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Effect of bicarbonate on uptake and translocation of ^{59}Fe in two grapevine rootstocks differing in their resistance to Fe deficiency chlorosis

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Summary

In order to study the effect of high bicarbonate concentration in the root medium on root Fe^{III} reduction, Fe uptake and its translocation to the leaves, two rootstocks (*Vitis riparia* Michx., susceptible, and 41 B (*Vitis vinifera* L. cv. Chasselas x *Vitis berlandieri* Planch.), resistant to Fe deficiency chlorosis) were pre-cultivated in nutrient solutions with high and low Fe supply. After three weeks of preculture at low Fe, chlorosis symptoms occurred in both, Fe-resistant and Fe-susceptible genotypes. The Fe^{III} reducing capacity by roots was enhanced at Fe deficiency in both genotypes, which was consistent with the increase of subsequent root uptake and translocation rates of ^{59}Fe. In the presence of bicarbonate in the solutions the Fe^{III} reducing capacity, ^{59}Fe uptake and translocation rate decreased in both genotypes precultured with low Fe supply. The ^{59}Fe uptake and translocation rate, however, were significantly higher in the Fe chlorosis-resistant rootstock 41 B. These results clearly indicate that bicarbonate-induced Fe chlorosis in grapevine rootstocks is obviously caused by an inhibition of Fe uptake and translocation due to an inhibition of Fe^{III} reduction by root cells. The fact that these processes were less inhibited in the chlorosis-resistant rootstock hints to genotypical differences in Fe acquisition by roots at high bicarbonate levels. These differences might be used in breeding programs to identify Fe chlorosis-resistant rootstocks.

K e y w o r d s : bicarbonate, iron deficiency chlorosis, ferric reduction, iron uptake, iron translocation, *Vitis* sp.

Introduction

High bicarbonate concentration in soil solution is considered to be the most important factor inducing iron (Fe) deficiency chlorosis in grapevine (Mengel *et al.* 1984; Römheld 1986; Kolesch *et al.* 1987) and other dicotyledonous plant species. The physiological basis for bicarbonate-induced Fe chlorosis is not yet completely understood (Mengel 1994), although many studies have shown bicarbonate-inhibited plasma membrane-bound Fe^{III} reduction, uptake by the roots and translocation to the shoots as main causal factors (review: Marschner and Römheld 1994). The Fe-deficiency-induced ATPase-mediated net H^+ extrusion results in the stimulation of root plasma membrane-bound Fe^{III} reductase activity by acidifying the micro-environment in the apoplast of various plant species (Schmidt 1999). Thus, the high bicarbonate concentration in calcareous soils buffers H^+ and increases the pH of the root apoplast, causing an inhibition of Fe^{III} reductase (Toulon *et al.* 1992).

At low Fe availability in soils, grapevine (*Vitis* sp.) responds as a typical so-called "Strategy I" plant. Various grapevine rootstocks show quantitative differences in root response reactions involved in "Strategy I", particularly with regard to rhizosphere acidification, root Fe^{III} reducing capacity and Fe uptake rate (Maggioni 1980; Varanini and Maggioni 1982; Bavaresco *et al.* 1991; Brancadoro *et al.* 1995). In spite of these studies, physiological response mechanisms of grapevine rootstocks to bicarbonate-induced Fe chlorosis are still lacking. According to several authors high bicarbonate in nutrient solution strongly inhibited Fe uptake by the roots and its translocation to the shoots during a short-term uptake period as observed in many other plant species such as sunflower, chrysanthemum, soybean, barley and orange trees (Willihan 1961; Rutland and Bukovac 1971; Venkatra u and Marschner 1981; Römheld *et al.* 1982; Fleming *et al.* 1984).

The objectives of this paper were to study the effect of high bicarbonate in root solution on root Fe^{III} reduction, Fe uptake and its translocation to the leaves of two grapevine rootstock genotypes differing in their resistance to Fe deficiency chlorosis, and to elucidate the different physiological response mechanisms to bicarbonate-inducing Fe deficiency in Fe chlorosis-resistant and -susceptible rootstocks.

Material and Methods

P l a n t c u l t u r e : Two grapevine rootstocks differing in Fe chlorosis resistance were used: *Vitis riparia* Michx. (susceptible) and 41 B (*Vitis vinifera* L. cv. Chasselas x *Vitis berlandieri* Planch.) (resistant). Segments of one-year-old canes with two buds were rooted in moist quartz sand in a greenhouse. After 4 weeks, rooted cuttings were transferred to plastic pots (4 plants per pot) with 2.5 l of a nutrient solution containing (M): 2×10^{-3} $Ca(N0_3)_2$; 0.7×10^{-3} K_2S0_4; 0.1×10^{-3} KH_2P0_4; 0.1×10^{-3} KCl; 0.5×10^{-3} $MgS0_4$; 10^{-5} H_3B0_3; 0.5×10^{-6} $MnS0_4$; 0.2×10^{-6} $CuS0_4$;

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10^{-8} $(NH_4)_6Mo_7O_{24}$ and 0.5 x 10^{-6} $ZnSO_4$. For 'high Fe plants' nutrient solution was supplied with 10^{-4} M FeEDDHA, while 2 x 10^{-7} M FeEDDHA was added to 'low Fe plants'. Nutrient solutions were replaced every 4 d and continuously aerated. The pH of the nutrient solutions was checked daily and kept in the range of 5.5-6.0. Vines were grown for three weeks in a controlled climate chamber with a day/night photoperiod of 16/8 h, temperature 24/20 °C, light intensity about 200 !Jmol m^{-2} s^{-1} and relative humidity 70 ± 5 %.

Fe^{III} reducing capacity of roots: Roots of intact plants were washed in 0.5 mM $CaSO_4$ for 30 min, and the reducing capacity was determined by incubating the roots in a solution containing 0.5 mM $CaSO_4$, 0.3 mM BPDS, 0.1 mM FeEDTA, buffered with 10 mM MES (pH 6.0) or 10 mM $NaHCO_3$ (pH ~ 8.0) in the darkness at room temperature and continuous aeration of the solution. After incubation (1 h), the $Fe^{II}(BPDS)_3$ red complex was measured (absorbance at 535 nm against blanks without roots). The extinction coefficient of 22.14 mM^{-1} cm^{-1} was used for calculation of the reducing capacity (CHANEY *et al.* 1972).

^{59}Fe uptake experiment: ^{59}FeEDDHA was prepared by mixing $^{59}FeCl_3$ with EDDHA (K-salt) the chelate was equilibrated overnight by stirring with filter paper flakes to absorb non-chelated Fe precipitates. Thereafter, the solution was filtered through a membrane filter (pore size 0.2 !Jm). The total specific activity was 5.1 GBq mol^{-1} Fe for the solution buffered with 10 mM MES (pH 6.0) and 25.6 GBq mol^{-1} Fe for the solution buffered with 10 mM $NaHCO_3$ (pH ~ 8.0). Before supplying labelled Fe chelate, each plant was adapted in a micronutrient-free non-labelled nutrient solution for 2 h, and then transferred to the aerated ^{59}Fe-labelled nutrient solution supplied with 10 !JM ^{59}FeEDDHA in both treatments. After an uptake period of 12 h, intact roots were rinsed in a solution containing 0.5 mM $CaSO_4$ and 5 mM MES (pH 5.5) for 10 min at vigorous aeration. Precipitated ^{59}Fe in the root apoplast (extraplasmatic Fe) was removed according to BIENFAIT *et al.* (1985) using 12.5 mM Na-dithionite and 1.5 mM a,a-bipyridyle under continuous N_2 flow for 7 min. After removal of extraplasmatic ^{59}Fe from roots, the plants were separated into roots and leaves, dried at 65 °C for 12 h, weighed, ashed at 550 °C for 8 h and dissolved in 10 ml 1 % HCl. The ^{59}Fe radioactivity was determined using a liquid scintillation counter.

Chlorophyll determination: Chlorophyll content in young leaves was determined using the portable Chlorophyll Meter SPAD-502 (Minolta Camera Co., 0saka, Japan).

Results

After one week of preculture in nutrient solution with low Fe supply slight symptoms of Fe deficiency chlorosis occurred only at young leaves of Fe chlorosis susceptible rootstocks (*Vitis riparia*) and after another two weeks, typical chlorosis symptoms occurred in both, *Vitis riparia* and 41 B. At the end of this period *Vitis riparia* was severely chlorotic, while 41 B was moderately chlorotic. Accordingly, the chlorophyll content determined as chlorophyll meter readings (SPAD) significantly decreased in both genotypes under Fe deficiency, being somewhat higher in leaves of the resistant genotype 41 B (Tab. 1).

An increase in the reducing capacity of the roots is one of the main response mechanisms to Fe deficiency in "Strategy I" plants. Thus, at the end of preculture (three weeks) both rootstocks at low Fe supply showed an increase in Fe^{III} reduction as compared to plants at high Fe supply, which was consistent with an increase in the subsequent ^{59}Fe uptake by roots (Figure, Tab. 3). Furthermore, in the resistant genotype (41 B) the Fe^{III} reducing capacity induced by low Fe supply measured under standard conditions was more than 7-times higher than the control supplied with adequate Fe. This increase was much lower (above 2-times) in *Vitis riparia* (Figure). The addition of bicarbonate (10 mM) to the test medium induced a significant decrease in the Fe^{III} reducing capacity of both low Fe precultured rootstocks (Figure). The inhibitory effect of bicarbonate on Fe^{III} reducing capacity was similar in both Fe deficient genotypes, *Vitis riparia* (68 %) and 41 B (70 %). In the presence of HCO_3^- in the assay solution, no significant differences among different Fe preculture were found for the Fe^{III} reducing capacity in *Vitis riparia*. Whereas, the Fe^{III} reducing capacity in 41 B plants with low Fe supply was still significantly higher compared to the control supplied with high Fe (Figure).

After 12 h of ^{59}Fe-labelled FeEDDHA supply the uptake of ^{59}Fe increased in both rootstocks with low Fe supply when compared to high Fe preculture. However, the enhance-

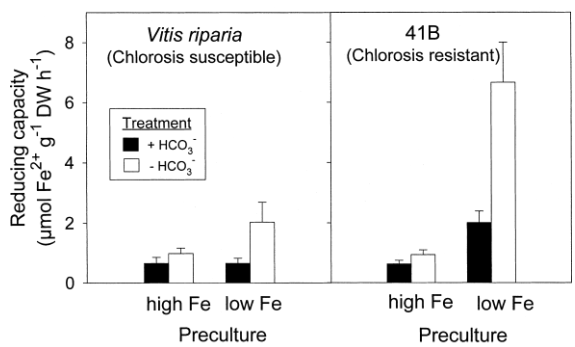

Figure: Effect of bicarbonate and high Fe (1 x 10^{-4} M) or low Fe (2 x 10^{-7} M FeEDDHA 3-week preculture) on the Fe^{III} reducing capacity of roots from rootstocks differing in their resistance to Fe deficiency chlorosis. Data represent mean ± SD (n=3).

Table 1

Chlorophyll content of leaves of two rootstock genotypes differing in their resistance to Fe deficiency chlorosis after three weeks growth in high (10^{-4} M) and low (2 x 10^{-7} M) FeEDDHA nutrient solutions. Data represent mean ± SD (n = 3)

	Chlorophyll (SPAD)	
Genotype	High Fe	Low Fe
Vitis riparia (Chlorosis susceptible)	27.7 ± 0.7	12.2 ± 0.4
41 B (Chlorosis resistant)	27.6 ± 1.3	16.2 ± 2.5

Table 2

Effect of bicarbonate and preculture (3 weeks) in high Fe (10^{-4} M FeEDDHA) and low Fe (2×10^{-7} M FeEDDHA) on distribution of ^{59}Fe between roots and shoot of rootstocks differing in their resistance to Fe deficiency chlorosis. Data represent mean ± SD (n = 3)

Preculture	HCO_3^- treatment (10 mM)	Amount (nmol ^{59}Fe organ^{-1}) Roots	Amount (nmol ^{59}Fe organ^{-1}) Shoot	Concentration (nmol ^{59}Fe g^{-1} DW) Roots	Concentration (nmol ^{59}Fe g^{-1} DW) Shoot
Vitis riparia (Chlorosis susceptible)					
High Fe	- HCO_3^-	6.67 ± 1.95	0.28 ± 0.03	7.35 ± 2.39	0.13 ± 0.04
	+ HCO_3^-	5.76 ± 1.92	0.08 ± 0.03	6.54 ± 1.73	0.07 ± 0.02
Low Fe	- HCO_3^-	10.28 ± 2.97	1.03 ± 0.06	12.54 ± 1.20	0.36 ± 0.14
	+ HCO_3^-	5.12 ± 2.49	0.13 ± 0.04	6.18 ± 2.24	0.08 ± 0.01
41 B (Chlorosis resistant)					
High Fe	- HCO_3^-	4.75 ± 1.00	0.22 ± 0.06	11.06 ± 3.28	0.28 ± 0.17
	+ HCO_3^-	4.06 ± 0.50	0.09 ± 0.03	8.17 ± 2.54	0.06 ± 0.03
Low Fe	- HCO_3^-	12.18 ± 2.58	1.96 ± 0.48	22.47 ± 2.38	0.72 ± 0.13
	+ HCO_3^-	6.64 ± 2.82	0.63 ± 0.31	12.63 ± 3.08	0.45 ± 0.20

ment of the uptake and translocation rate of ^{59}Fe was lower in the susceptible genotype, resulting in lower amounts and concentrations of ^{59}Fe in different plant organs (Tabs. 2 and 3). In agreement with the effect of bicarbonate on the Fe^{III} reducing capacity, the enhanced uptake rate of ^{59}Fe is also inhibited by bicarbonate in Fe deficient rootstocks.

Tab. 3 clearly shows that extraplasmatic Fe was significantly decreased in the roots of both genotypes in the presence of bicarbonate in ^{59}Fe uptake solution with respect to the control (without bicarbonate supply). However, bicarbonate inhibited ^{59}Fe uptake less than Fe^{III} reduction, particularly in the Fe susceptible genotype (Figure, Tab. 3). The presence of bicarbonate in the solution affected the rate of uptake less in the resistant 41 B (45 %) than in *V riparia* (58 %). Bicarbonate inhibited ^{59}Fe uptake by roots in both genotypes less than ^{59}Fe translocation from roots to shoots (Tabs. 2 and 3). Thus, bicarbonate reduced the translocation rate to more than 19 and 35 % of the control for susceptible and resistant genotype, respectively (Tabs. 2 and 3). However, the effect of bicarbonate was rather significant if based on the calculated amount or concentration of ^{59}Fe in the leaves (Tab. 2).

Discussion

The results presented confirm that there is a close correlation between the resistance to Fe deficiency chlorosis and the capacity for Fe^{III} reduction and ^{59}Fe uptake by roots

Table 3

Effect of bicarbonate and Fe preculture (high Fe = 10^{-4} M FeEDDHA, low Fe = 2×10^{-7} M FeEDDHA for 3 weeks) on extraplasmatic ^{59}Fe in roots, uptake and translocation of ^{59}Fe in grapevine rootstocks differing in their resistance to Fe deficiency chlorosis. Data represent mean ± SD (n = 3)

Preculture	HCO_3^- treatment (10 mM)	Extraplasmatic Fe (nmol ^{59}Fe g^{-1} root DW)	Fe uptake rate (nmol ^{59}Fe g^{-1} root DW 12 h^{-1})	Fe translocation rate (nmol ^{59}Fe g^{-1} root DW 12 h^{-1})
Vitis riparia (Chlorosis susceptible)				
High Fe	- HCO_3^-	360.3 ± 61.2	6.1 ± 1.2	0.36 ± 0.12
	+ HCO_3^-	131.2 ± 25.0	5.3 ± 1.7	0.46 ± 0.17
Low Fe	- HCO_3^-	225.9 ± 38.2	10.7 ± 3.5	1.87 ± 0.36
	+ HCO_3^-	66.1 ± 20.4	4.7 ± 1.8	0.42 ± 0.18
41 B (Chlorosis resistant)				
High Fe	- HCO_3^-	627.6 ± 90.9	10.7 ± 2.1	1.38 ± 0.34
	+ HCO_3^-	150.9 ± 40.2	7.3 ± 1.5	0.68 ± 0.09
Low Fe	- HCO_3^-	323.3 ± 61.9	27.9 ± 5.1	3.98 ± 0.64
	+ HCO_3^-	58.8 ± 2.07	15.2 ± 4.1	1.98 ± 0.42

of grapevine rootstocks (*V riparia*, 41 B) when grown under low Fe supply (see also BAVARESCO *et al*. 1991; BRANCADORO *et al*. 1995). Thus, these two parameters may be used in breeding programs to screen new rootstocks for resistance to lime-induced chlorosis (BAVARESCO *et al*. 1991).

The induction of Fe^{III} reduction activity of roots of peach rootstock was much stronger when plants had been precultured with low Fe supply than in Fe-free nutrient solution (GOGORCENA *et al*. 2000). The Fe deficiency-enhanced capacity for Fe^{III} reduction of roots of both grapevine rootstock genotypes at low Fe supply was strongly inhibited by bicarbonate supply (Figure). A similar pattern has also been observed in other plant species such as sunflower, soybean and cucumber (RÖMHELD *et al*. 1982; DOFING *et al*. 1989; ROMERA *et al*. 1992). While RÖMHELD *et al* (1982) and TOULON *et al*. (1992) found no differences in the inhibition of Fe^{III} reducing capacity caused by high pH (8.0) with either bicarbonate or buffered with Hepes or Hepes-Tris buffers, ROMERA *et al*. (1992) observed a higher inhibition by bicarbonate than by the treatment with the same pH buffer such as Hepes. Fe chlorosis in calcareous soils, however, may be caused to some extend by the high pH value of these soils and, more important, by the presence of a bicarbonate buffering system, which prevents root apoplast acidification due to the consumption of H^+ extruded by the proton ATPase (TOULON *et al*. 1992). The chlorosis resistant grapevine rootstocks showed a higher root acidification capacity in contrast to the susceptible *V riparia*, which was not able to acidify external medium (BRANCADORO *et al* 1995). The finding that in the resistant rootstocks Fe deficiency induced a decrease of the pH value of the nutrient solution for 2 units, may explain the lower inhibitory effect of bicarbonate shown on Fe^{III} reduction capacity and Fe uptake rate in the resistant genotype 41 B (Figure, Tab. 3).

The results shown in the Figure clearly indicate that bicarbonate inhibits Fe^{III} reduction in roots which in turn can result in higher precipitation of Fe^{III} as hydroxides or phosphates in the root apoplast (BIENFAIT *et al*. 1985). 0n the other hand no increase in extraplasmatic ^{59}Fe in roots was found after addition of bicarbonate to the solution as proposed by MENGEL (1994). Rather, the concentration of extraplasmatic ^{59}Fe was significantly lower in bicarbonate supplied to the roots as compared to the controls without bicarbonate (Tab. 3). Compared to our results in earlier studies with other plant species (see RUTLAND and BUKOVAC 1971; FLEMING *et al*. 1984) a much lower relative translocation rate of Fe to the shoots has been found. This can be explained by the removal of extraplasmatic Fe in the present study. In contrast to our results, MAGGIONI (1980) and VARANINI and MAGGIONI (1982) concluded that the rate of Fe absorbed by excised roots of grapevine rootstocks increased with increasing pH of the nutrient medium with some gradual differences between various genotypes. However, in these studies the higher Fe absorption rate from labelled $^{59}FeEDTA$ at a high pH value could be the consequence of the lower chelate stability of the used chelate above pH 6.5 and the precipitation of extraplasmatic Fe on the root surface (STRASSER *et al*. 1999; NIKOLIC and KASTORI 2000). 0bviously, the removal of extracellular and apoplastic Fe after the uptake experiment with ice-cold, non-active uptake solution was far from being complete, which agrees with unpublished results of 0. STRASSER (pers. comm.). FLEMING *et al*. (1984) found that bicarbonate (10 mM) decreased the ^{59}Fe translocation from roots to shoots by 69 and 83 % for a resistant and a susceptible soybean cultivar, respectively, which was in agreement with the results shown in Tab. 3.

In another study, a high bicarbonate concentration (28 mM) did not show any effect on long-distance transport of labelled ^{55}Fe in grapevines, but it did affect the distribution of Fe within a leaf with strong accumulation of Fe along the leaf veins (MENGEL and BÜBL 1983). In this experiment, however, plants showed severe growth inhibition due to high $C0_2$ supply to the root compartment with calcareous soil during preculture and further ^{55}Fe was not applied to the roots, but injected by very thin needles into the petiole of each leaf at different leaf positions. Thus, the data of this experiment are not directly comparable with the data presented here.

Besides the inhibitory effect of bicarbonate on Fe^{III} reduction and Fe uptake (Figure, Tab. 3; see also RÖMHELD *et al*. 1982; ROMERA *et al*. 1992), it has been proposed that high bicarbonate might also increase the pH value in the leaf apoplast, resulting in a decrease of Fe availability in the leaf tissue (MENGEL 1994). However, bicarbonate supplied to root up to 5-20 mM substantially affected neither the pH value of the xylem exudate and leaf apoplastic fluid nor the Fe^{III} reducing capacity of leaf tissue of faba bean or grapevine rootstocks (NIKOLIC and RÖMHELD 1999). These findings agree with our preliminary results on the pH of apoplastic leaf fluid which did not differ significantly between green and chlorotic leaves of grapevines grown in a calcareous soil under field conditions (NIKOLIC, unpubl. data).

The results presented in this study clearly indicate that the bicarbonate-induced Fe chlorosis of grapevine rootstocks is caused by an inhibition of Fe uptake by roots and translocation to leaves due to the buffering of released protons and an inhibition of the Fe^{III} reduction by root cells. In addition, the capacity for Fe acquisition, significantly expressed by the rate of Fe reduction and uptake is more expressed in the chlorosis-resistant rootstock. Furthermore, the bicarbonate-induced inhibition of Fe acquisition is lower in the chlorosis-resistant than in the -susceptible genotype.

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