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Effects of grafting on growth, root morphology and leaf physiology of pepino (*Solanum muricatum* Ait.) as affected by salt stress under hydroponic conditions

Firdes Ulas[1,*]

[1]Erciyes University, Faculty of Agriculture, Department of Horticulture, Kayseri, Turkey

[*]Corresponding Author: fulas@erciyes.edu.tr

Abstract

In this study, grafted and ungrafted pepino (*Solanum muricatum* Ait.) plants were tested under different saline conditions. The nutrient solution experiment was conducted within October – November 2016, by employing the technique of Deep-Water Culture (DWC) in an entirely operated automatically climate chamber found in the Plant Physiology Laboratory of Erciyes University, Agriculture Faculty, Kayseri, Turkey. Plants were examined under three various salt levels (i.e., 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}) by growing them in a 8 liter pots loaded constantly in an aerated Hoagland solution. The study was organized with completely randomized block design through three repetitions. The climate chamber study was performed to investigate effects of salt stress on plant growth, shoot- root fresh- dry weights, photosynthesis, leaf area formation, chlorophyll content of leaf (SPAD), leaf and root electrolyte leakage, total length of root, volume of root, and diameter of root in grafted and ungrafted pepino plants. The results showed that shoot growth, root morphological and leaf physiological responses were considerably (p<0.001) influenced by various levels of salt conditions at the nutrient solution. Increased salt level of the nutrient solution decreased significantly root and shoot growth, area of leaf, photosynthetic activity of both grafted and ungrafted plants. Irrespective of being grafted, significant declines were observed in shoot fresh weight (23.6%, 52.1%), root fresh weight (24.8%, 52.8%), leaf area (21.3%, 51.9%), shoot dry weight (24.3%, 53.0%), root dry weight (15.4%, 45.1%), SPAD (5.7%, 18.7%), photosynthesis rate (24.6%, 42.1%), total root length (6.7%, 16.4%), and root volume (3.8%, 5.8%) of pepino plants under 4 dS m^{-1} salt applications and 8 dS m^{-1} salt applications, respectively. Grafting promoted growth of plant in pepino plants under both control and saline conditions, furthermore it was noticed that under saline conditions biomass production of both grafted and ungrafted ones were significantly depressed. Grafted plants produced 54.1%, 43.0% and 9.6% higher shoot fresh weight; 52.0%, 42.0% and 12.8% higher root fresh weight; 52.5%, 40.7% and 8.7% higher leaf area; 60.0%, 46.6% and 11.1% higher shoot dry weight; 68.8%, 36.0% and 29.3% higher root dry weight; 19.9%, 9.2% and 8.2% higher SPAD; 8.0%, 5.1% and 10.8% higher photosynthesis rate; 8.6%, 3.6% and 6.6% higher total root length; 3.1%, 6.7% and 2.4% higher root volume than ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, correspondingly. Overall, our study showed that the effectiveness of grafting with respect to expansion of plants growth and development under salinity. Grafting was demonstrated to be an effective mean to achieve this goal.

Keywords: Nutrient solution, Pepino, Root volume, Salt stress, *Solanum muricatum*

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Introduction

Pepino (*Solanum muricatum* Ait.) a vegetable belonging to the Solanaceae family, it is innate to Andean regions of Columbia, Peru, Chile from South America, it was domesticated in pre-Hispanic times. It is, on the other hand, grown in several tropical and subtropical areas (Prohens et. al., 1996; Huyskens-Keil et. al., 2006), and it was an essential crop throughout the periods of the Inca Empire. It is a similar vegetable as phylogenetically with potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* L.). Pepino stalks root with ease, and it is vegetatively propagated by small cuttings. Ripening of pepino dulce fruit is characterized by a climacteric (El Zeftawi et al., 1988); develop parthenocarpic fruit (Hermann, 1988). The fruit is consumed fresh or cooked. When ripe it has a muskmelon-resembling flavor, tender, and juicy but vary greatly in shape, color, size (Hermann, 1988), and also in sugar (Dennis et al., 1981) SSC (El-Zeftawi et al., 1988; Hermann, 1988), and pH. The fruits are often exotically colored. Within, they are a little watery with pleasant flavor, but usually not over-sweet. At its finest, pepino fruit is soft and juicy and is utilized as a dessert fruit. They are high in vitamin C (45-70 mg/100 g) (Hermann, 1988; Morley-Bunker, 1983; Redgewell and Turner, 1986), K (>1000 mg kg^{-1}), and are considered more selenium than highly ordinary fruits and vary greatly in flavor (Dennis et al., 1981; Hermann, 1988), in many cases leaving an off-flavor sensation. Currently, little is known about pepino crop, as the crop remains underexplored or unexploited. In countries such as New Zealand, the USA (California), and Chile, pepino is being grown under contemporary and regulated conditions, but not on a wide scale. During the 1990s and first years of 2000s, Spain has been a reference of breeding and marketing of pepinos (Rodriguez-Burruezo et al., 2011). Pepino is one of the latest introduced vegetables to Turkey. In recent times, the pepino crop has developed an increasing interest in Turkey owing to its flavor, nutritional value, and attractive appearance. It is also known as a medicinal plant in the country. Pepino production has been rising in the last years in Turkey (Cavusoglu et al., 2009; Yalcin, 2010).

Salinity stress inhibits growth and expansion of the plant, causing osmotic stress and yield loses and might be toxic to plants (Mass and Nieman, 1977; Levitt, 1980). In addition, Na and chloride ions may inhibit absorption of essential minerals (Levitt, 1980). Defeating salt stress problems is crucial for future agriculture to meet the food needs of future populations. One feasible method to encourage salt stress tolerance by physical means is grafting of a productive scion on a salt stress-tolerant rootstock. Vegetable plants were first grafted in the late 1920s by Japan and Korea, where watermelon was grafted onto gourd rootstocks to enable for continuous cropping in fields that were prone to Fusarium wilt soil-borne disease (Davis et al., 2008). It also serves as a revolutionary procedure for the proper propagation of fruit-bearing vegetables including cucumber, tomato, eggplant, bean, and melon in Korea, Mediterranean region, Japan, and in Europe (Pogonyi et al., 2005). Grafting with vigorous rootstocks improves pest and disease resistance, yield, increases the nutrients uptake and the mineral content of plant aerial portion, drought, and cold tolerance, growth, fruit quality, tolerance to high and low temperatures and salinity tolerance as emphasized for several crops including tobacco, melon, watermelon, and tomato (Sarabi et al., 2017; Ulas et al., 2019; Ulas et al., 2019a, b).

Up to now, several studies connected to pepino propagation have been conducted throughout the world in countries including Italy (Vincenzoni, 1988), China (Luo, 1994), Spain (Seidel, 1974), Netherlands (Welles, 1992), France (Peron et al., 1989), the United States (Maynard, 1989), Turkey (Ercan and Akilli, 1995; Yalcin, 2010), Israel (Pluda et al., 1993), and Korea (Joo et al., 1990). However, to our knowledge, no detailed study concerning the grafting effect on growth, root morphology and leaf physiology of pepino plants under salt stress at hydroponic growth systems has been performed in Turkey in literature. Thus, this research is purposed at assessing impacts of grafting on shoot growth and root morphological and leaf physiological responses of pepino plants as affected by salt stress under hydroponic growth condition.

Materials and Methods

Plant material, treatments, and experimental design

A hydroponic experiment was conducted between October - November in 2016 for six weeks by using an aerated deep-water culture (DWC) technique in a fully automated climate chamber in the Plant Physiology Laboratory of Erciyes University, Faculty of Agriculture, central Anatolia in Turkey. For the vegetation period, the average day/night temperatures were 25/22 °C, the relative humidity was 65–70% and about 350 µmol m^{-2} S^{-1} photon flux was supplied in a photoperiod of 16/8 h of light/dark regimes in the controlled growth chamber. Pepino *(Solanum muricatum* Ait. seedlings were used as plant materials by an agricultural company. The pepino variety Miski, which is the most grown variety under greenhouse and field conditions in Turkey. The pepino seedlings were obtained in multipots filled with a mixture of peat (pH: 6.0-6.5) and perlite (2v:1v). The seedlings were grafted by “tube grafting method” described by Lee (1994), while ungrafted pepino seedlings were used as control plants. After grafting, plants were healed and acclimatized in the tunnel covered with double-layered plastic film and shade cloth in the climate chamber for one week. 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 three or four 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, grafted plants were transplanted to 8 liter plastic pots after roots were washed from growth media. Two plants were grown in each pot filled with 8 liter nutrient solution (modified Hoagland). During hydroponic study only distilled water with analytical grade (99% pure) chemicals contained were used. The solution was continuously aerated by an air pump to supply sufficient dissolved oxygen (8.0 mg/ L). The experiment was conducted with three different salt levels (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}). Each pot was filled with 8 liter 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. The experiment was in a completely randomized block design with three replications and six plants in each replication.

Harvest, Shoot- Root Fresh and Dry Weight, Shoot: Root Ratio Measurements

Grafted and ungrafted pepino plants were harvested by cutting them into the leaf, stem, and roots for the fresh weight determination at the end of the experiment. After measuring the fresh weights of each shoot and root fraction, samples were dried separately in paper bags in a ventilated oven at 70 °C for 72 hours. Shoot: root ratio was calculated from the dry weight measurements.

Root Morphological Measurements

At the end of the hydroponic experiment, root length (cm), root volume (cm^3) and root diameter (mm) of the plant root morphological parameters were analyzed by using a special image analysis software program WinRhizo (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Canada) in combination with Epson Expression 11000XL scanner. From harvested fresh root samples of pepino plants, almost 5.0 g sub-samples were taken. The samples were each (one after the other) placed in the scanner's tray.

Water was added and with the aid of a plastic forceps, the roots were homogenously spread across the tray; and the scanning and analysis done from the WinRhizo system's interface on a computer connected to the scanner. The total plant root length and volume was then determined as the ratio of sub-sampled root fresh weight to the total root fresh weight.

Leaf Physiological Measurements

At the hydroponic experiment, some of the leaf physiological parameters were determined destructively at final harvest, while some of them were measured non-destructively prior to the final harvest. The leaf chlorophyll index (SPAD) was determined non-destructively by using a portable chlorophyll (SPAD) meter (Minolta SPAD-502). During the growth period, SPAD readings were performed on 3rd and 4th week of the vegetation period at the center of the leaves on the fully expanded youngest leaf of whole plants for each treatment. 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 (cm^2).

The leaf-level CO_2 gas exchange (µmol CO_2 m^{-2} s^{-1}) measurements were done non-destructively in controlled growth chamber by using a portable photosynthesis system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA). The leaf photosynthesis measurement was performed on the most recent fully expanded leaves, using four replicate leaves per treatment on 3rd and 4th week of the vegetation period. All gas exchange measurements were carried out between 09:00 and 12:00 HR.

Leaf and Root Electrolyte Leakage Measurements

Electrolyte leakage (EL) in leaves and roots 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 hr every 48 hr 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.

EL determination in plant roots was done by taking fresh root tips (2 cm in length) from each treatment at the final harvest. The samples of root and leaf 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) × 100.

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 (F-Test). Differences between the treatments were analyzed using Duncan's Multiple Test ($p < 0.05$).

Results and Discussion

Shoot and Root Biomass Production and Partitioning

Shoot fresh weight, root fresh weight, leaf area of the grafted and ungrafted plants examined under different salt applications (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}) were presented in Table 1. Fresh weight of shoot, fresh weight of root and leaf area were significantly influenced by grafting, various salt applications, and grafting × salt interaction. Weak growth and development of plants were recorded in grafted and ungrafted pepino as salt level was rised in nutrient solution. Therefore, significant declines were observed in fresh weight of shoot (23.6%, 52.1%) and root (24.8%, 52.8%) and leaf area (21.3%, 51.9%) of pepino plants under 4 and 8 dS m^{-1} salt applications comparing with 1 dS m^{-1} (control) salt application, respectively. To date, several studies have indicated that salinity causes many types of harm, including growth inhibition, physiological disorders, and yield and quality reduction; nevertheless, the degree of salt impairment has been indicated to be reliant on cultivar, salinity level, salinity span, and plant developmental stage (del Amor et al., 1999). Fresh weight of shoot, fresh weight of root, dry weight of shoot, dry weight of root reduction under salt application has been observed in melon (Del Amor et al., 1999; Rouphael et al., 2012), watermelon (Colla et al., 2006; Yetisir and Uygur, 2010), cucumber (Colla et al., 2013), tomato (Gong et al., 2013), and eggplant (Yasar et al., 2006) under salt stress conditions.

Grafted plants had a greater fresh weight deposition. They produced significantly higher shoot fresh weight under both 4 dS m^{-1} (93.43 g $plant^{-1}$) and 8 dS m^{-1} (52.0 g $plant^{-1}$) salt applications comparing with ungrafted plants. Grafted plants produced 54.1%, 43.0% and 9.6% higher fresh weight of shoot than ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively. Root fresh weight was observed to be comparatively more in grafted ones than in ungrafted ones under 1 dS m^{-1} (control) salt application, though there was no substantial difference occurred among grafted and ungrafted pepino under 4 dS m^{-1} salt application, as well as under 8 dS m^{-1} salt application. Grafted plants significantly had higher root fresh weight under both 4 dS m^{-1} (19.16 g $plant^{-1}$) and 8 dS m^{-1} (10.88 g $plant^{-1}$) salt applications than ungrafted plants. Grafted plants produced 52.0%, 42.0% and 12.8% higher fresh weight of root than the ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively. In our study, grafting had encouraging impacts on pepino growth under control and salt stress conditions. Inhibitions of shoot growth in ungrafted plants are due to decline in leaves number, deposition of shoot fresh weight and shoot dry weight, extension of shoots and a decrease in the area and size of the leaf (Flexas et al., 2004; Zhang et al., 2009). Our outcomes were in line by research of grafted plants such as pepper (Penella et al., 2016; Al Rubaye et al., 2020), melon (Ulas et al., 2019a) and watermelon (Ulas et al., 2020) in a saline state.

The area of leaf measurements for different salt applications at the final harvest indicated that grafted ones had a greater leaf area than ungrafted ones. Though there were no substantial variations observed amongst grafted and ungrafted pepino under 4 dS m^{-1} salt application. Grafted plants produced 52.5%, 40.7% and 8.7% higher leaf area than the ungrafted plants under different salt applications (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}), respectively. Results obtained confirms with previous studies demonstrating that ungrafted watermelon leaf area showed significant improvement as grafting with two different commercial rootstocks, (*Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne) and Macis [*Lagenaria siceraria* (Mol.) Standl.] under salt stress and hydroponic conditions (Yamac, 2017). Decline in leaf was due to accumulation of ion in the leaves, especially the old leaves (Greenway and Munns, 1980). Wignarajah et al. (1975) underscored that salt application limited leaf expansion in beans, and it was mainly as a result of cell division inhibition instead of cell expansion. Under salinity, decline in leaf area was seen on grafted watermelon (Colla et al., 2006; Yamac, 2017) and grafted pepper (Al Rubaye et al., 2020).

Table 1. Fresh weight of shoot, fresh weight of root and leaf area formation of grafted and ungrafted pepino at hydroponic system under salinity (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1})

Salt levels	Shoot fresh weight (g $plant^{-1}$)		Root fresh weight (g $plant^{-1}$)		Leaf area (cm^2 $plant^{-1}$)	
	Ungrafted	Grafted	Ungrafted	Grafted	Ungrafted	Grafted
1 dS m^{-1}	81.80 a	126.00 b	17.24 a	26.18 b	1867 b	2846 a
4 dS m^{-1}	65.30 a	93.43 b	13.48 a	19.16 a	1542 a	2169 a
8 dS m^{-1}	47.46 a	52.00 b	9.64 a	10.88 a	1087 b	1182 a
Significance						
Salt	***		***		***	
Graft comb.	***		***		***	
Salt X Graft comb.	***		***		***	

Values indicated by various letters are substantially distinct from genotypes inside columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Dry weight of shoot, dry weight of root, and root to shoot ratio of the grafted and ungrafted plants examined under various salt applications (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}) were presented in Table 2. Dry weight of shoot, dry weight of root, and ratio of root to shoot were substantially ($p < 0.001$) influenced by grafting, changing levels of salt, and interaction of grafting × salt. Irrespective of the grafting, adverse effects were observed and recorded in growth and development of both grafted and ungrafted plants as salt application increases. Therefore, significant declines were observed in shoot dry weight (24.3%, 53.0%), and root dry weight (15.4%, 45.1%) of pepino plants under 4 and 8 dS m^{-1} of salt applications comparing with 1 dS m^{-1} (control) of salt application, respectively. Decline in dry weight of shoot and, dry weight of root were observed also in many crop species including tomato (Dasgan et al., 2002), pepper (Chartzoulakis and Klapaki, 2000), melon (Sivritepe et al., 2005), eggplant (Chartzoulakis and Loupassaki, 1997), strawberry (Awang et al., 1993), broad bean (de Pascale and Barbieri, 1997), and cucumber (van der Sanden and Veen, 1992) under salt application.

Grafted plants produced significantly higher shoot dry weight under both 4 dS m^{-1} (9.54 g $plant^{-1}$) and 8 dS m^{-1} (5.24 g $plant^{-1}$) salt applications comparing with ungrafted plants. Grafted plants produced 60.0%, 46.6% and 11.1% higher dry weight of shoot than the ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1}, and 8 dS m^{-1} salt applications, respectively. Khah et al., (2006) studied grafting effects have on tomato growth and yield in greenhouse and open-field conditions, 'Big Red' tomato was self-grafted (used both as scion and rootstock) and ungrafted served as control, and two hybrid tomatoes

'Primavera' and 'Heman' were selected as rootstocks. Grafted plants with rootstocks were more vigorous than ungrafted ones in the greenhouse as well as in the open field. On the other hand, higher plant height, stem fresh-dry weight, and leaves fresh-dry weight were recorded in self-grafted plants than in ungrafted control under both conditions of cultivation. The obtained results were in line with those of Yamac (2017), who underscored that salt stress under saline and hydroponic conditions decreased shoot dry weight in grafted and ungrafted watermelon. The combined osmotic stress impact could be the major cause of reduction of growth (Greenway and Munns, 1980), which is detrimental to plants in growth stage and uptake of ion (Dumbroff and Cooper, 1974). Penella et al. (2016) exhibited that grafted pepper plants were less susceptible to salt stress than the ungrafted ones, with respect to photosynthesis and subsequently growth and yield. To date, in several greenhouse crops grown under hydroponic conditions, the ameliorative grafting impact on plant growth under salt application has been entirely consistent with other studies in tomatoes and melons (He et al., 2009; Rouphael et al., 2012; Yamac, 2017).

Root dry weight was highly influenced by salt application in grafted and ungrafted plants. Dry weight of root of the grafted plants were substantially greater than ungrafted ones under 1 dS m^{-1} (control) salt application, though no considerable variation was observed among grafted and ungrafted pepino under 4 dS m^{-1} salt application, as well as under 8 dS m^{-1} salt application. Regarding dry weight of root, grafted plants produced significantly higher root dry weight under both 4 dS m^{-1} (1.79 g $plant^{-1}$) and 8 dS m^{-1} (1.13 g $plant^{-1}$) salt applications comparing with plants that were not grafted. The grafted plants produced 68.8%, 36.0% and 29.3% higher root dry weight than the ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively (Table 2). Regarding root-to-shoot ratios, no significance difference was reported among ungrafted and grafted plants under various salt applications. These results confirm with studies conducted by other researchers, which indicated that, under salt stress conditions, grafted pepper plants showed higher significance in root dry mass than ungrafted plants. In grafted plants, higher root development, independent of salt stress, might be the cause of more photosynthetic rate regulated in grafted plants (Penella et al., 2016). Improvement in crop growth and performance was observed when plants were grafted onto strong rootstocks with numerous root hairs and higher root length which facilitates additional water and mineral uptake from soil and transfer them to plant aerial parts (Yarsi and Sari, 2006).

Table 2. Dry weight of shoot, dry weight of root, and root to shoot ratio of grafted and ungrafted pepino at hydroponic system under salinity (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1})

Salt levels	Shoot dry weight (g $plant^{-1}$)		Root dry weight (g $plant^{-1}$)		Root to shoot ratio (g g^{-1})	
	Ungrafted	Grafted	Ungrafted	Grafted	Ungrafted	Grafted
1 dS m^{-1}	8.15 b	13.03 a	1.36 b	2.30 a	0.17 a	0.18 a
4 dS m^{-1}	6.50 b	9.54 a	1.31 a	1.79 a	0.20 a	0.19 a
8 dS m^{-1}	4.72 b	5.24 a	0.88 a	1.13 a	0.19 a	0.22 a
Significance						
Salt	***		***		***	
Graft comb.	***		***		*	
Salt X Graft comb.	***		***		***	

Values indicated by various letters are substantially distinct from genotypes inside columns at $p < 0.05$. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Leaf Physiological Development, Photosynthetic Activity of Leaves, Leaf and Root Ion Leakage

The results of leaf chlorophyll content (SPAD), photosynthesis, leaf and root ion leakage at final harvest of grafted and ungrafted pepino grown under various salt levels (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}) were shown in Figure 1. Obtained data clarify that chlorophyll content of leaf was considerably ($p < 0.001$) influenced by grafting, various salt applications, and grafting × salt interaction. Leaf chlorophyll content was considerably reduced by rising salt level of the nutrient solution. Regardless of grafting, reductions in comparable shoot- root dry weight under salinity resulted in a significant reduction in chlorophyll content (5.7%, 18.7%) of pepino plants under 4 and 8 dS m^{-1} salt applications comparing with 1 dS m^{-1} (control) salt application, respectively. This explains the reason why the production of shoot- root dry weight (Table 1 and 2) of grafted and ungrafted plants were negatively influenced by hydroponically saline condition, because biomass production and yield of crops are extremely contingent on leaf area formation and activities of leaf photosynthesis (Hirasawa and Hsiao, 1999). The inhibition of growth in several salinity-focused plant species is most often related to a decline in photosynthetic ability (He et al., 2009). Decreased photosynthesis due to increased salinity may be due to a number of causes, like smaller conductance of stomatal and depression in certain physiological activities associated with carbon uptake and fixation, or a combination of the two (Flexas et al., 2004; Zhang et al., 2009).

Grafted plants produced significantly higher leaf chlorophyll content under both 4 dS m^{-1} (66.2 SPAD) and 8 dS m^{-1} (56.8 SPAD) salt applications comparing to ungrafted plants. Grafted plants produced 19.9%, 9.2% and 8.2% higher SPAD than the ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively. Content of chlorophyll, that has a directly effect on leaves photosynthetic activity, is a significant index of photosynthetic potential and can be regarded as a source of food and energy. Trinchera et al. (2013) documented a relatively greater concentration of chlorophyll pigments in grafted plants comparing with ungrafted control plants. The data is also in accordance with those of Yamac (2017), who concluded that salinity diminished leaf chlorophyll contents in grafted and ungrafted watermelon at the hydroponic conditions.

Photosynthesis was influenced significantly (p < 0.001) by different levels of salt and grafting. A sharper drop in the rate of photosynthesis occurred because of salt application at grafted and ungrafted plants. Ungrafted and grafted plants receiving a 1 dS m^{-1} (control) salinity displayed maximum photosynthesis rate than 4 or 8 dS m^{-1} salt applications. Irrespective of graft combinations, significant declines were observed in photosynthesis rate (24.6%, 42.1%) of pepino plants under 4 and 8 dS m^{-1} salt applications comparing with 1 dS m^{-1} (control) salt application, respectively. Bethke and Drew (1992) considered the rationale for reduction in photosynthesis at salt-stressed pepper plants and proposed that photosynthesis reductions are largely non-stomatal, biochemical level and strongly associated with concentration of both Na^+ and Cl^- in plant leaves. Grafted plants produced significantly higher photosynthesis rate under both 4 dS m^{-1} (9.1 μ mol CO_2 m^{-2} s^{-1}) and 8 dS m^{-1} (7.2 μ mol CO_2 m^{-2} s^{-1}) salt application comparing by ungrafted ones. Grafted plants produced 8.0%, 5.1% and 10.8% higher photosynthesis rate than the ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively. Though, there were no substantial variations observed among grafted and ungrafted plants under 1 dS m^{-1} and 4 dS m^{-1} salt applications. These findings are corresponding to those of Penella et al. (2015) stated that salinity affects photosynthesis negatively as slightly at grafted pepper plants than ungrafted 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).

Regarding leaf ion leakage, it was substantially (p < 0.001) affected by different levels of salinity; though not grafting, and grafting × salt interaction. On the other hand, root ion leakage was significantly (p < 0.001) affected by grafting and various salt applications, though not grafting × salt interaction. Regardless of grafting mixture, an enhancement in salt content of nutrient solution resulted in a significant increase in leaf (17.1%, 36.7%) and root (20.3%, 48.9%) ion leakage of salt-treated pepino plants (4 dS m^{-1} and 8 dS m^{-1}) comparative to controls (1 dS m^{-1}) (Figure 1C and 1D). These are typical reactions of plants that generally express tolerance strategies which can be seen in studies of melon (Kuşvuran, 2010; Sarabi et al., 2017), rice (Lutts et al., 1996), cucumber (Mumtaz-Khan et al., 2013), pepper (Kaya et al., 2001), sugarbeet (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). Grafted plants produced significantly higher leaf and root ion leakage under both 4 dS m^{-1} (28.15, 39.05) and 8 dS m^{-1} (33.72, 48.44) salt applications comparing with ungrafted plants. Similar results were observed under saline conditions at grafted plants such as cucumber (Colla et al., 2013), watermelon (Yamac, 2017), melon (Ulas et al., 2019a), tomato (Ulas et al., 2019b), and pepper (Penella et al., 2016; Al Rubaye et al., 2020).

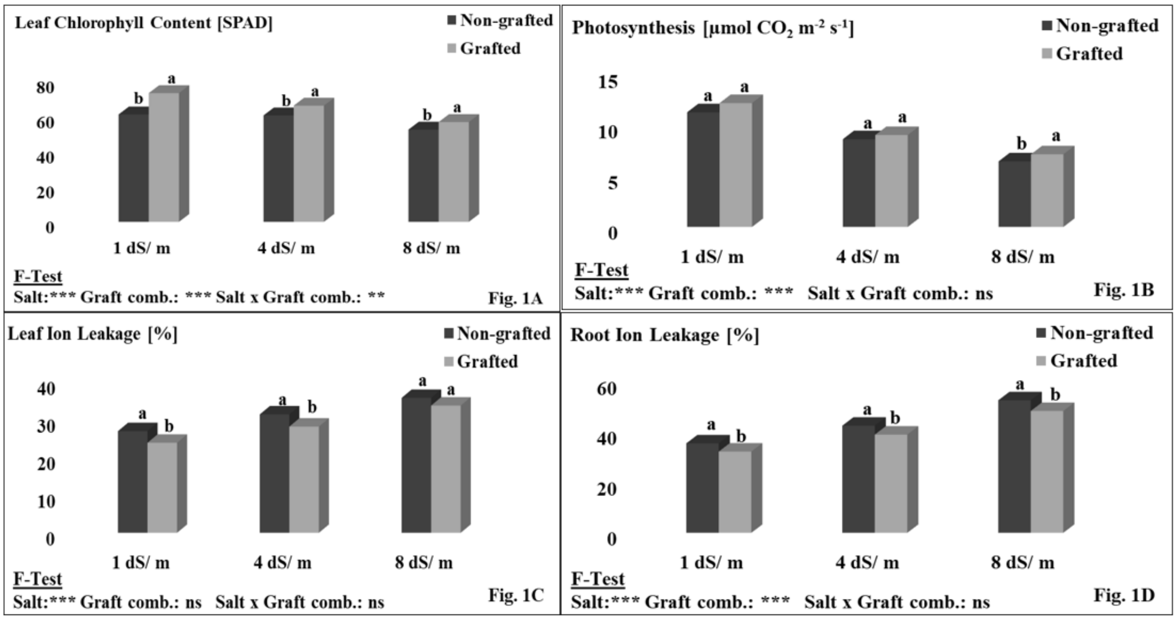

Figure 1. Leaf chlorophyll content (SPAD) (A), leaf photosynthesis (B), leaf (C) and root ion leakage (D) of grafted and ungrafted pepino at hydroponic system under salinity (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}). Values indicated by various letters are substantially distinct from genotypes inside columns at p < 0.05. ns, non-significant. *p < 0.05, **p < 0.01 and ***p < 0.001.

Root Morphological Development and Root Architecture

At the completion of grafting experiment, root morphological development and architecture of grafted and ungrafted plants tested at different salt levels (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}) were presented in Table 3. Total root length, and root volume were considerably ($p < 0.001$) influenced by grafting, various salt applications, and grafting × salt interaction. Irrespective of grafting, significant declines were observed in total root length (6.7%, 16.4%), and root volume (3.8%, 5.8%) of pepino plants under 4 and 8 dS m^{-1} salt applications comparing with 1 dS m^{-1}, respectively. Average root diameter was influenced substantially ($p < 0.001$) by grafting, different levels of salt, but not the interaction of grafting x salt. Average root diameter significantly increased (1.5, 6.2%) under 4 and 8 dS m^{-1} salt applications comparing with 1 dS m^{-1}, respectively. Grafted plants produced significantly higher overall root length (8602.08 cm $plant^{-1}$, 7818.52 cm $plant^{-1}$), volume of root (5.60 cm^3 $plant^{-1}$, 5.38 cm^3 $plant^{-1}$), diameter of root (0.28 mm $plant^{-1}$, 0.3 mm $plant^{-1}$) under both 4 dS m^{-1} and 8 dS m^{-1} salt applications comparing with ungrafted plants, respectively. The grafted plants produced higher total root length (8.6%, 3.6% and 6.6%), root volume (3.1%, 6.7% and 2.4%) than ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively.

Salt stress decreases transpiration and respiration, and also water absorption and root growth (Dölarslan and Gül, 2012). Like our research, Rastgeldi (2010) found that salinity induced decrease in total root length according on pepper genotypes comparing with control plants. In another study, the rootstocks experienced increment or decrement effects in root biomass depending on genotypes under salinity and drought (Penella et al., 2017).

Table 3. Total root length, total root volume and total root diameter of grafted and ungrafted pepino at hydroponic system under salinity (1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1}).

Salt levels	Total root length (cm $plant^{-1}$)		Total root volume (cm^3 $plant^{-1}$)		Average root diameter (mm)	
	Ungrafted	Grafted	Ungrafted	Grafted	Ungrafted	Grafted
1 dS m^{-1}	8690.88 b	9441.09a	5.55 a	5.73 a	0.29 a	0.28 b
4 dS m^{-1}	8307.34 b	8602.08a	5.25 b	5.60 a	0.29 a	0.28 b
8 dS m^{-1}	7331.37 b	7818.52a	5.25 b	5.38 a	0.30 a	0.30 b
Significance						
Salt	***		***		***	
Graft comb.	***		***		***	
Salt X Graft comb.	***		**		ns	

Values indicated by various letters are substantially distinct from genotypes inside columns at p < 0.05. ns, non-significant. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Conclusion

In conclusion, increasing salt level had a considerable negative impact on shoot growth, root morphological and leaf physiological responses of grafted and ungrafted plants. Although this negative impact was obvious in ungrafted plants than grafted plants. Irrespective of grafting, significant declines were observed in shoot fresh weight (23.6%, 52.1%), root fresh weight (24.8%, 52.8%), leaf area formation (21.3%, 51.9%), shoot dry weight (24.3%, 53.0%), root dry weight (15.4%, 45.1%), SPAD (5.7%, 18.7%), photosynthesis rate (24.6%, 42.1%), total root length (6.7%, 16.4%), and root volume (3.8%, 5.8%) of pepino plants under 4 and 8 dS m^{-1} salt applications comparing with 1 dS m^{-1} (control) salt application, respectively. Grafted plants produced 54.1%, 43.0% and 9.6% higher shoot fresh weight; 52.0%, 42.0% and 12.8% higher root fresh weight; 52.5%, 40.7% and 8.7% higher leaf area; 60.0%, 46.6% and 11.1% higher shoot dry weight; 68.8%, 36.0% and 29.3% higher root dry weight; 19.9%, 9.2% and 8.2% higher SPAD; 8.0%, 5.1% and 10.8% higher photosynthesis rate; 8.6%, 3.6% and 6.6% higher total root length; 3.1%, 6.7% and 2.4% higher root volume than ungrafted plants under 1 dS m^{-1}, 4 dS m^{-1} and 8 dS m^{-1} salt applications, respectively. Grafting encouraged plant growth in pepino plants under both control and salinity, also, significant decline in plant biomass under salinity was found in grafted and ungrafted pepino. Grafting technology has demonstrated to be a rapid alternative approach for conferring resistance to biotic and abiotic stresses, promoting plant vigor, increasing plant growth, and developing pepino production. Definitively, the use of grafting is a significant integrated approach for more developed cultivation forms, like hydroponics especially under salt stress conditions. As a next step, the same experiment may be performed with different Solanaceae rootstock genotypes to observe morphological, physiological, and biochemical responses among scion (pepino)/rootstock combinations under different salt treatments.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

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.

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