related Capsicum species are beneficial not only breeding disease resistance, although additionally for improving the yield, nutritional quality, and adaptation to abiotic stresses (Yoon et al., 2006). However, no comprehensive hydroponic studies were found in the literature that aimed to search the answer to the most critical questions of which agronomical, physiological and root morphological characteristics are contributing to salt tolerance, and which are the consistent traits for selecting salt-tolerant pepper genotypes. Therefore, the aim of this study was to determine the genotypic differences in salt tolerance of third backcrossed (BC3) peeper progenies and their respective parents [Sena (*Capsicum annuum* L.) and Kopan (*Chile penguin*)] through examining the changes in the shoot growth at agronomical, root growth at morphological and leaf development at physiological levels under hydroponic salt stress. This approach will help to identify plant traits that are suitable for the selection and breeding of salt-tolerant pepper varieties for sustainable agriculture in the future.

Materials and Methods

Plant material, treatments, and experimental design

This study was carried out in the Plant Physiology Laboratory of Erciyes University, Faculty of Agriculture, central Anatolia in Turkey. A hydroponic experiment was conducted by using an aerated deep-water culture (DWC) technique in a fully automated climate chamber. For the vegetation period, the average day/night temperatures were 25/22 °C, the relative humidity was 65–70% and about 390 μmol m^{-2} S^{-1} photon flux was sup-plied in a photoperiod of 16/8 h of light/dark regimes in the controlled growth chamber. The plant materials used were backcross lines obtained through consecutive backcrossing for three generations of Sena (*Capsicum annuum* L.) and Kopan (*Chile penguin*). Five selected BC3 plants (BC3-1, BC3-2, BC3-3, BC3-4, BC3-5) and their parents screened for salinity tolerance at 8 dS·m^{-1}. The seeds used in the experiment were produced by selfing from the parents and the plants selected from the backcross population. Kopan has small, hot pepper fruits that had lower strength for separation from fruit pedicel. Sena has fruits that are approximately 10 cm in length and 2.5 cm in diameter and appropriate for pepper powder and dry chili pepper production. The seeds were sown in 72-cell polystyrene trays (W 280 × L 540 × H 45 mm, IBK Iklim Bahce Co., Ltd., Turkey) filled with a mixture of peat (pH: 6.0-6.5) and perlite (2v:1v). To promote germination, the plug trays were wrapped with vinyl chloride resin film and then placed in a germination chamber at 28°C. After four days, the germinated seedlings were moved to a greenhouse. Seedlings were watered daily. When the seedlings developed three or four true leaves, they were transplanted to plastic pots after the root system of the plants was carefully washed in distilled water to clean the substrate.

For salinity treatments two electrical conductivity (EC) levels (control at 1.0 dS m^{-1} and salt at 8.0 dS m^{-1}) were applied two weeks after transplantation. Each pot was filled with 8 L cultivation solution that was aerated by an air pump. The nutrient solution contained 1.5 mM calcium nitrate ($Ca(NO_3)_2$,) 250 μM monopotassium phosphate (KH_2PO_4), 500 μM potassium sulfate (K_2SO_4), 325 μM magnesium sulfate ($MgSO_4.7H_2O$), 50 μM sodium chloride (NaCl). Micronutrients were 80 μM iron (Fe) (III)- ethylenediaminetetraacetic acid (EDTA)- sodium (Na), 0.4 μM manganese sulfate ($MnSO_4$), 0.4 μM zinc sulfate ($ZnSO_4$), 0.4 μM copper sulfate ($CuSO_4$), 8 μM boric acid (H_3BO_3), 0.4 μM sodium molybdate (Na_2MoO_4). Solutions were replaced completely every week in the first two weeks. In hydroponic experiment the total vegetation period from transplanting into 8 L plastic pots up to final harvest was almost six weeks. The experiment was in a completely randomized block design with three replications and four plants in each replication.

Biomass Determination

At the end of the experiment, the biomass of the aerial part of the plants was deter-mined. Pepper plants were harvested by separating them into stems, leaves, and roots and weighed for fresh biomasses determination. Immediately afterward, their tissues were dried in a forced-air oven at 70 ◦C for 72 h for dry biomass determination until the stable weight was reached. And then they were weighed on an electronic digital scale. Shoot biomass was equal to the sum of aerial vegetative plant parts (leaves + stems) and was expressed in g plant^{-1}. Root: shoot ratio was calculated by dividing the root dry weight by the shoot dry weight.

Root Morphological Measurements

The plant root morphological parameters such as total root length (m plant^{-1}), total root volume (cm^3 plant^{-1}) and average root diameter (mm) were measured by using a special image analysis software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc., Québec, QC G1V 1V4, Canada) in combination with a recording device of Epson Expression 11000XL scanner (Long Beach, CA, USA). It was recorded as cm plant-1, and then converted to m plant^{-1}.

Leaf Physiological Measurements

In the hydroponic experiment, the total leaf area of the plants was measured destructively during the harvesting process by using leaf-area meter (LI-COR Model 3100, LI-COR. Inc., Lincoln, NE, USA). Total leaf area was recorded in centimeter square (cm^2). Prior to harvest, non-destructive measurement of the leaf chlorophyll content (SPAD) was done in a controlled growth chamber by using the Minolta SPAD-502 chlorophyll meter. During the growth period, the leaf chlorophyll content measurement was performed on the youngest fully expanded leaves (3rd–4th leaf from the apex) of whole plants, using four replicate leaves per treatment in the third and fifth week of the vegetation period. The measurements were carried out at the temperature of 25/22°C (average day/night temperatures), the relative humidity of 65–70%. The supplied photon flux in the growth chamber was almost 390 µmol m^{-2} S^{-1} with an intensity of 16/8h (light/dark) photoperiod. All SPAD readings were carried out between 09:00 and 12:00 HR.

Leaf Ion Determination

Dried plant tissues (leaf) were ground separately in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 20-mesh screen; then 0.5 g of the dried plant tissues were analyzed for chloride concentration (Cl^-). Chloride was analyzed by precipitation as AgCl and titration according to Johnson and Ulrich (1959).

Statistical Analysis

All measured physiological and morphological parameters were analyzed using SAS Statistical Software (SAS 9.0, SAS Institute Inc., Cary, NC, USA). A two-factorial analysis of variance was performed to study the effects of genotypes and salt and their interactions on the variables analyzed. Levels of significance are represented by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and ns means not significant. Differences between the treatments were compared using Duncan's Multiple Test ($p < 0.05$).

Results and Discussion

Biomass Production and Partitioning

To evaluate whether plants provided a similar growth rate after NaCl treatment, fresh and dry biomass were measured in both roots and aerial organs at final harvest. According to the results shown in Table 1 generally, the growth and development of the pepper plants were negatively affected by increasing the salt level of the nutrient solutions. Plant growth significantly decreased as the NaCl concentration in the nutrient solution increased. The growth performance of the pepper plants was dependent on genotype and salinity. Fresh matter of leaf, shoot and root were significantly (p <0.001) affected by genotype, different salt levels and genotype × salt interaction (Table 1). Fresh matter of leaf, shoot and root decreased by different rates in each genotype with the salt application. Leaf fresh matter ranged between 9.10-16.27 g plant^{-1} under control conditions (1 dS m^{-1}) and between 4.22-10.92 g plant^{-1} under saline conditions. Compared to the control plants, 8 dS m^{-1} NaCl decreased the leaf fresh matter of BC3-3 and BC3-5 plants by 21.5 and 71.9%, respectively.

Regarding shoot fresh matter, it ranged between 10.60-20.00 g plant^{-1} under control conditions (1 dS m^{-1}) and between 4.99-13.62 g plant^{-1} under saline conditions. Under non-saline conditions, BC3-2 has a significantly higher leaf and shoot fresh matter, whereas Sena has significantly lower leaf and shoot fresh matter than other backcross plants. Salt stress decreased the shoot fresh matter of BC3-3 and BC3-5 plants by 26.3 and 74.5%, respectively as compared to unstressed plants. Regarding root fresh matter, BC3-1 plants developed a higher root system than respective parents and their progenies of third backcrosses under non-saline conditions. Root fresh matter ranged between 5.41-13.72 g plant^{-1} under control conditions (1 dS m^{-1}) and between 2.90-9.30 g plant^{-1} under saline conditions. Salt-stress caused a decrease in leaf, shoot and root fresh matter in all treated respective parents and their progenies of third backcrosses studied. 8 dS m^{-1} NaCl treatment decreased the root fresh matter of BC3-3 and BC3-4 plants by 19.1 and 56.0%, respectively compared to control plants. BC3-3 had a significantly higher leaf and root fresh matter, whereas BC3-5 has significantly lower leaf and shoot fresh matter than other respective parents and their progenies of third backcrosses under saline conditions. Sena had a significantly lower root fresh matter under saline conditions.

Results of the leaf, shoot and root dry matter of the pepper plants grown in different salt levels (1 dS m^{-1} and 8 dS m^{-1}) at the end of the growing cycle were shown in Table 2. Dry matter of leaf, shoot and root were significantly (p <0.001) affected by genotype, different salt levels and genotype × salt interaction. Salt stress applied through the nutrient solution significantly inhibited plant growth, reduce plant mass (Table 2), total leaf area (Fig. 1A) and leaf chlorophyll content (Fig. 1B). The exposure of pepper plants to NaCl salinity (8 dS m^{-1}) resulted in a marked suppression of the leaf, shoot and root dry matter. Salt stress adversely affects the growth and development of pepper plants, and the results of our study confirm that all growth variables of pepper genotypes drastically decreased with NaCl treatment. Under non-saline conditions, leaf, shoot and root dry matter in BC3-1 were found to be significantly higher than respective parents and their progenies of third backcrosses. On the other hand, under saline conditions, BC3-1, BC3-2, BC3-3, and BC3-4 had significantly higher leaf dry matter; though, BC3-5, Kopan and Sena had significantly lower leaf dry matter. Compared to the unstressed plants, salt stress decreased the leaf dry matter of BC3-4 and BC3-5 plants by 24.5

and 68.3%, respectively compared to control plants. There was a significant difference between pepper cultivars for shoot dry matter. The maximum accumulation of dry matter of the shoot was in BC3-1 under saline conditions, although the lowest accumulation of dry matter of the shoot was in Kopan. Salt stress decreased shoot dry matter from 26.5% in BC3-3 to 64.9% in BC3-5 as comparing with unstressed plants.

The root dry matter varied from 0.86 in BC3-3 to 0.25 in Sena of 8 dS m^{-1}. 8 dS m^{-1} NaCl decreased the root dry matter of BC3-3 and BC3-5 plants by 17.3 and 56.2%, respectively than control plants.

Table 1. The effects of different salt levels (1 dS m^{-1} and 8 dS m^{-1}) on fresh matter of leaf, shoot and root of pepper plants

	Leaf fresh matter (g $plant^{-1}$)			Shoot fresh matter (g $plant^{-1}$)			Root fresh matter (g $plant^{-1}$)		
Genotypes	Control	Salt	% R	Control	Salt	% R	Control	Salt	% R
BC3-1	15.88 b	10.60 B	33.3	19.61 b	13.62 A	30.5	13.72 a	7.93 C	42.2
BC3-2	16.27 a	10.38 C	36.2	20.00 a	12.69 C	36.6	13.09 b	8.95 B	31.7
BC3-3	13.90 d	10.92 A	21.5	17.52 d	12.92 B	26.3	11.49 c	9.30 A	19.1
BC3-4	11.90 e	7.81 D	34.3	17.72 c	10.22 D	42.3	10.00 d	4.40 D	56.0
BC3-5	15.02 c	4.22 G	71.9	19.60 b	4.99 F	74.5	8.98 e	3.99 E	55.6
Kopan	10.72 f	4.31 F	59.8	11.79 e	5.03 F	57.3	6.60 f	3.60 F	45.4
Sena	9.10 g	4.41 E	51.5	10.60 f	5.63 E	46.9	5.41 g	2.90 G	46.3
F-Test									
Genotype		***			***			***	
Salt		***			***			***	
Genotype × Salt		***			***			***	

%R: Reduction. Values denoted by different letters are significantly different between respective parents and their progenies of third backcrosses within both columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Table 2. The effects of different salt levels (1 dS m^{-1} and 8 dS m^{-1}) on dry matter of leaf, shoot and root of pepper plants

	Leaf dry matter (g $plant^{-1}$)			Shoot dry matter (g $plant^{-1}$)			Root dry matter (g $plant^{-1}$)		
Genotypes	Control	Salt	% R	Control	Salt	% R	Control	Salt	% R
BC3-1	1.74 a	1.12 A	35.3	2.16 a	1.45 A	32.8	1.26 a	0.74 C	41.6
BC3-2	1.61 b	1.09 A	32.3	1.95 b	1.33 B	31.9	1.21 b	0.83 B	31.3
BC3-3	1.46 d	1.09 A	25.6	1.74 c	1.28 B	26.5	1.04 c	0.86 A	17.3
BC3-4	1.43 d	1.08 A	24.5	1.89 b	1.36 AB	28.0	0.92 d	0.41 D	55.3
BC3-5	1.52 c	0.48 B	68.3	1.94 b	0.68 C	64.9	0.82 e	0.36 E	56.2
Kopan	1.18 e	0.46 B	61.4	1.28 d	0.53 D	58.3	0.61 f	0.33 F	46.4
Sena	0.97 f	0.52 B	46.6	1.14 e	0.60 CD	47.2	0.50 g	0.25 G	49.6
F-Test									
Genotype		***			***			***	
Salt		***			***			***	
Genotype × Salt		***			***			***	

%R: Reduction. Values denoted by different letters are significantly different between respective parents and their progenies of third backcrosses within both columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Total Leaf Area and Leaf Chlorophyll Content

The results shown in Figure 1A and 1B showed that the negative salinity effect resulted in a clear decrease in total leaf area and leaf chlorophyll content (SPAD) by increasing salt concentrations. Total leaf area and SPAD were significantly (p <0.001) affected by genotype, different salt levels and genotype × salt interaction. Total leaf area was significantly decreased by increasing salt level of the nutrient solution. Salt treatment with increasing concentrations of the nutrient solution negatively affected plant growth causing significant decreases in total leaf area and leaf chlorophyll content. Total leaf area ranged between 358-604 cm^2 $plant^{-1}$ in control conditions and between 159-386 cm^2 $plant^{-1}$ in the saline conditions. Significantly higher total leaf area formation was observed in BC3-2, whereas significantly lower total leaf area formation was observed in Sena. On the other hand, under salt stress conditions, BC3-3 had significantly higher total leaf area formation as compared to respective parents and their progenies of third back-crosses. Significantly lower total leaf area formation was observed in BC3-5 and Sena (Figure 1A). 8 dS m^{-1} NaCl decreased the total leaf area of BC3-3 and BC3-5 plants by 28.2 and 72.9%, respectively than control plants.

Based on the SPAD, the supply of saline nutrient solution to pepper plants restricted markedly the rate of SPAD during the whole growing period, and the suppression was consistently more profound under salinity. Leaf chlorophyll content ranged between 33.2-51.9 in control conditions and 32.7-53.8 in saline conditions. At control conditions, BC3-4 had a higher SPAD value and was significantly different from respective parents and their progenies of third backcrosses. Under saline conditions, the highest SPAD value was observed at BC3-2 and BC3-5 and significantly different from respective parents and their progenies of third backcrosses (Figure 1B). The lowest SPAD value was determined in the Kopan pepper genotype.

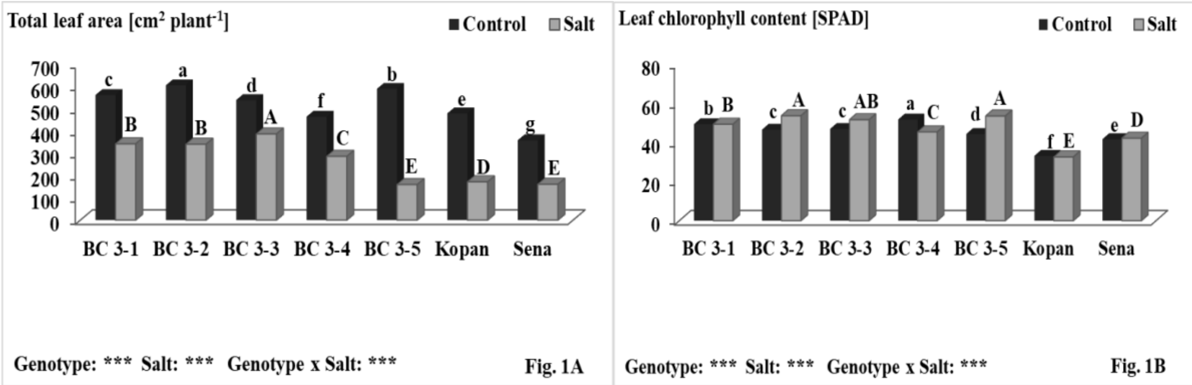

Figure 1. The effects of different salt levels (1 dS m^{-1} and 8 dS m^{-1}) on total leaf area (A), and leaf chlorophyll content (B) of pepper plants.

Values denoted by different letters are significantly different between respective parents and their progenies of third backcrosses within both columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Root Morphological Development and Root Architecture

The results of the total root length, total root volume and average root diameter at the end of the growing cycle of pepper plants grown in different salt levels (1 dS m^{-1} and 8 dS m^{-1}) were shown in Table 3. Total root length, total root volume and average root diameter were significantly (p <0.001) affected by genotype, different salt levels and genotype × salt interaction. The root growth of pepper plants was severely restricted at a salinity level of 8 dS m^{-1}. Pepper plants under 1 dS m^{-1} salt concentrations produced higher total root length and total root volume than plants grown under 8 dS m^{-1} salt concentrations. The effects of 8 dS m^{-1} NaCl on total root length in all pepper plants, respectively, showed a similar trend as root fresh weight. Under control conditions, significantly greater total root length was produced at progenies of third backcrosses BC3-2; however significantly lower total root length was produced at Sena.

The total root length was ranked between 8649 m $plant^{-1}$ and 3538 m $plant^{-1}$ under control conditions. Compared to the unstressed plants, salt stress decreased the total root length of BC3-3 and BC3-4 plants by 7.8 and 61.3%, respectively compared to control plants. It was ranked from 6361 m $plant^{-1}$ and 2283 m $plant^{-1}$ under salinity. The progenies of third backcrosses of BC3-3 have significantly higher total root length, while BC3-4 produced significantly lower total root length (Table 3). Regarding total root volume, it was ranked between 4.33 cm^3 $plant^{-1}$ and 3.00 cm^3 $plant^{-1}$ under non-saline conditions. On the other hand, under saline conditions it was ranked from 4.14 cm^3 $plant^{-1}$ to 1.77 cm^3 $plant^{-1}$. 8 dS m^{-1} NaCl decreased the total root volume of BC3-1 and BC3-4 plants by -30.2 and 49.9%, respectively than control plants.

The maximum root volume found were 4.33 cm^2 $plant^{-1}$ in Kopan grown under control conditions whereas B3-3 plant produced the highest root volume with 4.14 g under salt stress (Table 3). Concerning average root diameter, it was ranked among 0.336 mm and 0.223 mm under non-saline conditions; while, under saline conditions it was ranked among 0.353 mm and 0.288 mm.

Table 3. The effects of different salt levels (1 dS m^{-1} and 8 dS m^{-1}) on total root length, total root volume and average root diameter of pepper plants

	Total root length (m $plant^{-1}$)			Total root volume (cm^3 $plant^{-1}$)			Av. root diameter (mm)		
Genotypes	Control	Salt	% R	Control	Salt	% R	Control	Salt	% R
BC3-1	7684 b	5365 C	30.2	3.00 d	3.91 B	-30.2	0.223 g	0.305 D	-36.6
BC3-2	8649 a	5369 B	37.9	3.75 b	3.66 C	2.4	0.235 f	0.295 E	-25.4
BC3-3	6896 c	6361 A	7.8	3.46 c	4.14 A	-19.7	0.253 e	0.288 E	-13.9
BC3-4	5896 d	2283 G	61.3	3.53 bc	1.77 G	49.9	0.276 d	0.314 D	-13.8
BC3-5	5856 e	2461 F	58.0	3.75 b	2.08 F	44.5	0.286 c	0.328 C	-14.9
Kopan	5419 f	2773 D	48.8	4.33 a	2.61 E	39.8	0.319 b	0.346 B	-8.5
Sena	3538 g	2762 E	22.0	3.14 d	2.71 D	13.7	0.336 a	0.353 A	-5.2
F-Test									
Genotype		***			***			***	
Salt		***			***			***	
Genotype × Salt		***			***			***	

%R: Reduction. Values denoted by different letters are significantly different between respective parents and their progenies of third backcrosses within both columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Salinity significantly increased average root diameter in all tested respective parents and their progenies of third backcrosses; furthermore, a significantly higher average root diameter was produced at by recurrent parent Sena.

Leaf Ion Determination

Results of leaf Cl^- composition of pepper plants grown in different salt levels (1 dS m^{-1} and 8 dS m^{-1}) at the end of the growing cycle were presented in Figure 2. Leaf Cl^- composition was significantly (p <0.001) affected by genotype, different salt levels and genotype × salt interaction. Exposure to high NaCl concentrations disrupts ion homeostasis in plant cells (Pasternak, 1987). Thus, the evaluation of the ion concentration in leaf tissues after exposure to stress was crucial for this experiment. The Cl^- concentration in leaves increased with a higher NaCl concentration. Under saline conditions, Kopan accumulated the highest Cl^- levels in leaves.

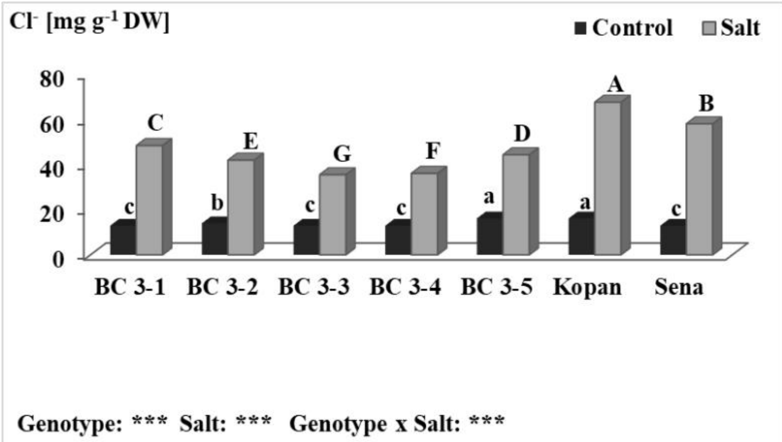

Figure 2. The effects of different salt levels (1 dS m^{-1} and 8 dS m^{-1}) on leaf Cl^- composition of pepper plants.
Values denoted by different letters are significantly different between respective parents and their progenies of third backcrosses within both columns at $p < 0.05$. ns, non-significant. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Discussion

To evaluate whether plants provided a similar growth rate after NaCl treatment, fresh and dry biomass were measured in both roots and aerial organs at final harvest. Our results showed that the growth, crop biomass and physiology of pepper plant decreased under salt stress. Compared to the control plants, 8 dS m^{-1} NaCl decreased the leaf fresh matter of BC3-3 and BC3-5 plants by 21.5 and 71.9%, respectively. The decline in fresh matter of leaves in response to salt stress has been studied for other crops and different reductions rate have been reported depending on salt stress and genotype (Penella et al., 2017). It is known that pepper is a salt-sensitive plant species (Navarro et al., 2006). It is also well-known that salinity stress reduces plant growth and that there are differences among cultivars with peppers (Penella et al., 2017). The lower growth rate of the plant is associated with the end of new leaf expansion and limited leaf growth by slowing the cellular division. Bojórquez-Quintal et al (2014) studying the tolerance mechanisms to salt in chili habanero (*Capsicum chinense* Jacq.) plants in two varieties that exhibit different sensibilities to the salt stress, between them the 'Rex' variety, more tolerant than the 'Chichen-Itza' variety testify a concentration of 150 mM of NaCl through seven days for a culture in hydroponic conditions, and observed high impact on the growth of the two varieties with significant reduction of dry and fresh weight induced by NaCl in both the genotypes, in the fresh weight the reduction was greater in 'Chichen-Itza' (75%), than the reduction in the 'Rex' variety (50%).

Several studies have indicated that shoot and root growth in most horticultural crops, including cucumber, tomato, watermelon, pepper, and citrus, are inhibited by elevated NaCl concentrations in the soil or growth medium solution (Gong et al., 2013).

The reduction in plant growth under salt stress conditions could be attributed to the decline in osmotic potential because of excess concentration of Na^+ and Cl^- ions in the root zone resulting in a nutritional imbalance (Pasternak, 1987). Also, inhibitory effects of salinity on vegetative growth may be because of the diversion of energy from growth to exclude Na^+ uptake and synthesis of compatible solutes to maintain cell turgor under hyperosmotic saline conditions (Munns and Tester, 2008).

Salt stress applied through the nutrient solution significantly inhibited plant growth, reduce plant mass (Table 2), total leaf area (Fig. 1A) and leaf chlorophyll content (Fig. 1B). The exposure of pepper plants to NaCl salinity (8 dS m^{-1}) resulted in a marked suppression of the leaf, shoot and root dry matter. Salt stress decreased shoot dry matter from 26.5% in BC3-3 to 64.9% in BC3-5 and the root dry matter of BC3-3 and BC3-5 plants by 17.3 and 56.2%, respectively as comparing with unstressed plants. This can be associated with salt stress which can cause morphological, physiological and biochemical alteration in critical levels, interfering with the absorption and transportation of water and nutrients to the plant (Ulas et al., 2020). The decline in plant growth under salinity has also been demonstrated in other studies in pepper (Abidalrazzaq Musluh Al Rubaye et al., 2021), melon (Ulas et al., 2019; Ulas et al., 2020), cucumber (Colla et al., 2012), tomato (Gong et al., 2013), lettuce (Lucini et al., 2015) and pepino (Ulas, 2021) grown hydroponically under greenhouse conditions.

The negative salinity effect resulted in a clear decrease in total leaf area and leaf chlorophyll content

(SPAD) by increasing salt concentrations (Figure 1A and 1B). Total leaf area were significantly decreased by increasing salt level of the nutrient solution. Variability in SPAD chlorophyll content due to abiotic stresses such as water and salt stresses were reported in earlier work, and the use of suitable genotypes to provide suitable environmental conditions for the crop was suggested (Ahmed and Hassan, 2015). Salt treatment with increasing concentrations of the nutrient solution negatively affected plant growth causing significant decreases in total leaf area and leaf chlorophyll content. Our results are inconsistent with Sagi et al., (1997) who observed the adverse effects of salinity stress on leaf area. It has been reported that, the decline in plant biomass may be due to excessive accumulation of NaCl in chloroplasts of sweet pepper, which affects growth rate, and is often associated with a decrease in the electron transport activities of photosynthesis and inhibition of PSII activity. Reduction in leaf area is caused by ion accumulation in the leaves, particularly the old ones. Leaf chlorophyll content is often well correlated in salt-stressed plants (Gong et al., 2013). Changes in chlorophyll content may be associated with genotypes, stress period and stress intensity. Özdemir et al., (2016) stated that an increase in chlorophyll degradation or the decrease in the synthesis of chlorophyll may affect a decline in chlorophyll content.

The root growth of pepper plants was severely restricted at a salinity level of 8 dS m^{-1} (Table 3). Pepper plants under 1 dS m-1 salt concentrations produced higher total root length and total root volume than plants grown under 8 dS m-1 salt concentrations. The effects of 8 dS m^{-1} NaCl on total root length in all pepper plants, respectively, showed a similar trend as root fresh weight. Salinity significantly increased average root diameter in all tested respective parents and their progenies of third backcrosses. Based on the study by Dölarslan and Gül (2012) transpiration and respiration as well as water uptake and root development decreased under saline conditions. Likewise in our study, Abidalrazzaq Musluh Al Rubaye et al., (2021) stated that salt stress caused a decline in total root length depending on genotypes as compared to control plants in pepper plants.

Exposure to high NaCl concentrations disrupts ion homeostasis in plant cells (Pasternak, 1987). Thus, the evaluation of the ion concentration in leaf tissues after exposure to stress was crucial for this experiment. The Cl^- concentration in leaves increased with a higher NaCl concentration (Figure 2). These results are in agreement with other studies explaining that plants suffer from ionic imbalance and nutrient deficiency under salt stress (Abidalrazzaq Musluh Al Rubaye et al., 2021). It is important to sustain ion homeostasis to prevent toxic accumulation, as plants come into contact with salt. Plants survive with this situation by various mechanisms that can contribute to salt tolerance, some of which are very well documented in the bibliography (Isayenkov and Maathuis, 2019). It is well documented that Cl^- may decline the leaf chlorophyll concentration and the activity of RuBP carboxylase without a corresponding decline in the concentration of this enzyme, which is accompanied by suppression of leaf photosynthesis. Efficient intracellular compartmentation of Cl^- may arise in salt tolerant plant species, thus defending chloroplasts from undesirably high Cl^- concentrations. Though, salt sensitive salt species are incompetent of providing low Cl^- concentrations in chloroplasts under salinity by means of effective compartmentation between cytoplasm and vacuole (Seemann and Critchley, 1985). Hence, part of growth reduction owing to salinity in pepper may be associated with Cl^- related decrease of leaf chlorophyll, which restricts net assimilation. Navarro et al., (2006) also reported negative effects of salinity on pepper growth, and they concluded that the yield reduction induced by salt stress can be linked to the toxic effects of Cl^- accumulation in the plant tissues.

Conclusion

Salt stress is one of the most widespread environmental hazards worldwide crop production, especially in arid and semi-arid regions, where land degradation, water deficit and population growth are already dominant concerns. Pepper is an important agricultural crop, because of its economic importance and the nutritional value of fruits (Navarro et al., 2006). It has been classified from moderately sensitive to sensitive under salinity conditions (Penella et al., 2017), even though cultivars with different salt tolerances have been reported in previous studies. In our research, significant differences for salt tolerance among mean of generations were observed in all the treatments. Leaf, shoot and root fresh and dry weights, total leaf area, total root length, and total root volume were generally reduced in different ratios under salinity stress. BC3-3 plants have significantly higher leaf and root fresh matter as compared to progenies of third backcrosses and their respective parents. The root dry matter varied from 0.86 g $plant^{-1}$ in BC3-3 to 0.25 g $plant^{-1}$ in Sena of 8 dS m^{-1}. 8 dS m^{-1} NaCl decreased the root dry matter of BC3-3 and BC3-5 plants by 17.3 and 56.2%, respectively than control plants. Up to now it was stated in many research that total leaf area formation is normally reduced when plants are under any case of stress. In our results, the significantly higher total leaf area formation was produced at the progenies of third backcrosses of BC3-3 plants. Salinity had a significant adverse effect not only on the plant growth and development, although also on leaf chlorophyll content of progenies of third backcrosses and their respective parents. Although this harmful effect was more obvious in respective parents than the progenies of third backcrosses. Leaf chlorophyll content are often well correlated in salt-stressed plants (Gong et al., 2013). Exposure to high NaCl concentrations disrupts ion homeostasis in plant cells (Munns and Tester, 2008). Thus, the evaluation of the ion concentration in leaf tissues after exposure to stress was crucial for this experiment. Nonetheless, although ionic and water homeostasis are crucial parameters in abiotic stress tolerance, the maintenance of shoot vigor and leaf function are vitally important. The Cl^- concentration in leaves increased with a higher NaCl concentration. In this way, Kopan accumulated high concentration of toxic ions (Cl^-) in plant tissues. Furthermore, regarding total root length, and total root volume, the significantly highest values were observed at the progenies of third backcrosses of BC3-3 plants. Summarizing, the progenies of third backcrosses of BC3-3 was more tolerant to salinity than their respective parents (Kopan and Sena). This was highly associated with vigorous root growth (root fresh and dry weight, total root length and volume) and photosynthetically active leaves (total leaf area, leaf chlorophyll index, chloride exclusion) under hydroponic salt stress.

Compliance with Ethical Standards

Conflict of interest

The author declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the author to the present study is equal. The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgments

The author would like to thank all the staff members of the Plant Physiology Laboratory of Erciyes University, Turkey, for their technical support and for supplying all facilities during the experiments. The author is grateful to Prof. Dr. Halit Yetisir to providing the seeds.

References

Al Rubaye, O.M., Yetisir, H., Ulas, F., Ulas, A. (2021). Enhancing salt stress tolerance of different pepper (*Capsicum annuum* L.) inbred line genotypes by rootstock with vigorous root system. Gesunde Pflanzen, 73, 375–389. [Google Scholar]

Ahmed, M. and ul Hassan, F. (2015). Response of spring wheat (*Triticum aestivum* L.) quality traits and yield to sowing date. PLoS ONE, 10, e0126097. [Google Scholar]

Bojórquez-Quintal, E., Velarde-Buendía, A., Ku-González, Á., Carillo-Pech, M., Ortega-Camacho, D., Echevarría-Machado, I., Pottosin, I., Martínez-Estévez, M. (2014). Mechanisms of salt tolerance in habanero pepper plants (*Capsicum chinense* Jacq.): Proline accumulation, ions dynamics and sodium root-shoot partition and compartmentation. Front. Plant. Sci., 5, 605. [Google Scholar]

Chartzoulakis, K. and Klapaki, G. (2000). Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Sci. Hortic. 86: 247-260. [Google Scholar]

Colla, G., Rouphael, Y., Rea, E., Cardarelli, M. (2012). Grafting cucumber plants enhance tolerance to sodium chloride and sulfate salinization. Sci. Hortic., 135, 177-185. [Google Scholar]

Colla, G., Rouphael, Y., Jawad, R., Kumara, P., Rea, E., Cardarellic, M. (2013). The effectiveness of grafting to improve NaCl and $CaCl_2$ tolerance in cucumber. Sci. Hortic., 164, 380-391. [Google Scholar]

Dölarslan, M. and Gül, E. (2012). Toprak bitki ilişkileri açısından tuzluluk. Türk Bilimsel Derlemeler Dergisi, 5(2): 56-59 [in Turkish]. [Google Scholar]

Gong, B., Wen, D., VandenLangenberg, K., Wei, M., Yang, F., Shi, Q., Wang, X. (2013). Comparative effects of NaCl and $NaHCO_3$ stress on photosynthetic parameters, nutrient metabolism, and the antioxidant system in tomato leaves. Sci. Hortic., 157, 1–12. [Google Scholar]

Huez-López, M.A., April, L., Ulery, A.L., Samani, Z., Picchioni, G., Flynn, R.P. (2011). Response of Chile pepper (Capsicum annuum L.) to salt stress and organic and inorganic nitrogen sources: II. Nitrogen and water use efficiencies, and salt tolerance. Tropical and Subtropical Agroecosystems, 14, 757-763. [Google Scholar]

Isayenkov, S.V. and Maathuis, F.J.M. (2019). Plant salinity stress: Many unanswered questions remain. Front. Plant Sci., 10. [Google Scholar]

Johnson, C.M. & Ulrich, A. (1959). Analytical methods for use in plant analysis. 1st Edn., California Agricultural Experiment Station, California, CA., USA. [Google Scholar]

Kumar, K., Kumar, M., Kim, S.R., Ryu, H., Cho, Y.G. (2013). Insights into genomics of salt stress response in rice. Rice, 6(1), 1-15. doi:10.1186/1939-8433-6-27. [Google Scholar]

Kurtar, E.S., Balkaya, A., Kandemir, D. (2016). Screening for salinity tolerance in developed winter squash (Cucurbita maxima) and pumpkin (Cucurbita moschata) lines. Yuz. Yil Univ. J. Agric. Sci., 26, 183–195. [In Turkish]. [Google Scholar]

Lucini, L., Rouphael, Y., Cardarelli, M., Canaguier, R., Kumar, P., Colla, G. (2015). The effect of a plant-derived biostimulant on metabolic profiling and crop performance of lettuce grown under saline conditions. Sci. Hortic., 182, 124-133. [Google Scholar]

Lycoskoufis, I.H., Savvas, D., Mavrogianopoulos, G. (2005). Growth, gas exchange and nutrient status in pepper (*Capsicum annuum* L.) grown in recirculating nutrient solution as affected by salinity imposed to half of the root system. Scientia Hort., 106(2), 147-161. [Google Scholar]

Munns, R. and Tester, M. (2008). Mechanisms of salinity tolerance. Annual Review of Plant Physiology, 59: 651–681. [Google Scholar]

Navarro, J.M., Flores, P., Garrido, C., Martinez, V. (2006). Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages as affected by salinity. Food Chemistry, 96: 66-73. [Google Scholar]

Özdemir, B., Tanyolac, Z.Ö., Ulukapı, K., Onus, A.N. (2016). Evaluation of salinity tolerance level of some pepper (Capsicum annuum L.) cultivars. Int. J. of Agriculture Innovations and Research, 5(2), 247-251. [Google Scholar]

Pasternak, D. (1987). Salt tolerance and crop production: a comprehensive approach. Annu. Rev. Phytopathol., 25, 271-291. [Google Scholar]

Penella, C., Nebauer, S.G., Lopez-Galarza, S., Oliver, A.Q. (2017). Grafting pepper onto tolerant rootstocks: An environmental-friendly technique overcome water and salt stress. Sci Hortic, 226, 33–41. [Google Scholar]

Sagi, M., Savidov, N.A., Vov, N.P.L., Lips, S.H. (1997). Nitrate reductase and molybdenum cofactor in annual ryegrass as affected by salinity and nitrogen source. Physiol Plant., 99,546-553. [Google Scholar]

Seemann, J.R., Critchley, C. (1985). Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, Phaseolus vulgaris L. Planta, 164, 151–162. [Google Scholar]

SAS Institute (2003). SAS for Windows 9.1. SAS Institute Inc., Cary, NC.

Shannon, M.C. (1998). Adaptation of plants to salinity. Adv. Agron., 60, 75-119. [Google Scholar]

Tripodi, P. and Kumar, S. (2019). The Capsicum Crop: An Introduction. In: Ramchiary N, Kole C, editors. The Capsicum genome. Switzerland: Springer; 1–8. https://doi.org/10.1007/978-3-319-97217-6_1. [Google Scholar]

Ulas, F., Aydın, A., Ulas, A. and Yetisir, H. (2019). Grafting for sustainable growth performance of melon (*Cucumis melo*) under salt stressed hydroponic condition. European J. of Sustainable Development, 8:201-210. [Google Scholar]

Ulas, A., Aydin, A., Ulas, F., Yetisir, H., Miano, T.F. (2020). Cucurbita rootstocks improve salt tolerance of melon scions by inducing physiological, biochemical and nutritional responses. Horticulturae, 6, 66. [Google Scholar]

Ulas, F. (2021). Effects of grafting on growth, root morphology and leaf physiology of pepino (*Solanum muricatum* Ait.) as affected by salt stress under hydroponic conditions. Int J Agric Environ Food Sci 5(2):203-212 [Google Scholar]

Wang, W., Vinocur, B., Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta, 218: 1-14. [Google Scholar]

Wu, G.Q., Liang, N., Feng, R.J., Zhang, J.J. (2013). Evaluation of salinity tolerance in seedlings of sugar beet (Beta vulgaris L.) cultivars using proline, soluble sugars and cation accumulation criteria. Acta Physiologiae Plantarum, 35(9), 2665–2674. doi:10.1007/s11738-013-1298-6. [Google Scholar]

Yoon, J.B., Yang, D.C., Do, J.W., Park, H.G. (2006). Overcoming two post-fertilization genetic barriers in interspecific hybridization between Capsicum annuum into Capsicum baccatum for introgression of anthracnose resistance. Breeding Science, 56:31-38. [Google Scholar]