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# Effect of Iron on the Transport of Citrate into the Xylem of Soybeans and Tomatoes

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## ABSTRACT

**Iron transport in soybeans (*Glycine max* [L] Merr.) and tomatoes (*Lycopersicum esculentum*) is controlled by factors that are altered manyfold as the plant experiences an iron stress (deficiency). Depending on their response to an Fe stress, plants in this study are classed (*a*) Fe-inefficient or (*b*) Fe-efficient. The Fe-efficient plants transport more Fe and concomitantly more citrate than the Fe-inefficient plants.**

**An available supply of Fe added to the plant root will facilitate the release of citrate to the stem exudate in both soybeans and tomatoes. Also, when the iron supply is decreased by trapping $Fe^{2+}$ at the root, Fe transport decreases with a concomitant decrease of citrate in the stem exudate. Factors other than citrate appear to affect movement of Fe from the external solution into root cells where Fe is chelated with citrate and moves thereafter as Fe citrate. This makes some of the citrate that is transported in the stem exudate dependent on the amount of Fe made available at the root.**

Iron deficiency in soybeans promotes the accumulation of citrate in the roots (2), and subsequent Fe transport (3), with maximal citrate accumulation being associated with maximal Fe stress in efficient varieties (13). This suggests that the citrate accumulating in the root may increase uptake and translocation of Fe by chelating Fe as Fe-citrate. Tiffin (10, 11, 12) studied the translocation of Fe in soybean, sunflower, cucumber, and tomato stem exudates and found that the electrophoretic migration of Fe as Fe-citrate was the same for all these plant species. His data indicate that Fe is chelated with citric acid and subsequently translocated as Fe-citrate in intact plants.

Iron and citrate transport in the stem exudate were strikingly parallel (3). Increase of iron in stem exudate paralleled an increase in translocation of citrate from the roots. Conversely, a decrease of iron in stem exudate was associated with a decrease of citrate in the exudate. However, the quantities of Fe and citrate translocated were not directly related to the citrate and Fe found in the root sap (2). These data suggest that Fe affects the amount of citrate transported into the xylem transport system. The objectives of this study were to clarify the role of citrate in Fe transport and in the Fe stress response of soybeans and tomatoes.

## MATERIALS AND METHODS

Iron-inefficient (highly susceptible to Fe deficiency) and -efficient (low susceptibility to Fe deficiency) soybeans (5, 13) and tomatoes (4) were used as test plants. Hawkeye (HA) soybean and T3238Fe(T-Fe) tomato are Fe-efficient and PI 54619-5-1 (PI) soybean and T3238fe(T-fe) tomato are Fe-inefficient. The plants were grown in a light chamber at 23 ± 2 C with 16 hr of light (1500 ft.-c) and 8 hr of dark.

Soybean and tomato seed were germinated for 72 hr between moist cheesecloth placed on stainless steel screens. Forty 3-day-old seedlings were transferred to holes in a plastic ring supported on a 10-liter battery jar that contained a one-fifth Steinberg solution (9) with 3 mg/liter P, but no Fe. T3238Fe tomato is known to be boron-inefficient; therefore, adequate boron (0.52 mg/liter) was supplied to the solution culture. When the plants were 7 days old, they were transferred to a "prenutrient solution" which was a conditioning treatment that preceded their transfer to a new solution (absorption nutrient) from which Fe uptake studies were made. For these studies soybeans were grouped 12 plants per bundle (2 bundles per treatment), whereas 4 separate tomato plants were used per treatment. Soybean and tomato plants were used for absorption experiments on their 18th and 30th day of growth, respectively.

In exudate studies, the roots were washed in demineralized water and then placed in 1 liter of absorption nutrient before they were topped. The cut ends of the stems were placed in half-inch Tygon tubing that extended into a glass vial in an ice bath. Exudate was collected in the dark for 20 hr at 24 C.

**Nutrient Cultures.** Two separate cultures were used: (*a*) "prenutrient" and (*b*) "absorption nutrient." In the prenutrient, plants were grown for an extended period of time to develop different degrees of Fe stress (Fe deficiency) in the plants. These plants were transferred to the absorption nutrient where absorption and subsequent transport of Fe were determined. The initial pH of the nutrient cultures was 6.3 and it was not adjusted during the course of the experiments.

*Prenutrient.* The prenutrient is a modified one-fifth Steinberg nutrient (9) containing in mg/liter: 76 Ca, 58 K, 11 Mg, 76 N (20 as $NH_4$ and 56 as $NO_3$), 2 P (3 P for tomato), 10 S, 9 Cl, 0.28 Mn, 0.17 B, 0.09 Zn, 0.02 Cu, 0.02 Mo, and variable Fe as FeEDDHA[1] for soybeans and variable FeHEDTA for tomatoes. Boron was increased in all tomato cultures to 0.52 mg/liter.

*Absorption Nutrient.* The absorption nutrient contained in mg/liter: 51 Ca, 41 K, 6.6 Mg, 52 N (13 as $NH_4$ and 39 as $NO_3$), 3.5 P, 0.14 Mn, 0.20 B, 0.032 Zn, 0.08 Mo, and 0.65 Fe as FeEDDHA for soybeans and variable Fe as FeEDDHA and FeHEDTA for tomatoes. The amounts of $^{59}Fe$ used in some experiments are given in the specific details for each experiment.

**Analysis.** Iron was determined by the *o*-phenanthroline

[1] Abbreviations: FeEDDHA: Fe-ethylenediamine di(*o*-hydroxyphenylacetic acid); FeHEDTA: Fe-hydroxyethylethylenediaminetriacetic acid; BPDS: bathophenanthrolinedisulfonate.

method (8) and citrate by the pentabromoacetone method (7). Root sap was obtained by freezing 10 g of roots overnight, allowing them to thaw partially, and then expressing the sap with a Carver press. The roots were washed in demineralized water for 1 min before they were frozen. Radioactive iron in root sap, exudate, and absorption nutrient was determined with a gas flow counter. Some exudate samples were too small to determine both citrate and Fe colorimetrically; thus $^{59}$Fe was measured and assumed to be a measure of the Fe being transported. Calculations were based on the specific activity of the $^{59}$Fe that was added to the absorption nutrient. Past experiments (3) indicate that Fe transported in xylem exudate above 6 $\mu$M is a measure of the Fe transported from the absorption nutrient. Three types of experiments were used to vary the amount of Fe transported in the plant.

**Experiment 1. Varied Fe Stress in PI and HA Soybeans.** PI and HA soybeans were grown at four levels of FeEDDHA (0.0, 0.25, 1.0 and 5.0 mg of FeEDDHA per liter) to produce plants having different degrees of Fe stress ranging from chlorotic to green. Eighteen-day-old plants were transferred to an absorption nutrient containing 0.56 mg of Fe + 20 $\mu$c $^{59}$Fe per liter as $^{59}$FeEDDHA (specific radioactivity, 750 cps/$\mu$g Fe) or no Fe. The plants were topped and stem exudate collected at 0 to 3, 3 to 6, 6 to 10, and 10 to 20 hr.

No attempt was made to relate citrate and Fe quantitatively, but only to show the relative amounts of citrate in stem exudate to that of $^{59}$Fe. One set of treatments received no Fe in the absorption nutrient to serve as a measure of the amount of citrate transported when external Fe was not available for transport. These treatments also served as a check regarding any residual Fe that might be transported from within the root.

**Experiment 2. Increasing Amounts of Iron to Tomatoes under Fe Stress.** Seventeen-day-old green T3238fe and T3238Fe plants (4) were transferred to prenutrient containing 0.2 $\mu$M FeHEDTA and grown for 5 days to produce an Fe stress. By this time all plants showed chlorosis and they were harvested. In harvesting, the plants were topped, the roots were placed in absorption nutrient, and stem exudate was collected. Stem exudate was collected at 0- to 4-, 4- to 8-, and 8- to 18-hr intervals from plants in absorption nutrient with Fe as FeHEDTA at 0.0, 0.2, 1, 10, and 40 $\mu$M, and Fe as FeEDDHA at 0.0, 1, 10, 30, and 60 $\mu$M. The specific radioactivity of the $^{59}$Fe (cps/m$\mu$mole Fe) in each treatment is shown in Table IV.

**Experiment 3. Ferrous Iron Trapped with BPDS.** This experiment was conducted exactly as experiment 2 except BPDS was added to a treatment containing 10 $\mu$M $^{59}$FeHEDTA. BPDS was used to trap ferrous iron at the root, which decreased the uptake of Fe and the quantity of Fe and citrate transported by the plant. BPDS forms a highly stable ferrous chelate, $Fe^{2+}(BPDS)_3$, log K = 21.8, which was used by Chaney, Brown, and Tiffin (unpublished data) to show that chemical reduction is an obligatory process in the utilization of iron from ferric chelates. The ferrous chelate is a colored solution with an absorption maximum at 535 nm; it is very soluble in water and strongly ionic.

## RESULTS

**Experiment 1. Varied Fe Stress in PI and HA Soybeans.** We are interested in the Fe that is absorbed and transported by the plant. If we take zero Fe in the prenutrient as maximal stress and 5.0 mg/liter FeEDDHA as minimal stress (Fig. 1), PI soybean translocates most $^{59}$Fe at minimal stress while HA soybeans transport most $^{59}$Fe at maximal stress (Fig. 1). But note, in each case, maximal transport of citrate is related to maximal transport of $^{59}$Fe. Where $^{59}$FeEDDHA was not added to the absorption nutrient, Fe stress had no effect on transport of citrate.

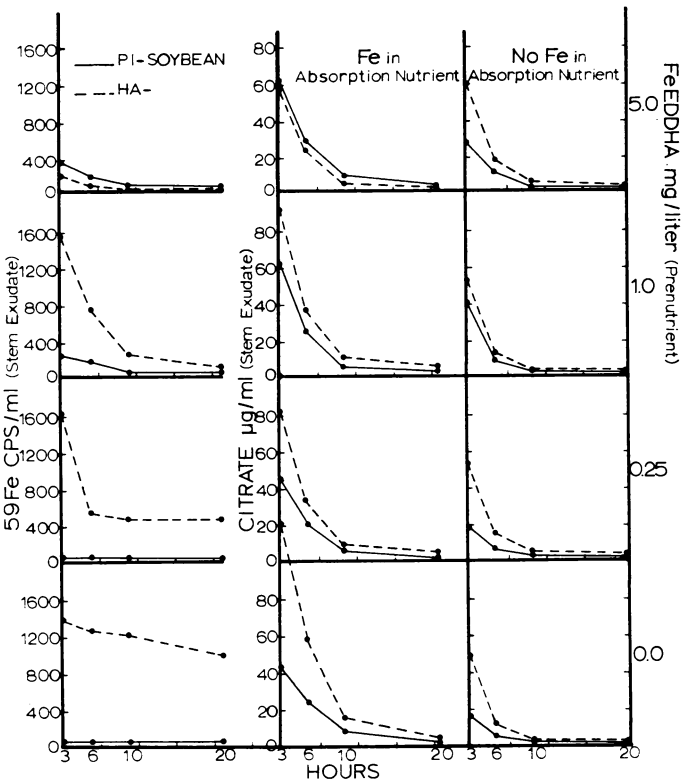


FIG. 1. Effect of Fe concentration in the prenutrient (varied Fe stress) on subsequent Fe transport and concomitant citrate transport in the stem exudate, with and without added Fe in the absorption nutrient of PI (Fe-inefficient) and HA (Fe-efficient) soybeans.

Table I. *Iron Concentration in Root Sap and Residue, and Citrate Concentration in Root Sap of PI and HA Soybeans at Different Degrees of Fe Stress, with and without Fe in Absorption Nutrient*

Absorption period was 20 hr.

| Treatments | | Root Sap | | | | Root Residue | |
|---|---|---|---|---|---|---|---|
| Prenutrient FeEDDHA | Absorption nutrient | Citrate | | $^{59}$Fe | | Fe | |
| | | PI | HA | PI | HA | PI | HA |
| *mg/liter* | | *µg/ml* | | *cps/ml* | | *µg/g dry wt* | |
| 0.00 | No Fe | 237 | 497 | None | | 130 | 109 |
| 0.25 | No Fe | 213 | 527 | None | | 130 | 125 |
| 1.00 | No Fe | 203 | 363 | None | | 196 | 142 |
| 5.00 | No Fe | 250 | 330 | None | | 318 | 297 |
| 0.00 | Fe[1] | 183 | 343 | 962 | 2463 | 347 | 318 |
| 0.25 | Fe[1] | 110 | 247 | 1047 | 1769 | 330 | 364 |
| 1.00 | Fe[1] | 140 | 280 | 1110 | 1571 | 385 | 359 |
| 5.00 | Fe[1] | 173 | 203 | 999 | 944 | 489 | 468 |

[1] Absorption nutrient contained 0.56 mg of Fe + 20 $\mu$c $^{59}$Fe as FeEDDHA per liter (specific radioactivity, 750 cps/$\mu$g Fe).

Thus, Fe transport increases citrate transport (Fig. 1), although the data do not indicate a quantitative relationship. Plants with maximal stress may lose more of the Fe in transit to the Fe-deficient plant parts than do less Fe-deficient plants. This may affect the ratio of Fe to citrate appearing in the stem exudate.

Iron concentration in the root was not a limiting factor in Fe transport because PI root residue contained as much Fe as HA root residue (Table I). Citrate was consistently higher in the

Table II. *Volumes of Exudate (with Time) as Affected by Fe Stress (Fe Deficiency) in PI and HA Soybean*

| Treatment: Prenutrient FeEDDHA | Exudate of PI Soybean | | | | | Exudate of HA Soybean | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0–3 hr | 3–6 hr | 6–10 hr | 10–20 hr | Total | 0–3 hr | 3–6. hr | 6–10 hr | 10–20 hr | Total |
| *mg/liter* | *ml* | | | | | *ml* | | | | |
| No Fe in absorption nutrient | | | | | | | | | | |
| 0.00 | 4.5 | 3.7 | 4.5 | 3.3 | 16.0 | 9.0 | 8.5 | 10.5 | 8.0 | 36.0 |
| 0.25 | 11.5 | 12.0 | 12.5 | 9.0 | 45.0 | 9.0 | 8.5 | 12.5 | 12.0 | 42.0 |
| 1.00 | 10.0 | 12.0 | 14.0 | 9.0 | 45.0 | 16.0 | 15.0 | 14.0 | 12.5 | 57.5 |
| 5.00 | 14.0 | 14.0 | 15.0 | 13.0 | 56.0 | 14.5 | 14.0 | 13.0 | 11.0 | 52.5 |
| $^{59}$FeEDDHA added (0.56 mg Fe/liter) | | | | | | | | | | |
| 0.00 | 2.4 | 3.1 | 4.5 | 5.5 | 15.5 | 6.5 | 7.0 | 8.5 | 10.5 | 32.5 |
| 0.25 | 4.0 | 6.5 | 8.5 | 10.5 | 29.5 | 9.2 | 9.5 | 10.5 | 12.5 | 41.7 |
| 1.00 | 4.0 | 5.0 | 6.5 | 6.0 | 21.5 | 11.5 | 11.0 | 10.0 | 11.0 | 43.5 |
| 5.00 | 4.0 | 7.5 | 9.0 | 11.0 | 31.5 | 9.5 | 9.5 | 11.0 | 10.0 | 40.0 |

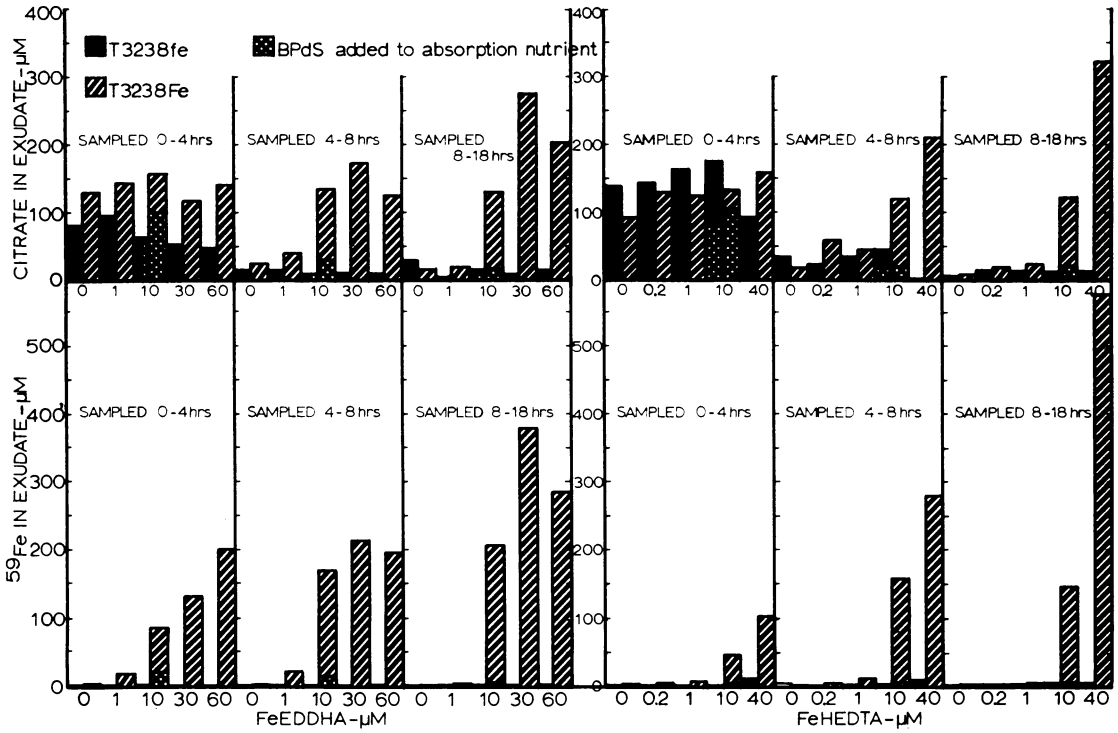


FIG. 2. Effect of increasing Fe in the absorption nutrient on Fe transport and concomitant citrate transport in the stem exudate of T3238fe (Fe-inefficient) and T3238Fe (Fe-efficient) tomatoes under an Fe stress. In one treatment, BPDS was added to trap $Fe^{2+}$ at the root.

root sap of HA than PI, with the concentration highest in the HA plants of the two most Fe-deficient treatments (Table I). All the plants were approximately the same size at the time of harvest. HA soybeans under maximal Fe stress transported more $^{59}$Fe, for a greater length of time, than plants under less Fe stress (Fig. 1). Most of the $^{59}$Fe, citrate (Fig. 1), and volume of exudate (Table II) were transported in the first 10 hr.

**Experiment 2. Increasing Amounts of Iron to Tomatoes under Fe Stress.** Treatments for tomatoes were different from those for soybeans in experiment 1. All tomato plants were under approximately the same degree of Fe stress, but soybeans were studied under different levels of Fe stress. In the latter, the amount of Fe transported was determined by Fe stress. But in tomatoes, increasing amounts of Fe were added to the absorption nutrient in order to increase Fe transport. When more $^{59}$Fe was transported, there was a concomitant increase in transport of citrate in the 4- to 8-hr samples and 8- to 18-hr samples for T3238Fe (Fig. 2). Citrate transported in the first 4 hr showed no relationship to the $^{59}$Fe transported during this time. Both $^{59}$Fe and citrate were at relatively low concentrations in the stem exudate of T3238fe tomato (Fig. 2). In most cases the volume of exudate was greater from T3238Fe than from T3238fe plants (Table III) and greater with than without BPDS for T3238Fe.

T3238Fe root sap contained approximately five times more citrate than T3238fe root sap (Table IV), but the citrate concentration in the roots did not appear related to the citrate transported in the stem exudate (Fig. 2). Likewise, Fe concentration in the root residue (Table IV) was not related to the Fe transported (Fig. 2).

**Experiment 3. Ferrous Iron Trapped with BPDS.** Ferrous iron produced at the root was trapped by adding BPDS to the

Table III. *Stem Exudate Collected at Indicated Time Intervals from T3238fe (Fe-inefficient) and T3238Fe (Fe-efficient) Tomatoes at Different Concentrations of FeHEDTA and FeEDDHA with BPDS in One of the 10 μM Treatments*

| Absorption Nutrient | 0–4 hr T3238 | | 4–8 hr T3238 | | 8–18 hr T3238 | | Total T3238 | |
|---|---|---|---|---|---|---|---|---|
| | fe | Fe | fe | Fe | fe | Fe | fe | Fe |
| | *ml* | | *ml* | | *ml* | | *ml* | |
| FeHEDTA-μM | | | | | | | | |
| 0.0 | 3.1 | 8.4 | 1.9 | 11.0 | 4.0 | 27.6 | 9.0 | 47.0 |
| 0.2 | 6.5 | 10.4 | 3.4 | 11.3 | 7.6 | 30.1 | 16.9 | 51.8 |
| 1.0 | 2.6 | 8.4 | 1.6 | 8.7 | 3.2 | 24.0 | 7.4 | 41.1 |
| 10 | 5.1 | 11.2 | 2.8 | 8.1 | 5.6 | 21.5 | 13.5 | 40.8 |
| 10 + BPDS[1] | 5.8 | 13.1 | 3.5 | 16.1 | 4.7 | 29.8 | 14.0 | 59.0 |
| 40 | 8.1 | 8.0 | 4.5 | 8.0 | 6.6 | 14.1 | 19.2 | 30.1 |
| FeEDDHA-μM | | | | | | | | |
| 0.0 | 7.3 | 5.8 | 4.5 | 6.9 | 5.1 | 19.8 | 16.9 | 32.5 |
| 1.0 | 7.7 | 8.2 | 4.2 | 9.4 | 5.3 | 27.0 | 17.2 | 44.6 |
| 10 | 8.5 | 7.5 | 6.4 | 7.3 | 7.4 | 11.9 | 22.3 | 26.7 |
| 10 + BPDS[1] | 7.8 | 7.7 | 6.5 | 8.6 | 8.4 | 24.3 | 22.7 | 40.6 |
| 30 | 6.6 | 9.3 | 6.0 | 8.7 | 11.5 | 14.8 | 24.1 | 32.8 |
| 60 | 8.4 | 8.2 | 7.6 | 8.4 | 11.5 | 15.4 | 27.5 | 32.0 |

[1] BPDS was 10% in excess of the Fe.

absorption nutrient. Iron trapped was less available for transport, causing decreased transport of $^{59}Fe$ and a concomitant decrease of citrate transported in T3238Fe tomato (Table V and Fig. 2). Citrate concentration in root sap was approximately the same with or without BPDS (Table IV). Most of the $Fe^{2+}(BPDS)_3$ remained in the absorption nutrient. When BPDS was not added to the absorption nutrient, T3238Fe removed 92 and 96% of the $^{59}Fe$ from $^{59}FeHEDTA$ and $^{59}FeEDDHA$, respectively; but when BPDS was added, only 17 and 9% of the Fe were removed. In trapping ferrous iron, BPDS also decreases the amount of $^{59}Fe$ incorporated into the root sap (Table IV). T3238fe translocated much less $^{59}Fe$ in its stem exudate than T3238Fe, with or without BPDS (Fig. 2).

These data support the hypothesis that Fe somehow moves from the external solution into the root cells where it is chelated with citrate and then translocated within root cells to the xylem for transport to the above-ground plant parts. Further study is needed to show how Fe moves into the root and exactly where Fe is chelated as Fe citrate.

## DISCUSSION

The data in this study show that Fe affects the release of citrate to the stem exudate of HA and PI soybeans and T3238Fe tomatoes. This was shown in several ways.

**Varied Fe Stress in PI and HA Soybeans.** PI and HA soybeans responded differently to Fe stress. HA transports most Fe at near maximal stress whereas PI transports most Fe at near minimal stress. In each case, where Fe transport increased there was a relative increase in transport of citrate. Fe-stress had no effect on citrate transport if Fe was not added to the absorption nutrient. This indicates that something other than citrate causes Fe to move into the root. When Fe movement into the root occurs, Fe-citrate is formed and it is transported into the xylem.

**Increasing Amounts of Fe to Tomatoes under Fe Stress.** Instead of treatments to produce a series of plants at different

Table V. *Decrease of Citrate and $^{59}Fe$ Concentrations in Stem Exudate of T3238Fe Tomato When $^{59}Fe^{2+}$ Was Trapped at Root by BPDS*

BPDS was 10% in excess of the Fe.

| Collection Intervals | Control | | | BPDS Added | | |
|---|---|---|---|---|---|---|
| | Citrate | $^{59}Fe$ | Exudate | Citrate | $^{59}Fe$ | Exudate |
| *hr* | *μM* | *μM* | *ml* | *μM* | *μM* | *ml* |
| $^{59}FeHEDTA$ (10 μM) | | | | | | |
| 0–4 | 130 | 42 | 11.2 | 99 | 0.50 | 13.1 |
| 4–8 | 115 | 161 | 8.1 | 16 | 0.50 | 16.1 |
| 8–18 | 120 | 147 | 21.5 | 16 | 0.52 | 29.8 |
| $^{59}FeEDDHA$ (10 μM) | | | | | | |
| 0–4 | 156 | 89 | 7.5 | 130 | 23 | 7.7 |
| 4–8 | 135 | 176 | 7.3 | 36 | 17 | 8.6 |
| 8–18 | 130 | 213 | 11.9 | 16 | 3 | 24.3 |

Table IV. *Effect of Increasing Iron and Addition of BPDS in Absorption Nutrient on Iron Concentration in Root Sap and Root Residue, and Citrate Concentration of Root Sap of T3238fe and T3238Fe Tomatoes*

| Absorption Nutrient | | T3238fe | | | | T3238Fe | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Root sap | | | Root residue Fe | Root sap | | | Root residue Fe |
| $^{59}Fe$ Treatment | Specific radio activity $^{59}Fe$ | Volume | Citrate | $^{59}Fe$ | | Volume | Citrate | $^{59}Fe$ | |
| *μM* | *cps/mμmole* | *ml* | *mM* | *μM* | *μg/g* | *ml* | *mM* | *μM* | *μg/g* |
| $^{59}FeEDDHA$ | | | | | | | | | |
| 0.0 | ... | 5.7 | 0.91 | ... | 297 | 15.7 | 8.73 | .... | 138 |
| 1.0 | 320.1 | 7.5 | 1.04 | 3.1 | 276 | 13.3 | 6.77 | 4.2 | 138 |
| 10.0 | 49.3 | 8.7 | 1.07 | 5.6 | 305 | 13.0 | 4.90 | 35.3 | 230 |
| 10.0 + BPDS | 51.9 | 7.8 | 0.89 | 1.8 | 247 | 15.5 | 5.37 | 3.8 | 180 |
| 30.0 | 19.8 | 13.0 | 0.86 | 8.0 | 222 | 20.0 | 6.51 | 101.7 | 447 |
| 60.0 | 9.89 | 10.6 | 1.07 | 14.2 | 322 | 18.7 | 8.86 | 143.0 | 502 |
| $^{59}FeHEDTA$ | | | | | | | | | |
| 0.0 | ... | 6.4 | 1.69 | ... | 263 | 16.0 | 6.15 | ... | 130 |
| 0.2 | 556.7 | 13.1 | 1.51 | 2.8 | 263 | 17.0 | 6.62 | 2.6 | 130 |
| 1.0 | 312.4 | 7.1 | 1.77 | 3.5 | 222 | 20.3 | 5.42 | 3.5 | 130 |
| 10.0 | 58.1 | 11.0 | 1.28 | 8.0 | 255 | 16.9 | 5.21 | 15.3 | 188 |
| 10.0 + BPDS | 58.3 | 7.1 | 0.81 | 1.7 | 247 | 19.0 | 5.05 | 1.5 | 138 |
| 40.0 | 16.0 | 11.8 | 0.94 | 16.8 | 372 | 14.5 | 7.35 | 178.3 | 585 |

degrees of Fe stress (soybean experiments), all the tomato plants were chlorotic with about the same Fe stress. Increasing amounts of Fe supplied in the absorption nutrient were used to increase Fe flow through the plant. But T3238fe was so Fe-inefficient under all conditions of these experiments as to make any comparison between T3238fe and T3238Fe tomato impractical. After 4 hr of collecting exudate, very little Fe or citrate was translocated in T3238fe. In contrast, T3238Fe plants, given the same treatments, translocated relatively large quantities of Fe and citrate. As Fe transport was increased by increasing Fe concentration on the absorption nutrient, the amount of citrate released to the stem exudate was also increased.

**Ferrous Iron Trapped with BPDS.** According to Chaney, Brown, and Tiffin (unpublished data), before iron from ferric chelates can be utilized efficiently it must be reduced at the root. Trapping ferrous iron with BPDS reduced the supply of Fe available for transport and caused a reduction in the transport of citrate in the stem exudate. Again, the amount of citrate released to the stem exudate was related to Fe flow.

In a previous study (3), both Zn and azide interfered with Fe translocation, and there was a corresponding decrease of citrate in the stem exudate.

The ratio of citrate to Fe has been observed to vary depending upon Fe stress in the plant (10), concentration of Fe in the absorption nutrient, and the time of sampling (11). It is doubtful that citrate/Fe ratios below 1 would be observed in nature, but this can occur, as in this study, when an Fe-efficient plant under Fe stress is given Fe. Data in this study indicate that the level of citrate in the root does not regulate the movement of Fe in the root. Thus, some other factor (5) must be responsible, such as reduction of $Fe^{3+}$ to $Fe^{2+}$ at the root aided by an efflux of hydrogen ions. Ambler *et al.* (1) observed reduction of $Fe^{3+}$ to $Fe^{2+}$ to be greatest between the regions of root elongation and root maturation of young lateral soybean roots. Tests for $Fe^{2+}$ showed reduced iron throughout the protoxylem of the smaller root to where it joined the metaxylem of the larger root.

Other studies (2–6) give more detail concerning plant factors that contribute favorably to Fe transport in Fe-efficient plants: (*a*) exudation of hydrogen ions into the growth medium, (*b*) reduction of $Fe^{3+}$ to $Fe^{2+}$ at the root, (*c*) accumulation of citrate in the root and the transport of Fe as Fe-citrate, and (*d*) a concomitant decrease of P in the root sap. Each of these factors needs to be characterized in order to understand the mechanism of Fe absorption and transport.

This study indicates that translocation of Fe in the plant involves more than citrate chelation of Fe in the root *per se*. Iron appears to move or be carried into the root after being reduced and separated from the chelating agent by the root. Here, some Fe is chelated by citrate, making some of the citrate that is transported into the xylem dependent on the amount of Fe made available by the root.

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