Response of *Ficus benjamina* and *Dracaena marginata* to Iron Stress

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Abstract. *Ficus benjamina* L. and *Dracaena marginata* Lam. were grown in a modified Hoagland's nutrient solution containing either 0, 0.22 or 5.52 mg Fe³⁺/liter (HEEDTA or EDTA). *F. benjamina* grew well at all Fe levels and showed mild chlorosis only at 0 mg Fe/liter. For *D. marginata*, growth decreased and chlorosis increased as solution Fe level decreased. *F. benjamina* exhibited a high capacity for Fe³⁺ reduction, which increased as Fe level decreased, reaching a maximum below 0.06 mg·liter⁻¹. *D. marginata* exhibited a low capacity for Fe³⁺ reduction, which was slightly enhanced at 0.1 to 0.15 mg·liter⁻¹. In both species, reduction occurred in the presence of roots, with minimal reduction in their absence. This result indicates that Fe³⁺ is reduced at the root surface and not by reductants released into the solution. *F. benjamina* increasingly lowered pH as solution Fe decreased, and always lowered pH more than *D. marginata* at all Fe levels. Total and extractable Fe concentration of leaves did not correlate well with chlorosis, whereas total Fe content per plant correlated highly with chlorosis. Chemical names used: N-hydroxyethyl-ethylene-diamine-triacetic acid (HEEDTA), ethylenediamine tetraacetic acid (EDTA).

Differences between species in susceptibility to Fe chlorosis are well-documented. Iron-efficient plants respond to Fe stress by inducing biochemical reactions in their root systems that make Fe available for absorption and metabolic use. These reactions include: 1) release of H⁺, 2) release of reductants, 3) reduction of Fe³⁺ to Fe²⁺ on their root surfaces, 4) increase in the endogenous concentration of organic acids, or 5) secretion of phytosiderophores (Bienfait, 1985; Hether et al., 1984; Landsberg, 1981; Marschner et al., 1986; Olsen and Brown, 1980; Romheld, 1987; Romheld and Marschner, 1983). Ferric (Fe³⁺) iron must be reduced chemically to ferrous iron (Fe²⁺)

to be absorbed by roots and used in metabolic processes (Olsen and Brown, 1980).

An earlier screening of foliage plants revealed that *Ficus benjamina* could grow well under very low amounts of Fe, while *Dracaena marginata* could not (Lang and Reed, 1987). The objective of this research was to investigate differences in the mechanism of Fe uptake between these two species.

Materials and Methods

Growth of plants. Cuttings were taken from greenhouse-grown stock plants and rooted in perlite under intermittent mist. After rooting, the root systems were washed in tap water, followed by a 0.1-N HEEDTA wash to remove adsorbed Fe, and then rinsed three times with double-distilled deionized water. In a first experiment for the determination of growth, solution pH changes, and iron adsorption, individual plants were placed in 3.8-liter containers of aerated, full-strength Hoagland's solution, pH 6.3, modified to contain 34 mg KH₂PO₄/liter and all N as NO₃⁻. The iron treatments were Fe³⁺-HEEDTA at 0, 0.22,

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or 5.52 mg·liter⁻¹, with 10 replicates per treatment in a completely randomized design. Plants were grown under greenhouse conditions of 30C day/25C night (± 5C) and 45% to 85% RH for 154 days. Periodically, plant height, leaf number, leaf chlorosis, and Fe concentration and pH of the nutrient solutions were determined. The nutrient solutions were replaced with fresh solutions about every 21 days. Containers having aerated nutrient solutions with no plants were maintained as controls for solution pH measurements. In a second experiment for determination of Fe³⁺ reduction, plants were grown as in the first experiment, except the Hoagland's solution was modified to contain 136 mg KH₂PO₄/liter, and the Fe treatments were Fe³⁺-EDTA at 0, 0.03, 0.06, 0.12, 0.24, 0.48, 0.96, and 2.78 mg·liter⁻¹. There were five replicates per treatment in a completely randomized design. *Ficus benjamina* was grown for 82 days (due to its initial smaller size) and *D. marginata* for 40 days.

Release of reductants. All plants were removed from the nutrient solutions and placed in fresh, aerated half-strength Hoagland's solution devoid of Fe and phosphate (volume ≈10× greater than the root volume). After 2 hr, cumulative release of reductants from roots was determined by a modification of the method of Bienfait et al. (1987) as follows: Duplicate 0.2-ml samples of nutrient solutions were placed in microplate wells and 0.05 ml of freshly mixed reagents were added to obtain the following final concentrations, i.e., 0.04 mM FeCl₃, 5 mM 2-(N-morpholino)ethanesulfonic acid (Mes) buffer at pH 5.5, and 0.3 mM bathophenanthroline-disulfonic acid (BPDS) (ferrous chelator). After 20 hr in the dark, the absorbance was measured at 490 nm with a microplate reader (Model MR650, Dynatech Laboratories, Alexandria, Va.), and compared to that of standard curves prepared by spiking nutrient solution with FeSO₄. Root volumes were determined by volume displacement. Reductant release was reported as nanomoles of Fe³⁺ reduced × cm³ root × min.

Reduction in the presence of roots. All plants were removed from the nutrient solution and their root volumes measured by volume displacement, then rinsed gently with distilled water. The roots were placed in light-tight containers filled with ≈10× the root volume of aerated solution containing 0.1 mM Fe³⁺ (EDTA), 5 mM Mes buffer at pH 5.5, and 0.3 mM BPDS. The Fe³⁺ concentration was increased compared to the released reductant assay due to the higher rate of reduction, and the more-stable EDTA was used. Samples (2 ml) were removed every 15 min and the absorbance was measured at 535 nm, which offered greater sensitivity than 490 nm. To compensate for autoreduction, samples were taken at each time interval from aerated nutrient solutions without plants; autoreduction was minimal. Reduction rates were calculated by linear regression from the linear portion of a plot of reduction vs. time and expressed as nanomoles of Fe³⁺ reduced × cm³ root × min.

Leaf analysis. At the termination of the experiment, fresh weights of the plants were taken. Leaves that developed during treatment were cleaned by agitation for 5 sec in 0.01 N HCl, followed by two rinses with double-distilled deionized water, then cut into ≈1-cm squares, mixed uniformly, and separated for analysis of chlorophyll, total Fe, or HCl-extractable Fe. Chlorophyll concentration was determined on fresh leaf tissue according to the procedure of Moran (1982). For HCl-extractable Fe (Lang and Reed, 1987), leaf sections were placed in flasks containing 0.1 N HCl (30 ml·g⁻¹ fresh weight tissue) and shaken for 24 hr at 23C. Extracts were collected, evaporated to near dryness, wet-ashed, and analyzed for Fe using an inductively coupled plasma (ICP) spectrophotometer (Model 3510,

Applied Research Laboratories, Dearborn, Mich.) (Lang, 1986). For total Fe, dried new leaves were ground to pass through a 40-mesh screen, wet-ashed, and analyzed for Fe by ICP (Lang, 1986).

Differences between Fe concentrations, within genera, were analyzed by analysis of variance and, when significant, were further analyzed by Duncan's multiple range test.

Results and Discussion

Growth differences. Growth and chlorosis ratings of *F. benjamina* and *D. marginata* differed in response to Fe level in solution (Table 1). *F. benjamina* showed significantly less growth and slight chlorosis only in the 0 mg Fe/liter treatment. *D. marginata* showed significant decreases for all growth criteria and chlorosis ratings with decreasing Fe levels.

Roots of *F. benjamina* grown without Fe showed increased root hair formation on the young lateral roots. This observation agrees with other findings that Fe-efficient species increase root hair number when placed under extreme Fe-deficiency stress (Brown, 1972). This growth response facilitates Fe uptake in the medium through increased root surface area and transfer cell formation (Romheld and Kramer, 1983). Transfer cell formation has been shown to be associated with the increased capacity to reduce Fe³⁺ in the epidermis (Romheld and Marschner, 1981). Very little root hair formation was noticeable on *D. marginata*.

Solution pH. *D. marginata* increased solution pH ≈1 pH unit over that of blank control containers (Fig. 1). Plants grown with NO₃⁻ as the sole N source increase the pH of the growing medium due to release of either OH⁻ or HCO₃⁻ upon NO₃⁻ uptake (Kirkby and Armstrong, 1980). The results with *D. marginata* agree with the current generalization that most monocots do not possess an Fe-stress-induced proton pump and cannot adequately lower the rhizosphere pH when placed under Fe-stress conditions (Olsen and Brown, 1980), at least when NO₃⁻ is the only form of N supplied.

For *F. benjamina*, the solution pH increased slightly with Fe at 5.52 mg·liter⁻¹, probably due to NO₃⁻ uptake (Fig. 1). Plants in the 0.22-mg·liter⁻¹ treatment reduced pH slightly. Plants grown without Fe lowered the solution pH by ≈2.5 to 3 pH units, despite the alkalizing effect of NO₃⁻ uptake. The release of H⁺ ions by Fe-stressed plant roots is one of the biochemical reactions that contribute to Fe-efficiency (Marschner et al., 1986).

Table 1. Growth and chlorosis rating of *F. benjamina* and *D. marginata* after 154 days in nutrient solution at different Fe levels.

Fe concn (mg·liter ⁻¹)	Increases ^{z,y}				
	Height (cm)	Leaf number	Fresh wt (g)	Dry wt (g)	Chlorosis rating
<i>F. benjamina</i>					
0	45	73	87	17	4.0
0.22	62	160	166	38	5.0
5.52	63	156	148	34	5.0
<i>P</i> > <i>F</i> ^x	***	***	***	***	***
<i>D. marginata</i>					
0	25	17	25	3	1.0
0.22	56	41	190	30	3.8
5.52	61	44	220	36	5.0
<i>P</i> > <i>F</i> ^x	***	***	***	***	***

^zCalculated as final measurement minus initial measurement; whole plant.

^y1 = completely yellow, 3 = interveinal chlorosis, 5 = green.

^xANOVA within columns for each species; significant at 0.1% (***).

Table 2. Leaf chlorophyll, total Fe, and extractable Fe from *F. benjamina* and *D. marginata* grown at different solution Fe levels.

Fe concn (mg·liter ⁻¹)	Chlorosis rating	Chlorophyll ^z (mg·g ⁻¹)	Total leaf Fe ^y (mg·g ⁻¹)	0.1 N HCl-Fe extract. leaf ^z (mg·g ⁻¹)	Total Fe per plant (mg/plant)	Fe absorption ^x (ng Fe/m ³ per day)
<i>F. benjamina</i>						
0	Slight	0.9	94	6.3	3.2	0
0.22	None	2.0	111	13.6	6.2	251 ± 7
5.52	None	2.0	133	11.5	17.5	3385 ± 324
Coefficient of correlation with chlorophyll			0.44	0.55*	0.95**	
<i>D. marginata</i>						
0	Severe	0.1	118	7.4	1.4	0
0.22	Moderate	0.5	60	4.5	4.7	181 ± 12
5.52	None	1.8	82	6.8	11.0	2022 ± 149
Coefficient of correlation with chlorophyll			-0.16	0.10	0.93**	

^zReported on a fresh-weight basis.

^yReported on a dry-weight basis.

^xDetermined by solution depletion.

**Significant at $P = 0.05$ or 0.01 , respectively, by linear regression analysis.

Fig. 1. Nutrient solution pH for *D. marginata* and *F. benjamina* over the last 23 days of the 154-day growth period at either 0, 0.22, or 5.52 mg Fe/liter and for blank control pots containing aerated nutrient solution. Nitrogen supplied only as NO_3^- .

Hydrogen release, an Fe-stress-inducible phenomenon, is turned off when adequate Fe is available (Bienfait, 1985; Brown, 1972). This response was exhibited by *F. benjamina*.

Fe³⁺ reduction by roots. Iron reduction was measured first in the absence of roots to quantify released reductants, then in the presence of roots to measure reduction at the root surface and reductants released into solution (Fig. 2). For both species and for all Fe concentrations tested, the amount of reductants released by the roots into solution was minimal compared to the amount of reduction observed in the presence of roots. These data indicate that both *F. benjamina* and *D. marginata* can reduce Fe³⁺, not by releasing reductants, but by a direct reduction at the cell wall or the plasmalemma (Bienfait, 1985).

The capacity of *F. benjamina* for Fe³⁺ reduction was up to 4-fold greater than that of *D. marginata*. *D. marginata* exhibited peak reduction at ≈ 0.1 to 0.15 mg·liter⁻¹, whereas *F. benjamina* exhibited peak reduction < 0.06 mg·liter⁻¹.

F. benjamina root growth was stimulated at very low Fe concentrations, while that of *D. marginata* was inhibited (data not shown), possibly partially explaining the differences in the peak of their reduction curves (Fig. 2).

Both species exhibited reduced rates of reduction at Fe concentrations above the peak value (0.06 mg·liter⁻¹ for *F. ben-*

Fig. 2. Rate of Fe³⁺ reduction by roots of *F. benjamina* and *D. marginata*, grown in nutrient solution containing 0 to 2.78 mg Fe/liter (EDTA). Figure shows reduction in the presence of roots and by release of reductants into the solution.

jamina, and 0.1 to 0.15 mg·liter⁻¹ for *D. marginata*). Thus, the Fe reduction mechanism is turned on only at very low Fe concentrations, which has been shown for other species (Bienfait, 1985; Brown, 1972).

Leaf analysis. *F. benjamina* showed no significant differences in total leaf Fe concentration (dry-weight basis) between treatments, although the concentration generally increased as Fe level in the treatment solutions increased (Table 2). There was a significant decrease in extractable Fe or total Fe per plant as solution Fe level decreased. *D. marginata* showed a significantly higher total leaf Fe concentration (dry-weight basis) with Fe at 0 mg·liter⁻¹. Chlorotic leaves have been reported to possess higher tissue Fe concentrations on a dry-weight basis (Lang, 1986). In this study, the higher leaf Fe concentrations observed

in *Dracaena* were due to reduced dry weight (Table 1) and not to increased whole Fe plant levels (Table 2). For both species, total Fe per plant, rate of Fe absorption, and chlorophyll content were drastically reduced as solution Fe level decreased.

In summary, it appears that *F. benjamina* is an Fe-efficient species eliciting various biochemical responses (acidification and reduction) that allow it to absorb Fe adequately under Fe stress. These responses were activated only at low Fe levels. Conversely, *D. marginata* appears to be Fe-inefficient, and grows well only if high amounts of Fe are available,

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