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## Effects of Bicarbonate Induced Iron Chlorosis on Selected Nutrient Contents and Nutrient Ratios of Shoots and Roots of Different Maize Varieties

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**Abstract:** The effects of  $\text{HCO}_3^-$  on chlorosis, some macro and micro nutrient uptake of different maize cultivars and some nutrient element ratios were examined. For this purpose a greenhouse experiment was conducted with thirteen different maize varieties and grown on five different nutrient solutions containing 20  $\mu\text{M}$  Fe-EDTA and  $\text{HCO}_3^-$  levels (7.5; 15 and 30 mM as  $\text{NaHCO}_3$ ). According to the  $\text{HCO}_3^-$  applications, all maize varieties gave response to iron chlorosis. Iron chlorosis was slightly developed in plants grown with S3 (7.5 mM  $\text{NaHCO}_3$ ), but it became severe in S4 (15 mM  $\text{NaHCO}_3$ ), especially S5 (30 mM  $\text{NaHCO}_3$ ), and control plots of all maize varieties. Application of 20  $\mu\text{M}$  Fe (Fe-EDTA) increased both shoots and roots dry weights of maize varieties in general. Sodium amounts of the plants both in shoots and roots increased with the increasing amounts of  $\text{NaHCO}_3$ . Potassium, phosphorous, calcium and magnesium contents on shoots and roots, decreased by the application of both Fe-EDTA and  $\text{NaHCO}_3$ . Zinc (Zn) contents were found to be highest in shoots and roots of maize grown with S2 (20  $\mu\text{M}$  Fe), but decreased with the increasing  $\text{HCO}_3^-$  amounts. Due to iron and increasing amounts of  $\text{HCO}_3^-$ , Manganese (Mn) contents of varieties decreased and found under sufficiency ranges. P/Fe and K/Ca ratios on shoots and on roots were found higher in chlorotic plants especially grown on control and  $\text{HCO}_3^-$  applied plots. In contrast, Fe/Mn ratio was found higher in 20  $\mu\text{M}$  Fe-EDTA than chlorotic plants.

**Key words:** Maize, bicarbonate, iron chlorosis, nutrient elements, nutrient ratios

### INTRODUCTION

Bicarbonate induced iron chlorosis is a widespread nutritional problem affects leaf and flower mineral composition and responsible for significant decreases in yield, crop size and the quality of many species. High  $\text{HCO}_3^-$  ion concentration makes Fe insoluble or limits Fe uptake, possibly affecting translocation of this element within the plant itself (Cinelli, 1995; Coulombe *et al.*, 1984) and affects the mineral composition and nutrient ratios (Cinelli, 1995; Coulombe *et al.*, 1984; Alcantara *et al.*, 2000; Römheld and Marschner, 1981).

Bicarbonate is considered determinant factor causing Fe chlorosis in calcareous soils (Alcantara *et al.*, 2000; Shi *et al.*, 1993; Romera *et al.*, 1991) and in solution culture (Shi *et al.*, 1993; Tisdale *et al.*, 1975). Nutrient solutions containing bicarbonate have been used in screening different species for tolerance to Fe chlorosis (Coulombe *et al.*, 1984; Alcantara *et al.*, 2000; Romera *et al.*, 1991; Chaney *et al.*, 1992). It has been shown that both bicarbonate and Fe concentration in the nutrient solution affect the degree of chlorosis

developed by a given cultivar (Alcantara *et al.*, 2000; Chaney *et al.*, 1992).

It is known that iron is indispensable for the biosynthesis of chlorophyll, but its total content in the plant was not associated with the occurrence of chlorosis (Köseoğlu and Açıkgöz, 1995) therefore, total iron concentrations of plants are not a valid criterion in evaluation of iron status of plants (Sönmez and Kaplan, 2004). Iron, which is the ferrous-iron ( $\text{Fe}^{+2}$ ) form termed active Fe and extracted with weak acids and some chelating agents, has been closely related to Fe chlorosis (Köseoğlu and Açıkgöz, 1995).

In recent studies, P/Fe and K/Ca ratios were correlated to the degree of chlorosis in peach grown in calcareous soil (Abadia *et al.*, 1985), but not when peach was grown in high pH nutrient solution (Rao and Rains, 1976). In another study, P/Fe ratio was thought reliable criteria for assessing Fe chlorosis in peach trees (Köseoğlu, 1995). Fe: Mn ratio in soybean without Fe application to soil is a reliable index for prediction of soybean response to Fe fertilization and tolerance to Fe deficiency provided both Fe and Mn concentration are in nutrient sufficiency range (Ghasemi *et al.*, 2003).

The objective of this greenhouse study was to examine the effect of  $\text{HCO}_3^-$  on chlorosis, some macro and micro nutrient uptake and some nutrient ratios of different maize cultivars as prediction criteria for iron chlorosis.

**MATERIALS AND METHODS**

Thirteen different hybrid maize varieties were grown in pots, which contain perlite and watered by five different nutrient solutions due to reservoir technique. Maize varieties and the nutrient solutions were shown in Table 1 and 2. To build up reservoir at the bottom of the pots, plastic bags in the pots were perforated at 4 cm high from the bottom of the pots and filled up with two liters of perlite. Six maize seeds were planted per pot with three replicates and the seedlings were thinned to four after the emergence. The composition of the basic half strength nutrient solution, modified from Römheld and Marschner (1981), was (in mM):  $\text{Ca}(\text{NO}_3)_2$ , 2;  $\text{K}_2\text{SO}_4$ , 0.75;  $\text{MgSO}_4$ , 0.65;  $\text{KH}_2\text{PO}_4$ , 0.5 and (in  $\mu\text{M}$ ):  $\text{KCl}$ , 25;  $\text{H}_3\text{BO}_3$ , 10;  $\text{MnSO}_4$ , 1;  $\text{CuSO}_4$ , 0.5;  $\text{ZnSO}_4$ , 0.5;  $(\text{NH}_4)_2\text{Mo}_7\text{O}_{24}$ , 0.05. Sodium bicarbonate ( $\text{NaHCO}_3$ ) was added to the basic nutrient solution to obtain 7.5; 15 and 30 mM bicarbonate levels. 10  $\mu\text{M}$  FeEDTA was used for iron supply with and without bicarbonate levels and a control solution used that includes neither iron nor bicarbonate. pH and some nutrient element concentrations of the solutions were analyzed and shown in Table 3. pH of the second nutrient solution was adjusted to 4.5 with  $\text{HNO}_3$  and two weeks later the concentration of iron was increased to 20  $\mu\text{M}$ .

Table 1: The hybrid maize varieties used in the experiment

No.	Variety	No.	Variety
V1	MF 714 Granada	V8	Progen 1550
V2	IsidoroMF	V9	Darva
V3	Tex MF	V10	H 2547
V4	Kendo MF	V11	Konsur
V5	Sancia	V12	Bursa beyazi
V6	C 955	V13	Otello
V7	Premier		

Table 2: Nutrient solutions used in the experiment

Remarks	Solution contents
S1	Basic nutrient solution Fe
S2	Basic nutrient solution + 20 $\mu\text{M}$ Fe
S3	Basic nutrient solution + 20 $\mu\text{M}$ Fe + 7.5 mM $\text{NaHCO}_3$
S4	Basic nutrient solution + 20 $\mu\text{M}$ Fe + 15 mM $\text{NaHCO}_3$
S5	Basic nutrient solution + 20 $\mu\text{M}$ Fe + 30 mM $\text{NaHCO}_3$

Table 3: pH and some nutrient element concentrations of the solutions

No.	pH	EC $\mu\text{S cm}^{-1}$	Na $\text{mg kg}^{-1}$	K $\text{mg kg}^{-1}$	Ca $\text{mg kg}^{-1}$	Mg $\text{mg kg}^{-1}$	Fe $\text{mg kg}^{-1}$
S1	6.86	966	6.7	80.8	111.5	34.44	0.04
S2	6.85	970	6.8	83.5	110.0	34.03	0.54
S3	7.38	1463	171.7	88.3	107.6	32.80	0.53
S4	7.65	1920	274.6	89.6	105.1	32.39	0.49
S5	7.88	2840	648.6	97.8	104.8	31.98	0.48
Tap Water	7.43	311	6.2	1.2	32.2	20.2	0.01

Maize plants kept 48 days, which was long enough for the appearance of effects of the treatments. Aerial parts of the plants were harvested on day 48. For evaluation of nutrient uptake of the plants, plant materials were washed with deionized water with 0.1 N HCl, rinsed with tap water and finally twice with deionized water, dried in a forced air oven at 70°C for 72 h; dry matter contents were determined and ground. The ground plant samples were wet digested using a  $\text{HNO}_3$ - $\text{HClO}_4$  mixture at volume ratios of 4:1; Fe, Zn, Mn and Mg contents in digest were determined by atomic absorption spectrophotometry (Philips PU 9200x, Pye Unicam Ltd. GB); Na, K and Ca by flame emission (Eppendorf Elex 6361); P by vanadomolybdophosphoric method. Active Fe contents were determined in dry plant parts by atomic absorption spectrophotometry after extraction with 1 N HCl solution (1:10) waiting in 24 h.

A complete randomized design was used with three replications. The mean values were compared using LSD (Least Significant Differences) multiple range test and simple correlations were measured with computer program Tarist (1994).

**RESULTS AND DISCUSSION**

Iron chlorosis was shown in control plots of all maize varieties at the beginning of the experiment. Chlorosis was slightly developed in plants grown with S3 (7.5 mM  $\text{NaHCO}_3$ ) and became severe by the application of increasing amounts of  $\text{HCO}_3^-$  in all maize varieties. Shi *et al.* (1993) and Cinelli (1995) reported that the treatment less than 10 mM  $\text{NaHCO}_3$  did not induce severe chlorosis.

In general means, application of 20  $\mu\text{M}$  Fe (Fe-EDTA) increased both shoot and root dry weights, total Fe, active Fe contents of maize varieties (Table 4). Total and active Fe was found to be highest in shoots and roots of maize grown with S2 (20  $\mu\text{M}$  Fe) and decreased by the application of  $\text{HCO}_3^-$ . Shi *et al.* (1993) were determined that the total amount of Fe taken up by the plants decreased as the  $\text{HCO}_3^-$  level was increased. Bicarbonate (> 5 mM) has been reported to inhibit Fe uptake in many crops (Coulombe, 1984; Boxma, 1972; Fleming *et al.*, 1984) and low  $\text{HCO}_3^-$  levels (< 5 mM) did not inhibit iron translocation. However, higher levels (> 10 mM) did (Coulombe, 1984; Boxma, 1972; Fleming *et al.*, 1984; Römheld and Marschner, 1986). Vizzotto *et al.* (1997) were found similar results in roots and shoots of Kiwi (*Actinidia deliciosa* cv. Hayward) by the application of 10  $\mu\text{M}$  Fe-EDTA. Although the other applications (S3-S5) include 20  $\mu\text{M}$  Fe, the amounts on shoots tend to decrease by the effect of  $\text{HCO}_3^-$  but in roots an accumulation was occurred. Cinelli (1995) found similar results and suggested that greater accumulation of the element occurs in the root system.

Table 4: Some macro, micro nutrient contents and ratios on shoots and roots of various maize varieties grown in different nutrient solutions

	Shoots					Roots				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Dry weight	5.19c	13.96ab	14.92a	13.61b	5.81c	2.71c	7.44b	8.32A	7.34b	3.05c
Total Fe (mg kg <sup>-1</sup> )	26.15b	29.39a	22.62c	16.10e	19.13d	109.59b	156.90a	94.18Bc	65.46cd	48.77d
Active Fe (mg kg <sup>-1</sup> )	11.95c	16.95a	13.73b	13.45b	11.60c	12.29c	17.12a	13.80B	14.73b	16.75a
Zn (mg kg <sup>-1</sup> )	30.54b	36.21a	21.97c	15.23d	13.85d	26.56b	41.13a	17.26C	27.03b	18.41c
Mn (mg kg <sup>-1</sup> )	13.49a	4.36bc	5.33b	3.72bc	3.26c	6.03a	4.56ab	4.46ab	3.13b	3.56b
Na (%)	0.04d	0.02d	0.28c	0.86b	1.74a	0.74d	0.58e	1.99c	2.39b	2.97a
K (%)	4.11a	2.01b	2.16b	2.09b	1.97b	1.34a	0.91b	0.63c	0.62c	0.65c
P (%)	0.62a	0.33b	0.19c	0.15c	0.15c	0.26a	0.18b	0.12c	0.11c	0.10c
Ca (%)	0.96a	0.72b	0.38c	0.23d	0.14e	1.05b	1.23a	0.55c	0.31d	0.16e
Mg (%)	0.76a	0.66b	0.44c	0.36d	0.32d	0.77a	0.47b	0.28c	0.28c	0.24c
P/Fe	239.45a	114.02b	89.83c	98.27bc	81.06c	28.61a	16.96b	21.10b	20.41b	22.00b
K/Ca	4.30d	2.83e	5.84c	9.62b	14.77a	1.41c	0.82d	1.33c	2.15b	4.32a
Fe/Mn	2.23c	8.03a	4.90b	4.87b	6.89a	20.26bc	37.30a	25.86b	21.71b	15.77c

Means followed by the same letter are not significantly different. Values are the means of varieties

The second high amounts of total Fe were taken from the control solution (S1) and also some varieties include higher amounts of total Fe in S1 than S2-S5. In agreement with previous observations (Jacobson and Oerti, 1956; Katyal and Sharma, 1980; Rashid *et al.*, 1990), it was confirmed that the total Fe does not always represent a suitable tool for detection of incipient leaf chlorosis and cannot be used to screen Fe efficiency.

Sodium amounts of the plants both in shoots and roots increased with the increasing amounts of NaHCO<sub>3</sub> (S3-S5) (Table 4). Concentration in roots found higher than shoots.

In general; potassium, phosphorus, calcium and magnesium contents on both shoots and roots of maize varieties (accept root Ca) grown on S1 (control plots) had the highest amounts resulted slow growth due to the severe chlorosis (Table 4). Dong (1987) and Özgümüş (1988) also pointed out that P, K and N contents of chlorotic leaves were found significantly higher than green leaves, respectively. Mengel *et al.* (1984) reported that this high P content result from the particular conditions prevailing in plants with Fe chlorosis and the high P content in chlorotic leaves is the result not the cause for Fe chlorosis.

Potassium contents on shoots and roots, decreased by the application of both Fe-EDTA and NaHCO<sub>3</sub>. Taban *et al.* (1999) found similar reduction by the application of NaCl in shoots and roots of maize in accordance with the antagonism between Na and K agreeing with the results of other researchers (Taban *et al.*, 1999; Siegel *et al.*, 1980; Cusido *et al.*, 1987; Cordovilla *et al.*, 1995; Güneş *et al.*, 1996).

Phosphorus contents also tend to decrease by the application of Fe-EDTA and NaHCO<sub>3</sub>. The decrease in P uptake was the result of the high pH due to the increasing HCO<sub>3</sub><sup>-</sup> of the nutrient solution and in agreement with the result taken before (Rasid *et al.*, 1990). The concentration of P was also reported to decrease in response to high HCO<sub>3</sub><sup>-</sup> levels in soybean (Coulombe, 1984).

Application of Fe-EDTA and NaHCO<sub>3</sub> affected the calcium and magnesium contents in shoot and roots of maize varieties. Decreasing Ca concentration with increasing HCO<sub>3</sub><sup>-</sup> level was reported in bean and soybean grown in solution culture (Coulombe, 1984; Tisdale *et al.*, 1975) and citrus plant grown in sand culture (Rutland and Bukovac, 1971).

The highest zinc contents of both shoots and the roots were found in the varieties grown with S2 and were found to be in sufficiency ranges (Jones and Mills, 1991) (Table 4). The second high amounts were taken from control plots and the zinc contents tend to decrease by adding increasing amounts of HCO<sub>3</sub><sup>-</sup>. The decrease in Zn uptake was the result of the high pH due to the increasing HCO<sub>3</sub><sup>-</sup> amounts of the nutrient solution and in agreement with the result taken before (Haynes and Swift, 1985).

Manganese contents on shoots and roots of the maize varieties were found to be high in control plots (S1). Due to iron and increasing amounts of HCO<sub>3</sub><sup>-</sup>, manganese contents of varieties decreased in the other solutions and found under sufficiency ranges (Jones and Mills, 1991). The results confirm the findings of other workers (Moraghan, 1985; Mordvet *et al.*, 1991; Moraghan, 1992). Mortvedt *et al.* (1991) reported that the interaction between Fe and Mn might be due to the reduction of Mn concentration by dilution effect, reduction in shoot: root ratio and reduced Mn uptake or toxic concentration of Fe in plants. Roomizadeh and Karimian. (1996) believed that Fe might interfere with Mn absorption and/or translocation of Mn root to shoot.

The plants on control plots grown with S1 had the highest P/Fe ratios on shoots and on roots. By the application of 20 µM Fe-EDTA the ratio started to decrease and the degradation continued by HCO<sub>3</sub><sup>-</sup> addition as a consequence of high pH. Roots grown on S2 (20 µM Fe-EDTA) gave the lowest P/Fe ratio (Table 4). Ji *et al.* (1985) also obtained similar results for the P/Fe ratio in apple trees.

K/Ca ratios on shoots and roots of maize plant grown on S1 (control plots) and  $\text{HCO}_3^-$  applied solutions S3-S5 found higher than S2 (20  $\mu\text{M}$  Fe-EDTA) (Table 4). The highest amounts were taken from S5 on both shoots and roots. Abadia *et al.* (1985) reported that P/Fe and K/Ca ratios of peach leaves were significantly affected by Fe chlorosis and both ratios were higher in the chlorotic than in the green leaves. Heras *et al.* (1976) and Dong. (1987) reported similar findings in chlorotic peach leaves.

Fe/Mn ratios were found highest on S2 (20  $\mu\text{M}$  Fe-EDTA) solution (Table 4). In control and  $\text{HCO}_3^-$  applied plots had less Fe/Mn ratio than S2 due to the lack of Fe in control plots (S1) and reduced Fe uptake in presence of  $\text{HCO}_3^-$  in other solutions (S3-S5). This situation is directly related with the antagonistic effects of Fe and Mn. Absence of one stimulated the uptake of the other. Ghasemi-Fasaei *et al.* (2003) found similar results on soybean by adding increasing amounts of Fe. Addition of Fe increased concentration and uptake of Fe but decreased Mn. Fe: Mn ratio were found high as a consequence of this and considered tolerant to Fe induced chlorosis.

### CONCLUSIONS

As a consequence total and active Fe, Zn, Mn, P, Ca and Mg contents of maize varieties were negatively affected by  $\text{HCO}_3^-$  applications. P/Fe and K/Ca ratios on shoots and on roots were found higher in chlorotic plants. In contrast, Fe/Mn ratio was found higher in green ones and these parameters can be used for evaluating the Fe nutrition status of the plants.

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