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Research Article

The effect of salinity and different rootstock on fruit and physiological parameters in Grafted-Cucumber

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Abstract

Salinity is a vital factor in reducing cucumber yield and quality. Grafting on the resistant rootstock could keep growth near to optimum condition. The present research aimed to investigate the characteristics of different rootstocks on the physiology and fruit characteristics of cucumber. Treatments were rootstocks [‘Rn’: Nongrafted (*Cucumis sativus* v. DAVOSII), ‘Rt’: Tanbal (*Cucurbita maxima*), ‘Rg’: Ghalyani (*Lagenaria siceraria*), ‘Rk’: Karela (*Momordica charantia*), ‘Rkh’: Khoreshi (*Cucurbita pepo*)] and salinity concentration included 0 (S1), 30 (S2) and 60 (S3) mM with three replications. Most of the fruit characteristics and physiology parameters like fruit weight and firmness, fruit length/diameter, photosynthesis rate, transpiration decreased with salinity. When grafted plants were used, physiological parameters improved in ‘Rkh’, ‘Rk’ at salinity compare with ‘Rn’. Transpiration decreased with salinity in Rkh and Rg and did not change in ‘Rn’. The highest transpiration was seen in ‘Rn’ in all salinity levels. Fruit quality is mostly improved at ‘Rg’ in salinity. TSS increased in ‘Rg’ and ‘Rkh’ in S2. Firmness decreased with S3 in ‘Rg’ and Rkh significantly. Na absorption was highest in ‘Rn’ and conversely, K concentration was lowest in ‘Rn’. Na concentration did not affect salinity levels in other rootstock and was lower than ‘Rn’. On the other hands, K concentration increased in ‘Rk’ and ‘Rkh’ in all salinity levels. Generally, using rootstocks like ‘Rk’, ‘Rkh’, and ‘Rg’ improved fruit quality and physiological aspect of grafted cucumber in saline soil.

Keywords: ***Cucurbita maxima, Momorida charantia, Lagenaria siceraria, Cucumis sativus, Cucurbita pepo***

Introduction

Salinity is currently one of the most disturbing environmental factors in agriculture (Manchanda and Garg, 2008). According to the FAO (FAO, 2012), more than 50% of irrigated land in the arid and semi-arid area is saline. Cucumbers are a sensitive plant to salinity and are one of the common vegetable used in Iran and mostly have to produce in saline soil.

One promising method for decreasing the detrimental effect of some stress in cucurbits could be the use of grafting. Grafting improved resistance to salinity, heavy metal, nutrient stress, thermal stress, drought stress (Colla *et al*., 2006a, b, 2005; Schwarz *et al*., 2010). They reported that decreasing Na^+ and/or Cl^- concentration in shoots is the main reason for resisted grafted-cucumber (Huang *et al*., 2009a, b; Zhu *et al*., 2008) and melon (Ruiz *et al*., 1997), to salinity. When they were grafted to *C. maxima* for melon, and *C. moschata*, *C. ficifolia*, and *Lagenaria siceraria* to cucumber, respectively. The first deleterious effects of salinity on plant growth are the low osmotic potential of the soil solution causing water stress, nutritional imbalance (Shannon, 1998; Rouphael *et al*., 2018) and accumulation of ions Na^+ and Cl^- (Parida and Das, 2005). Accumulating of toxic ions in the leaf apoplast lead to cell dehydration and turgor loss and stomata closer (Sudhir and Murthy, 2004; Rouphael *et al*., 2016). Additionally, photosynthesis decreased with salinity due to the reduced chlorophyll pigment (Rady, 2011), inhibition of Rubisco (Kahrizi *et al*., 2012) closure of stomata (Bethkey and Drew, 1992). Rouphael *et al* (2012) revealed that there was limited information on the physiological response cucumber grafted onto *Cucurbita rootstocks* to salinity and less in new rootstock like Karela (*Momorida charantia*).

Cucumber (*Cucumis sativus* L. cv. Akito) plants grafted onto the *Cucurbita maxima* Duch.×*Cucurbita moschata* Duch., ‘P360’, under 40 mmol L^{-1} of NaCl. Salinity decreases leaf area index, photosynthesis, stomata resistant, and shoot and fruit weight lower in grafted compared to the non-grafted plants. They believed that first, grafting improved cucumber photosynthetic and consequently, crop performance; secondly, grafting reduced concentrations of sodium in leaves in stress and resulting in more growth (Rouphael *et al*., 2012).

Conclusively, grafting of commercial cultivars onto different rootstocks could be a promising tool against abiotic stresses. Concerning salt tolerance, many studies have been carried out to determine the response of grafted plants to salinity (Santa-Cruz *et al*., 2002). Recently grafting has been used to improve yield and

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quality in Iran. This research was aimed to investigate whether different or/and new cucurbits could be an excellent rootstock to have consistent growth and fruit quality and have the potential of resistant to saline conditions for cucumber.

Materials and methods

Grafting plant and treatment preparation: The experimental design was a factorial experiment based on RCBD. The different factors which were studied included rootstocks [‘Rn’: nongrafted (*Cucumis sativus* v. DAVIS II), ‘Rt’: Tanbal (*Cucurbita maxima*), ‘Rg’: Ghalyani (*Lagenaria siceraria*), ‘Rk’: Karela (*Momordica charantia*), ‘Rkh’: Khoreshi (*Cucurbita pepo*)]. Commercial cultivar *Cucumis sativus* v. DAVIS II used for scion. Salinity concentration included 0 (S1), 30 (S2) and 60 (S3) mM with three replications in the greenhouse of Isfahan University of Technology, Iran with Longitude 18 '7° 23 Nort h, Latitude 2 '53° 51 East.

Grafting: Rootstock seed were grown in cocopeat/perlite (50: 50 V: V) 2 weeks earlier than scion growth hole insert ion grafting with 5-day old ‘DAVOSII’ cultivar scions have been done. The growing point of the rootstocks removed before grafting. A hole is made with a drill at a slant angle to the longitudinal direction in the removed bud region. The hypocotyl port ion of the scion was prepared by slant cutting to a tapered end for easy insertion into rootstock hole.

Healing period: In this experiment, grafted seedlings were placed in 95% humidity, temperature 26-28 during the day and 19-21 during the night, and complete darkness for initial 10 days.

Acclimatization of the grafted plants: After healing time, plants were put under a clear plastic cover for five days for acclimatization to prevent leaf burning and wilting. Grafted plants transferred to 4 liter container. Salinity stress was induced by the addition of three different concentrations of NaCl solution to each respective treatment followed by proper irrigation.

Parameters were measured: Plants were harvested after 60 days. Fruit length and fruit diameter were measured with caliper (Mitutoyo Corp, Japan) Fruit shape presented by the ratio of length/diameter of the fruit. Plant height was measured by the meter. Fruit firmness was measured with Pentameter and TSS with a portable refractometer (PAL-1 Brix, Japan) (Raeisi *et al*., 2014). The fresh and dry weight of shoot was measured in an oven at 70° C overnight.

Chlorophyll content: Chlorophyll content was measured by using a chlorophyll meter (SPAD-502 plus, Japan) and Fv/Fm was measured by chlorophyll fluorescence (RS232, Handy PEA) after 3 weeks. Photosynthetic properties were determined from the youngest fully expanded leaf by a calibrated portable gas exchange system (LCi, ADC Bioscientific Ltd., UK) from 10:00 to 11:00 am with photosynthetically active radiation (PAR) intensity of 1000 μmol $m^{-2}s^{-1}$ and references CO_2 concentration of 350 μmol·mol. Mesophyll conductance (mmol CO_2 $m^{-2}s^{-1}$) was calculated as Ahmadi and Siosemardeh (2005) revealed by following formula dividing the photosynthetic rate by the sub-stomatal CO_2 concentration.

Electrolyte leakage (EL): Electrolyte leakage was measured using an electrical conductivity meter by using the method described by Lutts *et al.* (1996). Relative water content (RWC) was calculated according to the following expression (Filella *et al*., 2007). The concentration of K, Na, and P was measured (Shield Torch System, Agilent 7500a). The determination of total nitrogen in the leaf samples was based on the Kjeldahl method (AOAC, 2000).

Proline: Proline accumulation was estimated using the method as described by Bates *et al*. (1973).

Phenol: Phenolic compound measured by (Kahkonen *et al*., 1999) methods. Samples (200 mg) were extracted with 80% (v/v) aqueous methanol containing 1% (v/v) HCl, with shaking for 2 hrs. at room temperature. Extracts were centrifuged at 1000×g for 15 mins. and 1 ml of each supernatant were mixed with 5 ml folin-ciocalteu and 4 ml and aqueous Na_2CO_3. Results were expressed as ferulic acid equivalents. The phenols were determined by spectrophotometer at 765 nm as gallic acid equivalents per gram (mg GAE g^{-1} DW) (Kahkonen *et al.,* 1999). By using DPPH compound total antioxidant activity was also measured, briefly, a solution of DPPH radicals (100 μL, 0.065 mM) was mixed with 20 μL leaf extract or a standard solution in a 96 well plate. DPPH and leaf extracts were dissolved in hexane/ethanol (1:1, v/v). The reaction was conducted at room temperature for 30 mins., at which time the absorbance was stabilized. After 30 mins. the absorbance of the solution was recorded at 515 nm by the spectrophotometer (V-530, JASCO, Japan), the antiradical activity was calculated by the following equation (Yu *et al.,* 2002).

% DPPH radical scavenging activity $=1-[A_{sample}/A_{control}] \times 100$

where A sample and $A_{control}$ are absorbance of sample and control (Yu *et al*., 2002).

All data were subjected to two-way ANOVA by using Statistix 8 software (Tallahassee FL, USA) and the means were compared for significance by the least significant difference (LSD) test at $P < 0.05$.

Result

Physical propert ies, Growth and fruit quality: Fruit weight decreased in ‘Rg’ although fruit length/diameter, TSS, and firmness increased. Conversely, fruit weight was higher in Rn and ‘Rt’ but in the aspect of quality, TSS, and firmness were lowest (Table 1). Fruit weight and firmness decreased in high levels of salinity (S3). Whereas TSS increased; fruit length/diameter decreased by salinity (Table 1).

Fruit weight decreased in S3 in all rootstocks. Fruit weight did not change significantly in low salinity level (S2) in grafted cucumber and decreased in Rn. The

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Table 1. The main effect of different rootstocks and salinity on fruit characteristics

Rootstock	Fruit weight (g)	Fruit length / diameter (cm)	TSS (Brix)	Firmness (kg/cm^3)
'Rn'	45.06 ^{a}	6.98 ^{b}	3.91 ^{b}	0.71 ^{b}
'Rt'	43.70 ^{a}	5.40 ^{c}	3.65 ^{c}	0.70 ^{c}
'Rg'	35.28 ^{b}	7.59 ^{a}	4.63 ^{a}	0.73 ^{a}
'Rkh'	38.44 ^{ab}	5.88 ^{c}	4.13 ^{b}	0.70 ^{c}
Salinity				
S1	40.77 ^{ab}	6.78 ^{a}	3.99 ^{b}	0.70 ^{b}
S2	46.40 ^{a}	6.22 ^{b}	4.22 ^{a}	0.73 ^{a}
S3	34.69 ^{b}	6.39 ^{b}	4.03 ^{ab}	0.70 ^{b}

Within a column means followed by the same letter are not significantly different at P < 5% according to least significant different test. 'Rn': Nongrafted (*Cucumis sativus v. DAVOSII*), 'Rt': Tanbal (*Cucurbita maxima*), 'Rg': Ghalyani (*Lagenaria siceraria*), 'Rkh': Khoreshi (*Cucurbita pepo*). (S1): 0, (S2): 30, (S3): 60 mM NaCl.

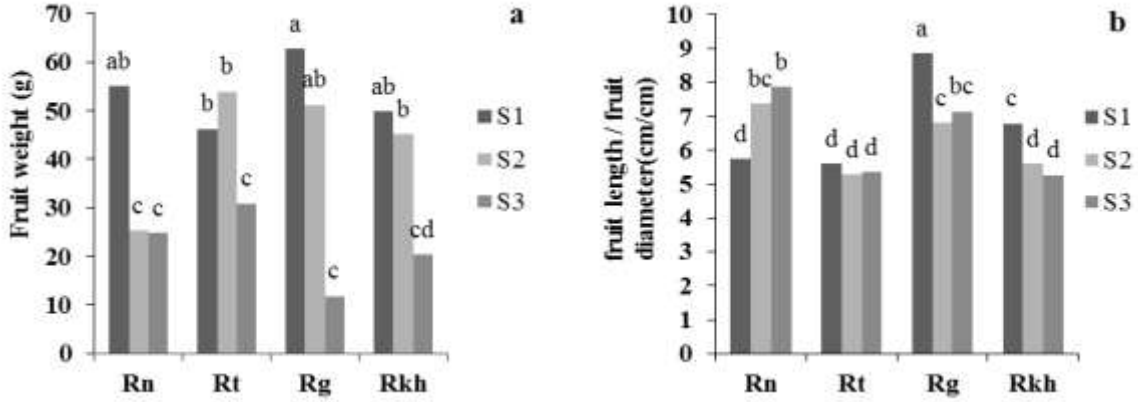

Figure 1. The interactive effects on salinity and rootstocks on fruit weight (a), fruit length / diameter (b). Rn': Nongrafted (*Cucumis sativus* v. DAVOSII), 'Rt': Tanbal (*Cucurbita maxima*), 'Rg': Ghalyani (*Lagenaria siceraria*), 'Rkh': Khoreshi (*Cucurbita pepo*). (S1): 0, (S2): 30, (S3): 60 mM NaCl

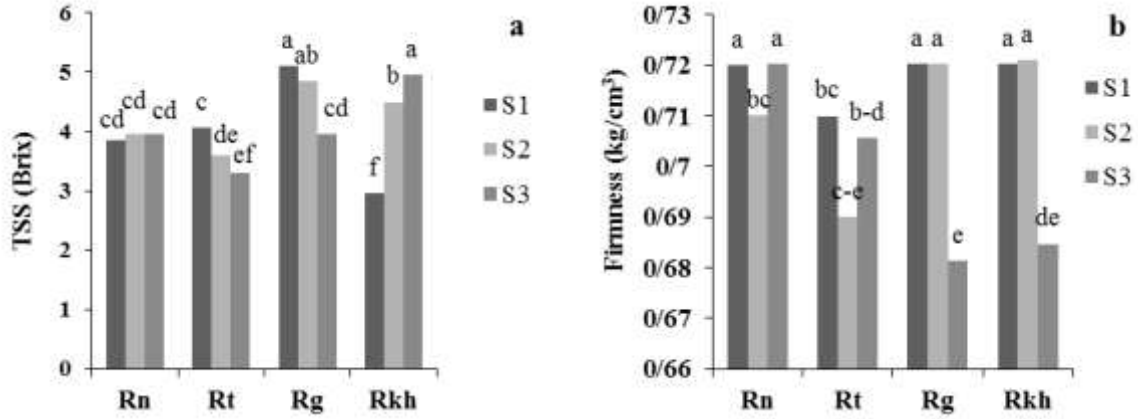

Figure 2. The interactive effects on salinity and rootstocks on TSS (a), Firmness (b). 'Rn': Nongrafted (*Cucumis sativus v.* DAVOSII), 'Rt': Tanbal (*Cucurbita maxima*), 'Rg': Ghalyani (*Lagenaria siceraria*), 'Rkh': Khoreshi (*Cucurbita pepo*). (S1): 0, (S2): 30, (S3): 60 mM NaCl

highest fruit weight and length/weight of fruit were at 'Rg' × S1 (Figure 1 a, b).

TSS increased in 'Rg' and 'Rkh' in S2. Firmness decreased with S3 in 'Rg' and 'Rkh' significantly (Figure 2 a and b).

Physiological changes, Photosynthesis traits: Photosynthesis rate and mesophyll conductance were the highest in 'Rg'. Transpiration increased in Rn and the lowest was in 'Rk' and 'Rd'. Chlorophyll fluorescence and chlorophyll content were the highest in 'Rt' and the lowest was seen in 'Rn' (Table 2). Photosynthesis rate and transpiration decreased with salinity. Mesophyll conductance increased in S3. Chlorophyll fluorescence and chlorophyll content did not change significantly between treatments (Table 2).

The interactive effects of salinity and rootstocks did not change the chlorophyll content (data was not shown). Photosynthesis decreased in line with

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Table 2. The main effect of different rootstocks and salinity on photosynthetic parameters

rootstocks	Chlorophyll fluorescence (Fv/fm)	Chlorophyll (SPAD value)	Transpiration	Mesophyll conductance ($mmolm^{-2}s^{-1}$)	Photosynthesis ($\mu mol\ m^{-2}\ s^{-1}$)
'Rn'	0.03 [b]	11.50 [c]	6.54 [a]	0.03 [c]	9.99 [c]
'Rt'	0.06 [a]	23.10 [a]	5.97 [c]	0.04 [c]	9.73 [c]
'Rg'	0.057 [ab]	18.43 [b]	6.21 [b]	0.07 [a]	17.64 [a]
'Rk'	0.04 [ab]	14.73 [bc]	4.18 [e]	0.05 [b]	14.31 [b]
'Rkh'	0.04 [ab]	16.10 [b]	4.53 [d]	0.07 [a]	17.17 [a]
Salinity					
S1	0.06 [a]	18.4 [a]	5.72 [a]	0.06 [a]	15.04 [a]
S2	0.05 [a]	15.10 [a]	5.02 [b]	0.05 [b]	12.64 [c]
S3	0.03 [a]	16.83 [a]	5.03 [b]	0.05 [a]	13.63 [b]

Within a column means followed by the same letter are not significantly different at P < 5% according to least significant different test. 'Rn': Nongrafted (*Cucumis sativus v.* DAVOSII), 'Rt': Tanbal (*Cucurbita maxima*), 'Rg': Ghalyani (*Lagenaria siceraria*), 'Rk': Karela (*Momorida charantia*), 'Rkh': Khoreshi (*Cucurbita pepo*). (S1): 0, (S2): 30, (S3): 60 mM NaCl.

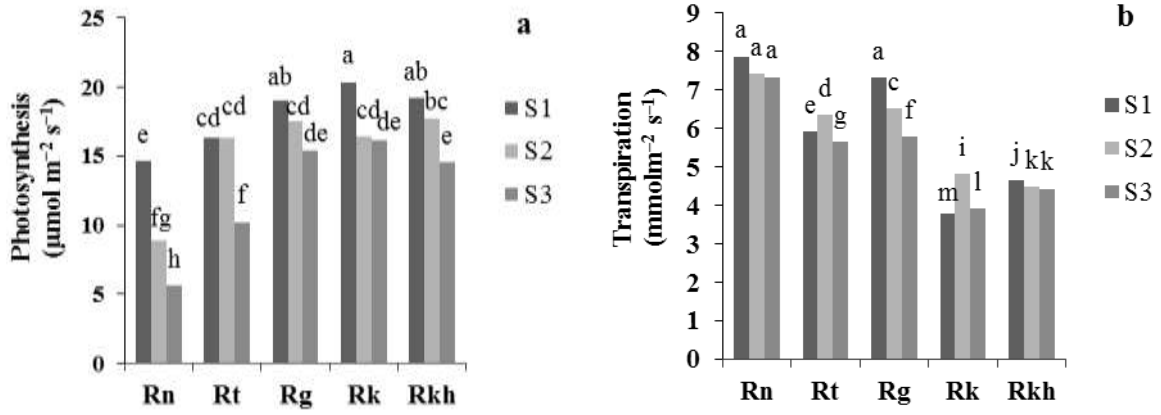

Figure 3. The interactive effects of salinity and rootstocks on photosynthesis (a) and transpiration (b). 'Rn': Nongrafted (*Cucumis sativus v.* DAVOSII, 'Rt': Tanbal (*Cucurbita maxima*), 'Rg': Ghalyani (*Lagenaria siceraria*), 'Rk': Karela (*Momorida charantia*), 'Rkh': Khoreshi (*Cucurbita pepo*). (S1): 0, (S2): 30, (S3): 60 mM NaCl

Table 3. The main effects of salinity on RWC, electrolyte leakage, proline and antioxidants

Salinity	RWC	Electrolyte leakage	Antioxidants	Proline (ppm)
	(%)			
S1	53.16 [a]	42.31 [c]	33.97 [c]	7.28 [b]
S2	51.57 [b]	44.31 [b]	35.63 [b]	9.54 [a]
S3	51.27 [b]	48.02 [a]	37.53 [a]	9.63 [a]

Within a column means followed by the same letter are not significantly different at P < 5% according to least significant different test. (S1): 0, (S2): 30, (S3): 60 mM NaCl

increasing salinity and the highest decrease was seen in Rn by 61% at S3, and the lowest reduction was seen in 'Rk' and 'Rkh' with salinity. Transpiration decreased with salinity in 'Rkh'and 'Rg' but did not change in Rn. The highest transpiration was in Rn in all salinity levels (Figure 3 a and b).

Biochemical compound, Proline, RWC and electrolyte leakage, and antioxidant: RWC, electrolyte leakage, proline, and antioxidant did not change significantly in rootstocks (data were not shown). RWC decreased, whereas electrolyte leakage, proline, and antioxidants increased with salinity (Table 3).

Proline concentration did not change significantly in different levels of salinity in each rootstock and was highest in 'Rk' × S3 (Figure 4).

Health-related compound, Antioxidant, phenol and nutrient uptake: The highest Na concentration was in Rn. The highest N and P concentration was seen in 'Rkh'. The K concentration increased in 'Rk' and 'Rkh' (Table 4). The Na concentration increased, whereas, K and N concentration decreased with salinity. P concentration and phenol content did not change significantly (Table 4).

Na absorption was highest in Rn and conversely, K concentration was lowest in Rn. In addition, Na concentration did not affect among salinity levels in other rootstock and was lower than Rn. On the other hands, K concentration increased in 'Rk' and 'Rkh' in all salinity levels (Figure 5 a and b).

The interactive effects of salinity and rootstocks on chlorophyll fluorescence, mesophyll conductance did not show any significant differences, as well as, N, P concentration did not change significantly in rootstock

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Table 4. The main effect of different rootstocks and salinity on nutrient element absorption.

Rootstocks	Na (ppm)	K	N	P	phenol
	(ppm)		(mmol/kg)	(mg/kg DW)	
'Rn'	32322 [a]	14065 [b]	2617.5 [c]	1.16 [b]	0.61 [a]
'Rt'	13625 [c]	14472 [b]	3931.3 [b]	1.31 [ab]	0.61 [a]
'Rg'	9376 [d]	14847 [b]	5610.4 [c]	1.01 [b]	0.61 [a]
'Rk'	21841 [b]	15757 [a]	3230.8 [bc]	0.91 [b]	0.61 [a]
'Rkh'	19741 [b]	15543 [a]	4957.2 [a]	1.72 [a]	0.61 [a]
Salinity					
S1	17497 [b]	15986 [a]	5464.[a]	1.41 [a]	0.61 [a]
S2	19989 [a]	14587 [ab]	3600.2 [b]	1.21 [a]	0.61 [a]
S3	20657 [a]	13038 [b]	3143.6 [b]	1.05 [a]	0.61 [a]

Within a column means followed by the same letter are not significantly different at P < 5% according to least significant different test. 'Rn': Nongrafted (*Cucumis sativus v.* DAVOSII, 'Rt': Tanbal (*Cucurbita maxima*), 'Rg': Ghalyani (*Lagenaria siceraria*), 'Rk': Karela (*Momorida charantia*), 'Rkh': Khoreshi (*Cucurbita pepo*). (S1): 0, (S2): 30, (S3): 60 mM NaCl

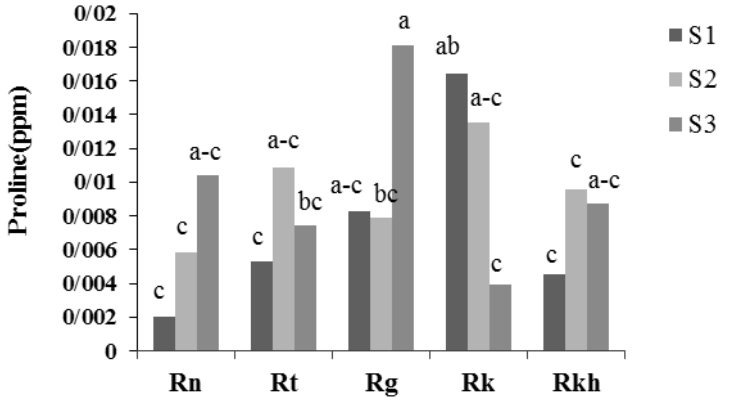

Figure 4. The interactive effects of salinity and rootstocks on proline. 'Rn': Nongrafted (*Cucumis sativus v.* DAVOSII, 'Rt': Tanbal (*Cucurbita maxima*), 'Rg': Ghalyani (*Lagenaria siceraria*), 'Rk': Karela (*Momorida charantia*), 'Rkh': Khoreshi (*Cucurbita pepo*). (S1): 0, (S2): 30, (S3): 60 mM NaCl

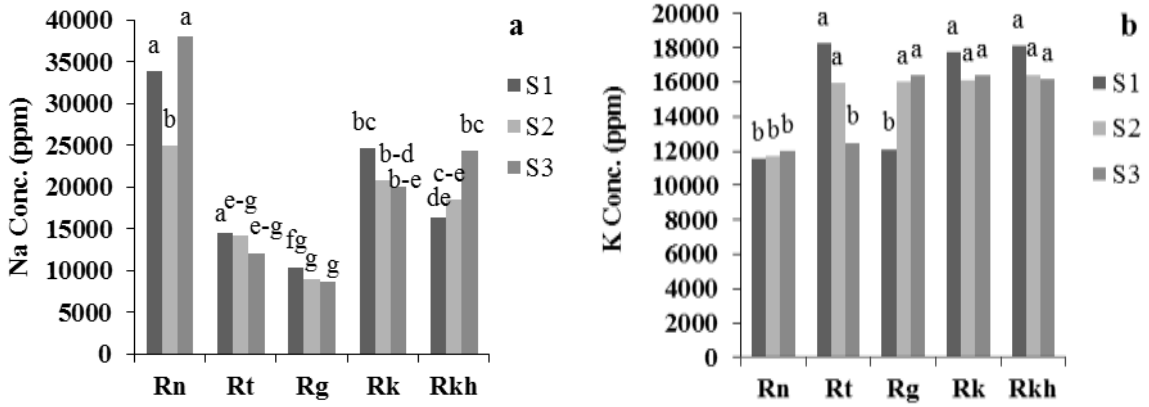

Figure 5. The interactive effects of salinity and rootstocks on Na concentration (a), potassium (b). 'Rn': Nongrafted (*Cucumis sativus v.* DAVOSII, 'Rt': Tanbal (*Cucurbita maxima*), 'Rg': Ghalyani (*Lagenaria siceraria*), 'Rk': Karela (*Momorida charantia*), 'Rkh': Khoreshi (*Cucurbita pepo*). (S1): 0, (S2): 30, (S3): 60 mM NaCl

with salinity (data were not shown).

Discussion

Physical properties, growth and fruit quality: Rootstocks affect scion differently for increasing yield and fruit size (Heidari *et al*., 2013), in this experiment, it was seen that fruit weight was the highest in 'Rt' and 'Rg.' The most upper fruit length/diameter was in 'Rg' with salinity. Fruit weight did not change in 'Rt' and 'Rkh' in 30 mM salinity, and also did not change in 'Rt' in high levels of salinity. It seems that 'Rt' was more tolerable to salinity via keeping the fruit weight and

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length/diameter even at a high salinity level. One possible mechanism could be a different root growth system, which is more significant in 'Rt' compared with the non-grafted (unpublished data). Heidari *et al*. (2013) showed that grafting decreased the length/diameter of the fruit (Heidari *et al*., 2013). The higher ratio of length/diameter of cucumber fruit is favorable regarding consumer taste in Iran. It mostly depends on species characteristics, so 'Rg' in salinity condition still keep weight and shape. TSS increased with salinity, especially in 'Rg' and 'Rkh'. Increasing total soluble sugar or some other osmulite as well as increasing K concentration in 'Rk' and 'Rkh' can increase osmotic potential result in keeping the turgor of cells and keeping growth (Pessarakli, 2010). Additionally, their increase resulting in improving the nutritional value of cucumber.

Physiological changes and photosynthesis traits: Salinity decreased photosynthesis and stomata conductance as well as mesophyll conductance (Mashouf *et al*., 2003; Mirmohammadi Meybodi and Ghareh yazi, 2002). The same results were seen in this experiment, the mesophyll conductance decreased almost in all rootstock and especially in 'Rk' and 'Rkh'. The decreased may be because of the closing of stomata in salinity, which results in lower stomata conductance and mesophyll conductance as well as lower CO_2 concentration and photosynthesis (Mirmohammadi Meybodi and Ghareh yazi, 2002; Wang and Nii, 2000). At the beginning of stress, the closing of stomata can be improved stress tolerance of plant via decreasing losing water by transpiration and keep better water potential and turgor, which resulted decrease of plant growth (Pessarakli, 2010). In line with this explanation, results revealed that, with decreasing mesophyll conductance in 'Rk' and 'Rkh', the transpiration also reduced. With increasing salinity, Na absorption increase and K decreased. K is essential elements in plant and with decreasing, K concentration stomata close and photosynthesis decreased, so growth diminished (Mirmohammadi Meybodi and Ghareh yazi, 2002). Grafting on squash rootstock resulting in higher increase due to: 1) The ability of squash root on water and nutrient absorption like N and P (Pogonyi *et al*., 2005) 2) High root activity according to furmasan concentration in dry weight, 3) Higher cytokinin in the root, and 4) Higher tolerant of squash root to low temperature compares with cucumber (Pogonyi *et al*., 2005 and Salehi, 2002). In addition to the information mentioned above, we added that squash rootstock keeps photosynthesis more active. Moreover, decreased water loss via reduced transpiration keeps growth well. Furthermore, RWC maintains unchanged via maybe osmoregulation in cucumber, as it will explain in the following.

Biochemical compound and health-related compound: Various reports have shown that antioxidant enzyme might increase or decrease with salinity; Salinity stress (50, 100, or 200 mM) increased electrolyte leakage; SOD, CAT, POD, enhanced in capsicum plants (Abu-Muriefah, 2015). The activities of many antioxidant enzymes did not change when the NaCl concentrations were low; However, they increased at higher levels in pea (Hernandez *et al*., 1999). The different report s could be related to the severity of salinity and duration of stress as well as plant sensitivity to salinity. In this research, the K efficiently absorbed with 'Rg', 'Rk', and 'Rkh'. It can be concluded that these rootstocks try to absorb K efficiently to keep the osmoregulation so keep the physiological activity of plants better in salinity stress (Javanmardi *et al*., 2001). The same results were seen in tolerate wheat varieties in saline conditions (Ashraf *et al*., 2004). The same results were seen it seems that in 'Rkh' and 'Rg' and 'Rk' with K, TSS and proline accumulation these rootstocks were more efficient in keeping growth via osmoregulation in 'Rg' and 'Rkh' (Pessarakli, 2010; Valliyodan and Nguyen, 2006). So RWC did not significantly change even in saline condition.

Conclusion

Conclusively, physiological parameters improved in 'Rkh', 'Rk' at salinity compare with Rn. Fruit quality is mostly improved at 'Rg' in grafted comparing non-grafted plants. Generally, using rootstocks like 'Rk', 'Rkh' and 'Rg' improved fruit quality and physiological aspect of grafted cucumber in saline soil.

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