BioMetals 1995, **8**, 297–300

Induction of ferric reductase activity in response to iron deficiency in *Arabidopsis*

Jennifer A. Saleeba & Mary Lou Guerinot

Department of Biological Sciences, Dartmouth College, Hanover, NH, USA

Received 7 July 1994; accepted for publication 4 February 1995

The response to iron deficiency was investigated in 16 ecotypes of *Arabidopsis thaliana* (L.) Heynh. and in *Arabidopsis griffithiana*. An increase in root ferric reductase activity was observed under conditions of iron deficiency in these ecotypes and in both species. This observation is consistent with a Strategy I response which is typical for dicot plants. *A. griffithiana*, however, showed a lower induction of ferric reductase activity in response to iron deficiency than that of the commonly studied *A. thaliana* Columbia ecotypes.

Keywords: *Arabidopsis*, iron, ferric reductase, iron deficiency

Introduction

Iron is essential to many life processes, including photosynthesis and respiration in higher plants. Although iron is abundant in the soil, it is generally present as ferric hydroxide compounds that are insoluble at neutral pH and are unavailable for uptake (Yi *et al.* 1994). Plants need soluble forms of iron for uptake, thus many plant species have a mechanism for responding to a lack of accessible iron. Plants, such as *Arabidopsis thaliana* (L.) Heynh. (Tingey *et al.* 1982), that are able to overcome chlorisis induced by iron deficiency are said to be chlorosis resistant due to their iron efficient physiology.

Two types of responses to iron deficiency have been documented. Graminaceous monocots generally show a Strategy II response. Ferric iron [Fe(III)] chelating compounds called phytosiderophores are released from the roots and complex with soil Fe(III) (Takagi *et al.* 1984). Uptake processes at the root surface are then able to transport these complexes into the plant (Römheld & Marschner 1986). Dicots and non-graminaceous monocots show a Strategy I response. Typical of this type of response are induction of ferric reductase activity at the root surface to convert rhizosphere Fe(III) chelates to ferrous iron [Fe(II)] chelates followed by separation of the Fe(II) from the chelate (Chaney *et al.* 1972), acidification of the rhizosphere to increase the solubility of Fe(III) compounds in the soil (Römheld *et al.* 1984), accumulation of organic acids in the root and release of reductants (Hether *et al.* 1984).

Assays have been developed to quantify responses to iron deficiency. Bienfait *et al.* (1983) used a colorometric assay to measure iron reduction by ferric reductase. Rhizosphere acidification has been visualized by laying roots on agar medium containing a pH indicator. Roots that show a net release of hydrogen ions will show an acid response in the pH indicator medium (Marscher & Römheld 1983). These and similar tools have been used to demonstrate a Strategy I response in a number of organisms including sunflower (Römheld & Marschner 1981a,b), bean (Bienfait *et al.* 1983, Ric de Vos *et al.* 1986), peanut (Römheld & Marschner 1983), tomato (Brown & Ambler 1974), soybeam (Chaney *et al.* 1972), cucumber (Zocchi & Cocucci 1990) and yeast (Lesuisse *et al.* 1987). Furthermore, Buckhout *et al.* (1989) have localized the ferric reductase activity in tomato to the plasma membrane and the NADH-dependent nature of the inducible enzyme activity has been reported (Holden *et al.* 1991).

In this investigation we studied the response of *A. thaliana* to iron deficiency. We have adapted a simple system to assay the ferric reductase activity of *A. thaliana* under iron sufficiency and deficiency and report evidence for a Strategy I type response in this organism. This information will be used for the isolation of mutants that show an altered response to iron deficiency, as has already been achieved in tomato (Brown *et al.* 1971). Isolation of mutants in *A. thaliana* will provide good material for molecular as well as genetic studies of iron deficiency due to the amenability of *A. thaliana* to these types of analyses.

Address for correspondence: M. L. Guerinot, Department of Biological Sciences, Dartmouth College, Hanover, NH 03755-3576, USA. Fax: (+1) 603 646 1347.

Materials and methods

Seeds of various *Arabidopsis* ecotypes and species were sterilized for 7 min in 95% ethanol, 7 min in 10% bleach, then rinsed four times in sterile distilled H_2O. Germination took place on Gamborg's B5 medium (Sigma, St Louis, MO), 0.7% Bacto-agar (Difco, Detroit, MI) at pH 5.8. At the six true leaf stage (10–14 days after germination), plants were transferred either to iron sufficient medium that contained 0.7% agar nutrient solution (Marschner *et al.* 1982) at pH 6.5 with 0.1 mM Fe(III)EDTA (NS + Fe) or to iron deficient medium (NS − Fe) that had the same composition as NS + Fe plates except that 0.3 mM FerroZine (Hach, Ames, IA) was substituted for the Fe(III)EDTA. FerroZine is a chelator of Fe(II), with a molar absorptivity of 28 600 at 562 nm (Gibbs 1976). Addition of FerroZine to the plant growth medium renders it free of plant available iron that may have been present in trace amounts in medium components. Plates were taped closed with micropore tape (3M, St Paul, MN).

Plantlets were incubated in constant light at 20°C under 65 $\mu Em^{-2}s^{-1}$ units of light from fluorescent and incandescent sources. A 3 mm thick yellow acrylic screen was placed between the light sources and plates to prevent light degradation of iron in the media and to prevent the formation of formaldehyde from culture media breakdown (Hangarter & Stasinopoulos 1991).

After plants were transferred from B5 medium and incubated on NS + Fe or NS − Fe medium, root ferric reductase activity was determined using a FerroZine assay. Plants were transferred to acid washed wells of microtiter dishes containing a solution of 0.1 mM Fe(III)EDTA, 0.3 mM FerroZine in distilled H_2O such that agar free roots were submerged in the assay solution. Plants were used for one assay only. After incubation for 1 h, plants were removed and the absorbance of the assay solution was determined. As the plants reduce Fe(III) to Fe(II), the Fe(II) is chelated by FerroZine; FerroZine changes from a colorless compound to form a purple colored complex which absorbs at 562 nm (Stookey 1970). Thus the absorbance of the assay solution is a measure of Fe(II) production and therefore estimates ferric reductase activity. At least 10 determinations were performed for each ecotype, and for each NS + Fe or NS − Fe incubation period, with samples taken from seeds germinated on at least two different days. Results were analyzed using *t*-tests with a 95% confidence level.

Results and discussion

The ferric reductase activity of *A. thaliana* ecotype Col-0 plants was assayed each day, for a total of 10 days, after transfer of plants from B5 medium to either NS + Fe or NS − Fe media (Figure 1). Ferric reductase activity of NS − Fe grown Col-0 roots was higher than the activity of NS + Fe grown roots on all days except day 8, according to *t*-tests. Induction of ferric reductase activity peaked around day 4.

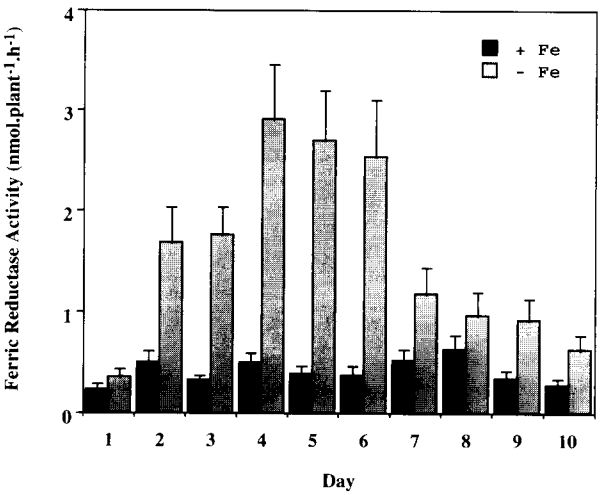

Figure 1. Root ferric reductase activity of *A. thaliana* ecotype Col-0 after transfer to iron sufficient (+Fe) or deficient (−Fe) growth conditions for 1–10 days. Ferric reductase activity was calculated from absorbance measurements and is expressed as nmol Fe(II) formed per plant per hour. Error bars indicate the standard error for activity determinations. 'Day' is the number of days plants were incubated on NS media.

The variation in ferric reductase activity among different ecotypes of *A. thaliana* and by *A. griffithiana* is shown in Figure 2. Levels of ferric reductase activity on either NS + Fe or NS − Fe media show little difference between ecotypes and are not different from that of the Columbia ecotypes, Col-0 and Col, as determined by *t*-tests. There is a statistically significant difference between activity levels on NS + Fe and NS − Fe for each *A. thaliana* ecotype. *A. griffithiana*, however, displayed significantly less activity on NS − Fe than the *A. thaliana* ecotypes Col-0 and Col, as determined by *t*-tests. *A. griffithiana* did show a significant difference in activity on NS + Fe compared to NS − Fe indicating that a response to iron deficiency does exist in this species. The ferric reductase activity of *A. griffithiana* was assayed over a 10 day period to see if the activity of plants grown on NS − Fe was similar to the activity of Col-0 and Col on a day after transfer other than day 3 (Figure 3). The ferric reductase activity for plants grown under iron deficiency was significantly less than the activity of iron deficient Col-0 and Col plants on all days. The reduced response to iron deficiency shown by *A. griffithiana* could be explained if this species was isolated from regions where a strong response to iron deficient conditions does not confer a selective advantage. *A. griffithiana* has been isolated from Pakistan, Afghanistan, the Western Himalayas (Jafri 1956), Palestine (Zohary 1966) and the lowlands of Iraq (Rechinger 1964).

We conclude that ecotypes of *A. thaliana* and *A. griffithiana* display a physiological process in response to conditions of deficient iron in a manner consistent with a Strategy I scheme. An understanding of the ferric reductase response of *A. thaliana* has allowed mutant screens to be devised for isolation of lines with an altered ferric reductase response to iron deficiency. Plants can be grown under iron

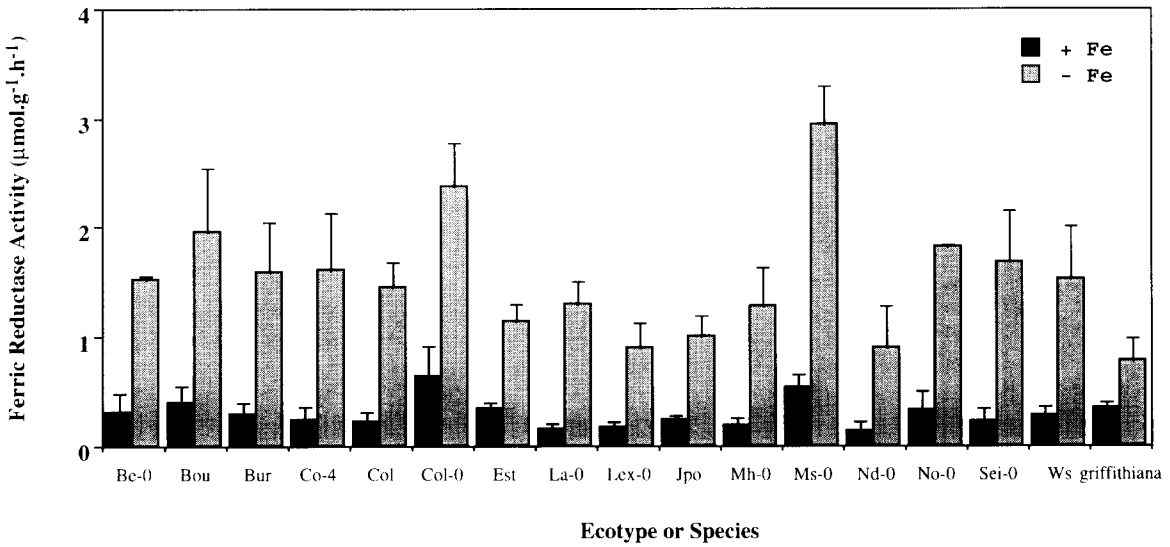

Figure 2. Root ferric reductase activity of 16 *A. thaliana* ecotypes and *A. griffithiana* after transfer to iron sufficient (+Fe) or deficient (−Fe) growth conditions for 3 days. Activity was calculated from absorbance measurements and expressed as μmol Fe(II) formed per gram wet weight root tissue per hour. Error bars indicate the standard error for activity determinations.

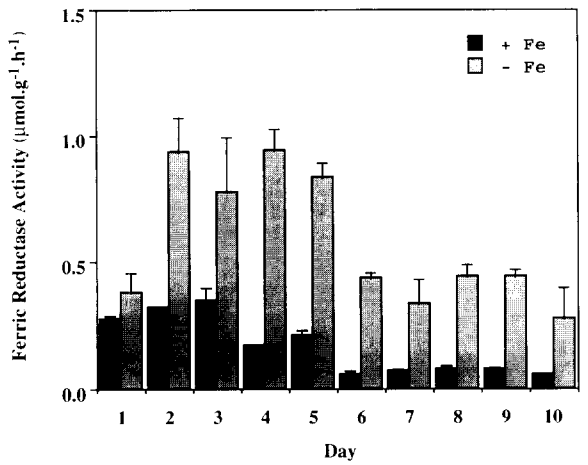

Figure 3. Root ferric reductase activity of *A. griffithiana* after transfer to iron sufficient (+Fe) or deficient (−Fe) growth conditions for 1–10 days. Ferric reductase activity was calculated from absorbance measurements and is expressed as μmol Fe(II) formed per gram wet weight root tissue per hour. Error bars indicate the standard error for activity determinations. 'Day' is the number of days plants were incubated on NS media.

deficient conditions and screened for low ferric reductase activity or plants can be grown under iron sufficient conditions and tested for high ferric reductase activity. These screens are currently being used for mutant isolation.

Acknowledgments

Arabidopsis stocks were kindly provided by F. Ausubel. Ellen R. Friday and Sacha M. Rajack are thanked for technical assistance. Comments on the manuscript from Ying Yi and Tim Littlejohn are appreciated. Supported by grants from the National Science Foundation of the USA (grant number 91-10080) and the US Department of Agriculture (grant no. 91-02987).

References

Bienfait HF, Bino RJ, van der Bliek AM, Duivenvoorden JF, Fontaine JM. 1983 Characterization of ferric reductase activity in roots of Fe deficient *Phaseolus vulgaris*. *Physiol Plant* **59,** 196–202.

Brown JC, Ambler JE. 1974 Iron-stress response in tomato (*Lycopersicon esculentum*) I. Sites of Fe reduction, absorption and transport. *Physiol Plant* **31,** 221–224.

Brown JC, Chaney RL, Ambler JE. 1971 A new tomato mutant inefficient in the transport of iron. *Physiol Plant* **25,** 48–53.

Buckhout TJ, Bell PF, Luster DG, Chaney RL. 1989 Iron stress-induced redox activity in tomato (*Lycopersicon esculentum* Mill.) is localized on the plasma membrane. *Plant Physiol* **90,** 151–156.

Chaney RL, Brown JC, Tiffin LO. 1972 Obligatory reduction of ferric chelates in iron uptake by soybeans. *Plant Physiol* **50,** 208–213.

Gibbs CR. 1976 Characterization and application of FerroZine iron reagent as a ferrous iron indicator. *Anal Chem* **48,** 1197–1201.

Hangarter RP, Stasinopoulos TC. 1991 Effect of Fe-catalyzed photooxidation of EDTA on root growth in plant culture media. *Plant Physiol* **96,** 843–847.

Hether NH, Olsen RA, Jackson LL. 1984 Chemical identification of iron reductants excluded by plant roots. *J Plant Nutr* **7,** 667–676.

Holden MJ, Luster DG, Cheney RL, Buckhout TJ, Robinson C. 1991 Fe^{3+}-chelate reductase activity of plasma membranes isolated from tomato (*Lycopersicon esculentum* Mill.) roots. *Plant Physiol* **97,** 537–544.

Jafri SMH. 1956 Some cruciferae of W. Pakistan, Afghanistan and N. W. Himalaya. *Edinb Roy Bot Gard Notes* **22,** 95–119.

Lesuisse E, Raguzzi F, Crichton RR. 1987 Iron uptake by the yeast *Saccharomyces cerevisiae*: involvement of a reduction step. *J Gen Microbiol* **133,** 3229–3236.

Marschner H, Römheld V. 1983 *In vivo* measurement of root-induced pH changes at the soil–root interface: effect of plant species and nitrogen source. *Z Pflanzenphysiol Bd* **111S,** 241–251.

Marschner H, Römheld V, Ossenberg-Neuhaus H. 1982 Rapid method for measuring changes in pH and reducing processes along roots of intact plants. *Z Pflanzenphysiol Bd* **105S,** 407–416.

Rechinger KH. 1964 *Flora of Lowland Iraq.* Weinheim: Cramer.

Ric de Vos C, Lubberding HJ, Bienfait HF. 1986 Rhizosphere acidification as a response to iron deficiency in bean plants. *Plant Physiol* **81,** 842–846.

Römheld V, Marschner H. 1981a Rhythmic iron stress reactions in sunflower at suboptimal iron supply. *Physiol Plant* **53,** 347–353.

Römheld V, Marschner H. 1981b Iron deficiency stress induced morphological and physiological changes in root tips of sunflower. *Physiol Plant* **53,** 354–360.

Römheld V, Marschner H. 1983 Mechanisms of iron uptake by peanut plants. *Plant Physiol* **71,** 949–954.

Römheld V, Marschner H. 1986 Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. *Plant Physiol* **80,** 175–180.

Römheld V, Muller C, Marschner H. 1984 Localization and capacity of proton pumps in roots of intact sunflower plants. *Plant Physiol* **76,** 603–606.

Stookey LL. 1970 Ferrozine — a new spectrophotometric reagent for iron. *Anal Chem* **42,** 779–781.

Takagi S, Nomoto K, Takemoto T. 1984 Physiological aspects of mugineic acid, a possible phytosiderophore of graminaceous plants. *J Plant Nutr* **7,** 469–477.

Tingey DT, Raba S, Rodecap KD, Wagner JJ. 1982 Vermiculite, a source of metals for *Arabidopsis thaliana*. *J Am Soc Hort Sci* **107,** 465–468.

Yi Y, Saleeba JA, Guerinot ML. 1994 Iron uptake in *Arabidopsis thaliana*. In: Manthey JA, Crowley DE, Luster DG, eds. *Biochemistry of Metal Micronutrients in the Rhizosphere*. Boca Raton: Lewis Publishers.

Zocchi G, Cocucci S. 1990 Fe uptake mechanism in Fe-efficient cucumber roots. *Plant Physiol* **92,** 908–911.

Zohary M. 1966 *Flora Palestina*. Jerusalem: Israel Academy of Sciences and Humanities.