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Fertigation with Fe-EDTA, Fe-DTPA, and Fe-EDDHA Chelates to Prevent Iron Chlorosis of Sensitive Species in High-pH Soilless Media

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Abstract. **Multiple studies have examined the use of chelates to *correct* pH-induced Fe chlorosis. Here we report the effects of three common chelates on *prevention* of Fe chlorosis in two sensitive species at high pH. *Calibrachoa* and soybean were grown in three media pH ranges (6.0 to 6.5, 7.0 to 7.2, and 7.6 to 7.8) and supplied with 1 mg·L^{-1} Fe as Fe-EDTA, Fe-DTPA, or Fe-EDDHA through fertigation. Chelate effectiveness was quantified by chlorosis rating and dry mass. In *Calibrachoa*, all three chelates prevented chlorosis at media pH up to 6.5, but above pH 7.2 only Fe-EDDHA was effective. Dry mass decreased as pH increased, but the decrease was less within the Fe-EDDHA treatment. Fe-DTPA was intermediate. There is a wide range in cost: Fe-EDDHA is currently four times, and Fe-DTPA is two times, the cost of Fe-EDTA. Fe-EDDHA binds Fe to pH 9, Fe-DTPA binds to pH 7.5, and Fe-EDTA binds to pH 6.5. Consistent with the stability constants for each chelate, the lower-cost Fe-EDTA chelate was effective in preventing chlorosis in *Calibrachoa* at media pH below 6.5. We conclude that the additional expense of Fe-DTPA and Fe-EDDHA is only necessary for *Calibrachoa* when the pH is above 6.5. However, Fe-EDDHA consistently resulted in greater dry mass of soybeans than Fe-EDTA in all pH levels. This suggests that Fe-EDDHA might improve growth of some species, even at a pH below 6.5.**

Iron (Fe) is an essential micronutrient critical to many processes including chlorophyll synthesis, heme protein production, and ferredoxin-mediated electron transfer in many enzymes (Briat et al. 2007; Buckhout and Schmidt 2013; Rout and Sahoo 2015; Schmidt et al. 2019). The bioavailability of Fe is pH-dependent and decreases with increasing pH (Aboulroos et al. 1983; Lindsay 1981). In acidic conditions (pH < 5.5), Fe is reduced, freeing ferric Fe from ferrous oxides, but in alkaline (pH > 7) conditions, Fe oxidizes to insoluble ferric oxide and hydroxide formations (Chen and Barak 1982; Morrissey and Guerinot 2009). In many arid regions and locations with limestone aquifers, the pH of irrigation water is naturally high, and reduces the solubility and bioavailability of Fe in the root zone solution leading to pH-induced Fe deficiency (Argo and Biernbaum 1996; Baudoin et al. 2013).

Iron deficiency inhibits chlorophyll synthesis resulting in interveinal chlorosis and, under severe deficiency, tissue necrosis (Anderson 1982; Fisher et al. 2003; Gibson et al. 2001; Imsande 1998; Wik et al. 2006). Symptoms are most evident in new growth due to the limited mobility of Fe in plant tissue (Fisher et al. 2003).

Chelates, ligands with a high affinity to bind metal cations, increase micronutrient solubility by reducing reactivity and preventing binding and precipitation with other elements like calcium (Lindsay and Schwab 1982; Orr et al. 2020). Synthetic chelates have been used in horticulture for decades to increase micronutrient bioavailability (Aboulroos 1983; Fisher et al. 2003; Tills 1987). Numerous Fe chelates are available, but they vary in cost. Ferric ethylenediaminetetraacetic acid (Fe-EDTA), ferric ethylenediamine-N,N′-bis(2-hydroxyphenylacetic acid (Fe-EDDHA), and diethylenetriaminepentaacetic acid (Fe-DTPA) are commonly used synthetic chelates. The stability of the ligand-metal ion complex is a function of pH (Fig. 1). Fe-DTPA remains chelated up to a pH of ≈7 and Fe-EDTA remains chelated up to a pH of 6. Compared with Fe-DTPA and Fe-EDTA, Fe-EDDHA is typically more expensive, but can remain almost chelated up to a pH of 9 (Krauskopf 2018).

Numerous studies (Bañuls et al. 2003; Holmes and Brown 1955; Papastylianou 1990) have examined the effectiveness of chelates in *correcting* chlorosis in calcareous and alkaline field soils, but relatively few have examined *prevention* of chlorosis in soilless media.

Fisher et al. (2003) examined *correction* of chlorosis by comparing drenches of Fe-EDDHA and Fe-DTPA and Fe concentration (0 to 80 mg·L^{-1}) on chlorotic *Calibrachoa* grown in a high pH (6.9 to 7.4) soilless media and found that Fe-EDDHA was superior to Fe-DTPA. Fe-EDDHA was more efficient with no differences in visual health between 20 and 80 mg·L^{-1} Fe. In contrast, chlorosis decreased with increasing Fe concentration up to 80 mg·L^{-1} when Fe was supplied as Fe-DTPA (Fisher et al. 2003). Broschat (2003) also studied *correction* of Fe chlorosis in dwarf *Ixora* in alkaline media and found that Fe-EDDHA and Fe-DTPA were superior to Hampshire iron (a mix of HEDTA and EDTA chelates).

Wik et al. (2006) compared Fe-EDDHA, Fe-EDTA, and $FeSO_4$ liquid fertilization and Fe concentration (1 to 4 mg·L^{-1}) to *prevent* chlorosis in *Calibrachoa* in a pH range of 6.3 to 6.9. They reported that Fe-EDDHA was more effective than Fe-EDTA and $FeSO_4$. Similar to the findings of Fisher et al. (2003), Fe-EDDHA was the most efficient Fe source requiring only 1 mg·L^{-1} Fe to achieve the same growth as Fe-EDTA applied at 4 mg·L^{-1} Fe.

Plants can chemically alter their rhizosphere to increase Fe solubility and availability (Rout and Sahoo 2015), but the efficiency is species dependent (de Vos et al. 1986; Fisher et al. 2003). However, species described as “Fe-inefficient” have minimal ability to increase Fe solubility by altering the rhizosphere when media pH is high (Argo and Fisher 2002; Fisher et al. 2003; Marschner 1995; Nelson 1994).

Calibrachoa (*Calibrachoa* ×*hybrida* Cerv.), an Fe-inefficient crop, is a popular annual bedding plant grown for its showy flowers, but, in greenhouse production, *Calibrachoa* is susceptible to pH-induced Fe deficiency and chlorosis (Fisher et al. 2003; Wik et al. 2006). The marketability of bedding plants is dependent on visual appeal, so it is critical to minimize chlorosis (Gibson et al. 2001).

Soybean (*Glycine max* L.) is not typically grown in greenhouses, but was included in this study because it is an Fe-inefficient species (Merry et al. 2022).

The objective of our study was to compare the effectiveness of fertigation with Fe-EDTA, Fe-DTPA, and Fe-EDDHA to prevent pH-induced Fe chlorosis in *Calibrachoa* and soybean grown in soilless media over a pH range from 6.0 to 7.8.

Materials and Methods

Environmental conditions

Studies were conducted in a glass greenhouse (Logan, UT, USA) with supplemental

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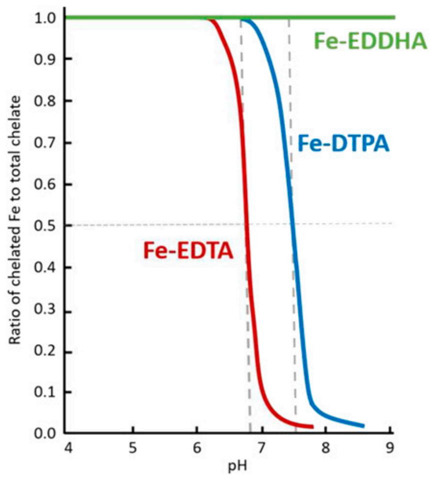

Fig. 1. The ratio of chelated to total Fe as a function of pH. Fe-EDTA binds 50% up to pH 6.8; Fe-DTPA at 7.5, whereas Fe-EDDHA maintains its complexation with Fe 100% up to pH 9.0 (graph modified from Norvell 1972).

light provided at 300 μmol·m^{-2}·s^{-1} (quantum sensor model SQ500-SS; Apogee Instruments, Logan, UT, USA) by LEDs (model LUXX-200 to 277-88/80R Spectrum; LUXX Lighting Systems Los Angeles, CA, USA). The daily light integral was 20 to 40 mol·m^{-2}·d^{-1} and the photoperiod was 16/8 d/night. Mean air temperature was 25/20 °C day/night and mean relative humidity was 40%/60%, day/night.

Media

Plants were grown in 1.7-L plastic containers (12.7-cm square pot) filled with soilless media composed of 75% sphagnum peatmoss (Premier Pro-Moss TBK; Premier Horticulture Inc., Quakertown, PA, USA), 13% expanded perlite (Hess®, Malad City, ID, USA), and 12% rice hulls (Riceland Foods, Inc., Stuttgart, AR, USA) by volume. Wetting agent (Aqua-Gro® 2000 G; Aquatrols, Paulsboro, NJ, USA) was added at 0.75 g·L^{-1} media.

pH treatment and maintenance

The base media was adjusted to the treatment pH using a combination of dolomitic (#65 AG Dolomite; Lhoist North America, Salinas, CA, USA) and hydrated lime (Chemstar® Type S lime; Chemstar Products, Minneapolis, MN, USA). Hydrated lime was added to achieve the initial media pH levels and dolomitic lime was added to maintain media pH over time. Hydrated and dolomitic lime were added at a rate of 1:1 (pH 6.0 to 6.5: 1.75 g·L^{-1} media for both hydrated and dolomitic lime; pH 7.0 to 7.2: 3 g·L^{-1} media; pH 7.6 to 7.8: 4.25 g·L^{-1} media).

Leachates were collected at every watering to monitor pH (Oakton® pH electrode; Oakton Instruments, Vernon Hills, IL, USA) and electrical conductivity [EC; mS·cm^{-1} (DiST 4 Waterproof EC Tester; Hanna Instruments®, Smithfield, RI, USA)].

The media pH was stable over the course of the study. Leachate EC was 1.5 to 2 over the course of each trial.

Nutrient solution

Nutrients were supplied by liquid fertilization using a modified Utah Hydroponic Dicot solution (Bugbee and Langenfeld 2022; Langenfeld et al. 2022) with added ammonium chloride (Table 1). Solutions were composed of deionized water and reagent grade chemicals. Copper (Cu) was also chelated to increase availability. Plants were fertigated twice weekly and all containers received 500 mL of nutrient solution at each irrigation (Table 1). The nutrient solution pH was 5.9 ± 0.1 and the EC was 1.3 ± 0.06 mS·cm^{-1} for all treatments.

Chelates

There were three chelate treatments in all trials: Fe-EDTA (Sigma-Aldrich Inc., St. Louis, MO, USA), Fe-DTPA (Sequestrene® 330 Fe; BASF Corporation, Research Triangle Park, NC, USA), or Fe-EDDHA (Ferriplus®; Miller Chemical and Fertilizer, LLC, Hanover, PA, USA). Each nutrient solution supplied 18 μM Fe (1 mg·L^{-1} Fe).

Plant material and experimental variation

The details for each trial are outlined as follows. *Calibrachoa* cultivars varied among trials due to availability. *Calibrachoa* were received as liners in 104-cell liner flats with 4-cm-deep cells. Soybeans were direct seeded into treatment medias. Pots were arranged in a completely randomized block design by combinations of chelate and media pH with a total of nine treatment combinations (pH × chelate) for each trial. Details for each trial are described as follows.

Calibrachoa

Trial 1. Calibrachoa ×hybrida Cerv. 'Superbells® Dreamsicle®' (Proven Winners, DeKalb, IL, USA) liners were transplanted into media adjusted to pH 6.5, 7.2, or 7.8. Plants received Fe chelate treatments for 25 d (8 Mar to 1 Apr 2022) with six replicate plants per treatment.

Trial 2. Calibrachoa ×hybrida Cerv. 'Superbells® Yellow Chiffon™' (Proven Winners) liners were transplanted into media adjusted to pH 6.5, 7.2, or 7.8. Plants received Fe chelate treatment for 24 d (18 Mar to 11 Apr 2022) with six replicate plants per treatment.

Trial 3. Calibrachoa ×hybrida Cerv. 'Superbells® Lemon Slice®' (Proven Winners) liners were transplanted into media adjusted to pH 6.0, 7.0, or 7.7. Plants received Fe chelate treatment for 23 d (n = 12).

Trial 4. Calibrachoa ×hybrida Cerv. 'MiniFamous® Neo Double Deep Yellow' (Ball Seed, West Chicago, IL, USA) liners were transplanted into media adjusted to pH 6.0, 7.0, or 7.7. Plants were received from the grower with preexisting chlorosis (a result of high media pH). Visual ratings for chlorosis severity of each plant were recorded before starting treatments. At the completion of the study, visual ratings were conducted to assess the correction of chlorosis by chelate. Plants received Fe chelate treatments for 28 d with either 1 or 3 mg·L^{-1} Fe with six replicate plants per treatment. Fe concentration did not affect visual score so, the 1 and 3 mg·L^{-1} Fe treatments within each chelate at each pH were pooled for analysis. The length of the trials varied from 23 to 28 d to allow all plants to fully express the treatment effects.

Table 1. Nutrient concentration for all studies. Chelated iron (Fe) was applied at a rate of 18 μM Fe (1 mg·L^{-1} Fe). Copper (Cu) was chelated to maintain Cu availability.

Element	mM	mg·L^{-1}
N	8.5	119
P	0.4	12
K	2	117
Ca	1.5	60
Mg	0.8	19
S	0.8	26
Si	0.6	17
	μM	
Fe Chelate	18	1
B	40	0.4
Cu-EDTA	4	0.3
Mn	3	0.2
Zn	3	0.2
Mo	0.1	9.6 μg·L^{-1}
Ni	0.1	5.9 μg·L^{-1}

Calibrachoa cultivar sensitivity

A separate study (Table 2) was conducted to assess cultivar sensitivity to pH-induced Fe chlorosis. Sixteen *Calibrachoa* cultivars (n = 16) were planted in media amended with lime to achieve a pH of 7.2 to 7.4 (n = 3 per cultivar). All containers received nutrient solution with 1 mg·L^{-1} Fe-EDTA for 22 d after transplant.

Soybeans

Trial 1. Seeds of *Glycine max* (L.) Merr. Hoyt, a dwarf cultivar, were sown in media adjusted to pH 6.5, 7.2, or 7.7. Plants were

Table 2. Sixteen *Calibrachoa* cultivars (n = 16) were planted in media with a pH of 7.2 to 7.4 to test cultivar sensitivity to pH-induced Fe chlorosis (n = 3 per cultivar). All containers received nutrient solution with 1 mg·L^{-1} Fe-EDTA. Table of the visual ratings ranks the cultivars (n = 16) from most to least sensitive. Visual ratings of chlorosis were given after growing for 22 d.

Calibrachoa cultivar	Visual rating
Mini Neon Deep Yellow	2.5 ± 0.5
Callie Burgundy	3 ± 1
Ombre Pink	3.5 ± 0
Cabaret Orange	3.83 ± 0.29
Mini Uno Double Red	4 ± 0.87
Callie Yellow	4 ± 0.87
Callie Coral	4.17 ± 0.76
Cabaret Deep Yellow	4 ± 1
Callie Rose Dark Center	4.17 ± 0.76
Candy Shop Fancy Berry	4.33 ± 0.29
Conga Orange Kiss	4.5 ± 0
Conga Rose Kiss	4.67 ± 0.29
Mini Vampire	4.67 ± 0.57
Cha Cha Diva Hot Pink	4.83 ± 0.29
Callie Dark Rose	5 ± 0
Cabaret Good Night Kiss	5 ± 0

Table 3. Statistical significance of visual ratings across four replicate trials of *Calibrachoa* ×*hybrida* Cerv.

Chloroisis visual rating			
	pH		
Chelate	6.0 to 6.5	7.0 to 7.2	7.6 to 7.8
Fe-EDDHA	a	a	a
Fe-DTPA	a	b	b
Fe-EDTA	a	b	c

thinned to one per pot after the first true leaves were fully expanded. Soybeans received Fe chelate treatments for 25 d after germination with six plants per treatment.

Trial 2. Seeds of *Glycine max* (L.) Merr. 'Hoyt' were sown in media adjusted to pH 6.0, 7.0, or 7.8. Plants were thinned to one per pot after the first true leaves were fully expanded.

Soybeans received treatment for 23 d after germination with six replicate plants per treatment. There were no detectable differences between treatments with and without chelate, so treatments were pooled for analysis for a total of 12 plants per chelate treatment.

Trial 3. Seeds of *Glycine max* (L.) Merr. Hoyt, a dwarf cultivar, and Minnesota 95, a large growing cultivar, were sown in media adjusted to pH 6.0. Plants were thinned to one per pot after the first true leaves were fully expanded.

Soybeans received Fe chelate treatment for 27 d after germination with three plants per treatment. Plant response to treatments was similar between cultivars so they were pooled for analysis for six replicate plants per treatment.

Harvest

Calibrachoa and soybean plants were visually rated for chlorosis then destructively harvested. Shoots were weighed and dried in an oven at 80 °C for 72 h to reach a constant weight. Fresh and dry masses were recorded for both species.

Visual rating

Visual assessment of plants is widely used in horticulture and often better represents treatment effects than measurements of chlorophyll on single small sections of leaves. Treatments were visually rated on a scale of 1 to 5 (1 being completely white or necrotic, no green; unhealthy and 5 being completely green, no yellow; healthy). Visual ratings were conducted by three to five people and were averaged for each chelate-pH combination. Visual ratings were collected before harvest on the same day.

Statistical analysis

Dry masses were normalized to the average of the media pH 6.0 and Fe-EDDHA treatment within each trial to account for seasonal growth differences. Results were analyzed with SAS® Studio (SAS Institute Inc., Cary, NC, USA) using the PROC GLM procedure with a two-way analysis of variance. An alpha level of 0.05 ($P < 0.05$) was used for all results to test for significance. Tukey's post hoc test was implemented to determine significantly different comparisons between treatments.

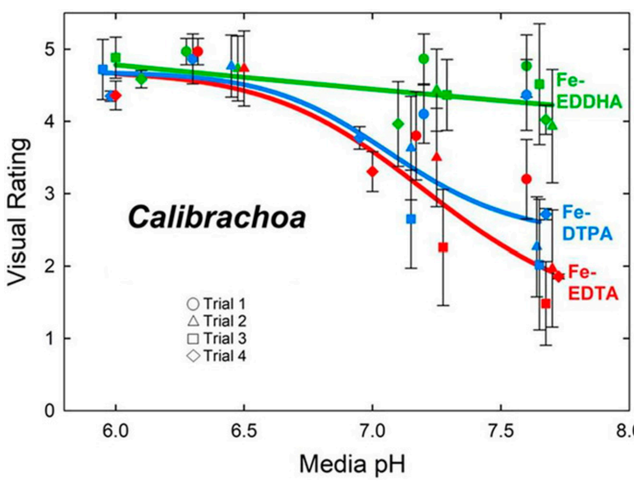

Fig. 2. Four *Calibrachoa* ×*hybrida* Cerv. cultivars (Trial 1: Superbells® Dreamsicle®, Trial 2: Superbells® Yellow Chiffon®, Trial 3: Superbells® Lemon Slice®, Trial 4: MiniFamous® Neo Double Deep Yellow) were visually rated (n = 3 to 5 raters) based on the intensity of iron (Fe) chlorosis. Data points represent the mean and error bars represent standard deviation of replicate containers (n = 6 to 12). Lines were fit with a sigmoidal logistics fourth parameter curve. The calculated r^2 values were as follows: Fe-EDDHA r^2 = 0.36; Fe-DTPA r^2 = 0.65; Fe-EDTA r^2 = 0.85.

Results

Chlorosis ratings for Calibrachoa. At pH 6.0 to 6.5 there were no visual differences in chlorosis between chelate treatments. At pH 7.0 to 7.2, Fe-EDDHA–treated plants were statistically less chlorotic than Fe-DTPA and Fe-EDTA ($P < 0.001$)-treated plants, but there was no difference between Fe-EDTA and Fe-DTPA (Table 3). At pH 7.6 to 7.8, Fe-EDDHA–treated plants were the least chlorotic, Fe-DTPA–treated plants were intermediate, and Fe-EDTA–treated were the most chlorotic ($P < 0.001$; Figs. 2, 3A, and 3B; Table 3). However, the severity of chlorosis varied by trial and plants in Trial 2 were less chlorotic (Fig. 3A) than plants in Trial 4 (Fig. 3B). There were no visual differences in chlorosis among treatments for soybeans.

Dry mass in Calibrachoa *and soybean.* In *Calibrachoa* (Figs. 3A, 3B, and 4A) total dry mass decreased with increasing pH,

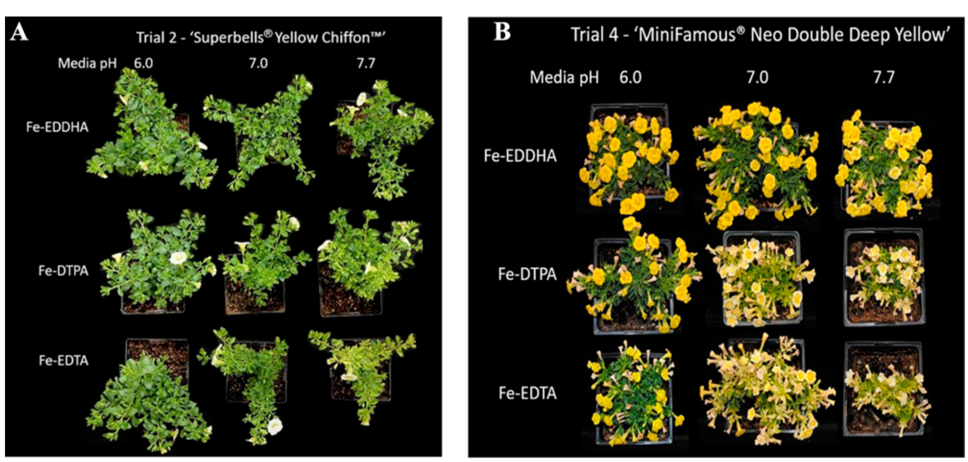

Fig. 3. (**A**) *Calibrachoa* ×*hybrida* Cerv. in Trial 2 ('Superbells® Yellow Chiffon®') had the least chlorosis and stunting. In the Fe-DTPA and Fe-EDTA treatments, plant size visually decreased as pH increased. Each replicate plant in the figures was selected to depict the average treatment response for each chelate and pH combination. (**B**) *Calibrachoa* ×*hybrida* Cerv. in Trial 4 had the most severe chlorosis and stunting ('MiniFamous® Neo Double Deep Yellow'). Each replicate plant in the figure was selected to depict the average treatment response for each chelate and pH combination.

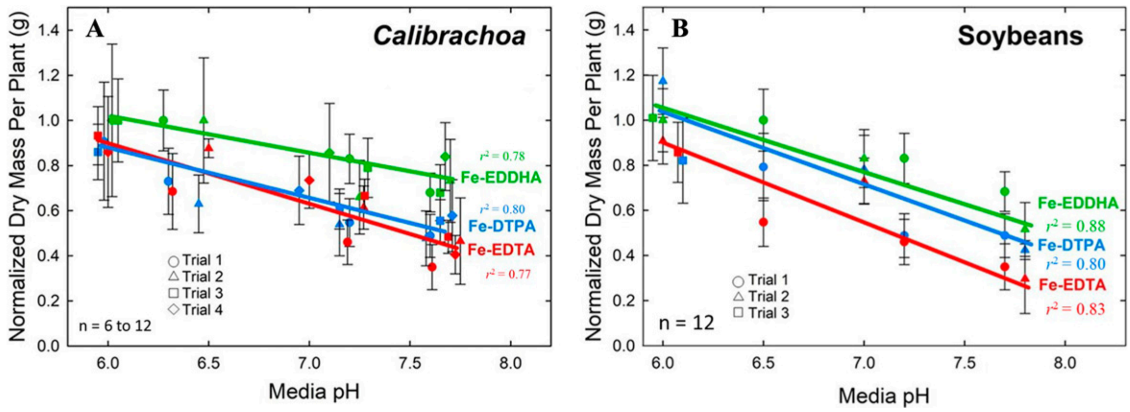

Fig. 4. (**A**) Normalized dry mass per plant mass of four *Calibrachoa* cultivars grown with three iron (Fe) chelates (Fe-EDTA, Fe-DTPA, Fe-EDDHA) in three media pH levels. Data points represent the mean and error bars represent standard deviation of replicate containers. (**B**) Normalized dry mass per plant mass of soybeans grown with three iron (Fe) chelates (Fe-EDTA, Fe-DTPA, Fe-EDDHA) in three media pH levels. Data points represent the mean and error bars represent standard deviation of replicate containers.

regardless of chelate treatment, but the difference in dry mass was only statistically significant above pH 7 (Fig. 4A; Table 4). The dry mass of *Calibrachoa* plants in media pH 6.0 was not statistically different from plants in media pH 7.2, regardless of chelate treatment (Table 4). However, at the highest media pH (7.6 to 7.8), Fe-EDDHA resulted in significantly larger plants than Fe-EDTA (Fig. 4A; Table 4).

In soybeans, total dry mass decreased with increasing pH, regardless of chelate treatment. Fe-EDDHA, however, resulted in significantly larger plants than Fe-EDTA at all pH levels ($P = 0.013$; Figs. 4B and 5; Table 4).

Flower color in Calibrachoa*.* In the orange-flowered *Calibrachoa* cultivar 'Superbells® Dreamsicle', media pH, severity of chlorosis, and chelate treatment affected flower color. In the highest pH treatment (7.6 to 7.8) the corollas of the most recently opened flowers presented varying degrees of pink pigmentation instead of the usual orange. Plants receiving Fe-EDTA had the most pink pigmentation, with some flowers being almost completely pink. Treatment with Fe-DTPA resulted in trace amounts of pink, whereas flowers on Fe-EDDHA–treated plants remained orange (Fig. 6). Differences in floral pigmentation lined to pH and chelate treatment were also observed in the yellow-flowered *Calibrachoa* 'MiniFamous® Neo Double Deep Yellow', which produced pale yellow to white flowers when treated with Fe-DTPA or Fe-EDTA in the media pH range of 7.0 to 7.7 (Fig. 6).

Discussion

Effects of chelate treatment on chlorosis. Argo and Fisher (2002) recommend a media pH between 5.4 and 6.2 when using Fe-EDTA for greenhouse production of *Calibrachoa* and other Fe-inefficient species. In contrast, we found that Fe-EDTA was as effective as Fe-EDDHA and Fe-DTPA in preventing chlorosis up to a pH of 6.5. However, Fe-EDDHA was the most effective chelate for chlorosis prevention in high-pH media (7.6 to 7.8). It is commonly accepted that Fe-EDDHA maintains higher Fe solubility across a wider range (pH 4 to 9) than Fe-EDTA (pH 4 to 6.3) and Fe-DTPA (pH 4 to 7.5) (Fig. 1). The chemistry of Fe solubility in the root zone is the same in both soil and soilless media. The ability of Fe-EDDHA to maintain Fe solubility at high pH was demonstrated by Aboulroos et al. (1983) in alkaline (pH 7.6 to 7.8) field soils. This aligns with Wik et al. (2006) who reported that Fe-EDDHA was the most effective chelate for chlorosis prevention in high-pH soilless media; however, the highest pH tested

Table 4. Statistical significance for dry mass in *Calibrachoa ×hybrida* Cerv. and soybeans [*Glycine max* (L.) Merr.].

		Dry mass		
		pH		
	Chelate	6.0 to 6.5	7.0 to 7.2	7.6 to 7.8
Calibrachoa	**Fe-EDDHA**	a	a	a
	Fe-DTPA	a	a	ab
	Fe-EDTA	a	a	b
Soybeans	**Fe-EDDHA**	a	a	a
	Fe-DTPA	ab	ab	ab
	Fe-EDTA	b	b	b

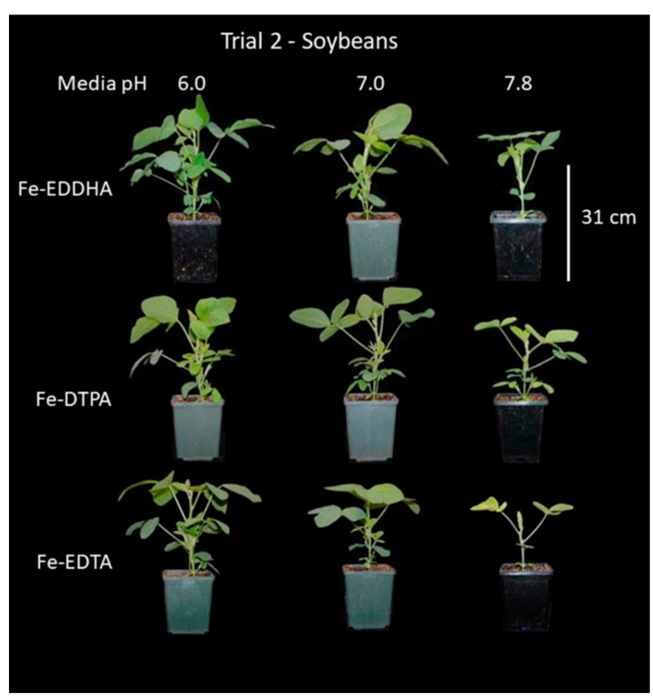

Fig. 5. Effect of pH-induced Fe deficiency on plant size in soybean [*Glycine max* (L.) Merr.].

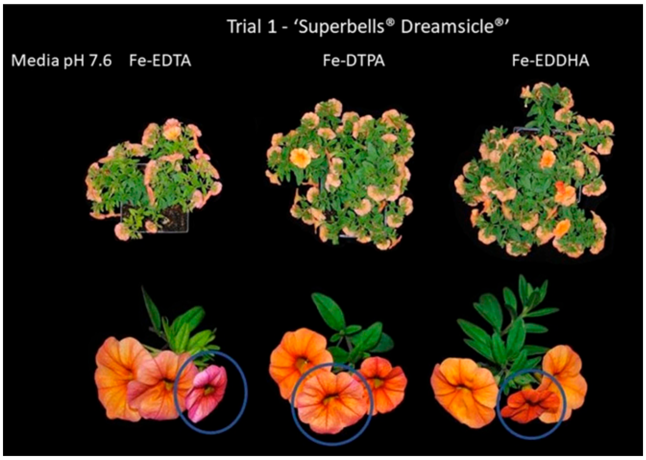

Fig. 6. Effect of pH and chelate on flower color was seen in *Calibrachoa* ×*hybrida* Cerv. 'Superbells® Dreamsicle®'. This cultivar is grown for orange flowers, but media pH and chelate treatment affected the corolla color of developing flowers. Flowers that developed cultiduring chelate treatment expressed pink pigmentations in the corolla in Fe-EDTA– and Fe-DTPA–treated plants, whereas plants grown in Fe-EDDHA remained orange.

was 6.9. In this research, we tested a wider pH range of 6.0 to 7.8 and found that Fe-EDDHA was most effective at preventing chlorosis up to a pH of 7.8.

Chlorosis severity varied among trials. This may be due to differences in cultivar sensitivity to pH-induced Fe deficiency (Dickson et al. 2016). In a preliminary study, we observed variation in the development and severity of chlorosis between cultivars receiving identical nutrition at the same media pH (Table 2; Figs. 4A, 4B, and 7). Cultivar variation is commonly observed in commercial greenhouses, and increasing pH tolerance is a primary goal in many *Calibrachoa* breeding programs (Yanik 2011).

In addition, liner health and nutrition status at the beginning of the trial can influence chlorosis severity at harvest. Bedding plants, like *Calibrachoa*, are commonly propagated vegetatively by cuttings and the nutritional profile of the stock plant from which cuttings were collected. Santos et al. (2011) reported that applying a complete fertilizer program with micronutrients to mature petunia [*Petunia* ×*hybrida* (Sweet) D. Don ex W. H. Baxter] stock plants and freshly stuck cuttings reduced the occurrence of future nutrient deficiency. In addition, subsequent nutrient management of cuttings during root development may impact liner health after transplant, increasing susceptibility to abiotic stresses like pH-induced Fe deficiency. Cultivar differences may also explain the lack of chlorosis we observed in soybean. In addition, soybean may be less sensitive to pH-induced Fe deficiency than *Calibrachoa*.

Effects of chelate treatment at different media pH values on dry mass. In *Calibrachoa* and soybean, dry mass decreased with increasing pH regardless of chelate treatment, which was also observed by Smith et al. (2004) in petunia. Furthermore, in *Calibrachoa*, at all pH levels, treatment with Fe-EDDHA resulted in larger plants at harvest, which aligns with the findings of Fisher et al. (2003) and Wik et al. (2006).

Soybean is not typically grown in soilless media but it is Fe-sensitive in calcareous field soils (Merry et al. 2022). The growth reduction we observed suggests that chelates with higher stability constants might be considered for some species in soilless media.

Flower color in Calibrachoa. The highest media pH (7.6 to 7.8) was associated with in changes in flower color and vibrancy in some *Calibrachoa* cultivars. Similar color changes were observed in snapdragon (*Antirrhinum majus* L.) and rose (*Rosa* ×*hybrida* L.) grown in Fe-deficient conditions (Laurie and Wagner 1940). Flower color in *Calibrachoa* is controlled by vacuole pH and the concentration and combination of anthocyanin, anthocyanidin, and carotenoid pigments (Kanaya et al. 2010; Waterworth and Griesbach 2001). Cultivars with red, red-violet, and orange flowers accumulate anthocyanin pigments (delphinidin and petunidin) and have low floral pH, whereas yellow-flowered cultivars accumulate carotenoids (Murakami et al. 2004). Anthocyanin and anthocyanidin are synthesized in the flavonoid biosynthetic pathway where Fe acts as an enzyme cofactor (Kejík et al. 2021; Martens et al. 2010; Wang et al. 2021). Low levels of bioavailable Fe can interfere with the flavonoid biosynthetic pathway resulting in decreased flavonoid synthesis and pigment production (Buckhout and Schmidt 2013; Kejík et al. 2021). Transgenic studies done by Murata et al. (2015) to introduce alkalinity tolerance to petunia using the Fe (III)-phytosiderophore transporter gene (HvYS1) isolated from barley found that the flowers of transgenic petunia were darker than the flowers of nontransgenic plants. Floral tissue analysis results concluded that the transgenic petunias produced more of

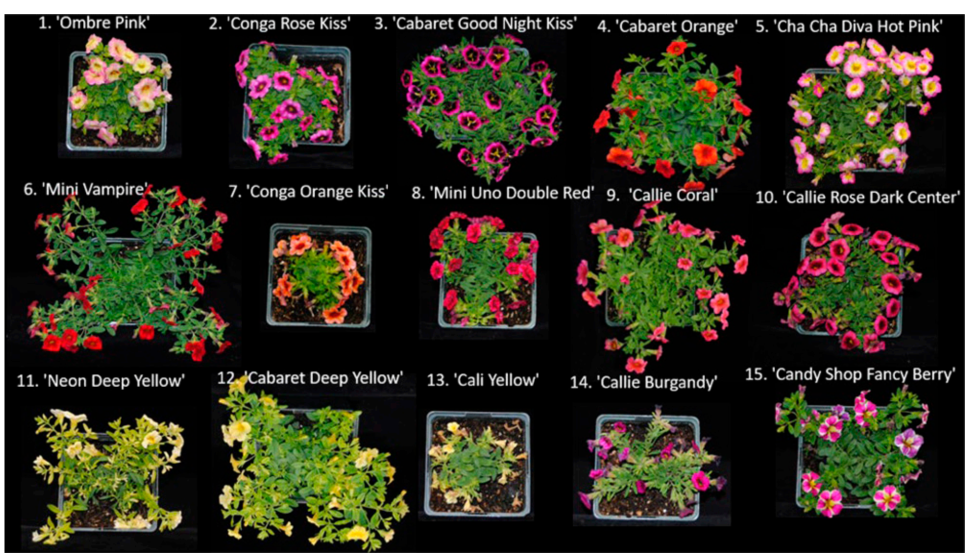

Fig. 7. Sixteen *Calibrachoa* cultivars (n = 16) were planted in media with a pH of 7.2 to 7.4 to test cultivar sensitivity to pH-induced Fe chlorosis (n = 3 per cultivar). All containers received nutrient solution with 1 $mg \cdot L^{-1}$ Fe-EDTA. Visual ratings of chlorosis were given after growing for 22 d. All plants of the cultivar Callie Dark Rose died, so this cultivar is not shown (n = 15 cultivars).

Table 5. The cost of bulk chelates provided by fertilizer manufacturers in 2021 in USD. Pricing varies depending on regional supply chains.

Chelate	Cost per fertilizer bag	lb per bag	Fe (%)	Dollars per lb Fe	Dollars per kg Fe	Cost relative to EDTA
Fe-EDDHA	$200 to $275	50	6	$66 to $92	$150 to $200	**3 to 4x**
Fe-DTPA	$260 to $340	50	10	$52 to $68	$114 to $150	**2 to 2.5x**
Fe-EDTA	$160 to $175	50	13.2	$24 to $27	$53 to $60	**1x**

the anthocyanidin pigment malvidin and had higher concentrations of Fe in their flowers (Murata et al. 2015). This suggests that pH-induced Fe deficiency could affect visual coloring and quality of flowers, which could impact marketability.

Implications for growers. The fertigation solutions in this study were maintained at pH 6, but Fe availability is also influenced by the pH of irrigation water. In arid regions, water typically has high alkalinity that can increase the pH of the fertigation solution (Baudoin et al. 2013). In-line acid injectors can be used to reduce the pH of irrigation water, but these add cost. Chelates vary in price and Fe-binding stability. Although Fe-EDDHA minimized chlorosis and increased biomass in both species, it is more expensive (Table 5). Fe-EDTA is three to four times less expensive than Fe-EDDHA and two times less expensive than Fe-DTPA (Table 5).

Fe-EDTA prevented chlorosis as well as Fe-DTPA and Fe-EDDHA up to a media pH of 6.5 for *Calibrachoa*, but Fe-EDDHA was necessary to prevent chlorosis above pH 7 (Figs. 2, 3A, and 3B; Table 3).

The dry mass reduction we observed with Fe-EDTA in soybean at pH 6.0 to 6.5 suggests that higher-stability chelates may improve growth of some Fe-inefficient species in soilless media at low pH.

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