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Communication

Control of the Development of Iron-Efficiency Reactions in Potato as a Response to Iron Deficiency Is Located in the Roots

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ABSTRACT

Roots of potato plants (*Solanum tuberosum* cv Bintje) growing on low Fe nutrient solution developed the characteristic Fe efficiency reactions, such as high ferric reductase activity, proton extrusion and increased root hair formation. Roots from a tuber with sprout removed, when grown on Fe-free nutrient solution, also expressed these reactions; transfer to iron-containing medium resulted in their complete disappearance within 10 days. Roots growing on 2% sucrose in sterile Murashige-Skoog medium increased their ferric reductase activity upon withholding Fe and formed transfer cells. It is concluded that potato roots themselves control the development of Fe-efficiency reactions, and that the shoot may exert a modulating influence on their expression.

Dicotyledons and non-grass monocotyledons, suffering from iron deficiency, develop Fe-efficiency reactions in the roots (15), such as a high reduction capacity for ferric chelates (1, 5, 14) rhizosphere acidification by proton excretion (16) and morphological changes such as increased root hair formation (13) and formation of rhizodermal transfer cells (12).

It is not known where the control on the development of these reactions is located. By grafting experiments, Brown showed that both in tomato and soybeans a gene controlling Fe-efficiency reactions exerts its effect in the root (3, 4). This does not imply that the control on Fe-efficiency reactions is located there. Conceivably, in both cases the roots might have lost the capacity to respond to a signal from the leaves to turn on the reactions ('chlorosis signal'). Indeed, Landsberg (8) observed that proton extrusion by roots of Fe-deficient sunflower was sensitive to cutting of some, not all, of the leaves. Addition of sucrose to the medium did not restore proton extrusion. We obtained comparable results with bean plants (HF Bienfait, unpublished). Furthermore, Landsberg (8) found that administration of triiodobenzoic acid to the leaves of Fe-deficient sunflower inhibited proton excretion by the roots. Landsberg concluded that Fe stress responses might be subject to hormone control (8–10).

We need to know whether a signal from the leaves is essential in the development or expression of Fe-efficiency reactions, and ideally this should be investigated with roots growing in tissue culture. However, as uninterrupted phloem transport seems to be essential for at least one of the Fe-efficiency reactions, such a system could at best yield ambiguous results. We therefore used potato roots growing from tubers, with an uninterrupted phloem connection, as the main experimental system. Roots growing in sterile culture were used for comparison. The results show that the roots themselves can control the development of the known Fe-efficiency reactions.

MATERIALS AND METHODS

Sterile Culture. Sterile plants of the potato cultivar 'Bintje,' a gift from Dr. Bokelman of ITAL, Wageningen, were cultivated in GA7 culture vessels (Magenta Corp., Chicago) on a Murashige-Skoog medium (11), pH 5.8, supplemented with 2% sucrose. The vessels were placed in a growth chamber at 28°C with a daylength (6 $W \cdot m^{-1}$) of 14 h. For cultivation of roots, a 0.5 cm long stem base carrying 1 week old roots was further cultivated in the dark in a vessel containing fresh medium with or without 100 μM Fe-EDTA, under slow shaking. One week later the roots were harvested.

Hydroponicaly grown potato plants were obtained by taking cuttings from sterile plants and growing these on Knop nutrient solution (containing N as nitrate only) with 1 μM ('-Fe') or 40 μM (control) Fe-EDTA as described earlier for bean plants (2).

Growth of roots on tubers. Potato tubers of the cv Bintje, stored for 1 year in the dark at 8°C, were a gift from Dr. van der Plas, Vrije Universiteit, Amsterdam. They were placed on perlite moistened with Knop nutrient solution without added Fe, and left in the dark at 23°C and 65% RH. After 2 to 3 weeks, potatoes which showed fresh sprout and root growth were placed on the openings of 200-ml glass pots filled with aerated nutrient solution with or without 40 μM Fe-EDTA, so that only the roots were hanging in the fluid. The sprouts were excised immediately above the origin of the roots; every day the plants were controlled to remove new sprouts (Fig. 1). For growth on agar, root systems attached to the potatoes were spread on a 0.5 cm thick layer of 0.75% agar containing 0.1 strength Fe-free Knop nutrient solution and 0.006% bromocresol purple at pH 5.8 (16).

Ferric reduction activity of root tips (5–7 in each assay) was measured in the dark in 1 ml 5 mM Mes, 0.5 mM $CaSO_4$, 100 μM Fe(III)-EDTA, 400 μM bathophenanthrolinesulfonate at pH 5.5 and 25°C. The assay was performed in a pasteur pipette, which was closed at the tip and aerated through a capillary, or in an Eppendorf reaction vessel which was continuously rotated. The $A_{535\text{-}600}$ was determined after 0.5 to 3 h. Controls contained no roots or no Fe-EDTA.

Reduction capacity of roots towards tetrazolium was assayed by immersing the roots in 0.1 strength Knop nutrient solution (pH 5.3) with 0.1 mg/ml nitro blue tetrazolium for 20 min at

25°C in the dark (17).

For EM, root tips were prefixed in 2% glutaraldehyde containing 50 mM Mes, pH 7, and further treated as described in Kramer *et al.* (7).

Bathophenanthrolinesulfonate was from Sigma, 4-nitro blue tetrazolium chloride from Serva (W. Germany).

RESULTS

Potato plants growing on nutrient solution with 40 μM Fe-EDTA had green leaves; the roots were without root hairs and had a low level of ferric reductase. Plants growing on 1 μM Fe-EDTA ('−Fe', Table I) developed chlorosis in the leaves, and in the roots zones, 3 to 10 mm behind the tip, carrying dense root hairs and with a high ferric reductase and nitro blue tetrazolium reduction activity. Control roots had a low ferric reduction activity and showed tetrazolium reduction only in the 0.5 mm tip region. Fe-deficient plants lowered the pH of the nutrient solution from 5.3 to 3.6 within 1 day after medium renewal, while Fe-sufficient controls always raised the pH. These results are summarized in Table I.

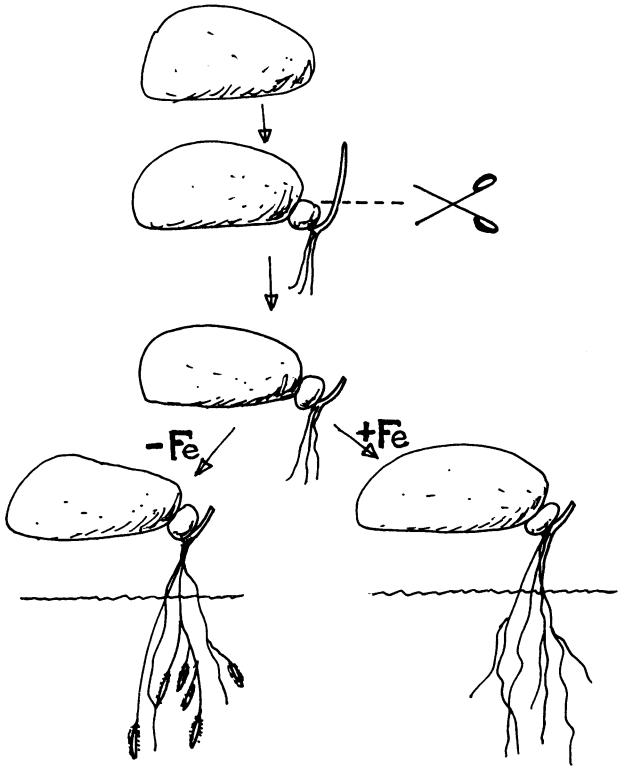

FIG. 1. Scheme illustrating the culture of roots from potato tubers without sprout on nutrient solution with and without Fe.

Roots growing on nutrient solution from a tuber developed zones with root hairs within 8 to 20 d after transfer to Fe-free medium, as in the intact plants. In these zones rhizodermal transfer cells were present, and tetrazolium was reduced. The ferric reductase activity of those root parts that showed these symptoms was increased with respect to the controls grown on Fe (Table I). When one-half of a root system grown without iron for 10 d was harvested for ferric reductase determination, and the other half was cultured further on medium with iron, this latter half decreased its ferric reductase to the control level; root hair formation ceased (Table I).

Roots growing from sproutless tubers on agar containing Fe-free medium and the pH indicator bromocresol purple showed yellowing of the agar around the tips within 2 d after layering of the roots on the gel, indicating a local pH less than 4. When a solution of Fe-EDTA was flooded over the agar leading to a final concentration of 200 μM, the intensity of the yellow spots increased for 1 day (*cf.* De Vos *et al.* [6]), after which they disappeared gradually to be replaced by the purple color indicating pH 6 or higher.

Finally, sterile roots were grown on liquid Murashige-Skoog medium with and without Fe. The roots were left attached to their stem bases in order to prevent them floating loosely around the medium. After 1 week the roots were assayed for ferric reductase and tetrazolium reduction. Ferric reductase activity was increased upon iron shortage, to the same degree as in the roots grown on tubers (Table I). The pattern of tetrazolium reduction was the same as in the roots on intact plants or from tubers, but the colored zones were shorter and less intensely blue. In Fe-free roots tetrazolium reduction was confined to the 2 to 5 mm zone behind the tip, which also carried the root hairs. In these zones rhizodermal transfer cells were formed (Fig. 2). Control roots growing with Fe also formed root hairs, but in more elongated zones (5–30 mm behind the tip). In these zones, however, no tetrazolium reduction occurred, not even after prolonged (3 h) incubation; only the 0.5 mm tip reduced tetrazolium, as in control roots grown on tubers or on intact plants.

Table I. *Fe-Efficiency Reactions Developed by Potato Roots Growing on Intact Plants, Tubers, and in Sterile Solution*

In experiment 3, the values are for incubations with separate parts of one root system.

Expt. No.	Growth Conditions	Ferric Reductase	n[a]	Tetrazolium Reduction	Root Hairs	Transfer Cells	pH Lowering
		$\mu mol \cdot h^{-1}\ g^{-1}\ FW \pm SD$					
1	Roots on intact plants	− Fe 1.85 ± 0.19	(2)	+	+	ND[b]	+
		+ Fe 0.10 ± 0.04	(2)	−	−	ND	−
2	Roots on tubers without sprout	− Fe 0.59 ± 0.39	(10)	+	+	+	+
		+ Fe 0.08 ± 0.04	(5)	−	−	ND	−
3	Roots on a tuber without sprout after 8 d further growth	− Fe 1.19 ± 0.11	(2)	+	+		
		+ Fe 0.07 ± 0.04	(3)	−	−		
4	Sterile roots on sucrose	− Fe 0.68 ± 0.27	(4)	+	+	+	ND
		+ Fe 0.10 ± 0.05	(4)	−	+	−	ND

[a] Number of experiments. [b] Not determined.

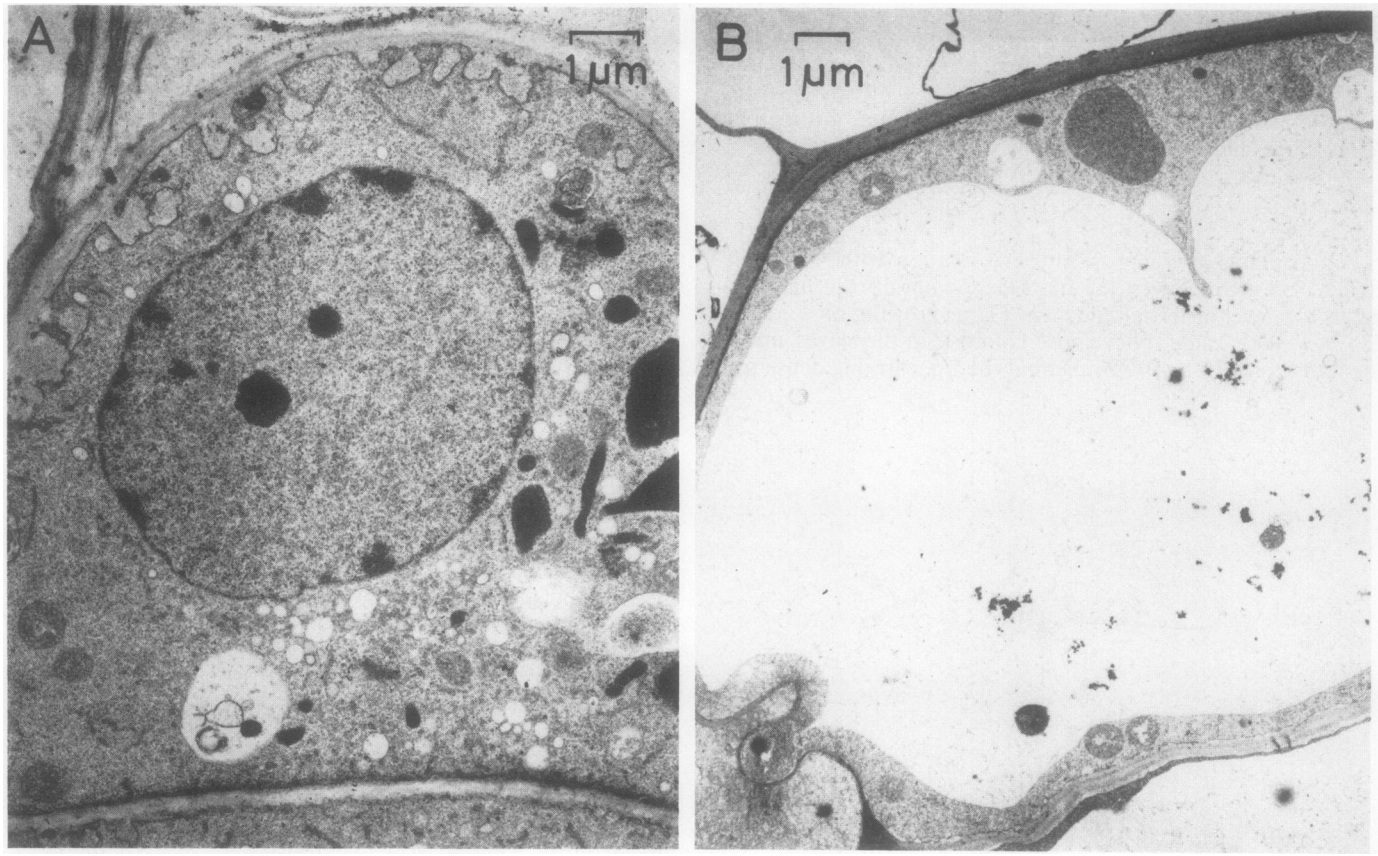

FIG. 2. EMs of the peripheral part of sterile roots growing on Murashige-Skoog medium + 2% sucrose. A, Fe; wall labyrinth at the peripheral wall. B, +Fe; no wall labyrinth.

No rhizodermal transfer cells were found in these roots (Fig. 2). The pH of the growth medium was not monitored.

DISCUSSION

Roots of potato plants showed the characteristic Fe-efficiency reactions of dicotyledons when grown on low-Fe medium (Table I) (*cf.* Römheld and Kramer [12]); it was shown earlier that potato roots also develop rhizodermal transfer cells upon iron deficiency (12). Roots growing on tubers with the sprouts cut off showed the same responses, both morphologically (root hairs, transfer cells) and biochemically (reduction of ferric chelate and tetrazolium, proton extrusion), although the expression was less vigorous (Table I). Fe-efficiency reactions, once developed, were turned off again upon further growth on Fe-containing nutrient solution (Table I). Roots growing in sterile culture showed essentially the same responses as the roots on tubers, insofar as they were measured.

Taken together, the results show that there was no qualitative difference in the capacity to develop Fe-efficiency reactions between roots growing on different sources of organic substrate. In the case of roots growing on tubers, it is improbable that the tubers would develop an Fe chlorosis, as they lost weight during the experiments, in parallel with a progressive breakdown of tissue and starch; we therefore assume that there was no 'chlorosis signal' coming from them.

The differences in the vigor at which proton extrusion and ferric reductase were expressed by the different roots may reflect a dependence on the vigor with which sugar is supplied via the phloem. Landsberg (8) showed that cutting of leaves stops proton extrusion within 1 h. De Vos *et al.* (6), who tapped the phloem sap of bean plants, noted that the sugar yields in the exudate from Fe-deficient plants were twice that from the controls. Recently, D van de Wetering and HF Bienfait (unpublished data) showed a close positive correlation between sugar yield in the phloem exudate and the ferric reductase activity in the roots of bean plants with different iron status. These results suggest that supply of sugar is a rate-determining factor for proton excretion and ferric reduction.

We conclude that potato roots are fully able to control the development of the known Fe-efficiency reactions of dicotyledonous plants. This fact does not exclude the existence of a modulating influence from the leaves.

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