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The rootstock potential of Citron *(Citrullus amarus)* for watermelon

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Abstract

Nowadays, global warming, abiotic and biotic stress factors pose significant challenges to the planet. Based on the increase in the population, the crop production of most agriculturally, economically, and nutritionally important crops as well as watermelon should be increased to their maximum. Grafting is a horticultural method by which the plant tissues are connected to enable the plants to continue growing together. It is usable technique to develop several horticultural traits in watermelon production such as yield and fruit quality and quantity. The main purpose of this study was to analyze the rootstock potential of citron (*Citrullus amarus)* genotypes for watermelon in terms of total yield, fruit characteristics and fruit quality (sugar and carotenoid contents). This study was conducted in the open field under low tunnel conditions within two successive years in Adana, Türkiye. The 11 *Citrullus amarus* genotypes (Kar 234, Kar 324, Kar 326, Kar 327, Kar 328, Kar 351, Kar 374, Kar 375, Kar 376, G 38, and G 40) were used as rootstocks and Crimson Tide F_1 was scion. Commercial rootstocks: Maximus a *Cucurbita* hybrid rootstock (*Cucurbita maxima* × *Cucurbita moschata*) and Argentario (*Lagenaria siceraria*) were utilized for comparison, and non-grafted Crimson Tide was considered as a control group. According to the results, plants grafted onto *C. amarus* genotypes produced the highest total yield compared to the control group, but lower than to the plants grafted onto commercial rootstocks. All fruit measurement values of plants grafted onto commercial rootstocks resulted higher or similar than plants grafted onto *C. amarus*. It was determined that using *C. amarus* rootstocks positively affected fruit sugar content, however the amount of total lycopene and carotenoid content varied among genotypes. Kar 328, Kar 234 and Kar 351 were found to be promising genotypes as rootstock in terms of total soluble solids, total lycopene, total sugar, and total carotenoid contents respectively. In conclusion citron genotypes could be applied as an alternative rootstock for watermelon grafting program.

Keywords: Watermelon, Genetic resources, Grafting, Lycopene, Fruit quality

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INTRODUCTION

Grafting is an alternative and useful method to solve problems associated with soil-borne diseases caused by intensive cultivation in limited agricultural areas (Adiguzel et al., 2022). Grafting is a vegetative growth technique that involves combining the rootstock (plant's underground root portion, with the scion (shoot part) (Goldschmidt, 2014). Besides, it is widespread in Japan and Korea at the same time other Asian and European countries (China, Spain, Italy, Greece, and Israel) where vegetables have edible fruit, are being cultivated intensively (Davis et al., 2008; Maurya et al., 2019). The first purpose of vegetable grafting is to provide resistance to diseases sourced from soil (Edelstein et al., 1999; Han et al., 2003; Yetişir et al., 2003; Lopez-Galarza et al., 2004; Yetişir et al., 2007; Keinath and Hassell, 2014; Toporek and Keinath, 2020; Aydin et al., 2022) and to increase yield (Passam, 2003; Yetişir and Sarı, 2003, Miguel et al., 2004; Davis et al., 2008; Yetişir et al., 2010). In addition, grafting is also used for enhancing tolerance and resistance to low and high temperatures (Rivero et al., 2003; Hu et al., 2006) to promote resistance/tolerance to the salinity, drought and flooding (Colla et al., 2006; Yetişir et al., 2006; Sakata et al., 2007; Colla et al., 2010), to induce water and nutrients uptake and efficient use in plant (Rouphael et al., 2008; Colla et al., 2011; Ozmen et al., 2015; Huang et al., 2016). Recently, grafting technique has been also applied for increasing yield and quality in seed production of seeded and seedless watermelons (Kombo and Sari, 2019; Aras, 2020; Hussein and Sari, 2020).

Cucurbitaceae as well as watermelons are important vegetable species grown nutritionally and economically in the worldwide. Türkiye is ranked the world's 3rd watermelon producer after China and India, with 3 147 921 tonnes in 640 700 da area (FAO, 2023). Watermelon has been cultivated in many regions of Türkiye, however, the serious yield losses occured due to soil-borne diseases, especially from *Fusarium* wilt, and to one after another cropping (Yetisir and Sari, 2003; Karaca et al., 2012). Grafting vegetable crops was introduced to Türkiye in the late 20th century (Sari et al., 1998) and nowadays watermelon and tomato are the primary vegetable crops in which grafted seedlings are used in Türkiye. Most of the watermelons are grafted mainly on bottle gourd, pumpkin and interspecific hybrid rootstocks (Davis et al., 2008; King et al., 2010; Karaağaç and Balkaya, 2013; Kong et al., 2014). The use of different rootstock has been indicated to affect watermelon plant vigor and yield (Yetisir and Sari, 2003). Watermelon grafting on *Lagenaria* or *Cucurbita* increases vigor, fruit size, and crop yield (Alexopoulos et al., 2007; Davis et al., 2008). Nevertheless, in certain cases grafting onto *Cucurbita* rootstocks may have a negative effect on fruit quality, such as less crispiness, harder fruit flesh with white strands, fruit deformation and changes in fruit size (Cohen et al., 2014). Frequently, it stated that grafting affects the fruit's morphological traits (size, shape, color), firmness, texture, flavor components (sugar, organic acids, and aroma), and compounds related to human health (minerals, vitamins, and carotenoids) (Rouphael et al., 2010; Edelstein et al., 2014).

Moreover, various studies have been demonstrated that grafting possess negative and postive impacts on the fruit quality (Yetişir and Sari, 2003; Lopez-Galarza et al., 2004; Davis and Perkins-Veazie, 2005-2006; Liu et al., 2006; Perkins-Veazie et al., 2008; Proietti et al., 2008; Bekhradi et al., 2011; Turhan et al., 2012; Çandır et al., 2013; Guler et al., 2014; Petropoulos et al., 2014; Soteriou et al., 2014; Aydin et al., 2022). The difference in grafting responses may be due to different agronomic practices and environments, genetics, scions, rootstocks, scion-rootstock combinations, growing conditions and the stage of fruit maturity (Yetisir and Sari, 2003; Davis et al., 2008; Rouphael et al., 2010). In addition to these, one way to compromise the quality of grafted watermelon varieties may be to use rootstocks that deviate from the *Citrullus* genes (Aydin et al., 2022). Yet, it has been mentioned that grafting watermelon onto wild type of watermelon rootstocks prevents the occurrence of *Fusarium* wilt (Davis et al., 2008) and it may overcome the decrease in fruit quality that resulting from the use of *Cucurbita* rootstocks (Huh et al., 2002; Cohen et al., 2014; Edelstein et al., 2014; Thies et al., 2015).

Citrullus amarus (previously expressed as "citron", "citron melon", and "preserving melon") known as *Citrullus lanatus* var. *citroides*. The wild and local citron forms exist in South Africa although it is known to be cultivated (Laghetti and Hammer, 2007; Chomicki et al., 2020). Its rind is used to make pickles, jam and fruits that are fed to livestocks (Wehner, 2008), its leaves are edible as green leafy vegetables. The citron's flesh is boiled in water, and mixing with maize meal to cook porridge, its name is "Kgodu", in South Africa and Botswana (Bultosa et al., 2020). Ripe citron seeds are dried in the sun, roasted and consumed as a snack (Mandizvo et al., 2021). Citron is also used in the jam and confectionery in France. Accessions of citron are precious rootstocks for grafting watermelon providing high grafting compatibility and resistance to root-knot nematodes (Thies et al., 2010; Thies et al., 2012; Thies et al., 2015). Fredes et al. (2016) also reported that *C. amarus* as hopeful alternative to the use of *Cucurbita* rootstocks which increases resistance against nematodes. Moreover, drought is one of the most important factors that lead to the limitation of agricultural production in regions with arid and semi-arid areas such as our country. One of the permanent methods to be applied in today's breeding to minimize the negative effects of drought stress is the development of drought-tolerant varieties or rootstocks and their utilization in agricultural production (Atakul, 2024). Citron can show high level of tolerance to drought thanks to grow mainly in arid and semi-arid regions. Because of that, citron expresses more tolerant character to drought than sweet watermelon and commercial hybrid rootstocks (Mandizvo et al., 2022; Metin et al., 2024).

The objective of this study was to investigate the effect of *C. amarus* genotypes rootstocks on Crimson Tide F_1 watermelon's total yield, fruit characteristics and fruit quality parameters such as sugar and carotenoid contents.

MATERIALS AND METHODS

Genetic material and field experiment

This study was carried out at the experimental field of the Department of Horticulture, Faculty of Agriculture, University of Cukurova, Adana-Türkiye (latitude 37°1'48.63"N, longitude 35°22'3.74" E, altitude 56 m) for two successive years.. The 11 *Citrullus amarus* genotypes (Kar 234, Kar 324, Kar 326, Kar 327, Kar 328, Kar 351, Kar 374, Kar 375, Kar 376, G 38, G 40) from watermelon genetic resources collection of Cukurova University, Faculty of Agriculture, Department of Horticulture, Türkiye were used as rootstock. The code, varieties and origins of the genotypes used in this study are given in Table 1. The rootstock potential of these *C. amarus* germplasms was compared with two commercial rootstocks [Maximus a *Cucurbita* hybrid rootstock (*Cucurbita maxima* × *Cucurbita moschata*) and Argentario (*Lagenaria siceraria*)]. Crimson Tide (CT) F_1, a commercial hybrid variety was used as a scion in all treatments and non-grafted Crimson Tide F_1 was used as control group.

Table 1. The code, varieties and origins of the genotypes

Code	Variety	Origin
Kar 234 (PI 296 341)	*Citrullus amarus*	Seminis
Kar 324 (PI 270563)	*Citrullus amarus*	USDA (USA)
Kar 326 (PI 295843)	*Citrullus amarus*	USDA (USA)
Kar 327 (PI 295843)	*Citrullus amarus*	USDA (USA)
Kar 328 (PI 482342)	*Citrullus amarus*	USDA (USA)
Kar 351	*Citrullus amarus*	INRA / Avignon / France
Kar 374 (PI 271769)	*Citrullus amarus*	Republic of South Korea
Kar 375 (PI 189225)	*Citrullus amarus*	Republic of South Korea
Kar 376 (PI 299379)	*Citrullus amarus*	Republic of South Korea
G 38	*Citrullus amarus*	Hungary
G 40	*Citrullus amarus*	Hungary
Kar 38 x Kar 234	*C. amarus* x *Citrullus amarus*	Çukurova University / Türkiye
Argentario	*Lagenaria siceraira*	Syngenta
Maximus	*Cucurbita maxima* x *Cucurbita moschata*	Antalya Tarim Company
Crimson Tide	*Citrullus lanatus* var. *lanatus*	Syngenta

Seed sowing, grafting applications and maintenance of the seedlings were carried out at Antalya Tarim Productive, Consultant and Marketing Co. in Antalya, Türkiye. Grafting was perfomed as described by Hassell et al., (2008). Grafted and control seedlings were planted under low tunnels conditions at a spacing of 2 m × 0.5 m in Adana, Türkiye (Figure 1). The field investigation was arranged in a complete randomized block design with four replications containing 10 plants for each rootstock-scion combination and control. Plants were drip irrigated and fertigated regularly, and weeds, insects and diseases were kept under control whenever the symptoms appeared, regularly. Fertilizers were implemented regularly at a rate of 200 kg P_2O_5 ha^{-1}, 180 kg N ha^{-1}, and 180 kg K_2O ha^{-1} (Yetisir and Sari, 2003). Phosphorus fertilizer was applied as an entire base fertilizer; nitrogen and potassium were given in 3 applications and the first application was applied as base fertilizer, the remaining applied at 3-week intervals with irrigation. All tunnels were held up one month after transplanting in both years.

Figure 1. Planting stages (setting tunnel wires, planting seedlings, closing the tunnels, appearance of the land after planting and closing the tunnels)

Harvest and fruit analysis

Mature fruits that have completely dried stipules and tendrils were harvested on the 11th of July in the first year and on the 25th of June in the second year (Figure 2). During harvest, all fruits were weighed in each plot by using a weighing balance to determine total fruit yield (kg/m^2). For pomological analysis, three fruits from each replication were selected. Fruit diameter (cm), and fruit length (cm) were measured by using a ruler. Fruit weight was measured using weighing balance (g). Data of the rind thickness of fruit (mm) was received by using a digital caliper (Mitutoyo CD-15D), and a digital refractometer (ATAGO Pocket PAL-3l) was used to determine the fruit total soluble solids content (%).

Figure 2. Harvested grafted fruits

Liquid chromatography analysis of sugar content

The extraction of samples was performed the methods reported by Kumar et al. (2012). A HPLC system (Agilent 1260 HPLC) consisted of a refractive index detector (G1362A RID), and a pump system was used for sugar amount analysis. The analytical conditions were 0.5 mL/min flow, eluent 0.09 mol/L H_2SO_4 with 6% acetonitrile (v/v). Sugar amounts were determined on an HPX-87H Aminex column (Bio-Rad - 300 × 7.8 mm), and fixed at 55°C. To detect the relationship between the concentration and peak area, a calibration curve was created using standards (Fredes et al., 2016). Sugar content analyses were performed only on samples from the second year of the study.

Extraction and analysis of carotenoids

For the analysis of carotenoids, samples were prepared through ethanol-hexane (5:4, v/v) as defined by Cucu et al. (2012). The homogeny extract was sonicated for 5 minutes after that centrifuged at 4500 rpm at 4°C for 15 minutes. The supernatant part was transferred into a falcon and the extraction process was duplicated. For LC-MS/MS analysis, two supernatants were mixed, evaporated, and resolved in 1 mL B mobile phase (methanol: Water: MTBE, 90:5:5, v/v/v). An Agilent 6430 LC-MS/MS spectrometer (integrated with an electrospray ionization source) was used for the analysis of carotenoids. Carotenoids were isolated and identified in accordance with the method described by Lv et al. (2015). A C30 column was used for the isolation of carotenoids (250 x 4.6 mm i.d., 5 µm, YMC). Carotenoid analyses were performed only on samples from the second year of the study.

Statistical analysis

The experiment was established as complete randomized block design with four replications, containing 10 plants for each combination, and the data were analyzed using JMP (v8.00) statistical software. ANOVA was performed to detect the effects of the years and rootstocks on exanimated features. To examine differences among the different groups, the least significant difference test was performed.

RESULTS AND DISCUSSION

Total fruit yield and average values of measured fruit parameters of grafted and non-grafted watermelons in two successive years are presented in Table 2 and Table 3. Analysis of variance indicated that grafted plants produced higher yield compared to non-grafted control and significant difference was found between rootstock and years. The highest yield was obtained from Argentario/CT (14.8 kg/m^2) and Maximus/CT (14.6 kg/m^2) while the lowest (5.84 kg/m^2) from non-grafted Crimson Tide. Citron (*Citrullus amarus*) genotypes performed better over non-grafted Crimson Tide but lower than those of two commercial rootstocks. Among the ten Citron rootstocks, total yield ranged between 9.01 kg/m^2 (Kar 375) and 11.20 kg/m^2 (G 38/CT). In the case of years first resulted higher than second. The reason for this situation was the difference in rainfall between the two years. Total rainfall during the season was 561 mm in 2012 and 272 mm in 2013.

Results of this present study indicated that grafting provides significant increase in total fruit yield inconsistent with several research reports (Yetisir et al., 2003; Yetisir and Sari, 2003; Colla et al., 2006; Alan et al., 2007; Alexopoulos et al., 2007; Cushman and Huan, 2008; Davis et al., 2008; Karaca et al., 2012; Mohamed et al., 2014; Alan et al., 2017). Davis et al. (2008) reported that even scion variety determines the mature stage size and yield in grafted plants, and rootstock types have significant effects. In our study, we have found higher yields when watermelons grafted onto commercial *Lagenaria* and *Cucurbita* interspecific hybrid (*Cucurbita maxima* × *Cucurbita moschata*) rootstocks compared to Citron (*Citrullus amarus*) rootstocks.

Despite the wide usage of bottle gourd and *Cucurbita* interspecific hybrids as a rootstock for watermelon, few studies (Thies et al., 2010; Edelstein et al., 2014; Thies et al., 2015; Fredes et al., 2016; Nkoana et al., 2022) have investigated the rootstock potential of Citron (*C. amarus*) and its effects on quality and yield. We demonstrated that Citron rootstocks provide an increase in fruit yield compared with non-grafted control plants. Similar to our findings Thies et al. (2010), Thies et al. (2012), and Thies et al. (2015) reported an increase in yield and declared that accessions of *C. amarus* are valuable and efficient rootstocks for watermelons grown in soils infested with nematodes. Also citron can be easily grown in arid regions due to its tolerance to abiotic and biotic stress conditions and can be used as a rootstock for cultivated watermelon to increase fruit quality (Nkoana et al., 2022). In previous studies, 2 different citron genotypes applied as rootstocks were determined as superior rootstocks in arid conditions and were found to have the characteristics that can be utilized as commercial rootstocks for watermelon (Yavuz et al., 2020).

Grafting did not alter the fruit weight statistically and the effect of the rootstock was found to be non-significant. The mean fruit weight ranged between 5176 g (non-grafted control) and 6974 g (Argentario/CT). In the agreement with our study, Petropoulos et al. (2014) observed no difference in mean fruit weight of two hybrid watermelon cultivars grafted onto *Cucurbita* interspecific hybrid and *Lagenaria* sp. rootstock. Similarly, in the study of Cushman and Huan (2008), fruit weight of four triploid watermelons was not affected by grafting onto five rootstock genotypes. However, Fredes et al. (2016) reported that both citron and *Cucurbita* hybrids have been improved fruit weight considerably compared to both non-grafted and self-grafted watermelons. Accordingly, Edelstein et al. (2014) described when the use of *Cucurbita* rootstocks as a rootstock for watermelon, plants produced heavier fruits than those from grafted on watermelon accessions. This situation can be explained as follows: Plants grafted onto strong rootstocks absorb more water and ions than non-grafted plants, and carry these water and ions to the scion (Aydin et al., 2022).

The content of TSS in watermelon is a prominent factor for consumers. We obtained statistical differences among the rootstock and years in terms of TSS. Both the highest and lowest TSS was observed in grafted watermelons. Watermelons grafted onto citron rootstock Kar 328/CT had the highest TSS (10.6%) while Maximus/CT combination had the lowest (8.93%) value. Other citron genotypes produced similar TSS content compared to non-grafted Crimson Tide. In agreement with our study, Fredes et al. (2016) reported the citron was the main rootstock material that notably increased (10.2 Brix) TSS compared to self-grafted (9.3 Brix) and non-grafted (9.6 Brix) watermelons. Several studies (Miguel et al., 2004; Liu et al., 2006; Yetisir et al., 2007; Huitron-Ramirez et al., 2009; Karaca et al., 2012; Turhan et al., 2012) indicated that total soluble solids of watermelon is highly affected by grafting, however increased, decreased or unchanged TSS contents are mainly due to the type of rootstock (Yetisir et al., 2003). Changes could be also attributed to different production environments, rootstock/scion combinations used and harvest periods (Davis et al., 2008).

Table 2. Total fruit yield, fruit weight and TSS of watermelons grafted on different rootstocks

Rootstock/ scion combination	Total fruit yield (kg/m^2)		Mean of rootstock	Fruit weight (g)		Mean of Rootstock	TSS (%)		Mean of rootstock
	Year			Year			Year		
	First	Second		First	Second		First	Second	
Kar 234/CT	11.0	9.4	*10.2 BC*	7236	4687	*5962*	10.0	9.4	*9.74 BCD*
Kar 324/CT	10.7	11.8	*11.2 B*	7548	4772	*6160*	10.7	10.1	*10.4 AB*
Kar 326/CT	11.7	10.2	*10.9 B*	6789	3651	*5220*	10.0	9.2	*9.60 CDE*
Kar 327/CT	10.0	10.2	*10.1 BC*	6756	5216	*5986*	10.0	8.9	*9.45 CDE*
Kar 328/CT	11.3	8.5	*9.92 BC*	6802	5623	*6212*	10.9	10.3	*10.6 A*
Kar 351/CT	10.3	9.6	*9.93 BC*	6747	4978	*5863*	9.96	8.6	*9.29 CDE*
Kar 374/CT	11.5	9.9	*10.7 BC*	6914	4818	*5866*	9.8	9.2	*9.49 CDE*
Kar 375/CT	8.7	9.4	*9.01 C*	6531	4620	*5576*	10.4	9.7	*10.0 ABC*
Kar 376/CT	10.9	9.7	*10.3 BC*	7815	4694	*6255*	10.7	9.2	*9.98 A-D*
G 38/CT	12.0	10.3	*11.2 B*	7885	4294	*6090*	10.7	9.4	*10.0 A-D*
G 40/CT	10.4	9.9	*10.2 BC*	7270	4777	*6024*	10.2	9.8	*10.0 A-D*
Argentario/CT	15.3	14.2	*14.8 A*	8049	5899	*6974*	9.8	8.7	*9.26 DE*
Maximus/CT	15.2	14.1	*14.6 A*	7997	5508	*6752*	9.7	8.2	*8.93 E*
Crimson Tide	6.3	5.4	*5.84 D*	6738	3614	*5176*	10.8	9.1	*9.95 A-D*
Mean of year	11.08 A	10.2 B		7220 A	4797 B		10.3 A	9.28 B	
LSD_{year} = 0.65**				LSD_{year}*** = 385.3			LSD_{year}*** = 0.30		
$LSD_{rootstock}$* = 1.73**				$LSD_{rootstock}$ = N.S.			$LSD_{rootstock}$** = 0.78		
$LSD_{year\ x\ rootstock}$ = N.S.				$LSD_{year\ x\ rootstock}$ = N.S.			$LSD_{year\ x\ rootstock}$ = N. S.		

Differences between the means of rootstock were shown with different letters
N. S: Not Significant, ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$

Table 3. Fruit length, diameter and rind thickness of watermelon grafted on different rootstocks

Rootstock scion combination	Fruit length (cm)		Mean of rootstock	Fruit diameter (cm)		Mean of rootstock	Fruit rind thickness (mm)		Mean of rootstock
	Year			Year			Year		
	First	Second		First	Second		First	Second	
Kar 234/CT	27.8	22.2	*25.0 BCD*	22.0	19.1	*20.6 BC*	11.6	12.6	12.1
Kar 324/CT	28.8	23.5	*26.2 AB*	22.2	19.3	*20.8 BC*	11.9	10.3	11.1
Kar 326/CT	27.3	20.4	*23.8 D*	21.8	18.3	*20.1 BC*	11.6	12.2	11.9
Kar 327/CT	27.1	24.3	*25.7 ABC*	21.7	19.7	*20.7 BC*	11.7	12.9	12.4
Kar 328/CT	26.5	24.1	*25.3 A-D*	21.9	20.2	*21.1 BC*	13.0	12.9	12.9
Kar 351/CT	26.9	22.9	*24.9 BCD*	21.4	19.1	*20.2 BC*	11.1	11.5	11.3
Kar 374/CT	28.3	22.6	*25.5 A-D*	21.0	19.3	*20.1 BC*	12.0	12.3	12.1
Kar 375/CT	27.3	22.8	*25.0 BCD*	21.4	18.9	*20.2 BC*	11.9	12.6	12.2
Kar 376/CT	29.6	22.9	*26.3 AB*	22.4	19.4	*20.9 BC*	12.2	12.5	12.3
G 38/CT	29.1	20.8	*24.9 BCD*	22.8	18.2	*20.5 BC*	12.4	11.6	12.0
G 40/CT	27.7	23.5	*25.6 A-D*	21.4	19.3	*20.3 BC*	11.6	11.5	11.5
Argentario/CT	28.4	25.0	*26.7 AB*	23.7	20.8	*22.2 A*	13.1	14.4	13.8
Maximus/CT	28.9	25.1	*26.9 A*	22.4	19.9	*21.2 AB*	13.4	11.6	12.5
Crimson Tide	26.7	21.7	*24.1 CD*	21.9	18.2	*20.1 C*	12.6	12.1	12.4
Mean of year	27.9 A	22.9 B		22.0 A	19.3 B		12.1	12.2	
LSD_{year}* = 0.68**				LSD_{year}*** = 0.42			LSD_{year}= N.S.		
$LSD_{rootstock}$* = 1.80				$LSD_{rootstock}$* = 1.12			$LSD_{rootstock}$= N.S.		
$LSD_{year\ x\ rootstock}$ = N.S.				$LSD_{year\ x\ rootstock}$ = N.S.			$LSD_{year\ x\ rootstock}$ = N.S.		

Differences between the means of rootstock were shown with different letters
N.S.: Not Significant, ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$

The significance of scion and rootstock interaction stated by Edelstein et al. (2014) has been indicated that grafting onto the same rootstocks decreases TSS in mini watermelon scion while increases in midi watermelon scion.

The findings of variance analysis showed that fruit length and diameter were significantly different among the rootstocks (Table 3). Regarding fruit length watermelons grafted onto Maximus had the longest fruits (26.9 cm) while citron rootstock Kar 326 (23.8 cm) had the shortest. Non-grafted Crimson Tide exhibited similar values to most of the citron rootstocks except Kar 324 and Kar 376. Based on the fruit diameter, larger fruits were obtained from plants grafted onto *Lageneria* rootstock Argentario (22.2 cm) compared to all citron rootstocks and non-grafted Crimson Tide. *Cucurbita* hybrid rootstock Maximus had higher values than non-grafted Crimson Tide but similar to citron rootstocks. Consistent with our results Fredes et al. (2016) demonstrated obtaining wider and longer fruits, without remarkable fruit shape variations using both citron and the two F_1 *Cucurbita* hybrids as a rootstock. Similarly, in the study of Yetisir et al. (2003), larger fruits were obtained from plants grafted onto *Lagenaria* than from non-grafted plants. Turhan et al. (2012) found that fruits were larger in plants grafted onto hybrid *Cucurbita* rootstocks Dynamo and RS- 841 as well.

No significant difference was found in rind thickness when grafted watermelons compared to non-grafted watermelons. The values ranged between 11.1 mm (Kar 324/CT) and 13.8 mm (Argentario/CT). Accordingly, Petropoulos et al. (2014) observed no difference in fruit rind thickness between grafted and non-grafted plants. However, in the study of Fredes et al. (2016) fruits with thicker rinds were obtained from plants grafted on both Citron melon and *Cucurbita* rootstocks than non-grafted and self-grafted plants. Also, various studies have proven that adverse effects like thicker fruit rind have been recorded in watermelon plants grafted on *Cucurbita* and *Lagenaria* rootstocks compared to non-grafted watermelons (Alexopoulos et al., 2007; Cushman and Huan, 2008; Turhan et al., 2012).

As a sugar component glucose, sucrose, and fructose were determined in examined extracts. Total amounts of sugar were between 75.54 g/kg (Maximus/CT) and 105.74 g/kg (Kar 234/CT) (Table 4). Most citron rootstocks had higher values than *Cucurbita* and *Lagenaria* rootstocks and non-grafted Crimson Tide. The main parts of carbohydrates in extracts are the three simple sugars: Glucose, sucrose, and fructose (Fig. 3). They represent many parts of the TSS of watermelon. The total amount of sugars detected was fructose>glucose>sucrose, respectively. According to Elmstrom and Davis (1981), cultivars or maturity that outcome from high fructose concentrations have a charming character since fructose has a greater effect on sweetness than sucrose. As reported in previous studies, also in our study, fructose was detected in the largest amounts and there was a significant difference between rootstocks and control. The highest content was found in Kar 234/CT and Kar 374/CT (57.99 g/kg and 55.86 g/kg respectively) and the lowest in Maximus/CT (40.00 g/kg). Glucose is the second most abundant sugar and significant differences were obtained between rootstocks. Its concentration ranged from 31.85 g/kg (Maximus/CT) to 43.43 g/kg (Kar 234/CT). Regarding sucrose, it constitutes about 4% of total sugar in all combinations.

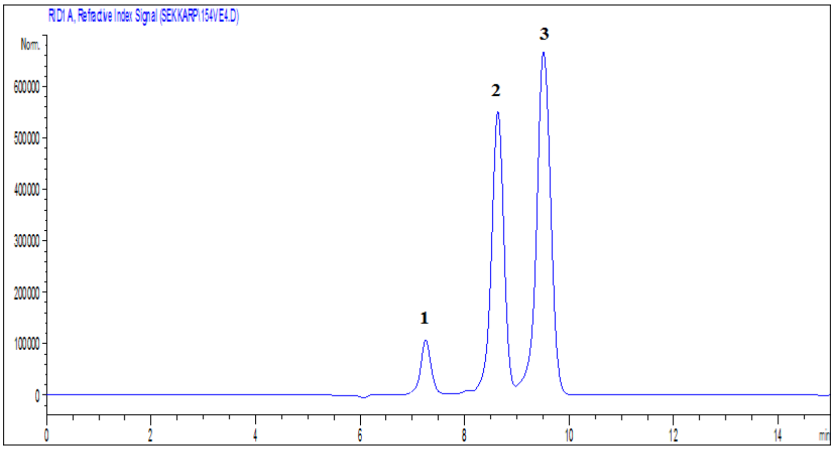

Figure 3. HPLC chromatogram of sugars in Kar 234/CT (Peaks: 1. Sucrose; 2. Glucose; 3. Fructose)

Table 4. Sugar concentration of watermelon fruits grafted on different rootstocks

Rootstock/Scion combinations	Sucrose (g/kg)	Glucose (g/kg)	Fructose (g/kg)	Total sugars (g/kg)
Kar 234/CT	4.32	43.43 a	57.99 a	105.74 a
Kar 324/CT	3.74	42.33 ab	55.14 ab	101.21 ab
Kar 326/CT	4.31	38.33 cd	47.64 e	90.28 de
Kar 327/CT	3.89	37.75 cde	51.27 cd	92.91 cde
Kar 328/CT	3.75	39.43 bcd	52.36 bc	95.55 bcd
Kar 351/CT	4.25	35.10 efg	51.49 cd	90.84 de
Kar 374/CT	3.99	39.91 bc	55.86 a	99.76 ab
Kar 375/CT	4.89	39.63 bcd	55.78 ab	100.30 ab
Kar 376/CT	3.79	39.45 bcd	55.22 ab	98.46 bc
G 38/CT	3.87	36.51 def	48.16 de	88.54 ef
G 40/CT	3.46	32.91 gh	45.74 e	82.11 fg
Argentario/CT	3.94	33.81 fgh	45.76 e	83.51 f
Maximus/CT	3.68	31.85 h	40.00 f	75.54 h
Crimson Tide	3.53	32.49 gh	40.80 f	76.82 gh
LSD	N.S.	3.15 ***	3.43***	6.48***

Differences between the means were shown with different letters
N.S.: Not Significant***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$

The total sugar ingredients, like sucrose, fructose, and glucose, are responsible for the watermelon sweetness (Yativ et al., 2010). Bianchi et al. (2018) reported that sucrose and glucose amount to 20-40% and fructose to 30-50% of total sugars in mature watermelons. Grafting often affects fruit attributes such as the content of sugars, acids, aroma volatiles, carotenoids, vitamins, and minerals (Rouphael et al., 2010). Our results indicated that grafting affects total sugars, glucose, and fructose content, however, changes in the grafted watermelons can be attributed to the type of the rootstock. We obtained mostly higher values from Citron rootstocks compared to *Cucurbita* hybrid and *Lagenaria* rootstock and non-grafted control. However, Fredes et al. (2016) reported that the Citron melon had no dramatic impact on sugars in comparison non grafted watermelons excluding a rise in glucose substance which also appeared in the *Cucurbita* rootstocks.

A total of five carotenoids were determined and quantified in watermelons, including cryptoxanthin, phytofluene, β-carotene, *cis*-lycopene and *trans*-lycopene. The total content of carotenoids in watermelon determined from different rootstock/scion combinations ranged from 41.60 (Kar 374/CT) to 102.01 (Kar 351/CT) mg/kg. The major carotenoid was *trans*-lycopene as it calculated for the largest part of the total carotenoid. The highest level of *trans*-lycopene was detected in Kar 351/CT (78.06 mg/kg), followed by non-grafted Crimson Tide (75.80 mg/kg) and Kar 376/CT (73.95 mg/kg) (Table 5).

Among the main carotenoids in watermelon, are lycopene and β-carotene (Bianchi et al., 2018), or lycopene is known to be higher than many other fruits and vegetables (Tadmor et al., 2005). Several studies (Davis and Perkins Veazie, 2005; Alan et al., 2007; Proietti et al., 2008; Turhan et al., 2012; Çandır et al., 2013; Petropolulos, 2014; Fredes et al., 2016; Kyricau et al., 2017) examined the effects of grafting on lycopene content, which is is the prevalent carotenoid and one of most important quality criteria in red-fleshed watermelons. However, these studies reported variable results in the lycopene content of fruits when grafted and non-grafted watermelons were compared. Nevertheless, in our case, the highest lycopene content was found both in fruits of plants grafted onto Kar 351 which is a Citron rootstock, and non-grafted control. Besides, the lowest value was also observed in a Citron rootstock (Kar 375). The amounts of β-carotene and lycopene were evaluated in mature watermelons according to stated by Perkins-Veazie et al. (2006) (35-112 mg/kg and 0.9-10.2 mg/kg, respectively).

Table 5. Carotenoid concentration (mg/kg) of watermelon fruits grafted on different rootstocks

Rootstock scion combinations	Cryptoxanthin	Phytofluene	*β*-carotene	*cis*-lycopene	*trans*-lycopene	Total lycopene	Total carotenoid
Kar 234/CT	0.54 a	1.19 b	4.68 a	11.75 g	52.86 d	64.61 fg	71.02 hi
Kar 324/CT	0.48 b	1.05 c	2.04 cd	20.26 a	60.34 c	80.59 cd	84.16 cd
Kar 326/CT	0.41 c	0.89 d	1.35 def	17.11 b	52.08 d	69.19 ef	71.83 gh
Kar 327/CT	0.34 d	0.74 ef	0.83 f	14.66 de	66.22 b	80.88 cd	82.79 cde
Kar 328/CT	0.35 d	0.93 cd	4.10 a	13.65 ef	68.23 b	81.88 c	87.26 c
Kar 351/CT	0.32 de	0.85 def	1.98 cde	20.80 a	78.06 a	98.86 a	102.01 a
Kar 374/CT	0.15 h	0.40 ı	0.92 f	8.45 ı	31.69 e	40.14 h	41.60 j
Kar 375/CT	0.21 fg	0.58 gh	1.34 ef	8.04 ı	35.60 e	43.64 h	45.77 j
Kar 376/CT	0.53 ab	1.44 a	3.34 b	16.08 c	73.95 a	90.03 b	95.35 b
G 38/CT	0.17 gh	0.45 hı	4.47 a	17.13 b	54.96 cd	72.09 e	77.18 efg
G 40/CT	0.49 ab	1.33 ab	1.24 f	9.86 h	52.64 d	62.50 g	65.56 i
Argentario/CT	0.22 fg	0.59 gh	2.56 c	13.48 f	59.82 c	73.30 e	76.68 fgh
Maximus/CT	0.26 ef	0.71 fg	2.46 c	15.05 d	59.99 c	75.04 de	78.48 def
Crimson Tide	0.32 d	0.88 de	3.37 b	17.38 b	75.80 a	93.18 ab	97.75 ab
LSD	0.06 ***	0.14***	0.69***	1.02***	5.59***	5.87***	5.85***

Differences between the means were shown with different letters
N.S.: Not Significant***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05

CONCLUSION

The results of this study showed that grafting supplies a radical increase in total fruit yield. Plants grafted onto *C. amarus* rootstocks had higher total yield in comparison to non-grafted plants, but lower than the commercial *Lagenaria* and *Cucurbita* hybrid rootstocks. All fruit characteristics from plants grafted onto commercial rootstocks yielded higher or comparable results to those from plants grafted onto *C. amarus* genotypes. Besides, it was determined that *C. amarus* rootstocks improved fruit sugar content, however the total lycopene and carotenoid content varied among genotypes. Regarding total sugar, total soluble solids, total lycopene, and total carotenoid levels, Kar 328, Kar 234, and Kar 351 were determined to be the most promising of the citron genotypes utilized as watermelon rootstock. Overall, our results indicated that Citron (*Citrullus amarus*) genotypes can be applied as an alternative rootstock and have a high potential for watermelon grafting.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript.

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