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Response of four grapevine (*Vitis* spp.) genotypes to direct or bicarbonate-induced iron deficiency

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Abstract

In Turkey, calcareous soil conditions usually cause significant decreases in grape yield. Understanding the physiological responses of grapevine genotypes under such conditions would yield invaluable knowledge to recover these problems. This study was thus conducted on the comparative evaluation of the responses of four *Vitis* spp. materials (a cultivar, 'Alphonse Lavallee', and three rootstocks, 'Fercal', '99 R' and '1613 C'), differing in tolerance potential to direct and lime-induced chlorosis. In greenhouse, rooted cuttings were grown in inert perlite using containers for two years with complete Hoagland nutrients solution except for Fe as variable. The experiment comprised five applications performed for three times per year. Iron applications (FeNaEDTA) in 9 and 36 mg L^{-1} doses and their combinations with 840 mg L^{-1} $NaHCO_3$ were compared with control (iron-free Hoagland solution). FeNaEDTA addition into nutrient solution induced significant increases in iron and chlorophyll contents across the genotypes. The highest Fe level was determined in 'Fercal' (169.8 mg kg^{-1}) with 36 mg L^{-1} FeNaEDTA. Bicarbonate additions restricted the vegetative development of '1613 C'. For instance, iron content of '99 R' was 137.9 mg kg^{-1} when treated with 9 mg L^{-1} FeNaEDTA, whereas the iron value reduced to 73.9 mg kg^{-1} when $NaHCO_3$ was added. 'Alphonse Lavallée' and 'Fercal' displayed their lime-tolerances by exhibiting little reduction of both iron and chlorophyll contents. Therefore, 'Fercal' would be proven as a favorable rootstock for regions with calcareous soil. Tolerance to $NaHCO_3$-induced Fe shortage appeared to be genotype-dependent. Chlorophyll content of young leaves positively correlated with Fe concentration, indicating the vital role of iron in chlorophyll content of leaves.

Additional key words: chlorophyll content, grape cultivar, grape rootstock, iron chlorosis.

Resumen

Respuesta de cuatro genotipos de vid (*Vitis* spp.) a deficiencias de hierro directas o inducidas por bicarbonato

Los suelos calcáreos causan normalmente disminuciones significativas en la producción de vid en Turquía. Para manejar este problema es importante conocer las respuestas fisiológicas de los genotipos de vid en tales condiciones. El presente estudio se condujo para evaluar comparativamente las respuestas de cuatro genotipos de *Vitis* spp. (un cultivar, 'Alphonse Lavallee', y tres portainjertos, 'Fercal', '99 R' and '1613 C') con diferente tolerancia potencial a la clorosis directa o férrica. Se cultivaron durante dos años consecutivos, en contenedores de perlita inerte en invernadero, esquejes enraizados con una solución completa de nutrientes de Hoagland, con el Fe como factor variable, mediante cinco aplicaciones realizadas tres veces por periodo vegetativo. Se compararon aplicaciones de hierro (FeNaEDTA) a dosis de 9 y 36 mg L^{-1} combinadas con 840 mg L^{-1} de $NaHCO_3$ con el grupo control (solución Hoagland sin Fe). La adición de FeNaEDTA en la solución nutritiva indujo un incremento significativo en los contenidos de Fe y clorofila de los genotipos. El nivel más alto de Fe se detectó en 'Fercal' (169,8 mg kg^{-1}) con 36 mg L^{-1} de FeNaEDTA. La adición de bicarbonatos restringió el desarrollo vegetativo de '1613 C' (el contenido de Fe en '99 R' fue 137,9 mg kg^{-1} cuando se trató con 9 mg L^{-1} de FeNaEDTA, y de 73,9 mg kg^{-1} cuando se añadió $NaHCO_3$). 'Alphonse Lavallée' y 'Fercal', tolerantes a la clorosis férrica, presentaron una disminución en su contenido en Fe y clorofila. 'Fercal' resultó ser un portainjerto favorable para regiones con suelos calcáreos predominantes. La tolerancia a escasez de Fe inducido por $NaHCO_3$ parece ser dependiente del genotipo. El contenido de clorofila de las hojas jóvenes correlacionó positivamente con la concentración de Fe, indicando el papel vital del Fe en el contenido de clorofila en las hojas.

Palabras clave adicionales: clorosis férrica, contenido en clorofila, cultivares de vid, portainjerto de vid.

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Abbreviations used: MSD (minimum significant differences), SPAD (soil plant analysis development).

Introduction

Turkey has an important proportion in world grape production with its approximate percentage of 7% (Anonymous, 2007). However, the production value per area is relatively lower due to certain mistakes in cultural practices such as selection of proper materials as well as the application of accurate fertilization particular to any genotypes and/or soil conditions. Vineyard soil in many part of Turkey is generally calcareous. This case decreases iron availability and expose the vines to severe chlorosis. The general physiological responses to Fe deficiency stress are release of H^+ ions and rising reduction of ferric Fe at the plasmalemma of roots or release of phytosiderophores from roots (Jolley *et al*., 1996) as well as chlorosis on leaves, reduction in vegetative and generative developments (Nikolic and Kastori, 2000; Lucena, 2003) since bicarbonate neutralizes the root H-ATPase activity (Rabotti and Zocchi, 1994). 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 iron status of the plant (Katyal and Sharma, 1980). Utilization of these physiological reactions for screening has been attempted using several grape cultivars and rootstocks under various growth conditions such as pots (Bavaresco and Poni, 2003; Ksouri *et al*., 2007), containers (Jiménez *et al*., 2007), *in vitro* culture (Tangolar *et al*., 2008) as well as field experiments (Bertamini and Nedunchezhian, 2005). Understanding such responses by practical techniques could be an invaluable aid for selecting the proper genotypes in breeding programs. Recent data highlight that grapevine genotypes differ in terms of capability to improve Fe-reductase activity and response to low availability level of iron in the soil (Ollat *et al*., 2003; Ksouri *et al*., 2007). Many American species are much more sensitive to lime-induced chlorosis than the varieties of *V. vinifera*. In American species, however, various hybrids of *V. berlandieri* have provided the most influential and practical control of lime-induced chlorosis as rootstocks (Winkler *et al*., 1974). In this perspective, comparative evaluation of valuable genotypes would aid to develop the grape productivity in especially regions where calcareous soil predominates.

Literature investigations reveal that experimental studies to detect evidence of macro and micro element deficiencies in grapes are insufficient to match necessity of clear knowledge to solve problems in genotype selection and vineyard nutrition practice. Furthermore, a fertilization program approved for certain area can not be necessarily applicable elsewhere, nor could be certain genotypes recommended for different regions since response of such deep-rooted and perennial crops to any element shortage would be very different. Due to many soil factors that impair iron nutrition, it is usually difficult to predict the possible chlorosis development of vines on the basis of soil parameters (Tagliavini and Rombola, 2001). On the other hand, Cook (1958) indicated that the leaf petiole is an ideal part to investigate the nutrient status of the vines.

The main objectives of this study was (a) to analyze the effects of iron (in FeNaEDTA form) on vegetative development as well as macro and micro element concentrations of grapevine genotypes differing in tolerance capacity to iron shortage, (b) to assess the contrasting interaction of iron and bicarbonate in plant physiology, and (c) to analyze the variability of morphological and physiological responses of four grapevine genotypes (tolerant to sensitive) to iron deficiency.

Material and methods

A greenhouse experiment was conducted in the 2006 and 2007 vegetation periods. Two bud-bearing rooted cuttings of grape genotypes were grown in inert perlite media in containers for two consecutive years, fertilization of the young grapes were performed with application of nutrient solutions by modifying Hoagland solution containing 24 g $MgSO_4.7H_2O$, 2.7 g KH_2PO_4, 15.7 g K_2SO_4, 47.23 g $Ca(NO_3)_2.4H_2O$, 0.1 g $CuSO_4$, 1.24 g H_3BO_3, 0.334 g $MnSO_4$, 0.57 g $ZnSO_4.7H_2O$, 0.048 g NH_4Mo_4 per liter. The experimental design was two factors randomized complete blocks with five applications (control, 9 mg L^{-1} iron chelates (FeNaEDTA), 36 mg L^{-1} iron chelates, 9 mg L^{-1} iron chelates plus 840 mg L^{-1} $NaHCO_3$, 36 mg L^{-1} iron chelates plus 840 g L^{-1} $NaHCO_3$). Four *Vitis* material, 'Alphonse Lavallée' (tolerant cultivar representing *V. vinifera* L.), 'Fercal' (tolerant rootstock of *Vinifera* × *Berlandieri*), '99 R' (moderate to sensitive rootstock of *Berlandieri* × *Rupestris*) and '1613 C' (sensitive rootstock of *Solonis* × *Othello*) were chosen on the basis of general knowledge about the tolerance/sensitivity to lime-induced iron chlorosis (Chauvet and Reynier, 1979; Saracco, 1992) as well as significance in worldwide viticulture. Each container, about 1.5 m long, 15 cm deep and wide, had single-trunk 30 plants with 10 cm intervals on two lines. Hoagland solutions, with iron and/or bicarbonate concentrations, were commonly applied for three times per year, while general irrigation was performed by using distilled water in equal amounts for each container with 1-3 days intervals depending on environmental conditions.

Investigations were performed in the second vegetative period when the shoot elongation was near to cease (Bavaresco, 1995). Chlorophyll contents of leaves (the 3rd and 4th leaf at the shoot tips) were calculated by using the portable chlorophyll meter (Minolta SPAD-502, Japan). After measurements on shoot parameters and SPAD (Soil Plant Analysis Development) readings, the plants harvested and divided into young leaves, stems plus old leaves, and roots. Plant samples were dried at 70°C for 48 h, and the dry matter production was measured. After being ground, samples were dry-ashed at 500°C for 5 h and dissolved in 3.3% HCl solution. Analysis was carried out for K, Ca, Mg, Fe, Mn, Zn, and Cu using atomic absorption spectrometry (Varian FS 220) and for P following the method of Barton (1948).

ANOVA analysis with interactions was used for all parameters by using SPSS program version 13.0 (SPSS Inc., Chicago, IL). Averages of the applications were compared by Tukey's MSD (Minimum significant differences) at 5% level.

Results

Iron applications increased the total Fe contents of leaves across the genotypes (Fig. 1a). The highest Fe level was determined in 'Fercal' (169.8 mg kg^{-1}) treated with 36 mg L^{-1} FeNaEDTA. However, the addition of $NaHCO_3$ into the nutrient solution resulted in a significant reduction of the leaf iron concentrations of '99 R' and '1613 C'. For instance, iron content of '99 R' was 137.9 mg kg^{-1} when treated with 9 mg L^{-1} FeNaEDTA, whereas the iron value reduced to 73.9 mg kg^{-1} when $NaHCO_3$ was added into the nutrient solution. Similar reductions occurred in '99 R' and '1613 C' when 36 L^{-1} iron plus bicarbonate was applied. But, the existence of $NaHCO_3$ had little effect on leaf iron contents of 'Alphonse Lavallée' and 'Fercal'. On the other hand, total Fe contents of the control plants were almost half compared to those of general Fe applications.

According to SPAD readings, Fe supplementation significantly increased the leaf chlorophyll contents of genotypes (Fig. 1b). Application of iron in 9 and 36 mg L^{-1} doses increased the chlorophyll contents of overall genotypes. In contrast, $NaHCO_3$ supplementation into nutrient solution caused a severe reduction in chlorophyll amounts of '99 R' and '1613 C'. But, 'Alphonse Lavallée' and 'Fercal' displayed remarkable tolerance to lime-chlorosis when irrigated with the solution containing bicarbonate.

The data presented in Table 1 indicate that the applications significantly affected the growth parameters of genotypes. Fe applications obviously induced to increase all values relevant to leaf, shoot and root observations across the genotypes. However addition of $NaHCO_3$ resulted in significant growth restrictions in especially '1613 C'. The highest values in leaf area among the applications were obtained from 9 and 36 mg L^{-1} Fe applications with 55.4 and 53.5 cm, respectively. But $NaHCO_3$ concentrations markedly inhibited the leaf area and shoot length of the genotypes. The longest shoot in 99 R was observed in samples treated with 9 mg L^{-1} Fe (30.4 cm) as was seen in 'Alphonse Lavallée' (32.9 cm) and 1613 C (29.4 cm). On the other hand, 36 mg L^{-1} Fe plus 840 mg L^{-1} $NaHCO_3$ resulted in the formation of the longest shoot in 'Fercal'. Among the growth parameters, leaf area appeared to be more sensitive to excessive bicarbonate than the others. The

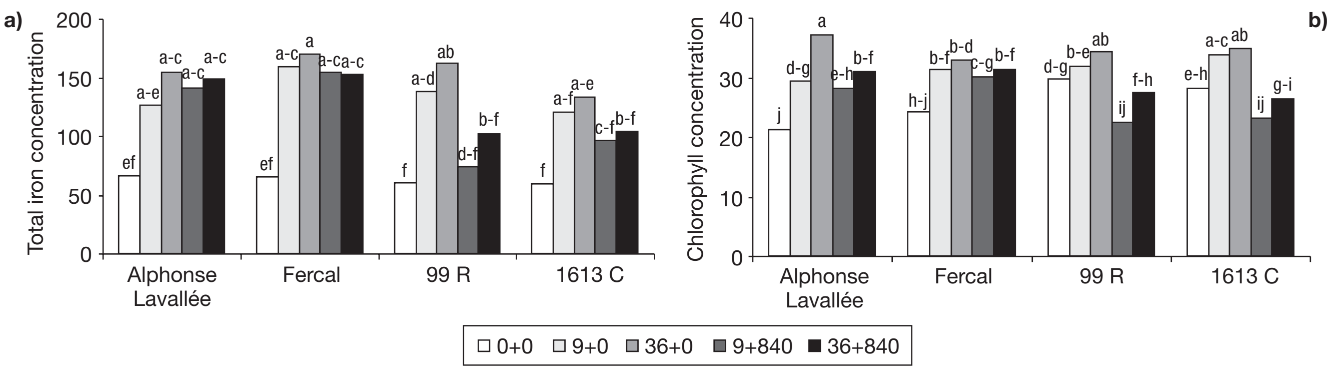

Figure 1. Total iron (a) and chlorophyll (b) contents of grapevine leaves (mg kg^{-1}).

Table 1. Growth parameters of grape genotypes in response to applications

Genotypes	Iron applications	Leaf area	Shoot length	Leaf no.	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight
Alphonse Lavallée	0+0	48.0^{e}	23.5^{bd}	17.6^{b-d}	9.6^{d-g}	2.97^{ef}	15.1^{d-g}	4.95^{fg}
	9+0	64.9^{ab}	32.9^{a}	19.6^{b}	14.2^{a}	3.78^{a-e}	23.8^{a}	6.24^{b-g}
	36+0	66.4^{a}	21.9^{c-e}	16.7^{b-f}	12.5^{a-c}	3.46^{c-f}	18.8^{b-f}	5.57^{e-g}
	9+840	56.5^{cd}	23.4^{bd}	16.8^{b-e}	13.7^{a}	4.56^{a}	19.9^{a-c}	7.82^{ab}
	36+840	60.3^{bc}	26.6^{a-c}	18.2^{bc}	13.1^{ab}	4.51^{ab}	18.8^{b-f}	7.22^{a-e}
Fercal	0+0	41.3^{f}	11.5^{hi}	7.6^{h}	7.04^{h}	2.90^{ef}	10.1^{h}	4.88^{g}
	9+0	63.6^{ab}	13.5^{hi}	12.3^{fg}	8.4^{gh}	3.22^{d-f}	14.0^{gh}	6.05^{b-g}
	36+0	52.3^{de}	13.6^{hi}	12.6^{e-g}	9.3^{e-g}	3.71^{a-e}	15.1^{d-g}	7.52^{a-c}
	9+840	52.8^{de}	14.1^{f-i}	13.5^{d-g}	9.7^{d-g}	3.35^{c-f}	15.0^{e-g}	7.79^{ab}
	36+840	50.1^{de}	15.0^{e-h}	18.9^{bc}	10.5^{d-f}	3.58^{b-e}	19.3^{b-e}	6.71^{a-f}
99 R	0+0	26.2^{j}	14.0^{g-i}	16.7^{b-f}	8.3^{gh}	3.01^{ef}	13.6^{gh}	5.76^{c-g}
	9+0	33.0^{hi}	30.4^{ab}	26.5^{a}	11.4^{b-d}	3.51^{c-f}	15.7^{c-g}	7.54^{a-c}
	36+0	32.9^{hi}	26.6^{a-c}	18.1^{bc}	9.1^{e-g}	3.12^{d-f}	14.5^{fg}	5.85^{c-g}
	9+840	30.1^{ij}	21.5^{c-f}	19.8^{b}	8.4^{gh}	3.10^{d-f}	13.6^{gh}	6.55^{a-g}
	36+840	36.4^{f-h}	21.2^{c-g}	15.0^{c-g}	9.2^{e-g}	3.08^{d-f}	15.5^{d-g}	6.31^{b-g}
1613 C	0+0	40.8^{f}	17.2^{d-h}	13.0^{e-g}	7.1^{h}	2.57^{f}	14.6^{fg}	5.72^{d-g}
	9+0	60.4^{bc}	29.4^{ab}	17.5^{b-d}	10.8^{c-e}	4.24^{a-c}	19.0^{b-e}	8.17^{a}
	36+0	62.1^{ab}	25.3^{bc}	15.5^{b-g}	9.3^{e-g}	3.17^{d-f}	22.7^{ab}	7.51^{a-d}
	9+840	40.2^{fg}	21.0^{c-g}	11.6^{gh}	8.9^{f-h}	4.00^{a-d}	17.6^{c-g}	7.40^{a-d}
	36+840	35.3^{gh}	17.5^{d-h}	11.2^{gh}	7.1^{h}	3.72^{a-e}	19.3^{b-d}	6.08^{b-g}
MSD 5% for appl.		*5.13*	*7.27*	*4.41*	*1.89*	*0.95*	*4.30*	*1.77*
Application means	0+0	39.1^{c}	16.3^{c}	13.7^{b}	8.0^{c}	2.86^{b}	13.4^{b}	5.33^{b}
	9+0	55.4^{a}	25.0^{a}	19.0^{a}	11.2^{a}	3.69^{a}	18.1^{a}	7.00^{a}
	36+0	53.5^{a}	21.8^{ab}	15.8^{b}	10.1^{b}	3.36^{a}	17.8^{a}	6.61^{a}
	9+840	44.9^{b}	20.0^{b}	15.5^{b}	10.1^{b}	3.75^{a}	16.5^{a}	7.39^{a}
	36+840	45.6^{b}	20.1^{b}	15.8^{b}	9.9^{b}	3.72^{a}	18.2^{a}	6.58^{a}
MSD 5% for means		*2.57*	*3.67*	*2.20*	*0.95*	*0.51*	*2.14*	*0.89*

Within column, means followed by a different letter differ significantly at $p < 0.05$ by Tukey. MSD: minimum significant differences.

highest leaf number was obtained from 9 g L^{-1} Fe application of '99 R' (26.5). When 9 mg L^{-1} Fe application was compared with its combination with 840 mg L^{-1} $NaHCO_3$ in terms of the decrease percentages of leaf number and shoot length in '1613 C', the decrease rates were about 29 and 34 %, respectively.

When the plants were nourished with iron-free Hoagland solution, fresh and dry weights of entire genotypes drastically restricted. Although the highest shoot fresh weight values were obtained from the application of 9 mg L^{-1} Fe in 'Alphonse Lavallée', '99 R' and '1613 C' (14.2, 11.4 and 10.8 g, respectively), the influence of bicarbonate addition on these criteria was not noticeable.

As seen in Table 2, Fe treatments increased macro element concentrations of genotypes. Especially 9 mg L^{-1} Fe application nearly doubled the general macro element status of 'Fercal'. The highest K concentrations were detected in samples of 'Fercal' treated by 36 mg L^{-1} Fe with and without $NaHCO_3$ (2.14 and 2.07%, respectively). These rates are fairly high in comparison with the application means, indicating the capability in nutrition utility of 'Fercal' under calcareous conditions. Surprisingly, the least K concentration was observed in control group of the same rootstock. The highest P concentration was observed in 'Fercal' treated with 9 mg L^{-1} Fe (0.230%). Actually, Fe application in 9 mg L^{-1} concentration resulted in the highest P concentration in most genotypes. The use of 9 mg L^{-1} Fe also yielded better results in terms of Ca and Mg concentrations (2.30 and 0.74%, respectively). However might it be interesting, there was no definite consequence whether the bicarbonate has restrictive effect on Ca uptake of general varieties, except for 9 mg L^{-1} Fe plus 840 mg L^{-1} $NaHCO_3$ application of '1613 C'. In the last case, addition of bicarbonate resulted in ob-

Table 2. Macro and microelement contents of grape petioles

Genotypes	Iron applications	K (%)	P (%)	Ca (%)	Mg (%)	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
Alphonse Lavallée	0+0	1.27^{a-f}	0.167^{a-e}	1.79^{a-g}	0.49^{b-g}	42.0^{c-e}	24.8^{b-d}	9.67^{a-e}
	9+0	1.42^{a-f}	0.202^{a-c}	2.52^{a-c}	0.59^{b-f}	44.6^{c-e}	48.3^{a}	11.35^{a-c}
	36+0	1.98^{ab}	0.193^{a-d}	2.64^{ab}	0.65^{b-d}	128.1^{a}	34.3^{a-c}	12.17^{ab}
	9+840	1.34^{b-f}	0.154^{a-e}	2.19^{a-f}	0.57^{b-g}	58.8^{b-e}	27.7^{a-d}	8.19^{b-e}
	36+840	1.74^{a-d}	0.197^{a-d}	2.52^{a-c}	0.67^{b-c}	90.2^{ab}	33.4^{a-c}	12.50^{ab}
Fercal	0+0	0.83^{f}	0.102^{de}	1.03^{g}	0.23^{g}	23.5^{e}	9.1^{d}	4.63e
	9+0	1.55^{a-f}	0.230^{a}	2.73^{a}	1.05^{a}	62.5^{b-e}	40.9^{ab}	10.89^{a-c}
	36+0	2.07^{ab}	0.184^{a-e}	2.24^{a-e}	0.64^{b-e}	69.6^{b-d}	34.2^{a-c}	10.19^{a-d}
	9+840	1.84^{a-c}	0.191^{a-e}	2.58^{a-c}	0.55^{b-g}	53.4^{b-e}	27.8^{a-d}	9.54^{a-e}
	36+840	2.14^{a}	0.219^{ab}	2.49^{a-d}	0.68^{bc}	71.7^{bc}	35.6^{a-c}	14.47^{a}
99 R	0+0	1.07^{d-f}	0.096^{e}	1.09^{f-g}	0.34^{d-g}	34.3^{c-e}	16.7^{cd}	5.68^{de}
	9+0	1.71^{a-d}	0.173^{a-e}	2.37^{a-e}	0.79^{ab}	56.2^{b-e}	42.8^{ab}	9.98^{a-d}
	36+0	1.50^{a-f}	0.118^{c-e}	1.39^{d-g}	0.48^{b-g}	58.5^{b-e}	16.2^{cd}	5.34^{de}
	9+840	0.89^{ef}	0.104^{de}	1.33^{e-g}	0.29^{fg}	27.7^{de}	28.7^{a-d}	5.71^{de}
	36+840	1.46^{a-f}	0.184^{a-e}	1.49^{c-g}	0.49^{b-g}	50.8^{b-e}	26.4^{b-d}	8.39^{b-e}
1613 C	0+0	1.40^{a-f}	0.114^{c-e}	0.99^{g}	0.30^{f-g}	31.9^{c-e}	16.5^{cd}	5.49^{de}
	9+0	1.74^{a-d}	0.153^{a-e}	1.58^{b-g}	0.52^{b-g}	27.0^{e}	29.1^{a-d}	9.19^{b-e}
	36+0	1.67^{a-d}	0.123^{b-e}	1.59^{b-g}	0.45^{c-g}	49.3^{b-e}	22.6^{b-d}	6.36^{c-e}
	9+840	1.36^{b-f}	0.125^{b}	0.92^{g}	0.31^{e-g}	29.7^{c-e}	19.2^{cd}	6.52^{c-e}
	36+840	1.59^{a-e}	0.135^{a-e}	1.74^{a-g}	0.46^{b-g}	47.8^{c-e}	23.3^{b-d}	7.49^{b-e}
MSD 5% for appl.		*0.747*	*0.094*	*1.01*	*0.33*	*42.03*	*21.0*	*5.13*
Application means	0+0	1.14^{c}	0.125^{b}	1.23^{b}	0.34^{c}	32.9^{c}	16.8^{c}	6.37^{b}
	9+0	1.61^{ab}	0.189^{a}	2.30^{a}	0.74^{a}	47.6^{bc}	40.3^{a}	10.35^{a}
	36+0	1.76^{a}	0.149^{ab}	1.96^{a}	0.56^{b}	76.4^{a}	26.8^{bc}	8.51^{ab}
	9+840	1.36^{bc}	0.144^{ab}	1.75^{ab}	0.43^{bc}	42.4^{c}	25.9^{bc}	7.48b
	36+840	1.73^{a}	0.184^{a}	2.06^{a}	0.58^{ab}	65.1^{ab}	29.7^{b}	10.71^{a}
MSD 5% for means		*0.363*	*0.048*	*0.49*	*0.16*	*21.01*	*10.52*	*2.56*

Within column, means followed by a different letter differ significantly at $p < 0.05$ by Tukey. MSD: minimum significant differences.

vious reduction in Ca at about 40% when 9 mg L^{-1} Fe application was compared with and without bicarbonate.

ANOVA analysis shows that the applications have different effects on the leaf microelement concentrations of genotypes. Among the application means, Mn concentration in young leaves ranged from 32.9 (control) to 76.4 mg kg^{-1} (36 mg L^{-1} FeNaEDTA). 'Alphonse Lavallée' was distinguished with its significantly higher Mn concentration detected in the application of 36 mg L^{-1} FeNaEDTA (128.1 mg kg^{-1}). Actually, Mn concentrations of the leaves reached up to maximum levels with the application of 36 mg L^{-1} iron. Among the genotypes, Mn concentrations were differently affected in the presence of bicarbonate. On the other hand, application of 9 mg L^{-1} iron resulted in the highest Zn concentration in all genotypes. Cu concentrations displayed a similar manner to Zn changes. Remarkable decreases in Cu levels of petioles were obvious in samples treated with 9 mg L^{-1} Fe containing 840 mg L^{-1} bicarbonate. However, the addition of bicarbonate into the solution had little effect on petiole Cu concentration, when the iron was increased to 36 mg L^{-1}.

According to regression analysis ($p < 0.01$), there is a significant correlation between the degree of chlorosis with total iron concentration of grapevine leaf (Fig. 2).

Discussion

The absence of Fe in the nutrient solution caused intensive deprivation in overall growth characteristics of grape genotypes. This case is in agreement with the assertion set forth by Jiménez *et al*. (2007) who interpreted the sensitivities of vegetative development to iron shortage in different genotypes of *Vitis*. According to the suggestion of Winkler *et al*. (1974), iron concen-

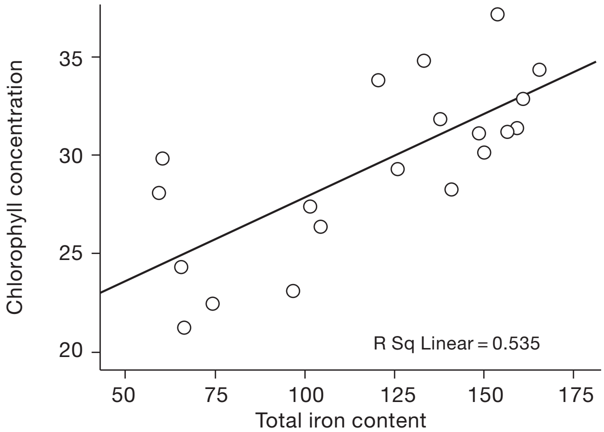

Figure 2. Relationship between leaf chlorophyll content and total iron concentration.

tration values of control plants are around the critical level of iron deficiency symptoms.

Considering the investigated criteria, tolerance to $NaHCO_3$-induced Fe shortage appears to be genotype-dependent. Such disparities among the genotypes relevant to bicarbonate restrictions were also reported before by numerous researchers using different genotypes (Bavaresco and Poni, 2003; Ksouri *et al*., 2005; Jiménez *et al*., 2007; Ozdemir and Tangolar, 2007). As previously demonstrated (Nikolic and Kastori, 2000; Ksouri *et al*., 2007), tolerance to Fe deficiency might physiologically rely on the plant aptitude to guarantee absorption of vital iron mediated by mechanism of «Strategy I». Therefore, chlorosis resulting from restrictive effect of bicarbonate on iron intake could be attributed to deprivation of such mechanism in sensitive genotypes like '1613 C'.

Chlorophyll concentration of genotypes increased with iron applications, similar to the same trend occurred in total iron concentration. Such relation was also reported by Chen *et al*. (2004) who examined the response of own-rooted one-year-old 'Concord' grape to iron supply. Application of iron in 9 and 36 mg L^{-1} doses increased the chlorophyll contents of overall genotypes in a linear relationship as demonstrated by Smith and Cheng (2006). Increases in chlorophyll concentrations after iron applications could be attributed to the inducing effect of iron for electron-transport chains in both mitochondria and chloroplasts (Bertamini and Nedunchezhian, 2005). In contrast, shortage of available iron in plant nutrition is accompanied by a decrease in the level of photosynthetic pigments (Val *et al*., 1987) that results in chlorosis. Such cases were particularly apparent in '1613 C' and '99 R', indicating their sensitivity levels to bicarbonate (Chauvet and Reynier, 1979; Saracco, 1992).

It is well known that Fe and Zn out of the others have a special significance in plant physiology as they undertake vital duty in photosynthesis reactions (Val *et al*., 1987). 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, 1986; Bertamini and Nedunchezhian, 2005). In accordance with these facts, restrictions in various morphological and nutritious characters in the examined genotypes in this study verified the fundamental roles of iron in a series of physiological processes. Supplementary bicarbonate in both doses caused to appear Fe deficiency symptoms and had depressive influences on physiological characteristics of grape genotypes, although its degree was obviously dependant on the tolerance level of genotypes. When the element concentrations of leaves were compared with the recommended levels (Winkler *et al*., 1974), almost entire of macro and micro elements determined in leaves of control plants are under normal levels. Particularly K, Mg and Zn concentrations of overall plants were also below the normal levels in the presence of $NaHCO_3$. Among the analyzed genotypes, 'Fercal' demonstrated remarkable tolerance to increased bicarbonate concentration regarding these elements. In contrast, existence of bicarbonate in the irrigation water obstructed solely the general parameters of '99 R' and '1613 C' rootstocks. As chlorosis progressed in severity along with the decrease in Fe concentration, these rootstocks exhibited premature senescence symptoms, in convenience to the recent study (Tangolar *et al*., 2008) in which some grapevines were tested for lime-induced iron chlorosis using *in vitro* technique. Veliksar *et al*. (1995) interpreted such case as Fe efflux from young tissues to perennial parts of plant in spite of relatively low mobility of iron in tissues.

The degree of chlorosis was significantly correlated with total Fe concentration of grape leaf. This case confirms the suggestion that early essay on iron status of leaves might be a useful evidence for estimation and selection of the resistant genotypes to iron deficit chlorosis (Bavaresco *et al*., 1992; Ksouri *et al*., 2005). Nevertheless, bearing in mind the fact known as «Fe chlorosis paradox» (Römheld, 2000), in some cases lime-induced chlorosis might be related with high levels of Fe in leaf, which is associated with restriction in leaf and shoot growth.

This study verifies the vital role of iron on vegetative development as well as macro and micro element concentrations of grapevines. Results also indicate the contrasting interaction of iron and bicarbonate in grapevine physiology and the existence of variability in morphological and physiological responses of four grapevine genotypes to iron deficiency. Therefore, selection of genuinely tolerant rootstocks represents a unique solution to get rid of iron deficiency problem in viticulture area where calcareous soil predominates. Finally, 'Fercal' was outstanding with its remarkable tolerance potential to lime-induced iron deficiency and was proven to use under such conditions.

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