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Changes in Growth, Mineral Nutrients, and Phenolic Compounds in Two Table Grape Cultivars and Their Graft Combinations Under Bicarbonate and Fe Deficiency Conditions

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ABSTRACT

Iron chlorosis is a concern in self-rooted and grafted vines cultivated in calcareous soils. Susceptibility to Fe chlorosis in grapevine usually varies, depending on genotype. The purpose of this study was to evaluate the morpho-physiological responses of grapevine cultivars ('Flame Seedless' and 'Thompson Seedless') and their graft combinations ('Flame Seedless'/'Thompson Seedless' (FS/TS) and 'Thompson Seedless'/'Flame Seedless' (TS/FS)) to bicarbonate (30 mM) application and Fe deficiency, while also examining the effects of different Fe sources (FeEDTA and $FeSO_4.7H_2O$) in soilless culture condition. In this study, a factorial experiment was conducted based on a completely randomized design with 4 replications. Results indicated that chlorophyll a, b, and total, as well as carotenoids in the grafting combination of FS/TS were less affected and decreased by about 20% under bicarbonate treatment. The 'Flame Seedless' cultivar and grafting combination of TS/FS was more affected and decreased by about 50% under this condition. Results indicated that shoot and root fresh weights decreased by about 25% and 32%, respectively, in all cultivars under bicarbonate treatment in response to both iron sources. Root volume decreased in 'Thompson Seedless', 'Flame Seedless', and TS/FS by about 30%, 38%, and 50%, respectively, under the bicarbonate treatment when FeEDTA was used as an iron source. Adding bicarbonate to the nutrient solution increased some of the phenolic compounds in 'Thompson Seedless' and the graft combination of FS/TS roots. Generally, bicarbonate had more adverse effects on 'Flame Seedless' and the TS/FS graft combination, compared to 'Thompson Seedless' and FS/TS, confirming that the use of more iron-efficient rootstocks in the graft combination can contribute to bicarbonate tolerance in the scions of cultivars with lower tolerance to bicarbonate

Introduction

Iron is an essential mineral nutrient for plant growth since it takes part in many primary metabolic processes, including chlorophyll synthesis, photosynthesis, respiratory electron transport, and nitrogen assimilation. Although

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the total iron content in soil is relatively high, its acquisition by crop plants is often limited because of its low bioavailability in well-oxygenated and alkaline soil conditions (Fu et al., 2017). Chlorosis in leaves and decreased yield usually result from iron deficiency, which can cause serious problems for crop production in alkaline and lime soils throughout the world.

Bicarbonate in soil and irrigation water is one of the main factors that cause Fe chlorosis in plants (Mengel et al., 1984). Bicarbonate is an anion that abounds in calcareous soils in arid and semi-arid areas. It is often created by dissolving carbonate minerals in plant materials and also by combining water and CO_2 (Zhao and Wu, 2017). In calcareous soils, bicarbonate concentrations can reach values of up to 15 mM (Boxma, 1972). It has been estimated that 30% of the world's arable land has soil properties that do not allow the uptake of sufficient Fe for optimal plant growth and yield. The earliest indications of iron deficiency date back more than 100 years ago. Since then, iron deficiency has been recognized as one of the most common yield-limiting nutrient disorders, posing a serious economic problem through damages, high costs, and often low efficiency of Fe fertilizers (Buckhout and Schmidt, 2013). Moreover, the supply of iron fertilizers not only increases production costs but also damages the environment.

Selecting iron-efficient cultivars and rootstocks is believed to be an effective and environment-friendly strategy to minimize iron chlorosis in crop production (Ksouri et al., 2007; Molassiotis et al., 2006). It is well known that plant susceptibility to Fe chlorosis in grapevine cultivars and rootstocks can be highly variable in function of the genotype and scion-rootstock interaction (Ksouri et al., 2006). Bavaresco et al. (2003) found that the shoot height of *V. vinifera cv.* ''Pinot blanc'' grafted onto 41B rootstock (Fe-deficiency tolerant) was less affected than when grafted on susceptible 3309C when cultivated on calcareous soils. De La Guardia et al. (1995) showed that peach shoot height and biomass production decreased to different extents by bicarbonate-induced Fe deficiency (De La Guardia et al., 1995). Grapevine belongs to strategy-I plants, and, therefore, under Fe deficiency, it can increase Fe reductase activity and net release of protons and organic compounds in root rhizospheres, e.g. organic acids, and phenolics, thereby lowering the pH and increasing the solubility of Fe(III) (Ksouri et al., 2006; Jiménez et al., 2008). The secretion of plant-derived chelators, chiefly phenolics compounds or flavins, is species-specific and may comprise heterogeneous compounds that could also affect the microbiome in the rhizosphere (Buckhout and Schmidt, 2013). Ksouri et al. (2006) found that '140 Ruggeri' is highly tolerant to Fe chlorosis because of its high root Fe(III)-reductase activity and its ability to release phenolic compounds in the medium (Ksouri et al., 2006). In a study carried out on grapevine rootstocks, Nikolic et al. (2000) concluded that bicarbonate-induced Fe chlorosis was caused by an inhibition of Fe uptake and translocation due to inhibited Fe(III) reduction by root cells, with these processes being less inhibited in chlorosis resistant rootstocks (Nikolic et al., 2000). In this context, a knowledge gap was identified under calcareous conditions, regarding the role of grapevine rootstocks and their responses to scions of different cultivars. Thus, the current research assessed the physiological and morphological status of two table grape cultivars, their graft combinations, and their ultimate response to bicarbonate and Fe deficiency.

Materials and Methods

Plant material, growth conditions, and treatments

This experiment was carried out in a research greenhouse, with diurnal temperatures of 27±3 ºC, and nocturnal temperatures of 16±3 ºC, in the Department of Horticultural Science, School of Agriculture, Shiraz University, Shiraz, Iran (52 ◦ 32'E and 29 ◦ 36'N, 1810 m) from March to August 2019. Hardwood cuttings of the 'Thompson Seedless' and 'Flame Seedless' were used as both scions and rootstocks and grafted by the bench grafting method reciprocally. They were placed in a greenhouse bench (with average relative humidity and daily temperatures of 45 ± 5% and 24±4 ºC, respectively). The plants were acclimated in perlite for 2 months (from March to April). Grafted cuttings (heterograft) and rooted cuttings ('Flame Seedless'/'Thompson Seedless' (FS/TS), 'Thompson Seedless'/ 'Flame Seedless'(TS/FS), 'Thompson Seedless', and 'Flame Seedless') were transferred into 7 L plastic pots filled with a mixture of cocopeat and perlite (1:1 v/v ratio). The vines were irrigated with half Hoagland nutrient solution for 3 weeks and then with complete Hoagland solution containing 6 mM KNO_3, 2 mM $MgSO_4$, 1 mM KH_2PO_4, 4 mM $Ca(NO_3)_2$, 9.2 µM $MnCl_2$, 46.2 µM H_3BO_3, 0.12 µM Na_2MoO_4, 0.8 µM $ZnSO_4$, 0.38 µM $CuSO_4$, and 23 µM FeEDTA (Covarrubias and Rombolà 2015). After the establishment of plants (about 3 months), when they had grown 10-12 fully expanded leaves, the treatments were applied and the experiment continued for 5 weeks. The treatments for each cultivar and graft

combination included Hoagland nutrient solution and the following treatments: (1-) 23 µM FeEDTA + 0 mM NaHCO3 (pH= 5.8±0.2) (FE-Bic), (2-) 23 µM FeEDTA + 30 mM $NaHCO_3$ (pH= 7.9±0.2) (FE+Bic), (3-) 23 µM $FeSO_4.7\ H_2O$ + 0 mM $NaHCO_3$ (pH= 5.8±0.2) (FS-Bic), (4-) 23 µM $FeSO_4.7\ H_2O$ + 30 mM $NaHCO_3$ (pH= 7.9±0.2) (FS+Bic), (5-) 2.3 µM FeEDTA + 0 mM $NaHCO_3$ (pH= 5.8±0.2) (FD-Bic).

Measurements

At the end of the experimental period, the leaf area was determined (Delta-t device, United Kingdom). Plants were harvested and their root volume was measured after the loss of water. The fresh weight was determined. The samples were then dried at 70 °C for 48 h and weighed for dry weight. After grinding the samples, they were ashed at 500 °C for 5 h and dissolved in an HCl solution (3.3%). An analysis was carried out on Ca, Mg, Zn, Cu, and Fe concentrations, using atomic absorption spectrometry (GBC Avanta Version 1/33; GBC Scientific, Hampshire, IL, USA). Phosphorous content was measured following the method of Barton (1948).

Absolute growth rate (AGR)

The rate of increase in growth was variable at the time 'T', called AGR. It was measured by a differential coefficient of 'H' concerning time 'T'. The absolute growth rate was calculated for the growth variable using the following formula:

$$AGR = \frac{H2 - H1}{T2 - T1}$$

AGR (plant height), H1 and H2 refer to plant height (cm) at time 'T1' and 'T2', respectively, expressed as cm per day (Ghule et al., 2013).

Chlorophyll content

Chlorophyll content was measured according to the method of Arnon (1949) using the following formula:

Chlorophyll a (mg g^{-1}. F. W)

$$= \frac{12.7(A_{663}) - 2.69\ (A_{645}) \times \text{Volume made}}{\text{Wt. of the sample} \times 10}$$

Chlorophyll b (mg g^{-1}. F. W)

$$= \frac{22.9\ (A_{645}) - 4.68\ (A_{663}) \times \text{Volume made}}{\text{Wt. of the sample} \times 10}$$

Total chlorophyll (mg g^{-1}. F. W)

$$= \frac{20.2\ (A_{645}) + 8.02\ (A_{663}) \times \text{Volume made}}{\text{Wt. of the sample} \times 10}$$

Carotenoid (mg g^{-1}. F. W)

$$= \frac{1000(A_{470}) - 1.82\ C_a - 85.02\ C_b}{198}$$

Total chlorophyll was extracted in the dark for 72 h in dimethyl sulphoxide. The absorbance of the leaf extract was measured at 663 nm and 645 nm using Epoch Microplate Spectrophotometer (Company Biotech, USA).

Proline content

Proline was quantified spectrophotometrically (UV-120-20, Japan) by the ninhydrin method according to Bates et al. (1973). Leaf samples (100 mg) from control and treated plants were incubated in 10 mL of 3% sulphosalicylic acid for 48 h. Then 2 ml of leaf extract was applied for assaying proline content. Ninhydrin (1.25 g) was dissolved in 30 mL of glacial acetic acid and then 20 mL of 6 M phosphoric acid was added and kept for 24 h at 40 °C. Together with 2 mL of plant extract, 2 mL of acid Ninhydrin, and 2 mL of glacial acetic acid were added and the mixture was boiled at 100 °C for 1 h in a water bath. Then, the solution was cooled and the reaction ended. Then, 4 mL of toluene was added to the solution and mixed vigorously for approximately one minute and OD values for the colored component were measured at 520 nm using toluene as the blank. From the OD values, proline content (µmoles g^{-1} fresh wt.) was calculated separately.

Malondialdehyde content (MDA)

The lipid peroxidation (MDA content) was measured using the method of Dhindsa and Matowe (1981) with some modifications. A 0.5 mL enzyme extraction was mixed with 1 mL of 20% trichloroacetic acid containing 0.5% (w/v) thiobarbituric acid. The tubes were placed in a preheated 95 ºC water bath for 20 min quickly cooled and then centrifuged at 10000 g for 10 min. The absorbance was measured at 532, 600, and 450 nm, respectively using Epoch Microplate Spectrophotometer (Company Biotech, USA). The concentration of MDA was calculated by subtraction of OD600 from OD532 and OD450.

HPLC analysis for phenolic compounds

Leaf samples were powdered (1 g per each sample) and were extracted with 10 ml methanol (80%) at room temperature for 24 h, by which time the extract was filtered through a filter paper for sample cleanup. Then, the sample solution was filtered through a 0.45 µm membrane filter and 20 µl of the obtained solution was injected into the HPLC system. Due to crowded peaks in the chromatogram, extracts were diluted in 1:10 ratios. HPLC analyses were performed on an Agilent Technologies 1200 series, liquid chromatograph (Germany) equipped with a diode-array detector (DAD). Zorbax Eclipse XDB-

C18 column was used at 30 ºC temperature and a flow rate of 1 ml min^{-1}. The mobile phase contained methanol (100%) (solvent A) and formic acid 1% (solvent B) according to the following linear gradient: 10% A + 90% B in 0 min, 25% A + 75% B in 10 min, 60% A + 40% B in 20 min, 70% A + 30% B in 30 min and 70% A + 30% B in 40 min. The injected volume for extracts and standards was 20 µl and chromatograms were observed at 280 nm. Flavonols and flavonol-glycosides (quercetin and rutin), flavanones (hesperidin and hesperetin), and flavon-3-ols (catechin) were identified via pure standard compounds and by comparing their retention times at 280 nm. Quantifications were achieved by establishing calibration curves for each compound with different concentrations of pure standards (0.1, 1, 25, 50, 100, 300, 600, and 1000 ppm). Linear calibration curves for standards (peak area vs. concentration) were obtained by R2 ¼0.99 (Pavlovic et al., 2013).

Statistical analysis

The factorial experiment was conducted as a completely randomized design with 4 replications. Statistical analyses were executed using the SAS statistical software (version 9.1) and mean values were compared using Duncan's Multiple Range Test ($p \leq 0.05$).

Results

Different morphological and physiological traits were measured to evaluate the scion and rootstock interaction in response to sodium bicarbonate and Fe deficiency treatment, while different iron sources were used.

Plant pigments

According to the main effects of each factor, chlorophyll and carotenoid content in the mature leaves decreased in plants subjected to bicarbonate and Fe deficiency treatments. However, this reduction was more pronounced with bicarbonate-induced deficiency than in direct Fe deficiency. Our findings showed that chlorophyll a, b and total, and carotenoid contents in the grafting combination of FS/TS were less affected (decreased by approximately 20%, compared to the control plant) under bicarbonate treatment. The 'Flame Seedless' cultivar and the grafting combination of TS/FS were more affected and decreased by approximately 50%, compared to the control, under this condition. The results also indicated that chlorophyll a, b and total, and carotenoid contents significantly decreased in FS/TS under the Fe deficiency treatment (Table 1).

Plant growth

The results indicated that shoot and root fresh weights decreased in all cultivars under bicarbonate treatment in response to both iron sources. From the data reported in Table 2, it is worth noting that the decrease in the shoot and root fresh weight was generally less affected in the grafting combination of FS/TS (which decreased by about 20% compared to the control plant) than in the grafting combination of TS/FS (which decreased by about 50% compared to the control) when bicarbonate was added to the nutrient solution with both iron sources. Also, shoot fresh weight decreased in the 'Thompson Seedless' cultivar and root fresh weight decreased in FS/TS under the Fe deficient treatment. Shoot dry weight significantly decreased in the 'Flame Seedless' cultivar and grafting combination of TS/FS when FeEDTA was used as the iron source. It was decreased in the 'Thompson Seedless' cultivar when $FeSO_4.7H_2O$ was used as the iron source under bicarbonate treatment (Table 2). Root dry weight decreased in the grafting combinations of FS/TS and TS/FS under bicarbonate and Fe-deficient treatments, regardless of iron source.

The results indicated that AGR decreased in both cultivars and their graft combination under bicarbonate treatment in both iron sources, except in the case of 'Thompson Seedless' under FS+Bic (0.75 cm day-1) treatment (Table 3). As presented in Table 3, leaf area decreased in cultivars under bicarbonate treatment in response to both iron sources, except in FS/TS under the FE+Bic treatment. Leaf area decreased in the 'Flame Seedless' cultivar and the grafting combination of TS/FS (which decreased by about 38% and 29%, respectively, compared to the control) under the Fe deficiency treatment.

Data in Table 3 revealed that root volume significantly decreased in 'Thompson Seedless', 'Flame Seedless', and their grafting combination of TS/FS under the FE+Bic treatment, and it was decreased in grafting combination of FS/TS, 'Flame Seedless', and the grafting combination of TS/FS under the FS+Bic treatment. Our results also showed that root volume decreased in both cultivars under the Fe deficiency treatment.

Table 1. Effect of bicarbonate and iron sources on chlorophyll a, b, and total, and carotenoid contents of two table grape cultivars and their graft combinations

Treatments	FS/TS	Thompson Seedless	Flame Seedless	TS/FS	*Mean*(±SD)
Chl a ($mgg^{-1}.F.W$)					
FE-Bic	3.14(±0.4)^{a*}	2.47(±0.5)^{bc}	2.29(±0.4)$^{b-d}$	2.1(±0.2)$^{b-d}$	**2.5(±0.5)^{A}**
FE+Bic	2.45(±0.5)^{bc}	2.03(±0.3)$^{b-e}$	1.24(±0.3)^{fg}	1.34(±0.3)$^{e-g}$	**1.76(±0.6)^{C}**
FS-Bic	2.27(±0.2)$^{b-d}$	1.93(±0.3)$^{b-f}$	2.6(±0.4)^{ab}	1.85(±0.5)$^{b-f}$	**2.16(±0.46)^{B}**
FS+Bic	2.08(±0.3)$^{b-d}$	1.61(±0.4)$^{d-g}$	1.78(±0.4)$^{c-f}$	1.03(±0.2)^{g}	**1.62(±0.49)^{C}**
FD-Bic	2.29(±0.4)$^{b-d}$	1.99(±0.9)$^{b-e}$	2.19(±0.7)$^{b-d}$	2.07(±0.5)$^{b-d}$	**2.14(±0.57)^{B}**
Mean	**2.45(±0.5)^{A}**	**2.01(±0.5)^{B}**	**2.02(±0.6)^{B}**	**1.68(±0.5)^{C}**	
Chl b ($mgg^{-1}.F.W$)					
FE-Bic	0.75(±0.12)^{a}	0.58(±0.12)^{bc}	0.55(±0.1)$^{b-d}$	0.5(±0.14)^{cd}	**0.6(±0.14)^{A}**
FE+Bic	0.58(±0.1)^{bc}	0.47(±0.08)$^{c-e}$	0.32(±0.13)^{ef}	0.39(±0.1)$^{d-f}$	**0.44(±0.14)^{B}**
FS-Bic	0.56(±0.05)$^{b-d}$	0.49(±0.08)$^{c-e}$	0.67(±0.08)^{ab}	0.47(±0.05)$^{c-e}$	**0.55(±0.1)^{A}**
FS+Bic	0.52(±0.03)$^{b-d}$	0.42(±0.1)$^{c-f}$	0.45(±0.09)$^{c-e}$	0.29(±0.04)^{f}	**0.42(±0.1)^{B}**
FD-Bic	0.55(±0.09)$^{b-d}$	0.47(±0.12)$^{c-e}$	0.57(±0.11)^{bc}	0.55(±0.05)$^{b-d}$	**0.53(±0.1)^{A}**
Mean	**0.59(±0.1)^{A}**	**0.49(±0.1)^{BC}**	**0.51(±0.15)^{B}**	**0.44(±0.12)^{C}**	
Chl t ($mgg^{-1}.F.W$)					
FE-Bic	3.89(±0.5)^{a}	3.05(±0.64)^{bc}	2.84(±0.46)$^{a-d}$	2.6(±0.1)$^{b-e}$	**3.1(±0.65)^{A}**
FE+Bic	3.04(±0.6)^{bc}	2.42(±0.37)$^{c-e}$	1.56(±0.5)^{gh}	1.72(±0.6)$^{f-h}$	**2.2(±0.7)^{C}**
FS-Bic	2.83(±0.25)$^{b-d}$	2.4(±0.39)$^{c-f}$	3.27(±0.5)^{ab}	2.32(±0.17)$^{c-f}$	**2.7(±0.5)^{B}**
FS+Bic	2.6(±0.4)$^{b-e}$	2.02(±0.5)$^{e-g}$	2.22(±0.44)$^{d-g}$	1.32(±0.2)^{h}	**2(±0.6)^{C}**
FD-Bic	2.84(±0.57)$^{b-d}$	2.46(±0.53)$^{c-e}$	2.76(±0.34)$^{b-e}$	2.6(±0.31)$^{b-e}$	**2.7(±0.43)^{B}**
Mean	**3(±0.6)^{A}**	**2.5(±0.55)^{B}**	**2.5(±0.7)^{B}**	**2(±0.6)^{C}**	
Car($mgg^{-1}.F.W$)					
FE-Bic	5.92(±0.39)^{a*}	4.74(±0.36)^{bc}	4.5(±0.7)$^{b-d}$	4.13(±0.38)$^{c-e}$	**4.82(±0.8)^{A}**
FE+Bic	4.78(±0.49)^{bc}	3.96(±0.79)$^{c-e}$	2.76(±0.52)^{g}	2.99(±0.56)^{fg}	**3.62(±0.9)^{C}**
FS-Bic	4.75(±0.4)^{bc}	4.05(±0.66)$^{c-e}$	5.3(±0.36)^{ab}	3.84(±0.26)$^{c-f}$	**4.49(±0.72)^{AB}**
FS+Bic	4.2(±0.65)$^{c-e}$	3.38(±0.57)$^{e-g}$	3.69(±0.52)$^{d-f}$	2.67(±0.3)^{g}	**3.48(±0.7)^{C}**
FD-Bic	4.46(±0.7)$^{b-d}$	3.8(±1.07)$^{c-f}$	4.6(±0.79)$^{b-d}$	4.35(±0.59)$^{c-e}$	**4.3(±0.79)^{B}**
Mean	**4.81(±0.78)^{A}**	**3.99(±0.79)^{B}**	**4.18(±1.05)^{B}**	**3.59(±0.77)^{C}**	

*Mean values with the same letters are not significantly different ($p \leq 0.05$), according to Duncan's test. FE-Bic: 23 µM FeEDTA + 0 Bic, FE+Bic: 23 µM FeEDTA + 30 mM Bic, FS-Bic: 23 µM $FeSO_4.7\ H_2O$ + 0 Bic, FS+Bic: 23 µM $FeSO_4.7\ H_2O$ + 30 Mm Bic, FD-Bic: 2.3 µM FeEDTA + 0 Bic.

Table 2. Effect of bicarbonate and iron source on the shoot and root dry and fresh weights of two table grapes cultivars and their graft combinations

Treatments	FS/TS	Thompson Seedless	Flame Seedless	TS/FS	*Mean*(±SD)
Shoot fresh weight (g)					
FE-Bic	101.3(±8)$^{a-d}$ *	88.7(±3.7)$^{c-f}$	108.9(±17)^{a}	100(±14) $^{a-d}$	**100.5(±13)^{A}**
FE+Bic	80.6(±6.5)^{fg}	69.6(±12.6)^{gh}	87.1(±1)$^{d-f}$	50.2(±11.5)^{i}	**72.6(±16)^{C}**
FS-Bic	105.8(±4)$^{a-c}$	81.8(±5.9)$^{e-g}$	98.8(±8.3)$^{a-e}$	87.2(±8.9)$^{d-f}$	**94.7(±11)^{AB}**
FS+Bic	78.3(±9.3)^{fg}	66.9(±4.2)^{gh}	79.2(±4.1)^{fg}	56.6(±11)^{hi}	**70.8(±12)^{C}**
FD-Bic	90.2(±13)$^{c-f}$	69.6(±16.2)^{gh}	106.5(±9.6)^{ab}	90.7(±8.4)$^{b-f}$	**88.1(±18.6)^{B}**
Mean	**91.2(±13)^{A}**	**74.9(±12.7)^{B}**	**96.9(±15)^{A}**	**77.8(±22.4)^{B}**	
Shoot dry weight (g)					
FE-Bic	30.8(±5)^{ab}	29.8(±2.7)$^{a-c}$	35.7(±6.1)^{a}	31.2(±1)^{ab}	**32.1(±4)^{A}**
FE+Bic	29.1(±2.5)$^{a-d}$	24.7(±0.9)$^{b-e}$	26.6(±3.3)$^{b-d}$	22(±4.1)^{de}	**25.8(±4.5)^{CD}**
FS-Bic	31.7(±5.6)^{ab}	28.9(±1)$^{a-d}$	29.2(±1.9)$^{a-d}$	29.2(±3.2)$^{a-d}$	**29.9(±3.1)^{AB}**
FS+Bic	25.2(±5.4)$^{b-d}$	20.3(±6.3)^{e}	22.7(±1)$^{b-d}$	22.7(±0.7)$^{c-e}$	**23.5(±4)^{D}**
FD-Bic	26.8(±3.7)$^{b-d}$	24.3(±5.7)$^{b-e}$	31.1(±3.6)^{ab}	27.6(±5.6)$^{b-d}$	**27.7(±3.7)^{BC}**
Mean	**28.9(±4.7)^{AB}**	**25.3(±5.2)^{C}**	**30.4(±4.4)^{A}**	**26.4(±4.7)^{BC}**	
Root fresh weight (g)					
FE-Bic	133(±11)^{a} *	104.3(±18)$^{c-e}$	109.8(±10)$^{b-d}$	131.8(±7)^{ab}	**119.7(±17)^{A}**
FE+Bic	100.2(±14)$^{c-e}$	82.5(±6)$^{e-g}$	86.2(±6)$^{d-f}$	75.1(±8)^{fg}	**85.1(±12)^{C}**
FS-Bic	115.5(±10)$^{a-c}$	114.6(±5.8)$^{a-c}$	109.1(±6.8)$^{b-d}$	109.2(±6.3)$^{b-d}$	**112.3(±7)^{A}**
FS+Bic	83.8(±15)$^{e-g}$	89.2(±17)$^{d-f}$	79.7(±20)$^{e-g}$	61.6(±21)^{g}	**77.4(±19)^{C}**
FD-Bic	97.6(±15)$^{c-f}$	96.1(±19)$^{c-f}$	100.6(±19)$^{c-e}$	96.9(±13)$^{c-f}$	**97.8(±14)^{B}**
Mean	**106.1(±20)^{A}**	**96.4(±17)^{B}**	**95.7(±18)^{B}**	**94.2(±27)^{B}**	
Root dry weight (g)					
FE-Bic	36.5(±1.6)^{a}	30.6(±4.2)$^{a-e}$	28.3(±5.7)$^{a-f}$	33.5(±7)$^{a-d}$	**32.2(±5.4)^{A}**
FE+Bic	25.6(±4.7)$^{d-f}$	22.9(±3.9)^{ef}	25.3(±4.3)$^{d-f}$	22.2(±3)^{ef}	**23.9(±3.9)^{B}**
FS-Bic	34.2(±1.8)$^{a-c}$	29.3(±4.1)$^{a-e}$	35.4(±1.6)^{ab}	29.6(±1.8)$^{a-e}$	**32.1(±3.6)^{A}**
FS+Bic	27.3(±3)$^{b-f}$	26.2(±5)$^{c-f}$	22.7(±7.3)^{ef}	20.4(±5)^{f}	**23.8(±5)^{B}**
FD-Bic	23.3(±2.5)^{ef}	23.2(±5.3)^{ef}	27.7(±5.5)$^{b-f}$	24.9(±6.5)^{ef}	**24.8(±4.8)^{B}**
Mean	**29.4(±5.8)^{A}**	**26.5(±4.9)^{A}**	**27.6(±6.2)^{A}**	**26.1(±6.1)^{A}**	

*Mean values with the same letters are not significantly different ($p \leq 0.05$), according to Duncan's test. FE-Bic: 23 µM FeEDTA + 0 Bic, FE+Bic: 23 µM FeEDTA + 30 mM Bic, FS-Bic: 23 µM $FeSO_4.7\ H_2O$ + 0 Bic, FS+Bic: 23 µM $FeSO_4.7\ H_2O$ + 30 Mm Bic, FD-Bic: 2.3 µM FeEDTA + 0 Bic.

Table 3. Effect of bicarbonate and iron sources on AGR, leaf area, and root volume of two table grapes cultivars and their graft combinations

Treatments	FS/TS	Thompson Seedless	Flame Seedless	TS/FS	*Mean*(±SD)
AGR (cm day^{-1})					
FE-Bic	1(±0.23)$^{a-c}$ *	1.1(±0.17)$^{a-c}$	1.25(±0.17)^{a}	1.03(±0.11)$^{a-c}$	**1.1(±0.17)^{A}**
FE+Bic	0.56(±0.2)^{ef}	0.56(±0.05)^{ef}	0.82(±0.16)$^{b-f}$	0.62(±0.2)$^{d-f}$	**0.65(±0.18)^{B}**
FS-Bic	1.2(±0.3)^{ab}	1(±0.2)$^{a-c}$	1.04(±0.27)$^{a-c}$	0.96(±0.2)$^{a-d}$	**1.05(±0.25)^{A}**
FS+Bic	0.7(±0.17)$^{c-f}$	0.75(±0.09)$^{c-f}$	0.58(±0.4)$^{d-f}$	0.44(±0.24)^{f}	**0.63(±0.23)^{B}**
FD-Bic	0.92(±0.09)$^{a-e}$	0.87(±0.12)$^{a-e}$	1(±0.25)$^{a-c}$	0.86(±0.17)$^{a-e}$	**0.92(±0.16)^{A}**
Mean	**0.9(±0.31)^{A}**	**0.89(±0.21)^{A}**	**0.95(±0.32)^{A}**	**0.82(±0.28)^{A}**	
Leaf area (cm^2)					
FE-Bic	95(±12)$^{a-d}$	91.3(±27)$^{b-e}$	114.4(±13)^{a}	104.3(±17)^{ab}	**101.3(±17.5)^{A}**
FE+Bic	78.6(±12)$^{d-f}$	56.5(±7.6)^{gh}	73.8(±21)$^{d-g}$	55.3(±6.4)^{gh}	**66.1(±17)^{C}**
FS-Bic	102.8(±10.6)$^{a-c}$	105.5(±15.8)^{ab}	104.8(±15)^{ab}	102.4(±9)$^{a-c}$	**103.9(±11.6)^{A}**
FS+Bic	68.6(±6.5)$^{f-h}$	60.3(±9)$^{f-h}$	76.8(±13)$^{d-g}$	51(±5.8)^{h}	**64.2(±11)^{C}**
FD-Bic	82.3(±11.7)$^{c-f}$	76.5(±18.7)$^{d-g}$	70.8(±13)$^{e-h}$	74.7(±18.6)$^{d-g}$	**76.1(±14)^{B}**
Mean	**85.5(±16)^{AB}**	**78(±24)^{B}**	**88.1(±22.6)^{A}**	**77.6(±24.7)^{B}**	
Root volume (ml)					
FE-Bic	150(±18)^{ab}	145(±26)^{ab}	153.3(±11.5)^{ab}	170(±18)^{a}	**154.7(±17)^{A}**
FE+Bic	130(±17)$^{b-d}$	102.5(±15)$^{d-f}$	96.7(±5.7)^{ef}	86.7(±15)^{f}	**103.8(±20.6)^{B}**
FS-Bic	162.5(±15)^{a}	150(±23)^{ab}	130(±18)$^{b-d}$	130(±18)$^{b-d}$	**143.1(±21)^{A}**
FS+Bic	133.3(±15)^{bc}	127.5(±15)$^{b-d}$	86.7(±15)^{f}	87.5(±15)^{ef}	**108.6(26)^{B}**
FD-Bic	115(±21)$^{c-e}$	113(±11.5)$^{c-f}$	100(±10)^{ef}	107.5(±10)$^{c-f}$	**109.3(±13)^{B}**
Mean	**138.9(±23)^{A}**	**128.4(±25)^{AB}**	**114.4(±27)^{C}**	**117.9(±34)^{BC}**	

*Mean values with the same letters are not significantly different ($p \leq 0.05$), according to Duncan's test. FE-Bic: 23 µM FeEDTA + 0 Bic, FE+Bic: 23 µM FeEDTA + 30 mM Bic, FS-Bic: 23 µM $FeSO_4.7\ H_2O$ + 0 Bic, FS+Bic: 23 µM $FeSO_4.7\ H_2O$ + 30 Mm Bic, FD-Bic: 2.3 µM FeEDTA + 0 Bic.

Mineral nutrients

The concentration of total Fe in grapevine shoot significantly decreased under high bicarbonate concentration in FS/TS, 'Flame Seedless', and TS/FS in both iron sources and 'Thompson Seedless' when $FeSO_4.7\ H_2O$ was used as an iron source (Table 4). The results also indicated that shoot total iron concentration significantly decreased in 'Thompson Seedless' (by approximately 25%) under the FD treatment. According to the main effects, total iron in the root significantly decreased in the FD treatment (a decrease of about 18% compared to the control). However, root and shoot Cu contents were not affected under the bicarbonate and Fe deficiency treatments. The results in Table 4 showed that Zn concentrations in grapevine shoots significantly decreased in FS/TS under the FE+Bic treatment

and in TS/FS under the FS+Bic treatment. The main effects showed that bicarbonate decreased shoot Zn concentrations by both iron sources. Also, Zn concentrations in the root significantly decreased in the grafting combination of TS/FS compared to the rootstocks (Table 4).

Table 4. Effect of bicarbonate and iron sources on the shoot and root micronutrient contents of two table grape cultivars and their graft combinations

Treatments	FS/TS	Thompson Seedless	Flame Seedless	TS/FS	*Mean*(±SD)
Fe(shoot) (mg kg^{-1}.D.W)					
FE-Bic	159.5(±18)^{a*}	163.7(±9.5)^{a}	148.2(±7)$^{a-c}$	153.2(±20)^{ab}	**156.1(±14)^{A}**
FE+Bic	122.8(±26)$^{b-e}$	132.5(±15)$^{a-d}$	102.9(±33)$^{d-f}$	95.4(±6)^{ef}	**113.4(±24)^{BC}**
FS-Bic	160.4(±5.7)^{a}	151.2(±20.8)^{ab}	148.4(±33)$^{a-c}$	132.6(±5.6)$^{a-d}$	**148.2(±20)^{A}**
FS+Bic	114(±16)$^{c-e}$	108.9(±27)$^{d-f}$	109.7(±27)$^{d-f}$	79.5(±22)^{f}	**103.1(±22)^{C}**
FD-Bic	132.3(±11)$^{a-d}$	122.9(±10)$^{b-f}$	130.2(±4)$^{a-d}$	121.9(±8)$^{b-e}$	**126.8(±9)^{B}**
Mean	**137.8(±24)^{A}**	**135.9(±25)^{A}**	**127.9(±27)^{AB}**	**116.5(±30)^{B}**	
Fe(root) (mg kg^{-1}.D.W)					
FE-Bic	530 (±33)$^{a-c}$	471 (±14)$^{a-e}$	450.7(±17)$^{b-e}$	508 (±116)$^{a-d}$	**490.2(±61)^{A}**
FE+Bic	577.7(±23.5)^{a}	496.5(±119)$^{a-e}$	382.1(±22)^{e}	449.3(±55)$^{b-e}$	**476.4(±94)^{A}**
FS-Bic	491.6(±25)$^{a-e}$	481.4(±33)$^{a-e}$	451.9(±48)$^{b-e}$	483(±12)$^{a-e}$	**477.1(±31)^{A}**
FS+Bic	484.6(±64)$^{a-e}$	545.6(±28)^{ab}	393.4(±68)^{de}	457.5(±111)$^{b-e}$	**477.3(±85)^{A}**
FD-Bic	416.3(±24)$^{c-e}$	396.1(±51)^{de}	424.7(±18)$^{c-e}$	401.9(±30)^{de}	**409.8(±31)^{B}**
Mean2	**500.1(±64)^{A}**	**478.2(±72)^{A}**	**422.5(±0.9)^{B}**	**460 (±75)^{AB}**	
Cu(shoot) (mg kg^{-1}.D.W)					
FE-Bic	2.1(±0.0)^{a}	2.8(±0.6)^{a}	3.2(±0.0)^{a}	2.5(±0.3)^{a}	**2.6(±0.5)^{A}**
FE+Bic	2.1(±0.5)^{a}	2.6(±1.8)^{a}	1.9(±0.3)^{a}	2.5(±0.8)^{a}	**2.3(±0.9)^{A}**
FS-Bic	2.6(±0.5)^{a}	2.6(±0.0)^{a}	2.6(±0.9)^{a}	2.9(±0.3)^{a}	**2.7(±0.49)^{A}**
FS+Bic	3.5(±0.8)^{a}	3.3(±1.8)^{a}	2.6(±1.05)^{a}	2.5(±1.09)^{a}	**2.9(±1.1)^{A}**
FD-Bic	2.6(±0.5)^{a}	2.8(±0.8)^{a}	2.6(±0.0)^{a}	2.5(±0.6)^{a}	**2.6(±0.5)^{A}**
Mean	**2.6(±0.7)^{A}**	**2.8(±1.08)^{A}**	**2.6(±0.67)^{A}**	**2.6(±0.62)^{A}**	
Cu(root) (mg kg^{-1}.D.W)					
FE-Bic	4.7(±0.9)^{ab}	5.9(±0.3)^{ab}	3.8(±0.8)^{ab}	5.2(±1.5)^{ab}	**4.9(±1.1)^{A}**
FE+Bic	5.1(±0.8)^{ab}	5.8(±0.5^{ab}	4.9(±1.2)^{ab}	4.7(±0.0)^{ab}	**5.1(±0.78)^{A}**
FS-Bic	4(±1.09)^{ab}	4.7(±1.5)^{ab}	3.3(±1.09)^{b}	4.6(±0.3)^{ab}	**4.2(±1.1)^{A}**
FS+Bic	5.1(±0.3)^{ab}	5.8(±3.2)^{ab}	4.2(±0.9)^{ab}	4.7(±0.9)^{ab}	**4.9(±1.6)^{A}**
FD-Bic	6.3(±3.6)^{a}	4.2(±0.0)^{ab}	5.3(±1.3)^{ab}	4.7(±0.52)^{ab}	**5.1(±1.9)^{A}**
Mean	**5.1(±1.6)^{A}**	**5.3(±1.5)^{A}**	**4.3(±1.1)^{A}**	**4.8(±0.76)^{A}**	
Zn(shoot) (mg kg^{-1}.D.W)					
FE-Bic	32.9(±0.6)$^{a-d}$	36.3(±3.2)$^{a-c}$	32.5(±4.4)$^{a-e}$	31.9(±1.2)$^{a-e}$	**33.4(±3)^{AB}**
FE+Bic	23.1(±9.4)^{e}	29.2(±3.4)$^{b-e}$	25.3(±4.7)^{de}	27.6(±0.78)$^{b-e}$	**25.9(±5.3)^{C}**
FS-Bic	29.8(±4.2)$^{b-e}$	27.1(±0.27)$^{c-e}$	30.9(±3.5)$^{a-e}$	30.5(±8.4)$^{b-e}$	**30.1(±4.6)^{B}**
FS+Bic	27.1(±1.8)^{de}	25.3(±5.2)^{de}	28.2(±2.7)$^{b-e}$	12.9(±3.4)^{f}	**24.2(±7)^{C}**
FD-Bic	31.6(±0.5)$^{a-e}$	36.8(±3.7)^{ab}	40.7(±3.7)$^{a-c}$	31.2(±2.6)$^{b-e}$	**35(±4.8)^{A}**
Mean	**28.9(±5.3)^{AB}**	**31.9(±5.8)^{A}**	**32.2(±6.3)^{A}**	**27.8 (±8.2)^{B}**	
Zn(root) (mg kg^{-1}.D.W)					
FE-Bic	55.9(±5.5)^{ab}	58.6(±3.6)^{ab}	60(±7.4)^{ab}	46.2(±0.5)^{ab}	**55.2(±7)^{A}**
FE+Bic	54.7(±7.7)^{ab}	53.9(±3.3)^{ab}	41.4(±8.5)^{ab}	42.2(±9.2)^{ab}	**48.1(±9.1)^{B}**
FS-Bic	40.5(±1.3)^{ab}	42.1(±6.4)^{ab}	41.8(±2.8)^{b}	42.6(±3.4)^{ab}	**41.7(±3.4)^{C}**
FS+Bic	50.5(±1.9)^{ab}	48.4(±8.7)^{ab}	36.7(±1.3)^{ab}	38.9(±1.05)^{ab}	**43.6(±7.3)^{BC}**
FD-Bic	54.7(±13.6)^{a}	46.8(±1.9)^{ab}	69.1(±6.1)^{ab}	56.7(±7)^{ab}	**56.8(±10.9)^{A}**
Mean	**51.9(±8.6)^{A}**	**49.9(±7.4)^{A}**	**50.4(±13.8)^{A}**	**45.3(±7.8)^{B}**	

*Mean values with the same letters are not significantly different ($p \leq 0.05$), according to Duncan's test. FE-Bic: 23 µM FeEDTA+ 0 Bic, FE+Bic: 23 µM FeEDTA + 30 mM Bic, FS-Bic: 23 µM $FeSO_4.7\ H_2O$ + 0 Bic, FS+Bic: 23 µM $FeSO_4.7\ H_2O$ + 30 Mm Bic, FD-Bic: 2.3 µM FeEDTA+ 0 Bic.

Our results showed that the concentration of Ca and Mg in grapevine shoot significantly decreased under bicarbonate treatment in both cultivars regardless of iron sources. Ca and Mg concentrations in grapevine shoots were not influenced by the Fe-deficient treatment. The addition of bicarbonate in the media did not affect Ca concentration in the roots of grapevine cultivars but, according to the main effects, Ca concentration in the roots increased under the Fe-deficient treatment. Mean values in Table 5 showed that P contents in grapevine shoots and roots were not affected by bicarbonate and Fe deficiency treatment. The results also indicated that Na content increased in the shoot and root of grapevine cultivars under the bicarbonate treatment, regardless of the type of iron source, because sodium bicarbonate was used as the source of bicarbonate in this experiment.

Table 5. Effect of bicarbonate and iron sources on root and shoot macronutrient contents of two table grapes cultivars and their graft combination.

Treatments	FS/TS	Thompson Seedless	Flame Seedless	TS/FS	*Mean*(±SD)
Ca(shoot) (%)					
FE-Bic	1.02(±0.0)^{ab*}	0.95(±0.1)$^{a-c}$	1.06(±0.12)^{ab}	0.93(±0.05)$^{a-c}$	**0.99(±0.08)^{A}**
FE+Bic	0.6(±0.22)^{d}	0.57(±0.01)^{d}	0.73(±0.2)^{cd}	0.61(±0.09)^{d}	**0.63(±0.14)^{B}**
FS-Bic	1.1(±0.03)^{a}	1.03(±0.12)^{ab}	1.05(±0.12)^{ab}	0.98(±0.07)^{ab}	**1.05(±0.9)^{A}**
FS+Bic	0.74(±0.22)^{cd}	0.54(±0.13)^{d}	0.72(±0.02)^{cd}	0.52(±0.13)^{d}	**0.63(±0.16)^{B}**
FD-Bic	0.95(±0.05)$^{a-c}$	1(±0.05)^{ab}	0.98^{a}(±0.07)^{b}	0.87(±0.21)^{bc}	**0.95(±0.11)^{A}**
Mean	**0.89(±0.23)^{A}**	**0.82(±0.23)^{AB}**	**0.9(±0.18)^{A}**	**0.78(±0.21)^{B}**	
Ca(root) (%)					
FE-Bic	0.81(±0.12)$^{a-d}$	0.85(±0.07)$^{a-d}$	0.92(±0.2)$^{a-d}$	0.75(±0.1)^{cd}	**0.8(±0.1)^{B}**
FE+Bic	0.75(±0.04)^{cd}	0.75(±0.09)^{cd}	0.88(±0.28)$^{a-d}$	0.82(±0.09)$^{a-d}$	**0.79(±0.14)^{B}**
FS-Bic	0.86(±0.03)$^{a-d}$	0.83(±0.1)$^{a-d}$	0.84(±0.07)$^{a-d}$	0.96(±0.05)$^{a-d}$	**0.87(±0.08)^{B}**
FS+Bic	0.87(±0.2)$^{a-d}$	0.75(±0.06)$^{b-d}$	0.87(±0.01)$^{a-d}$	0.73(±0.01)^{d}	**0.81(±0.12)^{B}**
FD-Bic	0.99(±0.1)$^{a-c}$	1(±0.17)^{ab}	1.01(±0.11)^{a}	1(±0.07)^{ab}	**1(±0.1)^{A}**
Mean	**0.85(±0.14)^{A}**	**0.84(±0.13)^{A}**	**0.91(±0.15)^{A}**	**0.85(±0.13)^{A}**	
Mg(shoot) (%)					
FE-Bic	0.89(±0.1)^{a}	0.72(±0.14)^{ab}	0.81(±0.07)^{a}	0.7(±0.15)$^{a-c}$	**0.78(±0.13)^{A}**
FE+Bic	0.4(±0.23)^{de}	0.37(±0.03)^{de}	0.49(±0.03)$^{c-e}$	0.4(±0.04)^{de}	**0.42(±0.11)^{B}**
FS-Bic	0.89(±0.1)^{a}	0.83(±0.15)^{a}	0.71(±0.13)^{ab}	0.75(±0.05)^{ab}	**0.79(±0.12)^{A}**
FS+Bic	0.54(±0.21)$^{b-e}$	0.39(±0.07)^{de}	0.54(±0.06)$^{b-d}$	0.31(±0.13)^{e}	**0.44(±0.15)^{B}**
FD-Bic	0.76(±0.03)^{ab}	0.9(±0.05)^{a}	0.75(±0.12)^{ab}	0.83(±0.11)^{a}	**0.81(±0.1)^{A}**
Mean	**0.69(±0.24)^{A}**	**0.64(±0.24)^{A}**	**0.66(±0.14)^{A}**	**0.6(±0.22)^{A}**	
Mg(root) (%)					
FE-Bic	0.83(±0.1)$^{a-e}$	0.83(±0.11)$^{b-e}$	1.07(±0.16)^{ab}	0.8(±0.08)$^{a-e}$	**0.88(±0.16)^{B}**
FE+Bic	0.6(±0.05)^{e}	0.57(±0.13)^{e}	0.73(±0.33)$^{c-e}$	0.67(±0.05)^{de}	**0.64(±0.17)^{C}**
FS-Bic	1.03(±0.1)$^{a-c}$	0.87(±0.05)$^{a-e}$	0.97(±0.05)$^{a-d}$	1.03(±0.06)$^{a-c}$	**0.98(±0.1)^{AB}**
FS+Bic	0.73(±0.16)$^{c-e}$	0.57(±0.11)^{e}	0.77(±0.13)$^{b-e}$	0.77(±0.17)$^{b-e}$	**0.7(±0.14)^{C}**
FD-Bic	1(±0.18)$^{a-c}$	1.07(±0.2)^{ab}	1.1(±0.17)^{a}	1.07(±0.24)^{ab}	**1.06(±0.18)^{A}**
Mean	**0.84(±0.19)^{AB}**	**0.78(±0.21)^{B}**	**0.93(±0.22)^{A}**	**0.87(±0.2)^{AB}**	

*Mean values with the same letters are not significantly different ($p \leq 0.05$), according to Duncan's test. FE-Bic: 23 µM FeEDTA + 0 Bic, FE+Bic: 23 µM FeEDTA + 30 mM Bic, FS-Bic: 23 µM $FeSO_4.7\ H_2O$ + 0 Bic, FS+Bic: 23 µM $FeSO_4.7\ H_2O$ + 30 Mm Bic, FD-Bic: 2.3 µM FeEDTA+ 0 Bic.

Table 5 Continued. Effect of bicarbonate and iron sources on root and shoot macronutrient contents of two table grapes cultivars and their graft combinations

Treatments	FS/TS	Thompson Seedless	Flame Seedless	TS/FS	*Mean*(±SD)
P(shoot) (%)					
FE-Bic	0.13(±0.006)^{ab}	0.1(±0.04)^{b}	0.15(±0.03)^{ab}	0.12(±0.01)^{ab}	**0.13(±0.02)^{B}**
FE+Bic	0.12(±0.02)^{b}	0.13(±0.03)^{ab}	0.12(±0.02)^{ab}	0.13(±0.03)^{ab}	**0.13(±0.02)^{B}**
FS-Bic	0.18(±0.05)^{a}	0.14(±0.04)^{ab}	0.16(±0.01)^{ab}	0.15(±0.03)^{ab}	**0.16(±0.04)^{A}**
FS+Bic	0.16(±0.02)^{ab}	0.14(±0.01)^{ab}	0.17(±0.01)^{ab}	0.13(±0.03)^{ab}	**0.15(±0.02)^{AB}**
FD-Bic	0.15(±0.06)^{ab}	0.14(±0.02)^{ab}	0.16(±0.04)^{ab}	0.14(±0.02)^{ab}	**0.15(±0.03)^{AB}**
Mean	**0.15(±0.04)^{A}**	**0.13(±0.03)^{A}**	**0.15(±0.03)^{A}**	**0.14(±0.02)^{A}**	
P(root) (%)					
FE-Bic	0.18(±0.03)^{a}	0.14(±0.03)^{a}	0.15(±0.03)^{a}	0.15(±0.005)^{a}	**0.16(±0.03)^{B}**
FE+Bic	0.19(±0.006)^{a}	0.14(±0.03)^{a}	0.16(±0.05)^{a}	0.16(±0.02)^{a}	**0.16(±0.03)^{A}**
FS-Bic	0.19(±0.02)^{a}	0.13(±0.01)^{a}	0.16(±0.04)^{a}	0.16(±0.05)^{a}	**0.16(±0.03)^{A}**
FS+Bic	0.15(±0.04)^{a}	0.18(±0.07)^{a}	0.18(±0.01)^{a}	0.15(±0.02)^{a}	**0.17(±0.04)^{A}**
FD-Bic	0.20(±0.07)^{a}	0.17(±0.02)^{a}	0.17(±0.02)^{a}	0.15(±0.01)^{a}	**0.17(±0.04)^{A}**
Mean	**0.18(±0.04)^{A}**	**0.15(±0.04)^{A}**	**0.17(±0.03)^{A}**	**0.15(±0.02)^{A}**	
Na(shoot) (%)					
FE-Bic	0.39(±0.04)^{c}	0.53(±0.07)^{c}	0.6(±0.3)^{c}	0.48(±0.06)^{c}	**0.5(±0.15)^{B}**
FE+Bic	0.82(±0.1)^{b}	1.13(±0.09)^{a}	1.08(±0.2)^{a}	1.**1**(±0.09)^{a}	**1.02(±0.17)^{A}**
FS-Bic	0.55(±0.02)^{c}	0.58(±0.09)^{c}	0.48(±0.08)^{c}	0.55(±0.05)^{c}	**0.54(±0.07)^{B}**
FS+Bic	0.81(±0.04)^{b}	0.95(±0.06)^{ab}	0.96(±0.05)^{ab}	1.07(±0.2)^{a}	**0.95(±0.13)^{A}**
FD-Bic	0.52(±0.03)^{c}	0.52(±0.02)^{c}	0.59(±0.03)^{c}	0.57(±0.02)^{c}	**0.55(±0.04)^{B}**
Mean	**0.62(±0.18)^{A}**	**0.75(±0.26)^{A}**	**0.74(±0.28)^{A}**	**0.75(±0.28)^{A}**	
Na(root) (%)					
FE-Bic	3.6(±0.11)^{d}	3.9(±0.53)^{d}	3.9(±0.23)^{d}	4.3(±0.53)^{cd}	**3.9(±0.44)^{B}**
FE+Bic	5.9(±0.4)^{ab}	5.5(±0.8)^{b}	6.15(±0.4)^{ab}	6.08(±0.96)^{ab}	**5.9(±0.65)^{A}**
FS-Bic	3.8(±0.47)^{d}	4.3(±0.35)^{cd}	3.4(±0.58)^{d}	3.7(±0.2)^{d}	**3.7(±0.51)^{B}**
FS+Bic	6.69(±0.23)^{a}	5.9(±0.73)^{ab}	5.3(±0.71)^{bc}	5.6(±0.42)^{b}	**5.8(±0.73)^{A}**
FD-Bic	3.9(±0.8)^{d}	4.2(±0.71)^{cd}	4.3(±0.4)^{cd}	4.3(±0.53)^{cd}	**4.2(±0.55)^{B}**
Mean	**4.7(±1.3)^{A}**	**4.7(±0.98)^{A}**	**4.6(±1.1)^{A}**	**4.8(±1)^{A}**	

*Mean values with the same letters are not significantly different ($p \leq 0.05$), according to Duncan's test. FE-Bic: 23 µM FeEDTA + 0 Bic, FE+Bic: 23 µM FeEDTA + 30 mM Bic, FS-Bic: 23 µM $FeSO_4.7\ H_2O$ + 0 Bic, FS+Bic: 23 µM $FeSO_4.7\ H_2O$ + 30 Mm Bic, FD-Bic: 2.3 µM FeEDTA+ 0 Bic.

Proline and Malondialdehyde contents (MDA)

Proline content significantly increased in the 'Flame Seedless' cultivar when FeEDTA was used as the iron source and it was increased in the grafting combination of TS/FS when $FeSO_4.7\ H_2O$ was used as the iron source under bicarbonate treatment. It increased by about 218% and 250%, respectively, compared to the control. In 'Flame Seedless' and the grafting combination of TS/FS under Fe deficiency, the proline content significantly increased. Leaf MDA content was not affected by bicarbonate and Fe deficiency in both cultivars (Table 6).

Table 6. Effect of bicarbonate and iron sources on proline content and MDA content of two table grapes cultivars and their graft combinations

Treatments	FS/TS	Thompson Seedless	Flame Seedless	TS/FS	***Mean***(±SD)
Proline (μmol g^{-1}.F.W)					
FE-Bic	0.29(±0.04)^{f*}	0.3(±0.06)^{f}	0.44(±0.2)^{ef}	0.28(±0.08)^{f}	**0.34(±0.1)^{D}**
FE+Bic	0.34(±0.1)^{f}	0.54(±0.2)$^{d-f}$	0.96(±0.3)$^{b-d}$	0.54(±0.2)$^{d-f}$	**0.60(±0.3)^{C}**
FS-Bic	0.54(±0.2)$^{d-f}$	0.36(±0.12)^{f}	0.46(±0.2)^{ef}	0.54(±0.13)$^{d-f}$	**0.48(±0.17)^{CD}**
FS+Bic	0.84(±0.35)$^{c-e}$	0.94(±0.25)$^{b-d}$	1.2(±0.4)^{ab}	1.4(±0.25)^{a}	**1.1(±0.38)^{A}**
FD-Bic	0.66(±0.45)$^{d-f}$	0.42(±0.05)^{ef}	1.1(±0.5)$^{a-c}$	1.4(±0.29)^{a}	**0.9(±0.5)^{B}**
Mean	**0.54(±0.3)^{B}**	**0.52(±0.26)^{B}**	**0.86(±0.46)^{A}**	**0.84(±0.53)^{A}**	
Leaf MDA (nmol g^{-1}.F.W)					
FE-Bic	4.9(±0.6)^{ab}	3.1(±0.8)^{b}	4.7(±1.4)^{ab}	4.9(±0.9)^{ab}	**4.3(±1.2)^{AB}**
FE+Bic	5.1(±0.56)^{a}	4.5(±1.5)^{ab}	4.9(±1.07)^{ab}	4.7(±1.6)^{ab}	**4.8(±1.1)^{A}**
FS-Bic	4.2(±0.69)^{ab}	3.6(±1.5)^{ab}	4.8(±0.19)^{ab}	3.91(±0.7)^{ab}	**4.1(±0.9)^{AB}**
FS+Bic	5.1(±1.1)^{a}	4.2(±0.9)^{ab}	4.7(±1.2) ^{ab}	4.9(±0.58)^{ab}	**4.7(±0.95)^{A}**
FD-Bic	4.1(±0.5)^{ab}	3.9(±1.3)^{ab}	3.6(±0.57)^{ab}	3.9(±0.77)^{ab}	**3.9(±0.78)^{B}**
Mean	**4.7**(±0.8)^{A}	**3.9(±1.2)^{B}**	**4.5(±1.02)^{AB}**	**4.5(±0.99)^{AB}**	

*Mean values with the same letters are not significantly different ($p \leq 0.05$), according to Duncan's test. FE-Bic: 23 μM FeEDTA + 0 Bic, FE+Bic: 23 μM FeEDTA + 30 mM Bic, FS-Bic: 23 μM $FeSO_4.7\ H_2O$ + 0 Bic, FS+Bic: 23 μM $FeSO_4.7\ H_2O$ + 30 Mm Bic, FD-Bic: 2.3 μM FeEDTA + 0 Bic.

Phenolic compounds

As shown in Table 7, p-coumaric acid and Hespredin increased in 'Thompson Seedless' and the graft combination of FS/TS under the bicarbonate treatment. Hesperetin increased in graft combinations of FS/TS and TS/FS (by approximately 200%). Catechin and Hespredin significantly increased in the graft combination of FS/TS under the Fe-deficient treatment.

The correlation analyses revealed moderate positive correlations between Fe concentration in the shoot and root dry weight, root volume, AGR, leaf area, and total chlorophyll. However, the Fe concentration had a negative correlation with Na content in the root (Table 8). The results also indicated that Na content in the root had a moderate, negative correlation with leaf area, root volume, AGR, and total chlorophyll.

Discussion

Considering the important role of iron in chlorophyll synthesis, chloroplast development, and electron transfer (Fu et al., 2017; M'sehli et al., 2009), a shortage of available iron in plant nutrition is accompanied by a decrease in the level of photosynthetic pigments, thereby causing chlorosis in young leaves (Sabir et al., 2010). Therefore, iron mediates the growth and development processes of plants based on the fact that chlorophyll synthesis and the photosynthetic chain are closely related to the iron status of plants (Sabir et al., 2010). Bavaresco et al. (2003) reported that in vines of *Vitis vinifera* cv. 'Pinot Blanc' grafted on the Fe-chlorosis susceptible '3309 C' rootstock, by cultivating in calcareous soils, Fe deficiency strongly reduced the leaf chlorophyll content and shoot length (Bavaresco et al., 2003). The morphological and physiological responses demonstrated that woody cuttings of 140 Ruggeri rootstock (not grafted) can withstand high concentrations of bicarbonate in the soil (10 mM), showing only slight/moderate decreases in leaf chlorophyll and plant biomass (Ksouri et al., 2005). Shahsavandi et al. (2020) reported that moderate and high bicarbonate concentrations, as well as Fe-deficient treatments, significantly decreased leaf chlorophyll index in 'Yaghouti', 'Thompson Seedless', 'Flame Seedless', and 'Rotabi' cultivars, regardless of the type of Fe source (Shahsavandi et al., 2020).

Table 7. Effect of bicarbonate and iron deficiency on root polyphenols in two table grapes cultivars and their graft combinations

Treatments	FS/TS	Thompson Seedless	Flame Seedless	TS/FS	***Mean***(±SD)
P-comaric acid (mg g^{-1}.D.W)					
FE-Bic	0.14(±0.03)$^{b-d}$ *	0.1(±0.03)$^{c-e}$	0.01(±0.01)^{f}	0.1(±0.04)^{f}	**0.09(±0.05)^{B}**
FE+Bic	0.3(±0.09)^{a}	0.2(±0.03)^{b}	0.08(±0.05)$^{d-f}$	0.03(±0.006)^{ef}	**0.15(±0.1)^{A}**
FD-Bic	0.17(±0.03)^{bc}	0.03(±0.009)^{ef}	0.08(±0.01)$^{d-f}$	0.17(±0.03)^{bc}	**0.11(±0.06)^{B}**
Mean	**0.2(±0.08)^{A}**	**0.1(±0.08)^{B}**	**0.06(±0.04)^{C}**	**0.1(±0.06)^{B}**	
Hespredin (mg g^{-1}.D.W)					
FE-Bic	0.21(±0.03)^{c}	0.07(±0.02)^{de}	0.09(±0.03)^{de}	0.24(±0.03)^{c}	**0.15(±0.08)^{B}**
FE+Bic	0.36(±0.14)^{b}	0.34(±0.03)^{b}	0.04(±0.02)$^{b-e}$	0.04(±0.02)^{e}	**0.19(±0.17)^{AB}**
FD-Bic	0.47(±0.03)^{a}	0.05(±0.02)^{de}	0.16(±0.1)$^{f-i}$	0.24(±0.03)^{c}	**0.23(±0.16)^{A}**
Mean	**0.35(±0.13)^{A}**	**0.16(±0.1)^{B}**	**0.09(±0.07)^{C}**	**0.17(±0.1)^{B}**	
Galic acid (mg g^{-1}.D.W)					
FE-Bic	8.3(±1.2)^{a}	8.5(±0.3)^{a}	8(±0.8)^{a}	8.5(±0.16)^{a}	**8.3(±0.67)^{A}**
FE+Bic	7.6(±0.69)^{a}	7.6(±0.32)^{a}	7.8(±0.73)^{a}	8.3(±0.26)^{a}	**7.8(±0.54)^{A}**
FD-Bic	8.3(±0.2)^{a}	7.8(±0.8)^{a}	8.3(±0.9)^{a}	7.6(±0.8)^{a}	**7.9(±0.7)^{A}**
Mean	**8.1(±0.8)^{A}**	**7.9(±0.6)^{A}**	**8(±0.76)^{A}**	**8.1(±0.6)^{A}**	
Hespretin (mg g^{-1}.D.W)					
FE-Bic	0.2(±0.03)^{b}	0.32(±0.14)^{ab}	0.2(±0.01)^{b}	0.26(±0.02)^{b}	**0.25(±0.08)^{B}**
FE+Bic	0.44(±0.17)^{a}	0.32(±0.07)^{ab}	0.37(±0.07)^{ab}	0.44(±0.11)^{a}	**0.39(±0.11)^{A}**
FD-Bic	0.2(±0.02)^{b}	0.33(±0.11)^{ab}	0.19(±0.006)^{b}	0.24(±0.03)^{b}	**0.24(±0.07)^{B}**
Mean	**0.28(±0.15)^{A}**	**0.32(±0.1)^{A}**	**0.26(±0.09)^{A}**	**9.5(±0.11)^{A}**	
Catechin (mg g^{-1}.D.W)					
FE-Bic	0.6(±0.01)^{b}	0.5(±0.03)^{bc}	0.57(±0.13)^{b}	0.47(±0.08)^{bc}	**0.54(±0.09)^{AB}**
FE+Bic	0.49(±0.03)^{bc}	0.57(±0.15)^{b}	0.59(±0.03)^{b}	0.37(±0.01)^{c}	**0.5(±0.11)^{B}**
FD-Bic	0.83(±0.02)^{a}	0.51(±0.01)^{bc}	0.61(±0.09)^{b}	0.39(±0.16)^{c}	**0.58(±0.18)^{A}**
Mean	**0.64(±0.14)^{A}**	**0.52(±0.08)^{B}**	**0.59(±0.08)^{AB}**	**0.41(±0.1)^{C}**	

*Mean values with the same letters are not significantly different (p≤0.05), according to Duncan's test. FE-Bic: 23 µM FeEDTA + 0 Bic, FE+Bic: 23 µM FeEDTA + 30 mM Bic, FD-Bic: 2.3 µM FeEDTA + 0 Bic.

Table 8. Pearson's correlation coefficients matrix for some parameters

	Fe (Shoot)	Na(Root)	Root Dry Weight	Leaf Area	Root Volume	AGR	Cht
Fe (Shoot)	1	-.629**	.503**	.570**	.604**	.605**	.630**
Na (Root)		1	-.422**	-.569**	-.433**	-.585**	-.487**
Root Dry Weight			1	.380**	.617**	.404**	.402**
Leaf Area				1	.544**	.625**	.392**
Root Volume					1	.470**	.456**
AGR						1	.419**
Cht							1

** Significance of correlation (p≤0.01).

Plant growth is significantly reduced by alkaline stress. It is mainly associated with a decrease in shoot growth, smaller leaves, and less leaf area, as well as reduced root growth and elongation (Pearce et al., 1999). In the grapevine cultivar 'Aurora', when grafted on the 'SO_4' rootstock, Fe deficiency reduced leaf and whole canopy photosynthesis, grape yield, and total dry matter production (Bavaresco and Poni, 2003). The decrease in leaf area and shoot growth has been previously described in grapevine and other woody species under bicarbonate and Fe deficiency conditions (Jiménez et al., 2008; Covarrubias and Rombolà, 2013). This may be due to decreased photosynthetic rate and stomatal conductance in bicarbonate-induced leaf chlorosis (Bie et al., 2004). The reduction in photosynthetic rate is due to impaired chlorophyll synthesis as a result of low Fe translocation. Ksouri et al. reported that shoot length, leaf expansion, and plant biomass production can be differently affected in varieties, decreasing on average from 20% in 'Khamri', 'Mahdaoui', and '140Ru' rootstocks to more than 50% in 'Balta4', 'Cardinal', and 'Beldi'. In *Parietaria diffusa*, bicarbonate supply induced a shorter root system, with the appearance of structures similar to proteoid roots that provide an enhanced surface of contact between plants and soil (Donnini et al., 2012). The presence of such structures in Fe-sufficient plants grown with bicarbonate but not in Fe-deficient plants suggested that this should not be a specific response to Fe deficiency but to a more general condition of low nutrient availability (Donnini et al., 2012; Covarrubias and Rombolà, 2013). Several essential micronutrients such as Fe, Zn, and Mn become less available to plants under alkaline stress (Valdez-Aguilar and Reed, 2010).

Bicarbonate is known as the main factor causing Fe deficiency in plants (Motesharezadeh et al., 2017). Several studies have shown that the presence of bicarbonate in the irrigation water and soil adversely affected nutrient uptake and plant nutrition status (Martínez-Cuenca et al., 2013). Wang et al. (2020) revealed that bicarbonate addition inhibited Fe translocation from roots to shoots. Moreover, Fe translocation from fine roots to the xylem of coarse roots was hampered under bicarbonate conditions indicating that bicarbonate may hinder axial transport and/or xylem loading of Fe in kiwifruit roots (Wang et al., 2020). It is well known that Fe and Zn, among other elements, have a special significance in plant physiology as they undertake vital duty in photosynthesis reactions. They also act either as metal constituents of essential enzymes or as functional, structural, or regulatory cofactors, and are thus associated with saccharide metabolism, photosynthesis, and protein synthesis (Marschner, 2012; Bertamini and Nedunchezhian, 2005). Bicarbonate can neutralize Zn, Fe, and other micronutrients in plants. Bicarbonate converts Fe to sediments in the leaf apoplast, and Fe concentration decreases subsequently (Motesharezadeh et al., 2017). It has been stated in numerous reports that bicarbonate ions decrease root volume, prevent Zn uptake, and decrease Fe mobility in the root, thereby reducing its transfer to the shoot (Motesharezadeh et al., 2017). In an experiment, it was observed that bicarbonate decreased shoot Zn content in rice varieties by decreasing organic acid synthesis and decreasing the root volume (Hajiboland et al., 2005). In addition to Fe and Zn, other nutrients that can become deficient in high pH values include calcium (Ca), magnesium (Mg), and phosphorus (P) (Valde-Aguilar and Reed, 2010). One of the main functions of Mg in green leaves is that the proportion of total Mg bound to chlorophyll depends on the amount of Mg supplied (Marschner, 2012). Sabir et al. (2010) reported that K, Mg, and Zn concentrations were below the normal levels in the presence of NaHCO3 (Sabir et al., 2010). Ca plays an important role in processes that preserve the structural and functional integrity of the plant membrane, stabilize cell wall structures, regulate ion transport and selectivity, control ion exchange behavior, and maintain cell wall and membrane enzyme activities (Rengel, 1992). These functions may be impaired by a decrease in Ca. One of the reasons for Ca and Mg reduction in the presence of bicarbonate was probably a decrease in root growth and volume.

Ahmad et al. (2014) reported that both proline and glycine betaine increased markedly with increasing external $NaHCO_3$ levels during the growth period. This pattern of accumulation of the two osmoprotectants clearly shows that they could be used as potential indicators of alkalinity tolerance in mulberry (Ahmad et al., 2014). Iron-efficient plants undergo both morphological and physiological changes in response to Fe deficiency, including enhanced root exudation of organic compounds when grown under Fe-limited conditions (Marschner, 2012). In non-graminaceous monocots and dicots (Strategy I plants), phenolic compounds are frequently reported to be the main components of root exudates in response to Fe deficiency (Hell and Stephan, 2003). Grapevine is a Strategy I plant. When exposed to Fe deficiency, it can increase Fe reductase activity and enhance the net excretion of protons and root organic compounds, such as organic acids and phenols, thereby lowering the

pH and increasing the solubility of Fe^{3+} (Jiménez et al., 2007; Covarrubias and Rombolà, 2013). Compared to other compounds in root exudates, phenolic compounds are particularly interesting because of their multiple chemical and biological functions. These functions include Fe chelation and reduction, radical scavenging, and antimicrobial activity (Jin et al., 2007). It has been suggested that the released phenolics function to enhance Fe availability in the rhizosphere soil as an alternative or supplement to the plasma membrane-bound ferric reductase through chelating and reducing insoluble Fe (Dakora and Phillips, 2002).

Conclusion

Iron chlorosis is a major concern in self-rooted and grafted vines cultivated in calcareous soils. The susceptibility to Fe chlorosis in grapevine is highly variable for different genotypes and rootstocks. Following these facts, restrictions in various morphological features and the nutrient profile in the examined cultivars verified the fundamental roles of iron in a series of physiological processes. Supplementary bicarbonate caused more Fe deficiency symptoms and had depressive influences on the physiological process of grape cultivars, although its degree depended on the tolerance level of cultivars. Our study indicated that the tolerant 'Thompson Seedless' cultivar as a rootstock can generally improve the tolerance of susceptible scions of the 'Flame Seedless' cultivar under bicarbonate treatment. Generally, the results showed that bicarbonate suppressed 'Flame Seedless' and graft combinations of TS/FS, compared to self-rooted 'Thompson Seedless' and graft combinations of FS/TS. This finding confirmed that grafting iron-inefficient cultivars on more iron-efficient and bicarbonate-tolerant rootstocks is a promising tool to alleviate the adverse effects of iron deficiency and excessive bicarbonate in grapevine.

Conflict of interest

The authors indicate no conflict of interest for this work.

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