PHOTOSYNTHETIC PERFORMANCE AND FRUIT QUALITY OF 'W.MURCOTT' MANDARIN VARIETY ON FOUR ROOTSTOCKS

Berken Cimen

Cukurova University, Faculty of Agriculture, Department of Horticulture, Adana 01330, Turkey

Abstract

Rootstocks play an important role in the rapid development of World citrus industry. The necessity of using rootstocks for citrus fruits is to have a profitable production against some limiting factors such as climate, bad soil conditions, diseases, etc. In addition, scion behavior depends in part on the rootstock-induced effects on leaf gas exchange. In the present study, photosynthetic performance and fruit quality of 'W.Murcott' mandarin variety grafted on three new citrus rootstocks (Bitters, Carpenter and Furr trifoliate hybrids) developed by a rootstock breeding program in USA together with local sour orange were evaluated under Adana ecological conditions. Effects of rootstocks on photosynthetic performance were assessed by Net photosynthesis (P_N), transpiration rate (E), stomatal conductance (g_S), leaf water use efficiency (WUE), chlorophyll concentration (SPAD readings), chlorophyll fluorescences (Fv'/Fm'). Rootstocks significantly affected ($p<0.05$) the scion leaf chlorophyll concentration and chlorophyll fluorescence (Fv'/Fm'). Leaf of trees on local sour orange had higher P_N than Bitters, Carpenter and Furr trifoliate hybrids. Slight increases in fruit weight (g) and diameter (mm) were observed from fruit samples on Furr trifoliate hybrid. In addition, rootstocks significantly affected total citric acid content, which ranged between 0.68% (Bitters) and 0.78% (local sour orange).

Keywords: *Citrus, fruit characteristics, chlorophyll concentration, net photosynthetic rate, chlorophyll fluorescence*

1. INTRODUCTION

Turkey's citrus-producing regions are located in the northern half of the world's citrus-producing region, and Turkey has exceptionally reasonable environmental conditions and citrus-producing prospective, with 4.769.726 tons of citrus fruit produced in 2017 (FAO 2019). The mandarin production of Turkey has expanded considerably over the years, approaching approximately 1.5 million tons in 2017. Currently, 65% of Turkey's fresh fruit exportation is citrus fruits and, there has been a large increase in the exportation of citrus fruits in recent years, especially for mandarins.

Mandarins are called 'easy-peelers' because of their sweet flavor and aroma, loose skins, relatively small fruit size among the edible citrus and are easy to peel and separate into segments (Demirkeser et al. 2004). Currently, mandarins remain the most consumed and demanded citrus species due to some important advantages, such as smaller fruit, thinner skins and easy peeling in all over the world. Mandarins are among the most popular citrus fruits in Turkey, accounting for approximately 30% of the total production (FAO 2019).

Fruit quality has been a considerable commercial and research concern for breeders, producers and consumers which can be manupilated by the usage of rootstocks (Castle 1995). Rootstocks play an important role in the rapid development of citrus in the world. The necessity of using rootstocks for citrus fruits is to have a profitable production against some limiting factors such as climate, bad soil conditions, diseases and etc. Beside these factors, use of the citrus rootstocks provides a large amount of choices to the growers to increase fruit quality and yield, obtain early fruiting, uniform cropping, avoidance of juvenility, control of the tree size and have the opportunity for high density planting etc. (Yildirim et al. 2010). In addition, rootstocks positively affect scion growth, fruit internal/external quality and fruit yield (Castle, Nunnallee & Manthey 2009; Forner-Giner et al. 2003), along with photosynthesis (González-Mas et al. 2009). Amongst other physiological progressions, photosynthesis (P_N) is one of the elementary factor of plant productivity and the ability to preserve the percentage of carbon assimilation under ecological stress (Lawlor 1995).

As mentioned above, citrus is the main fruit group growing in the Mediterranean region of Turkey where the fruit trees can suffer from iron deficiency. Fe deficiency is a common issue in fruit trees grown in the Mediterranean region due to high calcareous soils. Lime induced Fe chlorosis negatively affects many of the plants grown in this area with its high humidity and semi-humid climate. In this region including our country, 20-50% of the fruit trees face lime induced Fe chlorosis (López-Millán et al. 2000; Pestana et al. 2005).

Sour orange is commonly used as a rootstock for all citrus cultivars in Turkey. Although the sour orange has many excellent horticultural advantages, it has a very important disadvantage for its susceptibility to Citrus Tristeza Virus (CTV). This problem has severely reduced the use of this rootstock in many places especially Western Mediterranean. Castle and Gmitter (1999) reported that sour orange no longer has a secure place in today's rootstock portfolios because of its susceptibility to CTV. Castle (1987) indicated that sour orange is an excellent rootstock for areas free of CTV. Ollitrault (2002) reported that the arrival of Tristeza radically called into question of using sour orange in the Mediterranean area, whereas it had been almost the only rootstock in the region. More than 80 million trees grafted on sour orange (*Citrus aurantium*) rootstock have been killed or rendered unproductive by CTV-induced decline. The losses caused in Argentina (more than 10 million trees), Brazil (more than 6 million trees) and USA (more than 3 million trees) have been reported. In Spain alone, more than 40 million trees, mainly sweet orange (*Citrus sinensis*) and mandarin (*Citrus reticulata*) grafted on sour orange, have declined progressively. Citrus breeders try to find new citrus rootstock until 1900's. Some of the important targets of citrus rootstock breeding are tolerance to CTV, alkanity, cold weather, and possitive effect on high fruit yield and quality. So far, Carrizo citrange, Troyer citrange, Swingle citrumelo, C35 citrange, C32 citrange obtained by hybridization method and all of them using in citrus production areas successfully (Cimen & Yesiloglu 2016). In addition Federici et al. (2009) reported that three new citrus rootstocks were released by the University of California, Riverside. These three rootstocks were named as "Bitters," "Carpenter," and "Furr" trifoliate hybrids, tested as C22, C54, and C57, respectively, and obtained by sexual hybridization of Sunki mandarin × Swingle trifoliate orange. The main character of these three hybrids is they all show good tolerance to citrus tristeza virus.

Although sour orange (*Citrus aurantium* L.) is still a popular rootstock in the Mediterranean region of Turkey, producers have began to prefer using citranges (Carrizo and C-35 citranges) in most recently established mandarin orchards due to their possitive effects on fruit quality. Sour orange and citranges have generally been satisfactory and, thus, there are limited rootstock studies involving mandarin varieties. In Mediteranean basin, producers still try to find a good rootstock not only tolerant to alkanity but also tolerant to CTV.

Regarding these matters, the present study was carried out to evaluate fruit quality and photosynthetic performance of W. Murcott mandarin variety budded on three newly introduced rootstocks (Bitters, Carpenter, Furr) together with commonly used sour orange under calcareous soil condition of the Mediterranean region.

2. MATERIALS AND METHODS

2.1. Plant Material

Trees of W. Murcott mandarin variety grafted on 'Bitters' (C22), 'Carpenter' (C54), 'Furr' (C57) and sour orange (*C. aurantium* L.) were used as material. Bitters, Carpenter and Furr are new rootstocks derived by crosses of Sunki mandarin x Swingle trifoliate orange originally produced by a rootstock-breeding program in the USA. Each produces uniform seedlings due to its high nucellar embryony and they are compatible with satsuma mandarin. In addition, Bitters is very tolerant of calcareous soil (Federici, Kupper & Roose 2009). Fruit samples were collected from 8-year-old trees located on the production lands of the Atlas Tarim Company (Latitude, 36°54'08.06" N; Longitude, 35°33'03.80" E; Altitude 11 m). The sample fruits of W.Murcott grafted on different rootstocks were harvested at optimum maturity stage and randomly selected from trees.

2.2. Fruit characteristics

The fruit weight (g), height (mm), diameter (mm), fruit shape index (fruit height/diameter ratio), rind thickness (mm), juice content (%), total soluble solids (%), titratable acidity (%), and ripening index (RI) were determined. Mature fruits of W.Murcott mandarin grafted on four rootstocks randomly selected (25 fruits for each rootstocks) from six trees. Fruit samples were immediately transferred to citrus physiology laboratory for quality analysis at the Department of Horticulture, Faculty of Agriculture, University of Cukurova. Fruits were randomly selected (25 fruits for each cultivar) and weighed to determine the average fruit size. The fruit was sized at the equatorial diameter and height with a digital caliper (Mitutoyo CD-15CPX). The fruits were cut in half and the thickness of the rind was measured with a digital caliper. Using a standard juicer, 25 fruits were weighed and juiced. The juice was weighed and expressed as a percentage of the total fruit weight. The total soluble solids (TSS) content was determined with a portable refractometer using a few drops of juice. The total acidity (TA) of the juice was determined by titration with 0.1 N sodium hydroxide (NaOH) using phenolphthalein as the indicator. The ripening index (RI) was calculated as the relation between the TSS and TA.

2.*3. Leaf chlorophyll concentration and Photosystem II efficiency*

For the estimation of leaf Chl concentrations by SPAD-502 chlorophyll meter, the same leaves were used with the measures for gas exchange (Minolta Inc., Osaka, Japan). Because SPAD reading and chlorophyll levels in citrus leaves are strongly linked, SPAD reading was used to estimate the concentration of Chl leaves (Jifon, Syvertsen & Whaley 2005). Also PSII maximum efficiency (*Fv'/Fm'*) readings in a light-adapted leaf phase were measured at the same leaves by using a portable fluorimeter (FluorPen FP100, Photon System Instruments Ltd, Drasov, Czech Republic).

2.4. Gas exchange measurements

A portable photosynthesis system detected the leaf gas exchange parameters of fully developed 4th to 5th leaves from the shooting apex (model LCA-4, ADC Bioscientific Ltd., Hoddesdon, UK) per each rootstocks (Cimen et al. 2014). Portable photosynthesis system measured net photosynthetic rate, P_N [µmol(CO_2) $m^{-2}s^{-1}$]; transpiration rate, E (mmol $m^{-2}s^{-1}$); and stomatal conductance, g_S (mmol $m^{-2}s^{-1}$) in each single measurement. The instantaneous photosynthetic water use efficiency was predicted as 'WUE = P_N/E' according to Ribeiro et al. (2009). During the gas exchange measurements, leaf temperature ranged between 26 and 28°C and the relative humidity was 65% where PFD was detected as 800-950 µmol m^{-2} s^{-1}.

2.5. Statistical analysis

The experiment was organized as four rootstocks and six replicates for each rootstocks in a 'Randomized Block Design'. The data were tested by an analysis of variance (ANOVA). The means and calculated standard deviations were stated. Least significant difference (LSD) test was used for mean comparison within rootstocks when the F test was significant at $p < 0.05$. Data subjected to ANOVA by the SAS v9 statistical analyses software and SigmaPlot® v11 (Systat Software, San Jose, CA, USA) was used for data presentation.

3. RESULTS AND DISCUSSION

3.1. Fruit characteristics

Rootstocks significantly affected variables related to fruit ripening such as total acid ($p < 0.01$), ripening index ($p < 0.05$) and juice content ($p < 0.05$) but not significantly affected on variables related to fruit size such as fruit weight, diameter and height (Table 1). Fruit weight of W.Murcott mandarin grafted on Bitters, Carpenter, Furr and sour orange ranged between 103.17 g and 107.63 g. Fruit diameter varied from 63.50 mm to 64.93 mm. Rootstock had no significant effect on fruit height and fruit index. Fruit height ranged between 51.42-53.50 mm, whereas fruit index varied from 1.19 to 1.24. Similary there were no significant effects of rootstocks on seed number per fruit where the seed number per fruit values ranged between 12.93 to 15.07 (Table 1).

Rootstock	Fruit weight (g)	Fruit diameter (mm)	Fruit height (mm)	Fruit index	Seed number
Bitters	105.37 ± 3.70	63.72 ± 0.41	52.48 ± 1.81	1.21 ± 0.04	13.80 ± 1.81
Carpenter	103.17 ± 2.61	63.70 ± 0.46	51.42 ± 0.58	1.24 ± 0.01	14.53 ± 1.16
Furr	107.33 ± 3.71	64.93 ± 1.93	52.73 ± 1.66	1.23 ± 0.04	15.07 ± 0.66
Sour orange	107.63 ± 5.78	63.50 ± 0.92	53.50 ± 0.61	1.19 ± 0.02	12.93 ± 2.71
Prob>F	*0.0873*	*0.1246*	*0.1948*	*0.1442*	*0.1059*

Table 1. Fruit weight (g), diameter (mm), height (mm), index, and seed number of W.Murcott grafted on different rootstocks. For each variable, one-way analysis of variance was performed with rootstocks as the independent variable.

Rootstocks also did not affected total soluble solids (TSS) of W.Murcott mandarin, however fruit samples collected from those grafted on sour orange (11.90%) had slightly lower TSS values than those grafted on citrandarins. TSS values were recorded as 12.33%, 12.17% and 12.37% from fruit samples grafted on Bitters, Carpenter and Furr, respectively (Table 2). In contrast, significant rootstock effect ($p < 0.01$) on total citric acid concentration of W. Murcott mandarin variety was obtained according to a one-way ANOVA (Table2). Total acid values ranged from 1.14% to 1.32%. The lowest total acids were determined in fruit samples grafted on Bitters citrandarin, whereas TA values were higher in fruit samples collected from the rest of the rootstocks and were in the same group according to a LSD test at α=0.05 (Table 2). The fruit ripening index is a widely used indicator to determine the citrus fruit maturity level. Significantly high values of the RI (TSS/TA) ratio were confirmed in fruits of W.Murcott grafted on Bitters citrandarin (10.80), whereas fruit samples from Carpenter, Furr and sour orange had the lowest values (Table 2).

Rootstock	TSS (%)	TA (%)	TSS/TA	Juice content (%)
Bitters	12.33 ± 0.51	1.14 ± 0.02 ^{b}	10.80 ± 0.31 ^{a}	31.68 ± 2.66 ^{b}
Carpenter	12.17 ± 0.55	1.27 ± 0.06 ^{a}	9.64 ± 0.91 ^{b}	37.24 ± 1.87 ^{a}
Furr	12.37 ± 0.11	1.28 ± 0.01 ^{a}	9.64 ± 0.12 ^{b}	39.25 ± 3.67 ^{a}
Sour orange	11.90 ± 0.23	1.32 ± 0.04 ^{a}	9.01 ± 0.21 ^{b}	35.36 ± 0.98 ^{ab}
Prob>F	*0.1013*	*0.0025*	*0.0134*	*0.0310*
LSD$_{0.05}$	-	*0.074*	*0.930*	*4.711*

Table 2. Total soluble solids (TSS), total acids (TA), ripening index (TSS/TA), and fruit juice content of W.Murcott grafted on different rootstocks. For each variable, one-way analysis of variance was performed with rootstocks as the independent variable. Values within the columns followed by different letters are significantly different by LSD test at α=0.05

Fruit internal and external properties are the most important quality parameters and are affected by many factors, such as genetic variability, climate and environment, and rootstock. However, variables related to fruit size such as fruit weight and diameter did not significantly affected by rootstocks in the present study. Several researhers also mentioned rootstocks did not significantly affect fruit dimensions and shape (Bassal 2009). Similar results were indicated by Gregoriou and Economides (1993) on 'Ortanique' tangor, Georgiou and Gregoriou (1999) on 'Shamouti' orange, and Georgiou (2002) on 'Clementine' mandarin, who reported that Sour orange, Carrizo citrange and 'Swingle' citrumelo did not significantly affect the fruit diameter. On the contrary, Legua et al. (2011) reported significant rootstock effects on fruit size of 'Lane Late' navel orange. Concerning juice acidity percentage, Bassal (2009) reported that the fruits of Marisol mandarin from trees budded on sour orange had the highest citric acid concentration

as compared with Carrizo citrange, Swingle citrumelo and Cleopatra mandarin rootstocks, which is in agreement with the present study. In addition significant rootstock effects on fruit juice concentration were reported by several authors, similarly to this study (Demirkeser et al. 2004; Legua et al. 2011).

3.2. Chlorophyll concentration and PSII efficiency

In the present study, as a result of the positive linear relationship (r^2 >0.8) between SPAD readings and Chl leaf concentrations, SPAD measurements were used to predict cholorophyll (Chl) levels of leaves (Jifon, Syvertsen & Whaley 2005). Rootstocks had significant influence on leaf chlorophyll concentration of W. Murcott mandarin. Considering the calcareous soils of the Mediterranean region of Turkey, where the experiment orchard was located, a remarkable decrease of leaf Chl content in W.Murcott grafted on Carpenter and Furr citrandarins (Fig 1A). In contrast, the highest leaf Chl content was estimated in the leaves on sour orange (67.00), followed by Bitters rootstock (61.67). The concentration of Chl was not significantly differed in the leaves of W.Murcott mandarin on Carpenter and Furr citrandarins growing in calcareous soil. The present study indicated that the Bitters rootstock showed good tolerance to lime-induced Fe chlorosis likely to sour orange. Fe is especially important in the synthesis and stabilisation of chlorophyll (Pestana et al. 2011). A number of authors have classified Fe tolerance to citrus rootstocks based on shoot and leaf chlorosis parameters. In deprived Fe conditions, sour oranges kept their content significantly higher than trifoliate orange and hybrids, compared to chlorophyll (Byrne, Rouse & Sudahono 1995; Castle, Nunnallee & Manthey 2009).

PSII efficiency (*Fv'/Fm'*) of W. Murcott mandarin was affected significantly by rootstocks ($p < 0.05$). Leaves grafted on to sour orange and Bitters had significantly ($P \leq 0.05$) higher *Fv'/Fm'* values than those on the other rootstocks in calcareous soil (Fig 1B). The declines in chlorophyll fluorescence variables from Navelina grafted on Carrizo rootstock in calcareous soil have been reported by Gonzáles-Mas et al. (2009). In addition, Pestana et al. (2011) indicated that chlorophyll fluorescence of Newhall navel grafted on Troyer rootstock decreased under iron deficiency. In the present study, PSII activity, in the light adapted period of the leaves, was slightly lower in Furr in comparison to the rootstocks evaluated.

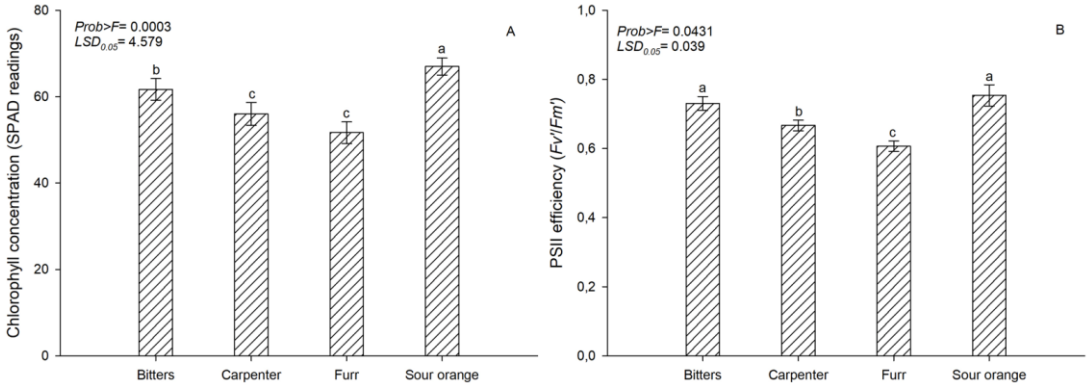


Fig. 1. Leaf chlorophyll (Chl) concentration (SPAD readings) and PSII efficiency of W. Murcott mandarin variety grafted on different rootstocks. Bars indicate standard deviations of the mean.

3.3. Gas exchange measurements

Leaf gas exchange measurements showed that rootstock effect was significant on photosynthetic variables of the mandarin variety evaluated in the present study. The results of an ANOVA indicated a significant effect ($p < 0.01$) on the net photosynthetic rate (P_N) of W. Murcott leaves (Fig. 2A). The highest P_N was recorded on the leaves of W. Murcott trees grafted on to sour orange (6.47 μmol-$m^{-2}s^{-1}$), followed by Bitters (5.95 μmol-$m^{-2}s^{-1}$). Leaf P_N was considerably on Furr (5.00 μmol-$m^{-2}s^{-1}$). The inhibition of P_N of the leaves on Furr citrandarin was found to be corresponding with the leaf Chl concentration and *Fv*'/*Fm*' of the leaves on same rootstock. A one-way ANOVA indicated that rootstock had also significant effect ($p < 0.05$) on leaf transpiration rate (*E*) of W. Murcott mandarin variety (Fig.

2B). Leaves on Carpenter and Furr had the highest E in contrast to P_N values of these rootstocks. Similary, significant rootstock effect ($p < 0.05$) on leaf stomatal conductance (g_S) was determined (Fig. 2C). The highest g_S was 96.05 mmol $m^{-2}s^{-1}$ in the leaves of shoots grafted on to sour orange followed by Bitters citrandarin (83.33 mmol $m^{-2}s^{-1}$). The lowest g_S was recorded in the leaves of shoots on Carpenter (72.00 mmol-$m^{-2}s^{-1}$) and Furr (65.67 mmol-$m^{-2}s^{-1}$). Leaf water use efficiency (WUE) significantly varied between leaf on different rootstocks ($p < 0.01$). The highest WUE was determined on the leaves grafted on sour orange and Bitters whereas lowesst WUE vales were determined from the leaves on Carpenter and Furr (Fig. 1D).

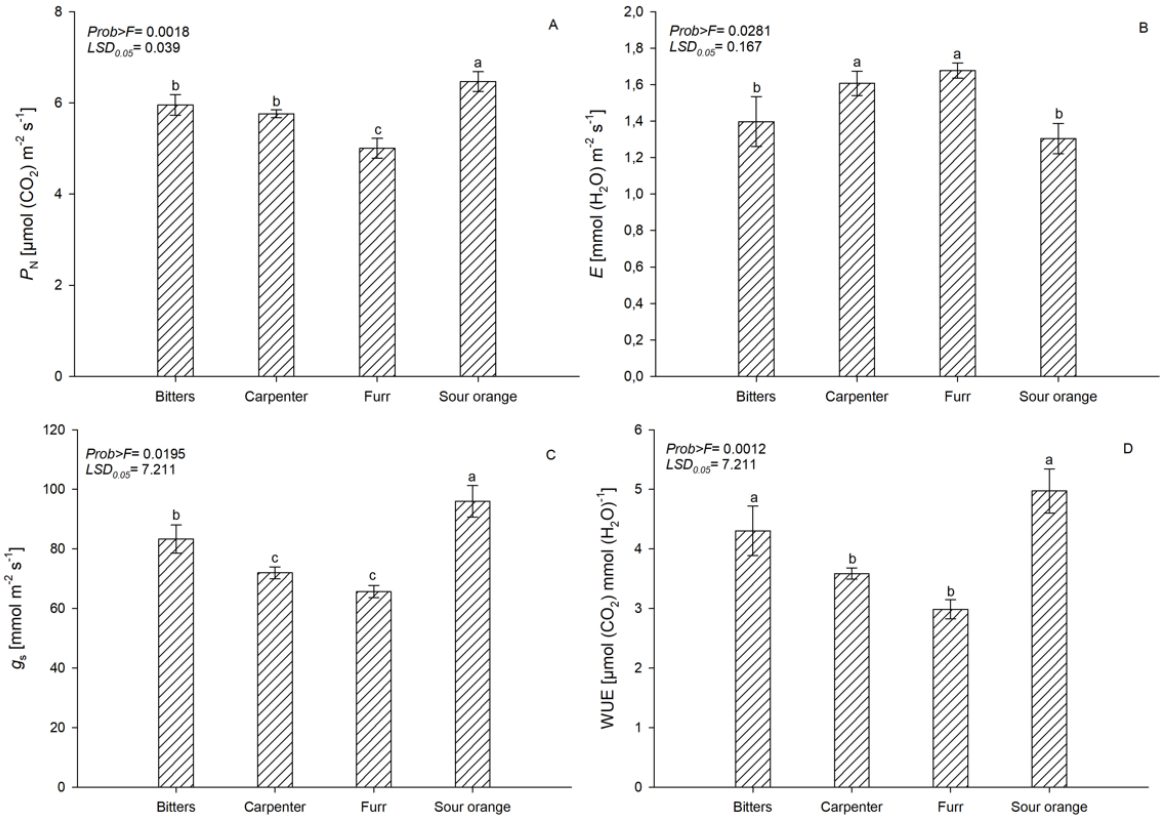


Fig. 2. Net photosynthetic rate, P_N [µmol(CO_2) $m^{-2}s^{-1}$] (A); transpiration rate, E (mmol $m^{-2}s^{-1}$) (B); stomatal conductance, g_S (mmol $m^{-2}s^{-1}$) (C); water use efficiency (D) of W. Murcott mandarin trees grafted on different rootstocks.

Physiological parameters like measurements of CO_2-gas exchange can confirm plant tolerance to Fe deficiency or high pH conditions (Bavaresco, Bertamini & Iacono 2006; Chouliaras et al. 2005; Larbi et al. 2006; Molassiotis et al. 2006; Morales et al. 2000; Nenova 2008). Gonzáles-Mas et al. (2009) indicated that P_N of the leaves of Navelina orange variety leaf on Carrizo was the lowest and different rootstocks significantly affected the P_N of Navelina variety growing in calcareous soils. The lime-induced iron deficiency has been reported to reduce Chl and P_N levels in citrus leaves (Byrne, Rouse & Sudahono 1995; Hamzé, Ryan & Zaabout 1986). In addition, Fe deficiency has discernible impact on the stomatal conduction of plants since of taking a part in numerous enzyme systems and for energy exchange during photosynthesis. On the other side, leaf water potential straightforwardly directs leaf transpiration, gas trade, and stomatal conduction in trees developed in calcareous soils enduring from Fe insufficiency (Brodribb & Holbrook 2003; Eichert et al. 2010; Meinzer 2002; Sperry 2000).

4. CONCLUSIONS

The present study assessed the effects of rootstock on some fruit quality characteristics and photosynthesis of W. Murcott mandarin which started to gain attention by Turkey's mandarin growers. Especially total acids and ripening index were remarkably affected by the usage of different rootstocks. Using Bitters citrandarin as a rootstock for W. Murcott mandarin under Mediterranean ecological conditions resulted slightly early ripening in comparison to other rootstocks investigated in this study. Besides, Carpenter and Furr significantly increased the juice content of W. Murcott mandarin. On the other hand, rootstocks were insignificant on fruit size, shape and seed number on W. Murcott mandarin. Since the Mediterranean region of Turkey has calcareous soils, concentration of leaf Chl in W. Murcott trees budded on Carpenter and Furr substantially reduced. Trees of W. Murcott budded on sour orange and Bitters performed much better on calcareous soil regarding photosynthetic variables. Thus using these rootstocks for W. Murcott mandarin should be beneficial. However, compatibility between rootstocks and scion should be further observed, especially in terms of fruit yield and productivity.

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