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Full Length Research Paper

Effects of rootstocks and irrigation levels on grape quality of *Vitis vinifera* L. cv. Shiraz

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The influence of two rootstocks (SO_4 and 1103P) on grape quality and berry chemical composition was studied in a factorial experiment, in field grown grapevines of cv. Shiraz (*Vitis vinifera* L.), subjected to five irrigation levels [0% (T1), 25% (T2), 50% (T3), 75% (T4) and 100% (T5) of irrigation depth (IW, mm): Class A pan evaporimeter (CPE)]. Spectrophotometric analyses of total anthocyanins (TA), total phenolics (TP) and total antioxidant activity (AA) in grape extracts were performed. Also, total soluble solids (TSS), total acidity, pH, total sugar content, ash, juice yield and color index of red grapes (CIRG) of berry samples were determined. TA, TP, AA, TSS, total sugar content, ash, and CIRG values decreased together with increasing irrigation levels. On the contrary, T4 and T5 irrigation treatments increased total acidity, pH and juice yield of samples compared to the effects of T1, T2 and T3 irrigation treatments for both rootstocks. Moreover, T1 or T2 treatments caused an increase in TA, TP, AA, TSS, total sugar content, ash, and CIRG index values of grape samples in comparison to that of vines irrigated with T3, T4 and T5 levels. Grape quality response to irrigation levels was altered by rootstocks and quality of grapes harvested from vines grafted on SO_4 was higher compared to those from 1103P under all irrigation treatments. Based on the findings, it was suggested that T2 irrigation level might be sufficient to guarantee Shiraz yield potential without significant loss in grape quality under the study conditions. Also, the results make it possible to recommend use of SO_4 rootstock under non-limiting water conditions because of its positive on grape quality parameters, while 1103P might be better choice under water-limiting conditions.

Key words: *Vitis vinifera* L., irrigation, rootstock, total phenolics, total anthocyanins, antioxidant activity.

INTRODUCTION

Water is the most important limiting resource for the vineyards of the South-Eastern Anatolia region of Turkey where water is supplied mostly by the winter rainfall that poorly matches the water requirement of vineyards in the region. Also, this region has the warmest climate in the country during the growing season and it is dry from early summer to late fall. Despite these conditions, grape is still one of the major horticultural crops grown in the region. The total area of vineyards in this region reached 12260 ha in 2006, which is about a quarter of the total grape production area of Turkey (Ozden and Karipcin, 2007).

Although grapevine (*Vitis vinifera* L.) is a traditionally non-irrigated crop grown in a range of natural environments, grape berry composition and vine development are highly dependent on vine water status (Jackson and Lombard, 1993). Thus recently, vineyards have been irrigated with drip irrigation system in the region. However, there is a controversy about the positive and negative impacts of irrigation on grapevine quality. It is commonly stated that excessive water application induces vegetative growth that causes lower fruit quality including low color, sugar content, phenolics and unbalanced acidity of berries (McCarthy, 1997; Esteban et al., 2001). On the contrary, low amount of water supplement can improve

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Abbreviations: TA, Total anthocyanins; **TP,** total phenolics; **AA,** total antioxidant activity; **TSS,** total soluble solids; **CIRG,** color index of red grapes.

grape quality due to reduction in vigor leading to an increase in slight interception in the cluster zone (Matthews and Anderson, 1988; Santos, et al., 2003, 2007; Chaves et al., 2007). Therefore, a rational use of water in irrigation in a given environment and cultivar is still unclear.

Rootstock utilization has been significantly increasing since the 1970s in the world. They vary in root distribution and affect scion responses in vigor, yield, fruit quality and other physiological parameters (Paranychianakis et al., 2004; Koundouras et al., 2008). Although vine response to irrigation treatments has been extensively investigated, rootstock effect on the quality components of grape berries under different irrigation treatments has not been sufficiently tested. In addition to their effect on yield, rootstocks also significantly affect fruit quality components such as total phenolic and anthocyanin content and vine productivity under different irrigation treatments (Hilal et al., 2000; Koundouras et al., 2008). The popularity of grape, as for wine making and fresh consumption, is getting higher as it is being a significant source of nutritional antioxidants such as phenolics and anthocyanins (Macheix et al., 1990). According to literature, the antioxidant activity of wine, fresh grape or grape juice stems from their phenolic compounds and anthocyanin content (Wang et al., 1997; Wang and Lin, 2000; Orak, 2007). Therefore, the objective of the study was to investigate the influences of rootstocks and irrigation treatments on total anthocyanins, total phenolics, total antioxidant activity and grape berry chemical compositions in Shiraz cultivar.

MATERIALS AND METHODS

Plant material and experimental conditions

The field experiment was carried out on an experimental vineyard of the agricultural research and training station of the agricultural faculty of Harran University in 2008. Shiraz cultivar grafted on 1103P (*Vitis berlandieri x Vitis. rupestris*) and SO_4 (*Vitis berlandieri x Vitis riparia*) was used as plant material. Rootstock is defined as the root system of the grapevine to which is grafted the desired variety of grapes. Two rootstock genotypes used in this study were characterized by different reported tolerance to dry conditions. 1103P was qualified as drought tolerant however, SO_4 was reported as less adapted to limited water conditions (Winkel and Rambal, 1993; Koundouras et al., 2008). Planting density of the vineyard, planted in 2004, was 3.0 x 1.5 m. Vines were trained to bilateral cordon system and spur pruned with 12 buds per plant. Row orientation was North-South. The experimental site had a clay soil with high permeability, pH and active lime content having low organic matter content. The climate of the region is classified as Mediterranean type, with hot and extremely dry summers and wet winters, with average annual rainfall of 400 mm during the autumn and winter months. July and August are the warmest months with almost no rainfall.

Irrigation treatment

In this study, five irrigation regimes were used and irrigation was applied weekly starting from the berry development (June) to harvest (August). Irrigation water applications were based on the ratio of irrigation water (IW, irrigation depth, mm), Class a pan evaporimeter (CPE) located in the vineyard. Irrigation treatments were 0% (T1), 25% (T2), 50% (T3), 75% (T4) and 100% (T5) of IW:CPE ratio. The total amounts of water supply for the T1, T2, T3, T4 and T5 treatments were 0, 218, 436, 654, and 873 mm, respectively. To determine the amount of water supplied for the irrigation treatments, total irrigated area was accepted as 33% of the vineyard and daily evaporation value corrected with 33% of the vineyard. All vines drip-irrigated by means of irrigation lines installed 30 cm above the soil surface, with drippers spaced 50 cm apart and set to supply water at a constant pressure of 152 kPa with 4.0 L/h. Amount of irrigation water was checked by flow-water-meter valves. Except for the irrigation treatments, all the other standard cultural practices in the vineyard were applied equally to all vines.

Sample preparation

Grape samples were harvested according to Rankine et al. (1962) at commercially ripe stage. According to Rankine et al. (1962), representative bunches from each vine were picked and berries were collected from each bunch in the laboratory. Subsequently, berries from each sub-sample were used for total soluble solids, ash, total sugar content, pH, total acidity and color index analyses. For sample extraction, berries were homogenized in an ice cold blender after removal of seeds and a 50 g of the homogenate were macerated in 100 ml of methanol containing 0.1% HCl and set aside overnight in darkness. Then the extract was filtered over Whatman No. 1 paper under vacuum and the residue was repeatedly extracted with the same solvent until it was colorless. A 50 ml extract was separated for determining anthocyanin content. The remaining portions were concentrated by a rotary evaporator at 50°C and used for determining total phenolics and antioxidant activities.

Determination of total anthocyanin content (TA)

TA content in the grape extracts were determined by the pH differential method described by Giusti and Wrolstad (2001) using a UV-vis spectrophotometer. Absorbance of the samples in 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) were measured at 520 and 700 nm using the equation: $A = (A_{\lambda 520} - A_{\lambda 700})_{pH\ 1.0} - (A_{\lambda 520} - A_{\lambda 700})_{pH\ 4.5}$. With a molar extinction coefficient of 28,000. Results were expressed as mg of malvidin-3-O-glucoside equivalent in per 1000 g fresh fruit (Wrolstad, 1976).

Determination of total phenolic contents (TP)

TP content in the grape extracts were estimated according to the method of Slinkard and Singleton (1977) with some modifications. Samples of 0.03 ml were introduced into test tubes, 2.370 ml distilled water and 0.15 ml Folin-Ciocalteu's reagent was added and the tubes were vortexed vigorously. After 8 min, 0.45 ml of saturated Na_2CO_3 was added to each tube and then each mixture was vortexed again and allowed to stand for 30 min at room temperature. Absorbance was measured at 750 nm. Tannic acid was used as standard. The results were expressed as mg tannic acid equivalent (mg/kg fresh weight).

Determination of total antioxidant activity (AA)

Free radical scavenging activity of the samples was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay with minor modifications as described by Blois (1958). A 0.1 mM solution of DPPH in ethanol was prepared. A 0.1 ml of various concentrations of the grape

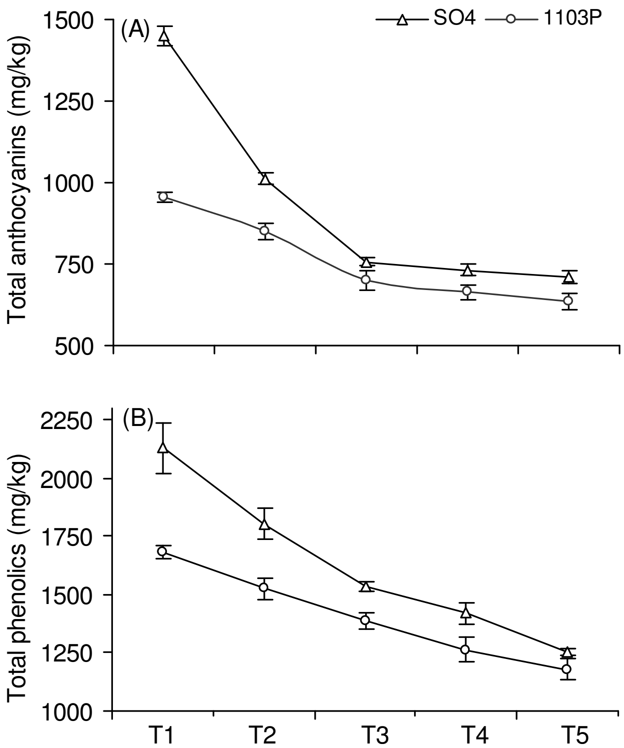

Figure 1. Total anthocyanins (A) and total phenolics (B) measured at harvest time in grape extracts from Shiraz grapevines grafted on SO_4 or 1103P under five irrigation levels (T1, T2, T3, T4, and T5). Each point represents the average of the three measurements with ± SEM.

extracts diluted in ethanol was added to 2.9 ml of DPPH solution. The decrease in absorbance at 517 nm was measured after 5 min of incubation at room temperature. Radical scavenging capacity of each extract was expressed as percent DPPH radical scavenging effect using the following equation.

$$\text{DPPH scavenging effect (\%)} = [(A_c - A_s)/A_c \times 100]$$

Where A_c is the absorbance of the control in ethanol and A_s is the absorbance of the grape sample. IC_{50}, the amount of sample extracted into 1 ml solution necessary to decrease by 50% the initial DPPH concentration, was calculated after constructing the percent inhibition versus extract concentration curve. Assays were carried out in triplicate.

Analyses of other quality parameters

TSS (Brix), pH, total acidity (g/l tartaric acid) and ash (%) were determined according to the standard methods of AOAC (1984). Total sugar content (%) of the samples was analysed by the Lane-Eynon method (Cemeroglu, 1992). Grape juice yield (cm^3/kg) was determined by using a manually operated packaged type press (AC Hydroulics P40H-40t, Viborg, Denmark). The pressing pressure was increased from 0 to 3,125 kg/cm^2 within 15 min (Yokotsuka, 1990). The color of grape pulp was measured with a colorimeter (Colour Quest XE, USA) calibrated with white and blank tiles provided by the manufacturer. The values of L*, a*, b* were converted to a color index for red grape (CIRG) defined by Carreno et al. (1995).

Statistical analysis

The experiment was arranged as a 2 x 5 factorial design (two rootstocks and five irrigation levels) with three replications. Each replication consisted of nine vines in a row (n = 27). Data were subjected to two-factor (rootstock and irrigation level) analysis of variance, using SAS (SAS Institute, 1995). Data presented here are means of three replicates with ± SEM. The significance of mean differences was determined by least significant differences (LSD) test using general linear model (GLM) procedure at $P \leq 0.05$.

RESULTS

Evaluation of TA, TP concentrations, and total antioxidant activities (AA) of grape samples

TA was affected by rootstocks and irrigation treatments with decreasing tendency from T1 to T5 (Figure 1A). When rootstock data were analyzed separately for each irrigation treatment, SO_4-grafted vines had a greater impact on TA in comparison to 1103P-vines. In addition, TA was significantly affected by irrigation treatments with decreasing means from T1 to T3, whereas no significant differences ($p < 0.05$) were found among T3, T4 and T5 treatments for any rootstock. Under T1 treatment, TA reached values up to 1452 mg/kg for SO_4-vines and up to 957 mg/kg for 1103P-vines. The TA of T5 treatment decreased by 51.2% compared to T1 in SO_4-vines and by 38.0% in 1103P-vines ($p < 0.05$). The effects of rootstocks and irrigation levels on TP content in Shiraz grapes are given in Figure 1B. Irrigation treatments had a significant effect on TP values of SO_4-grafted vines (ranged from 2130 to 1255 mg/kg). On this rootstock, TP of T1 treatment reduced by 15.3% compared to T2 treatment while the decrease was only 9.4% for 1103P-vines. Although T3, T4 and T5 treatments did not affect TP value of samples harvesting from 1103P-vines, each of the irrigation treatments was significantly effective ($p < 0.05$) on TP for SO_4-vines. Under the study conditions, rootstock effect on TP content, significant differences between rootstocks were found for all irrigation treatments except for T5 and samples of SO_4-vines displayed significantly higher TP values in comparison to 1103P-grafted vines. The method of scavenging stable DPPH radical is a widely used method to evaluate AA of samples in a short time. In this assay, the DPPH radical scavenging capacity of different extract volumes (0.1 to 1.0 ml) derived from grape samples in SO_4 or 1103P-grafted vines and subjected to five irrigation levels was measured (Figures 2A - B). Antioxidant capacity of samples was expressed as DPPH scavenging (%) versus extract concentrations.

Under five irrigation treatments, the DPPH radical scavenging capacity of different extract volumes for SO_4-grafted vines was different from each other especially at 0.1, 0.2, and 0.4 ml. Irrigation treatment effect on the DPPH scavenging capacity of SO_4-grafted vines decreased significantly ($p < 0.05$) in the following order: T1> T2>

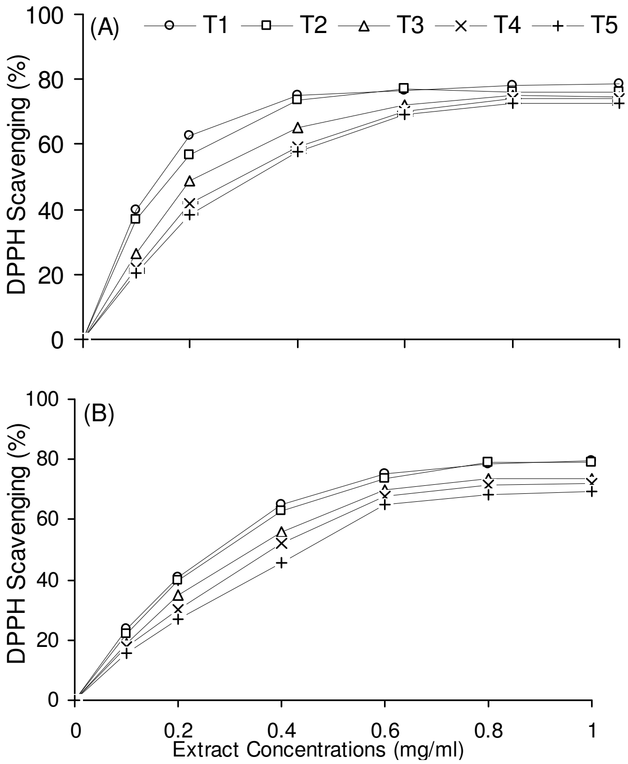

Figure 2 (A-B). DPPH radical scavenging capacity (%) of grape extracts at different volumes (0.1-1.0 ml) from Shiraz grapevines grafted on SO_4 (A) and 1103P (B) under five irrigation levels (T1, T2, T3, T4, and T5).

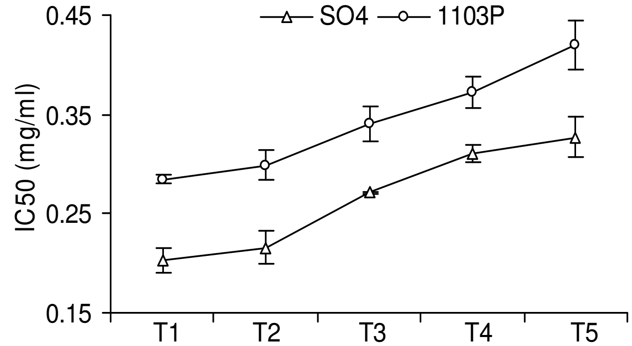

Figure 3. IC_{50} (mg/ml) of grape extracts from Shiraz grapevines grafted on SO_4 or 1103P under five irrigation levels (T1, T2, T3, T4, and T5). Each point represents the average of the three measurements with ± SEM.

T3> T4> T5 at 0.1, 0.2, and 0.4 ml extract volumes, respectively. However, under the same irrigation treatments, no significant difference was observed among 0.6, 0.8 and 1.0 ml samples for the inhibition percentage of DDPH (Figure 2A). Antioxidant capacity of grape samples in 1103P-grafted vines showed very similar variation pattern for the inhibition percentage of DDPH at the same concentrations (Figure 2B). However, the amount of fruit juice required to scavenge 50% of DPPH (IC_{50} values of samples harvested from SO_4-vines) was significantly lower compared to 1103P-vines. The highest IC_{50} values (ranging from 0.2853 to 0.4197 mg/ml) were obtained from vines on 1103P, whereas the lowest IC_{50} values (ranging 0.2033 to 0.3268 mg/ml) were measured in sample from SO_4-vines. Especially grape extracts from T1 treated vines grafted on SO_4 or 1103P showed the lowest IC_{50} values, 0.2033 and 0.2853 mg/ml, respectively (Figure 3). IC_{50} values decreased in the following order of irrigation treatments T1> T2> T3> T4> T5 and rootstocks SO_4>1103P. IC_{50} values were significantly affected by irrigation levels and rootstocks. SO_4 was much more sensitive decreasing in the IC_{50} value in comparison to 1103P. Lower IC_{50} value, indicating higher antioxidant capacity, was observed in samples obtained from SO_4-grafted vines as found with the inhibition percentage of DPPH as well.

Assessment of quality parameters

Influences of rootstocks and irrigation levels on TSS, color index, total sugar content and ash of samples are shown in Figures 4A - D. T1 grapes had higher TSS (25.5; 24.0 brix) than T2 and other irrigated vines in SO_4 or 1103P, respectively. Moreover, vines irrigated with T2 showed slightly higher TSS than the TSS value of T3, T4 and T5 (Figure 4A). However, the samples from T3, T4 and T5 treatments had similar TSS values in both rootstocks, indicating that those irrigation levels did not significantly delay ripening. At the time of harvest, significant reduction in color index of grape samples increased in parallel with increasing level of irrigation water (Figure 4B). Among the irrigation treatments, T1 treated vines showed the highest color index in both SO_4 and 1103P rootstocks in comparison to the other irrigation levels (Figure 4B). However, vines grafted on SO_4 exhibited higher color index compared to 1103P at all irrigation levels. Total sugar content of berries obtained from SO_4 grafted vines subjected to different irrigation treatments ranged from 23.93 to 21.78%, however, total sugar content varied from 22.96 to 19.66% in the berries collected from vines grafted on 1103P rootstocks (Figure 4C). It was also observed that increasing amount of irrigation water brought about a sharp decrease in total sugar content of berries from vines grafted on 1103P in comparison to vines grafted on SO_4 (Figure 4C). The evolution of ash percent in samples from vines grafted on 1103P treated with T1, T2 and T3 were very similar and the variations were found in T4 and T5 irrigation treatments. On the contrary, increasing irrigation levels resulted to a significant decrease in ash percent of berries collected from vines grafted on SO_4 (Figure 4D). There was no detectable difference between SO_4 and 1103P rootstocks at T4 and T5 irrigation levels. Total acidity, pH and grape juice yield are important parameters for evaluation of grape juice quality. Their values are illustrated in Figures 5A - C. While total acidity, pH and grape juice yield of T1 grapes

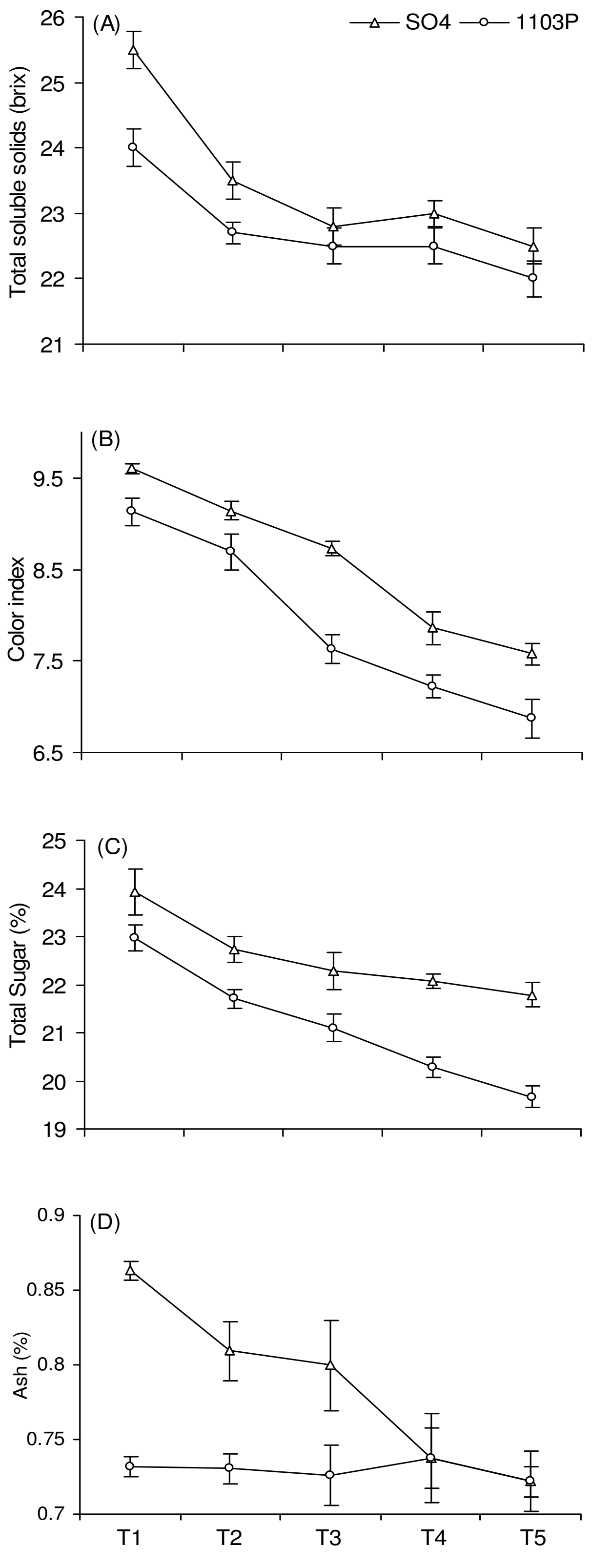

Figure 4. Total soluble solids (A), color index (B), total sugar (C), and ash (D) measured at harvest time in grape extracts from Shiraz grapevines grafted on SO_4 or 1103P under five irrigation levels (T1, T2, T3, T4, and T5). Each point represents the average of the three measurements with ± SEM.

were the lowest levels, the highest values of these parameters were measured in T5 grape samples in both rootstocks. The results showed that total acidity of samples significantly increased starting from T1 to T5 for both rootstocks (Figure 5A). However, the effect of T2, T3 and T4 on pH level of samples were negligible compared to T5. Also pH value of samples taken from 11O3P-grafted vines were significantly higher in comparison to pH value of SO_4-grafted vine samples (Figure 5B). According to the results, higher amount of juice yields (543, 480 cm^3/kg) were obtained with T5 treatment for both rootstocks, while the lower amount of juice yields (455, 480 cm^3/kg) were measured in T1 treated vines on SO_4 and 1103P, respectively. Also, grape juice yield of vines on 1103P was significantly higher than that of vines on SO_4 with for all irrigation treatments tested in this study (Figure 5C).

DISCUSSION

Irrigation increases photosynthesis, yield and grape quality depending on the amount of water applied, cultivar, environmental conditions and other cultural practices (Escalona et al., 2003; Cifre et al., 2005). The results of this study showed that increasing irrigation level decreased TA, TP, AA, TSS, total sugar content, ash and CIRG index values. However, it is interesting to note that the same irrigation treatments increased total acidity, pH and juice yield of berries collected from vines grafted on SO_4 or1103P rootstocks. Moreover, T1 or T2 irrigation treatments caused an increase in TA, TP, AA, TSS, total sugar content, ash, and CIRG index values of grape samples compared to that of vines irrigated at T4 or T5. Similar findings in different grape cultivars were reported by Chaves et al. (2007) and Santos et al. (2007) that fruits harvested from non-irrigated and lower-irrigated vines exhibited higher concentrations of TA and TP than those presented by fully irrigated vines. The significant decrease in TA, TP, AA, TSS, total sugar content, ash and CIRG index values of samples in increasing irrigated vines on both rootstocks may be attributed to the excess water flow into the berries which results to dilution of the sugar content, and also decreases flavor compounds (phenolics and anthocyanins) in the skin of Shiraz grapes. On the other hand, low amount of water supply (T2 or T2 treatments) displayed positive effects on grape quality by reducing grape juice and thus resulted in an increase in skin to juice ratio. This could increase the concentration of total anthocyanins and total phenolics in Shiraz grapes as indicated in earlier studies conducted by Medrano et al. (2003) and Chavez et al. (2007). In our study, quality parameters of Shiraz grapes decreased in parallel to the increasing amount of applied irrigation water. The higher amount of irrigation water supply, the lower TA, TP, AA concentrations was detected in the berries of Shiraz vines grafted on SO_4 or 1103P. These results showed that although higher amount of water

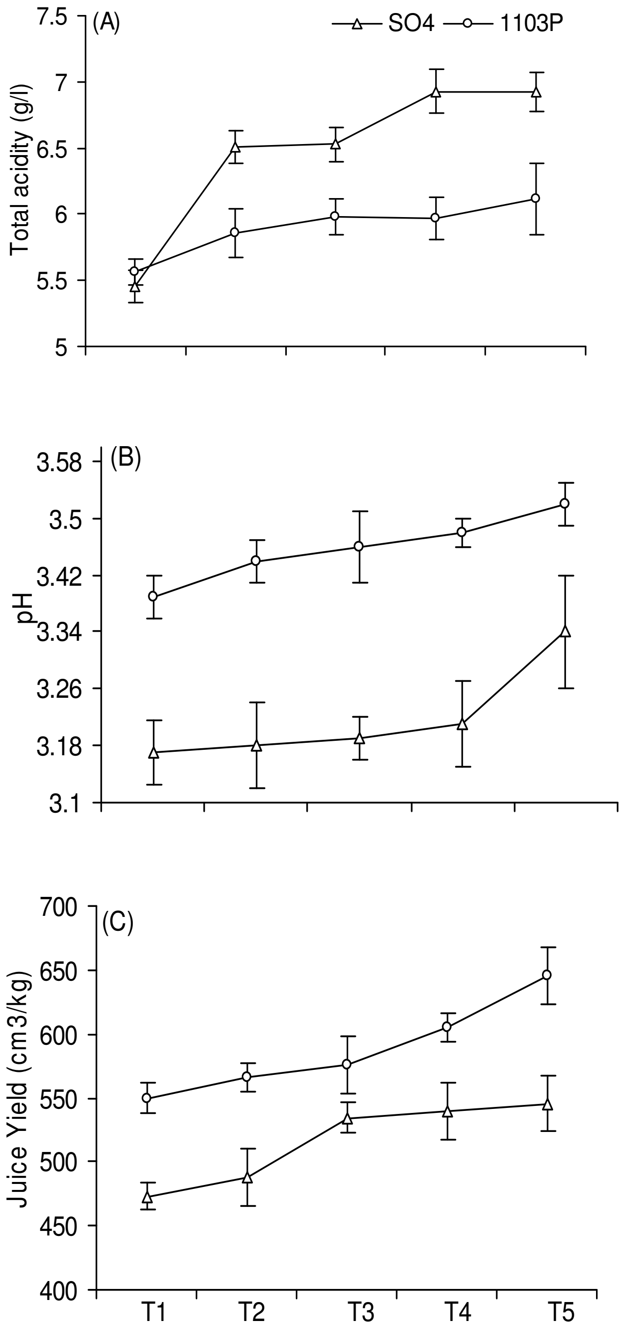

Figure 5. Total acidity (A), pH (B), and juice yield (C) measured at harvest time in grape extracts from Shiraz grapevines grafted on SO_4 or 1103P under five irrigation levels (T1, T2, T3, T4 and T5). Each point represents the average of the three measurements with ± SEM.

supply had a positive effect on fruit juice yield, it had a negative effect on quality, due to color loss and low sugar content. Similar findings regarding higher amount of water application were observed in different grape cultivars as well (Calo et al., 1997; Esteban et al., 1999, 2001). On the contrary, limited water supply positively affected quality of grape juice by increasing fruit color, soluble solids, total sugar content and pH as reported earlier studies. For the DPPH assay, the lower the IC_{50} value is, the better the radicals' scavenging capacity, particularly proxy radicals which are the propagators of the auto-oxidation of lipid molecules and thereby break the free radical chain reaction (Frankel, 1991). Based on our findings, the bleaching of DPPH solution increased regularly with increasing amount of fruit extract in a given volume of solution. Also, strong relationship was observed between TA, TP concentrations and IC_{50} values. The bleaching action of the samples was attributed to the presence of phenolic compounds in the grape extracts. Lower IC_{50} values were obtained from T1, T2 and T3 vines with the higher TA, and TP concentrations compared to the other irrigation treatments. Our findings are supported by Orak (2007) who presented the relationship between TA, TP concentrations and AA capacity of grapes. It was determined that grape cultivars with higher TA and TP contents had higher AA capacity. According to the data obtained, both SO_4 and 1103P rootstocks were found significantly effective on quality components and chemical compositions of berries under the same irrigation levels. However, these effects were altered by the amount of water applied, in terms of quality and berry components. Under the same irrigation treatments, TA, TP, AA, TSS, total sugar content, ash and CIRG index values of samples obtained from vines grafted on SO_4 were higher than those from 1103P. However, total acidity, pH and fruit juice yield of grape samples harvested from vines on 1103P was higher, compared to vines on SO_4. Based on the literature, the difference observed in rootstock effects on quality components and berry chemical compositions under the irrigation treatments could be explained with a high vegetative growth of vines on 1103P compared to SO_4 rootstock. It was reported that rootstocks affected characteristics of grapevine variety including amount of yield, yield quality, canopy growth and drought resistance. 1103P rootstock genotype is well adapted to warm regions and more resistant to drought conditions compared to SO_4. On the contrary, SO_4 rootstock genotype is more vigorous than 1103P under non-limiting water conditions. Rootstock impact on berry quality parameters could be caused by difference in their root structure and consequential difference in their water use efficiency. Because vine canopy is in balance with its root system, bigger canopies are associated with larger root system (Southey and Jooste, 1992). Also it is commonly known that 1103P has a dipper and larger root system compared to SO_4 rootstock genotype. In this context, higher vegetative growth of vines grafted on 1103P compared to SO_4 grapevine rootstock was associated with a larger root system (Swanepoel and Southey, 1989; Koundouras et al., 2008). Moreover, SO_4 was reported to have low biomass, shallow rooting and higher water flow resistance compared to 1103P (Herralde et al., 2006). Therefore, the results make it possible to use SO_4 rootstock under non-limiting water conditions because of its quality effect, while 1103P might be a better choice under water-limiting conditions.

Conclusion

Based on the results, it may be concluded that rootstocks and irrigation levels play direct role on grape quality in terms of TA, TP, AA concentration and berry chemical composition of Shiraz grapes. Moreover, T2 irrigation level for the both rootstocks might be sufficient to guarantee Shiraz yield potential without significant loss in grape quality under the study conditions. When aiming to achieve the quality standards for premium wines, the data of this study, may be great relevance in evaluating rootstocks for dry and non-limiting water conditions. Therefore, the results make it possible to recommend SO_4 rootstock under non-limiting water conditions, while 1103P might be a better choice under water-limiting conditions. Finally, the results implied that the choice of the appropriate rootstock might be crucial to improve grape quality and berry composition under limited water resource conditions.

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