Quantitative Separation of the Bicarbonate from the Counter-cation Effect in Bean Plants

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ABSTRACT. The effect of Rb^+ and Na^+ as counter-cations of HCO_3^- was evaluated on bean (*Phaseolus vulgaris* L. cv. Poncho) plants using mixture experiment statistical methodology in a series of experiments set up in a controlled environment chamber. Mixture experiments using three components (Rb^+ , K^+ , and Na^+) or two components (K^+ and Na^+) were conducted to delineate the toxicity of HCO_3^- versus the counter-cation effect. The quantitative separation of the toxic effects was possible only when the individual stress had an additive effect when combined with the other stress. Potassium mixtures were used as reference for comparison with other mixtures because plants did not respond to K^+ , probably because it was included at a minimum concentration of 2.5 mM K^+ or because it was supplied in the preestablishing solution. Rubidium caused a decrease in shoot dry weight (SDW), but SDW accumulation was even lower when HCO_3^- was added to the Rb^+ solutions. However, Rb^+ was not included in follow-up experiments because the response of plants to Rb^+ was very similar to that of Na^+ . The toxic effect of Na^+ caused SDW to decrease at a rate of 3.7% per millimolar increase of Na^+ . However, the effect of HCO_3^- was dependent on its concentration, because at 2.5 mM HCO_3^- , the decrease in SDW was 12.7% per millimolar HCO_3^- , whereas at 3.75, 5, and 5.65 mM, the decrease was 11.0%, 7.8%, and 10.7% per millimolar HCO_3^- , respectively. At 7.5 mM HCO_3^- , the decrease in SDW was 4.2% to 8.2% per millimolar increase in HCO_3^- , respectively. The decreasing HCO_3^- rate may be explained by the nonadditive effect between HCO_3^- and Na^+ at high alkalinity levels.

Saline and sodic soils cover $\approx 10\%$ of arable land globally (Tanji, 1990). The predominant ions in these soils include: HCO_3^- , CO_3^{2-} , Na^+ , and K^+ (Shi and Wang, 2005). In addition to salinity, HCO_3^- and CO_3^{2-} are the major soluble alkalis that cause alkalinity in water. Alkalinity measures the concentration of soluble alkalis and the buffering capacity of water, which results in neutralization of H^+ and increased pH. Irrigation with water of high alkalinity may induce stunted growth (Pearce et al., 1999a, 1999b) as a result of decreased chlorophyll concentration in young leaves in many plant species (Alcántara et al., 1988; Campbell and Nishio, 2000; McCallister et al., 1989; Nikolic and Kastori, 2000). Decreased chlorophyll concentration has been attributed to impaired iron uptake (Bertoni et al., 1992) or decreased solubility of iron (Alcántara et al., 1988) at high soil solution pH. High alkalinity may also inhibit growth of plants as a result of the direct effect of HCO_3^- on root growth and physiology (Alhendawi et al., 1997; Matkin and Petersen, 1971; Pearce et al., 1999b; Qi et al., 1994).

Most of the studies that investigate the effect of alkalinity on plant growth have been conducted using Na^+ as the counter-cation of HCO_3^- , although a lesser number of studies have used K^+ (Norvell and Adams, 2006). In these studies, the specific toxicity and osmotic effects of Na^+ have been ignored, probably

under the assumption that plants have mechanisms to exclude Na^+ from uptake or translocation or because the concentrations of Na^+ were considered to be relatively low, although some plant species such as bean may be very sensitive to low concentrations of Na^+ (Marschner, 1995). In addition, the use of K^+ as counter-cation of HCO_3^- may also interfere with experimental results as a result of the potential enhancement of K^+ nutrition and subsequently alter the response of plants to alkalinity (Kaya et al., 2002). Rubidium can also substitute K^+ , which in nature occurs mainly in acidic soils as a result of excessive K^+ leaching (Nyholm and Tyler, 2000). Because the counter-cations may prevent the delineation of the actual alkalinity effect, a series of mixture experiments using bean (*Phaseolus vulgaris*) as a model plant were conducted to quantitatively separate the effect of HCO_3^- from that of the counter-cation. Mixture experiments allow for the formulation of solutions in which only the proportions of the counter-cations can be modified while holding a constant HCO_3^- concentration. Repeating the mixtures at different concentrations of HCO_3^- , a mixture–amount experiment, allows the delineation of the HCO_3^- effect.

Materials and Methods

EXPT. 1: EFFECT OF MIXTURES OF Rb^+ , K^+ , AND Na^+ ON THE RESPONSE TO HCO_3^- . The objective of this experiment was to evaluate the effect of Rb^+ , K^+ , and Na^+ as counter-cations of HCO_3^- on the growth of bean plants. ‘Poncho’ pinto bean

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(Syngenta Biotechnology, Research Triangle Park, NC) seeds were germinated and kept at room temperature for 7 d, and uniform seedlings with fully expanded cotyledonary leaves were transferred to 1.6-L containers with one plant per container. Seedlings were allowed to establish for 7 d in a complete nutrient solution containing ($\text{mg}\cdot\text{L}^{-1}$): 105 N, 15.5 P, 107 K, 90 Ca, 24 Mg, 44 S, 5 Fe (Fe-DTPA), 0.02 Cu, 0.05 Zn, 0.5 B, 0.11 Mo, and 0.65 Mn. The nutrient solution was prepared with $\text{Ca}(\text{NO}_3)_2\cdot 4\text{H}_2\text{O}$ (Fisher Scientific, Pittsburgh, PA), $(\text{NH}_4)_2\text{SO}_4$ (Sigma, St. Louis, MO), KNO_3 (Sigma), KH_2PO_4 (Sigma), CaSO_4 (Sigma), $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ (Sigma), Fe-DTPA (Becker Underwood, Ames, IA), H_3BO_3 (Spectrum Chemical, Gardena, CA), $\text{MnCl}\cdot 4\text{H}_2\text{O}$ (Matheson Coleman & Bell Manufacturing Chemists, Norwood, OH), $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ (Matheson Coleman & Bell Manufacturing Chemists), MoO_3 (Sigma), and $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ (Fisher Scientific, Fair Lawn, NJ).

The containers were covered with a coextruded black/white plastic sheet to prevent increasing temperature and light to affect the nutrient solution and roots. Air was filtered and bubbled constantly in each container for the duration of the experiment. Plants were grown in a controlled environment chamber (model M96-10-5K-0750A-277/480; Environmental Growth Chambers, Chagrin Falls, OH) with temperature set at 27 °C day/16 °C night, 65% relative humidity, and daylength maintained from 0700 to 1900 HR. Average photosynthetically active radiation was $480\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

The experiment was a simplex-centroid design mixture experiment augmented with three interior points yielding a total of 10 mixtures (Fig. 1); HCO_3^- was used as a process variable with two levels, 0 and 7.5 mM. The components that varied in proportion were the counter-cations Rb^+ , K^+ , and Na^+ . The sum of the concentration of the $\text{Rb}^+:\text{K}^+:\text{Na}^+$ in the mixtures

Fig. 1. Simplex centroid design augmented with three interior points. Each design point indicates the proportion of $\text{Rb}^+:\text{K}^+:\text{Na}^+$ in a mixture of 7.5 mM total cation concentration. The design was a mixture-amount experiment replicated at 0 and 7.5 mM HCO_3^- . The axes for Rb^+ , K^+ , and Na^+ are the lines inside the triangle.

was 7.5 mM and HCO_3^- concentration was held constant at 7.5 mM. The mixtures were prepared with NaHCO_3 (Fisher Scientific), KHCO_3 (Sigma), and RbHCO_3 . Rubidium bicarbonate was synthesized by bubbling pure CO_2 gas into a solution of RbOH (Sigma) of a known concentration until pH was stabilized at 8.25 to 8.35 (R.H. Loeppert, personal communication). As a control, the experiment was repeated at 0 mM HCO_3^- using SO_4^{2-} as the anion and the solutions were prepared using Na_2SO_4 (Sigma), K_2SO_4 (Sigma), and Rb_2SO_4 (Sigma). Average initial solution pH and electrical conductivity (EC) was 6.34 and $2.18\ \text{dS}\cdot\text{m}^{-1}$, respectively, for mixtures containing 0 mM HCO_3^- and 7.90 and $2.13\ \text{dS}\cdot\text{m}^{-1}$, respectively, for mixtures containing 7.5 mM HCO_3^- . The speciation of carbonates at pH 7.90 is in equilibrium with a mole fraction of HCO_3^- of 0.968; thus, the actual concentration of HCO_3^- was 7.26 mM.

Nutrient solutions for mixtures with 0 mM HCO_3^- contained ($\text{mg}\cdot\text{L}^{-1}$): 195 N (6.5% NH_4^+ : 93.5% NO_3^-), 181 Ca^{2+} , 49 Mg^{2+} , and 120 S and were prepared with $\text{Ca}(\text{NO}_3)_2\cdot 4\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{SO}_4$, and $\text{Mg}(\text{NO}_3)_2\cdot 6\text{H}_2\text{O}$ (Fisher Scientific) (additional S was from the mixtures of Na_2SO_4 , K_2SO_4 , and Rb_2SO_4). The mixtures with 7.5 mM HCO_3^- had the same composition, except for S (14 $\text{mg}\cdot\text{L}^{-1}$). All the nutrient solutions contained micronutrients as reported previously in the preculture solution. Potassium was not added to the nutrient solutions because it was a component of the mixtures. To test for hidden K^+ deficiency, leaf K^+ concentration was determined by inductively coupled plasma mass spectrometry (SpectroCircus^{CCD} Type: 76004527 4LO076; Circus, Fitchburg, MA) at experiment termination at the Soil, Water and Forage Testing Laboratory, Soil and Crop Sciences Department, Texas A&M University, College Station.

At 7.5 mM HCO_3^- , treatment solutions contained no P to avoid precipitation as a result of alkalinity. To supply all treatments with adequate P, every 7 d for the duration of the study, solutions were replaced for 1 d with a 31 $\text{mg}\cdot\text{L}^{-1}$ P solution. This solution was prepared with $(\text{NH}_4)_2\text{HPO}_4$ (Sigma) and $\text{NH}_4\text{H}_2\text{PO}_4$ (Sigma) to render a final pH of 6.3. The 0 mM HCO_3^- treatment solutions contained a constant 31 $\text{mg}\cdot\text{L}^{-1}$ P. Average leaf P concentration in plants treated with 0 mM HCO_3^- was $0.28\% \pm 0.01\%$ and in plants treated with 7.5 mM HCO_3^- , it was $0.22\% \pm 0.01\%$. Thus, leaf P concentration was within the sufficiency range (0.2% to 0.4%) common for most plants, indicating that sufficient P was supplied by both protocols.

Each mixture treatment had four replicates randomly distributed in the growth chamber. The treatments were imposed 7 d after transplant and the plants were harvested 21 d later. At experiment termination, the shoots were washed, placed in individual paper bags, and dried in an oven at 75 °C for 72 h. Once dried, shoot dry weight (SDW) was measured. Shoot dry weight data were analyzed with Design Expert[®] version 6.0.4 software (Stat-Ease, Minneapolis, MN) computer program to obtain the three-dimensional response surface for the mixtures, analysis of variance, and the model that best fit the response. Model selection was based on highest R^2 , adjusted R^2 close to the R^2 , an adequate precision higher than 4.0 (a measurement of the signal-to-noise ratio), and the model with fewer terms. Shoot dry weight was also analyzed by multiple mean comparison with Tukey's test at $P < 0.05$.

EXPT. 2: EFFECT OF $\text{K}^+:\text{Na}^+$ BINARY MIXTURES ON THE RESPONSE TO HCO_3^- . The objective of this experiment was to assess the effect of $\text{K}^+:\text{Na}^+$ binary mixtures on the response of bean plants to HCO_3^- . Rubidium was not selected as a

component of the mixtures because in the previous experiment, it was observed that the response of bean plants was similar to that of Na^+ . The experimental design was a mixture–amount experiment with two components: K^+ and Na^+ , replicated at three total concentrations, 2.5, 5, and 7.5 mM, with or without HCO_3^- .

'Poncho' pinto bean seeds were germinated as in Expt. 1 and the seedlings were transferred to the hydroponic containers 7 d later. The preculture nutrient solution contained ($\text{mg}\cdot\text{L}^{-1}$): 210 N (10% NH_4^+ : 90% NO_3^-), 31 P, 235 K^+ , 200 Ca^{2+} , 49 Mg^{2+} , and 65 S and was prepared with $\text{Ca}(\text{NO}_3)_2\cdot 4\text{H}_2\text{O}$, NH_4NO_3 (Sigma), $\text{Mg}(\text{NO}_3)_2\cdot 6\text{H}_2\text{O}$, and $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$. Micronutrients, growing conditions, K^+ and P supply were as described in Expt. 1.

Mixtures with HCO_3^- were prepared with NaHCO_3 and KHCO_3 , and mixtures with no HCO_3^- were prepared with Na_2SO_4 and K_2SO_4 . To gain more precision in the model, five $\text{K}^+:\text{Na}^+$ proportions—1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75, and 0:1—were evaluated in each total concentration. All the mixtures contained N, Ca^{2+} , and Mg^{2+} as reported previously for the preculture solution and micronutrients for Expt. 1. Mixtures containing 2.5, 5, or 7.5 mM HCO_3^- had 32 $\text{mg}\cdot\text{L}^{-1}$ S, whereas in the 2.5, 5, or 7.5 mM total mixtures with 0 mM HCO_3^- , the concentration of S was 73, 116, and 153 $\text{mg}\cdot\text{L}^{-1}$, respectively (additional S from Na_2SO_4 and K_2SO_4).

Each mixture treatment had four replicates, which were randomly distributed in the growth chamber. The treatments were imposed 7 d after transplant and the plants were harvested 21 d later. At experiment termination, SDW was measured and analyzed as in Expt. 1; the best fit equation for each response surface was selected as in Expt. 1.

Initial solution pH was 7.95, 7.97, and 8.00 for the mixtures containing 2.5, 5, or 7.5 mM HCO_3^- , respectively. The speciation of carbonates at pH of 7.95, 7.97, and 8.00 is in equilibrium with a mole fraction for HCO_3^- at 0.971, 0.972, and 0.973, respectively; thus, the actual concentration of HCO_3^- for each of the total mixtures was 2.43, 4.86, and 7.30 mM. Initial EC ranged from 1.63 to 1.73, 1.90 to 1.91, and 2.00 to 2.10 $\text{dS}\cdot\text{m}^{-1}$ for the mixtures containing 2.5, 5, or 7.5 mM HCO_3^- , respectively. In mixtures with 0 mM HCO_3^- , the initial solution pH ranged from 6.57 to 6.64, and EC from 1.71 to 1.88, 1.90 to 2.00, and 1.99 to 2.10 $\text{dS}\cdot\text{m}^{-1}$ for 2.5, 5, and 7.5 mM HCO_3^- , respectively.

EXPT. 3: EFFECT OF $\text{K}^+:\text{Na}^+$ BINARY MIXTURES WITH A MINIMUM CONCENTRATION OF 2.5 mM K^+ . The objective of this experiment was to assess the effect of the $\text{K}^+:\text{Na}^+$ binary mixtures on the response of bean plants to HCO_3^- . To eliminate the possibility of K^+ deficiency in the Na^+ pure blends, all the solutions contained a minimum concentration of 2.5 mM K^+ . The experimental design was a mixture–amount experiment with two components: K^+ and Na^+ , and five levels of HCO_3^- (mM): 0, 1.88, 3.75, 5.65, and 7.5. The $\text{K}^+:\text{Na}^+$ proportions evaluated at each HCO_3^- concentration were 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75, and 0:1.

Mixtures with HCO_3^- were prepared with NaHCO_3 and KHCO_3 and the 0 mM HCO_3^- control mixtures were prepared with Na_2SO_4 and K_2SO_4 . To avoid K^+ deficiency, especially in the 0 K^+ mixtures, all the solutions contained a baseline of 2.5 mM K^+ , supplied as K_2SO_4 , which was not considered as part of the mixtures. Thus, the actual $\text{K}^+:\text{Na}^+$ concentrations were (mM) 10:0, 8.13:1.88, 6.25:3.75, 4.28:5.63, and 2.5:7.5. The mixtures containing 0 mM HCO_3^- had 189, 160, 131, 102, and 73 $\text{mg}\cdot\text{L}^{-1}$

S (additional S from Na_2SO_4 and K_2SO_4), respectively, whereas mixtures containing HCO_3^- had 32 $\text{mg}\cdot\text{L}^{-1}$ S. All the mixtures contained N, Ca^{2+} , Mg^{2+} , and micronutrients as explained in previous experiments. Initial solution pH was 6.54, 7.95, 8.04, 7.95, and 8.14 for the mixtures containing 0, 1.88, 3.65, 5.63, and 7.5 mM HCO_3^- , respectively. At these pH values, the speciation of carbonates is in equilibrium with a mole fraction for HCO_3^- at 0.971, 0.975, 0.971, and 0.977 for the mixtures containing 1.88, 3.65, 5.63, and 7.5 mM HCO_3^- , respectively; thus, the actual concentration of HCO_3^- was 1.83, 3.56, 5.47, and 7.33 mM, respectively. Initial EC ranged from 2.1 to 2.5 $\text{dS}\cdot\text{m}^{-1}$ for all the mixture treatments.

Germination of seeds and harvest were conducted as previously reported, whereas the preculture nutrient solution, growing conditions, and P supply were as described in Expt. 2. At experiment termination, SDW was measured and data analyzed as in Expts. 1 and 2.

Results

EXPT. 1: EFFECT OF MIXTURES OF $\text{Rb}^+:\text{K}^+:\text{Na}^+$ ON THE RESPONSE TO HCO_3^- . Shoot dry weight of plants was significantly affected by the $\text{Rb}^+:\text{K}^+:\text{Na}^+$ mixtures at 0 mM HCO_3^- ; however, at 7.5 mM HCO_3^- , there was a nonsignificant effect (Table 1). Eq. [1] is the best fit model ($R^2 = 0.714$, $P = 0.0018$) for the 0 and 7.5 mM HCO_3^- treatments (where K^+ , Rb^+ , and Na^+ equal the proportion of each in the solution and must sum to 1.0; the 0 mM HCO_3^- is solved with $\text{HCO}_3^- = -1$, and for 7.5 mM HCO_3^- , $\text{HCO}_3^- = +1$).

$$\text{SDW} = 3.97\text{K}^+ + 3.47\text{Rb}^+ + 3.68\text{Na}^+ - 1.14\text{K}^+ \times \text{HCO}_3^- - 0.37\text{Rb}^+ \times \text{HCO}_3^- - 0.25\text{Na}^+ \times \text{HCO}_3^- \quad [1]$$

The response surface for Eq. [1] is presented in Figure 2, in which the top plane corresponds to the response to the 0 mM HCO_3^- and the bottom plane corresponds to the 7.5 mM HCO_3^- .

Table 1. Effect of mixtures of varying proportions of Rb^+ , K^+ , and Na^+ as counter-cations of HCO_3^- on shoot dry weight of bean (*Phaseolus vulgaris* cv. Poncho) grown in hydroponics with a 7.5 mM total concentration of Rb^+ , K^+ , and Na^+ and two levels of HCO_3^- .

Mixtures ($\text{Rb}^+:\text{K}^+:\text{Na}^+$)	Shoot dry wt ^a (g)	
	HCO_3^- (mM)	
	0	7.5
Pure blends		
1: 0: 0	3.2 b	2.7
0: 1: 0	4.9 a	2.8
0: 0: 1	4.1 b	3.3
Binary blends		
0.5: 0.5: 0	4.7 a	3.2
0.5: 0: 0.5	3.8 b	3.2
0: 0.5: 0.5	4.6 ab	3.7
Centroid		
0.33: 0.33: 0.33	4.7 a	2.8
Tertiary blends		
0.66: 0.17: 0.17	4.9 a	3.9
0.17: 0.66: 0.17	4.5 ab	3.2
0.17: 0.17: 0.66	3.5 b	2.4

^aMeans within columns with the same letter indicates nonsignificant difference at $P \leq 0.05$ according to Tukey's multiple comparison test.

Fig. 2. Response surface of shoot dry weight of 'Poncho' bean (*Phaseolus vulgaris*) as affected by mixtures of $\text{Rb}^+:\text{K}^+:\text{Na}^+$ and HCO_3^- concentration. Top plane = 0 mM HCO_3^- response surface; bottom plane = 7.5 mM HCO_3^- response surface. Models correspond to Eqs. [1], [2], and [3].

Eqs. [2] and [3] are the best fit models when HCO_3^- is 0 and 7.5 mM, respectively (where K^+ , Rb^+ , and Na^+ equals the proportion of each in the solution and must sum to 1.0):

$$\text{SDW} = 5.10\text{K}^+ + 3.84\text{Rb}^+ + 3.93\text{Na}^+; 0\text{mM HCO}_3^- \quad [2]$$

$$\text{SDW} = 2.83\text{K}^+ + 3.10\text{Rb}^+ + 3.43\text{Na}^+; 7.5\text{mM HCO}_3^- \quad [3]$$

0 mM HCO_3^- MIXTURES. In the 0 mM HCO_3^- mixtures (top response surface in Fig. 2), SDW decreased linearly when the proportion of Na^+ and Rb^+ increased. The highest SDW was estimated in the K^+ pure blend followed by the Na^+ and Rb^+ pure blend (coefficients in Eq. [2]).

7.5 mM HCO_3^- MIXTURES. The response surface was relatively flat for the mixtures with 7.5 mM HCO_3^- (bottom response surface in Fig. 2). This is because there was very little difference in the toxicity of the counter-cations of HCO_3^- as indicated by the similar SDW (Table 1) and the comparable coefficients in Eq. [3].

EXPT. 2: EFFECT OF $\text{K}^+:\text{Na}^+$ BINARY MIXTURES ON THE RESPONSE OF BEAN PLANTS TO HCO_3^- . The models that best fit the response to the $\text{K}^+:\text{Na}^+$ mixtures were quadratic for the 2.5 (Fig. 3A) and 5 mM (Fig.

3B) total mixtures and linear for the 7.5 mM total mixture (Fig. 3C). The best fitting models for the 2.5, 5.0, and 7.5 mM total mixtures correspond to Eqs. [4], [5], and [6], respectively, with an $R^2 = 0.993$ ($P = 0.0002$), 0.952 ($P = 0.0098$), and 0.894 ($P = 0.0025$), respectively.

$$\text{SDW} = 3.68\text{K}^+ + 2.23\text{Na}^+ + 4.15\text{K}^+ \times \text{Na}^+ - 0.69\text{K}^+ \times \text{HCO}_3^- - 0.09\text{Na}^+ \times \text{HCO}_3^- - 0.85\text{K}^+ \times \text{Na}^+ \times \text{HCO}_3^-; 2.5 \text{ mM total mixture.} \quad [4]$$

$$\text{SDW} = 3.47\text{K}^+ + 2.14\text{Na}^+ + 3.79\text{K}^+ \times \text{Na}^+ - 0.84\text{K}^+ \times \text{HCO}_3^- - 0.12\text{Na}^+ \times \text{HCO}_3^- - 1.77\text{K}^+ \times \text{Na}^+ \times \text{HCO}_3^-; 5 \text{ mM total mixture.} \quad [5]$$

$$\text{SDW} = 4.25\text{K}^+ + 2.43\text{Na}^+ - 1.18\text{K}^+ \times \text{HCO}_3^- - 0.15\text{Na}^+ \times \text{HCO}_3^-; 7.5 \text{ mM total mixture.} \quad [6]$$

0 mM HCO_3^- . When the concentration of HCO_3^- was at 0 mM, SDW decreased as the proportion of Na^+ increased in all the mixtures regardless of the total mixture concentration (top lines of Fig. 3A–C). In the 2.5 and 5 mM total mixtures, the decrease was more pronounced when the proportion of Na^+ was greater than 0.5 (Fig. 3A–B). In the 7.5 mM total mixture, there was a linear decrease, suggesting a constant negative effect resulting from a higher Na^+ proportion (top line of Fig. 3C).

MIXTURES CONTAINING HCO_3^- . Bicarbonate caused a marked decrease in SDW as indicated by the separation between the top and bottom regression lines of Figure 3. Decreased SDW resulting from HCO_3^- ranged from 7% to 44% depending on the total concentration and proportion of $\text{K}^+:\text{Na}^+$. In Figures 3A and 3B, the separation between top and bottom regression lines is almost equidistant when the proportion of Na^+ was less than 0.5. However, the regression lines appear closer at higher proportions, suggesting that Na^+ and HCO_3^- are more detrimental. In mixtures containing 7.5 mM HCO_3^- (bottom line of Fig. 3C), the effect of increasing Na^+ was not apparent.

EXPT. 3: EFFECT OF $\text{K}^+:\text{Na}^+$ BINARY MIXTURES WITH AT LEAST 2.5 mM K^+ CONCENTRATION. The final equation, including the

Fig. 3. Effect of 2.5, 5, and 7.5 mM total mixtures of varying K^+ and Na^+ proportions at two levels of HCO_3^- on shoot dry weight of 'Poncho' bean (*Phaseolus vulgaris*). Models correspond to Eqs. [4], [5], and [6], respectively. Bars represent SE ($n = 4$).

process variable that best fit the response to the K^+Na^+ mixtures in all the HCO_3^- concentrations, was ($R^2 = 0.927$; $P < 0.0001$):

$$SDW = 5.70K^+ + 4.02Na^+ + 0.06Na^+ \times HCO_3^- + 0.50K^+ \times HCO_3^- - 0.20Na^+ \times (HCO_3^-)^2 - 0.43K^+ \times (HCO_3^-)^2 + 0.02Na^+ \times (HCO_3^-)^3 + 0.04K^+ \times (HCO_3^-)^3. \quad [7]$$

Independent analysis of the HCO_3^- concentrations, in terms of the actual factors, demonstrated that the best fitting models at the 0, 1.88, 3.75, 5.65, and 7.5 mM HCO_3^- were the linear blending models of Eqs. [8] to [12], respectively (Fig. 4).

$$SDW = 5.65K^+ + 4.00Na^+; 0 \text{ mM } HCO_3^- \quad [8]$$

$$SDW = 5.57K^+ + 3.77Na^+; 1.88 \text{ mM } HCO_3^- \quad [9]$$

$$SDW = 3.34K^+ + 2.14Na^+; 3.75 \text{ mM } HCO_3^- \quad [10]$$

$$SDW = 2.25K^+ + 2.00Na^+; 5.65 \text{ mM } HCO_3^- \quad [11]$$

$$SDW = 2.16K^+ + 2.14Na^+; 7.5 \text{ mM } HCO_3^- \quad [12]$$

0 mM HCO_3^- . Similar to Expt. 2, in solutions with 0 mM HCO_3^- , SDW decreased as the proportion of Na^+ increased (Fig. 4A). When compared with the 1:0 K^+Na^+ mixture, the decrease was 7%, 15%, 22%, and 29% when the proportion of Na^+ increased to 0.25, 0.5, 0.75, and 1.0, respectively.

MIXTURES CONTAINING HCO_3^- . The trend of decreased SDW with increasing proportion of Na^+ in mixtures with 0 mM HCO_3^- was also exhibited when plants were grown in solutions

containing 1.88 and 3.75 mM HCO_3^- (Fig. 4B–C) (comparable slopes). However, when the solutions had 5.65 and 7.5 mM HCO_3^- , increasing proportions of Na^+ apparently did not further decrease SDW (Fig. 4D–E).

Discussion

The addition of HCO_3^- to the mixtures caused a decrease in SDW, demonstrating the detrimental effect of alkalinity on bean plants. However, Rb^+ and Na^+ were also associated with a decrease in SDW, suggesting that these counter-cations may also detrimentally affect plant growth in addition to HCO_3^- when they are mixed together. Similar results were demonstrated by Navarro et al. (2000), who reported that dry weight of tomato plants treated with NaCl was reduced, but the addition of HCO_3^- promoted a more pronounced negative effect than NaCl salinity alone.

The response to HCO_3^- observed in the present experiments was independent of the K^+ concentration in the nutrient solutions. As indicated previously, the plants grown in the Rb^+ or Na^+ pure blends in Expts. 1 and 2 did not receive K^+ during the experimental period, raising the question as to what extent SDW was affected by the K^+ deprivation. This was ruled out because leaves sampled at experiment termination from plants grown in the Rb^+ and Na^+ pure blends had leaf K^+ concentrations of 1.42% and 1.58% in 0 mM HCO_3^- -exposed plants. Leaf K^+ tissue concentrations were at the lower level of the sufficiency range and were above the deficiency range for most crops (Bould et al., 1984). Our assumption that K^+ had no effect is also supported by Expt. 3 (in which all the solutions, including the Na^+ pure blend, contained a minimum of 2.5 mM K^+), since the effect on SDW was comparable to that of the previous experiments, as observed in the regression lines of Figures 4A and 4D (Expt. 3). The regression lines of Figures 4A and D show comparable tendencies as the regression lines of Figure 3C (Expt. 2) and estimated SDW surface heights above the K^+Na^+ edge of the triangular region in Figure 2 (Expt. 1).

Potassium sufficiency, despite the deprivation during the experimental period, was probably the result of sufficient K^+ accumulation when the plants were precultured in K^+ -containing solutions or a short exposure to K^+ -free solutions (21 d). The K^+ accumulated during preculture may also have been redistributed to developing organs during the deprivation period, allowing plant growth (Sudharmai and Padmaja, 1997).

In Expt. 1, the highest SDW of plants grown in the 0 mM HCO_3^- mixtures (top response surface in Fig. 2) was estimated to occur when they were grown in the K^+ pure blend. Plants grown in the Rb^+ and Na^+ pure blends had a decrease in biomass accumulation of 25% and 23%, respectively, compared with those grown in the K^+ pure blend. However, there was very little difference between the toxicity of the

Fig. 4. Effect of 0, 1.88, 3.75, 5.65, and 7.5 mM of HCO_3^- on shoot dry weight of 'Poncho' bean (*Phaseolus vulgaris*) as affected by mixtures of varying K^+ and Na^+ proportions. Models correspond to Eqs. [8] to [12], respectively. Total $K^+ + Na^+$ mixture is 7.5 mM. Bars represent SE ($n = 4$).

counter-cations at 7.5 mM HCO_3^- (bottom response surface in Fig. 2). The decrease in the position of the 7.5 mM HCO_3^- response surface when compared with the 0 mM HCO_3^- response surface in Figure 2 represents the toxicity effect of HCO_3^- . On average, this toxicity effect caused a 24% decrease in SDW over all the design points evaluated. However, the decrease in SDW depended on the predominant counter-cation, because it was of 19%, 13%, and 45% in the Rb^+ , Na^+ , and K^+ pure blends, respectively.

In Expts. 2 and 3, Rb^+ was not included in the study because the decrease in SDW was comparable to that of Na^+ . In Expt. 2, at 0 mM HCO_3^- , the Na^+ pure blend (0:1 $\text{K}^+:\text{Na}^+$) caused a decrease in SDW of 47%, 47%, and 52% in the 2.5, 5, and 7.5 mM total mixtures, respectively, whereas in mixtures containing HCO_3^- , the decrease was of 28%, 23%, and 26%, respectively (Fig. 3). In Expt. 3, at 0 mM HCO_3^- , SDW decreased 7%, 15%, 22%, and 29%, when the proportion of Na^+ was increased to 0.25, 0.5, 0.75, and 1.0, respectively (Fig. 4A). However, in mixtures containing HCO_3^- , the decrease associated with increasing Na^+ was observed when the plants were grown in solutions containing 1.88 and 3.75 mM HCO_3^- (Fig. 4B–C) (comparable slopes). When the solutions contained 5.65 and 7.5 mM HCO_3^- , increasing the proportions of Na^+ did not further decrease SDW, as suggested by the near to zero slope in the regression lines of Figures 4D and 4E.

The largest SDW was observed in plants grown in K^+ pure blends, indicating that there was no K^+ toxicity. Thus, the K^+ pure blends can be used as a reference solution, similar to a control, because there was no toxic effect and because K^+ concentration was close to that of Hoagland's nutrient solution. Assuming that the K^+ pure blend is the control solution, it is convenient to transform or reparameterize Eqs. [2] and [3] by removing the K^+ term from the models and substituting a constant or intercept term (Cornell, 2002). At 0 mM HCO_3^- , the reparameterized model for Eq. [2], which was $\text{SDW} = 5.10 \text{ K}^+ + 3.84 \text{ Rb}^+ + 3.93 \text{ Na}^+$, is:

$$\text{SDW} = 5.10 - 1.26\text{Rb}^+ - 1.17\text{Na}^+. \quad [13]$$

At the 7.5 mM level of HCO_3^- , the reparameterized model for Eq. [3], which was $\text{SDW} = 2.83 \text{ K}^+ + 3.10 \text{ Rb}^+ + 3.43 \text{ Na}^+$, is:

$$\text{SDW} = 2.83 + 0.27\text{Rb}^+ + 0.60\text{Na}^+. \quad [14]$$

The difference between the intercepts of Eqs. [14] and [13] ($2.83 - 5.10 = -2.27$) represents the effect of replacing 0 mM HCO_3^- with 7.5 mM HCO_3^- , which is the pure HCO_3^- effect. The coefficients for Na^+ (-1.17) and Rb^+ (-1.26) in solutions containing 0 mM HCO_3^- (Eq. [13]) represent the pure Na^+ and Rb^+ effects, but the coefficients of Eq. [14] for Na^+ ($+0.60$) and Rb^+ ($+0.27$) consider the interaction between the counter-cations and HCO_3^- .

In this article, we use the term additive, as defined by Welfare et al. (1996), when the effect of the combination of two stresses is equal to the sum of the effect of each individual stress, the term synergistic when it is greater than the sum of the two stresses, and the term antagonistic when it is lower than the sum of the two stresses. To visualize this in Expt. 1, let us position an imaginary third plane between the two planes of Figure 2, where the heights of the vertices of the third plane are midway between the heights of the vertices of the 0 and 7.5 mM HCO_3^- planes. Since plane 3 would be twisted because

HCO_3^- differently affects the response to K^+ , Rb^+ , and Na^+ , then one or more of the crossproduct terms in Eq. [1] is significant (nonzero) and we say that the presence of HCO_3^- interacts with the linear blending properties of the cations and that the effect is nonadditive. If raising HCO_3^- from 0 to 7.5 mM would have had planes separated by the same distance, then HCO_3^- and the counter-cations would have had an additive effect. Additionally, if the interaction between HCO_3^- and Rb^+ or Na^+ was additive, then the total stress of the mixtures would be equal to the sum of the pure HCO_3^- effect (-2.27) with the pure effect for the respective counter-cation ($\text{Na}^+ = -1.17$ and $\text{Rb}^+ = -1.26$). Because the Na^+ and Rb^+ coefficients of Eq. [14] are not equal to the sum of the HCO_3^- and the Na^+ effect ($-2.27 - 1.17 \neq 0.27$) or the HCO_3^- and the Rb^+ effect ($-2.27 - 1.26 \neq 0.60$), then the conclusion is that the combined effect of HCO_3^- plus the counter-cation effect was not additive.

The nonadditive interaction detected in Expt. 1 indicates that the stress caused by HCO_3^- and that of the counter-cation reaches a point that it is not equal to the sum of the individual stresses. Shi and Sheng (2005) mentioned that salinity and alkalinity stress cause a reciprocal enhancement of its individual components, suggesting that their effects are synergistic. Our results, however, indicate that for bean, the stress caused by Rb^+ or Na^+ is indeed enhanced by the addition of HCO_3^- , but they were not additive or synergistic at the concentrations evaluated in Expt. 1. In Expt. 2 (Fig. 3C) and Expt. 3 (Figs. 4A and 4D), the same trends were observed when similar concentrations of HCO_3^- were used. Welfare et al. (1996) explained the possible mechanisms for a nonadditive effect of salinity and ozone toxicity in alfalfa indicating that the affect was related to physiological processes such as the stomatal closure, which in turn decreased photosynthesis rate and yield. The authors suggest that stomatal closure also decreases the entry of ozone into the mesophyll and transpiration rate, thereby limiting the root uptake of salts and thus, the effect of salinity. Because the effects of HCO_3^- , Rb^+ , and Na^+ when they are mixed were nonadditive, it is impossible to quantitatively determine to what extent each ion is exerting its effect. Thus, the real separation of the HCO_3^- effect from its counter-cation effect should be addressed only when both effects are additive and can be estimated numerically.

The additive effects of Na^+ and HCO_3^- on SDW were observed only when HCO_3^- concentration was 3.75 mM or less as inferred by the parallel lines in Figure 4 A–C, which indicate comparable slopes and equidistant regression lines (see Eqs. [15] to [17]). When HCO_3^- concentration was 0 and 1.88 mM, there was only a Na^+ effect (Fig. 4A), but when the concentration of HCO_3^- was increased to 3.75 mM, there was both Na^+ and HCO_3^- effects (Fig. 4C). When HCO_3^- concentration was 5.66 mM, there was only a HCO_3^- effect, but with 7.5 mM neither HCO_3^- nor Na^+ increased the toxic effects (Fig. 4D). Therefore, the pure Na^+ effect can be estimated when HCO_3^- was at 0, 1.88, and 3.75 mM, whereas the pure HCO_3^- effect can be estimated when the concentration was 1.88, 3.75, and 5.66 mM. Thus, to separate the effects of Na^+ from that of HCO_3^- when both were mixed in the solutions, the analysis should be only when both affected plant growth. Using the reparameterized models of Eqs. [8] to [10], which are Eqs. [15] to [17], allows for the estimation of the HCO_3^- effect by comparing the intercepts and the estimation of the Na^+ effect using the coefficients.

$$\text{SDW} = 5.65 - 1.65\text{Na}^+; 0 \text{ mM HCO}_3^- \quad [15]$$

$$\text{SDW} = 5.57 - 1.80\text{Na}^+; 1.88 \text{ mM HCO}_3^- \quad [16]$$

$$\text{SDW} = 3.34 - 1.20\text{Na}^+; 3.75 \text{ mM HCO}_3^- \quad [17]$$

Thus, HCO_3^- at 3.75 mM caused a 2.31-g decrease in SDW (3.34 to 5.65), whereas Na^+ at 7.5 mM caused, on average, a decrease of 1.55 g [(1.65 + 1.80 + 1.20)/3]. Therefore, Na^+ is responsible for a SDW decrease of 0.21 g per mM increase of Na^+ (1.55/7.5) and HCO_3^- is responsible for a SDW decrease of 0.62 g per mM increase of HCO_3^- (2.31/3.75). These figures represent a 3.7% decrease in SDW [(0.21/5.65) × 100] per mM increase of Na^+ and an 11.0% decrease in SDW [(0.62/5.65) × 100] per mM increase of HCO_3^- , indicating that HCO_3^- is responsible for a larger decrease in SDW than Na^+ . Data from Expt. 2 is consistent with this approach. As observed in Figure 3A, the regression lines show a tendency to be parallel and equidistant, suggesting that the toxicity of the HCO_3^- and Na^+ stresses is additive when the concentration of the former is low (2.5 mM). However, the equidistance between regression lines tends to be lost at 5 and 7.5 mM HCO_3^- (Fig. 3B–C), indicating that the additive effect is lost at higher HCO_3^- concentrations. Furthermore, at 2.5 mM total mixtures, it seems that there was no significant Na^+ effect when its proportion was less than 0.5 (Fig. 3A), suggesting that low concentrations of Na^+ (1.25 mM) did not affect plant growth and that SDW was affected exclusively by HCO_3^- .

Despite no observed additive effect at high alkalinity, it is possible to estimate the detrimental effect of HCO_3^- on SDW using the predictions of the respective models. In Expt. 1, the decrease resulting from the pure HCO_3^- effect was of –0.30 g per mM HCO_3^- ; this represents a 5.9% decrease per mM HCO_3^- . In Expt. 2, the decrease resulting from the pure HCO_3^- effect at 2.5 mM was –0.56 g per mM HCO_3^- ; at 5 mM, the decrease was –0.33 g per mM HCO_3^- ; and at 7.5 mM, the decrease was –0.19 g per mM HCO_3^- . These figures represent a decrease of 12.7%, 7.8%, and 4.2% when HCO_3^- concentration was 2.5 mM, 5 mM, and 7.5 mM, respectively. In Expt. 3, there was a decrease in SDW when HCO_3^- concentration was 3.75 mM or more, which was of 0.62, 0.60, and 0.47 g per mM HCO_3^- at 3.75 mM, 5.65 mM, and 7.5 mM, respectively. The figures from Expt. 3 represent a decrease of 11.0%, 10.7%, and 8.2%, per mM HCO_3^- at 3.75, 5.65, and 7.5 mM, respectively. These SDW decrease rates attributable exclusively to HCO_3^- suggest a concentration-dependent effect because they tended to be higher at lower HCO_3^- concentrations and lower at higher HCO_3^- concentrations (Fig. 5). This tendency may explain the lack of additive effects when the concentration of HCO_3^- was high.

Conclusions

The effect of the counter-cations should be considered when investigating the response of plants to alkalinity because they affect the SDW when combined with HCO_3^- . This is particularly important at low HCO_3^- concentration because the separation of the counter-cation effect (Na^+) from the HCO_3^- effect was possible only when the toxicity resulting from the combination of both was additive, which was observed when

Fig. 5. Effect of increasing HCO_3^- concentration on the decreasing effect rate on shoot dry weight of 'Poncho' bean (*Phaseolus vulgaris*).

HCO_3^- was 3.75 mM or less. At this concentration, there was a 3.7% decrease in SDW per millimolar Na^+ and an 11.0% decrease per millimolar HCO_3^- .

Literature Cited

- Alcántara, E., F.J. Romera, and M.D. de la Guardia. 1988. Genotypic difference in bicarbonate-induced iron chlorosis in sunflower. *J. Plant Nutr.* 11:65–75.
- Alhendawi, R.A., V. Römhild, E.A. Kirkby, and H. Marschner. 1997. Influence of increasing bicarbonate concentration on plant growth, organic acid accumulation in roots and iron uptake by barley, sorghum, and maize. *J. Plant Nutr.* 20:1731–1753.
- Bertoni, G.M., A. Pissaloux, P. Morad, and D.R. Sayag. 1992. Bicarbonate-pH relationship with iron chlorosis in white lupine. *J. Plant Nutr.* 15:1509–1518.
- Bould, C., E.J. Hewitt, and P. Needham. 1984. Diagnosis of mixed disorders in plants. Vol. 1: Principles. Chemical Publishing, New York, NY.
- Campbell, S.A. and J.N. Nishio. 2000. Iron deficiency studies of sugar beet using an improved sodium bicarbonate-buffered hydroponic growth system. *J. Plant Nutr.* 23:741–757.
- Cornell, J.A. 2002. Experiments with mixtures. Designs, models, and the analysis of mixture data. 3rd Ed. Wiley, New York, NY.
- Kaya, C., D. Higgs, and A. Ikinici. 2002. An experiment to investigate ameliorative effects of potassium on salt and alkalinity stressed vegetable crops. *J. Plant Nutr.* 25:2545–2558.
- Marschner, H. 1995. Mineral nutrition of higher plants. 2nd Ed. Academic Press, San Diego, CA.
- Matkin, O.A. and F.H. Petersen. 1971. Why and how to acidify irrigation water. *Amer. Nurserymen* 133:14, 73.
- McCallister, D.L., R.A. Wiese, and N.J. Soleman. 1989. Effect of potassium salts on alleviation of lime-induced chlorosis in soybean. *J. Plant Nutr.* 12:1153–1174.
- Navarro, J.M., V. Martínez, and M. Carvajal. 2000. Ammonium, bicarbonate and calcium effects on tomato plants grown under saline conditions. *Plant Sci.* 157:89–96.
- Nikolic, M. and R. Kastori. 2000. Effect of bicarbonate and Fe supply on Fe nutrition of grapevine. *J. Plant Nutr.* 23:1619–1627.
- Norvell, W.A. and M.L. Adams. 2006. Screening soybean cultivars for resistance to iron-deficiency in culture solutions containing magnesium or sodium bicarbonate. *J. Plant Nutr.* 29:1855–1867.
- Nyholm, N.E.I. and G. Tyler. 2000. Rubidium content of plants, fungi and animals closely reflects potassium and acidity conditions of forest soils. *For. Ecol. Mgt.* 134:89–96.

- Pearce, R.C., Y. Li, and L.P. Bush. 1999a. Calcium and bicarbonate effects on the growth and nutrient uptake of burley tobacco seedlings: Hydroponic culture. *J. Plant Nutr.* 22:1069–1078.
- Pearce, R.C., Y. Li, and L.P. Bush. 1999b. Calcium and bicarbonate effects on the growth and nutrient uptake of burley tobacco seedlings: Float system. *J. Plant Nutr.* 22:1079–1090.
- Qi, J., J.D. Marshall, and K.G. Mattson. 1994. High soil carbon dioxide concentrations inhibit root respiration in douglas fir. *New Phytol.* 128:435–442.
- Shi, D. and Y. Sheng. 2005. Effect of various salt–alkaline mixed stress conditions on sunflower seedlings and analysis of their stress factors. *Environ. Exp. Bot.* 54:8–21.
- Shi, D. and D. Wang. 2005. Effects of various salt–alkaline mixed stresses on *Aneurolepidium chinense* (Trin.) Kitag. *Plant Soil* 271:15–26.
- Sudharmai, C.R. and P. Padmaja. 1997. Effect of substitution of potassium by sodium on the chlorophyll content, stomatal characteristics and relative water content in cassava. *J. Root Crops* 23:81–85.
- Tanji, K.K. 1990. Nature and extent of agricultural salinity, p. 1–17. In: Tanji, K.K. (ed.). *Agricultural salinity assesment and management*. Amer. Soc. Civil Engineers, New York, NY.
- Welfare, K., T.J. Flowers, G. Taylor, and A.R. Yeo. 1996. Additive and antagonistic effects of ozone and salinity on the growth, ion contents and gas exchange of five varieties of rice (*Oryza sativa* L.). *Environ. Pollut.* 92:257–266.