Pigment Accumulation and Micronutrient Concentration of Iron-deficient Chile Peppers in Hydroponics

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Abstract. Pigment and micronutrient concentrations of New Mexico 6-4 and NuMex R Naky chili pepper (Capsicum annuum L.) cultivars as affected by low Fe levels were studied under soilless culture. A custom-designed, balanced nutrient solution (total concentration <2 mm) was continuously recirculated to the plants potted in acid-washed sand (pot volume 15.6 L). Each set of plants from each cultivar received iron concentrations at 1, 3, 10, and 30 µmol Fe as Fe-EDDHA. The pigments of leaves, green fruit, and red fruit were extracted with acetone and measured with a spectrophotometer. Surface color of green and red fruit was measured with a spectrophotometer. Total concentrations of Fe, Cu, Zn, Mn, P, and K of leaf blades and red fruit were measured by inductively coupled plasma emission spectroscopy (ICP). Ferrous iron in leaf blades, and NO₃⁻ in petioles also were determined. Iron nutrition level affected total leaf chlorophyll and carotenoid content at early season, and the level of these pigments in green fruit at second harvest. No differences in extractable or surface color of red fruit were found among iron treatments in the nutrient solution, despite variations in red fruit iron content, total foliar iron, and foliar ferrous iron. Higher levels of iron in the nutrient solution increased both ferrous and total iron of the leaves, but depressed foliar Cu and P. High iron levels in the nutrient solution were associated with higher concentrations of leaf pigments at early season and higher pigment concentration in green fruit.

Iron deficiency decreases chlorophyll and carotenoid accumulation in leaves (Abadía and Abadía, 1993). This plant disorder affects the total and relative concentration of photosynthetic pigments, namely chlorophylls and carotenoids. Carotenoids are responsible for the yellow color of leaves and are less affected than chlorophylls by iron deficiency, thus leaves lacking iron have a typical yellowish-green, chlorotic appearance along the interveinal region (Abadía et al., 1991; Abadía and Abadía, 1993).

Chlorophyll and carotenoid concentrations correlate strongly with color, quality, and nutritional parameters of horticultural commodities, such as green and red peppers. The green color of chili pepper (Capsicum annuum L.) fruit is due to high levels of chlorophylls, while red color is a result of carotenoids (Camara and Brangeon, 1981; Davies et al., 1970). Some carotenoids used as natural colorings are found almost exclusively in pepper fruit (Almela and López-Roca, 1990). Fruit production is significant economically for New Mexico, the main producer of chili peppers in the United States (Bosland et al., 1993). Chili fruit color is important because nonpungent red chile is used principally as a source of pigments for food and other products. The iron status of chiles may influence the color attributes of both green and ripe fruit, which constitutes an important quality parameter for chili pepper marketing.

Martínez-Sánchez et al. (1989) found a highly significant correlation between leaf and stem iron and red color of field grown chile fruit, but not between fruit iron and color. More studies are needed to determine the specific contribution of iron to fruit color. The objective of this study was to evaluate the response in fruit color, leaf pigments, ferrous iron and micronutrient concentration of chili pepper under varying iron nutrition levels.

Materials and Methods

Plant material and root substrate. Seeds of ‘New Mexico 6-4’ and ‘NuMex R Naky’ chile pepper (C. annuum L.) were germinated at room temperature in paper towels periodically moistened with a 0.1 mol·m⁻³ CaSO₄ solution to preserve membrane function (Gutschick, 1993). After 9 d, germinated seeds were transferred to plastic trays filled with 40-mesh white silica sand that had been washed with hydrochloric acid (HCl) to remove iron and other micronutrients.

The trays were watered as needed in the greenhouse during the 34 d of initial growth with the same nutrient solution used later in the hydroponic system described below. From these trays, 24 seedlings (43 d old) from each cultivar were randomly selected and individually transplanted into 15.7-L plastic pots filled with the acid-washed silica sand. The pots were painted white to reduce heat loading.

The sand was rinsed several times with reverse osmosis (R.O.) water mixed with 4 L concentrated HCl (commercial grade) and shaken within a manual, plastic cement tumbling mixer for 20 min. After standing 2–3 d, the mix of acid and sand was drained, rinsed several times with R.O. water, neutralized with KOH, and rinsed again. Control of the washing process was achieved by sampling, extraction with diethylene triamine pentaacetic acid (DTPA), and analysis of the sand batches for iron in a JY70 inductively coupled plasma spectrophotometer (Jovin Yvon, Edison, N.J.).

Hydroponic system and nutrient solution. In 1996–97, the recirculating hydroponic system was established in a greenhouse at the Fabian García Agricultural Science Center of the New Mexico State Univ. at Las Cruces. The greenhouse had natural light with a transmission factor near 70% for photosynthetically active radiation (PAR). Temperature was controlled within a range of 18 to 37°C (12 to 35°C in early April, to favor fruit set) by evaporative cooling and natural gas heating. The latter raised CO₂ mixing ratios to as much as 450 µmol·mol⁻¹ episodically.

Four, 200-L plastic drums contained the nutrient solution (described below) for the 48 potted plants of the experiment. Each drum served a set of 12 randomly placed pots, half of which were planted to each cultivar of chili pepper under varying iron nutrition levels. Each 15.7-L pot received a nutrient solution flow of 75 mL·min⁻¹. The nutrient solution was recirculated between the drums and the pots by 1/40 HP submersible pumps (Little Giant, Oklahoma City) until red fruit were harvested (231 d after germination). Every drum contained the same basic nutrient solution, plus one of four different iron concentrations [1, 3, 10, and 30 µmol Fe-EDDHA, ferric ethylene-diamine di(o-hydroxyphenyl-acetate)].

The composition of the nutrient solution was custom-designed according to the elemental composition of chili pepper (Winsor and
The concentration was determined on the basis of the expected growth rate of the crop and the replacement schedule of the nutrient solution, trying also to resemble the diluted concentrations of macronutrients often found in natural, high-fertility soils (Clarkson, 1985; Gutschick, 1987).

The nutrient solution was prepared with R.O. water and contained 1 mm KNO₃, 125 μM Ca(H₂PO₄)₂, 250 μM MgSO₄, 250 μM CaSO₄, 20 μM KCl, 25 μM H₂BO₃, 10 μM MnSO₄, 3 μM ZnSO₄, 1 μM CuSO₄, 0.5 μM Na₂MoO₄, and four varying levels of iron (1, 3, 10, and 30 μM Fe-EDDHA) that represented the four treatments of the experiment.

The pH of the nutrient solution was kept at 6.5 ± 0.3 by addition of HNO₃. Both N and K, as well as pH, were adjusted daily after monitoring with ion selective electrodes and combination electrodes, respectively. Iron levels in the nutrient solution were not adjusted between replenishments, because the solution volume was large enough to account for depletion. The nutrient solution was discarded and replaced with fresh solution every 2 weeks at the beginning of the season, and at shorter intervals when plants were larger, to avoid depletion of elements other than Fe that could not be measured practically for replenishment.

Iron was suppressed intentionally to treatments 1 and 3 (3 μM Fe-EDDHA) from days 96 to 106, to test the system after no visible deficiency of iron was evident during the iron shortage. When iron was supplied to treatments 1 and 3 (1 μM Fe), produced lower relative growth rates and photosynthetic rates, and less total dry matter accumulation and red fruit yield than high iron treatments (10 and 30 μM Fe) (Anchondo-Najera, 1999).

Statistical Analysis. Analysis of variance (ANOVA) of all variables was performed using the general linear models procedure (PROC GLM) of the SAS® system. Mean separation for those variables that had significant differences among treatments also was performed in SAS® and followed a cluster-based method (Bautista et al., 1997). This method produces a clearer, unambiguous separation of treatments, without placing a given treatment mean in two or more different groups.

Results and Discussion

The cultivar × treatment interaction was nonsignificant for all variables considered, therefore, the iron treatment means across cultivars are reported. Also, the growth and yield depressing effects of iron deficiency induced in this study are reported elsewhere (Anchondo-Najera, 1999).

Pigment Accumulation. No plant, regardless of the iron treatment, showed chlorosis or any other visible symptom of deficiency during the first 100 d. Leaves from plants receiving Fe at 1 and 3 μM had less extractable chlorophylls and carotenoids at 93 d (Table 1), showing also a relative enrichment of carotenoids (as shown by the chlorophyll a to carotenoid ratio). At the same date, the ratio of chlorophyll a to b in 1 μM Fe plants was higher than that of other treatments. The reduction of total chlorophylls, the relative enrichment in carotenoids, and the increased chlorophyll a/b ratio represent typical responses of plants to iron deficiency (Abadía et al., 1993).

Differences in leaf pigment concentration between low iron (1 and 3 μM Fe) and high iron (10 and 30 μM Fe) plants were more evident after the intentional, temporary, iron shortage initiated on day 96. When iron was resupplied to treatments 1 and 3 μM Fe, 10 d later (day 106), the total chlorophyll and carotenoid content of 3 μM Fe, and the ratio between the these pigments, appeared to drop drastically (Table 1). At 106 d, the low-iron treatments not only the lowest chlorophyll and carotenoid accumulation, but also the lowest ferrous iron concentration (Table 1).
Plants grown with iron treatments (data not shown). The green fruit from the Fe-EDDHA treatment had the highest concentration of total chlorophyll and carotenoids. Pale green fruit, as a result of lower chlorophyll content, represent a marketing disadvantage. The concentrations of total chlorophyll, chlorophyll $a/b$, total carotenoids, and chlorophyll/carotenoid ratio of green fruit grown in hydroponics at four iron levels are in Table 2. Iron and phosphorus are antagonistic to each other (Gutschick, 1987). The classical antagonism between leaf iron and manganese (Marschner, 1986) was not evident in this study. Moraghan (1980) alleviated manganese toxicity in flax plants with a soil application of 2 ppm Fe (as Fe-EDDHA), which not only reduced foliar manganese but also leaf zinc concentrations and slightly increased foliar phosphorus content.

All elemental nutrients in the leaf blades were within the sufficiency range reported for greenhouse chile crops (Winsor and Adams, 1987) for all treatments except the 1 µM Fe plants. However, the levels of foliar iron and copper in the present study were considerably lower than those reported by Guzman and Romero (1998) for commercial greenhouse-grown chile.

Nitrates-nitrogen from leaf petioles did not follow a clear, definite pattern (Table 4). With low iron, a lower nitrates reduction was expected. There was high variability among replicates. Lower levels of nitrates-N in the

Table 1. Surface color and extractable pigment content of green chile pepper fruit grown in hydroponics at four iron levels.

| Iron treatment (µM Fe-EDDHA) | Lightness (L*) | Chroma (C) | Hue (°) |
|-----------------------------|----------------|----------|--------|
| 1                           | 92.5           | 19.6     | 108.1  |
| 3                           | 78.2           | 22.8     | 125.1  |
| 10                          | 62.1           | 23.6     | 127.5  |
| 30                          | 47.8           | 23.6     | 127.5  |

Table 2. Surface color and extractable pigment of green chile peppers at three sampling times after germination. Plants were hydroponically-grown at four iron concentrations.

| Iron treatment (µM Fe-EDDHA) | Extractable pigment content (µg cm$^{-2}$) |
|------------------------------|-------------------------------------------|
|                             | Mg Carot | Fe | Mn | Zn | Cu | P | K |
| 1                           | 145.8   | 39.7 | 29.0 | 33.6 |
| 3                           | 125.1   | 38.1 | 25.3 | 33.6 |
| 10                          | 147.2   | 38.9 | 27.9 | 33.8 |
| 30                          | 120.4   | 39.1 | 27.6 | 34.9 |

Table 3. Extractable color, surface color and nutrient concentration of chile pepper fruit at red harvest time.

| Iron treatment (µM Fe-EDDHA) | Extractable pigment (µg cm$^{-2}$) | Fe (µg g$^{-1}$) | Mn (µg g$^{-1}$) | Zn (µg g$^{-1}$) | Cu (µg g$^{-1}$) | P (µg g$^{-1}$) | K (µg g$^{-1}$) |
|------------------------------|-----------------------------------|----------------|--------------------|----------------|----------------|---------------|---------------|
| 1                           | 145.8                             | 39.7           | 29.0               | 33.6           |                |               |               |
| 3                           | 125.1                             | 38.1           | 25.3               | 33.6           |                |               |               |
| 10                          | 147.2                             | 38.9           | 27.9               | 33.8           |                |               |               |
| 30                          | 120.4                             | 39.1           | 27.6               | 34.9           |                |               |               |

**Crop Production**

**Table 1.** Leaf pigment content of chile peppers at three sampling times after germination. Plants were hydroponically-grown at four iron concentrations.

| Iron treatment (µM Fe-EDDHA) | Leaf pigment content (mg cm$^{-2}$) |
|------------------------------|-----------------------------------|
| 1                           | 45.3                             | 19.9 | 108.1 |
| 3                           | 47.8                             | 22.8 | 125.1 |
| 10                          | 45.6                             | 20.3 | 127.4 |
| 30                          | 45.0                             | 19.2 | 127.5 |

5). No differences in pigment concentration occurred during the subsequent sampling dates. For this reason, the pigment contents at the only the last sampling date (day 181) are reported (Table 1).
petioles of higher iron treatments could perhaps be explained by the higher depletion rate of nitrogen from the solution in the tanks. Plants grown under 3 µM Fe, however, showed an apparent trend towards nitrate accumulation in the petiole ($P = 0.06$). Like many cultivated Solanaeaceae species, chile is reported to reduce nitrates mainly in the shoot (González and Salas, 1999).

It is not clear if iron level had some effect on nitrate levels, via enzyme activity. Nenova and Stoyanov (1995) showed that iron-deficient maize plants had a reduced activity of the enzyme nitrate reductase, which reduces nitrate to nitrite.

Nitrate-N levels from petioles were, on the average, considerably below the standards (6,000 to 10,000 ppm N) followed for some commercial laboratories for field chile crops at the fruit set and development stage (Laboratory Consultants, Ltd.). However, crops at the fruit set and development stage standards (6,000 to 10,000 ppm N) followed for treatments 1 and 3 µM Fe-EDDHA from days 96 to 106 and to treatments 10 and 30 µM Fe from days 96 to 100.

There is little information available on the micronutrient concentration of red chile fruit to be used as reference, and almost all that information comes from field experiments. The average fruit iron (from the 1 µM Fe plants), zinc and copper concentrations of this study were, on the average, only half of those measured in Israel by Navrot and Levin (1976) in field grown Capsicum annuum cv. Vinedale. We are aware of no information on field fruit-manganese. Similar differences existed with regard to fruit iron from field cultivated chile in Spain (Martinez-Sanchez et al., 1989), although the differences for manganese and zinc were smaller.

Besides differences in fruit nutrient status likely introduced by cultivar, environment, culture medium, and crop management, there is also the issue of frequent differences in the protocols used for cleaning, grinding, digestion and analysis of the fruit samples.

### Conclusions

Low iron levels (1 and 3 µM Fe) decreased the pigment accumulation of leaves (at early season only), while 3 µM Fe reduced total chlorophyll and carotenoids of green fruit at final harvest. However, the total extractable pigments of red fruits and their surface color remained unaffected by iron treatment.

Iron supply favorably impacted leaf Fe and Fe**, while reducing copper, phosphorus and zinc leaf concentrations. Similarly, higher solution and fruit iron were associated with low copper.

Differences in leaf ferrous iron among the treatments were detected only after the iron shortage (106 d). At this date, ferrous iron, total chlorophyll and total carotenoids were all lower in the low-iron treatments (1 and 3 µM Fe) than in the high-iron ones (10 and 30 µM Fe).

The intentional suppression of iron to treatments 1 and 3 µM Fe during 10 d (days 96 to 106) to test the system for iron stress induced short-term effects on leaf pigment accumulation. Absence of chlorosis indicates that plants acclimatize new tissue growth rapidly to minimize tissue nutrient deficiencies.

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