Relative Salt Tolerance of 22 Pomegranate (*Punica granatum*) Cultivars

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Abstract. A greenhouse experiment was conducted to determine the relative salt tolerance of pomegranate (*Punica granatum*) cultivars. Twenty-two pomegranate cultivars were irrigated weekly with a saline solution at an electrical conductivity (EC) of 10.0 dS·m⁻¹ for 4 weeks and subsequently with a saline solution at an EC of 15.0 dS·m⁻¹ for another 3 weeks (salt treatment). Another group of uniform plants was watered with a nutrient solution without additional salts at an EC of 1.2 dS·m⁻¹ (control). No visual foliar salt damage (leaf burn, necrosis, or discoloration) was observed during the entire experimental period; however, salt treatment impacted pomegranate growth negatively, with a large variation among cultivars. Salt treatment reduced shoot length by 25% and dry weight (DW) by 32% on average for all cultivars. Cluster analysis classified the 22 tested pomegranate cultivars in two groups. The group consisting of ‘Arturo Ivey’, ‘DeAnda’, ‘Kazake’, ‘Russian 8’, ‘Apseronski’, ‘Purple Heart’, ‘Carolina Venum’, ‘Chiva’, ‘Kunduzski’, ‘Larry Ceballos 1’, ‘ML’, ‘Salavatski’, ‘Spanish Sweet’, and ‘Wonderful’ was more salt tolerant than the group including ‘Al-Sirin-Nar’, ‘Kandahar’, ‘Surh-Anor’, ‘Early Wonderful’, ‘Angel Red’, ‘Ben Ivey’, ‘Utah Sweet’, and ‘Mollar’. The sodium (Na) concentration in the leaf tissue of all 22 pomegranate cultivars was less than 1 mg·g⁻¹ on a DW basis. All pomegranate cultivars in the salt treatment had an average leaf chloride (Cl) content of 10.03 mg·g⁻¹ DW—an increase of 17% from the control. These results indicate that pomegranate plants have a strong capability to exclude Na and Cl accumulation in leaf tissue. In conclusion, the pomegranate plant is very tolerant to saline water irrigation up to an EC of 15 dS·m⁻¹ with little foliar salt damage and a slight growth reduction. Investigation is needed to determine the effects of saline water on the fruit yield and nutritional quality of pomegranate.

Pomegranate (*Punica granatum*, Lythraeeae) is a bushy shrub or small tree native to Iran to the Himalayas in northern India. It has been cultivated since ancient times throughout the Mediterranean regions of Asia, Africa, and Europe. The fruit is rich in nutrition, with a unique flavor and taste, and medicinal properties. Recent scientific findings corroborate the traditional use of the pomegranate as a medical remedy for its antimicrobial properties and for its health benefits, such as the ability to reduce blood pressure and to act against serious diseases such as diabetes and cancer (Holland et al., 2009). Increased public awareness of the benefit of the pomegranate, particularly in the western world, has led to

a prominent increase in its consumption. In the United States, California produces more than 90% of U.S. pomegranates, with 26,935 acres, yielding 10.5 tons/acre according to the California Department of Food and Agriculture (Marzolo, 2015).

Pomegranate plants adapt to a wide range of environmental and soil conditions, but perform best in areas with long, hot, and dry summers (Castle et al., 2011; Holland et al., 2009). Although it is an ancient crop, pomegranate has not been studied systematically with regard to cultural practices, fertilization requirements, and salinity and drought tolerance. Salinity is a major environmental constraint in many pomegranate-growing areas such as India,

Mediterranean countries, and the southwestern United States. Saline brackish groundwater, treated municipal or industrial effluents, and recycled agricultural runoff water are the major alternative water sources for crop irrigation in many regions of the world, including those growing pomegranate (Qadir et al., 2008). These water sources often contain high salt levels that are detrimental to many species. Salt damage depends on the levels of salts and the degree of salt tolerance of crops. Therefore, the use of alternative waters for irrigation requires an adequate understanding of how salts impact plant performance and soil characteristics.

Limited literature shows that pomegranate is relatively tolerant to salt stress, with variations among cultivars (Bhantana and Lazarovitch, 2010; El-Khawaga et al., 2013; Okhovatian-Ardakani et al., 2010). ‘Malas-Saveh’ pomegranate is less tolerant than ‘Shishe-Kab’ (Khayyat et al., 2014). Irrigation with saline groundwater at 6.0 dS·m⁻¹ increased Na and Cl accumulation in leaves; reduced growth, flowering, and yield; and increased incidence of fruit cracking but did not change the total sugar and acidity percentages of fruit in 7-year-old ‘Manfalouty’, ‘Wonderful’, and ‘Nab-Elgamal’ pomegranates, with different responses to saline water irrigation among cultivars (El-Khawaga et al., 2013). ‘Malas Shirin’ pomegranate was tolerant up to 40 mM sodium chloride (NaCl) in 1:1 sand–perlite medium irrigated with complete Hoagland’s solution (Naeini et al., 2006). Okhovatian-Ardakani et al. (2010) compared 10 Iranian commercial cultivars in a pot experiment irrigated with saline water at three levels of salinity (4, 7, or 10 dS·m⁻¹) and found that salt tolerance is cultivar dependent, based on vegetative growth and tissue Na and Cl concentrations. However, salt tolerance of many existing cultivars in the United States is unknown. Identifying salt-tolerant cultivars is of great importance in pomegranate production. The aim of this study was to determine the relative salt tolerance of 22 pomegranate cultivars and their morphologic and physiologic responses to saline water irrigation in greenhouse conditions.

Materials and Methods

Plant materials. On 12 Mar. 2014, hardwood cuttings (≈15 cm) in RL98 Ray Leach Cone-tainers (SC10 Super, Stuewe and Sons., Inc., Tangent, OR; diameter, 3.8 cm; depth, 21 cm; volume, 164 mL) were received from Marcelino’s Nursery (Tornillo, TX). On 5 May 2014, rooted cuttings were transplanted in 5-L treepots (CP512CH, Stuewe and Sons., Inc.; width, 12.7 cm; height, 30.5 cm) containing commercial substrate Metro-Mix 902 (50% to 60% composted bark, Canadian sphagnum peatmoss, vermiculite and coarse perlite, starter nutrient charge with gypsum and slow-release nitrogen, and dolomitic limestone; SunGro®, Agawam, MA). All plants were grown in a greenhouse in El Paso, TX (lat. 31°41’45”N, long. 106°16’54”W; elevation, 1139 m) for 3 months

and irrigated with a nutrient solution at an EC of 1.2 ± 0.1 dS·m⁻¹ (mean and sd). The nutrient solution was prepared by adding 15N-2.2P-12.5K (Peters 15-5-15 Ca-Mg Special; Scotts, Marysville, OH) to reverse osmosis water at a nitrogen concentration of 150 mg·L⁻¹.

Treatments. On 5 Aug. 2014, all plants were pruned to 30 cm tall. One week later (i.e., 11 Aug.), uniform plants were chosen and assigned to two groups, and treatment was initiated. One group of plants was irrigated weekly with a saline solution at an EC of 10.0 dS·m⁻¹ (actual EC is 9.9 ± 0.4 dS·m⁻¹) for 4 weeks and subsequently with a saline solution at EC 15.0 dS·m⁻¹ (actual EC is 14.9 ± 0.6 dS·m⁻¹) for 3 more weeks (salt treatment). This was because plants irrigated with saline solution did not show any damage. A greater salinity treatment was needed to distinguish the differences among the 22 cultivars. Another group of plants was watered with the aforementioned nutrient solution without additional salts (control). Saline solutions at an EC of 10 dS·m⁻¹ and 15 dS·m⁻¹ were prepared by adding 57.2 mM NaCl and 28.7 mM calcium chloride (CaCl₂), and 86.4 mM NaCl and 43.3 mM CaCl₂, respectively, to the nutrient solution. This mixture was used because NaCl is the common salt in reclaimed water (Niu and Cabrera, 2010), and CaCl₂ forestalls potential calcium deficiencies (Carter and Grieve, 2006). Both nutrient and saline solutions were prepared in 100-L tanks with EC confirmed using an EC meter (Model B173; Horiba, Ltd., Kyoto, Japan) before irrigation. Between treatment solutions, plants were irrigated with the nutrient solution whenever the substrate surface became dry. Irrigation frequency varied with environmental condition and treatment. For example, plants at a high salinity level used less water and needed irrigation less often compared with those plants in the control. At each irrigation, plants were irrigated with 1 L treatment solution/plant, resulting in a leaching fraction of $\approx 29 \pm 11\%$.

Greenhouse environmental conditions. The average air temperature in the greenhouse was 30.9 ± 5.2 °C during the day and 23.0 ± 4.3 °C at night during the entire experimental period. The average daily light

integral was 16.3 ± 3.2 mol·m⁻²·d⁻¹, and the average relative humidity was $41.4 \pm 17.2\%$.

Leachate EC. Leachate EC was determined using the pour-through method according to Cavins et al. (2008). In brief, a saucer was placed under the container that had drained for at least 30 min right after treatment solution was applied. A total of 100 mL distilled water was poured on the surface of the substrate to get leachate in the saucer. The leachate solution was collected and tested using an EC meter. One plant per treatment per cultivar was chosen for measurement each time after treatment solutions were applied. Leachate EC readings were averaged across cultivars.

Growth parameters. At the end of the experiment, plant height (measured in centimeters) was recorded from the pot rim to the top growing point. New growth of shoots (visibly distinguishable from the old growth before pruning) were harvested, and the length of all new shoots (>5 cm) was measured as shoot length. Then, all leaves of the new shoots were separated from the stems. Both leaves and stems were oven-dried at 70 °C for 7 d, and the leaf and stem DW was determined.

Foliar salt damage evaluation. One week before harvest, foliar salt damage was rated visually using a reference scale from 0 to 5, where 0 = dead, 1 = more than 90% foliar damage (leaf burn, necrosis, or discoloration), 2 = moderate (50% to 90%) foliar damage, 3 = slight (less than 50%) foliar damage, 4 = good quality with minimal foliar damage, and 5 = excellent with no foliar damage (Sun et al., 2015b). The foliar salt damage visual rating did not account for plant size.

Chlorophyll fluorescence and performance index. Maximal photochemical efficiency (F_v/F_m) and performance index (PI) were measured according to Strasser et al. (2000, 2004) using a Hansatech Pocket PEA chlorophyll fluorimeter (Hansatech Instruments Ltd., Norfolk, UK) to examine the effect of elevated salinity on leaf photosynthetic apparatus of pomegranate plants 1 week before harvest. Healthy and fully expanded leaves of three plants per treatment per cultivar were chosen for the measurements. Measurements were taken on sunny days between 0900 and 1600 HR, and plants were well watered to avoid drought stress. The leaves were dark-acclimated for at least 30 min before F_v/F_m and PI measurements were acquired. Minimal fluorescence values in the dark-adapted state (F_0) were obtained by application of a low-intensity red light emitting diode light source (627 nm) at 50 μs, whereas maximal fluorescence values (F_m) were measured after applying a saturating light pulse of 3500 μmol·m⁻²·s⁻¹. The parameter F_j is fluorescence intensity at the j th step at 2 ms; V_j is relative variable fluorescence at 2 ms calculated as $V_j = (F_j - F_0)/(F_m - F_0)$. M_0 represents the initial slope of fluorescence kinetics, which can be derived from the equation $M_0 = 4 \times (F_{300\mu s} - F_0)/(F_m - F_0)$. Maximum quantum use efficiency (F_v/F_m) of photosystem II (PS II) in the dark-adapted

state was calculated as $F_v/F_m = (F_m - F_0)/F_m$. PI was calculated as follows (Strasser et al., 2000; Živčák et al., 2008):

$$PI = \frac{1 - (F_0/F_m)}{M_0/V_j} \times \frac{F_m - F_0}{F_0} \times \frac{1 - V_j}{V_j}.$$

Gas exchange. Leaf net photosynthesis (P_n), stomatal conductance (g_s), and transpiration (E) of three plants per treatment per cultivar were measured 1 week before harvest using a CIRAS-2 portable photosynthesis system (PP Systems, Amesbury, MA) with an automatic universal PLC6 broadleaf cuvette. A fully expanded leaf at the top of the plant was chosen for measurement. The environmental conditions within the cuvette were maintained at a leaf temperature of 25 °C, a photosynthetic photon flux of 1000 μmol·m⁻²·s⁻¹, and a carbon dioxide concentration of 375 μmol·mol⁻¹. Data were recorded when the environmental conditions and gas exchange parameters in the cuvette became stable. These measurements were taken on sunny days between 0900 and 1600 HR, and plants were well watered to avoid water stress.

Mineral analysis. Four pomegranate plants per cultivar per treatment were selected randomly to analyze leaf Na, Cl, calcium (Ca), and potassium (K) concentrations. All leaves of each plant were dried and ground with a stainless Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 40-mesh screen. Powder samples were extracted with 2% acetic acid (Fisher Scientific, Fair Lawn, NJ) to determine Cl levels using the method described in Gavlak et al. (1994). The concentration of Cl was determined with an M926 Chloride Analyzer (Cole Parmer Instrument Company, Vernon Hills, IL). Powder samples were submitted to the Soil, Water and Forage Testing Laboratory at Texas A&M University (College Station, TX) to determine Na, Ca, and K concentrations. In brief, powder samples were digested in nitric acid following the protocol described by Havlin and Soltanpour (1989). Na, Ca, and K in digested samples were analyzed by inductively coupled plasma-optical emission spectrometry (SPECTRO Analytical Instruments Inc., Mahwah, NJ) and reported on a dry plant basis as described by Isaac and Johnson (1975).

Experimental design and statistical analysis. A split-plot design with salinity treatment as the main plot and 22 cultivars as the subplot was used. As a result of plant material availability, four, five, or seven plants (replications) per treatment per cultivar were grown. Analysis of variance was used to test the effects of soil salinity and cultivar on plant growth. Means separation between treatments was conducted using Student's *t* test.

Because of the large number of cultivars, measurements took 2 weeks to complete. To minimize differences caused by different days, measurements started by rep number across the cultivars and treatments. Relative shoot DW was calculated for each plant in salt treatment as: Relative shoot DW (%) = (Shoot DW in salt treatment/Shoot DW in

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control) $\times 100$. Similarly, relative values for height, shoot length, leaf DW, and stem DW were calculated. These relative values and visual scores were used as salt tolerance indices for hierarchical cluster analysis (Zeng et al., 2002). The dendrogram of the 22 pomegranate cultivars is based on the Ward linkage method and squared Euclidian distance on the means of the salt tolerance indices for six multivariate parameters, including visual scores and all relative growth data. All statistical analyses were performed using JMP (version 12; SAS Institute Inc., Cary, NC).

Results

Leachate EC. The average leachate EC for the control (nutrient solution at EC of 1.2 $\text{dS}\cdot\text{m}^{-1}$) ranged from 2.8 to 3.9 $\text{dS}\cdot\text{m}^{-1}$ during the entire experimental period (Fig. 1). For salt treatment, the leachate EC increased from 10.5 to 23.4 $\text{dS}\cdot\text{m}^{-1}$ (for EC values >20 $\text{dS}\cdot\text{m}^{-1}$, samples were diluted before the final measurement). The data indicated that more salts accumulated in the root zone of pomegranate plants irrigated with saline solution compared with nutrient solution.

Foliar salt damage and growth parameters. Regardless of cultivar, all pomegranate plants had no foliar salt damage (leaf burn, necrosis, or discoloration), with a visual score of 5, during the entire experimental period (i.e., 66 d; Tables 1 and 2). Salt treatment affected plant height, shoot length, leaf DW, stem DW, and shoot DW of all pomegranate cultivars, but no interactions between salinity and cultivar were observed (Table 1). This indicates that all pomegranate cultivars responded similarly to the saline solution applied in this study. Salt treatment did not inhibit the plant height of all pomegranate cultivars except 'Mollar', 'Purple Heart', and 'Russian 8' (Table 2). Of all tested cultivars, the average reduction in plant height was 6%, with 'Mollar' having the greatest reduction of 14%. Salt treatment reduced the shoot length of 'Arturo Ivey', 'Al-Sirin-Nar', 'DeAnda', 'Early Wonderful', 'Kandahar', 'Purple Heart', 'Russian 8', 'Surh-Anor', and 'Utah Sweet' pomegranate.

Fig. 1. Leachate electrical conductivity (EC) measured using the PourThru technique during the experimental period. Control represents a nutrient solution at an EC of 1.2 $\text{dS}\cdot\text{m}^{-1}$, whereas Salt represents a saline solution at an EC of 10.0 $\text{dS}\cdot\text{m}^{-1}$ for the first 4 weeks and 15.0 $\text{dS}\cdot\text{m}^{-1}$ for the latter 3 weeks. Vertical bars represent SDs of 22 samples (cultivars) per treatment.

'Early Wonderful' pomegranate had the greatest reduction of 46%, whereas 'ML' had the least reduction of 10%. The average reduction of shoot length of all cultivars was 25%.

Salt treatment decreased the leaf, stem, and shoot DW of 'Al-Sirin-Nar', 'Angel Red', 'Apseronski', 'DeAnda', 'Early Wonderful', 'Kandahar', 'Kazake', 'Purple Heart', 'Russian 8', 'Salavatski', 'Surh-Anor', and 'Utah Sweet' (Table 3). Salt treatment also reduced the leaf DW of 'Carolina Venum', and stem and shoot DW of 'Chiva'. Although no significant differences were observed for the remaining cultivars, salt treatment slightly decreased their leaf, stem, and shoot DW. The reductions of leaf, stem, and shoot DW on average for all cultivars were 32%, 32%, and 32%, respectively, with large variations among cultivars. The greatest reduction in leaf DW, stem DW, and shoot DW was 52% for 'Al-Sirin-Nar', 49% for 'Kunduzski', and 48% for 'Al-Sirin-Nar', respectively. The least reduction in leaf DW, stem DW, and shoot DW was 19% for 'Kunduzski', 21% for 'Mollar', and 25% for 'ML', respectively.

A dendrogram was developed using the means of the salt tolerance indices for six multivariate parameters including visual scores and relative height, shoot length, leaf DW, stem DW, and shoot DW of all pomegranate cultivars (Fig. 2). Two major clusters were identified. The cluster of 'Arturo Ivey', 'DeAnda', 'Kazake', 'Russian 8', 'Apseronski', 'Purple Heart', 'Carolina Venum', 'Chiva', 'Kunduzski', 'Larry Ceballos 1', 'ML', 'Salavatski', 'Spanish Sweet', and 'Wonderful' was more salt tolerant than the other cluster of 'Al-Sirin-Nar', 'Kandahar', 'Surh-Anor', 'Early Wonderful', 'Angel Red', 'Ben Ivey', 'Utah Sweet', and 'Mollar'.

Chlorophyll fluorescence, PI, and gas exchange. Salt treatment affected F_v/F_m , PI, P_n , g_s , and E (Table 1). All parameters except F_v/F_m were significant among cultivars, and no interactions occurred between salt treatment and cultivar. Salt treatment reduced the F_v/F_m values of 'Al-Sirin-Nar' and 'Kunduzski' only (Table 4). The averaged F_v/F_m values for all pomegranate cultivars were 0.80 and 0.78 for the control and salt treatment, respectively. Salt treatment also reduced the PI value of 'Al-Sirin-Nar'. The mean PI values for all pomegranate cultivars were 3.31 and 2.46 for the control and salt treatment, respectively, with a 26% reduction.

The P_n , g_s , and E of all pomegranate cultivars irrigated with saline solution were similar to those with nutrient solution with the exception of 'Apseronski' (Table 5). On average, the P_n , g_s , and E of all pomegranate cultivars were 11.2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 237.2 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and 3.8 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for plants irrigated with nutrient solution, respectively; and 9.2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 158.0 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and 2.9 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for plants irrigated with saline solution, respectively.

Mineral analysis. Salt treatment increased leaf Na concentration significantly by 3.2, 3.3, 5.1, 2.8, 18.8, 6.3, 8, 0.97, and 8.2 times for 'Al-Sirin-Nar', 'Angel Red', 'Kazake',

'Kunduzski', 'Russian 8', 'Salavatski', 'Surh-Anor', 'Utah Sweet', and 'Wonderful', respectively, compared with the control (Table 6). No significant difference in leaf Na concentration of the remaining 13 pomegranate cultivars was observed between control and salt treatment. The averaged leaf Na content of all tested pomegranate cultivars was 0.07 and 0.28 $\text{mg}\cdot\text{g}^{-1}$ DW for plants in the control and salt treatment, respectively. 'Angel Red' pomegranate in salt treatment had the greatest Na concentration of 0.71 $\text{mg}\cdot\text{g}^{-1}$ DW.

Salt treatment also increased leaf Cl concentration of 'Al-Sirin-Nar', 'Apseronski', 'Carolina Venum', 'Kazake', 'Kunduzski', 'Mollar', and 'Russian 8' pomegranate by 51%, 33%, 16%, 32%, 35%, 42%, and 37%, respectively, compared with their respective controls (Table 6). The averaged leaf Cl concentration of all pomegranate cultivars was 8.56 and 10.03 $\text{mg}\cdot\text{g}^{-1}$ DW for plants in the control and salt treatment, respectively. 'Angel Red' pomegranate in salt treatment showed the greatest Cl content of 12.04 $\text{mg}\cdot\text{g}^{-1}$ DW.

Saline solution prepared with NaCl and CaCl_2 increased the leaf Ca concentration of 'Purple Heart', 'Russian 8', 'Salavatski', and 'Surh-Anor' pomegranate by 34%, 64%, 51%, and 36%, respectively (Table 6). However, leaf Ca concentration of 'Arturo Ivey', 'Al-Sirin-Nar', 'Angel Red', 'Apseronski', 'Ben Ivey', 'Chiva', 'Carolina Venum', and 'Early Wonderful' pomegranate was less in salt treatment than in the control. No significant difference in the leaf Ca concentration between control and salt treatment was observed for the remaining 10 pomegranate cultivars. The averaged leaf Ca concentration of all pomegranate cultivars was 4.76 and 4.49 $\text{mg}\cdot\text{g}^{-1}$ DW for plants in the control and salt treatment, respectively.

Leaf K concentration decreased significantly with increasing EC in 'Carolina Venum', 'Kazake', and 'Kunduzski' pomegranate (Table 6). Although leaf K content of 'Arturo Ivey', 'Al-Sirin-Nar', 'Apseronski', 'Ben Ivey', 'Chiva', 'DeAnda', 'Early Wonderful', 'Kandahar', 'Larry Ceballos 1', and 'ML' tended to decrease, no significant difference between control and salt treatment was observed. However, leaf K content of 'Purple Heart', 'Russian 8', 'Surh-Anor', 'Utah Sweet', and 'Wonderful' tended to increase, but no significant difference between control and salt treatment occurred.

Discussion

Salt accumulation in the substrate. To quantify the salinity levels throughout the experiment, we used the PourThru method (Cavins et al., 2008) to check the EC of the leachate solution, which is an indication of salt accumulation. More salts accumulated in the root zone of pomegranate plants irrigated with saline solution compared with those with nutrient solution (Fig. 1). El-Khawaga et al. (2013) also observed that saline

Table 1. A summary of analysis of variance for the effects of salt treatment (Trt), cultivar (Cv), and their interactions on visual score, height, shoot length, leaf dry weight, stem dry weight, shoot dry weight, chlorophyll fluorescence (F_v/F_m), performance index (PI), net photosynthesis (P_n), stomatal conductance (g_s), and transpiration (E) of 22 pomegranate cultivars that were grown and irrigated with nutrient solution or saline solution in the greenhouse.

Source	Analysis of variance										
	Visual score	Ht	Shoot length	Dry wt			F_v/F_m	PI	P_n	g_s	E
				Leaf	Stem	Shoot					
Trt	NS	*	***	***	***	***	*	***	***	***	***
Cv	NS	***	***	***	***	***	NS	**	***	**	***
Trt × Cv	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, *, **, ***Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

Table 2. Visual score, height, and shoot length of 22 pomegranate cultivars irrigated with nutrient solution (Control) or saline solution (Salt). Reduction (%) in height and shoot length was calculated as a percentage of the control.

Cultivar	Visual score		Ht (cm)			Length of new shoots (cm)		
	Control	Salt	Control	Salt	%	Control	Salt	%
Arturo Ivey	5	5	72.4	71.8	0.9	384.0 a	247.3 b	35.6
Al-Sirin-Nar	5	5	95.7	85.4	10.7	383.7 a	238.3 b	37.9
Angel Red	5	5	76.3	71.0	6.9	308.0	266.3	13.5
Apseronski	5	5	93.4	91.1	2.4	289.1	226.7	21.6
Ben Ivey	5	5	88.1	79.9	9.4	449.6	336.3	25.2
Chiva	5	5	89.4	86.0	3.8	473.2	410.4	13.3
Carolina Venum	5	5	84.6	82.6	2.4	393.0	294.4	25.1
DeAnda	5	5	77.6	76.6	1.3	398.4 a	269.3 b	32.4
Early Wonderful	5	5	76.4	72.2	5.5	438.0 a	237.4 b	45.8
Kandahar	5	5	79.0	74.1	6.1	454.9 a	297.4 b	34.6
Kazake	5	5	81.4	79.0	2.9	401.0	296.8	26.0
Kunduzski	5	5	87.0	85.6	1.6	492.0	413.2	16.0
Larry Ceballos 1	5	5	83.4	83.2	0.2	355.2	311.4	12.3
ML	5	5	92.6	92.0	0.6	423.8	380.2	10.3
Mollar	5	5	84.7 a ^z	72.9 b	14.0	584.7	426.7	27.0
Purple Heart	5	5	82.0 a	78.6 b	4.1	400.6 a	296.8 b	25.9
Russian 8	5	5	92.0 a	82.7 b	10.1	466.8 a	292.6 b	37.3
Salavatski	5	5	85.0	80.4	5.4	408.7	334.8	18.1
Spanish Sweet	5	5	88.3	79.0	10.5	364.0	316.5	13.1
Surh-Anor	5	5	92.4	84.0	9.1	351.0 a	244.7 b	30.3
Utah Sweet	5	5	82.8	75.6	8.7	414.4 a	329.2 b	20.5
Wonderful	5	5	91.4	84.6	7.4	386.2	321.2	16.8
Mean (CV)	5 (0%)	5 (0%)	85.3 (7.5%)	80.4 (7.5%)	5.7% (69.7%)	410.1 (15.5%)	308.5 (18.9%)	24.5% (40.4%)

^zMeans with different lowercase letters within a row for the same variable are significantly different between treatments by Student's *t* test at $P < 0.05$.

Table 3. Leaf, stem, and shoot dry weight (DW) of 22 pomegranate cultivars irrigated with nutrient solution (Control) or saline solution (Salt). Reduction (%) in leaf, stem, and shoot DW was calculated as a percentage of the control.

Cultivar	Leaf DW (g)			Stem DW (g)			Shoot DW (g)		
	Control	Salt	%	Control	Salt	%	Control	Salt	%
Arturo Ivey	17.6	11.0	37.5	11.6	9.0	22.0	29.2	20.1	31.4
Al-Sirin-Nar	23.6 a	11.4 b	51.9	16.1 a	9.4 b	41.4	39.7 a	20.8 b	47.6
Angel Red	18.3 a	12.1 b	33.6	13.5 a	8.0 b	40.8	31.8 a	20.1 b	36.7
Apseronski	20.8 a	13.1 b	36.7	12.7 a	8.7 b	31.2	33.5 a	21.9 b	34.7
Ben Ivey	26.3	18.7	29.2	19.0	12.0	36.8	45.3	30.7	32.3
Chiva	27.6	21.1	23.6	22.9 a	14.8 b	35.4	50.6 a	35.9 b	29.0
Carolina Venum	21.9 a ^z	13.1 b	40.1	18.0	12.1	32.6	39.9	25.2	36.7
DeAnda	20.2 a	14.8 b	26.8	14.6 a	10.2 b	30.0	34.8 a	25.0 b	28.1
Early Wonderful	20.3 a	11.0 b	45.8	15.1 a	9.7 b	35.9	35.4 a	20.7 b	41.6
Kandahar	12.4 a	6.9 b	44.5	14.9 a	9.2 b	38.1	27.2 a	16.1 b	40.9
Kazake	21.4 a	16.4 b	23.4	16.8 a	12.2 b	27.6	38.2 a	28.6 b	25.2
Kunduzski	20.1	16.4	18.5	21.9	11.3	48.5	42.0	30.6	27.0
Larry Ceballos 1	23.7	18.3	22.6	16.0	11.4	28.6	39.7	29.7	25.1
ML	23.4	17.8	24.1	18.9	14.1	25.2	42.3	31.9	24.5
Mollar	24.5	14.2	41.9	14.1	11.2	20.5	38.6	25.5	34.0
Purple Heart	21.5 a	13.8 b	35.7	14.2 a	10.1 b	28.6	35.7 a	23.9 b	32.9
Russian 8	21.1 a	15.4 b	26.8	17.8 a	12.8 b	28.1	38.9 a	28.2 b	27.4
Salavatski	18.0 a	14.0 b	22.3	16.3 a	11.8 b	27.6	34.3 a	25.8 b	24.8
Spanish Sweet	21.0	15.7	25.7	11.4	8.1	29.4	32.4	23.7	26.9
Surh-Anor	17.6 a	10.2 b	42.1	13.5 a	7.8 b	42.0	31.1 a	18.0 b	42.2
Utah Sweet	20.7 a	13.7 b	33.7	14.4 a	9.2 b	36.4	35.1 a	22.9 b	34.7
Wonderful	23.8	17.6	26.2	18.0	12.8	28.7	41.8	30.4	27.1
Mean (CV)	21.2 (15.6%)	14.4 (22.8%)	32.4% (28.5%)	16.0 (18.8%)	10.7 (18.5%)	32.5% (21.4%)	37.2 (14.7%)	25.3 (20.0%)	32.3% (20.3%)

^zMeans with different lowercase letters within a row for the same variable are significantly different between treatments by Student's *t* test at $P < 0.05$.

groundwater irrigation at an EC of 1.8 dS·m⁻¹ and 6.0 dS·m⁻¹ raised the salt accumulation in the root zone at a soil depth of 60 to 90 cm

from 3.7 dS·m⁻¹ to 4.8 dS·m⁻¹ and 7.7 dS·m⁻¹, respectively, when pomegranate plants were grown in sandy clay loam soil. In addition,

salts accumulated less rapidly in this experiment compared with those reported previously (Sun et al., 2015b; Wu et al., 2016),

which might result from different substrates used. Metro-Mix 902 with 50% to 60% composted bark was used in this experiment, whereas Metro-Mix 360 with 45% to 55% Canadian sphagnum peatmoss was used in others. Metro-Mix 902 may retain fewer salts and hold less water compared with Metro-Mix 360 because composted bark has a lesser cation exchange capacity and container capacity than peatmoss (Altland et al., 2014; Gabriel et al., 2009). This substrate could be suitable for long-term pomegranate production to prevent salt accumulation.

Salinity effect on growth. Salinity can inhibit plant growth and cause deleterious effects on plant foliage such as leaf burn, necrosis, or discoloration (Munns, 2002; Wahome et al., 2001). Previous studies have

shown that elevated salinity levels decrease leaf and shoot biomass in a variety of plant species (Cai et al., 2014; Niu and Rodriguez, 2006; Niu et al., 2013; Sun et al., 2013, 2015a, 2015b). Salt treatment decreased leaf, stem, and shoot DW significantly in all cultivars, with large variations among cultivars. However, all pomegranate cultivars had no foliar salt damage. In addition, shoot length reduced by 25% on average. These results are in line with previous work that consistently reported that increasing salinity level will inhibit pomegranate growth in terms of shoot length, leaf area, shoot biomass, or yield. Seven-year-old ‘Manfalouty’, ‘Wonderful’, and ‘Nab-Elgamal’ pomegranate grown in sandy clay loam soil and under environmental conditions in upper Egypt had greater reductions in growth, flowering, and yield, with greater fruit cracking, when they were irrigated with saline groundwater at an EC of 6.0 dS·m⁻¹ than at an EC of 1.8 dS·m⁻¹ (El-Khawaga et al., 2013). Naeini et al. (2006) reported that ‘Malas Torsh’ and ‘Alak Torsh’ pomegranate had reduced stem length, internode length and number, and leaf surface when irrigated with saline water spiked with 40, 80, or 120 Mm NaCl. Net productivity and crop yield of pomegranate would be expected to reduce as growth reduction occurred as a result of saline water irrigation.

Salinity effect on photosynthetic apparatus. Salinity also impairs the plant photosynthetic apparatus (PS II) (Taiz and Zeiger, 2015). Salt treatment affected F_v/F_m , PI, P_n , g_s , and E, and all pomegranate cultivars showed similar responses to salt treatment. Salt treatment decreased the F_v/F_m , PI, P_n , E, and g_s of pomegranate cultivars by 2%, 25%, 18%, 34%, and 23%, respectively. This result indicated that salt treatment affected the photosynthetic appa-

atus of pomegranate. Khayyat et al. (2016) reported that the photosynthetic efficiency of ‘Malas-e-Saveh’ and ‘Shishe-Kab’ pomegranates was reduced under salinity stress. Hasanpour et al. (2015) also observed that salinity treatment decreased the chlorophyll index and chlorophyll fluorescence.

Salinity effect on mineral contents. Plants can adapt to salt stress through excluding or tolerating Na or Cl accumulation in their shoots (Munns and Tester, 2008). A total of 77% of pomegranate cultivars tested in our experiment increased or tended to increase the Na in leaf tissue when irrigated with saline solution; however, Na concentration of all 22 cultivars was less than 1 mg·g⁻¹. This result is similar to previous work done on pomegranate plants by Karimi and Hasanpour (2014, 2017), Khayyat et al. (2014, 2016), Naeini et al. (2004, 2006) and Okhovatian-Ardakani et al. (2010), who all observed an increase in Na in plant tissue with increasing NaCl concentration in irrigation water. This result indicates that pomegranate plants have greater ability to minimize tdoie transport of Na into the shoots to avoid foliar salt damage (Karimi and Hassanpour, 2014, 2017). Leaf Na content in pomegranate is similar to that in rose rootstocks (*Rosa ×hybrida* ‘Dr. Huey’, *R. ×fortuniana*, *R. multiflora*, and *R. odorata*) that experienced foliar salt damage (Niu and Rodriguez, 2008). But, leaf Na content in pomegranate is less than in other woody plants, such as *Sophora secundiflora* (Niu and Rodriguez, 2010) and *Jatropha curcas* (Niu et al., 2012).

On average, leaf Cl content of all pomegranate cultivars in salt treatment was 10.03 mg·g⁻¹ DW, or a 17% increase compared with that of the control. Previous researchers have documented that mineral concentration of Cl in plant tissue increased with increasing

Fig. 2. The dendrogram of cluster analysis of 22 pomegranate cultivars based on the Ward linkage using squared Euclidian distance on means of multivariate parameters including visual scores and relative height, shoot length, leaf dry weight, stem dry weight, and shoot dry weight.

Table 4. Leaf chlorophyll fluorescence (F_v/F_m), and performance index (PI) of 22 pomegranate cultivars irrigated with nutrient solution (Control) or saline solution (Salt). Reduction (%) in F_v/F_m and PI was calculated as a percentage of the control.

Cultivars	F_v/F_m			PI		
	Control	Salt	%	Control	Salt	%
Arturo Ivey	0.80	0.80	0.0	3.65	3.26	10.8
Al-Sirin-Nar	0.80 a ²	0.78 b	3.5	3.22 a	1.10 b	66.0
Angel Red	0.79	0.79	0.4	3.39	2.82	16.8
Apseronski	0.78	0.77	0.9	1.73	1.21	30.1
Ben Ivey	0.80	0.79	1.7	4.14	3.26	21.1
Chiva	0.80	0.78	2.5	3.62	3.05	15.8
Carolina Venum	0.80	0.78	2.1	4.37	2.98	31.9
DeAnda	0.80	0.79	2.1	4.00	2.18	45.5
Early wonderful	0.79	0.78	0.8	2.46	2.21	10.2
Kandahar	0.79	0.79	0.8	3.21	2.16	32.6
Kazake	0.81	0.81	1.6	3.50	3.44	1.8
Kunduzski	0.81 a	0.77 b	4.9	2.79	1.70	38.9
Larry Ceballos 1	0.80	0.80	0.4	3.89	2.46	36.9
ML	0.80	0.79	1.3	2.95	2.37	19.8
Mollar	0.78	0.78	0.0	2.77	2.46	11.1
Purple Heart	0.80	0.80	0.4	3.80	3.26	14.1
Russian 8	0.80	0.76	4.6	3.64	2.06	43.5
Salavatski	0.79	0.79	0.4	2.39	1.91	20.2
Spanish Sweet	0.80	0.78	2.5	3.10	1.63	47.3
Surh-Anor	0.79	0.78	1.7	2.66	2.24	15.8
Utah Sweet	0.80	0.79	2.1	3.91	2.86	26.9
Wonderful	0.81	0.79	1.7	3.60	3.70	2.7
Mean (CV)	0.80 (1.1%)	0.78 (1.2%)	1.7 (81.9%)	3.31 (19.8%)	2.46 (28.7%)	25.4 (63.5%)

²Means with different lowercase letters within a row for the same variable are significantly different between treatments by Student's *t* test at *P* < 0.05.

Table 5. Leaf net photosynthesis (P_n), stomatal conductance (g_s), and transpiration (E) of 22 pomegranate cultivars irrigated with nutrient solution (Control) or saline solution (Salt). Reduction (%) in P_n , g_s , and E was calculated as a percentage of the control.

Cultivars	P_n ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)			g_s ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)			E ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)		
	Control	Salt	%	Control	Salt	%	Control	Salt	%
Arturo Ivy	12.2	8.9	26.9	248.3	123.5	50.3	4.0	2.6	36.0
Al-Sirin-Nar	8.5	8.5	0.7	149.4	138.8	7.1	2.8	2.7	2.5
Angel Red	13.0	11.2	13.8	332.7	213.3	35.9	4.8	3.7	22.3
Apseronski	10.1 a ^z	7.1 b	29.8	213.8 a	112.0 b	47.6	3.6 a	2.3 b	37.0
Ben Ivy	11.2	8.2	26.7	203.3	127.0	37.5	3.6	2.6	26.2
Chiva	12.3	9.5	22.4	319.5	146.7	54.1	4.5	2.9	35.5
Carolina Venum	11.5	9.8	15.0	284.7	159.0	44.1	4.1	3.2	21.9
DeAnda	12.5	10.3	17.6	272.0	171.3	37.0	4.4	3.2	26.4
Early wonderful	12.1	10.0	17.9	319.0	146.3	54.1	4.6	2.9	37.0
Kandahar	10.2	9.8	3.9	210.0	138.0	34.3	3.5	2.9	17.0
Kazake	8.5	6.2	27.9	154.0	92.0	40.3	2.9	2.1	26.9
Kunduzski	11.7	11.5	1.1	247.3	217.0	12.2	3.9	3.7	6.7
Larry Ceballos 1	12.2	11.7	3.7	233.3	231.0	1.0	3.9	3.9	1.0
ML	11.7	11.2	3.7	266.7	251.2	5.8	3.9	3.8	1.9
Mollar	14.9	12.2	18.0	343.8	258.5	24.8	4.7	4.0	16.6
Purple Heart	12.5	9.8	21.7	250.4	176.3	29.6	4.2	3.1	26.6
Russian 8	8.1	5.4	33.7	139.6	66.7	52.2	2.7	1.6	41.5
Salavatski	11.4	9.1	20.1	217.2	153.3	29.4	3.8	2.9	25.0
Spanish Sweet	10.0	6.7	33.0	185.4	91.5	50.6	3.2	2.1	34.8
Surh-Anor	13.8	9.6	30.5	328.7	189.0	42.5	4.8	3.3	31.0
Utah Sweet	8.6	6.3	27.0	163.3	96.0	41.2	3.1	2.1	33.0
Wonderful	9.6	9.2	4.4	163.7	150.3	8.2	3.1	2.9	9.2
Mean (CV)	11.2 (16.0%)	9.2 (20.8%)	18.1 (60.6%)	238.4 (27.1%)	156.8 (33.7%)	33.6 (50.4%)	3.8 (17.3%)	2.9 (22.1%)	23.4 (53.4%)

^zMeans with different lowercase letters within a row for the same variable are significantly different between the treatments by Student's *t* test at $P < 0.05$.

Table 6. Leaf sodium (Na), calcium (Ca), chloride (Cl), and potassium (K) concentrations of pomegranate cultivars irrigated with nutrient solution (Control) or saline solution (Salt).

Cultivar	Mineral concn (mg/g DW)							
	Na		Cl		Ca		K	
	Control	Salt	Control	Salt	Control	Salt	Control	Salt
Arturo Ivey	0.12	0.11	10.07	11.39	5.14 a	3.16 b	21.35	20.48
Al-Sirin-Nar	0.13 b ^z	0.52 a	7.74 b	11.69 a	5.83 a	3.18 b	17.86	16.24
Angel Red	0.17 b	0.71 a	9.33	12.04	4.54 a	3.60 b	21.55	22.06
Apseronski	0.10	0.25	8.02 b	10.67 a	5.08 a	3.72 b	18.51	16.82
Ben Ivey	0.08	0.19	8.12	8.79	4.26 a	3.04 b	20.67	19.09
Chiva	0.07	0.07	9.31	9.30	5.70 a	4.03 b	21.51	20.62
Carolina Venum	0.04	0.05	8.95 b	10.34 a	4.75 a	2.31 b	22.12 a	18.61 b
DeAnda	0.07	0.06	9.32	9.27	4.63	4.08	22.41	21.07
Early Wonderful	0.07	0.06	10.72	9.75	4.26 a	2.73 b	22.46	20.92
Kandahar	0.02	0.39	8.47	10.21	4.78	3.73	21.03	17.94
Kazake	0.11 b	0.65 a	7.03 b	9.29 a	5.38	3.88	18.32 a	14.57 b
Kunduzski	0.12 b	0.45 a	7.70 b	10.37 a	4.34	5.35	18.39 a	16.03 b
Larry Ceballos 1	0.07	0.13	7.93	9.57	4.26	4.23	20.44	19.95
ML	0.02	0.10	9.88	9.93	4.42	3.13	23.43	21.20
Mollar	0.01	0.07	7.63 b	10.80 a	4.87	4.93	15.94	16.36
Purple Heart	0.05	0.34	7.86	9.40	4.06 b	5.42 a	19.82	21.59
Russian 8	0.02 b	0.45 a	7.34 b	10.03 a	4.37 b	7.19 a	17.52	18.33
Salavatski	0.05 b	0.35 a	7.74	8.38	4.46 b	6.71 a	17.30	17.37
Spanish Sweet	0.15	0.61	9.31	10.36	4.74	6.60	17.21	17.69
Surh-Anor	0.04 b	0.34 a	7.63	9.51	4.55 b	6.18 a	16.88	18.13
Utah Sweet	0.08 b	0.15 a	9.54	9.50	5.55	4.94	22.46	24.01
Wonderful	0.02 b	0.21 a	8.75	10.12	4.78	6.73	20.74	22.47
Mean (CV)	0.07 (61.6%)	0.28 (73.8%)	8.56 (11.7%)	10.03 (9.0%)	4.76 (10.4%)	4.49 (32.4%)	19.90 (11.1%)	19.16 (12.8%)

^zMeans with different lowercase letters within a row for the same variable are significantly different between treatments by Student's *t* test at $P < 0.05$.

salinity (Karimi and Hassanpour, 2014, 2017; Khayyat et al., 2014, 2016; Naeini et al., 2004, 2006; Okhovatian-Ardakani et al., 2010). The Cl content in pomegranate leaves was also less than in other woody plants, such as rose rootstocks (*Rosa ×hybrida* 'Dr. Huey', *R. ×fortuniana*, *R. multiflora*, and *R. odorata*) at an EC of 8.2 dS·m⁻¹ (Niu and Rodriguez, 2008), *Sophora secundiflora* at an EC of 6.0 dS·m⁻¹ (Niu and Rodriguez, 2010), and *Jatropha curcas* at an EC of 3.0 dS·m⁻¹ or greater (Niu et al., 2012). These results indicate that pomegranate plants are capable of restricting either

the uptake or transport of Cl (Karimi and Hassanpour, 2014, 2017).

Salinity dominated by Na salt reduces Ca availability, transport, and mobility to growing regions of the plant, which subsequently affects the quality of both vegetative and reproductive organs (Grattan and Grieve, 1999). In our study, 64% of pomegranate cultivars in salt treatment had a significant or a slight decrease in Ca concentration, which agreed with the results of Khayyat et al. (2016). Salinity dominated by Na salts also reduces K acquisition (Grattan and Grieve, 1999;

Hasegawa et al., 2000). Thirteen of 22 pomegranate cultivars in salt treatment had a significant or slight reduction in leaf K content. This is probably a strategy for plants to reduce salt stress, as K plays an important role in adjusting the osmotic potential of plant cells, as well as activating enzymes related to respiration and photosynthesis (Taiz and Zeiger, 2015). In our study, we observed that 41% of pomegranate cultivars tended to increase leaf K content, which agreed with the results of Karimi and Hassanpour (2014) and Naeini et al. (2004).

Conclusions

Pomegranate plants are very tolerant to a saline water up to an EC of 15.0 dS·m⁻¹ with little foliar salt damage and slight growth reduction. Like previous reports, pomegranate plants are capable of restricting either the uptake or transport of Na and Cl to leaves to reduce salt damage. Pomegranate plants can be grown in hot, arid and semiarid regions, and irrigated with saline groundwater with high salinity. Future research to quantify the effect of salinity on fruit yield and quality is needed.

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