



Enhancing Salt Stress Tolerance of Different Pepper (*Capsicum annuum* L.) Inbred Line Genotypes by Rootstock with Vigorous Root System

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Received: 15 January 2021 / Accepted: 19 April 2021

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Abstract

In this study, pepper (*Capsicum annuum* L.) inbred lines were grafted onto different rootstock genotypes and tested under saline conditions. A hydroponic experiment was conducted in nutrient solution growth system in a growth chamber of Erciyes University, Agricultural Faculty in Kayseri, Turkey. The experiment was conducted in spring 2017 growth season. Two pepper inbred lines (ERÜ-462 and ERÜ-1227) were grafted onto three different pepper rootstocks/genotypes (Scarface F1, 11B14, and Yaocali F1) and grown in 8 L pots filled with continuously aerated nutrient solution under saline conditions (8 dS m⁻¹) with three replications. The growth chamber experiment was carried out to determine the effects of salt stress on plant growth, shoot and root dry weights, leaf area, photosynthesis, leaf total chlorophyll (a + b) and carotenoid content, proline content, glycine betaine content, leaf electrolyte leakage, leaf and root macro element concentration in grafted and non-grafted pepper plants. The results indicated that ERÜ-462 grafted on to Scarface and 11B14 rootstock genotypes were more tolerant to salinity than ERÜ-1227 in term of leaf chlorophyll (a + b) content and leaf carotenoid content, photosynthesis, and proline content. Though, higher shoot and root biomass, leaf area formation, root K⁺, Na⁺, Cl⁻ contents were observed when ERÜ-1227 grafted on to Scarface and 11B14 rootstock genotypes. Strong rootstock promoted plant growth in pepper plant both under control and saline conditions and significant depression of plant biomass production under saline conditions was observed in both grafted and non-grafted plants. However, grafting onto vigorous rootstocks alleviated negative effects of salinity stress on pepper plants. Scarface and 11B14 were found more tolerant to salinity than non-grafted pepper plants and the other genotypes used as regard to investigated parameters.

Keywords Grafting · Hydroponic system · Pepper · Proline · Salinity

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Verbesserung der Salzstresstoleranz verschiedener Genotypen von Parika-Inzuchtlinien (*Capsicum annuum* L.) durch Wurzelstöcke mit kräftigem Wurzelsystem

Zusammenfassung

In dieser Studie wurden Paprika-Inzuchtlinien (*Capsicum annuum* L.) auf verschiedene Wurzelstockgenotypen gepfropft und unter salzhaltigen Bedingungen getestet. Ein hydroponisches Experiment wurde in einem Nährlösungs-Wachstumssystem in einer Wachstumskammer der Erciyes-Universität der Landwirtschaftlichen Fakultät in Kayseri, Türkei, durchgeführt. Das Experiment fand in der Wachstumsaison im Frühjahr 2017 statt. Zwei Paprika-Inzuchtlinien (ERÜ-462 und ERÜ-1227) wurden auf drei verschiedene Wurzelstöcke (Scarface F1, 11B14 und Yaocali F1) gepfropft und in 8-Liter-Töpfen, die mit kontinuierlich belüfteter Nährlösung gefüllt waren, unter salzhaltigen Bedingungen (8 dS m^{-1}) angebaut; das Experiment wurde drei Mal wiederholt. Das Wachstumskammerexperiment wurde durchgeführt, um die Auswirkungen von Salzstress auf das Pflanzenwachstum, das Spross- und Wurzeltrockengewicht, die Blattfläche, die Photosynthese, den Gesamtchlorophyll- (a + b) und Carotinoidgehalt, den Prolingehalt, den Glycinbetaingehalt, den Elektrolytverlust des Blatts und die Konzentration von Blatt- und Wurzel-Makroelementen bei gepfropften und nicht gepfropften Paprikapflanzen zu bestimmen. Die Ergebnisse zeigten, dass ERÜ-462, das auf Scarface- und 11B14-Wurzelstock-Genotypen gepfropft wurde, hinsichtlich des Blattchlorophyllgehalts und des Blattcarotinoidgehalts, der Photosynthese und des Prolingehalts salztoleranter war als ERÜ-1227. Allerdings wurden höhere Spross- und Wurzelbiomasse, Blattflächenbildung, Wurzel-K⁺-, -Na⁺- und -Cl⁻-Gehalte beobachtet, wenn ERÜ-1227 auf Scarface- und 11B14-Wurzelstock-Genotypen gepfropft wurde. Ein starker Wurzelstock förderte das Pflanzenwachstum der Paprikapflanzen sowohl unter Kontroll- als auch unter salzhaltigen Bedingungen und eine signifikante Verringerung der Pflanzenbiomasseproduktion unter salzhaltigen Bedingungen wurde sowohl bei gepfropften als auch bei nicht gepfropften Pflanzen beobachtet. Das Pfropfen auf kräftige Wurzelstöcke milderte jedoch die negativen Auswirkungen von Salzstress auf Paprikapflanzen Scarface und 11B14 erwiesen sich in Bezug auf die untersuchten Parameter als salztoleranter als nicht gepfropfte Paprikapflanzen und die anderen verwendeten Genotypen.

Schlüsselwörter Pfropfung · Hydroponisches System · Paprika · Prolin · Salzstress

Introduction

Salinity stress is the major environmental factor that severely limits plant growth and productivity in arid and semiarid regions (Kronzucker and Britto 2011). Currently, more than 800 million hectares of land throughout the world are salt-affected, accounting for more than 7% of the world's total land area (Ferreira-Silva et al. 2010). In Turkey, 4 million hectares of land was affected by salinization (Sönmez 1990). Cevik (1986) reported that the total area of land in Turkey is 78 million hectares; 36% of this land is cultivated, and 3.2% of 36% has salinity problems. Climate change, saline irrigation water, low-level rainfall, rise in sea levels, high-level evaporation, excessive irrigation without proper drainage in inland, and underlying rocks rich in harmful salts can cause salinity problems in agricultural areas (Kumar et al. 2013). In addition to soil salinization, ever-increasing human population poses serious challenges to world agriculture (Mittler and Blumwald 2010) and global food requirements are projected to increase 70% by 2050 (Fischer et al. 2011).

A high concentration of salts in the root zone impairs most physiological functions and cellular metabolism, which eventually leads to reduced growth and crop productivity (Isayenkov and Maathuis 2019). It causes reduction in the plant's water uptake and root development. Con-

sequently, the hormonal balance is affected negatively; reduction in protein synthesis, photosynthesis, the amount of chlorophyll, and plant height occur because of the decrease in nitrate uptake; the number of flowers, fresh and dry matter, and yield is decreased (Penella et al. 2014, 2015, 2016). If salinity stress occurs, a specific ionic effect appears, mediated by the accumulation of toxic ions in cellular tissues (De Pascale et al. 2003) with imbalances between nutrients (Hasanuzzaman et al. 2013). The deleterious effects of soil salinity on plants growth are associated with excessive accumulation of Na⁺ and Cl⁻, as most saline soils are dominated by these ions and K⁺ deficiency caused by competition with the high external Na⁺ concentration (Wu et al. 2013). All these factors have adverse effects on both plant growth and development at physiological and biochemical levels (Munns and James 2003). Therefore, improving plant tolerance to salt stress would be critical to allow crop cultivation in salinized areas (Wu 2018).

The pepper (*Capsicum annuum* L.) is a member of the family *Solanaceae* and well known for its high bioactive compound content and strong antioxidant capacity. It is one of the most popular fresh fruits worldwide because of its combination of color, flavor, and nutritional value (Blanco-Ríos et al. 2013). It has been classified from moderately sensitive to sensitive under salinity conditions (Penella et al. 2015), even though cultivars with different salt tolerances

have been reported in previous studies (Ruggiero et al. 2019). To decrease the effect of salinity on agricultural productivity and to facilitate the development and utilization of saline soil, several strategies have been recommended and applied in the past decades. Reclamation of saline soil and adjustment of soil salinity are essential strategies in agriculture; however, these are temporary and relatively expensive. Breeding resistant genotypes have also been considered as means for improving salt tolerance in horticultural crops, but it is laborious and complex due to the polygenic nature of salt resistance (Yan et al. 2018). One possible method to induce salt stress tolerance by physical means is grafting of a productive scion on a salt stress-tolerant rootstock. The salinity resistance mechanism in grafted plants are various pepper plants grafted onto tolerant rootstock (e.g., *Capsicum chinense* Jacq., *Capsicum baccatum* L.) can achieve better plant efficiency owing to restricting of Cl^- transport to the leaves by the rootstock and reduction of the Na^+ load in the roots and leaves. This allows the uptake of other cations (i.e., K^+ , Ca^{2+} , Mg^{2+}), for lower osmotic potential at lower energy cost (Penella et al. 2016). However, these effects cannot be generalized due to the great specificity of scion/rootstock interactions (Giuffrida et al. 2013; Penella et al. 2016; Serrano et al. 2017). Grafting on vegetable plants was first performed in Korea and Japan in the late 1920s by grafting watermelon onto gourd rootstocks to allow for continuous cropping in areas prone to soil-borne diseases such as *Fusarium* wilt (Davis et al. 2008). It is also an innovative technique for the suitable cultivation of fruit-bearing vegetables such as tomatoes, bean, eggplant, cucumber, melon in Japan, Korea, the Mediterranean basin, and several European countries (Pogonyi et al. 2005). Grafting with vigorous rootstocks can enhance pest and disease resistance, yield, to increase the absorption of nutrients and the mineral content in the aerial portion of the plant, drought, and cold tolerance, growth, fruit quality, to confer tolerance to high and low temperatures and salt tolerance as reported for different crops such as watermelon, melon, tobacco, and tomato (Gungor and Balkaya 2016; Sarabi et al. 2017; Ulas et al. 2019a, b, 2020). Current studies have investigated the response of grafted horticultural crops to salt stress and have revealed that grafting is an effective strategy for increasing salt tolerance in horticultural crops, as well as cucumber (Zhen et al. 2010), melon (Ulas et al. 2019a), tomato (Ulas et al. 2019b), pepper (Penella et al. 2014, 2015, 2016), potato (Etehadnin et al. 2010), citrus (Balal et al. 2011), cashew (Ferreira-Silva et al. 2010), and grape (Upreti and Murti 2010). Former studies have established that higher photosynthetic capacity, carbon assimilation rates, proline, sugar, betaine accumulation, antioxidant capacity, and lower accumulation of Na^+ and/or Cl^- in the leaves constitute possible explanations for grafting-induced salt tolerance (Ferreira-Silva et al. 2010). These

results indicate that the utilization of rootstocks with high salinity tolerance can result in highly salt stress-resistant scion plants (Yan et al. 2018). As far as we know, up to now the effects of grafting under salt stress conditions on pepper plants were investigated by very few authors (Giuffrida et al. 2013; Penella et al. 2014, 2015, 2016; Serrano et al. 2017). Though, more extensive studies on the pepper plants must be examined to better understand whether grafting could improve salinity tolerance. Inbreeding effect due to selfing causes depression in plant growth and development in pepper inbred lines. Therefore, research on the alleviated effect of strong root system rootstocks on plant growth and development of pepper (*Capsicum annuum* L.) inbred lines under salinity conditions should be continued. In this study, the effects of strong pepper rootstocks/genotypes selected in a preliminary study on morphological and physiological parameters of weak pepper inbred pepper lines under salt stress were investigated. The aim of the study was to assess whether grafting with different pepper rootstock genotypes could improve the salt tolerance of pepper inbred lines and to determine the physiological, biochemical, and nutritional responses induced by the rootstocks under salt stress.

Materials and Methods

Plant Material, Treatments, and Experimental Design

This study was carried out in spring 2017, 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 350 $\mu\text{mol m}^{-2} \text{S}^{-1}$ photon flux was supplied in a photoperiod of 16/8 h of light/dark regimes in the controlled growth chamber. Two pepper inbred lines (ERÜ 1227 and ERÜ 462) were used as scion and three different pepper Rootstocks/cultivars (Scarface F1, 11B14 and Yao-cali F1) were used as rootstock materials. The rootstock and inbred line genotypes were selected from prior screening experiment based on the agronomical, morphological, and physiological parameters (Ulas 2019). The seeds were sown in 72-cell polystyrene trays (W 280 × L 540 × H 45 mm, IBK İklim 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, scions were grafted

onto rootstocks by “tube grafting method” described by Ulas (2019), while non-grafted scion inbred lines were used as control plants. Grafting was carried out in a laboratory conditions, in a shady place sheltered from the wind, to avoid wilting of the grafted plants. Briefly, the shoot tip of the rootstock was cut off below the cotyledons at an angle of 45 with the same for the scion above the cotyledons. The grafting position was fixed firmly with a tubular silicone clip (1.5 mm hole diameter). After grafting during the formation of callus, plants were healed and acclimatized in the tunnel covered with double-layered plastic film and shade cloth in the climate chamber for five days with relative humidity between 60 to 90% and air temperature around 20–25°C (Ulas 2019). In order to prevent grafted plants from wilting by the excessive transpiration and to enhance healing, the tunnel was closed for the first three or four days of the healing and acclimatization period. For the next five or six days, the opening and closing of the tunnel were done depending on the conditions of grafted plants and the growth room. This was done for the acclimatization of grafted plants to environmental conditions outside the tunnel. The grafted plants stayed at the healing and acclimatization period totally for ten days. After the end of healing and acclimatization, the seedlings with eight mature leaves were transplanted to plastic pots after root system of the plants were carefully washed in raw water to clean the substrate on 07.03.2017. The experiment was terminated on 20.04.2017. Two salinity treatments were applied starting two weeks after transplantation (March 21, 2017), 1 and 8 dS · m⁻¹. 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(NO₃)₂), 250 μM monopotassium phosphate (KH₂PO₄), 500 μM potassium sulfate (K₂SO₄), 325 μM magnesium sulfate (MgSO₄ · 7H₂O), 50 μM sodium chloride (NaCl). Micronutrients were 80 μM iron (Fe) (III)- ethylenediaminetetraacetic acid (EDTA)- sodium (Na), 0.4 μM manganese sulfate (MnSO₄), 0.4 μM zinc sulfate (ZnSO₄), 0.4 μM copper sulfate (CuSO₄), 8 μM boric acid (H₃BO₃), 0.4 μM sodium molybdate (Na₂MoO₄). Solutions were replaced completely every week in the first two weeks. The experiment was in a completely randomized block design with three replications and six plants in each replication.

Harvest, Shoot and Root Dry Weight Measurements

At final harvest, 44 d after transplanting in 2017, respectively, the biomass of the upper part of the plants was determined. Grafted and non-grafted plants were harvested by separating them into stems, leaves, and roots. Their tissues were dried in a forced-air oven at 80°C for 72 h for 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 to shoot ratio was calculated by dividing the root dry weight by the sum of leaf and stem dry weights.

Leaf Area and Photosynthetic Activity Measurements

The physiological and biochemical parameters were obtained by measurements and analysis of leaf samples taken from twenty-four plants for each salinity treatment and grafting combination (i.e., non-grafted/grafted plants), as there were six plants per replicate and three replicates of the salinity/grafting combination (experimental unit, *n* = 3).

In the hydroponic experiment, the leaf area of the plants was measured destructively during the harvesting process by using a portable leaf-area meter (LI-3100, LI-COR. Inc., Lincoln, NE, USA). Total leaf area was recorded in centimeter square (cm²). Prior to harvest, non-destructive measurements of the leaf-level CO₂ gas exchange (μmol CO₂ m⁻²s⁻¹) were done in a controlled growth chamber by using a portable photosynthesis system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA). The leaf net photosynthesis measurement was performed on the youngest fully expanded leaves (3rd–4th leaf from the apex), using four replicate leaves per treatment in the third and fifth week of the vegetation period. The LI-6400 was equipped with a well-stirred 2.5 × 10⁻⁵ m³ leaf chamber with constant area inserts (1.2 × 10⁻³ m²) and fitted with a variable intensity red source (Model QB1205LI-670; Quantum Devices, Barneveld, WI). Leaf temperature within the chamber was 30 ± 2 °C, vapor pressure difference between the leaf and air was 2.6 ± 0.3 °C, and CO₂ concentration was 365 ± 10 μL L⁻¹. All gas exchange measurements were carried out between 09:00 and 12:00 HR.

Leaf Total Chlorophyll (a + b) and Carotenoid Content Measurements

A day before harvesting, extraction of the photosynthetic pigments from 100 mg (0.1 g) of fresh leaf samples from each replication of the two treatments were taken for measuring the leaf total chlorophyll and carotenoid contents using UV-VIS Spectroscopy. The leaf samples used for chlorophyll and carotenoid determinations were of the same physiological age with those used for the leaf net photosynthesis measurements. The samples were put into 15 ml capped containers where 10 ml of ethylene alcohol of 95% concentration was added. They were then kept in darkness at room temperature for overnight, to allow the extraction of the leaf pigments. Measurements were done using the spectrometer (UV/VIS T80+ of PG Instruments Limited, UK) at wavelengths of 470 nm, 648.6 nm, and 664.2 nm.

Total chlorophyll (Total-Chlo) and total carotenoids (TC) were then estimated from the spectrometric readings using the formulae of Lichtenthaler (1987).

$$\begin{aligned} \text{Total-Chlo(mg/gplant sample)} \\ &= [(5.24 \text{ WL664.2} + 22.24 \text{ WL648.6}) \times 8.1] / \\ &\text{weight of plant sample(g)} \\ \text{TC(mg/gplant sample)} \\ &= [(4.785 \text{ WL470} + 3.657 \text{ WL664.2}) \\ &- 12.76 \text{ WL648.6}) \times 8.1] / \text{weight of plant sample(g)} \end{aligned}$$

Note: WL470, WL648.6 and WL664.2 refers to spectrometric readings at wavelength 470 nm, 648.6 nm and 664.2 nm respectively.

Proline and Glycine Betaine Contents Measurements

The proline contents were estimated by the method of Bates et al. (1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate mixture was centrifuged at 10,000 rpm. The supernatant was used for the estimation of proline contents. The reaction mixture consisted of 2 ml acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at 100 °C for 1 h after termination of reaction in an ice bath. The reaction mixture was extracted with 4 ml of toluene and absorbance was read at 520 nm. Estimation of endogenous glycine betaine content in pepper was carried out by modifying a method previously described by Grieve and Grattan (1983). Dried and ground plant leaf tissue (0.5 g) was incubated in tubes containing 20 ml of deionized water for 48 h at 25 °C. The samples were filtered and mixed with 2 N H₂SO₄. An aliquot (0.5 ml) was transferred into a test tube and cooled in icecold water for 1 h. Cold potassium iodide-iodine reagent (0.2 ml) was added, vortexed, and then centrifuged at 1000 × g for 15 min at 4 °C. The sample was incubated for 24 h at 4 °C. The formed periodite crystals were dissolved in 9 ml of 1,2-dichloroethane and shaken at room temperature for 48 h. The absorbance was then read at a wavelength of 365 nm using a FLUOstar Omega UV-visible spectrophotometer (BMG LabTech GmbH, Ortenberg, Germany).

Leaf Electrolyte Leakage Measurements

Electrolyte leakage (EL) was determined as described by Lutts et al. (1995). EL measurements were done on the youngest fully expanded leaves between 11:00 and 15:00 h every 48 h using three replicates per treatment. 1 cm leaf disks were excised from young fully expanded leaves using a cork borer. To remove surface contamination, leaf samples were washed 3 times with distilled water and then placed in individual stoppered vials containing 10 mL of distilled

water. The samples were incubated at room temperature (25 °C) on a shaker (100 rpm) for 24 h. The electrical conductivity of the bathing solution (EC1) was read after incubation. The same samples were then placed in an autoclave at 120 °C for 20 min and a second reading of the EC (EC2) was made after cooling the solution to room temperature. The EL was expressed as: $EL = (EC1)/(EC2) \times 100$.

Mineral Analysis Measurements

For the 2017 growing season, dried plant tissues (leaf and root) 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 K, Ca, Na. Their concentrations were determined by dry ashing at 400 °C for 4.5 h, dissolving the ash in 5 ml of HCl with 20% concentration was added to the ashed samples and filtered. The filtered solutions were then diluted with distilled water to a volume 50 ml. Out of which 10 ml was taken for Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) analysis. The ICP-AES results were converted into percentages (%) and parts per million (ppm). Chloride was analyzed by precipitation as AgCl and titration according to Johnson and Ulrich, (1959). Chloride analysis was performed on the water extracts of dry materials. The sample (0.5 g DW) was incubated in a water bath at 60 °C for 30 min. Following centrifugation, the supernatant was collected and Cl⁻ was determined in anion chromatograph (DX-100 ion chromatograph Dionex™, ThermoScientific).

Statistical Analysis

Statistical analysis of the nutrient solution experiments data was performed 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 genotype or grafting combination 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 analyzed using Duncan's Multiple Test ($P < 0.05$).

Results and Discussion

Biomass Production and Partitioning

Results of the shoot and root dry weight and shoot to root ratio of the grafted and non-grafted 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 1. Shoot dry matter was significantly ($p < 0.001$) affected by rootstock, different salt levels, scion × rootstock ($p < 0.01$), scion × salt levels inter-

Table 1 The effects of graft combination and different salt levels (1 dSm⁻¹ and 8 dSm⁻¹) on shoot dry weight, root dry weight and shoot to root ratio of pepper plants

Graft combination (S/R)	Shoot dry weight (g plant ⁻¹)		Root dry weight (g plant ⁻¹)		Shoot to root ratio (g g ⁻¹)	
	Control	Salt	Control	Salt	Control	Salt
ERÜ-1227	7.35 b	5.50 de	1.49 b	1.38 c	4.93 de	3.98 f
ERÜ-1227/Scarface	5.68 d	4.53 f	1.35 c	1.21 de	4.20 ef	3.74 f
ERÜ-1227/Yaocali	6.87 c	4.20 f	0.95 f	0.68 h	7.23 b	6.17 c
ERÜ-1227/11B14	5.38 de	5.15 e	0.79 g	1.37 c	6.81 bc	3.75 f
ERÜ-462	5.65 d	2.37 g	0.76 gh	1.36 c	7.43 b	1.74 g
ERÜ-462/Scarface	7.73 b	4.29 f	1.22 de	1.14 e	6.33 c	3.76 f
ERÜ-462/Yaocali	6.78 c	4.10 f	1.27 cd	0.94 f	5.33 d	4.36 ef
ERÜ-462/11B14	8.33 a	4.47 f	1.89 a	0.53 i	4.40 ef	8.43 a
<i>F-Test</i>						
Scion		n. s.		n. s.		n. s.
Rootstock		***		**		**
Salt		***		*		***
Scion × Rootstock		**		n. s.		**
Scion × Salt		***		*		n. s.
Rootstock × Salt		n. s.		***		***

Values denoted by different letters are significantly different between graft combination within both columns at $P < 0.05$. ns, non-significant.

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

actions; however, it was not significantly affected by scion, and rootstock × salt levels interaction.

Vegetative growth was affected negatively under salt stress. Shoot and root dry matter decreased by different rates in each genotype with salt application. Under non-saline conditions, shoot dry matter increased in grafted plants as compared to non-grafted plants. The graft combination of ERÜ-462/11B14 (8.33 g plant⁻¹) had significantly higher shoot dry matter under control conditions; though, the graft combination of ERÜ-1227/11B14 (5.15 g plant⁻¹) and the non-grafted plants of ERÜ 1227 (5.50 g plant⁻¹) had significantly higher shoot dry matter under saline conditions. On the other hand, ERÜ-462 presented increased shoot dry matter, when grafted with all three rootstock genotypes compared to non-grafted control plants. The graft combination of ERÜ-1227/11B14 (5.15 g plant⁻¹) had significantly higher shoot dry matter, though the non-grafted plants of ERÜ-462 (2.37 g plant⁻¹) had lower shoot dry matter. Compared to the unstressed plants, salt stress decreased shoot dry matter from 3.48 to 58.11%. 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 lower accumulation of plant biomass in pepper plants by salinity application is associated with marked inhibition of photosynthesis (Table 1). This result confirms the findings of previous investigators, which indicated that pepper is a salt-sensitive plant species (Navarro et al. 2002). Our results are in line with those of Yamac

(2017) reported that salt stress decreased shoot dry matter in grafted watermelon plants under saline conditions under hydroponic conditions. The reduction in growth could be a combined effect of osmotic stress (Greenway and Munns 1980), which is more harmful to plants during the growth stage and the higher ion uptake (Dumbroff and Cooper 1974). Penella et al. (2016) demonstrated that grafted pepper plants were less sensitive to salt stress compared to their non-grafted counterparts, in terms of photosynthesis and consequently growth and yield. Up to now it was stated in many greenhouse crops grown in hydroponic conditions that the ameliorative effect of grafting on plant's growth under salinity conditions fully agrees with other findings in tomato, melon, and watermelon (Rouphael et al. 2012; Yamac 2017; Ulas et al. 2019a, b, 2020).

Root dry matter was significantly ($p < 0.001$) affected by rootstock ($p < 0.01$), different salt levels ($p < 0.05$), scion × salt levels interaction ($p < 0.05$), rootstock × salt levels interaction; however, it was not significantly affected by scion, scion × rootstock interaction. Root dry matter was increased when ERÜ-462 plants grafted onto all three rootstock genotypes. The graft combination of ERÜ-462/11B14 (1.89 g plant⁻¹) had significantly higher root dry matter under control conditions. Under salt stress conditions, the graft combination of ERÜ-1227/11B14 (1.37 g plant⁻¹) had significantly higher root dry matter. Compared to the unstressed plants, salt stress decreased root dry matter of the ERÜ-462/11B14 and ERÜ 1227/Scarface F1 plants by 6.36% and 38.07%, respectively (Table 1). These findings are in line with those of Penella et al. (2016) who found

Table 2 The effects of graft combination and different salt levels (1 dSm⁻¹ and 8 dSm⁻¹) on leaf area, photosynthesis, and leaf electrolyte leakage of pepper plants

Graft combination (S/R)	Leaf area (cm ² plant ⁻¹)		Photosynthesis (μmol CO ₂ cm ⁻² s ⁻¹)		Leaf electrolyte leakage (%)	
	Control	Salt	Control	Salt	Control	Salt
ERÜ-1227	1134 ef	1149 e	9.47 def	8.15 fgh	40.4 ef	56.2 a
ERÜ-1227/Scarface	1255 d	1246 d	10.28 de	5.55 i	43.9 def	50.8 abc
ERÜ-1227/Yaocali	1493 b	1275 d	10.62 cd	7.40 h	45.6 cde	55.3 ab
ERÜ-1227/11B14	1590 a	1075 f	9.00 efg	6.68 hi	43.1 ef	55.6 ab
ERÜ-462	1388 c	280 i	11.78 bc	8.07 fgh	42.0 ef	39.6 ef
ERÜ-462/Scarface	1405 c	977 g	13.13 ab	8.07 fgh	37.4 f	51.3 abc
ERÜ-462/Yaocali	1270 d	928 gh	13.05 ab	7.70 gh	39.5 ef	53.8 ab
ERÜ-462/11B14	1254 d	867 h	13.27 a	7.55 gh	40.8 ef	49.6 bcd
<i>F-Test</i>						
Scion		***		***		***
Rootstock		***		n. s.		*
Salt		***		***		***
Scion × Rootstock		***		n. s.		n. s.
Scion × Salt		***		***		n. s.
Rootstock × Salt		***		*		**

Values denoted by different letters are significantly different between graft combination within both columns at $P < 0.05$. ns, non-significant. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

that the grafted pepper plants have significantly higher root dry mass than non-grafted plants under salt stress conditions. In grafted plants, higher root development might be the reason of the higher photosynthetic rate determined in grafted plants independently to salt stress. Similar results were observed in many crop species such as melon (Ulas et al. 2019a, 2020), cucumber (Zhen et al. 2010), strawberry (Awang et al. 1993), tomato (Dasgan et al. 2002), eggplant (Chartzoulakis and Loupassaki 1997), pepper (Chartzoulakis and Klapaki 2000), and broad bean (de Pascale and Barbieri 1997). Crop growth and performance were enhanced in grafted plants onto strong rootstocks with more root hairs and root length which maintain more water and mineral elements uptake from soil and transfer them to aerial parts of the plant (Yarsi and Sari 2006).

Shoot-to-root ratio was significantly ($p < 0.001$) affected by rootstock ($p < 0.01$), different salt levels, scion × rootstock ($p < 0.05$), rootstock × salt levels interaction; however, it was not significantly affected by scion, and scion × salt levels interaction. Regarding shoot-to-root ratios, the significantly higher shoot-to-root ratios of ERÜ-462/11B14 graft combinations under salt stress. Non-grafted ERÜ-462 showed significantly lower shoot-to-root ratios in salt stress conditions. All the results clearly indicated that grafting with 11B14 rootstocks significantly improved the salt tolerance of ERÜ-462 plants. Because of strong and vigorous root systems of grafted plants crop growth enhanced at grafted plants than non-grafted control plants. The reason might be uptake of more water and nutrient caused an increase in leaf area and photosynthetic activity of leaves under salt

stress conditions (Colla et al. 2006). It is suggested that growth stimulation or no adverse impacts on growth were due to accumulation of proline and lower accumulation of Na in the meristem that inhibit growth (Hmidi et al. 2018). However, accumulation of such amounts of salts can affect the hormonal balance or osmolyte accumulation which may initiate salt adaptive responses (Albacete et al. 2008). The lower growth of the plant is associated to the end of new leaf expansion and a limited leaf growth by slowing the cellular division (Albacete et al. 2008).

Leaf Area, Photosynthesis, and Leaf Electrolyte Leakage

The results of the leaf area, photosynthesis, and leaf electrolyte leakage at the end of the growing cycle of graft combination and control plants grown in different salt levels (1 dS m⁻¹ and 8 dS m⁻¹) were shown in Table 2. Leaf area was significantly ($p < 0.001$) affected by scion, rootstock, different salt levels, scion × rootstock, scion × salt levels interaction and rootstock × salt levels interaction. Leaf area, photosynthesis and leaf electrolyte leakage were significantly decreased by increasing salt level of the nutrient solution. Grafted plants produced significantly higher leaf area and photosynthesis rate as compared to non-grafted plants. Under control conditions, significantly higher leaf area formation was observed when inbred line ERÜ-1227 grafted with all rootstock genotypes. The graft combination of ERÜ-1227/11B14 (1590 cm²) had a significantly higher leaf area, whereas the non-grafted plant of ERÜ-

1227 (1134 cm²) had a significantly lower leaf area. On the other hand, under salt stress conditions, the graft combination of ERÜ-1227/Yaocali F1 (1275 cm²) and ERÜ-1227/Scarface (1246 cm²) had significantly higher leaf area formation as compared to the non-grafted plants. Significantly lower leaf area formation was observed in the non-grafted control plant of ERÜ 462 (280 cm²) (Table 2). Reduction in leaf area is caused by ion accumulation in the leaves, particularly the old ones (Greenway and Munns 1980). Wignarajah et al. (1975) stated that salt stress limited leaf expansion in beans and it was largely due to an inhibition of cell division rather than to cell expansion. Under salt stress conditions, decline in leaf area has been observed on grafted watermelon (Colla et al. 2006; Yamac 2017; Ulas et al. 2020) and pepper (Chartzoulakis and Klapaki 2000) plants.

Photosynthesis rate was significantly ($p < 0.001$) affected by scion, different salt levels, scion \times salt levels interaction, rootstock \times salt levels interaction ($p < 0.05$); however, it was not significantly affected by rootstock, scion \times rootstock. Photosynthesis rate was significantly decreased following NaCl treatment in all graft combinations. Under control conditions, significantly higher photosynthesis rate was produced when ERÜ 462 scion genotype plants grafted onto all rootstock genotypes. Under control conditions, inbred line ERÜ-462 inbred line grafted with all rootstocks significantly enhanced its photosynthesis rate. With the longer duration of salt stress, photosynthesis rate in the non-grafted plants always showed a decrease with increased salt stress. Under salt conditions significantly higher photosynthesis rate was produced by ERÜ-1227/Scarface F1 graft combination and non-grafted plants of ERÜ-1227 and ERÜ-462. On the other hand, significantly lower photosynthesis rate was produced in ERÜ-1227/11B14 (Table 2). Compared to the unstressed plants, salt stress decreased the photosynthesis rate of the ERÜ-462/11B14 and ERÜ-1227/Scarface F1 plants by 16.3% and 41.2%, respectively. Reduced photosynthesis can be caused by stomatal closure and/or non-stomatal inhibition, and the latter is associated with damage in photosynthetic machinery (Flexas et al. 2004). Bethke and Drew (1992) examined the reason of photosynthesis reduction in pepper under salt stress and concluded that reductions in photosynthesis are primarily at non-stomatal, biochemical level and closely correlated with the concentration of both Na⁺ and Cl⁻ in leaf tissue. These findings are corresponding to those of Penella et al. (2015) who stated that salt stress affects photosynthesis negatively as slightly at grafted pepper plants than non-grafted plants. Similar results were observed at pepper genotypes under salt stress conditions by Chartzoulakis and Klapaki (2000). In another study, in grafted watermelon plants, photosynthesis level was decreased as salt level increased in the nutrient solution (Yamac 2017).

Leaf electrolyte leakage was significantly ($p < 0.001$) affected by scion, rootstock ($p < 0.05$), different salt levels, rootstock \times salt levels interaction ($p < 0.05$); however, it was not significantly affected by scion \times salt levels interaction and scion \times rootstock. It ranged from 45.6 to 37.4%. Graft combination of ERÜ-1227/Yaocali F1 (45.6%) had significantly higher leaf electrolyte leakage under control conditions. However, graft combination of ERÜ-462/Scarface (37.4%) had significantly lower leaf electrolyte leakage. Under saline conditions, significantly higher leaf electrolyte leakage was found in all graft combinations and non-grafted control plants of ERÜ-1227 (Table 2). These results agree with those of Yamac (2017) who observed that non-grafted plants had higher electrolyte leakage than grafted plants in watermelon under saline conditions. Inbred line of ERÜ-462 showed increased electrolyte leakage when grafted with all rootstock genotypes under saline conditions. On the other hand, ERÜ-1227 decreased its electrolyte leakage when grafted with all rootstock genotypes, though it was not significantly different. These are typical reactions of plants that generally express tolerance strategies which can be seen in studies of melon (Sarabi et al. 2017), rice (Lutts et al. 1995), cucumber (Mumtaz-Khan et al. 2013), pepper (Kaya et al. 2001), sugar beet (Ghoulam et al. 2002), barley (Perez-Lopez et al. 2008), okra (Saeed et al. 2014), watermelon (Li et al. 2017) and tomato (Kaya et al. 2001).

Leaf Chlorophyll (a + b) Content, Leaf Carotenoid Content, Proline Content and Glycine Betaine Content

The results of the leaf chlorophyll (a+b) content, leaf carotenoid content, proline content and glycine betaine content at the end of the growing cycle of graft combination and control plants grown in different salt levels (1 dS m⁻¹ and 8 dS m⁻¹) were shown in Fig. 1. Leaf chlorophyll (a + b) content was significantly ($p < 0.001$) affected by different salt levels ($p < 0.05$); however, it was not significantly affected by scion, rootstock, scion \times rootstock, scion \times salt levels, and rootstock \times salt levels interaction (Fig. 1a). Chlorophyll and carotenoids are the main photosynthetic pigments of higher plants. Salt stress increased the amount of chlorophyll a and b in both grafted and non-grafted plants. However, there was a not significant difference between graft combinations (Fig. 1a). Chlorophyll a and b content increased with different ratios (9–31%) with increasing salt stress. This agrees with the findings of Yamac (2017) in grafted watermelon plants under salt stress conditions. Concentrations of Na⁺, K⁺, and chlorophyll are often well correlated in salt-stressed plants (Gong et al. 2013). Rao et al. (2013) reported that tolerant varieties had less chlorophyll degradation than sensitive ones. Changes on chlorophyll content may be related with

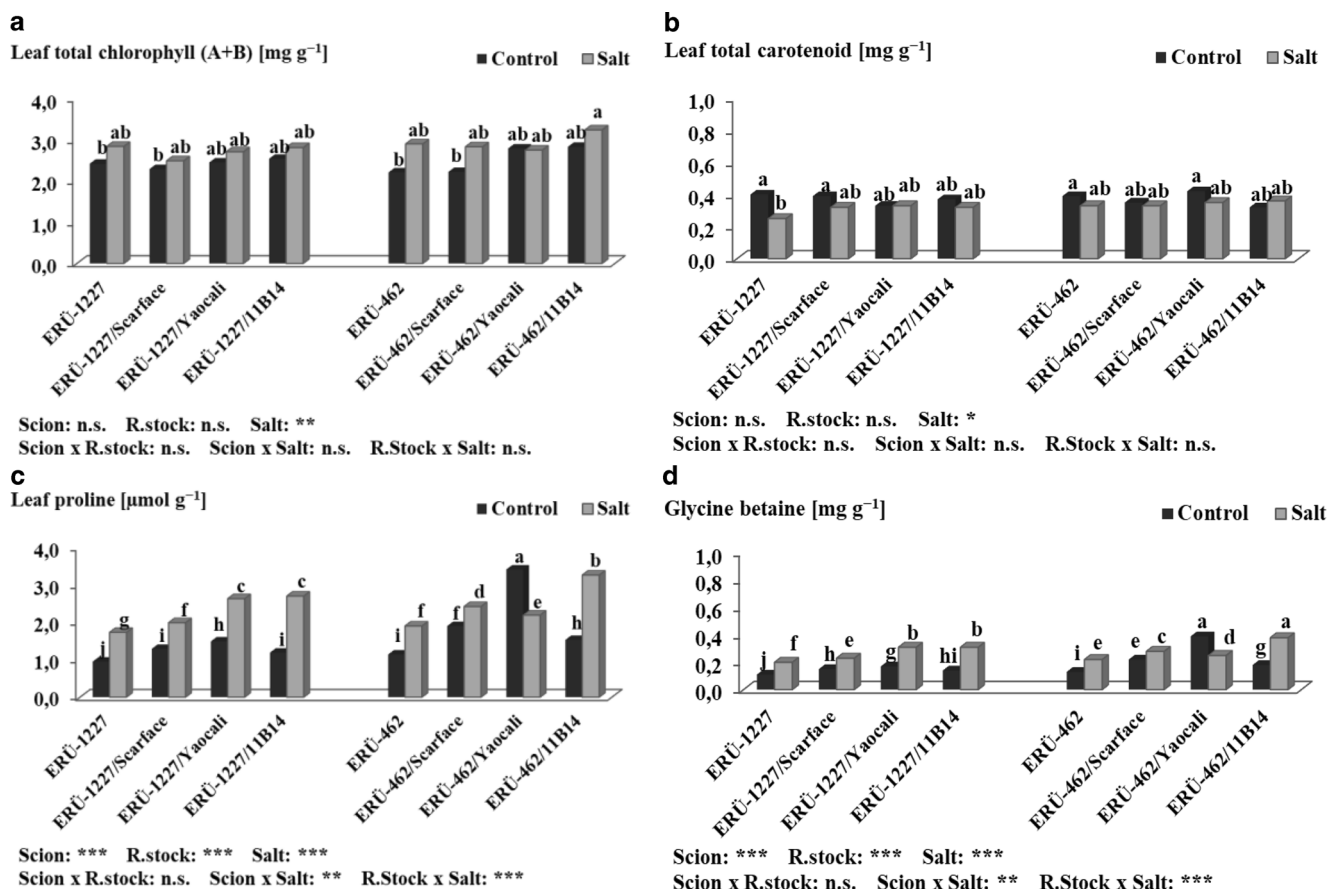


Fig. 1 The effects of graft combination and different salt levels (1 dSm^{-1} and 8 dSm^{-1}) on leaf total chlorophyll content (a), leaf total carotenoid content (b), leaf proline (c), glycine betaine (d) of pepper plants. Values denoted by different letters are significantly different between graft combination within both columns at $P < 0.05$. ns, non-significant. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

genotypes, stress period and intensity of stress. It was reported that increase on chlorophyll degradation or the decrease on synthesis of chlorophyll might cause reduction on chlorophyll content (Özdemir et al. 2016). Contrastingly, conflicting results were observed by Yarsi et al. (2017) salt stress decreased the amount of chlorophyll a and b in both grafted and non-grafted melon plants.

Carotenoid content was significantly ($p < 0.001$) affected by different salt levels ($p < 0.05$); however, it was not significantly affected by scion, rootstock, scion \times rootstock, rootstock \times salt levels, and scion \times salt levels interaction (Fig. 1b). Carotenoid content was decreased by increasing salt stress. Although carotenoid content decreased in 7 different graft combinations, it increased in the graft combinations of ERÜ-462/11B14 and ERÜ-462/Yaocali F1 by 14%. Under salt stress conditions, the higher carotenoid content was observed in ERÜ-462 inbred lines grafted on to Yaocali F1 (0.36 mg g^{-1}) and 11B14 (0.35 mg g^{-1}), though lower carotenoid content was observed in non-grafted plants of ERÜ-1227 (0.25 mg g^{-1}). Increasing in carotenoid content was more evident in ERÜ-1227 plants by grafting under salt stress (Fig. 1b). Similar results were

reported at grafted melon plants under salt stress conditions by Yarsi et al. (2017) and Ulas et al. (2020). In another study, Öztekin (2009) observed that the use of different tomato rootstock genotypes plays an important role on total chlorophyll quantity and photosynthesis metabolism influencing plant growth and development under salt stress conditions.

Salt-induced proline accumulation was also evident in the present study. Proline accumulation was significantly ($p < 0.001$) affected by scion, rootstock, different salt levels, scion \times salt levels, rootstock \times salt levels interaction; however, it was not significantly affected by scion \times rootstock interaction (Fig. 1c). Proline accumulation ranged from 0.95 to $3.41 \mu\text{mol g}^{-1}$ under control conditions and it was lower in the non-grafted plants than grafted plants. Significantly higher proline accumulation was observed in graft combination of ERÜ-462/Yaocali F1 ($3.41 \mu\text{mol g}^{-1}$), whereas significantly lower proline accumulation was found in the non-grafted plants of ERÜ-1227 ($0.95 \mu\text{mol g}^{-1}$). Under saline conditions, proline accumulation was enhanced by 27% and 127% in all graft combinations and non-grafted plants except graft combination of ERÜ-462/Yaocali F1.

Proline accumulation was higher in the grafted plants than non-grafted plants and it ranged from 1.73 to 3.26 $\mu\text{mol g}^{-1}$. Significantly higher proline accumulation was recorded in graft combination of ERÜ-426/11B14 (3.26 $\mu\text{mol g}^{-1}$), whereas significantly lower proline accumulation was found in the non-grafted plants of ERÜ-1227 (1.73 $\mu\text{mol g}^{-1}$) (Fig. 1c). Proline is one of the compounds that can accumulate in plant tissues, most frequently as an osmolyte or protective substance under unfavorable environmental conditions, such as drought and salt stress (Bojórquez-Quintal et al. 2014). Similar results have been observed in several plant species such as *Cakile maritima* (Hmidi et al. 2018), sugar beet (Wu et al. 2013), and sorghum (Bavei et al. 2011). Under these conditions, proline could be considered as an osmoregulator. It can also improve salinity tolerance by protecting and stabilizing membranes and enzymes under saline conditions (Ashraf and Harris 2004). Recently, several researchers have specified that accumulation of proline occurs in stressed plants and can be mediated by signaling molecules, including H_2O_2 (Wen et al. 2013). Accumulation of proline is a well-known adaptive mechanism in plants against salt stress conditions (Szabados and Savaure 2010). High proline levels might protect plants by scavenging the oxygen-free radicals caused by salt stress (Colla et al. 2010). The amount of proline level observed in the grafted and non-grafted plants were related to the greater salt sensitivity of pepper genotypes, as reported for other species such as wheat (Colmer et al. 2005), barley (Chen et al. 2007), *Centaurea ragusina* (Radic et al. 2013) or rice (Lutts et al. 1995). On the other hand, contrary results reported that there were no significant differences between grafted and non-grafted pepper plants in proline metabolism under saline conditions (Penella et al. 2015).

Glycine betaine content was significantly ($p < 0.001$) affected by scion, rootstock, different salt levels, scion \times salt levels, rootstock \times salt levels interaction; however, it was not significantly affected by scion \times rootstock interaction (Fig. 1d). Glycine betaine content increased by increasing salt level in the nutrient solution. From results of the control treatment, ERÜ-462/Yaocali F1 graft combination had significantly higher glycine betaine content. However, non-grafted plants of ERÜ-1227 (0.11 mg g^{-1}) had significantly lower glycine betaine content. Under salt treatment, graft combination of ERÜ-462/11B14 (0.38 mg g^{-1}) had significantly higher glycine betaine content; on the other hand, non-grafted plants of ERÜ-1227 (0.20 mg g^{-1}) had significantly lower glycine betaine content (Fig. 1d). In our study increase in leaf glycine betaine contents in response to salt stress are in agreement with those observed in different plant species such as lentil (Bandeoglu et al. 2004), corn (Yakit and Tuna 2006), switchgrass (Wang et al. 2012), strawberry (Garriga et al. 2015) and watermelon (Zhiping et al. 2008). As reported by Yamac (2017) concluded that

glycine betaine content in leaf tissues of grafted watermelon plants was higher than non-grafted plants under saline conditions.

Mineral Composition and Partitioning of Leaf and Root

Results of leaf and root K^+ , Ca, Na^+ , and Cl composition of the grafted and non-grafted 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 Tables 3 and 4, respectively. Leaf K^+ concentration was significantly ($p < 0.001$) affected by different salt levels, rootstock \times salt levels interaction ($p < 0.05$); however, it was not significantly affected by scion, rootstock, scion \times rootstock, scion \times salt levels interaction. Leaf K^+ concentration decreased with salt treatments in all graft combinations. Leaf K^+ concentration was higher in non-grafted plants compared to all graft combinations under saline conditions.

Ca concentration in leaf tissue was significantly affected by rootstock ($p < 0.01$), rootstock \times salt levels interaction ($p < 0.001$); however, it was not significantly affected by scion, salt levels, scion \times rootstock interaction, scion \times salt levels interactions. Generally, leaf Ca concentration increased as salt level increased in nutrient concentration. ERÜ-462/Scarface had the highest Ca levels in leaves, whereas non-grafted control plants of ERÜ-462 plants showed the lowest Ca accumulation under salt stress conditions.

Leaf Na^+ concentration was significantly ($p < 0.001$) affected by rootstock, different salt levels, scion \times salt levels ($p < 0.05$), rootstock \times salt levels interaction ($p < 0.01$); however, it was not significantly affected by scion, scion \times rootstock interaction. Na concentration in leaves increased under salt stress conditions in all control plants and graft combinations. The highest values recorded in control plants of ERÜ-462 under both saline and non-saline conditions.

In leaves, Cl^- concentration was significantly ($p < 0.001$) affected by different salt levels, rootstock ($p < 0.01$), rootstock \times salt levels interaction; however, it was not significantly affected by scion, scion \times salt levels, scion \times rootstock interaction. The Cl^- concentration in leaves increased with a higher NaCl concentration. Under saline conditions, non-grafted plants of ERÜ-462 accumulated the highest Cl^- levels in leaves.

These results agree with other studies showing that plants growing under salinity conditions suffer from ionic imbalance and nutrient deficiency (Hmidi et al. 2018). It is known that the addition of external Ca^{2+} and K^+ can significantly mitigate salinity stress symptoms in many species (Rengel 1992). Better maintenance of K^+ homeostasis in plant tissue is another salt tolerance mechanism that grafting can affect (Colla et al. 2010). Wu et al. (2013) stated that salinity

Table 3 The effects of graft combination and different salt levels (1 dSm⁻¹ and 8 dS⁻¹ m) on leaf K⁺, Ca²⁺, Na⁺ and Cl⁻ composition of pepper plants

Graft combination (S/R)	K ⁺ (%)		Ca ²⁺ (%)		Na ⁺ (%)		Cl ⁻ (mg gr ⁻¹)	
	Control	Salt	Control	Salt	Control	Salt	Control	Salt
ERÜ 1227	6.53 a	5.01bcd	1.26 bc	1.22bcd	0.057 d	0.81 b	16.8 c	51.0 b
ERÜ 1227/Scarface	5.48 abc	4.92bcd	1.18bcd	1.46 ab	0.040 d	0.48 c	13.9c	39.0b
ERÜ 1227/Yaocali	5.66 abc	5.00bcd	1.11bcd	1.27 bc	0.047 d	0.56 bc	14.4c	40.4 b
ERÜ 1227/11B14	5.92 ab	4.55 cd	0.99cd	1.08bcd	0.060 d	0.71 bc	15.4c	45.3 b
ERÜ 462	6.31 a	4.00 d	1.11bcd	0.84 d	0.063 d	1.23 a	16.5 c	66.0 a
ERÜ 462/Scarface	5.89 ab	5.57abc	1.14bcd	1.69 a	0.043 d	0.56 bc	15.0c	45.5 b
ERÜ 462/Yaocali	6.13 ab	4.96bcd	1.14bcd	1.09bcd	0.043 d	0.82 b	15.4c	50.1 b
ERÜ 462/11B14	5.62 abc	5.34abc	1.17bcd	1.15 bcd	0.040 d	0.58 bc	14.4 c	40.1 b
<i>F-Test</i>								
Scion		n. s.		n. s.		n. s.		n. s.
Rootstock		n. s.		**		***		**
Salt		***		n. s.		***		***
Scion × Rootstock		n. s.		n. s.		n. s.		n. s.
Scion × Salt		n. s.		n. s.		*		n. s.
Rootstock × Salt		*		*		**		*

Values denoted by different letters are significantly different between graft combination within both columns at $P < 0.05$. ns, non-significant. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

raises Ca²⁺ concentration of shoot in sugar beet (*Beta vulgaris* L.) cultivars. Penella et al. (2015) studied the effects of salt stress on pepper plants and reported that leaf K⁺ and Ca²⁺ levels was enhanced by increasing salt level in grafted plants. It has been suggested that Ca²⁺ could be redistributed from roots to shoots during salinity stress (Ramoliya et al. 2006). Present study strongly supports this hypothesis. In another study, increased Na and Cl⁻ concentration in leaf tissue has been reported in grafted pepper plants under salinity stress in the nutrient solution (Chartzoulakis and Klapaki 2000). Penella et al. (2016) found that grafting a salt tolerant pepper rootstock on a commercial pepper variety increased yield and photosynthesis parameters but did not decrease Na⁺ and Cl⁻ leaf ion content as compared to non-grafted pepper. Penella et al. (2017) determined increased salt tolerance of grafted pepper as compared to non-grafted pepper and significantly greater Cl⁻ but not Na⁺ in the leaves of non-grafted plants. Also, Bai et al. (2005) reported that eggplant grafted onto *Solanum torvum* had lower leaf Na⁺ and Cl⁻ contents than did self-rooted plants under NaCl stress. These results contrast with Almeida et al. (2014) who in a three-week experiment did not find a correlation between Na⁺ concentrations in the leaves and vegetative growth of 23 tomato accessions. They concluded that the relationship between Na⁺ concentration in the cells and tissue tolerance to salinity may vary among accessions. Romero et al. (1997) also mentioned that grafted plants developed many mechanisms to avoid the physiological damage caused by excessive accumulation of Cl⁻ and Na⁺ in the leaves, as well as prohibiting Cl⁻ and/or decreasing Cl⁻ absorption by the

roots, and the replacement or substitution of total K⁺ by total Na⁺ in the foliage. The decreases in leaf K⁺ and Ca²⁺ concentrations in pepper plants the salinity treatments were also observed by Lycoskoufis et al. (2005). This is due to their physicochemical similarity, which promotes ionic competition for binding sites on membrane transporters (Munns and James 2003). So, K⁺ would be preserved for the vital functions of the plant, namely activation of enzymes, protein synthesis and photosynthesis, as well as for the transport of solutes in the phloem, although, it plays an important role in expanding cells particularly for young leaves (Olías et al. 2009).

Root K⁺ concentration was significantly affected by rootstock ($p < 0.05$), and different salt levels ($p < 0.01$); however, it was not significantly affected by scion, scion × salt levels, rootstock × salt levels interaction, scion × rootstock interaction. K⁺ concentration in roots lowered under salt conditions compared to the values recorded in non-saline conditions. In the root compartment under salt treatment, the K⁺ concentration increased in ERÜ-1227/Scarface plants.

Root Ca²⁺ concentration in root tissue was not significantly affected by scion, rootstock, different salt levels, scion × salt levels, rootstock × salt levels interaction, and scion × rootstock interaction. Root Ca²⁺ concentration pepper genotypes were decreased with salt treatments except for non-grafted ERÜ-462. Under salt stress, lower level of Ca²⁺ content was observed in all grafted plants compared to non-grafted control plants.

Root Na⁺ concentration was significantly affected by different salt levels ($p < 0.001$), and rootstock × salt levels inter-

Table 4 The effects of graft combination and different salt levels (1 dSm⁻¹ and 8 dSm⁻¹) on root K⁺, Ca²⁺, Na⁺, and Cl⁻ composition of pepper plants

Graft combination (S/R)	K ⁺ (%)		Ca ²⁺ (%)		Na ⁺ (%)		Cl ⁻ (mg gr ⁻¹)	
	Control	Salt	Control	Salt	Control	Salt	Control	Salt
ERÜ 1227	2.96 a	2.34abc	0.30 ab	0.27ab	0.04 c	1.47 a	9.9 d	48.0 a
ERÜ 1227/Scarface	2.40 abc	2.52 ab	0.32 ab	0.21 b	0.04 c	1.45 a	10.6 d	51.8 a
ERÜ 1227/Yaocali	2.13 a–d	1.98 a–d	0.24 b	0.22 b	0.04 c	1.26 a	7.8 d	40.5 ab
ERÜ 1227/11B14	2.39 abc	1.24 d	0.23 b	0.21 b	0.04 c	0.97 b	7.6 d	25.4 c
ERÜ 462	1.78 bcd	1.46 cd	0.30 ab	0.35 a	0.08 b	1.27 a	9.9 d	29.8 bc
ERÜ 462/Scarface	2.40 abc	2.18 a–d	0.26 ab	0.23 b	0.05 c	1.48 a	8.6 d	44.7 a
ERÜ 462/Yaocali	2.74 ab	1.88bcd	0.27 ab	0.27ab	0.04 c	1.53 a	8.9 d	38.5 abc
ERÜ 462/11B14	2.38 abc	1.91bcd	0.30 ab	0.25 ab	0.04 c	1.37 a	9.9 d	39.2 abc
<i>F-Test</i>								
Scion		n. s.		n. s.		n. s.		n. s.
Rootstock		*		n. s.		n. s.		*
Salt		**		n. s.		***		***
Scion × Rootstock		n. s.		n. s.		n. s.		n. s.
Scion × Salt		n. s.		n. s.		n. s.		n. s.
Rootstock × Salt		n. s.		n. s.		*		n. s.

Values denoted by different letters are significantly different between graft combination within both columns at $P < 0.05$. ns, non-significant.

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

action ($p < 0.05$); however, it was not significantly affected by scion, rootstock, scion × salt levels, scion × rootstock interaction. ERÜ-462 increased root Na⁺ concentration when grafted with Scarface F1 and Yaocali F1 rootstock genotypes under salinity stress, but it decreased in ERÜ-1227/11B14 and ERÜ-1227/Yaocali F1 combinations. Root Na⁺ accumulation may provide a mechanism to manage salt stress in rooting medium and/or might indicate the existence of an inhibition mechanism of Na⁺ transport to leaves to protect shoot from adverse effects of increased level of Na⁺.

In roots, Cl⁻ concentration was significantly affected by rootstock ($p < 0.05$), and different salt levels ($p < 0.001$); however, it was not significantly affected by scion, scion × salt levels, rootstock × salt levels interaction, scion × rootstock interaction. Cl⁻ concentration was increased when ERÜ-1227 was grafted on Scarface F1 rootstock under saline conditions. On the other hand, ERÜ-462 had increased Cl⁻ concentration when grafted with all three rootstock genotypes. Among grafting combinations, the highest Cl⁻ concentration was recorded in ERÜ1227/Scarface F1 under saline conditions. The lowest Cl⁻ concentration was recorded in graft combinations of ERÜ-1227/11B14 under saline conditions.

Regulating intracellular K⁺, Ca²⁺, and Na⁺ homeostasis is of critical importance for crop plants adaptation to salinity stress (Wu and Wang 2012). Our results are in line with Wu et al. (2013) that salinity raises Na⁺ concentration of both shoot and root in sugar beet (*Beta vulgaris* L.) cultivars; whilst reduced K⁺ and Ca²⁺ concentration. Penella

et al. (2016) examined the grafted and non-grafted pepper plants under saline conditions that Na⁺ and Cl⁻ increased in both roots and shoots tissues in both grafted and non-grafted plants. Effects of increasing salt level of nutrient solution on K⁺ concentration was less evident than those of Na⁺ and Cl⁻. In another study, increased Na⁺ and Cl⁻ concentration in leaf tissue has been reported in grafted pepper plants under salinity in the nutrient solution. The concentration of K⁺ in leaf tissue was not significantly influenced by salinity, while roots displayed lower K⁺ concentration than the leaves with the trend to decrease with increasing salinity of nutrient solution (Chartzoulakis and Klapaki 2000). Navarro et al. (2002) 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

The productivity of several commercial pepper crops is limited by salinity stress in many areas of the world (Kurunc et al. 2011). Grafting has been recommended as an effective approach that improves yields and quality of many crops under salt stress (Ulas et al. 2019; 2019a). Most research conducted on pepper grafted plants have been focused on both obtaining resistances to biotic stresses and obtaining yield increments, but few works have been done to study the effect of grafting on salt stress in pepper plants, despite it is known that grafted plants show tolerances to abiotic

stresses and improve yields in other crops like melon (Ulas et al. 2019a) or tomato (Ulas et al. 2019b).

In our research, under saline conditions, grafted pepper plants have shown that this technique leads to producing plants with superior attributes compared to the non-grafted ones. Despite the negative effects of salinity on vegetative growth, the effect of grafting onto pepper rootstocks on shoot and root biomass production was increased with the rootstock genotype of 11B14. Plants grafted on to 11B14 rootstock have significantly higher shoot and root biomass than non-grafted control plants. The ERÜ-462 grafted on to 11B14 rootstocks has had a higher leaf chlorophyll (a + b) content and leaf carotenoid content compared to other rootstock genotypes and non-grafted control plants. It is known that photosynthesis is normally reduced when plants are under any case of stress. In our results, photosynthesis has been found as good indicator to distinguish between tolerant and sensitive genotypes to salt stress. The significantly higher photosynthesis was produced for the ERÜ-462 grafted on to Scarface F1. Salinity had a significant adverse effect not only on the plant growth and development, although also on leaf area formation of both grafted and non-grafted plants. Though this adverse effect was more evident in non-grafted plants than grafted plants. In terms of leaf area, the significantly highest leaf area formation was produced when ERÜ-1227 grafted on to Scarface F1 and Yaocali F1. Under saline conditions, pepper plants of ERÜ-462 grafted plants onto 11B14 rootstock activated tolerance mechanisms based on ion exclusion or retention under salinity. We speculate that under a stress stimulus proline represents the key metabolite by which plants could face salinity conditions. The ERÜ-462 grafted on to 11B14 rootstocks has had a higher proline content than non-grafted control plants. Several studies have attributed a dual role to proline: compatible osmolyte and antioxidant compound (Hayat Hayat et al. 2012). Free proline is considered an important osmoprotectant and accumulation following salt, drought, and heavy metal exposure is well documented (Gill and Tuteja 2010). The maintenance of scion homeostasis under salinity was achieved through the restriction of Cl⁻ transport to leaves and to diminished Na⁺ loading in roots and leaves, thus favoring K⁺ uptake. Nonetheless, although ionic and water homeostasis are crucial parameters in abiotic stress tolerance, the maintenance of shoot vigor and leaf function are vitally important. In this way, ERÜ-462 plants grafted onto Scarface accumulated high concentration of toxic ions in plant tissues. As a further confirm, grafted plants onto Scarface under salinity accumulated in leaves even more ions (both Na⁺ and Cl⁻) than the non-grafted one. Furthermore, regarding root K⁺, Na⁺, Cl⁻ contents, the significantly highest root K⁺, Na⁺, Cl⁻ contents were observed at the ERÜ-1227 grafted on to Scarface F1. Summarizing, strong rootstock promoted plant growth

in pepper plants under both control and saline conditions. Scarface and 11B14 rootstock were found more tolerant to salinity than non-grafted pepper plants and the other genotypes used as regard to investigated parameters. These both rootstocks were more tolerant to salinity due to vigorous root system that transmits water and nutrients better to the scion. As a result of the present study, it can be concluded that the negative effect of salt stress can be alleviated by using strong rootstocks under salt stress conditions.

Conflict of interest O. Abidalrazzaq Musluh Al Rubaye, H. Yetisir, F. Ulas and A. Ulas declare that they have no competing interests.

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