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'*Momordica charantia*' introducing a new rootstock for grafted cucumber under low-temperature stress

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Key words: *Cucurbita maxima,* female flower, Karela, photosynthesis, rootstock, yield.

Abbreviation: Rma= grafted onto *Cucurbita maxima;* Rmo= grafted onto *Momordica charantia*; Rn= non-grafted; Rs= self-grafted; Tc= control temperature; Ts= stress temperature.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: Cucumber is a sensitive vegetable to low temperatures. Grafting vegetables on different rootstocks can decrease the harmful effects of environmental stresses, including low-temperature stress. An experiment was performed to evaluate grafting cucumbers on different rootstocks at low temperatures. Cucumber growth and yield and photosynthesis traits were examined. Treatments were the optimum temperature (25±2°C), and cold temperature (15±3°C, Ts), and rootstocks, were *Momordica charantia* (Rmo), *Cucurbita maxima* (Rma), non-grafted (Rn) and self- grafted (Rs) with 4 replications. Shoot fresh and dry weight, chlorophyll, RWC, transpiration, decreased with temperature stress. The number of female flowers, electrolyte leakage, photosynthesis, stomatal conductance increased with Ts. First fruit emergence per plant, N, P, K, Mg concentration decreased with Ts stress. Transpiration, female flower, RWC, and stomata conductance, N, P, K, Ca, and phenol increased in Rma and Rmo. Mg was at the highest concentration in Rma and Na in Rn. All in all, using Rmo as well as Rma is recommended for rootstock as it causes more reproductive growth.

1. Introduction

Recently, the greenhouse culture of plants has widely expanded (Yan *et al.,* 2013). Cucumbers (*Cucumis sativus*) are commonly grown in the greenhouse. The optimal temperature for aerial growth and roots are about 28-23/18-15°C (day/night) and 20°C, respectively (Ikeda and Kawashiro, 2005). Thus, cucumbers are cold-sensitive vegetables. Low-temperature stress can occur in plastic culture or non-equipped greenhouses, which are very common in Iran. It may also affect field culture cucumbers and spring culture cucumbers. Low temperatures have different deleterious effects on cucumbers, including affecting the nutrient uptake of plants (Pregitzer and King, 2005), and inducing greater H_2O_2, MDA, and soluble sugar content in cucumbers, causing damage to plants and inhibiting plant growth (Qiu-yan *et al.,* 2013).

There are several ways to control low-temperature damage to plants, including controlling root temperatures by warming nutrient solution or soil. Still, these ways require excess energy (Willits and Peet, 1998). Finally, excess chemical nutrient applications for promoting growth can prevent damage to plants, which unfortunately involves environmental risks. Therefore, vegetable grafting can be a proper way to increase plant resistance to environmental stresses in addition to expanding its yield, quality, and growth. Furthermore, if any energy-efficient way is introduced, which can raise the growth rate of plants in greenhouse conditions at lower temperatures, vast amounts of energy and money will be saved. Vegetable grafting has been used to increase plant tolerance under environmental stresses, including high temperatures (Rivero *et al.,* 2003), low temperatures (Rivero *et al.,* 2003), salinity stresses (Estan *et al.,* 2005), drought stresses (Bhatt *et al.,* 2002) and heavy metal stresses (Edelstein *et al.,* 2005).

Fig leaf gourd and bur cucumbers (*Sicyos angulatus*) are used for increasing resistance against low-temperature stresses for cucumber rootstocks (Venema *et al.,* 2008). These rootstocks increase plant resistance using different ways. First, low-temperature stresses decrease Co_2 assimilation in cucumbers and this reduction is improved by grafting (Zhou *et al.,* 2009). Secondly, a previous study done by Zhou e*t al.* (2007) showed that fig leaf gourd rootstocks enhanced vegetative growth and yield of cucumbers under low-temperature stresses.

There is a hypothesis stating that grafted plant responses to temperature stresses are related to scion species (Venema *et al*., 2005). Many researchers have shown beneficial effects of vegetable grafting on the growth, fruit yield, and quality of plants. Some of the studies have used different rootstocks for cucumbers under different stresses showing better growth rate for grafted plants under stress conditions compared to non-grafted ones like: heavy metal absorption (Rouphael *et al*., 2008; Kumar *et al*., 2015), low soil temperatures (Tachibana, 1982), salinity (Huang *et al*., 2010), improve NaCl and $CaCl_2$ tolerance in Cucumber (Colla *et al.,* 2013), Al toxicity in cucumber (Rouphael *et al.,* 2016).

Different rootstocks were used in normal and stress conditions to improve yield and growth traits like different kinds of squash (Yang *et al*., 2006), (Massai *et al*., 2004), (Rouphael *et al*., 2008), Fig leaf guard (Tachibana, 1982), pumpkins and bottle gourd and P360 (*Cucurbita maxima* Duch.×*Cucurbita moschata* Duch.) (Colla *et al*., 2010 and 2013). To the best of our knowledge, few studies have investigated the use of *Momordica charantia* as a rootstock for cucurbits, or more explicitly grafting, to examine the possibility of cucumber cultivation at low temperatures in the whole growth period. Hence, the goal of the present study was the use of *Momordica charantia* and *Cucurbita maxima* as rootstocks and *Cucumis sativus* var. DavosII*,* which is a common variety of cucumbers in Iran as a scion under low-temperature stress.

2. Materials and Methods

Experimental design and plant preparation

This experiment was conducted in a plastic greenhouse in the Department of Horticulture Science at the Isfahan University of Technology, Isfahan, Iran. An investigation was arranged as a combined analysis involving data from two locations, simultaneously collected, based on CRD with 4 replications. Treatments were optimum (25±2°C, Tc) and low temperatures (15±3°C, Ts). Cucumbers *(Cucumis sativus* var. DavosII*)* were grafted to *Momordica charantia* (Rmo), *Cucurbita maxima* (Rma). Non-grafted (Rn) and self-grafted (Rs) consider as a control. Scion seeds had been cultivated 10 days before rootstock seeds in cocopeat: perlite 1:1. Scion plants and rootstocks were cut beneath and above the first true leaves, respectively. Hole-insertion grafting was used and grafted plants were transferred to a recovery greenhouse with high relative humidity. Plants were kept for 2 weeks in recovery conditions and gradually adapted to normal greenhouse conditions. Grafted plants were transferred to 5kg pots, including soil. Irrigation was used when the plant needed it. Chemical fertilizer NPK (20, 20, 20) 2 mg/pot was applied every 10 days. Plants were conducted to wire above the greenhouse, and there was no use of any pesticide.

Plant growth and fruit properties

The male and female flower, node numbers, and shoot numbers were counted during the experiment;

root and internode length was determined with a ruler. The time and node of male and female flower emergence were recorded. Fruit diameter, fruit firmness, and TSS were measured with a caliper (Mitutoyo Corp, Japan), Pentameter (model OSK-I-10576), and portable Refractometer (PAL^{-1} Brix, Japan), respectively (Raeisi *et al.,* 2014).

Shoots were excised from the roots using a steel blade and then fresh weights of roots and shoots were measured. All the samples were oven-dried at 70˚C for 48 hours and the dry weights were estimated. During the experiment and finally 124 days after transplanting, fruits were harvested and washed using tap water and were weighed by an analytical balance.

SPAD value and photosynthesis trait assay

Chlorophyll content was measured using a chlorophyll meter (SPAD-502 plus, Minolta, Japan). Fv/Fm was measured by chlorophyll fluorescence (OS- 30, USA) after 3 weeks. Photosynthetic properties were determined from the youngest fully-expanded leaf for 3 replications per treatment by Portable photosynthesis systems for gas exchange and chlorophyll fluorescence measurements (LI-COR-6400, USA) from 10:00 to 11:00 AM on a clear day (without clouds). The measurements were conducted with photosynthetically active radiation (PAR) intensity of 1000 µmol m^{-2} s^{-1} and CO_2 concentration of 350 µmol·mol. Mesophyll conductance (µmol $m^{-2}s^{-1}$) was calculated by dividing the photosynthetic rate by the sub-stomatal CO_2 level (Ahmadi and Siosemardeh, 2005).

Antioxidant activity

Antioxidant activity was measured and expressed as gallic acid (equivalents l of gallic acid/g) with UV-VIS spectrophotometer (Shimadzu UV160A-Japan). Three mg of sample were dissolved in 5 mL methanol stock, and 1.4 ml of this solution was blended with 0.6 mL of DPPH solution. After 30 min, the absorbance of the solution was recorded at 515 nm by the spectrophotometer (UV 160A- Shimadzu Corp., Kyoto, Japan) against methanol as a blank. The 0.2 mM of DPPH solution in methanol was used as a stock of DPPH for the determination of the free radical scavenging activity of the samples (Koleva *et al.*, 2002).

Phenolic content

Total phenolic content was determined using the Folin-Ciocalteu method. The absorbance was measured at 725 nm with a spectrophotometer (UV 160A- Shimadzu Corp., Kyoto, Japan). The results were expressed in gallic acid equivalents (mg/100 g fresh weight) using gallic acid (0-0.1 mg/mL) standard curve (Singleton *et al*., 1965).

Proline

Proline accumulation was determined using the method proposed by Bates *et al*. (1973). After the extraction of toluene, the clear phase was recovered and spectrophotometrically estimated at 520 nm using toluene as a blank. Purified proline was used for standardization (0-50 mg/mL) and was expressed as mol proline g^{-1} fresh weight.

Electrolyte leakage

Electrolyte leakage (EL) was measured using an electrical conductivity meter employing the method described by Lutts *et al.* (1995)

Relative water content

Relative water content (RWC%) was determined using ten 7 mm-diameter leaf discs. The leaf discs of each treatment were weighed (FW). They were then hydrated until saturation was reached (constant weight) for 48 h at 5°C in darkness (TW). The leaf discs were dried in an oven at 105°C for 24 h (DW). Relative water content was calculated according to the following equation (Filella *et al*., 1998):

$$RWC\% = (FW - DW)/(TW - DW) \times 100$$

The determination of total nitrogen in the leaf samples was based on the Kjeldahl method (Estan *et al.,* 2005). The concentrations of K, Ca, Mg, and P were measured (Shield Torch System, Agilent 7500a). Meanwhile, phosphorus was estimated by the vanadomolybdo phosphoric acid colorimetric method at 460 nm (Estan *et al.,* 2005). P was colorimetrically determined using a spectrophotometer (UV 160A- Shimadzu Corp., Kyoto, Japan).

Statistical analysis

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

3. Results and Discussion

Analysis of variance of temperature and rootstock on some characteristics of cucumber

Temperature effect shoot fresh and dry weight, chlorophyll index, and the number of the female flower, photosynthetic rate, transpiration, stomatal conductance, RWC, EL, phenol content, and all nutrient concentration (Table 1 a, b). Rootstock changes all cucumber characteristics except for average fruit weight, fruit diameter, TSS, firmness, mesophyll conductance, proline, and antioxidant (Table 1 a, b). The interactive effect of temperature and rootstock showed that all measured parameters change significantly (Table 1 a, b).

The main effect of temperature on cucumber

Shoot fresh and dry weight, chlorophyll, RWC, transpiration, decreased with temperature stress. The number of the female flower, EL, photosynthesis, and stomatal conductance increased with Ts (Table 2 a). First fruit emergence per plant, N, P, K, Mg concentration decreased with Ts stress (Table 2 b). Shoot fresh and dry weight and fresh root weight was high-

Table 1 a - Analysis of variance of the effect of temperature and rootstock on some characteristics of cucumber

Source	df	Shoot fresh weight	Shoot dry weight	Root fresh weight	Chl	Number of female flowers	Number of male flowers	Fruit number	Fruit yield	First fruit per plant	Average fruit weight	Fruit diameter	TSS	Firmness
Temperature	1	3203.42 *	230. 9 **	244.56 NS	43.12 **	78.12 *	6.12 ns	230. 9 NS	1441.5 NS	152.91 *	1971.09 NS	0.002 NS	0.30 NS	7.65 NS
Rootstock	3	2467.99 *	23.45 *	150.01 *	35.93 **	602.45 **	13.08 **	23.45 *	19335 *	92.57 *	2094.59 NS	0.10 NS	0.18 NS	2.07 NS
T×R	3	1327.25 *	26.54 **	111.77 **	4.65 **	81.12 **	4.20 *	26.54 **	11769.1 **	4.81 **	2133.88 *	0.04 **	0.01 **	1.23 **
Error	18	708.05	5.27	67.38	3.23	76.32	1.35	5.27	5110.2	17.23	1292.36	0.04	0.12	4.26
CV		10.30	25.62	13.04	15.50	13.97	14.38	25.62	18.03	26.05	18.65	6.77	13.40	25.25

NS= no significant, * significant at 5% and ** significant at 1%.

Table 1 b - Analysis of variance of the effect of temperature and rootstock on some characteristics of cucumber

Source	df	Photo-synthetic rate	Tran-spiration	Stomata conduc-tance	Mesophyll conduc-tance	RWC	EL	Proline	Anti-oxidant activity	Phenol content	N Conc.	P Conc.	K Conc.	Ca Conc.	Mg Conc.	Na Conc.
Temperature	1	47.52 *	10.52 **	0.002 **	240063 NS	0.006 **	257.36 **	3.27 NS	0.06 NS	55824.1 **	0.72 **	0.036 **	0.06 **	0.15 **	0.16 **	0.039 **
Rootstock	3	25.08 *	2 **	0.001 *	26980 NS	0.001 **	60.38 **	1.31 NS	0.006 NS	5561.8 **	1.39 **	0.03 **	2.06 **	0.65 **	0.07 **	0.01 **
T×R	3	14.25 **	1.12 **	0.002 **	4898 **	0.0008 **	72.71 **	2.82 **	0.02 **	4553.6 **	0.54 **	0.01 **	0.62 **	0.54 **	0.06 **	0.008 **
Error	18	6.17	0.97	0.001	14354	0.00003	9.38	1.05	0.03	52.2	6.53	4.71	5.08	3.85	1.05	2.78
CV		14.41	14.82	17.80	38.05	0.74	7.56	11.03	5.81	5.01	12.1	26.7	21.5	7.87	6.23	16.9

NS= no significant, * significant at 5% and ** significant at 1%.

Table 2 a -The effect of temperature on some characteristics of cucumber

Temperature (° C)	Shoot fresh weight (g)	Shoot dry weight (g)	Chl (SPAD value)	Number of the female flower	El (%)	RWC (%)	Transpiration (mmol H_2O m^{-2} s^{-1})	Photosynthetic rate (mmol H_2Om^{-2} s^{-1})	Stomata conductance (mmol H_2Om^{-2} s^{-1})
Tc	76.52 a	11.71 a	12.7 a	2.18 b	37.1 b	0.79 a	2.83 a	4.16 b	61.31 b
Ts	55.53 b	6.21 b	10.4 b	3.06 a	43.91 a	0.75 b	1.57 b	7.02 a	177.34 a

Tc= optimum temperature, Ts= low temperature.
Within a column means followed by the same letter are not significantly different at P<5% according to LSD test.

Table 2 b - The effect of temperature on some characteristics of cucumber

Temperature (° C)	First fruit emergence per plant (day)	Phenol (mgg^{-1} FW)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)
Tc	19.57 a	17.69 b	3.51 a	0.43 a	3.11 a	1.54 b	0.74 a	0.14 b
Ts	12.30 b	271.06 a	3.37 b	0.39 b	2.54 b	1.66 a	0.68 b	0.21 a

Tc= optimum temperature; Ts= low temperature.
Within a column means followed by the same letter are not significantly different at P<5% according to LSD test.

est in Rma, the number of male flower increased in Rn and Rs and female flower increased in Rmo and Rma.

The main effect of rootstock on cucumber

The highest El and photosynthesis were observed in Rmo. The RWC and stomata conductance increased in Rma and Rmo, however, transpiration increased in Rs, Rmo, and Rma (Table 3 a). N, P, K, Ca, and phenol increased in Rma and Rmo. Mg was at the highest concentration in Rma and Na in Rn (Table 3 b). Rma has the highest fruit number, fruit yield, although, first fruit emerges in Rmo (Table 4).

The interaction effect of temperature and cucumber grafting on some growth characteristics of cucumber

K concentration was the highest in Rmo in both temperatures and P concentration increased in Rma×Ts. The highest N, K and Ca, concentrations were seen in Rma×Ts. P concentration increased in Rma and Rmo at Ts. Mg increased in Rs×Tc and Na in Rs×Ts (Table 5).

Table 3 a -The effect of rootstock on some characteristics of cucumber

Rootstock	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Number of male flowers	Number of female flower	El (%)	RWC (%)	Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	Transpiration (mmol H_2O m^{-2} s^{-1})	Stomata conductance (mmol H_2O m^{-2} s^{-1})
Rn	61.28 b	8.23 b	16.78 b	23.50 a	2.37 b	38.98 bc	0.76 b	4.94 b	1.65 b	78.79 b
Rs	55.48 b	9.29 ab	17.96 ab	23 a	1.75 b	41.67 ab	0.76 b	3.83 b	2.69 a	71.94 b
Rma	92.34 a	11.17 a	18.21 a	5.5 b	4.5 a	37.10 c	0.78 a	4.84 b	1.86 ab	89.96 ab
Rmo	55b	7.14 b	17.95 b	12.75 b	3.87 ab	44.38 a	0.78 a	8.75 a	2.61 ab	126.62 a

Rn= nongrafted (*Cucumis sativus* var. DavosII);
Rs= self grafted;
Rma= *Cucurbita maxima*;
Rmo= *Momordica charantia*.
Within a column means followed by the same letter are not significantly different at P<5% according to LSD test.

Table 3 b - The effect of rootstock on some characteristics of cucumber

Rootstock	Phenol (mgg^{-1} FW)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)
Rn	124.54 bc	3.19 c	0.45 b	2.52 d	1.05 d	0.70 b	0.19 a
Rs	112.19 c	3.08 d	0.38 c	2.55 c	1.32 c	0.69 c	0.20 a
Rma	133.90 b	3.85 a	0.47 a	3.65 a	2.11 a	0.51 d	0.14 c
Rmo	206.87 a	3.64 b	0.33 d	2.57 b	1.89 b	0.93 a	0.18 b

Rn= nongrafted (*Cucumis sativus* var. DavosII);
Rs= self grafted;
Rma= *Cucurbita maxima*;
Rmo= *Momordica charantia*.
Within a column means followed by the same letter are not significantly different at P<5% according to LSD test.

Table 4 - The effect of rootstock on some characteristics of cucumber

Rootstock	Fruit number	Fruit yield (g)	First fruit emergence per plant (day)
Rn	0.8 b	63.88 b	21.51 a
Rma	2.00 a	159.50 a	15.29 ab
Rmo	1.37 ab	91.88 ab	11.00 b

Rn= nongrafted (*Cucumis sativus* var. DavosII);
Rs= self grafted did not have any fruit;
Rma= *Cucurbita maxima*;
Rmo= *Momordica charantia*.
Within a column means followed by the same letter are not significantly different at P<5% according to LSD test.

Shoot fresh weights decreased in Rn and Rs at low temperatures (Ts), but shoot dry weights did not significantly change in Rma and Rmo at both temperatures (Fig. 1). Shoot dry weights followed the same trend as the fresh shoot weight was the highest in Rn, Rs, Rma at Tc (Fig. 1). Root fresh weights decreased in Rn, Rs, and Rma at Ts compared with Tc, but did not significantly change in Rmo at both temperatures, although the fresh root weight was the lowest in Rmo at Tc. The highest root fresh weight was seen in Rma×Tc (Fig. 2 a).

SPAD value was the highest in Rs, Rma, and Rmo at Tc (Fig. 2 b). The interaction effect of the tempera-

Table 5 - The interaction effect of temperature and cucumber grafting on some elements concentration (%) of leaves

Rootstock	N		P		K		Ca		Mg		Na	
	Tc	Ts	Tc	Ts	Tc	Ts	Tc	Ts	Tc	Ts	Tc	Ts
Rn	3.36 d	3.03 e	0.37 c	0.35 c	2.50 f	2.75 e	1.28 c	0.83 g	0.81 b	0.58 c	0.18 c	0.21 b
Rs	3.08 e	3.08 e	0.33 c	0.34 c	3.00 d	2.15 g	1.65 b	1.00 e	0.88 a	0.51 e	0.12 f	0.28 a
Rma	3.78 c	4.2 a	0.34 c	0.52 a	3.45 b	3.69 a	1.24 d	1.75 a	0.51 e	0.57 d	0.12 f	0.14 e
Rmo	3.98 b	3.78 c	0.42 b	0.48 a	3.25 c	3.10 cd	0.83 g	0.87 f	0.56 d	0.50 e	0.09 g	0.16 d

Rn= nongrafted (*Cucumis sativus* var. DavosII);
Rs= self grafted;
Rma= *Cucurbita maxima*;
Rmo= *Momordica charantia*.
Within a column means followed by the same letter are not significantly different at P<5% according to LSD test.

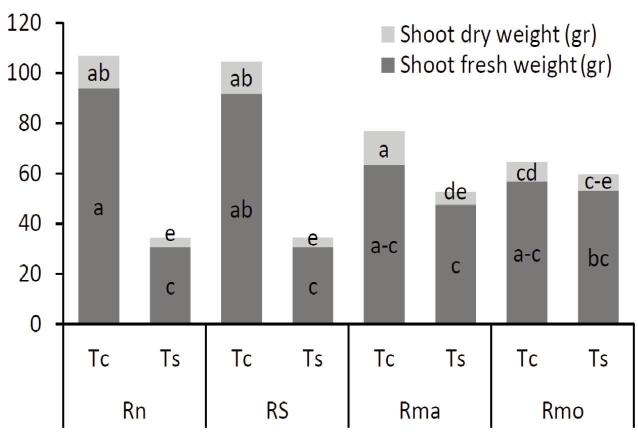

Fig. 1 - The interaction effect of temperature and cucumber grafting on shoot fresh and dry weight. Tc= optimum temperature; Ts= low temperature; Rn = nongrafted (*Cucumis sativus* var. DavosII); Rs= self grafted; Rma= *Cucurbita maxima*; Rmo= *Momordica charantia*.

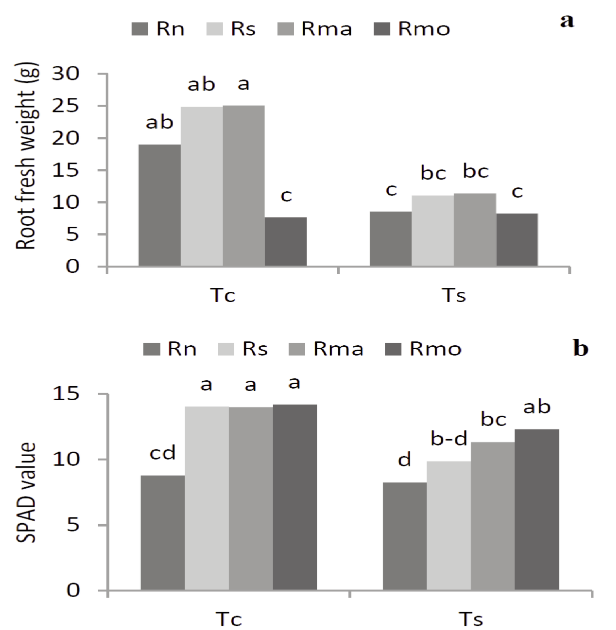

Fig. 2 - The interaction effect of temperature and cucumber grafting on root fresh weight (a) and SPAD value (b) Tc= optimum temperature; Ts= low temperature; Rn = nongrafted (*Cucumis sativus* var. Davos II); Rs= self grafted; Rma= *Cucurbita maxima*; Rmo= *Momordica charantia*.

ture on grafted cucumber did not significantly affect the time of the emergence of the first female flowers (data did not show). The highest and the lowest number of the male flower were seen in Rs×Tc and Rma, respectively. The female flower was affected by temperature variations in different rootstocks. The female flower increased in the Rn and Rma at Ts and did not change in Rs and Rmo at Tc and Ts (Fig. 3).

Fruit number was the highest in Rma×Tc, although the same results were seen in Rmo×Tc and Rmo and Rma at Ts (Fig. 4 a). Fruit yield was the highest in Rma×Tc; the same result was statically seen in Rma ×Ts (Fig. 4 b).

First, fruit emergence time significantly decreased in grafted plants at this temperature (Fig. 5). The highest fruit weight was in Rma×Tc; fruit diameter did not significantly change between treatments (Fig. 6). TSS increased in Rn and Rmo at Ts (Fig. 7 a). Firmness decreased in Rmo×Ts (Fig. 7 b) results, Goreta *et al.* (2008) found that shoot weight reduction in watermelon grafted onto *Cucurbita maxima* Duch.× *Cucurbita moschata* Duch was less) than nongrafted plants under salt stresses. The grafted cucumber plants showed less change in shoot and root weights under Cu stress conditions and this might be due to the lower accumulation of Cu in leaves through their squash rootstocks (Rouphael *et al.*, 2008).

The lowest male flower was seen in Rma at both temperatures (Fig. 3). Conversely, the highest number of female flowers was observed in Rma at Ts and Tc compared with other rootstocks at each temperature. On the other hand, at the optimum temperature (Tc), each rootstock had a lower female flower

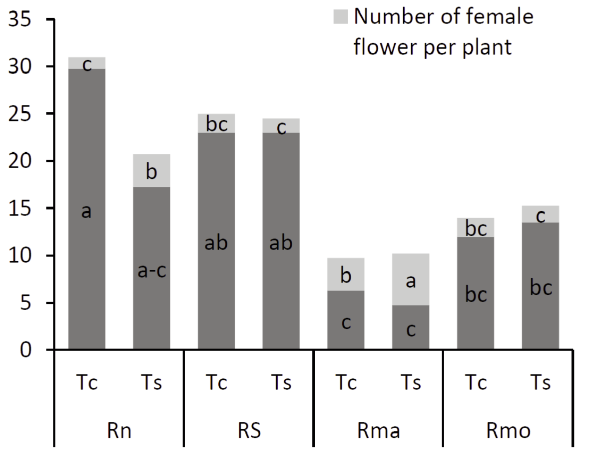

Fig. 3 - The interaction effect of temperature and cucumber grafting male and female flower number. Tc= optimum temperature; Ts= low temperature; Rn = nongrafted (*Cucumis sativus* var. DavosII); Rs= self grafted; Rma= *Cucurbita maxima*; Rmo= *Momordica charantia*.

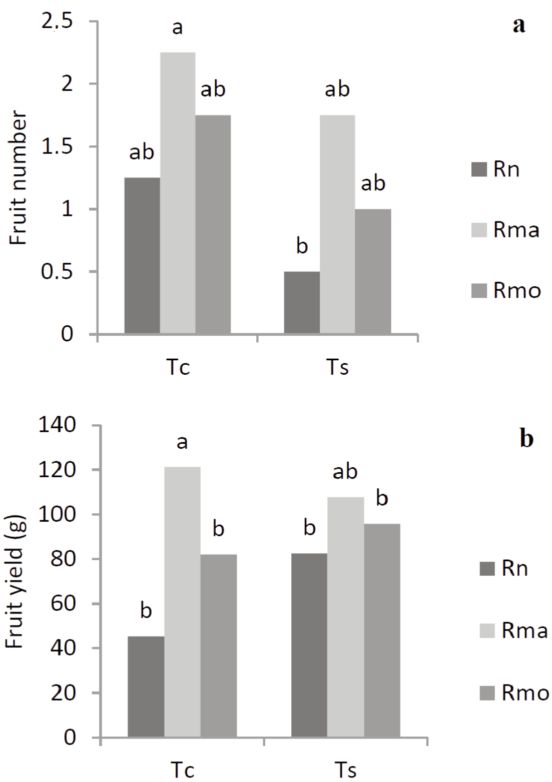

Fig. 4 - The interaction effect of temperature and cucumber grafting on fruit number (a) and fruit yield (b) *Momordica charantia* Rs did not have fruit. Tc= optimum temperature; Ts= low temperature; Rn = nongrafted (*Cucumis sativus* var. DavosII); Rs= self grafted; Rma= *Cucurbita maxima*; Rmo= *Momordica charantia.*

showed the highest male flower. Van der Ploeg and Heuvelink (2005) reported that low temperatures reduced the tomato fruit set through poor pollen quality and increased the period between anthesis and fruit maturity resulting in lower fruit yields. The result of Khah *et al.* (2006) showed that tomatoes cv. Big Red grafted onto cv. Heman and Primavera produced more fruit at low temperatures compared to non-grafted plants in greenhouse conditions. In our study, fruit number decreased at Ts and grafting had a beneficial effect on fruit number, but did not influence fruit yields. In agreement with our study, commercial tomato grafting was not able to improve the reduction of tomato yields under low light stresses by shading (Krumbein and Schwarz, 2013).

The economic increase of yield imparted by select vigorous commercial rootstocks (Kyriacou *et al.*, 2017) like increasing tomato yields was observed by

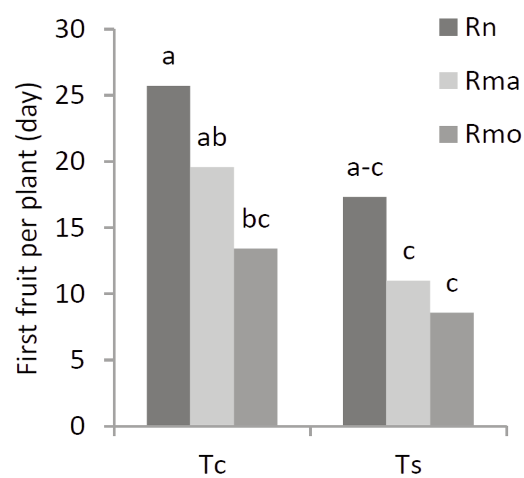

Fig. 5 - The interaction effect of temperature and cucumber grafting on the first fruit emergence of a plant. Tc= optimum temperature; Ts= low temperature; Rn = nongrafted (*Cucumis sativus* var. DavosII); Rs= self grafted; Rma= *Cucurbita maxima*; Rmo= *Momordica charantia*.

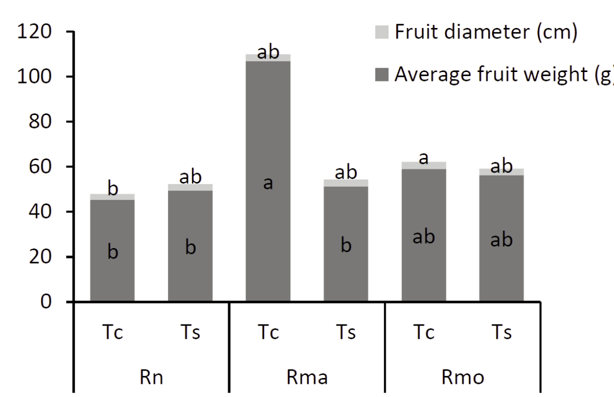

Fig. 6 - The interaction effect of temperature and cucumber grafting on average fruit weight and fruit diameter. Tc= optimum temperature; Ts= low temperature; Rn = non-grafted (*Cucumis sativus* var. DavosII); Rs= self grafted; Rma= *Cucurbita maxima*; Rmo= *Momordica charantia*.

grafting tomatoes onto Kagemusia and Helper's rootstocks by Lee *et al.* (2007). Santa-Cruz *et al.* (2001) grafted tomato cv. Moneymaker onto Pera and observed that growth and yield of tomato increased under salt stress conditions. Similar results were observed by Estan *et al.* (2005), who found that tomato yield increased through grafting under salt stresses and also reported that fruit yield in hetro-grafted plants increased more compared with self-grafted plants. Ruiz *et al.* (1997) believed that increase yield and growth of the grafted plants might be due to an increase in the nutrient and water uptake fed by vigorous rootstock. Huang *et al.* (2010) reported that cucumbers grafted had a higher fruit yield than non-grafted plants under salt stresses. Colla *et al.* (2010) found that increasing CO_2 assimilation by cucumber grafting increased fruit yields.

The interaction effect of temperature and cucumber grafting on some physiological characteristics and nutrient concentration of cucumber

The photosynthesis rate decreased in Rn and Rs at Ts compared with Tc. However, it did not significantly change in Rma and Rmo (Fig. 8 a). Transpiration decreased at Ts in all rootstocks compared with Tc (Fig. 8 b). Stomata conductance gradually increased in Rs, Rma, and Rmo at Ts compared with Tc and reached the highest in Rmo×Ts (Fig. 8 c). Mesophyll conductance did not significantly change in each rootstock at Tc and Ts, except for ungrafted cucumbers in which it increased at Ts compared with Tc (Fig. 8 d).

El and RWC were generally the lowest and the highest at TS, respectively. the highest EL was seen in Rmo×Ts. The maximum RWC, at Ts, was observed in Rn, Rma, and Rmo (Fig. 9 a, b). Antioxidant increased in Rn and Rs at Ts and did not change in other treat-

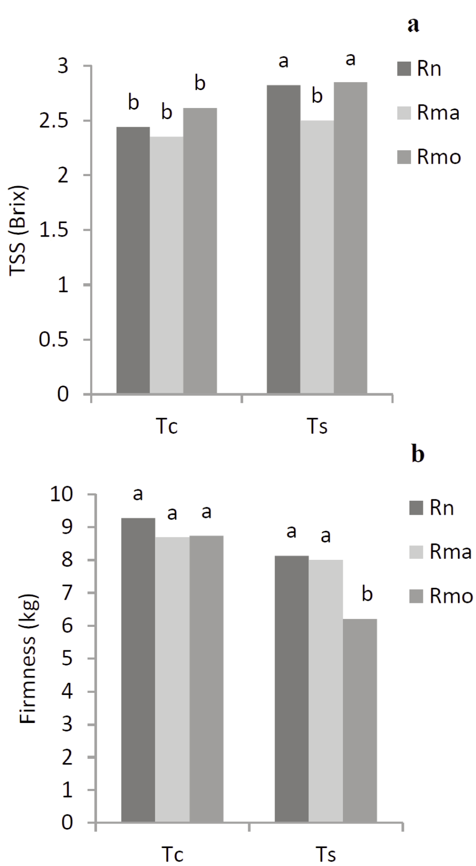

Fig. 7 - The interaction effect of temperature and cucumber grafting on TSS (a) and firmness (b). Tc= optimum temperature, Ts= low temperature; Rn = nongrafted (*Cucumis sativus* var. DavosII); Rs= self grafted; Rma= *Cucurbita maxima*; Rmo= *Momordica charantia.*

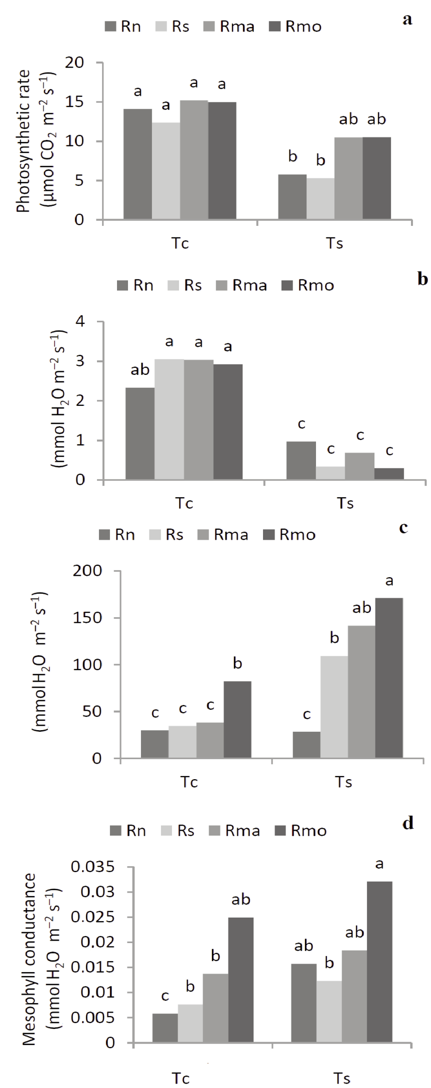

Fig. 8 - The interaction effect of temperature and cucumber grafting on photosynthesis (a), transpiration (b), stomata conductance (c), mesophyll conductance (d) Tc= optimum temperature; Ts= low temperature; Rn = nongrafted (*Cucumis sativus* var. DavosII); Rs= self grafted; Rma= *Cucurbita maxima*; Rmo= *Momordica charantia*.

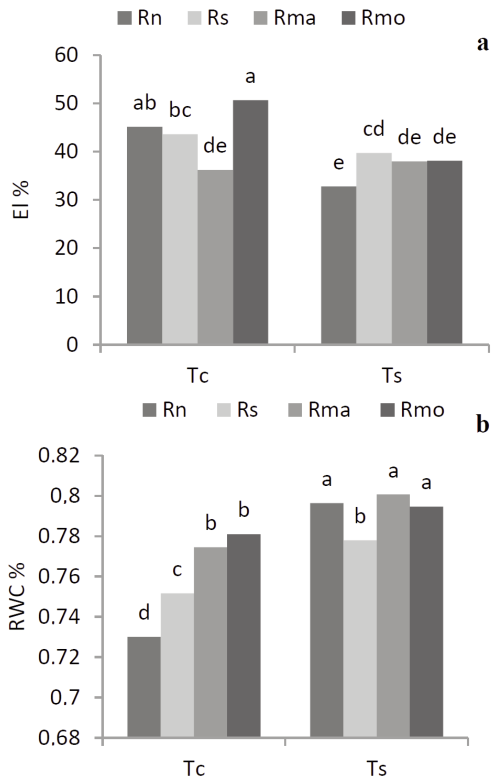

Fig. 9 - The interaction effect of temperature and cucumber grafting on El (a) and RWC (b) Tc= optimum temperature, Ts= low temperature, Rn = nongrafted (*Cucumis sativus* var. DavosII); Rs= (self grafted); Rma= *Cucurbita maxima*, Rmo= *Momordica charantia*.

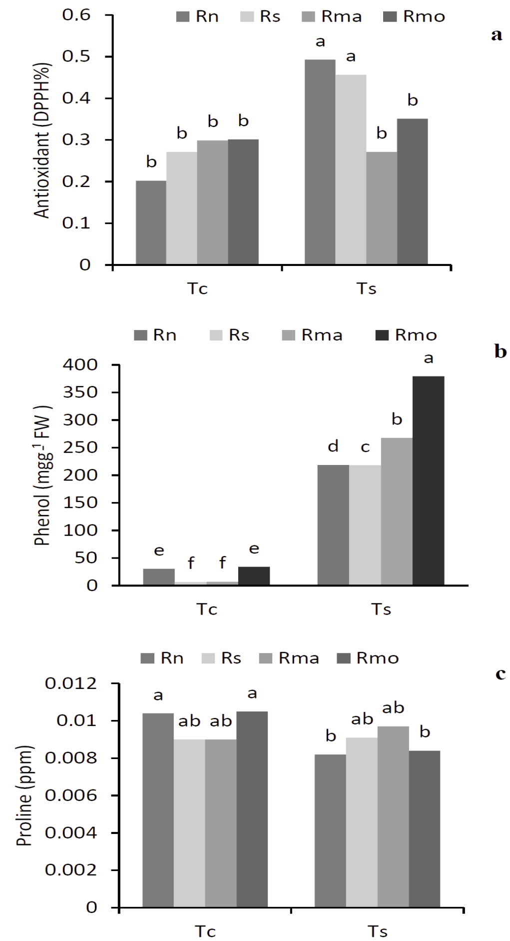

Fig. 10 - The interaction effect of temperature and cucumber grafting on antioxidant (a) and phenol (b) and proline (c) Tc= optimum temperature; Ts= low temperature; Rn = nongrafted (*Cucumis sativus* var. DavosII); Rs= self grafted; Rma= *Cucurbita maxima*; Rmo= *Momordica charantia*.

ments (Fig. 10 a). Phenol content increased in all plants at Ts and reached the highest point in Rmo×Ts (Fig. 10 b). Proline did not significantly change among treatments (Fig. 10 c).

Increasing antioxidant content with using rootstock was reported by Rouphael *et al*. (2018); this may help grafted plant to have a better function under different stress.

The photosynthesis rate and SPAD value showed that the SPAD value did not change in Rmo. Thus, photosynthesis did not change. However, the same result was not seen in Rma. Therefore, it seems that another mechanism interfered with the stability of photosynthesis in Rma in this condition rather than changes of chlorophyll content (Fig. 6 a and 7a).

Some researchers demonstrated that the photosynthesis rate was influenced by leaf area, stomatal conductance, and chlorophyll content. In our study, low-temperature stress caused a reduction in SPAD value and resulted in photosynthesis reduction. On the other hand, the photosynthesis in grafted cucumbers having high SPAD value and stomata conductance improved under low-temperature stresses. Davis *et al.* (2008) reported that grafting increased photosynthesis under salt stress conditions too.

Grafted cucumbers showed higher photosynthesis rates, stomatal conductance, and intercellular CO_2 under salt stresses compared with non-grafted plants (Yang *et al.,* 2006). Massai *et al.* (2004) and Moya *et al.* (2002) reported that grafting improved photosynthesis under salt stress conditions, too. Rootstock-induced changes in stomatal development, as reduced transpiration relates to lower stomatal density in grafted plants (Kumar *et al*., 2017).

Zhou *et al.* (2009) reported that increasing the photosynthesis rate by grafting plants onto a different rootstock might be due to a decrease in the ROS concentration in the leaves. Beside, grafting increased water and nutrient uptake of the plant and could enhance its photosynthesis (Martinez-Ballesta *et al.,* 2010). Salehi *et al.* (2010) reported that melon grafting had high CO_2 assimilation due to increasing stomata conductance and Ci and resulted in an increase in photosynthesis.

The photosynthesis rate and the fresh shoot weight of Rmo and Rma did not significantly change at both temperatures. Thus, low temperatures could not affect the photosynthesis rate in Rmo and Rma. On the other hand, decreasing the fresh weight was seen at Ts in Rn due to a decrease of photosynthesis rate in these rootstocks (Fig. 7).

Overall, low stomata and mesophyll conductance of un-grafted cucumbers which have adverse effects on water relation, mineral nutrient uptake and transport (Kumar *et al*., 2017), carbohydrate and hormone relationship, photosynthesis and respiration rates result in a decline in yields (Kozlowiski, 1984; Barrick and Noble, 1993; Bacanamwo and Purcell, 1999). These results were in line with our results at low-temperature stresses. Still, when cucumbers were grafted onto Karella they were kept under proper conditions for more efficient photosynthesis, and consequently, a better yield was produced.

The result of the study showed that salinity stresses reduced the K concentration in melons and cucumbers and grafted plants had higher nutrient concentrations than non-grafted plants. Many rootstocks are capable of increasing the uptake and translocation of nutrients (Kumar *et al*., 2017). In agreement with our result, Colla *et al.* (2013) reported that cucumber grafting had no influence on K and P concentrations in the leaves, root, and fruit under salt stresses. Our result showed that the highest K concentration was in Rmo at both temperatures and P concentration increased by grafting at Tc.

4. Conclusions

It seems that the photosynthetic traits of grafted cucumbers were not affected mainly by Rmo and Rma at stress conditions. On the other hand, hormonal changes or nutrient uptake of these rootstocks seemingly caused lesser root and additional vegetative growth but stimulated more fruit and yields. The effect of these rootstocks on productivity growth resulted in more male flowers than female ones, which was predictable according to the productive growth model of Cucurbitaceae in which male flowers and then female flowers emerged in the bush. Accordingly, if this experiment lasted more, it might result in more female flowers, suggesting that further investigations might be needed. All in all, using Rmo as well as Rma is recommended after testing the fruit quality and the economic yield.

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