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Effect of Increasing Concentration of Bicarbonate on Plant Growth and Nutrient Uptake by Maize Plants

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Abstract: Increasing concentration of HCO_3^- ions in the nutrient medium progressively decreased the growth of maize plants as measured by decrease in shoots and root weight, these effects becoming more distinct with time, particularly at the two higher levels of bicarbonate supply (10 and 20 mM). Various parameter of growth including plant height, root and shoot measurements, nutrient composition of shoots and roots and nutrient uptake were all affected in this manner as was also the appearance of iron chlorosis. In the cation-anion balance data of the roots and shoots, mineral cation totals exceeded anion totals for each HCO_3^- treatment and in the same pattern was observed in the roots with high concentration levels, but it was more pronounced in the excess of Ca^{2+} in the roots. These high Ca^{2+} values suggest that the Ca^{2+} may not have been taken up but rather accumulated on the root surface. Therefore, the specific effect on roots and shoots growth as compared with control may be due to inhibited respiration caused by high levels of bicarbonate treatments.

Key words: Bicarbonate nutrition · Cation-anion balance · Shoots: roots ratio

INTRODUCTION

It has long been known that presence of an excessive amount of bicarbonate adversely affects the absorption of ions and causes chlorosis in plants [1-3]. Following these earlier findings [4, 5] have concluded that high concentrations of bicarbonate ion in soils and irrigation water seem to inhibit the metabolic process of plant which ultimately affect plant growth and the uptake of nutrients. Within the roots bicarbonate promotes dark fixation of CO_2 which is of consequence in relation to mineral nutrition since the primary products of dark fixation in the roots are malate and other organic acids [6, 7]. However, the mode of action of bicarbonate is not yet totally understood.

The purpose of this experiment was to investigate the effects of increasing concentration of bicarbonate in the nutrient medium on the growth of maize as measured at a single harvest and to determine the uptake and distribution of the main mineral nutrients. In this work cation and anion uptakes and distributions within maize plants are reported in relation to the HCO_3^- supply in the nutrient medium.

MATERIALS AND METHODS

Plant Growth and Harvesting: Seeds of maize (*Zea mays* L.) plants were germinated for 6 days in a moist compost / Perlite mixture and after germination similar sized seedlings were selected and transferred to aerated nutrient solutions held in plastic containers of 10 litre volume. Each of these containers held 5 plants that were maintained upright by a foam rubber collar wrapped round the plant stem and inserted into holes in a polystyrene sheet, placed over the containers. To prevent the possible occurrence of any anoxia compressed air was bubbled vigorously into the solution. For the first 2 days after transfer a half strength nutrient solution was used (to prevent the plants suffering from osmotic stress). This was then replaced by a full strength solution for a

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further 2 days immediately prior to the beginning of the experiment. The composition of the nutrient solution was as follows: (mM) $Ca(NO_3)_2$, 2; K_2SO_4, 0.75; $MgSO_4$, 0.65; KH_2PO_4, 0.5; (µM) Fe-EDDHA, 250; H_3BO_3, 10; $MnSO_4$, 1.0; $ZnSO_4$, 0.5; $CuSO_4$, 0.5; $Na_2MoO_4.H_2O$, 0.05. The plants were then split into four treatments each with 4 replicates and grown for a further 16 days in a growth room. All plants were supplied with the complete nutrient solution as described above but with different concentrations of $NaHCO_3$ (mM); (0, 5, 10 and 20). The sodium concentrations was maintained the same in all treatments (20 mM) by appropriate addition of Na_2SO_4 solution (0.5 M). The pH of the nutrient solution of the control plants was 7. The pH of the nutrient solution in all bicarbonate treatments (5,10 and 20 mM) was adjusted daily to 8 with a few drops of 0.1M H_2SO_4 or NaOH as required. Nutrient solution were changed every two days.

The conditions in the growth room were as follow; a temperature maintained at 25°C day (maximum) and 18°C night (minimum), photo period of 16 hour light and 8 hour dark, at a photon flux density of 250 µmol $m^{-2}.s^{-1}$; and a relative humidity of 70-80%.

Analytical Methods: At harvest plant heights were measured in (cm). Plants were carefully separated into roots and shoots and fresh and oven dried (24 hours at 75°C) weights determined. The number of roots and root length of individual plants from fresh roots were measured by the modified line-intercept method of Tennant [8].

Total K, Ca^{2+}, Mg^{2+}, Na^+, S, P were determined on dried plant material using the method outlined in MAFF [9] and analysed by inductively-coupled plasma-spectrometry (ICP) after ashing at 500°C. For the determination of NO_3^-, hot water extracts of dried and ground 0.5 g subsamples were analysed using the method of Cataldo *et al*. [10].

RESULTS

Growth Observations: The effect of HCO_3^- on maize growth was dependent upon the concentration of HCO_3^- supply. At harvest, plant heights and shoot and root weights were severely decreased by increasing HCO_3^- ion concentration in the growth medium as compared with the control (Table 1). In case of 20 mM HCO_3^- treatment, total dry weights of shoots and roots were reduced by 37% and 45% respectively as compared with control plants. This reduction in growth was also seen in plants grown at 10 mM HCO_3^- where 27% and 36% reductions in dry weight of shoot and roots respectively were obtained as compared with the control (Table 1). In general shoot dry weight was less affected than roots, so that the effect of increasing HCO_3^- concentration was reflected in a slight decrease in root: shoot ratio.

Bicarbonate supply in the nutrient medium, regardless of concentration (5-20 mM) resulted in shortened thickened lateral roots with swollen tips, the effect being more pronounced of the highest concentration and increasing with duration of growth. These morphological effects are clearly evident from the data which show a symptoms of iron deficiency in young leaves and a decrease in the number of root tips and in root length as the HCO_3^- concentration increased (Table 1).

Mineral Nutrient Composition: In the shoots total cation (K^+, Na^+, Ca^{2+}, Mg^{2+}) and total anion (NO_3^-, $H_2PO_4^-$ and SO_4^{2-}) concentrations were relatively little affected by HCO_3^- level in the nutrient medium and reasonably close cation-anion balances were obtained (Table 2). Of the individual ions, K^+ concentration was slightly depressed and the NO_3^- concentration in the 20 mM HCO_3^- was 50% lower than the control. Sulphur and phosphorus were also depressed at the highest concentration of HCO_3^- treatments (Table 2).

In the roots the major impact of HCO_3^- in the nutrient medium indicates that balance of total cation and anion concentrations were not so close as for the shoots and in each treatment excess cation charge was recorded. The higher total charge values in response to HCO_3^- occurred despite a marked fall in K^+ concentration from 3588 to 2535 ppm and a fall in NO_3^- concentration from 1984 to 1178 ppm (Table 3). The higher charge values with increasing HCO_3^- concentration were the result of a more than four fold increase in Ca^{2+} concentration (from 400 to 1780 ppm) as shown in the experiment (Table 3).

For K^+, S^{2-} and P^- the effect of HCO_3^- treatment was to decrease these concentrations in both shoots and roots (Tables 2-5). For Ca^{2+} this was only true for the shoot but in contrast an enormous increase occurred in the roots uptake (Tables 3 and 5). In comparison with the enormous effect of HCO_3^- in increasing the Ca^{2+} uptake of the roots, Na^+ and Mg^{2+} uptake was little affected (Table 5). Because of this great difference in behaviour between Ca^{2+} and other mineral nutrients it may be questioned whether Ca^{2+} uptake has actually

Table 1: Effect of increasing bicarbonate supply on various measurements on shoots and roots of young maize plants 16 days after application of the treatments (26 DAS).

	HCO_3^- (mM)			
Plant measurements	0	5	10	20
Plant height (cm plant^{-1})	56 a	53 a	42 b	38 bc
Shoot dry wt (g plant^{-1})	0.51 a	0.46 b	0.37 c	0.32 d
Root dry wt (g plant^{-1})	0.11 a	0.09 a	0.07 b	0.06 bc
Root / shoot dry wt ratio	0.22a	0.20 a	0.19 a	0.18 a
Root tips (number plant^{-1})	16 a	12 b	9 bc	7 c
Symptoms of iron deficiency	*	*	**	***

Results in columns (Table 1) are means of 4 replicates. Means in the same column with different letters are significantly different ($p < 0.05$). (*): green leaves; (**) : leaves had slightly yellow; (***) : leaves showed distinct yellowing over most of the leaf.

Table 2: Effect of increasing bicarbonate supply on the cation-anion balances in shoots of young maize plants 16 days after application of the treatments (26 DAS).

HCO_3^-	Shoots-Cation Concenration (ppm)				Shoots-Anion Concentration (ppm)		
mM	K^+	Na^+	Ca^{2+}	Mg^{2+}	NO_3^-	S (1)	P (1)
0	2886 a	32.2 b	240 a	120 d	1426 a	416 a	395 a
5	2691 b	34.5 b	260 a	144 b	1116 b	384 a	301 b
10	2496 c	46.0 a	200 b	156 a	806 c	320 b	341 b
20	2262 d	50.6 a	160 c	132 c	620 c	288 b	434 a

Results in columns (Table 2) are means of 4 replicates. Means in the same column with different letters are significantly different ($p < 0.05$). (1) = Total element concentrations of S and P assigned charges of 2 and 1 respectively.

Table 3: Effect of increasing bicarbonate supply on the cation-anion balances in roots of young maize plants 16 days after application of the treatments (26 DAS).

HCO_3^-	Roots-Cation Concentration (ppm)				Roots-Anion Concentration (ppm)		
mM	K^+	Na^+	Ca^{2+}	Mg^{2+}	NO_3^-	S (1)	P (1)
0	3588 a	414 bc	400 d	156 b	1984 a	864 a	755 b
5	3081 b	368 c	860 c	168 b	1763 b	768 ab	961 a
10	2535 d	552 a	1780a	192 a	1240 c	672 b	620 c
20	2535 d	552 a	1780a	216 a	1178 c	640 b	558 c

Table 4: Effect of bicarbonate treatment as compared with the control without HCO_3^- on the uptake and distribution on the cation-anion balances in shoots of young maize plants 16 days after application of the treatments (26 DAS).

HCO_3^-	Shoots-Cation Uptake (ppm)				Shoots-Anion Uptake (ppm)		
mM	K^+	Na^+	Ca^{2+}	Mg^{2+}	NO_3^-	S (1)	P (1)
0	1471 a	16 a	122 a	61 a	727 a	212 a	201a
5	1237 b	15 a	119 a	66 a	513 b	176 ab	199 a
10	923 c	17 a	74 b	57 ab	298 c	118 bc	126 b
20	723 d	16 a	51 b	42 c	198 d	92 c	96 c

Table 5: Effect of bicarbonate treatment as compared with the control without HCO_3^- on the uptake and distribution on the cation-anion balances in roots of young maize plants 16 days after application of the treatments (26 DAS).

HCO_3^-	Roots-Cation Uptake (ppm)				Roots-Anion Uptake (ppm)		
mM	K^+	Na^+	Ca^{2+}	Mg^{2+}	NO_3^-	S (1)	P (1)
0	394 a	45 a	44 c	17 a	218 a	95 a	83 a
5	277 b	33 b	77 b	15 ab	159 b	69 b	86 a
10	191 c	34 b	89 b	13 b	87 c	47 c	43 b
20	152 d	31 b	107 a	12 b	71 c	38 c	33 b

Results in columns (Tables 3-5) are means of 4 replicates. Means in the same column with different letters are significantly different ($p < 0.05$). (1) = Total element concentrations of S and P assigned charges of 2 and 1 respectively.

occurred in the HCO_3^- treatment or whether Ca^{2+} was present in some form precipitated on the root surface (Table 5).

DISCUSSION

The presence of HCO_3^- in the nutrient medium retarded the growth of maize to a considerable extent and this detrimental effect was closely dependent on the HCO_3^- concentration in the nutrient medium, causing chlorosis and symptoms of iron deficiency (Table 1). Results indicate the effects becoming progressively greater as the concentration increased from 0 to 20 mM. It has been demonstrated previously by many authors that bicarbonate reduces the accumulation of Fe in the shoots of plants [11-13].

High pH per se can inhibit root and shoot growth of some sensitive plants [14]. In the present experiment, however, the pH of the nutrient solution of bicarbonate treatments and control (without bicarbonate) was kept always at 8.0 and 7.0 respectively. Thus, in most instances it is not the high pH per se but the high bicarbonate concentrations in particular in the rhizosphere which can be regarded as the major factor in calcareous soils for inhibiting root and shoot growth of sensitive plant species and cultivars [7].

The height of the plant and the measurements of root and shoot were particularly adversely affected by the two higher concentrations of 10 and 20 mM HCO_3^- supply (Table 1). This finding is also in accordance with various previous studies reporting effect of high levels of HCO_3^- in inhibiting growth and metabolic processes of plants [15-17]. As compared with the control treatment, root growth as measured by dry weight was more adversely affected than shoot growth so that the root : shoot dry weight ratio decreased slightly with increasing HCO_3^- supply up to the 20 mM level (Table 1).

The adverse effect of HCO_3^- on root growth as measured by dry weight (Table 1) is in accordance with earlier finding reported in our previous study [15] and confirms similar convincing evidence presented by [18]. In addition a dramatic effect of HCO_3^- ion in decreasing root length and the number of individual root tips per plant was also observed (Table 1). This depression may be due to inhibited respiration caused by the high levels of HCO_3^- supply in the nutrient medium [19]. Both root length and the number of root tips per plant play an important role in the acquisition and uptake of mineral nutrients particularly in plants growing in soil. Root tips are highly active in uptake particularly for some nutrients such as Ca^{2+} [20, 21] and root length is closely correlated to root surface area thus favouring nutrient acquisition. This is especially so for nutrients like P and K which are present in the soil solution in low concentration and relatively immobile in the soil. The amount of root produced by the plant under such conditions becomes important for total uptake of these nutrients. The extension of the depletion zone of K and P in soils is also more or less a reflection of root hair length [22, 23].

It is of interest that in the shoots and roots of all bicarbonate treatments, mineral cation totals exceeded anion totals (Tables 2 and 3). Anion totals were already overestimated as total S and total P values were reported rather than SO_4^{2-} and $H_2PO_4^-$ and it would therefore appear that chloride and ash alkalinity values which was not estimated can underestimate the contribution of organic acid anions (not estimated) to the cation-anion balance data. Even though not provided in the nutrient solution chloride may have been present as a contaminant; and taken up by the plants. Additionally in the high HCO_3^- treatments the very high Ca^{2+} concentrations in the roots (Tables 3 and 5) may have been associated with other anions not determined such as oxalate or carbonate.

In general the increasing concentrations of HCO_3^- in the nutrient medium lowered the uptake of all nutrients K, Mg^{2+}, S and P with the exception of Ca^{2+} as find in this experiment. This is in accordance with the depressing effect of HCO_3^- on the root pressure driven solute flux as measured by the rate of sap flow from the decapitated plants [11]. This effect of HCO_3^- in the nutrient medium in depression of root pressure has been previously observed [24]. The cause of this depression is not totally clear but may be dependent on the effect of HCO_3^- in decreasing root size relative to the shoot (Table 1) as well as a detrimental effect on root membranes. This damage probably is turn affects K^+ and NO_3^- uptake, the major ions responsible in depressing the osmotic potential of the sap.

From the results in Tables 2 and 3 little can be said with certainity about NO_3^- uptake since total N was not determined and NO_3^- tissue concentrations do not provide a reliable indicator of NO_3^- uptake since after uptake NO_3^- is rapidly assimilated. However, the lower NO_3^- concentration both in the roots and shoots of the high HCO_3^- treatments are consistent with the concept of depressed NO_3^- uptake and with experimental finding of ion competition for uptake [25].

Increasing HCO_3^- ion concentration had the most prominent influence on Ca^{2+} concentrations in the roots (Table 3). The high concentration of Ca^{2+} in the roots of the plants of the highest bicarbonate treatment of the total Ca^{2+} uptake may possibly indicate the precipitation of large amounts of Ca^{2+} as $CaCO_3$ on root surfaces. It has been suggested by many author that increasing Ca^{2+} levels in root tissues occurs in cell walls or on the root surface as a result of precipitation of Ca^{2+} as $CaCO_3$ or as Ca -oxalate [26]. The Ca^{2+} concentrations in the living parts of the cell, the symplasm are of course extremely low and in the order of 0.1-0.2 µM of free Ca^{2+} [27].

The concentration of Na in the nutrient medium in all treatments of the experiment was maintained at 20 mM, a much higher concentration than any of the major nutrients. Despite the high concentration in the root environment the uptake of Na was compared with the major nutrients was relatively low. This has been previously observed [28] who reported that maize belonged to a group of plant natrophobic species in which it was not possible to substitute Na for K to enhance growth.

The effect of increasing concentration of HCO_3^- in the nutrient medium was to depress K uptake to a much greater extent than Na uptake (Tables 4 and 5). This finding which has not been previously reported in the literature, supports the concept that a major detrimental effect of bicarbonate is to modify membrane function in the roots so that the normally highly selective uptake for K is greatly impaired whereas Na uptake is much less affected. The reason why bicarbonate should have this effect on the root cell membrane is not clear but is in accordance with the damaging role of HCO_3^- on root morphology as considered by many authors [29].

The uptake of K^+ and NO_3^- in the roots (Table 5) were markedly decreased by high bicarbonate concentrations, either due to a decrease in influx or an increase in efflux or to both of these events. Inhibition in root respiration should decrease the activity of the plasma membrane-bound H^+-ATPase and the uptake of cations and anions like K^+ and NO_3^- via the H^+-NO_3^- symport [30] and H^+-K^+ symport [31].

It might be suggested that impairment of the plasma membrane integrity after long-term exposure of roots to elevated bicarbonate concentrations and the capacity of apical root zones for synthesis of organic acids and their sequestration in the vacuoles is limited under conditions of simultaneously inhibited root respiration.

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