Comparison of Growth and Alkalinity-induced Responses in Two Cultivars of Hibiscus (*Hibiscus rosa-sinensis* L.)

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Abstract. Response to alkalinity was evaluated in two hibiscus cultivars, Bimini Breeze and Carolina Breeze, grown in a soilless growing medium and in hydroponic culture. For soilless growing medium, plants were potted in a sphagnum peat–perlite-based substrate and irrigated with solutions containing 0 to 10 mM NaHCO₃ for 12 weeks. In hydroponic culture, bare-rooted plants were transferred to a 9-L tray containing a Hoagland's nutrient solution prepared with NaHCO₃ at the concentrations previously indicated. In soilless growing medium, shoot dry weight was minimally affected by NaHCO₃ concentration for 'Bimini Breeze', but 'Carolina Breeze' exhibited a significant decrease in shoot mass with increasing NaHCO₃ concentration. In hydroponic culture, increasing concentration of NaHCO₃ induced a decrease in shoot and root mass in both cultivars, but root mass decrease was more pronounced in 'Bimini Breeze'. In soilless growing medium, increasing the concentration of NaHCO₃ caused an increase in growing medium pH. The pH increase was less pronounced for 'Bimini Breeze' than for 'Carolina Breeze', indicating a higher capacity for root zone acidification by 'Bimini Breeze'. Newly developed leaves of both cultivars showed increasing chlorosis with increasing NaHCO₃ concentration. However, 'Bimini Breeze' was more tolerant because, according to regression models, 5.7 mM NaHCO₃ would be required to reduce chlorophyll levels by 10%, compared with 2.2 mM for 'Carolina Breeze', when grown in soilless medium. Fe reductase activity decreased when 'Carolina Breeze' plants were grown in 5 mM NaHCO₃. However, in 'Bimini Breeze', Fe reductase activity was enhanced. These observations indicate that the increased tolerance of 'Bimini Breeze' to increasing alkalinity is the result of enhanced Fe reductase activity and increased acidification of the root zone.

Alkalinity is a measure of the buffering capacity of an aqueous solution and is the sum of the concentration of soluble alkalis in water. In irrigation water, the main components of alkalinity are HCO₃⁻ and CO₃²⁻ (Whipker, 2001). High alkalinity in irrigation water results in increased growing medium pH resulting from the neutralization of H⁺ by HCO₃⁻ and CO₃²⁻. Alkalinity of irrigation water is considered a critical quality parameter because of its direct effect on growing medium solution pH and its direct and indirect effects on plant growth, quality, and marketability. Alkalinity of water is a severe problem in many areas. In the United States, Argo et al. (1997) reported that only 22% of

the waters surveyed contained alkalinity concentrations within the recommended range (40–100 mg·L⁻¹), and the mean (160 mg·L⁻¹) and median (130 mg·L⁻¹) alkalinity levels were more than the recommended range.

One of the major consequences of high alkalinity, and the resultant pH, is decreased availability of Fe for plant acquisition. The most evident responses of plants to high alkalinity are interveinal chlorosis in the youngest leaves and stunted growth (Lucena, 2000; Pearce et al., 1999a,b), which have been attributed to Fe deficiency resulting from decreased Fe uptake (Bertoni et al., 1992; McCallister et al., 1989) or Fe availability. At high pH values, Fe is in the form of hydroxides or other insoluble compounds. Leaf chlorosis also may be the result of tissue Fe not being available or inactive (Marschner, 1995). In hydroponic culture, HCO₃⁻ has been demonstrated to inhibit Fe utilization, inducing chlorosis (Fleming et al., 1984). Plants have developed mechanisms to overcome alkalinity-induced Fe deficiency. These include increased Fe reductase capacity, net extrusion of H⁺, release of phenolic compounds or phytosiderophores to act as Fe³⁺ chelators, and formation of proteoid roots with a high reductase activity and high capacity to release H⁺ (Marschner, 1995).

Most studies on the effect of alkalinity on the performance of greenhouse ornamental plants have been carried out on plants cultivated in a soilless growing medium. The buffering capacity of artificial growing media often causes an increase in pH to occur slowly, as a result of alkalinity. Thus, newly transplanted plants may grow under a non-detrimental growing medium pH for at least some weeks, as reported for impatiens by Argo and Biernbaum (1996). For this reason, to determine the direct and immediate response of plants to alkalinity, it would be desirable to use hydroponic culture.

Valdez-Aguilar and Reed (2007) screened several greenhouse crops for response to alkalinity in irrigation water. Two cultivars of hibiscus exhibited differential alkalinity tolerance. Based on growth and leaf chlorosis, 'Bimini Breeze' was more tolerant than 'Mango Breeze'. The objectives of this study were to compare the growth responses of one tolerant and one susceptible hibiscus cultivar grown in both soilless growing medium and hydroponic culture, and to determine the role of Fe reductase and increased proton extrusion as mechanisms of tolerance to alkalinity.

Materials and Methods

Determination of tolerance to alkalinity in plants grown in sphagnum peat–perlite-based soilless growing medium. Hibiscus 'Bimini Breeze' and 'Carolina Breeze' (Yoder Brothers, Salinas, Calif.; substituted for 'Mango Breeze') liners were transplanted on 2 Nov. 2000 into 10.2-cm-diameter standard pots containing Sunshine #2 sphagnum peat–perlite mix (Sun Gro Horticulture, Bellevue, Wash.), a commercial soilless growing medium adjusted to pH 6.3 with lime. The soilless growing medium was preplant amended with 4.7 kg·m⁻³ 14–14–14 Osmocote® (Scotts Sierra, Marysville, Ohio) and 1 kg·m⁻³ Micromax trace element mix (Scotts Sierra). The experiment was conducted in a glass greenhouse, and the average photosynthetically active radiation, temperature, and relative humidity were 354 ± 26 μmol·m⁻²·s⁻¹ noontime, 25/13 °C day/night, and 65% respectively. The plants were allowed to establish for 1 week and treatments started on 9 Nov. 2000. A 0.5 to 1-cm soft pinch was carried out 1 week after treatments started. The experimental design was a single factorial completely randomized design with five replications; one pot containing one plant constituted a replication. The five alkalinity treatments were 0, 2.5, 5, 7.5, and 10 mM NaHCO₃ (0, 153, 305, 458, and 610 mg·L⁻¹ HCO₃⁻) prepared with reverse osmosis-deionized purified water. Solutions were prepared 24 h before use to allow pH stabilization, under ambient conditions, and the corresponding treatment solution pH values were 5.61 ± 0.14, 8.28 ± 0.07, 8.51 ± 0.09, 8.61 ± 0.09, and 8.65 ± 0.05 (pH ± SE, n = 5) respectively. Plants were top watered as needed by hand to achieve a leaching fraction

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between 0.2 and 0.3. On irrigation day, a plant from each treatment was randomly selected and the volume of irrigation water needed was determined by adding small increments of the respective solutions until a leaching fraction of 0.2 to 0.3 was achieved.

The experiment was ended on 9 Feb. 2001 (day 92). The parameters measured were chlorophyll concentration (as determined by the SPAD index) of newly formed leaves, shoot and root dry weight, and growing medium pH. The SPAD index was determined with a model 501 SPAD meter (Minolta Camera Co., Ltd., Osaka, Japan). Root dry weight was determined by separating the container medium into three horizontal layers: top, middle, and bottom third. Roots were separated from the growing medium under running water, blotted dry, and dried in an oven at 75 °C for 72 h before dry weight determination. Samples of growing medium from each layer were collected to determine growing medium pH. The growing medium was thoroughly mixed with nanopure water in a 1:2 proportion (30 mL growing medium: 60 mL water), allowed to sit for 60 min, and then filtered. The filtrate pH and electrical conductivity (EC) were determined with a Twin pH meter (Spectrum Technologies, Plainfield, Ill.) and EC meter (model B-173; HORIBA Ltd., Kyoto, Japan). For shoot dry weight determination, plants were cut at the growing medium surface, placed in individual paper bags, dried in an oven at 75 °C for 72 h, and then weighed.

Data were analyzed by analysis of variance (ANOVA; SAS Institute, Cary, N.C.) and regression analysis to determine linear, quadratic, and cubic regression models. The best fit regression model selected was the simplest significant model with the highest R^2 value and a nonsignificant lack of fit. Subsequently, the best fit model was used to estimate the NaHCO_3 concentration yielding a 10%, 20%, and 30% decrease in SPAD index, and shoot and root dry weight compared with the 0 mM NaHCO_3 controls.

Growth responses and Fe reductase activity in hibiscus plants grown in hydroponic culture under high alkalinity. Hibiscus liners were grown in aerated hydroponic solutions in a controlled environment growth room (model M96-10-5K-0750A-277/480; Environmental Growth Chambers, Chagrin Falls, Ohio). Temperature was set at 27/16 °C day/night, average relative humidity was 60%, and average photosynthetically active radiation was 480 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Day length was 0700 to 1900 HR.

Individual 10 to 12.5-cm liners (Yoder Brothers, Salinas, Calif.) were transplanted on 3 Nov. 2000, and plants were harvested on 14 Dec. 2000 (day 41). Five plants of each cultivar were inserted into Styrofoam floats in 9-L plastic containers containing the respective nutrient solutions. Filtered air was bubbled constantly in the nutrient solution of each container. Containers were covered with a bicolor plastic sheet (black inside, white top) to prevent light from reaching nutrient

solutions. Treatments started as soon as plants were transferred to the containers.

The complete nutrient solution contained 15 mM N (80% NO_3^- N and 20% NH_4^+ N), 1 mM P, 6 mM K, 4.5 mM Ca, 2 mM Mg, 4.5 mM S, 5 $\text{mg}\cdot\text{L}^{-1}$ Fe as Fe-diethylenetriamine pentaacetate (Fe-DTPA), 0.02 $\text{mg}\cdot\text{L}^{-1}$ Cu, 0.5 $\text{mg}\cdot\text{L}^{-1}$ B, 0.11 $\text{mg}\cdot\text{L}^{-1}$ Mo, and 0.65 $\text{mg}\cdot\text{L}^{-1}$ Mn. The solutions were prepared with nanopure water and $\text{Ca}(\text{NO}_3)_2\cdot 4\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{SO}_4$, KNO_3 , $\text{CaSO}_4\cdot 2\text{H}_2\text{O}$, KH_2PO_4 , $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, Fe-DTPA, $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$, $\text{ZnSO}_4\cdot 5\text{H}_2\text{O}$, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$, $\text{MnSO}_4\cdot \text{H}_2\text{O}$, and H_3BO_3 . The concentrations of NaHCO_3 treatments were 0, 2.5, 5, 7.5, and 10 mM. The pH of the solutions was 6.14 ± 0.02 , 7.93 ± 0.02 , 8.39 ± 0.04 , 8.53 ± 0.05 , and 8.66 ± 0.03 (pH $\pm$ SE, $n = 5$) respectively, and EC was $2.50 \pm 0.1 \text{ dS}\cdot\text{m}^{-1}$, $2.45 \pm 0.2 \text{ dS}\cdot\text{m}^{-1}$, $2.65 \pm 0.1 \text{ dS}\cdot\text{m}^{-1}$, $2.70 \pm 0.2 \text{ dS}\cdot\text{m}^{-1}$, and $2.80 \pm 0.2 \text{ dS}\cdot\text{m}^{-1}$ (EC $\pm$ SE, $n = 5$) respectively. It should be noted that even though an iron chelate (Fe-DTPA) was used in the hydroponic solutions, alkalinity-induced iron stress can still occur in the higher pH solutions (Marschner, 1995). Nutrient solutions were maintained at the original volume in the containers with nanopure water and were changed every 2 weeks.

The parameters measured were SPAD index, shoot dry weight, root dry weight, root diameter, root length, root surface area, and Fe reductase activity. The SPAD index and shoot and root dry weight were measured as indicated in the previous experiment. Harvested roots were scanned and the image analyzed using WinRHIZO v4.1b (Reagent Instruments, Quebec) to determine root diameter, length, and surface area.

The Fe reductase assay was performed on entire root systems according to Lang et al. (1990) and Rosenfield et al. (1991). The intact root system from each treatment was immersed in 250 mL of a continuously aerated pH 5.5 buffer solution consisting of 0.1 mM Fe-ethylenediamine tetraacetic acid (Fe-EDTA), 5 mM 2-[N-morpholino]ethanesulfonic acid (MES), 0.5 mM CaSO_4 , and 0.3 mM 4,7-diphenyl-1, 10-phenantrolinedisulfonic acid (BPDFS). The glass assay containers were painted black to prevent light from reaching the solution. The reaction was carried out at 21 °C and under greatly reduced light to avoid photoreduction. After 60 min, a sample of the solution was withdrawn and its absorbance was measured (spectrophotometer model 21; Bausch and Lomb, Rochester, N.Y.) at 535 nm. The readings were compared

with a standard curve prepared with Fe^{2+} , and the data were expressed as micrograms of iron per gram root dry weight per hour.

Data were analyzed using ANOVA (SAS Institute) and regression analysis to produce linear, quadratic, and cubic regression models.

Results and Discussion

Shoot dry weight. A significant cultivar and NaHCO_3 concentration effect on shoot dry weight was detected in plants grown in soilless medium (Table 1). The response of shoot dry weight in 'Carolina Breeze' grown in soilless growing medium best fit a quadratic model, with a slight beneficial effect at low NaHCO_3 concentration and a significant decrease at higher concentrations of NaHCO_3 (Fig. 1A). The increased sensitivity to high concentrations of NaHCO_3 and the beneficial effect of a low concentration in hibiscus 'Carolina Breeze' is similar to the response exhibited by hibiscus 'Mango Breeze' in previous studies (Valdez-Aguilar and Reed, 2007). The response of shoot dry weight in 'Bimini Breeze' best fit a quadratic model, with only a moderate decrease in shoot dry weight with increased NaHCO_3 concentration. This greater tolerance of 'Bimini Breeze' to NaHCO_3 is similar to previous studies (Valdez-Aguilar and Reed, 2007). The predicted 10%, 20%, and 30% decrease in shoot dry weight, according to the best fit models, was at 6.6, 8.4, and 9.8 mM NaHCO_3 for 'Carolina Breeze' respectively, and was at 7.9, 14.1, and 19.4 mM NaHCO_3 for 'Bimini Breeze' respectively. Thus, at higher concentrations, 'Bimini Breeze' exhibited much greater tolerance, relative to shoot dry weight, to NaHCO_3 concentration.

A significant cultivar and NaHCO_3 concentration effect on shoot dry weight was detected in plants grown in hydroponic culture (Table 2). The best fit models were linear, with a steep slope for both cultivars, indicating that high concentrations of NaHCO_3 induced a severe decrease in shoot growth (Fig. 2A). In hydroponic culture, where exposure to alkalinity was direct and immediate, 'Bimini Breeze' did not exhibit greater tolerance to increasing NaHCO_3 concentrations. The greater tolerance of 'Bimini Breeze' to higher NaHCO_3 concentrations demonstrated in the peat-perlite growing medium (Fig. 1A) was not evident in hydroponic culture (Fig. 2A).

With regard to the peat-perlite growing medium, other studies reported that irrigating

Table 1. Analysis of variance of shoot dry weight, root dry weight, SPAD index, and growing medium pH of two cultivars of hibiscus grown in soilless growing medium in response to increasing concentrations of NaHCO_3 .

	Shoot dry weight (g)	Root dry weight (g)	SPAD index	Growing medium pH		
				Top layer	Middle layer	Bottom layer
ANOVA						
Cultivar	*	NS	***	NS	***	***
NaHCO_3	***	***	***	***	***	***
Interaction	NS	NS	**	NS	NS	NS
R^2	0.53	0.71	0.90	0.97	0.97	0.98

NS,***,****,*****Nonsignificant or significant at $P < 0.05$, <0.01 , and <0.001 respectively.

Fig. 1. (A–C) Effect of increasing concentrations of NaHCO_3 in irrigation water on shoot dry weight (A), root dry weight (B), and SPAD index (C) of hibiscus ‘Bimini Breeze’ and ‘Carolina Breeze’ grown in soilless growing media. Bars represent SE ($n = 5$).

impatiens with water containing $320 \text{ mg}\cdot\text{L}^{-1}$ of alkalinity (as CaCO_3) only caused a measurable increase in growing medium pH after 4 weeks, and reached its maximum level (pH 8.5) in 8 weeks (Argo and Biernbaum, 1996). Thus, there is a period of time in peat-based growing media when plants are exposed to a nondetrimental pH level even though they are being irrigated with water of high alkalinity. It is possible that the buffering capacity of the limed sphagnum peat moss–perlite-based medium used for the current experiment allowed enough time for both cultivars to establish a larger functional root systems. This might allow ‘Bimini Breeze’ to elicit strategies of alkalinity tolerance in soilless growing media that are not possible in hydroponic culture, which will be addressed later.

Root mass and growth. Root dry weight of both hibiscus cultivars was affected by high alkalinity in plants grown in soilless medium (Table 1) and in hydroponic culture (Table 2). In soilless medium, the decrease in root dry weight was similar in both cultivars (Fig. 1B), which is verified by a nonsignificant cultivar $\times$ NaHCO_3 interaction (Table 1). In hydroponic culture, the decrease in root mass exhibited by ‘Bimini Breeze’ had a greater slope compared with ‘Carolina Breeze’, indicating a higher sensitivity of the root system of ‘Bimini Breeze’ (Fig. 2B). This is verified by a significant cultivar $\times$ NaHCO_3 interaction (Table 2). Thus, the root systems of both cultivars were highly sensitive to indirect (in soilless growing medium) and direct (in hydroponic culture) effects of NaHCO_3 .

In hydroponic culture, increasing levels of alkalinity caused a significant decrease in total root length and surface area in both cultivars (Table 3). The cultivar $\times$ NaHCO_3 interaction was significant, indicating that cultivars responded differently to increasing alkalinity. Root diameter remained unaffected in ‘Bimini Breeze’, but in ‘Carolina Breeze’ there was a 34% increase at 10 mM NaHCO_3 compared with the control plants. Similar results were reported in *Lupinus angustifolius*, where root growth was inhibited by high pH resulting from a decrease in cell elongation, leading to increased root diameter (Marschner, 1995). An increase in root tip diameter has been reported for plants growing under Fe deficiency conditions, which is associated with an enhanced H^+ extrusion confined to the swollen tips (Dell’Orto et al., 2002; Zouari et al., 2001). Increased root diameter in maize, sorghum, and barley has been reported when grown in solutions containing a high concentration of HCO_3^- (Alhendawi et al., 1997). The alterations in root morphology may be the result of increased synthesis of ethylene (Schikora and Schmidt, 2002) and auxins (Schikora and Schmidt, 2002; Schmidt et al., 2000). In the current study, we report the average diameter of roots, not discriminating between tips or any other portion of the roots.

These observations indicate that the greater tolerance of ‘Bimini Breeze’ to higher NaHCO_3 concentrations, based on shoot dry weight (Fig. 1A), was not the result of quantitative growth parameters of the root system (Figs. 1B and 2B, Table 2). Other

qualitative or physiological factors must be involved.

Growing medium EC. The highest EC readings were obtained in the bottom layer of the growing medium in all treatments, with the following values: 1.16 ± 0.10 , 1.34 ± 0.20 , 1.31 ± 0.12 , 1.21 ± 0.13 , and $1.08 \pm 0.10 \text{ dS}\cdot\text{m}^{-1}$ for the 0, 2.5, 5, 7.5, and 10 mM NaHCO_3 treatments respectively. All these EC readings are within the range or less than the recommended maximum EC of 1.2 to $1.5 \text{ dS}\cdot\text{m}^{-1}$ for established plants (Lang, 1996; Warncke and Krauskopf, 1983). Therefore, high growing medium EC levels were not a contributing factor to the plant responses observed.

Growing medium pH. The growing medium pH was significantly altered by the NaHCO_3 concentration in irrigation water in all three layers (Table 1). There also was a significant cultivar effect, indicating that the two cultivars affected the pH of the growing medium differently.

Both cultivars acidified the growing medium in the control treatment (0 mM NaHCO_3) by 0.7 to 1.5 pH units, depending on the cultivar and growing medium layer (Fig. 3). As the concentration of NaHCO_3 increased, only ‘Bimini Breeze’ roots in the bottom layer were able to acidify the growing medium enough to counteract even the lowest concentration of NaHCO_3 tested (2.5 mM, Fig. 3C). In the bottom layer, where most of the roots occur, ‘Bimini Breeze’ maintained a pH 0.5 to 1.0 units lower than ‘Carolina Breeze’. The greater acidification by ‘Bimini Breeze’ became most evident at higher NaHCO_3 concentrations (Fig. 3C), despite a significant decrease in root mass (50%; Fig. 1B), total root length (79%), and root surface area (80%; Table 3). This indicates a qualitative change in the root system of ‘Bimini Breeze’ with increasing alkalinity. Increased acidification of the rhizosphere is correlated to enhanced synthesis and activity of the H^+ -ATPase in the plasmalemma of root cells (Dell’Orto et al., 2002; Rabotii and Zocchi, 1994) when plants are Fe deficient. Acidification of the rhizosphere may also be the result of the extrusion of H^+ coupled with

Table 2. Analysis of variance of shoot and root dry weight, SPAD index, and Fe reductase activity of two cultivars of hibiscus grown in hydroponic culture in response to increasing concentrations of NaHCO_3 in nutrient solution.

	Shoot dry weight (g)	Root dry weight (g)	SPAD index	Fe reductase activity ($\mu\text{g Fe}^{3+}\cdot\text{g}^{-1}\text{dry wt}\cdot\text{h}^{-1}$)
ANOVA				
Cultivar	***	***	***	***
NaHCO_3	***	***	***	***
Interaction	NS	***	*	***
R^2	0.95	0.96	0.89	0.95

NS,***Nonsignificant or significant at $P < 0.05$ and <0.001 respectively.

Fig. 2. (A–C) Effect of increasing concentrations of NaHCO₃ in nutrient solution on shoot dry weight (A), root dry weight (B), and SPAD index (C) of hibiscus 'Bimini Breeze' and 'Carolina Breeze' grown in hydroponic culture. Bars represent se (n = 5).

Table 3. Effect of increasing concentrations of NaHCO₃ in nutrient solution on root growth parameters of two cultivars of hibiscus grown in hydroponic culture.

NaHCO ₃ (mM)	Root length (cm)		Root surface area (mm ²)		Root diameter (mm)	
	Bimini Breeze ^a	Carolina Breeze	Bimini Breeze	Carolina Breeze	Bimini Breeze	Carolina Breeze
0	2646 a	1950 a	615 a	600 a	0.71	0.99 c
2.5	2269 a	1477 b	522 a	520 ab	0.74	1.14 bc
5	1318 b	1132 b	317 b	443 b	0.75	1.24 b
7.5	1160 bc	655 c	303 b	290 c	0.82	1.49 a
10	564 c	642 c	131 c	276 c	0.76	1.33 ab
ANOVA ^b						
Cultivar	***		***		***	
NaHCO ₃	***		***		***	
Interaction	***		***		***	

^aWithin columns, means followed by the same letter indicates a nonsignificant difference at $P < 0.05$.

***Significant at $P < 0.001$.

NH₄⁺ uptake (Marschner, 1995) or NH₄⁺ nitrification (Havlin et al., 1999). The higher acidification exhibited by 'Bimini Breeze' is a characteristic of Fe-efficient plants (Marschner, 1995).

Iron Reductase activity. In Fe-efficient plants, the activity of Fe reductase increases in response to Fe deficiency (Li et al., 2000; Zouari et al., 2001). Conditions such as high alkalinity in water result in increased grow-

ing medium pH, which in turn results in the formation of insoluble Fe. For this reason, Fe-efficient plants exhibit increased Fe reductase activity when exposed to Fe deficiency or an alkalinity-induced decrease in Fe availability. Fe reductase activity was significantly affected by both cultivars and NaHCO₃ concentration in the nutrient solution (Table 2). In 'Carolina Breeze', Fe reductase activity decreased as the concentration of NaHCO₃

increased, but in 'Bimini Breeze' the Fe reductase activity increased as NaHCO₃ concentration increased (Fig. 4). There was greatly enhanced Fe reductase activity at 10 mM NaHCO₃ in 'Bimini Breeze', up to three-fold the activity of control plants. This type of response is characteristic of Fe-efficient plants. Enhancement of root Fe reductase activity may be another mechanism explaining the increased tolerance of hibiscus 'Bimini Breeze' to high NaHCO₃ concentrations.

Chlorophyll concentration. Leaves showed increased chlorosis with increasing concentrations of NaHCO₃. As indicated by SPAD index measurements, chlorophyll concentration decreased significantly with increasing concentrations of NaHCO₃ in plants grown in both soilless medium (Table 1, Fig. 1C) and in hydroponic culture (Table 2, Fig. 2C). For soilless growing medium, the predicted 10%, 20%, and 30% decrease in leaf chlorophyll concentration (as indicated by SPAD readings), according to the best fit models, was at 2.2, 4.5, and 6.6 mM NaHCO₃ for 'Carolina Breeze' respectively, and was at 5.7, 7.6, and 9.0 mM NaHCO₃ for 'Bimini Breeze' respectively. In plants grown in hydroponic culture, similar trends were

Fig. 3. (A–C) Effect of increasing concentrations of NaHCO₃ in irrigation water on pH in the top (A), middle (B), and bottom (C) container layers for hibiscus 'Bimini Breeze' and 'Carolina Breeze' grown in soilless growing media. Bars represent SE (n = 5).

Fig. 4. Effect of increasing concentrations of NaHCO_3 in nutrient solution on Fe reductase activity of intact root systems of hibiscus 'Bimini Breeze' and 'Carolina Breeze' grown in hydroponic culture. Bars represent SE ($n = 5$).

observed (Fig. 2C). The increased tolerance of 'Bimini Breeze' is also verified by the significant cultivar $\times$ NaHCO_3 interaction in plants grown in both soilless medium (Table 1) and in hydroponic culture (Table 2). The maintenance of higher leaf chlorophyll concentrations in 'Bimini Breeze' is probably the result of enhanced Fe reductase activity and Fe acquisition by the root systems (Fig. 4).

In summary, compared with 'Carolina Breeze', 'Bimini Breeze' was able to maintain higher shoot dry weight and lower leaf chlorosis as NaHCO_3 concentration increased. This response was associated with the higher capacity of 'Bimini Breeze' to acidify the root zone (Fig. 3C), which allows greater Fe availability for plant uptake, and the enhancement of Fe reductase activity (Fig. 4), which allows increased root absorption of Fe. These responses indicate that 'Bimini Breeze' exhibits characteristics of Fe-efficient plants. These data also indicate that Fe efficiency exists within hibiscus cultivars, which should allow for selection of cultivars adapted to alkaline soils and irrigation water.

The experimental design tested the response of hibiscus to increasing NaHCO_3 concentrations. The responses reported herein could be the result of either the in-

direct effect of alkalinity-induced pH increases or increased salinity resulting from either Na^+ or HCO_3^- , and the experimental design does not allow differentiation between the two. However, the chlorosis and Fe reductase activity responses reported herein are classic plant responses to increased pH (Marschner, 1995), which indicates that the differential responses between the two hibiscus cultivars are primarily the result of alkalinity-induced increased pH.

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