Iron Translocation
II. Citrate/Iron Ratios in Plant Stem Exudates

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Summary. Stem exudates of sunflower, soybean, cucumber, and tomato were analyzed for citrate and iron content. Generally, the lowest iron treatments given at decapitation were associated with lowest citrate levels in the exudate and intermediate treatments with the highest citrate levels. Citrate reached 6.2×10^{-4} M in sunflower exudate, 7.3×10^{-4} M in soybean, 9.4×10^{-4} M in cucumber, and 1.8×10^{-4} M in tomato. Some of these values represent a doubling or tripling of citrate when compared to the lowest ones obtained. High Fe depressed the citrate content of exudate in some cases.

As iron was raised in the nutrient, the increases of iron in the exudate were proportionately greater than those of citrate. The highest Fe treatments led to exaggerated uptake of iron by sunflower. In some cases iron was translocated in molar excess of citrate. A time-response experiment with tomato showed a rapid flooding of iron into the roots but a relatively slow release of iron into xylem exudate.

Similar electrophoretic patterns of iron were obtained despite changes in citrate and iron with time. Exudates from sunflower, cucumber, and tomato gave iron concentrations in the position of iron-citrate.

In the previous paper (2) it was shown that preculture had pronounced effects on the iron and citrate content of sunflower exudate. But despite these effects, the analysis of exudate from both green and chlorotic plants indicated that iron was carried by citrate.

The effect of iron supplied in the exudation period is considered in the present paper. The plant groups in each experiment were precultured alike but were given different iron treatments at decapitation. Assays were carried out to determine citrate/iron ratios in the exudates.

Another consideration has been the length of the exudation period. The primary concern was whether citrate and iron change drastically as exudation proceeds. In experiments with tomato, the exudates were collected at several intervals to determine citrate and iron variability with time.

Materials and Methods

The experimental plants were soybean, *Glycine max* L., Merr., var. Hawkeye; sunflower, *Helianthus annuus* L. var. Greystripe; cucumber, *Curcurbita sativas* L. var. Burpee's Sunnybrook; and tomato, *Lycopersicon esculentum* Mill., var. Marglobe.

Exudate sampling and analytical procedures given previously (2) were employed here. Eight liters of standard nutrient (2) were used in the extended growth period after germination and up to the time of decapitation. This nutrient contained Fe as 10^{-6} M FeEDDHA. The absorption nutrient is the standard nutrient with Fe concentration varied as shown in the tables.

Experiments and Results

Sunflower Experiments. Table I shows the distribution of iron and citrate in 20-hour exudation periods of sunflower. The nutrient iron probably was limiting in the first 2 treatments of experiment 1 and the first treatment of experiment 2. This was suggested by the relatively higher percentages of iron left in the nutrient after 20 hours. Associated with the low iron supply were the low exudate iron levels, low citrate levels, and high citrate/iron ratios.

In general, intermediate iron treatments were associated with highest citrate production. The results show clearly that up to certain limits of nutrient iron it is possible to triple citrate concentration in the exudate. The highest treatments depressed the citrate content of the exudates and gave citrate/iron ratios near unity or considerably lower. Other than the quantitative tests (table I), the only attempt to characterize these exudates was by electrophoresis. The radiograph (fig 1) shows 4 samples from the second experiment (table I). The streaking of radio-iron on the paper was associated with the low citrate/

iron ratios. Probably a less reactive support than paper will be necessary to resolve the binding patterns of samples that contain excess iron. The iron spots closest to the anode are in the position of iron citrate.

Soybean Experiment. Results from soybean (table II) were similar to those reported for sunflower. Nutrient iron probably was limiting up to the fourth level (5×10^{-6} M). This is suggested by the quantities of iron left in the nutrient. Plant group 4 left only 10% of the iron after 20 hours. A fairly consistent relationship was noted in the ability of the roots to translocate iron at concentrations considerably above those of the nutrient. The ratio of exudate Fe/nutrient Fe was 20 for group 1. The other groups gave ratios from 29 ot 36. Exudate with the highest concentration of iron still contained citrate in molar excess.

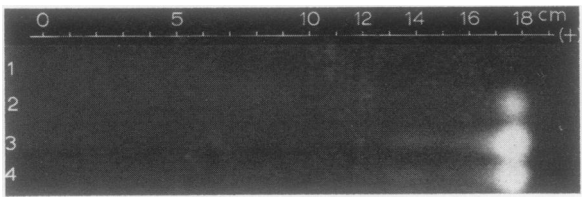

FIG. 1. Electrophoretic distribution of ^{59}Fe in sunflower stem exudates. The 4 exudates are characterized in table I, experiment 2. Electrophoretic conditions: Whatman No. 3 paper, 0.05 M acetate buffer, pH 5.4, 450 v, 2.5 hours.

Cucumber Experiment. Results from cucumber are not given in detail because of similarities to sunflower and soybean. The plants were given iron treatments from 1 to 50×10^{-6} M FeEDDHA. Iron in the exudate ranged from 6.7 to 235×10^{-6} M. Citrate was from 5.1 to 9.4×10^{-4} M. The citrate/iron ratios were from 76 down to 4.

Table I. *Effect of Variable Fe Supply on the Distribution of Fe and Citrate in 20-Hour Exudation Periods of Sunflower*

Plant age (days) and treatment (expt 1): 0, germination; 3, seedlings into standard nutrient and shaded; 6, groups into full light and standard nutrient; 13 and 20, renewed nutrient; 25, transferred each group (3 plants) to 1 liter absorption nutrient (1 μc $^{59}Fe/\mu$mole Fe) and decapitated. Preculture in both experiments was identical, but plants in experiment 2 were decapitated the twenty-sixth day and received 0.5 μc $^{59}Fe/\mu$mole Fe.

Plant group	Absorption nutrient FeEDDHA	Fe left in nutrient	Nutrient Fe into exudate	Exudate volume	Fe in exudate	Citrate in exudate	Ratio: citrate / Fe
	M $\times 10^{-6}$	%	%	ml	M $\times 10^{-6}$	M $\times 10^{-4}$	
Expt 1							
1	1.0	33	24	29	8	2.0	25.0
2	2.5	35	47	36	33	3.6	10.9
3	5.0	7	62	49	63	1.9	3.0
4	7.5	12	54	18	225	6.0	2.7
5	10.0	15	49	26	190	6.0	3.1
6	25.0	33	35	21	425	5.0	1.2
7	50.0	60	21	22	465	4.6	1.0
Expt 2							
1	1	39	12	21	6	2.0	33.3
2	10	9	52	23	220	3.0	1.3
3	50	41	38	18	1050	6.2	0.6
4	100	51	18	21	870	3.6	0.4

Table II. *Effect of Variable Fe Supply on the Distribution of Fe and Citrate in 20-Hour Exudation Periods of Soybean*

Plant age (days) and treatment: 0, germination; 3, seedlings into standard nutrient and shaded; 17, groups into full light and standard nutrient; 18, transferred each group (10 plants) to 1 liter absorption nutrient (5 μc $^{59}Fe/\mu$mole Fe) and decapitated.

Plant group	Absorption nutrient FeEDDHA	Fe left in nutrient	Nutrient Fe into exudate	Exudate volume	Fe in exudate	Citrate in exudate	Ratio: citrate / Fe
	M $\times 10^{-6}$	%	%	ml	M $\times 10^{-6}$	M $\times 10^{-4}$	
1	0.5	33	14	7.0	10	4.0	40
2	1.0	18	28	9.3	30	3.8	12.7
3	2.5	13	41	12.8	80	5.0	6.3
4	5.0	10	48	13.4	180	5.7	3.2
5	7.5	28	35	11.0	240	7.3	3.0
6	10.0	32	35	12.3	290	7.1	2.5

Table III. *Distributions of Fe and Citrate in an Exudation Period of Tomato*

Plant age (days) and treatment: 0, germination; 7, seedlings into standard nutrient and shaded; 7, plants into full light and standard nutrient; 25 and 31, renewed nutrient; 36, transferred 2 plants into 1.9 liters absorption nutrient (2.5×10^{-6} M FeEDDHA, 4 μc ^{59}Fe/μmole Fe) and decapitated.

Sampling time	Absorption nutrient Fe	Nutrient Fe into exudate*	Nutrient Fe into exudate (cumulative)	Fe in exudate	Citrate in exudate	Ratio: citrate / Fe
hr	%	%	%	M × 10^{-6}	M × 10^{-6}	
0	100					
3	19	10.1	10.1	20.0	120	6.0
5	7	12.3	22.4	24.3	140	5.8
8	3	12.4	34.8	24.5	110	4.5
12	1	8.7	43.5	17.3	20	1.2
17	1	4.8	48.3	9.5	6	0.6
22.5	1	3.2	51.5	6.3	7	1.1

* Exudate volume for each collection was 24 ml.

Table IV. *Distributions of Citrate and Fe in Exudates of Tomato Plants Treated with Different Nutrient Fe Levels*

Plant age (days) and treatment: 0, germination; 6, seedlings into standard nutrient and shaded; 12, plants into full light and standard nutrient; 22 and 29, renewed nutrient; 35, transferred 8 plants to 8 liters absorption nutrient (5 μc ^{59}Fe/μmole Fe, group 1; 1 μc ^{59}Fe/μmole Fe, group 2) and decapitated.

	Group 1 Treatment: 2×10^{-6} M FeEDDHA				Group 2 Treatment: 10×10^{-6} M FeEDDHA			
Exudate sampling time	Exudate volume	Exudate citrate	Exudate Fe	Ratio: citrate / Fe	Exudate volume	Exudate citrate	Exudate Fe	Ratio: citrate / Fe
hr	ml	M × 10^{-5}	M × 10^{-5}		ml	M × 10^{-5}	M × 10^{-5}	
3	47	10.8	1.5	7.2	41	11.0	4.8	2.3
6	55	11.1	4.5	2.4	50	13.5	12.5	1.1
9	45	10.9	6.6	1.6	39	13.2	18.8	0.70
12	51	9.0	4.6	1.9	41	14.0	18.4	0.76
15	49	6.0	3.1	1.9	40	14.8	17.3	0.85
18	42	4.8	2.2	2.1	40	15.0	14.7	1.0
21	46	5.0	1.1	4.5	38	16.3	11.8	1.4
24	38	4.8	0.9	5.3	31	14.5	9.7	1.5
27	24	2.8	0.9	3.1	21	9.6	9.5	1.0

Tomato Experiments. Table III shows results from a tomato experiment in which exudate was collected at 6 intervals. The plants lowered nutrient iron rapidly. Less than 10 % remained at 5 hours. This pattern indicates flooding of root iron pools. Of the total nutrient iron (zero time) 81 % had moved into the roots at 3 hours, 10 % had moved into the exudate and, by difference, 71 % was left in the roots. In contrast to the rapid uptake by the roots, the release into the xylem was relatively slow.

The concentration of iron in the exudate held at about 24×10^{-6} M (ca. 10-fold over the original nutrient) for 8 hours and then decreased to about one-fourth that value. About one-half the total iron supplied to the roots was in the exudate at the end of the experiment.

Citrate reached a peak in the second collection, but in the final collection was about one-twentieth its highest concentration. Citrate concentrations declined more rapidly than iron. This was reflected in the drop in citrate/iron values from 6 to approximately 1.

These results suggested that a higher initial supply of iron would have extended the high citrate curve for a longer time. This was confirmed by treating plants with different levels of iron (table IV). The citrate from plants treated with 10^{-5} M Fe continued to increase up to the twenty-first hour. The citrate from plants given 2×10^{-6} M Fe began to decrease after the nineth hour. Compared quantitatively, the low-Fe plants produced 30.4 μmoles of citrate, and the high-Fe plants produced 46.7 μmoles. Electrophoresis of the exudates gave patterns very similar to those given by sunflower in figure 1. All 18 exudates gave a fast-moving spot in the position of iron citrate.

Discussion

Citrate/Fe Ratios Above and Below Unity. As discussed previously (2), green sunflower plants, precultured on 10^{-5} M Fe and treated with the same level for exudate collection, produced exudate with a citrate/Fe ratio of 15. The iron in the exudate of the plants was 2×10^{-6} M. A ratio in which citrate is in considerable excess is believed to be characteristic of exudates from normal plants.

In contrast, the experiments with sunflower and other species reported here involved preculture with 10^{-6} M Fe. The plants were green but under iron deficiency stress as shown by their response when iron was restored at decapitation. In general, the magnitude of the citrate/Fe ratio in exudates depended on iron treatment. The sunflower data in table I show the ratios obtained under a wide range of treatments. On low Fe the citrate/Fe ratio was 74, but on high Fe the ratio was 0.4. Thus manipulation of iron supply can invert a ratio to give iron in excess of citrate.

One implication of these findings is the restriction placed on the translocation concept. It is not possible to generalize under all conditions that iron is carried by citrate. On the other hand, an excess of iron over citrate in stem exudate does not change the concept that citrate is the agent normally involved in iron transport. Apparently the control of iron influx by iron-deficient plants is not the same as that of normal plants. Restoration of high iron to deficient plants therefore results in overloading and, consequently, a translocation of iron in abnormal amounts.

If the combining quantities of citrate and iron are 1:1, then an excess of iron in the exudate would require that considerable iron be carried by other ligand(s). If true, this could have important implications for iron translocation and possibly for the translocation of several other trace metals. It would be necessary of course to prove 1) that citrate carries other metals and 2) that iron can displace them when it reaches certain levels in the exudate.

Changes in Exudate with Time. The iron and citrate curves (tables III, IV) indicate continuous change as exudation proceeds. Nutrient iron accumulated rapidly by tomato (table III) was released relatively slowly into the xylem exudate. Citrate increased until about the fifth hour, but fell sharply after the eighth hour. By providing additional iron, the decline in citrate could be delayed a considerable time, although not indefinitely (table IV). Apparently a certain minimum iron content in root pools is required to maintain a high rate of citrate release.

The tomato exudates (table IV) reveal a sharp decrease in citrate at about 21 hours; exudate volume also declined. The total citrate released by group 1 in the last 3 hours was 0.67 μmole compared to 1.82 μmoles in the previous 3 hours. For group 2 these values were 2.0 and 4.5 μmoles of citrate. Because iron concentrations were still high in the final collections, the citrate decline does not appear to be associated with iron shortage.

Effect of Iron on Citrate Release. It has been shown that the citrate content of exudates can be increased by providing additional iron to plant roots. This was true for all the plants studied. This effect is clearly shown in the exudates of tomato (table IV) where high-Fe plants gave increases of citrate for about 21 hours. The citrate in the exudates of low-Fe plants began to decrease at about 9 hours.

Although a release of additional citrate into the xylem can be induced by iron treatment, the source of the acid is not known. The difference in the quantities of citrate released by the tomato groups (table IV) could represent a difference in citrate production and release from respiratory pools, or possibly a difference in its release from remote compartments (1).

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