Translocation of Iron from Soybean Cotyledons

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ABSTRACT

Soybean seeds, Glycine max L. Merrill, were produced by plants treated from anthesis to seed maturity with *Fe supplied as ferric ethylenediaminedi(o-hydroxyphenylacetae). Seed coats accounted for 7.4% of dry seed weight and had Fe concentrations 5 times greater than the embryos. After germinating 2 days, cotyledons contained 69.6% and radicles 5.0% of original seed Fe. Fractions of seed Fe unavailable to seedlings were: 19.8% in seed coats, 1.7% in germination paper, 0.1% in the water under germinating seeds, and 3.8% unaccounted for. Every 3 days seedlings received nutrient solution without Fe or with 10 μM ferric ethylenediaminedi(o-hydroxyphenylacetae) and developed as deficient Fe or normal Fe plants. The deficient Fe cotyledons on day 18 retained 13% of the labeled Fe originally present. Cotyledons of normal Fe plants retained 50 to 70% of their original Fe. Moreover, cotyledons of the normal Fe plants accumulated externally supplied Fe and finally contained twice the quantity of Fe originally present. Stem exudate collected above cotyledons of deficient Fe plants contained 5.3 μM *Fe. Electrophoresis of exudate showed that most of the *Fe migrated anodically as a single band and was in the position of ferric citrate.

Knowledge of Fe translocation in plants generally focuses on movements of this metal from roots to leaves. Much less has been done to study conditions that induce or suppress translocation of Fe stored internally, e.g. in cotyledons.

Obviously, the seedling that depends on cotyledonary Fe for its establishment must have an efficient transport mechanism, for it must export the stored Fe before its cotyledons are shed, and the available time for this may be only a few days.

This study had three objectives: to determine Fe distribution from soybean cotyledons to other plant parts, to measure effects of externally applied Fe on that distribution, and to identify the form of Fe in vascular exudate of seedlings whose main source of Fe was in the cotyledons. A brief preliminary report of this work has been given (9).

MATERIALS AND METHODS

Plant Culture before Anthesis. Hawkeye soybean, Glycine max L. Merrill, was the experimental plant. General cultural methods have been described (7). In the prelabeling period, 32 plants were grown in complete nutrient solutions (4 plants/8 liters) containing 2 μM FeEDDHA.

Radioiron Treatment at Anthesis. At anthesis (approximately 30 days after germination) plants were given nutrient solutions containing 5 μM *FeEDDHA (2 μc *Fe/μ mole Fe). These solutions received isotope periodically and contained 3 to 5 μM Fe throughout the labeling period (from 30 to 100 days after germination). Each 8 liters of nutrient solution received 120 μ moles of Fe in the labeling period. Mature seeds dried on the vine and were harvested over the period of 70 to 100 days from germination.

Analysis of Seeds. The distribution of *Fe between seed coats and embryos and the specific activity of *Fe in whole seeds were determined on oven-dry (70 C) seeds. All assays of seeds and plant parts were made with a γ-scintillation spectrometer.

Germination and Treatments. A total of 145 seeds of about equal size were assayed for *Fe and germinated (24 ± 2 C) between moist Whatman No. 1 papers. The paper extended down the sides of a stainless steel support into 2 cm of water in a covered glass dish. After germination for 2 days, the seed coats, embryos, germination papers, and water were analyzed for *Fe.

On day 2 all seedlings with radicles 1 to 2 cm long were again assayed for radioiron and, except for five seedlings, were returned to the growth chamber under 8 hr dark, 16 hr light.

1 This work was partially supported by United States Atomic Energy Commission funds available to Lee O. Tiffin.

2 Contribution from the Agricultural Environmental Quality Institute and Plant Physiology Institute, Agricultural Research Center, ARS, United States Department of Agriculture, Beltsville, Md. 20705.

Abbreviations: FeEDDHA: ferric ethylenediaminedi(o-hydroxyphenylacetae); —Fe: deficient Fe; +Fe: normal Fe.
(1500 ft-c), and 22 ± 2 °C. The seedlings were placed and identified by position in black lucite frames (7) with radicles extending into full nutrient solutions without Fe. The five seedlings mentioned above made up the first harvest of plants grown without Fe in the experimental period. The second harvest was on day 4.

Seedlings were cultured in elongation frames until day 3. At that time all seedlings were identified by numbered tape, and 40 of them received complete nutrient solutions (20 plants/8 liters) containing 10 μM FeEDDHA. The other plants received nutrients without Fe. Subsequent nutrient replacements were made on day 6, 9, 12, and 15. Five —Fe plants and four +Fe plants were harvested on day 6, 9, 12, 15, and 18.

Up to eight fractions of seedling material were analyzed for radioiron. The hypocotyl plus radicle and the cotyledons (including plumule) were the two fractions analyzed on day 2. On day 4, the primary leaf laminae were separated as a third fraction. Two additional fractions on day 9 were the first trifoliate leaf (with apical tissue) and the epicotyl stem plus petioles. In later harvests the second and third trifoliate leaves were excised and analyzed separately; also, hypocotyls and roots were analyzed separately. The *Fe results for several plant parts were summed for graphical presentation.

Iron in Vascular Exudate. Root systems of seedlings were placed in nutrient solutions (5 plants/liter) without Fe, and all epicotyl tissue was excised 2 cm above the cotyledons. Exudate was collected for 12 hr from 15 plants on three harvest dates: 9, 12, and 15 days. Exudates were analyzed for radioiron, and exudate from the 9-day-old plants was analyzed electrophoretically.

Total Iron Determination. Total Fe was determined by a variation of the ferrozine method of Stookey (6). Seeds and other tissues were ashed 15 hr in Pyrex beakers at 500 °C, treated with several drops of HNO₃ dried, and reashed 6 hr at 500 °C. The ash was dissolved in 10 ml of a solution containing 0.5 mM ferrozine, 72 mM NH₄OH·HCl, and 600 mM HCl. The solutions in covered beakers were placed for 2 hr on a steam plate and then transferred to 25-ml volumetric flasks. Color was developed by adding 3.5 ml of a solution containing 1.25 mM NH₄OH and 1.25 mM NaOAc and then diluting to volume. The absorbance of samples, appropriate blanks, and standards was determined at 562 nm. Results were calculated based on a molar absorptivity of 27,900.

RESULTS AND DISCUSSION

Total and Radioiron in Seeds. Radioiron measurements of eight selected seeds gave 39 to 69 μg Fe/g dry weight. Total Fe measurements gave 44 to 74 μg Fe/g. The seed showing the greatest deviation had a specific radioactivity of 0.82 relative to original nutrient Fe. The other seeds gave a range of 0.88 to 0.96. The mean relative specific radioactivity for all seeds was 0.91. Radioiron assays therefore underestimated total seed Fe about 9%.

Radioiron Distribution in Embryos and Seed Coats. Results from 10 replicate assays showed that seed coats accounted for about 7.4% (range, 6.9–9.2%) of the total seed weight. However, the mean value for Fe in seed coats was 29.9% (range, 27.5–32.0%) of the seed Fe, indicating that Fe concentration in seed coats was 5 times greater than in embryos (dry weight basis).

Seed and Cotyledonal Iron Distribution. Figure 1A shows that on day 2 cotyledons contained 69.6% and hypocotyls 5.0% of the original seed Fe. This gives a total of 74.6% of the seed Fe in seedlings at the first harvest. Distribution of the remaining 25.4%, designated as early Fe loss (Fig. 1A), was:

19.8% in seed coats, 1.7% in germination papers, 0.1% in the water under germinating seeds, and 3.8% unaccounted for.

Our results show very little diffusion of *Fe into the paper and water under the seeds. In contrast, Witt (10) and Bidulph (1) observed that bean seeds germinated for 2 days in paper lost half their Fe to the paper. Their seeds were germinated at about 33 °C, which might account for the large Fe loss. Our seeds were germinated near 24 °C.

A summary of the final harvest data in Figure 1A shows 14.7% of the seed Fe in the hypocotyl and roots. Tissue above the cotyledons contained 50.9% of the seed Fe. We emphasize that the ordinate in Figure 1A shows percentages of seed Fe, the reference being the *Fe in dry seeds at zero time. The curve for cotyledons of —Fe plants shows 69.6% of the seed Fe in the cotyledons on day 2 and only 9% remaining on day 18. Figure 1B gives the same curve on a different scale; the ordinate shows cotyledonal Fe set at 100% (instead of 69.6%) on day 2. On that scale, 13% of the cotyledonal Fe present on day 2 remained on day 18.

We have observed routinely, as in the present study, that Fe-deficient soybeans display some chlorosis at about day 12 but continue to grow several more days without necrosis and with only moderately less dry matter production than green plants. The fact that Fe-deficient seedlings export 90% of their cotyledonal Fe suggests an adaptive response that provides physiologically important Fe to developing cells remote from the storage site.

The +Fe plants, treated with chelated Fe continuously from day 3, gave a very different Fe export pattern (Fig. 1B). Several contrasts are obvious. The —Fe plants exported cotyledonal Fe fairly steadily and rapidly from day 4 to 18, but export by +Fe plants was decidedly suppressed. The mean values for the last three harvests of the +Fe cotyledons indicate that Fe export was approaching a limit on day 12. The greatest fraction of Fe exported by an individual —Fe plant (day 15) was 50%. Fe export for all individuals in the last two harvests ranged from 30 to 50%.

Other contrasts are noticeable. The variability among replicates of —Fe plants, shown by vertical bars, was quite limited. Fe deficiency apparently affected all plants similarly and in-
duced export responses that were much alike. In contrast, "Fe export by +Fe plants varied widely among replicates, particularly in the last three harvests. This could have resulted from different nutrient Fe accumulations in cotyledons of these plants. The suppressed export of cotyledonary "Fe seems clearly related to accumulation of nutrient Fe in the experimental period, for the +Fe plants accumulated high Fe concentrations (discussed below) in the cotyledons.

Cotyledonary Weight and Iron Changes. Table 1 shows weight and total Fe changes in cotyledons of plants grown with and without Fe. The weight of 10 cotyledons for both plant groups was about 820 mg on day 2. Cotyledons from both treatments lost dry matter at similar rates and finally contained about 20% of their original dry matter.

Export of cotyledonary Fe from -Fe plants lagged behind weight loss until about day 9, and the Fe concentration increased to 65 µg/g. Thereafter, the Fe concentration decreased. Despite this rise and fall in concentration, the absolute Fe content steadily decreased, and the final Fe concentration was not greatly different from that on day 2.

The cotyledons of +Fe plants provided a striking contrast in Fe relationships. The quantities of Fe in +Fe cotyledons increased over twofold, which amounted to a tenfold increase in Fe concentration. The Fe concentration ratio of the two groups (Table 1) increased from 1.4 on day 6 to 12.9 on day 18.

The 10 cotyledons of +Fe plants contained approximately 40 µg Fe on day 4. We estimated this from cotyledons of -Fe plants because +Fe plants were not harvested before day 6. Figure 1B shows that roughly 60% of the original Fe, i.e., 24 µg, remained in the cotyledons on day 18. Subtracting this amount from 128.5 µg Fe, the final value for +Fe cotyledons (Table 1), gives approximately 100 µg of externally supplied Fe loaded into the cotyledons in 15 days. We present this estimate to give some idea of the Fe loading pattern, but we have no information about turnover. It is reasonable that the cotyledons exported some of the newly acquired Fe in the time intervals shown, but we have no information about quantity.

Iron in Vascular Exudate. Exudate volumes collected on days 9, 12, and 15 were 0.9, 5.5 and 8.6 ml per 15 plants, respectively. Exudate collected on day 9 contained 5.3 µM Fe. Exudates collected on days 12 and 15 contained only about 0.1% of that Fe concentration, which was too low for radiographic analysis. Figure 2 shows the electrophoretic distribution of "Fe in exudate collected on day 9. Most of the Fe migrated anodically as a single band and was in the position of ferric citrate.

Although we did not analyze for citrate, we would expect from other soybean studies (7-9) that the exudate would contain from 300 to 1000 µM citrate. High citrate-Fe ratios occur particularly in exudates from low Fe plants. Assuming citrate to be much higher than Fe (5.3 µM) in the exudate, we conclude that citrate was the Fe carrier.

The channel in which Fe was moving when intercepted was not identified. We recognize that part of the Fe carried to the roots in phloem tissue could have been diverted upward in the xylem. Such diversion probably would account for only a small part of the Fe in the exudate, but our data are not explicit. We thus refer to the stem fluid as "vascular" exudate and do not specify the transporting channel.

It is generally understood, however, that phloem tissue differentiates early in cotyledons and functions in exporting minerals and other solute throughout the seedling (2). We assume, therefore, that Fe translocated from cotyledons, as shown in this study, was moving, at least initially, in the phloem.

Forms of Iron in Cotyledons. We made no attempt to study forms of Fe in cotyledons themselves. It is reasonable, however, to assume that two Fe fractions (in addition to Fe enzymes) are present, at least in the early stages of germination. The first, which is physiologically important in the developing seedling, is the mobile, chelated form. This is the species presumably exported. We did not determine citrate in the cotyledons used in this study, but we would expect this acid to exceed the Fe and to be the carrier of Fe leaving this tissue.

Another fraction of Fe, perhaps the principal one in early germination, would be immobile and possibly crystalline, such as that represented by phytoferritin. Ferritin, the Fe-protein complex found universally as the Fe storage form in animals, exists as micelles of ferric phosphate and hydrated ferric oxide enclosed in a protein shell (3). Hyde et al. (4) identified phytoferritin in ungerminated pea cotyledons. They propose that phytoferritin reserves in the cotyledons are mobilized for young seedling axis development. Marinos (5) has shown that plastids in the meristematic dome and leaf primordia of a dormant potato tuber bud store phytoferritin and other materials, all of which tend to disappear when the bud sprouts. He proposed that the Fe and other materials become physiologically important in the initial stages of potato bud sprouting.

Knowledge of Fe storage and transport capability raises several questions. Knowledge of the principal forms of Fe in dry and germinated seeds and, particularly, the mechanism for Fe release from the germinating cotyledon would be of funda-
mental importance. Assuming phytoferritin is a storage form, we would like to know whether protease is involved in releasing Fe from its protein shell. If protease is involved, would an inhibitor of the enzyme suppress cotyledonian Fe export? These and other questions about forms of Fe in cotyledons and the chemistry of its release from such tissue deserve further study.

LITERATURE CITED


