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Full Length Article

Hybrid *Cucurbita* Rootstocks Improve Root Architecture, Yield, Quality, and Antioxidant Defense Systems of Cucumber (*Cucumis sativus*) Under Low Temperature Conditions

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Abstract

Cucumber (*Cucumis sativus* L.) is one of the sensitive vegetable species to low temperature stress. In recent years, grafting technology has been utilized to meet consumer demand for better price in off-season growing periods. In this study, the performance of two cucumber cultivars grafted onto 12 C*ucurbita* hybrid rootstocks were investigated at low temperature in Samsun, Turkey. The root morphology and biochemical characteristics of grafted and non-grafted cucumber plants in temperature-controlled and light-controlled greenhouses were determined. In addition, in the late autumn period, under unheated greenhouse conditions, the effects of rootstocks on yield and quality of cucumber plants were investigated. The root system architecture (RSA) (root length, root surface area, and root volume) of the grafted plants under low-temperature conditions was found to be approximately four times higher than the non-grafted plants. Fruit skin thickness, fruit flesh-skin firmness values increased as a result of grafting. Fruit color, total soluble solids (TSS), fruit appearance, and taste values were not affected by grafting. Under low-temperature conditions, the number of fruits per plant, yield and late yield values were significantly affected and increased with rootstock use. The average yield of the non-grafted cucumber cultivars was 24.8 t/ha while the highest total yield was 55.2 t/ha and 49.2 t/ha in cucumber cultivars grafted onto HMO21 and HMO20 rootstocks, respectively. The average superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) enzyme activities of the grafted combinations were better under low-temperature conditions and increased by 5%, 15%, and 7%, respectively compared with non-grafted plants. Furthermore, malondialdehyde (MDA) concentration in scion × rootstock combinations was 40% lower and the hydrogen peroxide (H_2O_2) content was also found 30% lower than non-grafted plants. It was determined that HMO9, HMO20 and HMO21 hybrid *C. moschata* rootstocks showed better performance under low-temperature conditions. It was also determined that in the selection of low-temperature tolerant cucumber rootstocks, APX enzyme activity and root surface area may be an important selection and breeding criteria. © 2020 Friends Science Publishers

Keywords: Abiotic stress; *Cucurbita*; ROS; Rootstock breeding; Yield

Introduction

Many vegetable species are sensitive to low temperature and can be grown in a short period. However, consumers are increasingly demanding access to vegetables out of growing season. Although vegetables can be produced by providing heat under protected cultivation system, however, the costs are high. Growth and yield performance of cucumber decreases below 16°C (Kozik and Wehner 2008). Below 12°C, photosynthetic activity, cytokinin production and the uptake of mineral substances are limited, having a significant negative effect on vegetative and reproductive development (Hetherington and Smillie 1989). Low temperature tolerance in cucumber has low degree of heritability (Nienhuis and Lower 1981; Wehner and Kozik 2007). Therefore, the low temperature tolerant cultivar related breeding studies are limited (Wehner 1984; Yan *et al.* 2010).

The production of grafted transplants is a technique in which rootstock and scion are combined to form a new plant. It has been reported that the yield in cucumber can be increased by 8.8% to 57.0% using rootstocks (Bie *et al.* 2017; Nawaz *et al.* 2017). Grafted cucumber plants grown at 10°C during night had higher yield and survival rate compared with non-grafted plants (Nijs 1981). Today, one of the most effective ways to expand the production season and increase yield is to use low-temperature tolerant rootstocks. Under low-temperature conditions, the roots of *Cucurbita ficifolia* rootstock have higher water and nutrient uptake capacity compared with cucumber roots, and cytokinin production is also higher (Tachibana 1988). In many studies, it has been reported that grafted cucumbers under low-temperature conditions are more tolerant than non-grafted cucumber plants (Tachibana 1982; Bulder *et al.* 1991; Schwarz *et al.* 2010). Currently, the rootstocks of

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C. moschata, *C. maxima* × *C. moschata*, and *C. ficifolia* are widely used for cucumber (Davis *et al.* 2008a; Nawaz *et al.* 2016; Bie *et al.* 2017). It has been reported that *C. ficifolia* rootstocks are tolerant to low temperatures (Tachibana 1988; Ahn *et al.* 1999; Lee *et al.* 2005). However, in these rootstock cultivars; there are some problems, including graft incompatibility (Lee *et al.* 2010), grafting difficulties (Davis *et al.* 2008a), lack of resistance to *Fusarium oxysporum* f. spp. *cucumerinum* (Lee 2003; Louws *et al.* 2010), low germination rate, and non-uniformity (Davis *et al.* 2008a). Therefore, its use at global level is low except for East Asian countries. Hybrid *C. moschata* rootstocks have gained popularity during the recent years due to their positive effect on fruit quality and shelf life (Sakata *et al.* 2008a; Rouphael *et al.* 2017). However, in this species, rootstock selection studies related to low-temperature tolerance are limited.

The ability of the roots to absorb water and nutrients under low-temperature conditions directly affects the performance of above ground plant parts. In general, root structures (Zijlstra *et al.* 1994) and polygenic variations (Schiefelbein and Benfey 1991) in the plant should be further investigated for rootstock breeding programs, and the selections should be made accordingly (Koevoets *et al.* 2016; Zhou *et al.* 2018). However, it is difficult to examine the structure of roots, being underground. Therefore, rootstock selection studies based on root phenotypic characteristics under low-temperature stress conditions are also limited (Schwarz *et al.* 2010). Using digital imaging systems, detailed investigation of root structures can be carried out (Paez-Garcia *et al.* 2015; Cáceres *et al.* 2017). In this study, the root structure of cucumber-grafted hybrid *Cucurbita* rootstocks grown under low-temperature conditions was examined and their relationship with cucumber yield was investigated.

In plants, the biochemical degradation in membrane lipid composition, and variations in proline and antioxidant enzymes is observed under low-temperature conditions (Chen 2012; Lang *et al.* 2017). The detection of plants that are less affected by reactive oxygen species (ROS) by producing more antioxidant enzymes under stress conditions has made important contributions to the ongoing plant breeding studies during the recent years (Loudet and Hasegawa 2017). The majority of literature on the biochemical contents of *Cucurbita* rootstocks and cucumbers under low-temperature conditions revealed that the analyses were carried out during the seedling stage and there were differences in terms of enzyme activities (Li *et al.* 2008; Lee *et al.* 2009; Zhang *et al.* 2012; Li *et al.* 2015). It was not clearly determined whether the tolerance detected during the seedling stage included the subsequent growth phases of the cucumber, or the yield and quality performance; particularly during the autumn growing season, where temperatures decreases gradually. In addition, the studies examining the relationship among ROS, RSA, and yield using a large number of grafted cucumber combinations under low-temperature conditions is limited.

In this study the effect of suitable hybrid *Cucurbita* rootstocks on fruit yield and quality for cucumber plants in late autumn under unheated greenhouse conditions was investigated. In addition, the root architecture and some biochemical characteristics of rootstock × scion combinations grown under controlled conditions are presented, and their effect on the yield characteristics of cucumbers are discussed.

Materials and Methods

Plant materials

This study was carried out in the physiology laboratory and greenhouses at the Black Sea Agricultural Research Institute, Samsun, Turkey during 2016 to 2017. As the rootstock, intraspesific *C. moschata* and interspesific *C. maxima* × *C. moschata* hybrid rootstocks were used considering their resistance to *Fusarium oxysporum* f. spp. *cucumerinum* (Göçmen *et al.* 2014) and grafting success rate for cucumber (Karaağaç *et al.* 2018) (Table 1). Their parents were also selected by morphological and physiological properties (Karaağaç *et al.* 2016, 2017) under low-temperature stress conditions.

The grafting was performed at the Genetika Seeds Co. Ltd., Antalya, Turkey. The rootstock seeds were sown on 15 August 2016 in a peat + perlite (3:1 V/V) medium 4 days after the scion seeds. The seedlings were grown at 25/20°C day/night temperatures in a controlled greenhouse until the grafting stage. Gordion F_1 and Asar F_1 cucumber cultivars were grafted onto twelve rootstocks by using the splice grafting method (Davis *et al.* 2008b) on 1 September 2016. Grafting was made for 150 seedlings of each combination. Post-grafting maintenance procedures were carried out according to Karaağaç (2013).

Root system architecture (RSA)

The seedlings of 24 grafted combinations and two non-grafted controls were transferred to 12 L pots in a temperature controlled and light controlled greenhouse. Peat, perlite, sand and loamy soil were used as the growing media. The experiment was carried according to randomized block experimental design with three replications. Each replicate was comprised of 10 plants. Cultivation was carried out until the first fruit harvest stage (approximately 40 days) under controlled photoperiodic conditions of a 16 h daytime (12°C and 270 mol m^{-2} s^{-1} low light intensity) and an 8 h night time (8°C, dark) (Smeets and Wehner 1997; Zhou *et al.* 2004). The roots of the plants were removed from the pots, washed and dried. The roots were scanned (Epson Expression 10000XL, Epson America Inc., Long Beach, CA, USA) at 400 dpi resolution. WinRHIZO (version 2013, Regent Instruments, QC, Canada) software was used to calculate the total root length

Table 1: Sources of investigated hybrid rootstock genotypes/cultivars and cucumber cultivars

Species	Code/cultivar name	Source
C. moschata × *C. moschata*	HMO9, HMO11, HMO13, HMO17, HMO18, HMO20, HMO21, HMO24	BSARI
C. maxima × *C. moschata*	ISR1	
C. moschata × *C. moschata*	New Super Unryu F_1 (NSU)	Kurume Vegetable Breeding Co., Japan
C. maxima × *C. moschata*	Maximus F_1 (MAX)	Antalya Tarim, Turkey
C. ficifolia	Triumph F_1 (TRI)	Hild Samen GMBH, Germany
C. sativus (Scions)	Asar F_1 (A)	Genetika Seeds, Turkey
	Gordion F_1 (G)	Antalya Tarim, Turkey

BSARI: Black Sea Agricultural Research Institute, Samsun, Turkey

(m), root volume (cm^3), and root surface area (cm^2). Subsequently, the roots were dried at 70°C for 48 h and weighed (Bekar *et al.* 2016).

Biochemical analyses

Five mid-aged leaves from the central part of three plants grown under controlled low temperature and photoperiod conditions were taken from each plot. Subsequently, 0.5 g leaf sample was crushed in 2 mL of 50 m*M* of phosphate buffer (pH 7.0) containing 1% (w/v) polyvinylpolypyrrolidone (PVPP) and 1 m*M* of ethylene diamine tetra acetic acid (EDTA) with liquid nitrogen and glass dust. The extract was centrifuged at 15000 g for 20 min at 4°C and the resulting filtrate was stored at −20°C (Drążkiewicz *et al.* 2004). H_2O_2 analysis was performed according to Patterson *et al.* (1984). MDA analysis was carried out according to Heath and Packer (1968). For proline analysis, 0.2 g fresh leaf sample was homogenized with 4 mL of 3% sulfosalicylic acid solution. Then 1 mL of acid-ninhydrin and 1 mL of glacial acetic acid was added to 1 mL of the homogenized sample and filtered and the mixture was kept in a water bath at 100°C for 60 min. The absorbance of the samples was measured at a wavelength of 546 nm and the amount of proline was calculated in μmol g^{-1} FW (Claussen 2005). For SOD analysis 50 m*M* of K-phosphate buffer (pH 7.8), 0.1 m*M* of Na-EDTA, 75 μm of NBT, 2 μm of riboflavin and 13 m*M* of methionine were used. The control and reaction solutions were measured at a wavelength of 560 nm (Dhinsa *et al.* 1981). APX values were analyzed according to the method of Nakano and Asada (1981). GR values were calculated by determining the oxidation of NADPH at 340 nm (ε: 6.2 m*M*.cm^{-1}) according to the method described by Rao (1992).

Yield and fruit quality experiments

The experiment was conducted in Samsun, Turkey (41°23'12.36'' N and 36 49'61.54'' E) on 20 September 2016 in an unheated greenhouse. The experiment was designed according to randomized complete block design; and randomization was ensured by placing the rootstocks on the sub-plots and scions on the main plots, and replicated three times. Each plot had 20 plants. The plants of 24 grafting combinations and two non-grafted controls were transplanted in double row with a distance of 100 × 50 × 50 cm. According to the soil analysis results, 150 kg.ha^{-1} of P_2O_5, 300 kg.ha^{-1} of K_2SO_4, 500 kg.ha^{-1} of $Ca(NO_3)_2$, and microelement fertilizers were applied. Fruit harvestings was started on October 25, 2016 and lasted for about two months.

Fruit dimensions were measured for 10 fruits taken from each plant. The fruit skin thickness (mm) was determined by measuring the distance between the outside of the fruit and the point where the tonal green color started using a digital Vernier caliper. To determine TSS values, 40 juice samples were taken from 20 fruits from each repeated section. A digital refractometer (Hanna HI 96801, Woonsocket, RI, USA) was used for TSS measurement. The fruit skin and flesh firmness were measured using 11.3 mm and 8 mm diameter head on a digital penetrometers (PCA-PT 200, PC Instruments, Lucca, Italy). Skin firmness was measured at the center of the fruit. Flesh firmness was determined from a horizontal section taken at a thickness of 12 mm (Sakata *et al.* 2008a). Fruit sensory properties were determined by panel of ten tasters. Panelists used a scale of 0 (very poor) to 5 (very good) for fruit taste and appearance (Edelstein *et al.* 2014). Fruit skin color measurement was carried out using the CIE $L^*a^*b^*$ color classification system using a CR-410 (Konica Minolta Co., Osaka, Japan) digital colorimeter. A total of three readings were taken from the center, lower and upper parts from 20 fruits of each combination, and their averages were taken. The number of fruits/plant and total yield (t/ha) were determined from the total amount of fruit harvested from each plot. The yield obtained during the last month of the harvesting period was taken as the late season yield rate.

Statistical analysis

The analysis of variance (ANOVA) was performed using SAS-JMP (version 5.01) statistical software. Significant differences among the groups were determined by Tukey's multiple range test at $P < 0.01$ and $P < 0.05$ (Tukey *et al.* 1985). Moreover, correlation analysis was used to determine whether there was a statistical relationship among RSA, cucumber yield characteristics and biochemical contents.

Results

Low temperature tolerance

Root system architecture (RSA): RSA parameters were significantly affected by the rootstocks ($P < 0.01$) under low-temperature conditions (Table 2). However, these parameters were found to be non-significant in terms of the scion cultivars and rootstock × scion interactions. The root volumes were found to be 1.52 cm^3 in non-grafted plants and 9.91 cm^3 in grafted combinations. HMO11 (11.78 cm^3), HMO24 (10.50 cm^3) and ISR1 (10.32 cm^3) rootstocks had the highest values and were placed in the same group. Root length ranged from 0.79 m (non-grafted cucumber) to 3.81 m for HMO11. The average lengths of the roots of the grafted combinations were 3.75 times longer compared with non-grafted plants (Fig. 1). In grafted combinations, root surface areas ranged from 453 to 749 cm^2. In non-grafted genotypes, the average root surface area was 119 cm^2. In terms of root surface area, cucumber grafted onto HMO11, HMO24 and ISR1 rootstocks had higher values. The highest root dry matter was observed in combinations grafted onto HMO11 (1.95 g) and ISR1 (1.81 g) rootstocks (Table 2). The lowest root dry weight among the grafted combinations was found with the HMO13 rootstock. In non-grafted genotypes, this average value was 0.56 g.

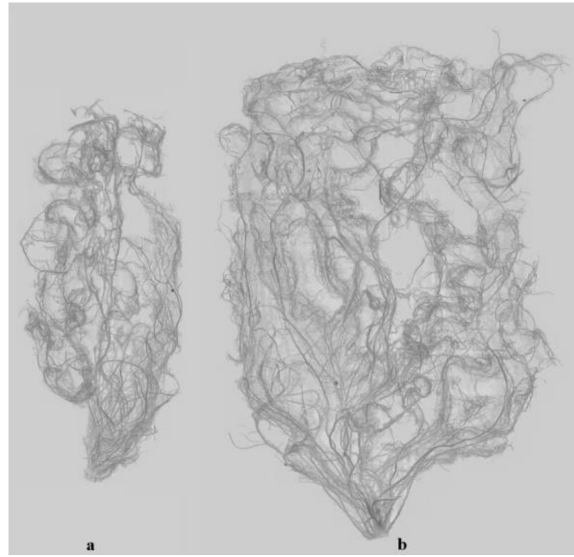

Fig. 1: Screening of roots of non-grafted Gordion F_1 (**a**) and Gordion / HMO11 (**b**) combination using the WinRhizo program

ROS and antioxidant defence system

MDA, H_2O_2, proline, SOD, APX, and GR contents were evaluated using the average values in grafted and non-grafted plants. In addition, another group was formed with HMO9, HMO20, and HMO21 rootstock combinations which are superior in terms of high yield and late yield ratio (Fig. 2). In high-yield grafting combinations, the MDA concentration was 40% lower than those of non-grafted cucumbers. Similarly, H_2O_2 content was found to be 30% lower in grafted plants compared with non-grafted plants. The average proline content were 9.90 μmol/g FW in non-grafted plants and 11.30 μmol/g FW in grafted plants. It has been observed that the combinations investigated under low-temperature conditions showed changes in antioxidant enzyme activities. In general, there were no significant differences between low-yield rootstock × scion combinations and the non-grafted cucumbers. However, SOD, APX and GR enzyme values increased by 5%, 15% and 7%, respectively, in the grafted cucumber combinations, with better results in terms of yield (Fig. 2).

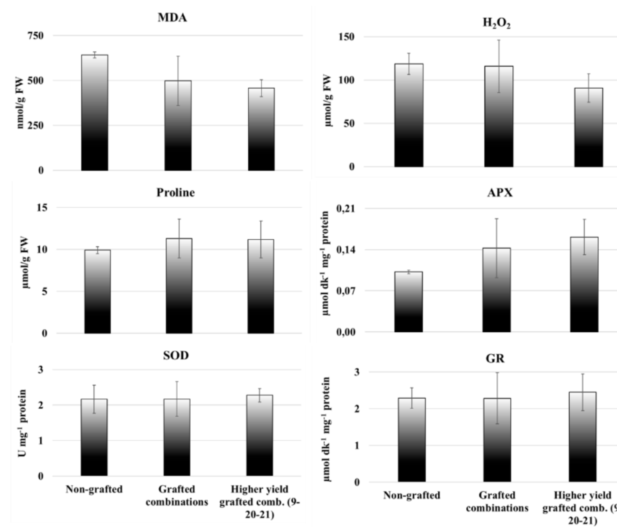

Fig. 2: Average values for MDA, H_2O_2, Proline, SOD, APX and GR contents of the non-grafted, all grafted and high-yielding grafted combinations

Yield components and fruit quality

The effect rootstocks and the scions on cucumber fruit dimensions was significant ($P < 0.01$). However, the rootstock × scion interaction was non-significant. Fruit length ranged between 14.82 cm (MAX) and 16.83 cm (HMO21) (Table 3). The average fruit length of non-grafted cucumbers was 15.93 cm. Fruit diameter ranged from 2.73 cm (TRI) to 3.29 cm (HMO21). As a result of reduced fruit diameter, the highest fruit shape index was found in TRI (*C. ficifolia*) rootstock (5.80). The lowest values were determined in plants grafted onto MAX (5.05) rootstock. This average value was 5.31 in non-grafted genotypes. The effect of scion and rootstock × scion interactions on the fruit skin thickness was non-significant. Fruit skin thickness (0.85 to 0.97 mm) increased for all grafted combinations. This value was 0.82 mm in non-grafted plants (Table 3). The average TSS value of non-grafted cultivars was 4.21% and the average TSS value of grafted cultivars was 4.18% (Table 4). In this study, both the rootstocks and the scions significantly affected the fruit skin firmness and fruit flesh firmness. The fruit flesh firmness was 24.03 N in non-grafted cultivars and 25.16 N to 33.86 N in grafted combinations (Table 4). These values ranged from 14.45 N (HMO18) to 17.84 N (TM-1). In non-grafted genotypes, this

Table 2: Root characteristics in grafted and non-grafted cucumbers under low-temperature conditions

Combinations	Root volume (cm^3)	Root length (m)	Root surface area (cm^2)	Root dry matter (g)
HMO9	9.08 ± 1.10 bc	3.33 ± 0.21 bc	550 ± 43 de	1.31 ± 0.06 de
HMO11	11.78 ± 1.41 a	3.81 ± 0.24 a	749 ± 55 a	1.95 ± 0.14 a
HMO13	8.69 ± 1.29 bc	3.17 ± 0.29 bc	623 ± 58 b-d	1.13 ± 0.19 e
HMO17	9.28 ± 0.69 bc	3.26 ± 0.11 bc	618 ± 69 b-d	1.38 ± 0.11 d
HMO18	9.36 ± 1.01 bc	3.27 ± 0.09 bc	634 ± 40 b-d	1.42 ± 0.09 d
HMO20	8.35 ± 0.32 b-d	3.02 ± 0.10 cd	563 ± 39 c-e	1.24 ± 0.08 de
HMO21	8.61 ± 1.24 bc	3.02 ± 0.08 cd	583 ± 42 b-d	1.31 ± 0.08 de
HMO24	10.50 ± 0.74 ab	3.56 ± 0.16 ab	684 ± 54 a-c	1.69 ± 0.16 bc
ISR1	10.32 ± 1.27 a-c	3.44 ± 0.09 ab	697 ± 52 ab	1.81 ± 0.09 ab
NSU	6.33 ± 0.89 d	2.64 ± 0.17 d	453 ± 41 e	1.46 ± 0.17 cd
MAX	8.21 ± 1.00 cd	2.98 ± 0.04 cd	552 ± 55 de	1.23 ± 0.04 de
TRI	8.48 ± 0.78 b-d	3.03 ± 0.13 cd	555 ± 44 de	1.25 ± 0.13 de
NG	1.52 ± 0.18 e	0.79 ± 0.05 e	119 ± 14 f	0.56 ± 0.05 f
Ave. A	8.38	3.03	566	1.35
Ave. G	8.62	3.02	569	1.37
R	**	**	**	**
S	NS	NS	NS	NS
R × S	NS	NS	NS	NS
CV (%)	18.0	9.0	15.0	11.7

A: Asar F_1 (*C. sativus*); G: Gordion F_1 (*C. sativus*); NSU: New Super Unryu F_1 (*C. moschata* × *C. moschata*); MAX: Maximus F_1 (*C. maxima* × *C. moschata*); TRI: Triumpf F_1 (*C. ficifolia*); NG: Non-grafted; R: Rootstock, S: Scion; Mean ± standard deviation; **: $P < 0.01$; NS: Non-significant

Table 3: Fruit dimension values of grafted and non-grafted cucumbers for the late autumn period

Combinations	Fruit length (cm)	Fruit diameter (cm)	Fruit shape index	Fruit skin thickness (mm)
HMO9	15.81 ± 0.38 bc	3.04 ± 0.07 c	5.39 ± 0.19 b-d	0.92 ± 0.06 cd
HMO11	15.99 ± 0.40 bc	2.97 ± 0.08 c	5.52 ± 0.27 a-c	0.94 ± 0.04 a-c
HMO13	16.18 ± 1.01 bc	2.99 ± 0.12 c	5.54 ± 0.21 a-c	0.97 ± 0.04 a
HMO17	16.50 ± 0.83 ab	3.21 ± 0.09 b	5.26 ± 0.20 cd	0.90 ± 0.04 d
HMO18	16.71 ± 0.92 ab	3.01 ± 0.07 c	5.69 ± 0.19 ab	0.85 ± 0.03 e
HMO20	15.90 ± 0.91 bc	2.99 ± 0.12 c	5.47 ± 0.50 a-c	0.90 ± 0.07 cd
HMO21	16.83 ± 0.77 a	3.29 ± 0.11 a	5.25 ± 0.21 cd	0.93 ± 0.04 a-d
HMO24	16.56 ± 0.75 ab	3.08 ± 0.08 bc	5.53 ± 0.52 a-c	0.93 ± 0.07 a-d
ISR1	16.34 ± 1.08 bc	2.96 ± 0.10 c	5.66 ± 0.58 ab	0.97 ± 0.06 a
NSU	16.51 ± 0.80 ab	2.95 ± 0.13 c	5.76 ± 0.70 a	0.86 ± 0.05 e
MAX	14.82 ± 0.50 d	2.96 ± 0.11 c	5.05 ± 0.35 d	0.96 ± 0.05 ab
TRI	15.45 ± 1.14 cd	2.73 ± 0.09 d	5.80 ± 0.54 a	0.86 ± 0.08 e
NG	15.93 ± 0.85 bc	3.08 ± 0.16 bc	5.31 ± 0.26 cd	0.82 ± 0.02 e
Ave. A	16.75 A	2.97 B	5.78 A	0.91
Ave. G	15.49 B	3.06 A	5.18 B	0.90
R	**	**	**	**
S	**	**	**	NS
R × S	NS	NS	NS	NS
CV (%)	3.9	3.7	4.4	3.0

A: Asar F_1 (*C. sativus*); G: Gordion F_1 (*C. sativus*); NSU: New Super Unryu F_1 (*C. moschata* × *C. moschata*); MAX: Maximus F_1 (*C. maxima* × *C. moschata*); TRI: Triumpf F_1 (*C. ficifolia*); NG: Non-grafted; R: Rootstock, S: Scion; Mean ± standard deviation; **: $P < 0.01$; NS: Non-significant

average value was 16.13 N. Fruit appearance and taste values were not different; however, many of the grafted combinations received higher scores compared with non-grafted cultivars (Table 4). The effect of the cucumber cultivar used on brightness, a, b, saturation (C) and hue angle values was significant, whereas the effect of rootstock and rootstock/scion interaction was non-significant (Table 5).

The rootstocks were found to have an obvious effect on the number of cucumber fruits (Table 6). Cucumber cultivars grafted onto HMO21 and HMO20 rootstocks had 22.4 and 20.1 fruits/plant, respectively. The lowest performance was observed for non-grafted plants having 10.5 fruits/plant. The total yield was affected by the rootstocks ($P < 0.01$), whereas scions and scion × rootstock interactions were non-significant (Table 6). The cucumber cultivars grafted onto HMO21 and HMO20 rootstocks were statistically in the same group, reaching the highest total yield of 55.2 t/ha and 49.2 t/ha, respectively. TRI and non-grafted cucumber cultivars formed the lowest yield group with 29.1 t/ha and 24.8 t/ha, respectively. In terms of late yield ratio, only the effect of the cucumber cultivars was observed.

A correlation analysis was carried out between the yield components and root properties for all combinations. The relationship between root length, surface area and root volume and the number of fruits and yield was positive ($P < 0.01$) (Table 7). There was no relationship between root dry weight and yield characteristics. Examining the relationship between RSA and ROS, it was found that only the H_2O_2 values were negatively correlated (r: -0.62) (Table 7).

Table 4: Fruit quality properties of grafted and non-grafted cucumbers for the late autumn period

Comb.	TSS (%)	Fruit skin firmness (N)	Fruit flesh firmness (N)	Fruit appearance (1–5)	Taste index (1–5)
HMO9	4.72 ± 0.31	30.15 ± 1.88 bc	15.54 ± 0.37 c-e	2.45 ± 0.07	2.65 ± 0.49
HMO11	4.20 ± 0.27	30.68 ± 2.16 b	15.40 ±1.59 de	2.70 ± 0.14	3.10 ± 0.14
HMO13	4.20 ± 0.30	34.50 ± 1.41 a	16.59 ± 1.07 bc	2.68 ± 0.04	3.20 ± 0.09
HMO17	4.15 ± 0.09	25.16 ± 1.69 fg	15.86 ± 0.56 cd	2.45 ± 0.21	2.80 ± 0.05
HMO18	4.06 ± 0.15	27.89 ± 0.72 de	14.45 ± 0.50 e	2.80 ± 0.14	2.90 ± 0.57
HMO20	4.46 ± 0.33	29.03 ± 0.78 b-d	16.53 ± 0.66 bc	2.70 ± 0.05	2.70 ± 0.06
HMO21	4.06 ± 0.20	30.62 ± 2.09 b	16.33 ± 0.46 b-d	2.68 ± 0.10	2.95 ± 0.07
HMO24	4.02 ± 0.13	33.99 ± 1.23 a	17.26 ± 0.49 ab	2.68 ± 0.11	2.75 ± 0.49
ISR1	4.06 ± 0.16	33.86 ± 1.28 a	17.84 ± 0.41 a	2.75 ± 0.07	3.00 ± 0.42
NSU	4.13 ± 0.09	27.89 ± 0.42 de	14.52 ± 0.40 e	2.53 ± 0.18	2.70 ± 0.14
MAX	4.22 ± 0.18	28.23 ± 1.62 c-e	17.77 ± 1.33 a	2.63 ± 0.40	2.70 ± 0.14
TRI	3.88 ± 0.32	26.53 ± 0.18 ef	15.76 ± 0.58 cd	2.65 ± 0.14	2.85 ± 0.35
NG	4.21 ± 0.04	24.03 ± 0.88 g	16.13 ± 1.18 cd	2.45 ± 0.07	2.50 ± 0.14
Ave. A	4.15	28.64 B	15.80 B	2.69	2.82
Ave. G	4.20	30.21 A	16.50 A	2.56	2.85
R	NS	**	**	NS	NS
S	NS	**	**	NS	NS
R × S	NS	NS	NS	NS	NS
CV (%)	3.76	4.97	3.03	10.8	9.8

A: Asar F_1 (*C. sativus*); G: Gordion F_1 (*C. sativus*); NSU: New Super Unryu F_1 (*C. moschata* × *C. moschata*); MAX: Maximus F_1 (*C. maxima* × *C. moschata*); TRI: Triumpf F_1 (*C. ficifolia*); NG: Non-grafted; R: Rootstock, S: Scion; Mean ± standard deviation; **: $P < 0.01$; NS: Non-significant

Table 5: Digital color values of the grafted and non-grafted cucumber fruits for the late autumn period

Combinations	L	a	b	C*	H°
Average R	33.2	-10.4	13.6	17.2	127.3
Average A	34.4 A	-10.9 A	14.6 A	18.2 A	126.7 B
Average G	31.9 B	-9.8 B	12.7 B	16.1 B	127.8 A
R	NS	NS	NS	NS	NS
S	**	**	**	**	*
R × S	NS	NS	NS	NS	NS
CV (%)	7.6	10.7	12.5	11.6	1.3

A: Asar F_1 (*C. sativus*); G: Gordion F_1 (*C. sativus*); R: Rootstock, S: Scion; **: $P < 0.01$; *: $P < 0.05$; NS: Non-significant

Table 6: The yield components of the grafted and non-grafted cucumbers for the late autumn period

Combinations	Fruit number/plant	Total yield (t/ha)	Late season yield (%)
HMO9	19.3 ± 0.61 bc	46.3 ± 3.14 bc	33.9 ± 5.69
HMO11	16.8 ± 0.83 c-e	41.9 ± 2.81 c-e	27.6 ± 2.14
HMO13	15.6 ± 2.67 e	37.7 ± 3.90 de	28.5 ± 3.45
HMO17	19.3 ± 0.22 bc	47.5 ± 2.11 bc	28.7 ± 4.01
HMO18	18.7 ± 1.73 b-d	47.0 ± 2.85 bc	28.5 ± 5.51
HMO20	20.1 ± 1.81 ab	49.2 ± 3.06 ab	29.1 ± 3.64
HMO21	22.4 ± 1.21 a	55.2 ± 1.84 a	29.0 ± 3.16
HMO24	18.1 ± 0.70 b-e	44.6 ± 1.16 b-d	28.3 ± 1.51
ISR1	19.2 ± 3.62 bc	47.4 ± 1.25 bc	28.3 ± 2.58
NSU	18.1 ± 0.68 b-e	44.0 ± 2.52 b-e	29.4 ± 3.67
MAX	16.0 ± 3.18 de	37.2 ± 4.96 e	27.9 ± 6.36
TRI	12.2 ± 2.68 f	29.1 ± 5.57 f	27.4 ± 4.85
NG	10.5 ± 1.38 f	24.8 ± 3.02 f	28.9 ± 2.03
Average A	17.3	43.8	30.6 A
Average G	17.4	41.1	27.6 B
R	**	**	NS
S	NS	NS	**
R × S	NS	NS	NS
CV (%)	3.4	11.6	15.1

A: Asar F_1 (*C. sativus*); G: Gordion F_1 (*C. sativus*); NSU: New Super Unryu F_1 (*C. moschata* × *C. moschata*); MAX: Maximus F_1 (*C. maxima* × *C. moschata*); TRI: Triumpf F_1 (*C. ficifolia*); NG: Non-grafted; R: Rootstock, S: Scion; Mean ± standard deviation; **: $P < 0.01$; *: $P < 0.05$; NS: Non-significant

Discussion

RSA parameters of all grafted cucumber combinations except *C. ficifolia* rootstock were found to be higher than non-grafted cucumbers. In many studies where the *C. ficifolia* was used as rootstock under low temperature conditions, it has been reported to produce more biomass compared with non-grafted plants (Tachibana 1982, 1986; Ahn *et al.* 1999). However, in this study, root biomass was less because the *C. ficifolia* rootstock was affected by

Table 7: Correlation values among RSA, yield components, and biochemical contents for cucumber in low temperature stress condition

	Root length	Root surface area	Root volume	Root dry weight
Fruit number/plant	0.58**	0.63**	0.51**	0.29
Yield	0.57**	0.58**	0.50**	0.31
MDA	-0.12	0.04	-0.08	0.21
H_2O_2	-0.44*	-0.62**	-0.50**	-0.45*
Proline	0.06	0.01	0.03	0.11
SOD	-0.08	0.09	-0.09	0.04
APX	0.03	0.09	-0.05	-0.21
GR	-0.09	-0.10	-0.14	-0.37

**: $P < 0.01$; *: $P < 0.05$

fusarium wilt disease. Fita *et al.* (2008) investigated the root structures in 10 different species of the *Cucurbita* genus. In this study, *C. moschata* was identified as the longest rooting species after *C. argyrosperma* with a root length of approximately 3 m. Fita *et al.* (2007) determined that melon grafted onto *C. melo* ssp. *agrestis* had a two-fold surface area increase and a four-fold total root length increase compared with that of non-grafted melon. Ntatsi *et al.* (2014) reported that cold tolerant *Solanum habrochaites* (LA1777) cultivar was used as rootstock under low-temperature conditions and the root dry weight, length and projected area values were higher than those of non-grafted tomato. In another study, the root biomass ratio was higher for LA1777 rootstock under low-temperature conditions (15/15°C) (Venema *et al.* 2008). In low-temperature tolerant pepper rootstock breeding studies, the root dry weight and root volume of grafted plants were higher than those of non-grafted plants (Shu *et al.* 2016; Aidoo *et al.* 2018). In terms of root structures, it was determined that HMO11 (*C. moschata* F_1) and ISR1 (*C. maxima* × *C. moschata* F_1) were the strongest rootstocks for cucumber under low-temperature conditions.

High ROS levels formed during stress may cause lipid, nucleic acid, protein and membrane damage (Chen 2012). It was observed that with rootstock use, under low-temperature stress conditions, cell membranes were less damaged and ROS production was reduced, positively affecting the yield. Similar results were observed in other studies under low-temperature stress conditions (Zhou *et al.* 2007; Yan *et al.* 2013; Li *et al.* 2015). In general, proline content of high-yielding grafted combinations were also found higher. Feng *et al.* (2003) and Li *et al.* (2015) reported that proline contents are high in tolerant plants under low temperature conditions. The high levels of antioxidative enzyme provide stabilization by reducing the ROS damage (Gill *et al.* 2012). Examining the studies conducted under low-temperature conditions, it was observed that SOD, APX, and GR values were higher in *C. ficifolia* than in cucumber seedlings (Zhang *et al.* 2012; Li *et al.* 2015). Also, Zhou *et al.* (2006) found that low temperature tolerant seedlings were found to have higher APX and GR contents than sensitive ones. Lin *et al.* (2013) determined higher SOD and APX enzyme activity in pumpkin seedlings tolerant to low temperature. However, in these studies, the relationship between low-temperature tolerance provided by high enzyme content and yield was not investigated. In this study, tolerance varied depending on the rootstock used, and the antioxidant enzyme levels were generally higher in combinations with the highest yield under low-temperature conditions. It is thought that the APX enzyme level can be used as a biochemical marker in the rapid and early detection of cucumber rootstocks which best adapt to low temperature.

Davis *et al.* (2008b) reported that fruit taste and quality depended on many parameters and taste and quality may change in a positive or negative direction because of grafting. Staub *et al.* (2008) reported that, in general, the fruit shape index value is preferred to be 4 or higher. Therefore, it can be said that the fruit shape index of all combinations examined under low-temperature conditions were within normal limits. Our rootstocks did not affect the TSS ratio under low-temperature conditions. The results of studies on the effect of grafting on TSS values had different results. For example, Li *et al.* (2006) and Farhadi *et al.* (2016) reported that rootstock use decreased the TSS of cucumber fruits, whereas Colla *et al.* (2012) reported that rootstock use increased the TSS values; Rouphael *et al.* (2008), Huang *et al.* (2009) and Bekar *et al.* (2017) reported that there were no changes, and Yarsi *et al.* (2008), Huang *et al.* (2010) and Chao and Yen (2013) reported that TSS of cucumber fruits changed depending on the rootstock. In this study, fruit skin of grafted cucumbers was 12% firmer compared with non-grafted cucumbers. In particular, interspecific hybrid rootstocks increased the flesh firmness more than pumpkin rootstocks. The hard fruit skin in cucumber increases resistance to transport and mechanical damage, while the thicker skin may not be preferred by consumers. Firmer fruit flesh is preferred for crispiness and longer shelf-life. Leonardi *et al.* (2017) reported that fruit firmness values may vary depending on the rootstock. In some of the studies on the effects of rootstock use on fruit firmness, values did not change (Sakata *et al.* 2008a; Colla *et al.* 2013; Bekar *et al.* 2017), whereas some studies reported an increase (Davis *et al.* 2008a; Sakata *et al.* 2008b; Savvas *et al.* 2012). No effect of rootstock use on cucumber fruit color and taste were found in the study. Velkov and Pevicharova (2016) reported that the taste and overall appearance values of cucumber cultivars grafted on different rootstocks differed depending on the combinations used. Zhou *et al.* (2006) and Savvas *et al.* (2012) found that the effect of rootstock on fruit color was not significant. On the contrary, Colla *et al.* (2012) reported that rootstock use increased the brightness value by 2%. These reports on the changes conferred by grafting onto cucumber fruit quality provide different information. Most of the quality traits exhibit a polygenic inheritance and are influenced by adverse environmental conditions or rootstock-scion incompatibility (Leonardi *et al.* 2017). Even though they were under low temperature stress, most of the grafted combinations showed good performance in terms of cucumber fruit quality.

Number of fruits and yield (t/ha) of grafted cucumber combinations were found to be higher than non-grafted cucumbers grown under low temperature conditions. But *C. ficifolia* rootstocks did not show the expected performance. Because fusarium disease reduced the yield of cucumbers grafted onto TRI (*C. ficifolia*) rootstock. Louws *et al.* (2010) and Liu *et al.* (2015) reported that this species can be sensitive to *F. oxysporum* f. spp. *cucumerinum*. The studies reported that grafting increased the number of fruits in cucumber (Huang *et al.* 2009; Maršić and Jakše 2010). Nijs (1984) found that the use of rootstock increased the number of cucumber fruits under low temperature conditions. In another study on grafted tomatoes grown under low-temperature conditions, the effect of the rootstock on yield was negative (Ntatsi *et al.* 2014); however, in another study carried out under similar stress conditions, it was determined that the pepper rootstocks increased the yield (Shu *et al.* 2016). Nijs (1981) in their study on cucumber yield in the late autumn period, reported that all grafted applications resulted in higher yields. Nijs (1984) reported that rootstock use increased the yield by one-and-a-half times under low-temperature conditions. Guan *et al.* (2018) reported that, in early cucumber growing period, the use of *C. moschata* rootstocks positively affected the yield.

Conclusion

It was determined that hybrid rootstocks with higher root length and root surface area had a positive effect on cucumber yield under low-temperature conditions. However, there was no significant relationship between root dry weight and the yield characteristics of the rootstocks. Therefore, it is suggested that in rootstock breeding studies, it will be better to examine the root surface area, root length and root volume rather than root dry weight. In addition, negative relationship was found between H_2O_2 content and root biomass. High-yielding grafted combinations had higher APX enzyme activity compared with other grafting combinations and non-grafted plants. Use of rootstocks for cucumber cultivation under autumn conditions at Samsun extended the harvesting period for a month. Under low-temperature conditions, HMO9, HMO20, and HMO21 hybrid *C. moschata* rootstocks proved superior and doubled the yield and late yield compared with the non-grafted plants under low-temperature conditions. It is suggested that these hybrid rootstocks that are resistant to the fusarium wilt and have a high grafting success rate, can be substituted for *C. ficifolia* rootstocks under low-temperature conditions.

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