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The Efficacy of Grafting on Alkali Stressed Watermelon Cultivars Under Hydroponic Conditions

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Abstract

The goal of the present study was to determine whether grafting of watermelon on gourd rootstocks could improve alkalinity tolerance and to investigate the physiological and morphological response mechanisms of the grafted plants under different pH levels. The experiment was carried out in a climate chamber to investigate growth, leaf chlorophyll content (SPAD), leaf area, stem length, shoot and root dry weight, root length, electrolyte leakage, leaf mineral composition, total chlorophyll and carotenoid contents. Two watermelon cultivars (Crimson tide, CT, and Crisby) were grafted onto three commercial *Cucurbita maxima* × *C. moschata* hybrid rootstocks under climate-chamber conditions (Strong tosa, ST, Ercole and Nun 9075). The grafted seedlings were transplanted onto 8 L continuously aerated pots containing nutrient solution with two different pH levels (8.5 and 6.5) and replicated three times. The results showed that in both grafted and non-grafted plants, there was a substantial reduction in shoot and root biomass production at high pH levels. At high pH level, the significantly highest leaf area, stem length, SPAD, concentration of P, Ca, S and Mn in leaf tissues were recorded in graft combination 'Crisby/Ercole', whereas the significantly highest concentration of Fe in leaf tissues, shoot dry weight were recorded in graft combination 'Crisby/Nun 9075'. Moreover, at high pH, the significantly highest concentration of Mg and Cu in shoot under high pH levels was significantly found in graft combination of 'CT/ST'. These results suggest that the use of interspecific *Cucurbita maxima* × *C. moschata* hybrid rootstocks can improve crop performance in watermelon plants under alkaline conditions.

Keywords Alkalinity · Gourd genotypes · Grafting · Nutrient solution · pH tolerance · Watermelon

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Die Wirksamkeit der Pfropfung auf Wassermelonensorten unter Alkalistress und hydroponischen Bedingungen

Zusammenfassung

Das Ziel der vorliegenden Studie war es zu bestimmen, ob das Pfropfen von Wassermelonen auf Kürbiswurzelstöcke die Alkalitoleranz verbessern kann und die physiologischen und morphologischen Reaktionsmechanismen der gepfropften Pflanzen bei verschiedenen pH-Werten zu untersuchen. Das Experiment wurde in einer Klimakammer durchgeführt, um Wachstum, Blattchlorophyllgehalt (SPAD), Blattfläche, Stammlänge, Spross- und Wurzeltrockengewicht, Wurzellänge, Elektrolytverlust, Blattmineralzusammensetzung, Gesamtchlorophyll- und -carotinoidgehalt zu untersuchen. Zwei Wassermelonensorten (Crimson tide, CT, und Crisby) wurden unter Klimakammerbedingungen (Strong tosa, ST, Ercole und Nun 9075) auf drei kommerzielle *Cucurbita maxima* × *C. moschata*-Hybridwurzelstöcke gepfropft. Die gepfropften Sämlinge wurden in kontinuierlich belüftete 8-Liter-Töpfe gepflanzt, die Nährlösung mit zwei verschiedenen pH-Werten (8,5 und 6,5) enthielten; der Versuch wurde dreimal repliziert. Die Ergebnisse zeigten, dass sowohl bei den gepfropften als auch bei den nicht gepfropften Pflanzen eine erhebliche Verringerung der Spross- und Wurzelbiomasseproduktion bei hohen pH-Werten auftrat. Bei hohem pH-Wert wurden die signifikant höchsten Werte bzgl. Blattfläche, Stammlänge, SPAD, Konzentration von P, Ca, S und Mn im Blattgewebe bei der Pfropfkombination ‚Crisby/Ercole' aufgezeichnet, während die signifikant höchste Fe-Konzentration im Blattgewebe und das höchste Sprosstrockengewicht bei der Kombination ‚Crisby/Nun 9075' registriert wurden. Außerdem wurde bei hohem pH-Wert die signifikant höchste Konzentration von Mg und Cu im Spross bei der Pfropfkombination ‚CT/ST' gefunden. Diese Ergebnisse legen nahe, dass die Verwendung von interspezifischen *Cucurbita maxima* × *C. moschata*-Hybridwurzelstöcken die Ertragsfähigkeit von Wassermelonenpflanzen unter alkalischen Bedingungen verbessern kann.

Schlüsselwörter Alkalinität · Kürbisgenotypen · Pfropfung · Hydroponische Lösung · pH-Toleranz · Wassermelone

Introduction

Salt affected soils have been categorized into three different types such as saline, saline-alkali, and alkaline soils depending on total concentration (electrical conductivity) of soluble salts they possess, pH of the soil solution, and exchangeable sodium percentage of the soil (Rasool et al. 2012). Alkalization and salinization of soils have become global environmental problems and are important factors limiting agricultural productivity. There are 831 million hectares of soil in the world that are affected by salt stress. Of this area, alkalinized soils underlie 434 million hectares, while saline soils underlie 397 million hectares (Wang et al. 2012). Some essential and nonessential plant nutrients are highly dependent on the level of soil pH on their solubility and possible availability or phytotoxicity. Some plants prefer acidic soils and are not bothered by nutrient deficits, whereas others tolerate pH levels above 7 and are adapted to micronutrient deficiencies (Kulak 2015). The "ideal" pH of the soil is similar to neutral, and it is considered that neutral soils fall into a range from a slightly acid pH of 6.5 to a slightly alkaline pH of 7.5. Most plant nutrients are optimally available to plants within this pH range of 6.5 to 7.0, and this pH range is generally very consistent with plant root growth (Brady and Weil 2000). The increase in soil pH associated with high HCO_3- and CO_3^{2}-concentrations adversely affects seed germination, plant growth and productivity by reducing nutrient solubility and availability (Roosta 2011; Wang et al. 2011; Lin et al. 2012; Zhang et al. 2012). In particular, bicarbonate ions interfere with the absorption of macro elements P, K and Mg (Pissaloux et al. 1995). Typically, due to the formation of metal complexes (e.g., Ca-P and Mg-P), P is largely unavailable to plants, as it is rendered sparingly soluble. In addition, HCO_3-concentration strongly interacts with the availability of many micro ions, resulting in significant yield and efficiency losses (Colla et al. 2010). Bicarbonate has been shown in many plant species to be a direct or indirect cause of iron (Fe)-deficiency chlorosis (Bertoni et al. 1992).

Watermelon is one of the most widely cultivated crops in Turkey. In terms of global production Turkey is the second largest watermelon producer after China in the world with a production of around 4.0 million tons per year (FAO 2017). Watermelon cultivation has been carried out intensively for many years in the watermelon production areas of Turkey. One of the production areas in the southern Turkey is the Çukurova region where the watermelon cultivation is performed mostly under low tunnels for early production (Yetisir and Sari 2003). There are several biotic and abiotic problems in watermelon cultivation. Some of these problems are the decrease in fruit yield and quality caused by soil diseases, mainly Fusarium wilt and continuous cropping (Miguel et al. 2004). There are different ways to prevent the attacks of soil-borne pathogens to plants: crop rotation, genetic improvement, and soil fumigation (Alan et al. 2007); however, there are limitations to each of these techniques. The prospect of breeding new disease resistant varieties takes a long time and is very costly (Passam 2003). Breeding for resistance against some pathogens has been futile because the pathogen may quickly overcome resistance. Grafting onto resistant rootstocks may be alternative solution to these problems (Lee 1994). Grafting limits contribution of agrochemicals against soilborne pathogens and is, thus, regarded as an environment friendly cultivation method, which is highly suggested for integrated crop management systems (Jabnoun-Khiareddine et al. 2019). The use of grafted vegetables (tomatoes, bean, eggplant, cucumber, melon, and watermelon) is a common technique in many parts of the world such as Japan, Korea, the Mediterranean basin, and several European countries. Reports differ on whether grafting effects are beneficial or deleterious, but most accept that for optimum fruit quality, the rootstock/scion combination must be carefully selected. A hard and fibrous flesh is shown by watermelon grafted on *Cucurbita* spp (Ceylan et al. 2018). Alan et al. (2007) reported no adverse effects in fruit quality and soluble solid content on grafted watermelon plants. Miguel et al. (2004) found no variations compared to controls in the soluble solids concentration of watermelon fruit from scions grafted to a *C. maxima* × *C. moshata* hybrid rootstock. Yetisir and Sari (2003) reported that quality factors such as Brix, firmness, thickness of rind, and watermelon fruit shape were significantly affected by grafting. However, the force of grafting on vegetables consists of not only a stronger resistance against pathogens and optimal fruit quality but also a higher tolerance to abiotic stress conditions such as salinity, heavy metal, nutrient stress, thermal stress, water stress, organic pollutants, and alkalinity (Colla et al. 2010, 2011; Savvas et al. 2010; Schwarz et al. 2010; Roosta and Karimi 2012). Roosta and Karimi (2012) reported that using *C. moschata* rootstock can reduce harmful effects of alkalinity stress on cucumber plants. Aydın (2018) suggested that the use of interspesific Cucurbita hybrid rootstocks can improve crop performance in melon plants under alkaline conditions. Most of the information about the effect of alkalinity in plants has been gained in field crops, but there is slight research regarding to adequate or threshold levels in vegetable greenhouse plants in hydroponic systems. The small number of published reports in considers to the effect of HCO_3– in vegetable greenhouse crops stresses the requirement of more research on these crops.

Therefore, the aim of the present study was to examine whether grafting with different *Cucurbita* rootstocks might improve the alkalinity tolerance of watermelon scions and to determine the physiological, biochemical, and nutritional responses induced by the rootstocks under different pH levels.

Materials and Methods

Plant Material, Treatments, and Experimental Design

A hydroponic experiment was conducted during in 2015–2016 growing season by using a non-flow nutrient film technique (NFT) in a controlled growth chamber for four weeks. For the vegetation period, the average day/night temperatures were 25/22 °C, the relative humidity was 60–80% and a photoperiod of 16 h of light. Two watermelon cultivars (Crimson Tide and Crisby) were used as scion varieties and 3 different Lagenaria and Cucurbita genotypes (landraces and commercial rootstocks (Strong tosa, Nun 9075 and Ercole)) were used as rootstock materials. The seeds were sown in multipots in a mixture of peat (pH: 6.0–6.5) and perlite in a 2:1 ratio and then the appropriate seedlings were selected for the grafting process using the procedure of "cleft grafting" described by Lee (1994), while non-grafted Crimson Tide and Crisby were used as control plants. Plants were left to heal and acclimatized for one week in a wide container protected by double-layered plastic film and shade cloth in the climate chamber after grafting (Lee et al. 2010). To improve healing, the container was closed for the first three or four days of the healing and acclimatization period to prevent grafted plants from wilting due to excessive transpiration. The opening and closing of the container were done for the next three or four days, depending on the conditions of the grafted plants and the growth space. This was required for the acclimatization of grafted plants outside the container to environmental conditions. After the healing and acclimatization, grafted plants were transferred to 8 L plastic containers after roots were washed from growth media, each pot was filled with nutrient solution and aerated by an air pump. The experiment was conducted with two different pH levels (8.5 and 6.5). 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.

Plant Growth Measurements

After four weeks of growing, plants were harvested and separated into shoot and roots. Stem length (cm) was measured using a meter rule. Leaf, stem and roots were weighed to obtain the fresh biomasses. In order to determine shoot and root dry weight, plant materials were dried in a forced-air oven for 48 h at 70 °C. Total leaf area of a plant was measured by a leaf area meter (LI-COR Model 3100, LI-COR. Inc., Lincoln, NE, USA). The root length of the plants was measured by using the special software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Canada). It was recorded as cm plant^{-1}, and then converted to m plant^{-1}.

Chlorophyll Meter Measurements

The Minolta SPAD-502 chlorophyll meter was used to take SPAD readings. During the growth period, fully expanded leaves of whole plants for each treatment were twice measured for SPAD data.

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

A day before harvesting, 100 mg (0.1 g) of fresh leaf samples were taken from each replication for measuring leaf total chlorophyll and carotenoid contents using UV-VIS Spectroscopy. 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 for the extraction of the leaf pigments. Measurements were carried out with spectrometer (UV/VS T80+ from PG Instruments Limited, UK) at wavelengths of 470 nm, 648.6 nm and 664.2 nm. From the spectrometric readings, total chlorophyll (Total-Chlo) and total carotenoids (TC) were then calculated using Lichtenthaler (1987) formulae.

$$\begin{aligned}
&\text{Total-Chlo (mg/gplant sample)}\\
&= [(5.24\,\text{WL}664.2 - 22.24\,\text{WL}648.6 \times 8.1]\\
&\quad /\text{weight of plant sample (g)}\\
&\text{TC(mg/gplant sample)}\\
&= [(4.785\,\text{WL}470 + 3.657\,\text{WL}664.2) - 12.76\,\text{WL}648.6)\\
&\quad \times 8.1]/\text{weight of plant sample (g)}
\end{aligned}$$

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

Electrolyte Leakage Determination

Electrolyte leakage (EL) was determined as described by Lutts et al. (1995). EL measurements were performed every 48 h between 11:00 and 15:00 h on the youngest completely expanded leaves using three replicates per treatment. Using a cork borer, 1 cm leaf discs were taken from fully expanded leaves. Leaf samples were washed 3 times with distilled water to eliminate surface contamination, and then put in individual stopper vials containing 10 mL of distilled water. The samples were incubated on a shaker (at 100 rpm) for 24 h at room temperature (25 °C). After incubation, the electrical conductivity of the solution (EC1) was measured. The same samples were then autoclaved for 20 min at 120 °C and allowed to cooled to room temperature and then a second EC (EC2) measurement was done. The EL was expressed as:

$$\text{EL} = (\text{EC1})/(\text{EC2}) \times 100.$$

Mineral Analysis Measurements

0.5 g of dried plant sample (leaf) was analyzed for K, Ca, and Na. Their concentrations were determined ashing at 400 °C for 4 h; dissolving the ash in 5 ml of HCl with 20% concentration was added to the ashed samples and filtered. The filtered solution was then diluted to a volume of 50 ml with distilled water and 10 ml taken for Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) analysis. ICP-AES results were converted into parts per million (ppm) and percentages (%) where necessary. Chloride was analyzed by precipitation as AgCl and titration according to Johnson and Ulrich (1959).

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 graft combination or grafting combination and pH 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

Rootstock Impact On Shoot and Root Growth

The results of the shoot dry matter, root dry matter and total root length at the end of the growing cycle of graft combinations and watermelon cultivars in different pH levels (6.5 and 8.5) were shown in Table 1. Shoot dry matter was significantly ($p < 0.001$) affected by scion, rootstock, pH levels, scion × rootstock ($p < 0.05$), scion × pH levels ($p < 0.05$), rootstock × pH levels interactions. The shoot biomass at the end of the growing cycle of the grafted

Table 1 The effects of graft combination and different pH levels (pH 6.5 and pH 8.5) on shoot dry matter, root dry matter and total root length of watermelon plants

Graft combination	Shoot dry matter (g plant^{-1})			Root dry matter (g plant^{-1})			Total root length (m plant^{-1})		
(S/R)	pH 6.5	pH 8.5	% R	pH 6.5	pH 8.5	% R	pH 6.5	pH 8.5	% R
Crisby	5.02 f	6.52 de	–29.9	0.56 def	0.78 a	–39.8	81.2 g	126.7 d	–56.0
Crisby/Nun9075	8.77 a	7.63 bc	12.93	0.75 ab	0.57 def	23.56	155.5 a	105.6 e	32.07
Crisby/Ercole	8.13 ab	7.23 cd	11.07	0.62 de	0.63 cde	–1.62	138.4 c	121.5 d	12.15
Crisby/ST	8.23 ab	5.30 f	35.63	0.55 bcd	0.51 f	8.43	144.2 bc	88.2 fg	38.85
CT	3.24 g	2.27 E	30.04	0.31 g	0.32 g	–3.19	41.7 i	21.6 j	48.26
CT/Nun9075	7.90 bc	6.38 e	19.20	0.52 f	0.56 def	–7.74	135.9 c	96.2 f	29.28
CT/Ercole	7.80 bc	6.65 de	14.74	0.63 cd	0.70 bc	–10.5	142.2 c	151.3 ab	–6.36
CT/ST	7.75 bc	5.47 f	29.46	0.53 f	0.35 g	34.38	87.3 fg	67.9 h	22.21
F-Test									
Scion	***			***			***		
Rootstock	***			***			***		
pH	***			n. s.			***		
Scion × R. Stock	*			**			***		
Sion × pH	*			n. s.			n. s.		
R. Stock × pH	***			***			***		

S Scion, *R. Stock* Rootstock, *CT* Crimson tide, *ST* Strong tosa, *% R* Reduction, *ns* non-significant
Values denoted by different letters are significantly different between graft combination within both columns at $P < 0.05$
* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

and non-grafted plants differed in response to solution pH. Under control conditions, all graft combinations produced significantly higher shoot biomass than non-grafted control plants. Compared to the unstressed plants, high pH level increased the shoot dry matter of the Crisby and Crisby/ST plants by –29.90 and 35.63%, respectively. Under alkaline conditions, the graft combination of 'Crisby/Nun9075' has significantly higher shoot biomass. The significantly lower shoot dry matter was observed by non-grafted control plants of 'CT' under alkaline conditions (Table 1). Regarding root dry matter, grafted and non-grafted watermelon plants altered differentially the pH of their root environments. Root dry matter was significantly ($p < 0.001$) affected by scion, rootstock, scion × rootstock ($p < 0.01$), rootstock × pH levels interactions; however, it was not significantly affected by different pH levels and scion × pH levels interactions. The watermelon cultivar of 'Crisby' has a vigorous root system as compared to the other watermelon cultivars and rootstocks. However, accumulation of root biomass was lower with the watermelon cultivar of 'CT' under high and neutral pH levels. The graft combination of 'Crisby/Nun9075' (0.75 g) produced a significantly greater root mass than other graft combinations under neutral pH levels. While lowest performance in root growth under high and neutral pH levels was shown by watermelon cultivar of 'CT'. At high pH level, the 'Crisby' (0.78 g) was ranked highest for root dry weight, while the combinations of 'CT/ST' and the watermelon cultivar of 'CT' ranked lowest. The reduction in the root dry biomass was ranked between –39.88 and 34.38%. The minimum reduction was observed at the non-grafted control plant of 'Crisby' (–39.88%), while the maximum reduction was observed in 'CT/ST' (34.38%) (Table 1). Generally, root length decreased as the solution pH increased. Total root length was significantly ($p < 0.001$) affected by scion, rootstock, pH levels, scion × rootstock, rootstock × pH levels interactions; however, it was not significantly affected by scion × pH levels interactions. The root length was ranked between 41.67 m plant^{-1} and 155.48 m plant^{-1} under non-saline conditions. The graft combination of 'Crisby/Nun9075' produced significantly longer roots (155.48 m plant^{-1}) under control conditions. However, the watermelon cultivar of 'CT' has the significantly lowest root length under control (41.67 m plant^{-1}) and alkaline (21.56 m plant^{-1}) conditions. The root length was ranked between 21.56 m plant^{-1} and 151.26 m plant^{-1} under alkaline conditions. Under alkaline conditions, 'CT/Ercole' graft combination produced significantly longer roots (151.26 m plant^{-1}) (Table 1).

Rootstock Impact On Leaf Area, Leaf Chlorophyll Index (SPAD) and Stem Length

In general plant growth was negatively affected by increasing the pH level of the nutrient solutions. Leaf area, leaf chlorophyll index (SPAD) and stem length of the grafted and non-grafted watermelon plants varied in response to solution pH. The result of final leaf area formation, SPAD and stem length at the end of the growing period of graft-

Table 2 The effects of graft combination and different pH levels (pH 6.5 and pH 8.5) on leaf area, leaf chlorophyll content (SPAD) and stem length of watermelon plants

Graft combination	Leaf area (cm^2 plant^{-1})			Leaf chlorophyll content (SPAD)			Stem length (cm plant^{-1})		
(S/R)	pH 6.5	pH 8.5	% R	pH 6.5	pH 8.5	% R	pH 6.5	pH 8.5	% R
Crisby	976.7 hg	1170.0 d	–19.80	48.6 ab	47.8 ab	1.60	87.5 g	63.3 h	27.62
Crisby/Nun9075	1277.2 b	1010.5 g	20.88	48.7 ab	47.7 ab	2.03	145.8 b	136.2 cd	6.63
Crisby/Ercole	1260.3 bc	1140.3 de	9.52	49.6 a	48.3 ab	2.73	158.5 a	145.0 b	8.52
Crisby/ST	1197.5 bcd	916.7 h	23.45	49.9 a	47.5 ab	4.98	141.7 bc	106.8 f	24.59
CT	497.2 i	554.3 i	–11.50	43.0 d	43.4 cd	–0.96	65.0 h	41.7 i	35.90
CT/Nun9075	1109.7 def	1062.5 efg	4.25	45.8 bcd	46.5 abc	–1.48	126.2 e	106.3 f	15.72
CT/Ercole	1175.0 cd	1018.3 g	13.33	46.9 ab	48.0 ab	–2.34	138.3 bc	113.2 f	18.19
CT/ST	1374.8 a	1038.5 fg	24.46	49.2 ab	45.9 bcd	6.64	127.7 e	130.0 de	–1.83
F-Test									
Scion	***			***			***		
Rootstock	***			***			***		
pH	***			n. s.			***		
Scion × R. Stock	***			n. s.			***		
Sion × pH	n. s.			n. s.			n. s.		
R. Stock × pH	***			n. s.			n. s.		

S Scion; *R. Stock* Rootstock, *CT* Crimson tide, *ST* Strong tosa, *% R* Reduction, *ns* non-significant
Values denoted by different letters are significantly different between graft combination within both columns at $P < 0.05$
* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

ing combinations and watermelon cultivars in different pH levels (6.5 and 8.5) was shown in Table 2. The final leaf area was significantly ($p < 0.001$) affected by scion, rootstock, pH levels, scion × rootstock, rootstock × pH levels interactions; however, it was not significantly affected by scion × pH levels interactions. Under control conditions, ‘CT/ST’ graft combination (1174.8 cm^2 plant^{-1}) produced significantly higher leaf area formation, whereas non-grafted plants of ‘CT’ (497.2 cm^2 plant^{-1}) significantly produced the minimum leaf area formation. Conversely, under alkaline conditions the graft combination of ‘Crisby/Ercole’ and non-grafted control plants of ‘Crisby’ produced significantly higher leaf area formation. It was followed by ‘Crisby/Nun9075’, ‘CT/Nun9075’, ‘CT/Ercole’ and ‘CT/ST’. High pH causes a decline in leaf area between –19.8% and 24.46% depending on the rootstocks (Table 2). Regarding leaf chlorophyll content, it was significantly ($p < 0.001$) affected by scion, rootstock; however, it was not significantly affected by pH levels, scion × rootstock, scion × pH levels, rootstock × pH levels interactions. Under control conditions the graft combinations of ‘Crisby/Ercole’, and ‘Crisby/ST’ produced significantly higher leaf chlorophyll content. On the contrary, under alkaline conditions significantly higher leaf chlorophyll content was produced at the graft combination of ‘Crisby/Ercole’, while the non-grafted plants of ‘CT’ has significantly lower leaf chlorophyll content. The reduction in the leaf chlorophyll content was ranked between –2.34 and 6.64%. The minimum reduction was observed in the graft combination of ‘CT/Ercole’ (–2.34%), while the maximum reduction was observed in ‘CT/ST’ (6.64%) (Table 2).

The stem length was significantly ($p < 0.001$) affected by scion, rootstock, pH levels, scion × rootstock; however, it was not significantly affected by scion × pH levels and rootstock × pH levels interactions. In comparison to the plants grown under unstressed conditions, high pH significantly decreased the stem length of non-grafted and grafted plants. Although the highest values of stem length were recorded on the graft combination of ‘Crisby/Ercole’ in both stressed (145.0 cm plant^{-1}) and unstressed conditions (158.5 cm plant^{-1}). However, the non-grafted watermelon cultivar of ‘Crisby’ had the overall shortest stem length under both stressed and unstressed conditions. High pH causes a decline in stem length between –1.83% and 35.90% depending on the rootstocks. The stem length was ranked between 41.67 cm plant^{-1} (‘CT’) and 145.0 cm plant^{-1} (‘Crisby/Ercole’) under alkaline conditions, respectively (Table 2).

Rootstock Impact On Leaf Total Chlorophyll Content, Leaf Total Carotenoid Content, Electrolyte Leakage in Leaves and Roots

The leaf total chlorophyll content, leaf total carotenoid content, leaf electrolyte leakage and root ion leakage in graft combinations and watermelon plants as a function of pH

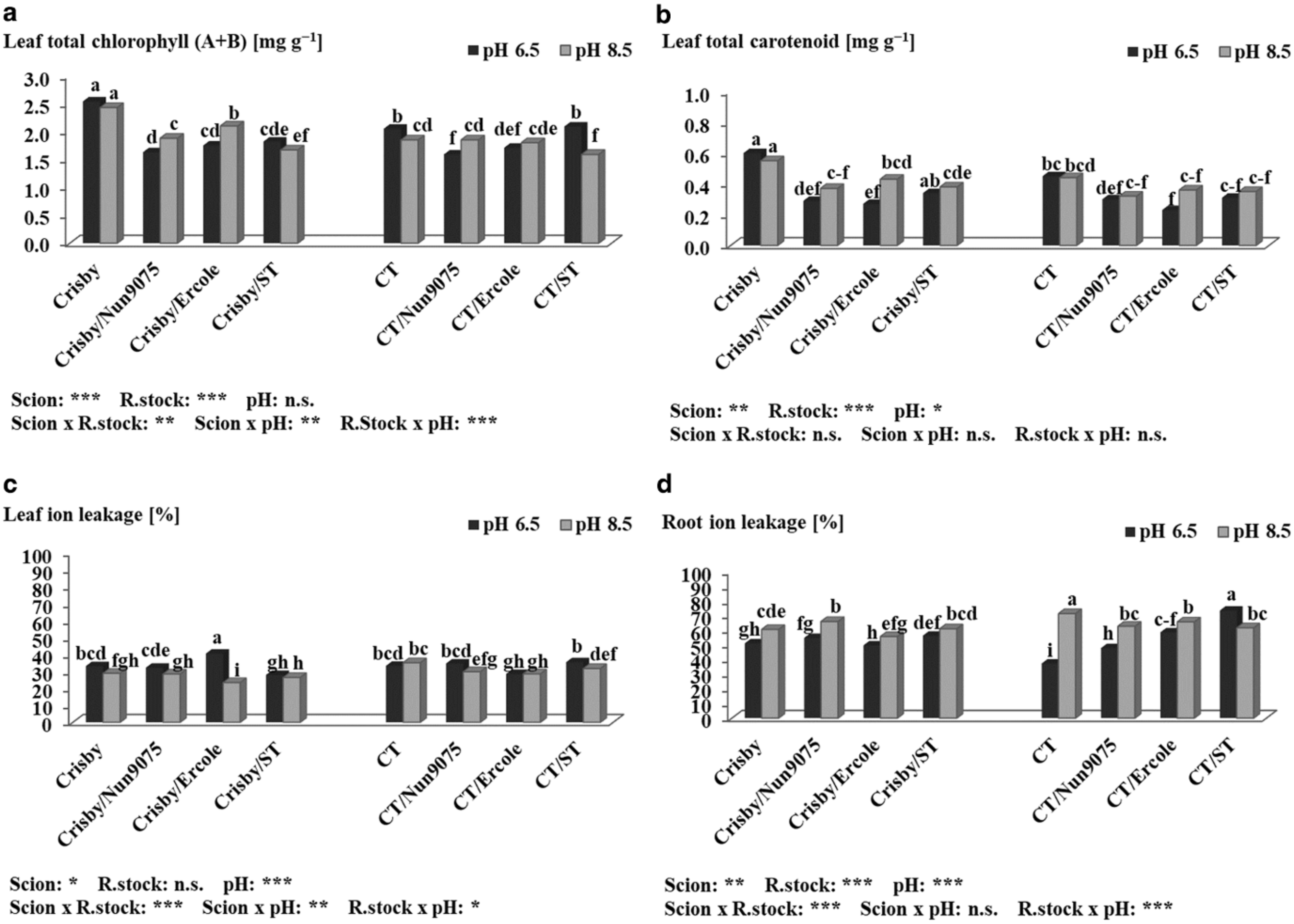

Fig. 1 The effects of graft combination and different pH levels (pH 6.5 and pH 8.5) on leaf total chlorophyll content (**a**), leaf total carotenoid content (**b**), leaf electrolyte leakage (**c**), root ion leakage (**d**) of watermelon plants. *CT* Crimson tide; *ST* Strong tosa, *% R* Reduction. 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 *** $P < 0.001$

level are displayed in Fig. 1. The leaf total chlorophyll content was significantly ($p < 0.001$) affected by scion, rootstock, scion × rootstock, scion × pH levels, rootstock × pH levels interactions; however, it was not significantly affected by different pH levels. The total chlorophyll content of the different graft combinations and watermelon cultivars differed in response to solution pH. The watermelon cultivar of ‘Crisby’ has the maximum total chlorophyll content under both neutral and high pH levels. However, under high pH level the graft combination of ‘CT/ST’ has the minimum total chlorophyll content. Regarding the total carotenoid content, it was significantly ($p < 0.001$) affected by scion, rootstock, pH levels; however, it was not significantly affected by scion × rootstock, scion × pH levels, rootstock × pH levels interactions. The watermelon cultivar of ‘Crisby’ has the maximum total carotenoid content under both neutral (0.60 mg g^{-1}) and high (0.55 mg g^{-1}) pH levels. Under high pH level, it was followed by ‘Crisby/Ercole’ and ‘CT’. The total carotenoid content of grafting combinations regarding to the different pH levels was enhanced in different ratios (8.56–58.64%) in six grafting combinations, whereas the total carotenoid content was decreased in different ratios (3.08–7.87%) in two grafting combinations (Fig. 1).

The leaf electrolyte leakage was significantly ($p < 0.001$) affected by scion, pH levels, scion × rootstock, scion × pH levels, rootstock × pH levels interactions; however, it was not significantly affected by rootstock. The leaf and root electrolyte leakage of the different grafting combinations and watermelon cultivars differed in response to solution pH. The leaf electrolyte leakage was ranked between 23.41% and 35.13% under high pH. The grafting combination of ‘Crisby/Ercole’ (40.53%) has the maximum leaf electrolyte leakage under neutral pH level, while under high pH level the watermelon cultivar of ‘CT’ (35.13%) has the maximum leaf electrolyte leakage. On the other hand, ‘CT/Ercole’ (28.48%) and ‘Crisby/ST’ (27.92%) have the minimum leaf electrolyte leakage under neutral pH level, while under high pH level the grafting combination of ‘Crisby/

Ercole' (23.41%) has the minimum leaf electrolyte leakage. Regarding root electrolyte leakage, generally, it was higher in the plants at high pH than plants at neutral pH. The root electrolyte leakage was significantly ($p < 0.001$) affected by scion, rootstock, pH levels, scion × rootstock, rootstock × pH levels interactions; however, it was not significantly affected by scion × pH levels interactions. The root electrolyte leakage was ranked between 55.59% and 71.28% under high pH. The grafting combination of 'CT/ST' (73.30%) has the maximum root electrolyte leakage under neutral pH level, though under high pH conditions the watermelon cultivar of 'CT' (71.28%) has the maximum root electrolyte leakage (Fig. 1).

Rootstock Impact On Mineral Composition and Partitioning

The leaf macro and micro element concentrations and distributions in grafting combinations and watermelon plants as a function of pH levels are shown in Tables 3 and 4, respectively. The results indicated that leaf P and Na concentrations were significantly ($p < 0.001$) affected by scion, rootstock, pH levels, scion × rootstock, scion × pH levels, rootstock × pH levels interactions. Leaf K concentrations was significantly ($p < 0.001$) affected by rootstock, pH levels, scion × rootstock, rootstock × pH levels interactions; however, it was not significantly affected by scion and scion × pH levels interactions. Leaf Ca concentrations was significantly ($p < 0.001$) affected by rootstock, scion × rootstock, rootstock × pH levels interactions; how-

Table 3 The effects of graft combination and different pH levels (pH 6.5 and pH 8.5) on leaf macronutrient composition of watermelon plants

		Leaf macro element content					
Graft combination (S/R)	pH	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Na (%)
Crisby	6.5	0.54 g	5.21 ef	3.10 ef	0.18 g	0.07 f	0.01 e
	8.5	0.45 i	5.81 bcd	2.86 fg	0.33 d	0.12 e	0.15 b
	Mean	0.50	5.51	2.98	0.25	0.09	0.08
Crisby/Nun9075	6.5	0.94 b	5.44 de	3.62 cde	0.23 f	0.20 c	0.01 e
	8.5	0.57 fg	6.02 abc	3.93 bcd	0.43 ab	0.15 d	0.07 d
	Mean	0.76	5.73	3.78	0.33	0.18	0.04
Crisby/Ercole	6.5	0.93 b	4.96 fg	3.14 ef	0.18 g	0.20 bc	0.01 e
	8.5	0.66 e	6.17 ab	4.22 bc	0.41 bc	0.25 a	0.12 c
	Mean	0.80	5.56	3.68	0.30	0.23	0.07
Crisby/ST	6.5	0.76 d	4.40 h	4.88 a	0.27 e	0.16 d	0.01 e
	8.5	0.47 hi	4.97 fg	3.87 bcd	0.39 c	0.22 bc	0.25 a
	Mean	0.61	4.69	4.38	0.33	0.19	0.13
CT	6.5	0.84 bc	4.57 gh	3.65 cde	0.20 fg	0.12 e	0.01 e
	8.5	0.29 j	5.00 fg	4.27 b	0.38 c	0.17 d	0.12 c
	Mean	0.56	4.78	3.96	0.29	0.14	0.07
CT/Nun9075	6.5	1.13 a	6.00 abc	5.37 a	0.31 d	0.23 ab	0.01 e
	8.5	0.63 ef	5.83 bcd	3.90 bcd	0.41 bc	0.21 bc	0.06 d
	Mean	0.88	5.92	4.64	0.36	0.22	0.03
CT/Ercole	6.5	0.89 bc	4.69 gh	2.33 g	0.18 g	0.17 d	0.01 e
	8.5	0.52 gh	5.75 bcd	2.51 g	0.33 d	0.16 d	0.02 e
	Mean	0.71	5.22	2.42	0.26	0.17	0.02
CT/ST	6.5	0.95 b	5.70 cd	4.22 bc	0.23 f	0.21 bc	0.01 e
	8.5	0.45 i	6.40 a	3.41 def	0.45 a	0.23 abc	0.14 bc
	Mean	0.70	6.05	3.81	0.34	0.22	0.07
F-Test	Scion	***	n. s.	n. s.	n. s.	**	***
	Rootstock	***	***	***	***	***	***
	pH	***	***	n. s.	***	**	***
	Scion × R. Stock	***	***	***	**	***	***
	Sion × pH	***	n. s.	n. s.	n. s.	n. s.	***
	R. Stock × pH	***	**	***	n. s.	***	***

S Scion, *R. Stock* Rootstock, *CT* Crimson tide, *ST* Strong tosa, % *R*: Reduction. *ns* non-significant
Values denoted by different letters are significantly different between graft combination within both columns at $P < 0.05$
* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

ever, it was not significantly affected by scion, pH levels, and scion × pH levels interactions. Leaf Mg concentrations was significantly ($p < 0.001$) affected by rootstock, pH levels, scion × rootstock; however, it was not significantly affected by scion, scion × pH levels, rootstock × pH levels interactions. Leaf S concentrations was significantly ($p < 0.001$) affected by scion, rootstock, pH levels, scion × rootstock, rootstock × pH levels; however, it was not significantly affected by scion × pH levels interactions. Increasing the pH level from 6.5 to 8.5 in the nutrient solution significantly decreased the P concentration in leaf; though, leaf K, Ca, Mg, S and Na concentrations increased significantly. The significantly highest values for the concentration of P, K, Ca, Mg, and S in leaf recorded under neutral pH level by the grafting combination of ‘CT/Nun9075’. Under high pH level, significantly highest values at the concentration of P in leaves were observed by the graft combination of ‘Crisby/Ercole’. Moreover, under high pH level, the leaf K concentration ranged from 4.97 to 6.40% in the non-grafted and grafted plants, respectively. The significantly highest values for the concentration of Ca in leaf recorded under high pH level by the grafting combination of ‘Crisby/Ercole’ and the non-grafted watermelon plants of ‘CT’. Averaged leaf Mg concentrations showed variation across watermelon plants from about 0.45 to 0.33% in grafted and non-grafted plants, respectively. The graft combination of ‘CT/ST’ produced significantly higher leaf Mg concentrations under high pH level. The concentration of S in leaves under high pH level was significantly affected in grafting combination of ‘Crisby/Ercole’. Increasing pH level in the nutrient solution significantly increased Na concentration in leaves. The significantly

Table 4 The effects of graft combination and different pH levels (pH 6.5 and pH 8.5) on leaf micronutrient composition of watermelon plants

	Leaf micro element content					
Graft combination (S/R)	pH	Cu (ppm)	Zn (ppm)	Fe (ppm)	B (ppm)	Mn (ppm)
Crisby	6.5	26.90 c	57.40 a	52.55 def	34.05 cd	27.05 cd
	8.5	25.53 c	42.60 ef	46.20 g	36.60 c	7.50 h
	Mean	26.22	50.00	49.38	35.33	17.28
Crisby/Nun9075	6.5	31.75 b	49.70 c	48.45 fg	31.78 de	40.70 b
	8.5	12.60 g	28.70 ih	79.65 a	45.50 b	18.50 ef
	Mean	22.18	39.20	64.05	38.64	29.60
Crisby/Ercole	6.5	32.30 b	43.70 de	54.43 de	29.65 e	31.40 c
	8.5	18.15 e	28.95 ih	65.53 c	50.25 a	27.25 cd
	Mean	25.23	36.33	59.98	39.95	29.33
Crisby/ST	6.5	33.90 b	44.25 de	34.80 h	20.15 g	36.15 b
	8.5	25.55 c	31.20 h	73.53 b	36.55 c	16.50 ef
	Mean	29.73	37.73	54.16	28.35	26.33
CT	6.5	26.40 c	46.49 d	47.90 fg	31.33 de	21.10 e
	8.5	22.97 d	40.20 f	56.38 d	45.70 b	9.80 gh
	Mean	24.68	43.35	52.14	38.51	15.45
CT/Nun9075	6.5	37.87 a	53.85 b	74.35 b	32.98 cde	57.65 a
	8.5	14.41 fg	34.60 g	68.95 c	33.30 cde	18.57 ef
	Mean	26.14	44.23	71.65	33.14	38.11
CT/Ercole	6.5	31.90 b	49.47 c	29.70 i	14.58 h	26.00 d
	8.5	15.13 f	26.15 ij	50.65 efg	32.92 cde	13.97 fg
	Mean	23.51	37.81	40.18	23.75	19.98
CT/ST	6.5	32.95 b	44.55 de	39.10 h	24.66 f	26.77 cd
	8.5	38.35 a	24.55 j	45.93 g	43.50 b	18.35 ef
	Mean	35.65	34.55	42.51	34.08	22.56
F-Test	Scion	*	n. s.	**	***	n. s.
	Rootstock	***	***	***	***	***
	pH	***	***	***	***	***
	Scion × R. Stock	**	***	***	***	***
	Sion × pH	n. s.	n. s.	***	n. s.	n. s.
	R. Stock × pH	***	***	***	***	***

S Scion, *R. Stock* Rootstock, *CT* Crimson tide, *ST* Strong tosa, *% R* Reduction, *ns* non-significant

Values denoted by different letters are significantly different between graft combination within both columns at $P < 0.05$

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

greatest Na concentration was found in 'Crisby/ST' while the lowest values in 'CT/Ercole' under high pH level (Table 3).

Leaf Cu and B concentrations were significantly ($p<0.001$) affected by scion, rootstock, pH levels, scion × rootstock, rootstock × pH levels interactions; however, they were not significantly affected by scion × pH levels interactions. Leaf Fe concentrations was significantly ($p<0.001$) affected by scion, rootstock, pH levels, scion × rootstock, scion × pH levels, rootstock × pH levels interactions. Leaf Zn and Mn concentrations were significantly ($p<0.001$) affected by rootstock, pH levels, scion × rootstock, rootstock × pH levels interactions; however, they were not significantly affected by scion and scion × pH levels interactions. The tissue concentrations of Cu, Zn, and Mn declined significantly as the pH level from 6.5 to 8.5 in the nutrient solution increased; though, leaf Fe and B concentrations increased significantly. The significantly highest values for the concentration of Cu, Fe, B and Mn in leaf recorded under neutral pH level by the grafting combination of 'CT/Nun9075', though significantly highest value for the concentration of Zn recorded under neutral pH level by non-grafted watermelon plants of 'Crisby'. The highest values for the concentration of Cu in leaf recorded under high pH level by the grafting combination of 'CT/ST'. The non-grafted watermelon plants of 'CT' and 'Crisby' produced significantly higher leaf Zn content under high pH level. Under high pH level, significantly highest values at the concentration of Fe in leaves were observed by the graft combination of 'Crisby/Nun9075'. Increasing pH level in the nutrient solution increased significantly the B concentration in leaves. Under high pH level, the leaf Mn concentration ranged from 7.5 to 27.25 ppm in the non-grafted and grafted plants, respectively. The significantly highest values for the concentration of Mn in leaf recorded under high pH level by the grafting combination of 'Crisby/Ercole' (Table 4).

Discussion

Researchers have concluded that plants counter to high pH conditions in soil or in nutrient solution with decreased shoot and root growth (Zribi and Gharsalli 2002). Shoot growth inhibition is associated with a reduction in the number of leaves, reduction in biomass productions and shoot elongation (Valdez Aguilar 2004). Similarly, in the present study, in comparison to the plants grown under stress free conditions, shoot and root growth of non-grafted plants and grafted plants decreased significantly under high pH (Table 1). In many studies, reduction in shoot growth under increased pH concentration in growing medium solution or in soil has been detailed on crop plants such as in tomato, petunia, pea, lettuce, rice and mulberry plants (Zribi and Gharsalli 2002; Roosta 2011; Wang et al. 2012; Ahmad et al. 2014). This might be due to either HCO_3^- or Na^+ (Pearce et al. 1999). Colla et al. (2010) stated that grafts were used as a tool to overcome the detrimental effects of alkaline stress. Similar results were observed on cucumber plants (Roosta and Karimi 2012) and melon plants (Aydın 2018).

Grafted and non-grafted watermelon plants altered differentially the pH of their root environments. The watermelon cultivar of 'Crisby' has a vigorous root system compared to the other watermelon cultivar and rootstocks (Table 1). Similarly, in the present study, root dry mass increased in non-grafted cucumber plants as compare to grafted plants as solution pH increased (Tachibana 1982). Similar results were also observed in melon plants under alkaline conditions. Non-grafted plants produced significantly higher root biomass as compare to grafted plants (Aydın 2018). Conversely, higher root dry mass in grafted watermelon plants than non-grafted plants was observed under alkaline conditions (Pulgar et al. 2000). Generally, root length decreased as the solution pH increased. Like the present study, reduction in root length/increase in root diameter in tomato, maize, sorghum and barley are observed under alkaline conditions (Kang et al. 2010). Inhibition of growth and development in a plant owing to alkali stress might be ensue caused by regulation of a multitude of physiological and biochemical processes such as water relations (Ahmad et al. 2012), ion transport and accumulation (Hasegawa et al. 2000), photosynthesis (Ashraf et al. 2010), accumulation of osmoprotectants (Koyro et al. 2012; Katare et al. 2012), synthesis of antioxidant enzymatic and nonenzymatic molecules (Patade et al. 2011; Bano et al. 2013; Kaya et al. 2013; Rasool et al. 2013), synthesis of plant growth regulators (Ashraf et al. 2010), mineral nutrient metabolism (Marschner 1995).

In this study, the growth and development of watermelon plants were negatively affected by increasing pH of the nutrient solution. Leaf area, leaf chlorophyll index (SPAD) and stem length of the grafted and non-grafted watermelon plants varied in response to solution pH (Table 2). Limitation of leaf area formation may be the result of the restricted net photosynthetic rates; as the concluding effect decreases the available assimilates for growth of the leaf. A leaf area reduction has been detailed for numerous greenhouse crops (mini-rose, chrysanthemum, hibiscus and vinca) irrigated with alkaline water (Valdez-Aguilar 2004). Likewise, in the current study, under alkaline conditions reduction in leaf area in tomato plants were observed (Kang et al. 2011). Aydın (2018) studied the effect of high solution pH on grafted and non-grafted melon plants, and he observed that grafted melon plants produced significantly higher leaf area, SPAD index and stem length as compare to non-grafted plants.

Similar results also observed by Roosta and Karimi (2012) at grafted cucumber plants.

The leaf and root electrolyte leakage significantly ($p < 0.001$) affected by graft combination, pH levels and graft combination × pH interaction. The leaf and root electrolyte leakage of the different grafting combinations and watermelon cultivars differed in response to solution pH. The grafting combination of 'Crisby/Ercole' (40.53%) has the maximum leaf electrolyte leakage under neutral pH level, while under high pH level the watermelon cultivar of 'CT' (35.13%) has the maximum leaf electrolyte leakage. Regarding root electrolyte leakage, generally, it was higher in the plants at high solution pH values than plants at neutral pH values. The grafting combination of 'CT/ST' (73.30%) has the maximum root electrolyte leakage under neutral pH level, though under high pH conditions the watermelon cultivar of 'CT' (71.28%) has the maximum root electrolyte leakage (Fig. 1). Similar results were observed by Kaya et al. (2003) on melon plants and Colla et al. (2010) on watermelon plants under alkaline conditions.

Bicarbonate ions could interfere with crucial nutrient uptake and translocation and hence disturbing nutrient composition of plants (Marschner 1995). In the present study, a significant reduction of P concentration in leaf tissue; though, leaf K, Ca, Mg, S and Na concentrations increased significantly (Table 3). Tremblay et al. (1989) stated that, in celery, increased HCO_3^- levels increased the concentration of P but did not affect K. Despite Tremblay et al. (1989) results, P, K, and Mg concentrations were found to have reduced significantly in leaf tissues of watermelon under high pH levels induced by HCO_3^- (Colla et al. 2010). In another study, P concentration remained unchanged in tomato seedlings treated with HCO_3^- (Bialczyk et al. 1994). Likewise, in the current study, Mg levels increase when HCO_3^- is high, as reported for peach rootstocks (De la Guardia and Alcántara 2002), sunflower (Liu et al. 2010), and white lupinus (Bertoni et al. 1992). Mg concentration remained unchanged in tobacco (Pearce et al. 1999) and decreased in olive (De la Guardia and Alcántara 2002) when HCO_3^- concentration was high. This suggests that different elements might limit growth at different pH values. Grafted and non-grafted plants responded differently to pH levels on the basis of the elemental composition of their tissues, as was observed for growth parameters in the present study. The tissue concentrations of Cu, Zn, and Mn declined significantly as the pH level from 6.5 to 8.5 in the nutrient solution increased; though, leaf Fe and B concentrations increased significantly (Table 4). Alkalinity affects plant growth via a reduction in solubility of nutrients, micronutrients especially, due to an increased pH of the solution because of increasing HCO_3^- concentrations (Zhang et al. 2012). Reduced uptake in Fe, Mn, Zn and Cu/Mg, Ca, K, and P in maize plants (Walter et al. 2000) is typically observed under high pH conditions. High alkalinity caused substantial decreases in the Zn, Mn, and Cu concentrations of grafted watermelon plants in accordance with the present findings (Colla et al. 2010).

Conclusions

Different watermelon cultivars grafted onto different gourd rootstocks and non-grafted ones behaved significantly ($p < 0.001$) different in growth, leaf area, leaf chlorophyll content (SPAD), stem length, shoot and root dry weight, root length, electrolyte leakage, leaf mineral composition, total chlorophyll and carotenoid contents. Under high pH levels, significant reduction of shoot and root biomass production was recorded in both grafted and non-grafted plants Among different grafting combinations, highly significant ($p < 0.001$) genotypic variation in shoot and root development was recorded under high pH levels. The significantly higher leaf area, stem length, SPAD, concentration of P, Ca, S and Mn in leaf tissues were recorded in graft combination 'Crisby/Ercole', whereas the significantly higher concentration of Fe in leaf tissues, shoot dry weight were recorded in 'Crisby/Nun 9075' graft combination under high pH levels. Moreover, at high pH, the significantly highest concentration of Mg and Cu in shoot under high pH levels was significantly found in graft combination of 'CT/ST'. The use of interspecific *Cucurbita maxima* × *C. moschata* hybrid rootstocks play a major role in improvement of crop growth performance in watermelon plants under alkaline conditions.

Conflict of interest F. Ulas, A. Aydın, A. Ulas and H. Yetisir declare that they have no competing interests.

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