

ROOTSTOCK INFLUENCES ON SEASONAL CHANGES IN LEAF PHYSIOLOGY AND FRUIT QUALITY OF RIO RED GRAPEFRUIT VARIETY

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Abstract. Citrus is a conventionally produced fruit crop in extensive agricultural areas in Mediterranean-type agroecosystems. The use of rootstocks for citrus fruits is necessary for profitable production under some limiting factors, such as climatic factors, bad soil conditions, and diseases. In addition, the use of the citrus rootstocks provides a large number of choices to growers to increase fruit quality and yield, obtain early fruiting, uniform cropping and high-density planting, avoid juvenility, and control tree size. The aim of the present study was to evaluate the effects of several citrus rootstocks on the fruit yield, quality, physiological changes in leaves and leaf mineral composition of Rio Red grapefruit. Thus, seasonal changes in the leaf chlorophyll concentration, PSII efficiency (F_v / F_m), stomatal conductance (g_s), leaf temperature, leaf mineral nutrient, fruit yield and fruit quality traits of the Rio Red grapefruit variety grafted onto six commonly used rootstocks (Carrizo citrange, citremon, sour orange, Swingle citrumelo, Troyer citrange and Volkameriana) in citriculture were evaluated. The physiological responses of fully expanded young leaves to rootstocks were significantly affected by seasonal changes. Two-way ANOVA indicated significant main effects of rootstock and season and their interaction ($p \leq 0.05$) on the leaf Chl concentration. Leaf Chl concentrations were lowest in April for all rootstocks. The PSII efficiency slightly decreased in the leaves of Rio Red grafted onto Volkameriana in February. The rootstocks significantly affected seasonal changes in the leaf stomatal conductance. The highest g_s was recorded in October in the leaves of a Rio Red variety grafted onto the Volkameriana rootstock. Significant rootstock effects on leaf Ca, Mg, Mn, Zn and Cu concentrations were observed in the Rio Red variety. The highest leaf Zn concentration (ppm) was recorded in plants on Volkameriana, whereas the lowest Zn concentrations were recorded in plants on Troyer citrange. There were no significant rootstock effects on leaf N, P, K and Fe concentrations. The rootstocks significantly affected the fruit yield of the Rio Red variety in the 2013 and 2014 harvest years. The highest fruit yield was observed in Rio Red grafted onto Carrizo citrange in 2013, whereas it was highest in plants on Troyer citrange in 2014. The fruit weight (g), fruit diameter (mm), total soluble solids (%), juice content (%) and juice color (hue^o) of Rio Red grapefruit were significantly affected by the rootstocks.

Keywords: *stomatal conductance, chlorophyll concentration, fruit quality, fruit juice color*

Introduction

The citrus growing areas of Turkey are situated in the northern hemisphere of the citrus belt. Turkey has very suitable ecological conditions and a high potential for citrus production; in fact, 4.293.007 t of citrus fruit was produced in Turkey in 2016 (TUIK, 2017). The eastern Mediterranean Region produces 80% of Turkey's total citrus fruit and 99% of its total grapefruit. In recent years, there has been a large increase in the exportation of citrus fruits, especially grapefruit. For example, Turkey produced 253.120 t of grapefruit in 2016 (TUIK, 2017) and exported 183.329 t of grapefruit in the

same year (AKIB, 2017). Star Ruby is the dominant grapefruit variety of Turkey. It is followed Rio Red, which is the latest variety and was derived from ‘Redblush’ by bud irradiation.

Rootstocks have a substantial role in the development of the citrus industry. It is necessary to use rootstocks for citrus fruits to have profitable production under some limiting factors, such as climate, bad soil conditions, and diseases. In addition to these factors, the use of citrus rootstocks provides a large amount of choices to growers, enabling growers to, among other things, increase fruit quality and yield, obtain early fruiting and uniform cropping, avoid juvenility, control the tree size and perform high-density planting (Tuzcu et al., 2005). Choosing a rootstock is an important decision, and local climatic and soil conditions are important factors in rootstock selection. Although any citrus variety can be used as a rootstock, some of them are better suited to specific conditions than the others (Davies and Albrigo, 1994; Lawrence and Bridges, 1974).

Citrus rootstocks have a large impact on scion growth, fruit quality and yield (Castle, 1987; Georgiou, 2002; Forner-Giner et al., 2003; Castle et al., 2009; Hussain et al., 2013). In addition, scion behavior depends in part on the rootstock-induced effects on leaf gas exchange (González-Mas et al., 2009) and chlorophyll content (Garci-Sanchez et al., 2002). The influence of rootstocks on scion photosynthetic capacity may play a key role in citrus plant performances in terms of vigor, crop load, and fruit characteristics and should be considered (Jover, 2012). In the grafted tree, rootstock type affects many traits such as leaf mineral elements (Jr et al., 2003; Toplu et al., 2008). Rootstocks directly affect the ability of plants to uptake water and nutrients from soil. Plant nutrient concentrations in scion cultivars may differ, even though they are grown under the same conditions. For this reason, it is important to determine the effects of rootstocks on plant nutrient statuses to optimize fertilization programs.

Sour orange is the main rootstock (approximately 85%) used in citrus growing in Turkey, because of the high soil clay content and high pH (causing to iron, zinc and manganese chlorosis) of many soils in the country. Trees on sour orange produce excellent quality fruit but are susceptible to citrus tristeza virus (CTV) and poor compatibility with some citrus cultivars; in some cases, these trees produce low fruit yields compared to trees with other rootstocks (Castle et al., 2010; Shafieizargar et al., 2012). To address these problems, researchers and citrus growers have made an effort to look for alternative rootstocks. In the selection of a suitable rootstock, its adaptability to soil conditions and interactive effects with the scion cultivar have to be carefully considered (Shafieizargar et al., 2012). Given that rootstocks are unlike genotypes and modify growth, fruit production, photosynthetic capacity and leaf mineral composition, the present study aimed to determine the effect of several citrus rootstocks on the fruit yield, quality and leaf mineral composition of Rio Red grapefruit. In addition, the rootstock effects on the photosynthetic performance of the scion were assessed based on leaf chlorophyll concentration (Chl), chlorophyll fluorescence, stoma conductance and gas exchange measurements.

Materials and methods

Plant material and growing conditions

‘Rio Red’ grapefruit which is a normal appearing grapefruit (shape and rind texture) variety with attractive dark pink flesh was grafted on the following rootstocks: local sour orange (*Citrus aurantium* L.), ‘Troyer’ and ‘Carrizo’ citranges (*Citrus sinensis*

Os. × *Poncirus trifoliata* (L.) Raf.), Volkameriana (*Citrus volkameriana* Tan. and Pasq.), Swingle citrumelo (*Citrus paradisi* Macf. × *Poncirus trifoliata*), and Citremon (*Citrus limon* × *Poncirus trifoliata*). The grafted trees were planted in 1997 with 8 × 8 m spacing at the Research Station of Cukurova University, Agricultural Faculty, Citrus Experiment Station, Adana (Latitude, 35°23' N; Longitude, 36°50' E, altitude 27 m), Turkey. A completely randomized experimental design was used, with five replicates for each combination. In the experimental area, the soil was a clay-loam (57% clay, 21% silt, and 22% sand, containing 12% CaCO₃), and the soil pH was in the range of 7.39-7.46 at a depth of 0-90 cm and considered slightly alkaline. The salt content of the soil was 0.22 EC (mmhos cm⁻¹). The area has a mean maximum and a mean minimum temperature of 26 and 14.5 °C, respectively, and an average annual rainfall of 465 mm (Fig. 1). The trees were irrigated weekly from May to October using drip irrigation. Nitrogen (N) was applied as Ammonium nitrate at a rate of 1.5 kg N tree⁻¹ (2/3 in mid-February and 1/3 in mid-May), phosphorus (P) was applied as triple superphosphate at a rate of 1 kg P tree⁻¹ (December) and potassium was applied as potassium nitrate (K) at a rate of 1 kg K tree⁻¹ (January).

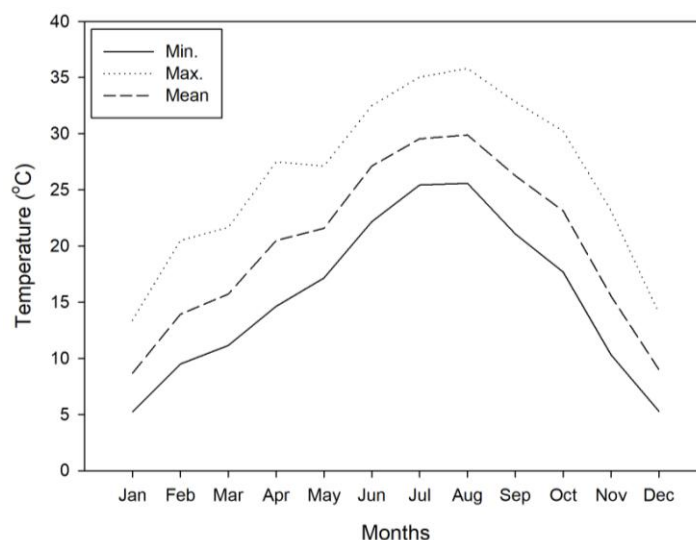


Figure 1. Minimum, maximum and mean temperature data of the experimental field in 2016

Fruit yield and quality

Rio Red grapefruit is mid to late-season in maturity with juicy and well pigmented flesh. The effects of different rootstocks on fruit yield were evaluated in 2013 and 2015. Each year, the fruit yield of each tree was determined during the harvesting period. Fruits were harvested at the optimum harvest time (at the end of December or at the beginning of January). The yield per tree (kg/tree) was obtained by weighing the harvested fruit.

A random sample of 25 grapefruits was evaluated for fruit quality analysis. The fruit samples were weighed, the fruit diameter at the equator was measured with a digital caliper, and the rind thickness was measured after cutting the fruit in half with a digital caliper (Mitutoyo CD-15CPX, Mitutoyo America Corporation, USA). The fruit samples were weighed and juiced using a standard juicer; then, the juice was weighed and expressed as a percentage of the total fruit weight. The total soluble solids (TSS) values

were determined with a portable refractometer (FG-103/113) using a few drops of juice. The total acidity (TA) of the juice was determined by titrating 5 ml of the juice sample with 0.1 N sodium hydroxide (NaOH) and phenolphthalein as the indicator. Fruit juice color determinations were made at 25 ± 1 °C using a Hunterlab Colorflex (Hunterlab, Reston, Virginia, USA). This spectrophotometer uses an illuminant D65 and a 10° observer as references. A sample cup for the reflectance measurements was used (5.9 cm internal diameter \times 3.8 cm height) with a path length of light of 10 mm. The results were expressed as the Hue angle [$h_{ab} = \arctan(b^*/a^*)$], which is determined as starting at the $+a^*$ axis and is expressed in degrees; 0% is $+a^*$ (red), 90% is $+b^*$ (yellow), 180% is $-a^*$ (green), and 270% is $-b^*$ (blue).

Leaf chlorophyll concentration and photosystem II efficiency

The leaf chlorophyll concentration was estimated using a portable SPAD meter (Minolta, Japan), and the chlorophyll fluorescence parameter ((Fv' / Fm') = quantum yield in light-adapted leaves) was measured with a portable fluorometer (FluorPen FP100, Photon System Instruments Ltd, Drasov, Czech Republic). The evaluations of the chlorophyll concentration and fluorescence measurements were taken in six periods between 2015 and 2016. The same leaves were used to estimate the leaf chlorophyll (Chl) concentrations and PSII efficiency, based on the quantum yield of light-adapted leaves (Fv' / Fm'). The chlorophyll concentration and maximum chlorophyll fluorescence efficiency readings for the light-adapted leaves were measured on 10 fully expanded young leaves (third and fourth leaves from the shoot apex) of each replicate at the four chosen time points. Measurements were performed before midday (08⁰⁰-11⁰⁰ h).

Leaf stomatal conductance measurements (g_s) and leaf temperature (°C)

After the leaf chlorophyll concentration and fluorescence measurements, the same leaves were analyzed by a portable SC-1 leaf porometer (Meter Group, Inc. USA) to estimate leaf stomatal conductance ($\text{mmol m}^{-2}\text{s}^{-1}$) and leaf temperature (°C). The stomatal conductance evaluations were conducted in five periods between 2015 and 2016. The stomatal conductance efficiency of leaves in the light-adapted stage was measured on 10 fully expanded young leaves (third and fourth leaves from the shoot apex) from each replicate at the four chosen time points (Fig. 2). Measurements were performed before midday (08⁰⁰-11⁰⁰ h).



Figure 2. A general view of the examined orchard and stomatal conductance measurements by porometer

Leaf mineral content

Leaf samples were taken from the fruitless shoots in autumn for the analysis of plant nutri-elements in 2015, according to Chapman (1960). The leaves were washed in a detergent solution, rinsed several times in distilled water, and then dried at 70 °C for 48 h using a thermoventilated oven. The dried leaves were ground (<0.5 mm) and ashed in a muffle furnace at 550 °C for 8 h. The ash samples were digested in HNO₃-HClO₄ (3:1 v/v) and filtered through a blue band paper filter (Kacar, 1972). In the extract solutions, sodium (Na), potassium (K), magnesium (Mg), iron (Fe), manganese (Mn) and copper (Cu) concentrations were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Varian Liberty Series II), and the concentrations of phosphorus (P) were determined by UV-VIS spectrophotometry (Barton, 1948). The leaf total nitrogen (N) content was determined by the semi-micro Kjeldahl method (Lees, 1971).

Statistical analysis

Data were subjected to two-way analysis of variance (ANOVA), and significant differences between means were evaluated using Tukey's multiple range test at $p \leq 0.05$ and $p \leq 0.01$. All statistical analyses were performed by using SAS v9.00 statistics software procedures (SAS, 2006), and SigmaPlot® version 11.00 was used for the data presentation.

Results

Fruit yield and quality

Rootstocks significantly affected the cumulative yield. The highest cumulative yield of the Rio Red grapefruit in the three-year period was obtained from the trees on Carrizo and Troyer citrange, followed by those on Swingle citrumelo. Trees on Citremon, sour orange and *Volkameriana* had the lowest cumulative yield (Fig. 3). There were statistically significant differences in the fruit yield per tree between rootstocks, except for in 2015 (Fig. 3).

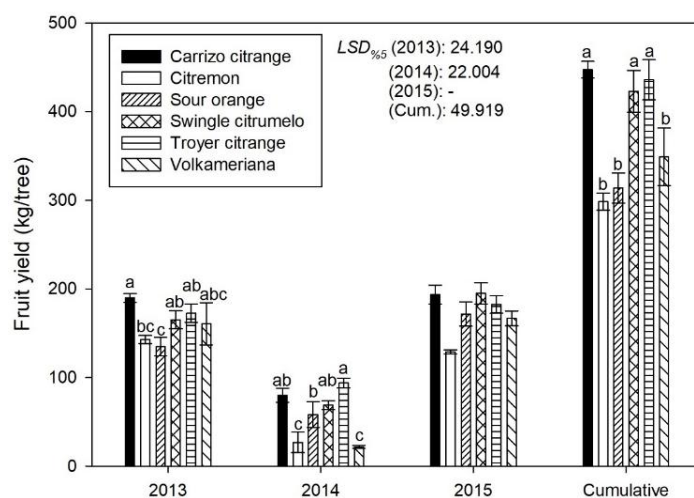


Figure 3. Effect of rootstock on the annual fruit yield and the cumulative fruit yield over three consecutive years (kg tree^{-1}) of 'Rio Red' grapefruit. Error bars represent \pm standard error

In 2013, the highest yield was obtained from trees on Carrizo citranges, followed by trees on Troyer citrange and Swingle citrumelo. In 2014, the highest yield was obtained from trees on Troyer citrange, followed by trees on Carrizo citrange and Swingle citrumelo. In 2013, the lowest yield was obtained from trees on sour orange, followed by those on Citremon; in 2014, the lowest yield was obtained from trees on Volkameriana and Citremon, followed by those on Sour orange.

According to the results obtained from this study, Carrizo and Troyer citranges followed by Swingle citrumelo generally had the highest fruit yields during the three-year study period. The lowest yield was found for the trees on Citremon (Fig. 3).

The present study showed rootstocks had a significant effect ($p \leq 0.05$) on the fruit weight, fruit diameter, TSS and juice content of 'Rio Red' grapefruit (Table 1). The effects of rootstocks on the rind thickness, TA and TSS/TA ratio were not statistically significant, and all of the rootstocks gave similar values (Table 1). Fruits from trees on sour orange were significantly heavier than those on the other rootstocks, followed by fruits from trees on Swingle citrumelo and Troyer citrange. The lightest fruits were obtained from the trees on Citremon. The fruits from the trees on Troyer citrange had the highest fruit diameters. Trees on Citremon produced fruit with a small diameter. Rootstock had a significant effect on the total soluble solids (TSS). The highest TSS was detected in the fruits from the trees on Swingle citrumelo rootstock and the lowest TSS in the juice was obtained from the trees on Volkameriana. There were significant differences among the juice contents of fruits with different rootstocks. The juice content was the highest for fruits from trees on Swingle citrumelo and the lowest for fruits on other rootstocks.

Table 1. Effects of rootstocks on the fruit quality parameters of 'Rio Red' grapefruit

Rootstock	Fruit weight (g)	Fruit Diameter (mm)	Rind thickness (mm)	TSS (%)	TA (%)	TSS/TA	Juice content (%)
Carrizo	354.35 bcd	93.30 ab	6.76	9.50 ab	2.70	3.54	33.95 b
Citremon	335.15 d	90.85 b	6.80	9.58 ab	2.51	3.85	30.29 b
Sour orange	445.60 a	99.14 ab	7.23	8.93 b	2.47	3.67	29.73 b
Swingle	418.40 ab	98.36 ab	6.85	9.95 a	2.69	3.71	42.56 a
Troyer	415.10 abc	101.94 a	7.24	9.40 ab	2.64	3.56	31.84 b
Volkameriana	344.47 cd	94.98 ab	7.70	7.87 c	2.24	3.52	31.73 b
<i>Prob>F</i>	0.0155	0.0362	0.6066	0.0062	0.1403	0.8517	0.0374
<i>LSD%5</i>	62.369	8.730	-	0.904	-	-	7.165

The fruit juice color was significantly affected by rootstock ($p \leq 0.05$) (Fig. 2), and the juice hue^o values ranged between 43.11 and 51.84. The juice from trees on Carrizo citrange had the lowest hue^o (most red), followed by the juice from trees on Swingle citrumelo. The highest hue^o values were obtained from the trees on Sour orange and Volkameriana (Fig. 4).

Leaf chlorophyll concentration and fluorescence measurements (PSII efficiency)

The SPAD measurements were used as an estimate of leaf chlorophyll concentration because there is a positive linear relationship between these two parameters ($r^2 > 0.8$, Jifon et al., 2005). Two-way analysis of variance (ANOVA) indicated a significant main effect of rootstocks, sample date and their interaction ($p \leq 0.05$) on the Chl concentration (Table 2).

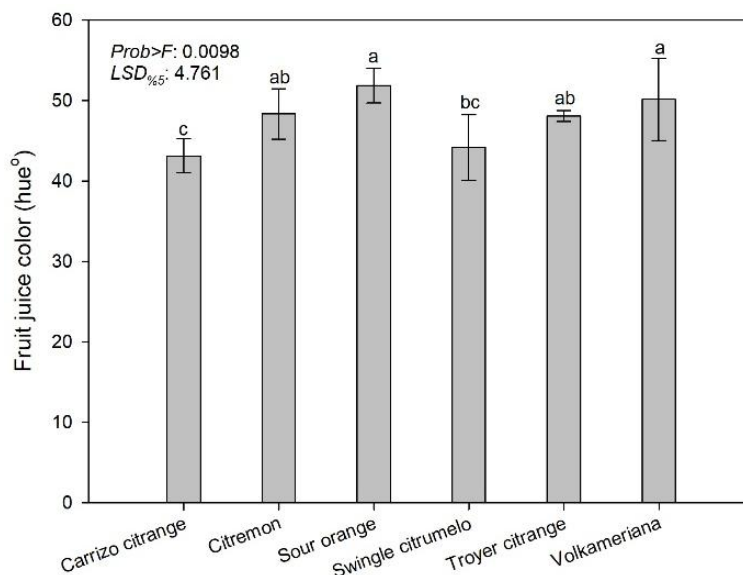


Figure 4. Effects of different rootstocks on the fruit juice color of 'Rio Red' grapefruit. Error bars represent \pm standard error

Table 2. Results of two-way analysis of variance (ANOVA) of the effects of rootstock (R) and season (S) and their interaction (R \times S) for the dependent variables considered. Numbers represent F values. * $p < 0.05$

Dependent variable	Independent variable		
	S	R	S \times R
SPAD	296.73*	5.17*	1.84*
F_v/F_m'	134.14*	4.29*	3.90*
gs	250.27*	14.27*	6.80*
Leaf temperature	196.23*	4.08*	10.33*

As shown in Figure 5a, in the present study, the leaf chlorophyll concentration ranged between 36.20 and 72.25. Rootstocks significantly affected the leaf chlorophyll (Chl) concentration. The scion leaves of shoots grafted onto Troyer citrange and Volkameriana had the lowest Chl concentrations, whereas the leaves of shoots budded on to Citremon and Swingle citrumelo had the highest Chl concentration for nearly all periods. In terms of Chl measurements, statistically significant differences were found between the measurement periods. The lowest Chl was observed in April 2015 and 2016. The highest Chl was observed in February and October every year. According to

the interaction between the rootstocks and the sampling date, Troyer citrange has the lowest value of Chl in all periods (Fig. 5a).

At the end of the experiment, the PS II efficiency ranged between 0.503 and 0.737. Two-way analysis of variance (ANOVA) indicated a significant main effect of rootstocks and sampling date and their interaction ($p \leq 0.05$) on the PS II efficiency (Table 2). Rootstocks significantly affected the PS II efficiency of Rio Red grapefruit. The maximum quantum yield of PSII was the smallest in the leaves of shoots grafted onto Volkameriana and Troyer citrange; the highest maximum quantum yield of PSII was observed in the leaves of shoots grafted onto Sour orange and Citremon (Fig. 5b). In terms of measurement period and the maximum quantum yield of PSII, in 2015 and 2016, the highest values were in July and October, followed by those in April. The smallest values were observed in February 2015 and 2016.

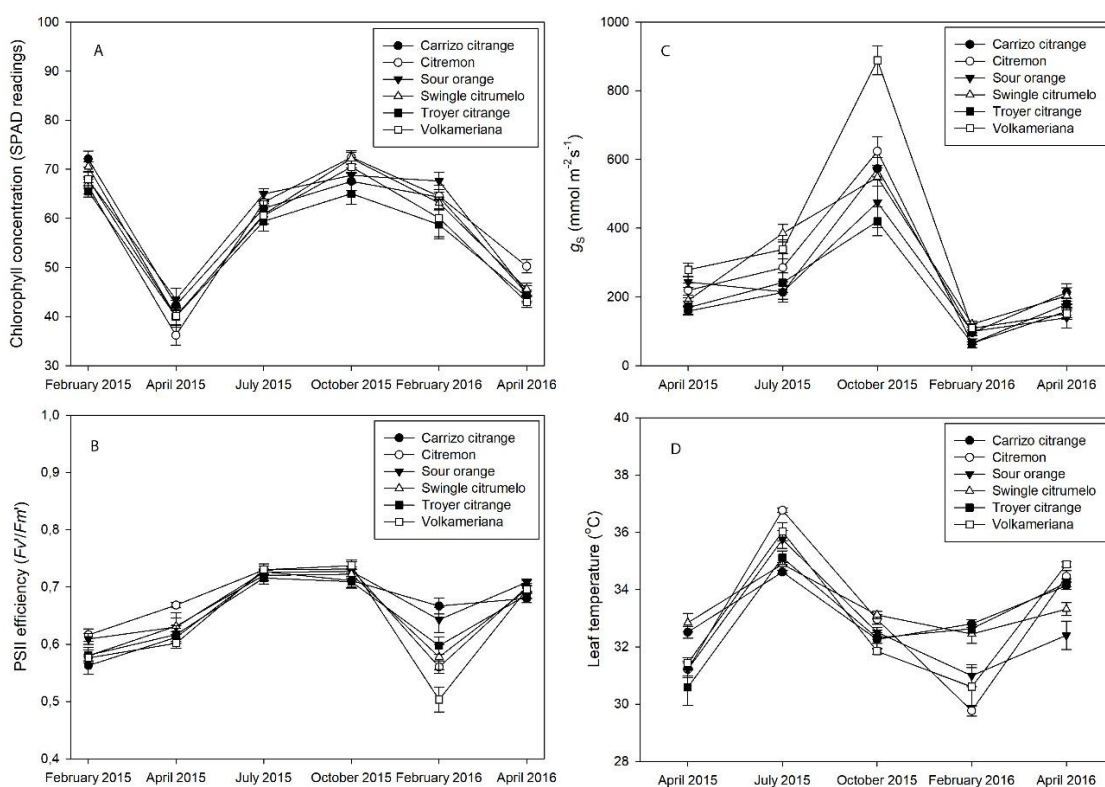


Figure 5. Rootstock effects on the Chlorophyll concentration (A), PSII efficiency (B), stomatal conductance (C), and leaf temperature (D) of 'Rio Red' grapefruit. Error bars represent \pm standard error

Leaf stomatal conductance measurements (g_s) and leaf temperature ($^{\circ}C$)

As shown in Table 2 in the present study, two-way analysis of variance (ANOVA) indicated a significant main effect of rootstocks and sampling date and their interaction ($p \leq 0.05$) on the leaf stomatal conductance and leaf temperature ($^{\circ}C$). There were also significant differences among rootstocks in terms of leaf stomatal conductance (g_s). The highest leaf stomatal conductance values were recorded in the trees on Volkameriana in almost all measurement periods. The lowest g_s values were recorded in the leaves of shoots on Troyer citrange, followed by those in the leaves of shoots on Carrizo. In terms

of measurement period, the highest g_s was recorded in October 2015; the lowest g_s was recorded in February 2016, followed by April 2016. According to the interaction between the rootstocks and the sampling date, Troyer citrange had the lowest g_s value in all periods (Fig. 5c). When all the measurement periods were examined, a decrease in leaf temperature due to the decrease in air temperature was clearly identified in the leaves of the trees grafted onto the Volkameriana and Citremon rootstocks. Decreases in stoma conductance were observed during periods when leaf temperature increased (Fig. 5d).

Leaf nutrients concentration

Leaf macro nutrients

The impact of rootstocks on the macronutrient concentrations of Rio Red leaves were significant, except for the N, P and K values (Table 3). There was no significant difference between the leaf N-K contents and rootstocks, but high N and K contents in the leaves of trees grafted onto the Volkameriana rootstock were found. The Ca and Mg contents in the leaves were affected by rootstocks. The leaf Ca content was significantly highest in trees on sour orange, while it was the lowest in trees on Volkameriana, followed by trees on Troyer citrange. The magnesium (Mg) contents of the trees on the Troyer and Carrizo citranges were significantly higher than those of trees on all other rootstocks, while the minimum Mg contents were recorded in trees on Volkameriana and Swingle citrumelo.

Table 3. Rootstock effects on the leaf mineral nutrients of 'Rio Red' grapefruit

Rootstock	%N	% P	% K	% Ca	% Mg	ppm Fe	ppm Mn	ppm Zn	ppm Cu
Carrizo	2.42	0.11	0.74	5.13 ab	0.34 a	85.70	46.10 cd	24.86 bc	13.82 a
Citrumelo	2.50	0.11	0.88	5.03 ab	0.32 c	71.54	48.53 bc	26.12 b	11.35 ab
Sour orange	2.56	0.10	0.76	5.35 a	0.25 b	73.17	51.17 ab	25.27 bc	12.67 a
Swingle	2.46	0.10	0.81	4.96 ab	0.23 c	74.22	49.32 b	26.05 b	13.78 a
Troyer	2.58	0.11	0.85	4.62 bc	0.35 a	70.36	44.82 d	24.47 c	13.10 a
Volkameriana	2.73	0.11	1.00	4.16 c	0.21 c	77.04	53.47 a	28.76 a	8.37 b
<i>Prob>F</i>	0.6518	0.1466	0.1477	0.0244	0.0001	0.6620	0.0002	0.0005	0.0230
<i>LSD%5</i>	-	-	-	0.560	0.022	-	2.786	1.381	2.781

Leaf micro nutrients

The concentration of micro-nutrients in the leaves of the Rio Red grapefruit cultivar was also significantly affected by rootstock-scion combinations, except for Fe (Table 3). The Rio Red/Volkameriana combination accumulated the highest scion leaf Mn and Zn concentrations, but the lowest concentrations of these micronutrients were recorded in the Rio Red/Troyer citrange combination. The leaf Cu content was significantly highest in trees on the Carrizo and Troyer citrange, Swingle citrumelo and sour orange rootstocks, while it was the lowest in trees on the Volkameriana rootstock, followed by the Citremon rootstock.

Discussion

Yield is the leading agronomic property for producers (Koepke and Dhingra, 2013). Most horticultural characters, such as tree growth, yield and quality, are influenced by rootstocks (Davies and Albrigo, 1994). In this study, rootstocks induced significant changes in fruit yield and some quality parameters. The highest cumulative yield of the 'Rio Red' grapefruit in the study period of three consecutive years (2013, 2014 and 2015 seasons) was obtained from the trees on the Carrizo and Troyer citranges and the Swingle citrumelo. Trees on the Citremon, Sour orange and Volkameriana rootstocks had the lowest cumulative yield. Previous studies have found that Carrizo citrange has positive effects on the fruit yield of Redblush and Marsh seedless grapefruits (Fallahi, 1992; Yalcin and Hizal, 1994; Tuzcu ve Toplu, 1999). In addition, in their study of Marsh seedless grapefruits, Mehrotra et al. (1999) reported that the Carrizo citrange rootstock had positive effects on yield. Despite these results, Ramin and Alirezanezhad (2005) reported that Volkameriana was the most productive rootstock for Marsh seedless and Redblush grapefruit. Volkameriana is a productive rootstock, and trees on Volkameriana may show high productivity. In this study, the low yield of trees on Volkameriana was probably due to the extremely low winter temperatures in the 2014 and 2015 seasons. The results obtained for sour orange productivity are in agreement with those of Louzada et al. (2008), who reported that 'Rio Red' trees on sour orange had the lowest yields compared to the other rootstocks evaluated, except for Goutou sour orange. In addition, Sharma et al. (2016) reported that sour orange was a low-yield rootstock for the Redblush grapefruit variety. Yield is controlled by rootstocks in fruit crops (Bassal, 2009) and the rootstocks induced changes in yield depending on the crop and type of rootstocks (Whiting et al., 2005). Based on the results of the current study on Rio Red grapefruit scions, Carrizo and Troyer citranges are very promising alternative rootstocks.

Fruit size is a valuable trait for both producers and consumers. Fruit size is one of the most important factors affecting the marketability of fresh citrus fruits, and medium- to large-sized fruits contribute maximum consumer returns in fresh markets. The present study showed rootstocks had significant effects on the fruit weight. The heaviest fruits were obtained from the trees on Sour orange, followed by the trees on Swingle citrumelo and Troyer citrange, while the Citremon rootstock produced the lightest fruit. Fruit diameter was significantly affected by rootstocks, and fruits from the trees on Troyer citrange had the highest fruit diameters. The trees on Citremon produced fruit with a small diameter. The results obtained in the present study are in agreement with those described in a previous report by Sharma et al. (2016), who studied the effects of rootstocks on the fruit quality of Marsh seedless grapefruit and indicated that Marsh seedless on Troyer citrange produced the heaviest fruit. Similar results have been reported for Redblush (Fallahi et al., 1989; Yalcin and Hizal; 1994), Marsh seedless (Mehrotra et al., 1999), and Lane Late (Emmanouilidou and Kyriacou, 2017), for which the highest fruit weights were recorded from trees on Carrizo and Troyer citranges. In their study of Marsh seedless grapefruit, Economides and Gregoriou (1993) concluded that the largest and heaviest fruits were produced by trees on the rough lemon group, Palestine sweet lime, Rangpur lime, Carrizo citrange and *C. volkameriana*. The TSS contents were higher in fruits from trees on Swingle and lower in fruits from trees on Volkameriana, followed by sour orange. The juice content per unit weight was higher for trees on Swingle citrumelo and lower for those on *C. volkameriana*. These results are in agreement with those of previous work, in which the fruits with the highest TSS

contents were from trees on Swingle citrumelo (Economides and Gregoriou, 1993; McCollum et al., 2002), and the lowest TSS contents were from trees on Volkameriana (Fallahi and Rodney, 1992; Stuchi et al., 2002; Ramin and Alirezanezhad, 2005; Emmanouilidou and Kyriacou, 2017). Variations in juice content due to rootstocks have also been reported in citrus (Economides and Gregoriou, 1993; Sharma et al., 2016). Fruit quality characters are important variables, as grapefruit is grown for fresh consumption and processed for its deep red coloration (Castle, 2012), which is highly influenced by the rootstock (Forner-Giner et al., 2003). Carrizo citrange and Swingle citrumelo produced fruit with more color, and the lowest color values were obtained from the trees on Sour orange and Volkameriana. Other researchers have also reported (Tuzcu et al., 1999; García-Sánchez et al., 2006; Bassal, 2009) that the fruits with the best color values were obtained from trees grafted on citranges.

Chlorophyll fluorescence is very useful to study the effects of environmental stresses on photosynthesis in plants (Bron et al., 2004). The maximum quantum yield of PSII was the smallest in the leaves of shoots grafted onto Volkameriana and Troyer citrange. Pestana et al. (2001) indicated that the chlorophyll fluorescence parameters of 'Newhall' orange grafted on 'Troyer' citrange slightly decreased under Fe-deprived conditions. González-Mas et al. (2009) reported clear decreases in the leaf chlorophyll fluorescence parameters of Navelina orange trees grafted onto Carrizo citrange growing in calcareous soil. In addition, Pestana et al. (2001) indicated that the chlorophyll fluorescence parameters of Newhall orange grafted onto Troyer citrange were slightly decreased under Fe-deprived conditions.

Our results demonstrate that rootstocks significantly affected leaf chlorophyll (Chl) concentrations. The scion leaves of shoots grafted onto Troyer citrange and Volkameriana had the lowest Chl concentrations, whereas the leaves of shoots budded onto Citremon and Swingle citrumelo had the highest Chl concentrations for nearly all periods. The results obtained in the present study are in agreement with those described in a previous report by Aboutalebi et al. (2012), who reported that rootstocks had significant effects on the leaf chlorophyll content of Valencia orange. In addition, González-Mas et al. (2009) indicated that the leaf Chl concentration of Navelina orange grafted on Carrizo citrange and grown in the calcareous soils of the Mediterranean region was lower than those of the other rootstocks evaluated. Considering the soil of the study area had 12% CaCO₃ and a slightly high pH level, the decreases in the leaf Chl concentrations of Rio Red grafted onto Troyer citranges are in agreement with the results of González-Mas et al. (2009).

There were also significant differences between the rootstocks in terms of leaf stomatal conductance (g_s). The highest the g_s value was recorded in the trees grafted onto Volkameriana; the lowest g_s values were recorded in the leaves of shoots on 'Troyer' citrange followed by the leaves of shoots on Carrizo. Chouliaras et al. (2004) indicated that g_s was significantly reduced in two orange cultivars on Swingle citrumelo. Similarly, Incesu et al. (2015) reported that 'Valencia' scion grafted on 'Swingle' citrumelo had a significantly lower stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$) that scion grafted onto other rootstocks grown in calcareous soil. Fe deficiency has a noticeable effect on the g_s of leaves because of the importance of Fe in many enzyme systems and in energy transfer during photosynthesis. Trees that were not affected by the calcareous soil had higher chlorophyll contents and photosynthesis values, which were associated with increased g_s values, as previously observed (Flexas et al., 2001; Afrousheh et al., 2010). Regarding the measurement periods, g_s values decreased in

periods when the leaf temperature became too high (>35 °C). The highest response of stoma conductance to changes in leaf temperature was detected for the Citremon and Volkameriana rootstocks. This may be due to the lemon hybridization of both rootstocks. In citrus plants, the optimum temperature for g_s is ≈ 30 °C (Khari and Hall, 1976). In addition, Ribeiro et al. (2004) indicated that the g_s values of sweet orange seedlings decreased as leaf temperature increased.

Rootstock had a meaningful impact on nutrient absorption, and the nutrient uptake efficiency varied with rootstock (Parvaneh et al., 2011). Taylor and Dimsey (1993) indicated that rootstock type and scion cultivar had different effects on the mineral element concentrations of scion leaves. In our experiment, there was no significant difference between the leaf N-K contents and rootstocks, but considerably high N and K contents in the leaves of the trees grafted onto the Volkameriana rootstock were observed. Barakat et al. (2013) reported that Volkamer lemon generally produced significantly higher N, P, K and Mg contents compared to sour orange. Georgiou (2000) showed that P and K in Nova mandarin leaves were induced by the Volkamer lemon rootstock. Similar results have been detected for Ca in previous studies, and higher Ca contents have been reported for trees on sour orange than for trees on other rootstocks (Toplu et al., 2008; Barakat et al., 2013; Dubey and Sharma, 2016). Citrange rootstocks produced higher concentrations of leaf Mg. These findings are in agreement with those of Taylor and Dimsey (1993) in Valencia and Navel oranges, Toplu et al. (2008) in Rhode Red Valencia and Valencia late, and Dubey and Sharma (2016) in Kagzi Kalan lemon.

Rootstocks significantly influenced the leaf Mn, Zn and Cu contents. The lower foliar Mn in trees on Troyer citrange reported by Iyengar et al. (1982), Toplu et al., (2008), Dubey and Sharma, (2016) is also supported by our results. Similar to previous studies, trees on Volkameriana had sufficient levels of leaf Zn, which were significantly higher than those in trees on Troyer citrange (Platt, 1981; Fallahi and Rodney, 1992). When compared to the budding of trees on Volkameriana, the budding of trees on citranges and other trifoliolate orange hybrids accumulated the highest leaf Cu contents in Rio Red. This result is compatible with the findings of Fallahi and Rodney (1992). Differences in leaf nutrient concentrations might be due to the alteration of root morphology and hydraulic conductance due to scion morphology and physiology (Save et al., 2000; Sharma et al., 2016).

Conclusion

In this study, we evaluated the influences of rootstock on the fruit yield, quality, leaf mineral composition and photosynthetic performances of 'Rio Red', which is an economically important grapefruit cultivar in Turkey. The results indicated that rootstock had significant effects on the evaluated variables. Fruit yield and quality were affected by the use of rootstocks. Carrizo and Troyer citrange and Swingle citrumelo rootstocks achieved the highest cumulative yields and improved the quality of fruits. The fruits with the lowest quality were obtained from trees on Volkameriana. In addition, fruit juice color is an important variable because grapefruit is grown for fresh consumption and processed because of its deep red color. Carrizo citrange produced fruit with more color than the fruit of other rootstocks. Rootstocks had different abilities to utilize plant nutrient elements; considering the yield, fruit quality and mineral nutrient uptake, Carrizo and Troyer citranges are suitable rootstocks for Rio Red

grapefruit grown under Mediterranean region conditions. Based on the chlorophyll content, gas exchange and the chlorophyll fluorescence parameters, “Rio Red” scion grafted onto the ‘Troyer’ and Carrizo citranges were affected by calcareous soil. On the other hand, trees on the sour orange rootstocks were shown to perform much better in calcareous soil. Considering its overall performance and its CTV resistance, Carrizo is a promising rootstock alternative to Sour orange, particularly in calcareous soils.

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