Calcium, Iron and Cobalt Accumulation in Root Hairs of Soybean *(Glycine max)*

Dietrich Werner, Klaus-Peter Kuhlmann

Botanisches Institut, Fachbereich Biologie der Philipps-Universität Marburg, Lahnberge, D-3550 Marburg/L.

Frank Gloystein, Friedrich-W. Richter

Fachbereich Physik der Philipps-Universität, D-3550 Marburg/L.

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Root hairs of soybeans *(Glycine max)*, target cells for infection by *Rhizobium japonicum* accumulate iron more than 10-fold, cobalt more than 8-fold and calcium more than 7-fold compared to the other parts of the root system. In root hairs of wheat *(Triticum aestivum)* a much smaller accumulation of these elements was found. The symbiont of *Glycine max, Rhizobium japonicum*, is known to have a high requirement for iron, cobalt and calcium.

Introduction

Root hairs of higher plants are forming the largest biological surface to the environment besides microorganisms. A single mature cereal plant can develop between 10^9 and 10^{10} root hairs, with a surface in one hectar of cereals in the order of 20 to 200 km^2. Despite the ecological and physiological importance of this cell type, the special biochemistry of root hairs is almost completely unknown, since they have not been isolated on a preparative scale. We have developed such a method, using the different fragility of root hairs and roots after freezing in liquid nitrogen and brushing the root hairs from the roots. From 10^4 soybean seedlings, 10^9 root hairs and about 100 mg root hair protein could be isolated. Three root hair specific proteins have been separated from soybeans, present only in rather small quantities or absent in the other root tissue [1].

In most legumes, root hairs are in addition to their function for uptake of water and nutrients from the soil and excretion of root exudates the target cells for infection by *Rhizobium* species [2]. In the signal chain of the *Rhizobium*-legume symbiosis especially the primary events in the root hair (signal uptake) are unknown on a biochemical basis. An exception is the lectin-EPS/CPS/LPS hypothesis [3, 4] for recognition which is however not undisputed [5, 6]. More than 95% of the primary infections in the root hair also in effective host-symbiont combinations are abortive. The root hair cells apparently recognize the *Rhizobium* cells more as a parasite than a symbiont. The same situation can be found in later stages of nodule development, where lysis of bacteroids in the vicinity of the host cell nucleus was obvious with a *Rhizobium japonicum* mutant, apparently lacking a defense mechanism against this attack [7]. From these observations it is assumed that the invading *Rhizobium* can find in the root hair a nutrient supply which allows such a fast growth of the bacteria, that at least a sufficient number of infecting rhizobia survive the attack by the invaded plant cell. As nutrient supply so far only the carbon/energy source has been considered to be decisive. We asked whether the root hair of legumes might also be a source for minerals for which rhizobia have a special large requirement, as for calcium, iron and cobalt [8, 9].

Reprint requests to Prof. Dr. D. Werner.

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Materials and Methods

Seeds of *Glycine max* cv. Maple Arrow were received from Agriculture Canada, Ottawa Research Station, seeds of *Triticum aestivum* cv. Kolibri from v. Lochow-Petkus, Northeim (Germany). The seeds were sterilized with 30% H_2O_2 for 30 min, thoroughly washed and incubated for 10–20 h in sterile tap water.

For germination 60–80 seeds were incubated on large agar plates (30 × 40 cm) covered with aluminium foil with ½ concentrated Hoaglands mineral solution and 3% agar. The temperature regime was 25 °C for 14 h and 20 °C for 10 h in phytotrons with 50% humidity.

After 4 to 8 days 5–8 cm roots were separated from the seeds and spread on metal plates, frozen with liquid nitrogen. The flexible root hairs were immediately frozen and could be separated from the main roots with a thin brush.

Root hairs and other parts of the roots were stored at −80 °C and later homogenized to a powder by using only plastic material. The mineral composition of the material was determined in three independent batches as described previously [11].

Results and Discussion

With root hairs isolated on a preparative scale, we analyzed the mineral composition by the PIXE

Element	Soybean root hair	Soybean root	Wheat root hair	Wheat root
K	11740 ± 2450	12840 ± 2640	4670 ± 1010	4780 ± 990
S	530 ± 165	560 ± 170	180 ± 55	190 ± 60
P	1326 ± 410	2590 ± 790	526 ± 165	1570 ± 475
Fe	414 ± 138	31 ± 5	120 ± 35	44 ± 26
Co	7.9 ± 3.8	0.88 ± 0.4	2.6 ± 0.8	1.3 ± 1.1
Ca	2200 ± 460	287 ± 70	246 ± 60	288 ± 70
Mo	3.1 ± 0.5	5.4 ± 0.7	0.6 ± 0.12	0.5 ± 0.3

Table I. Mineral salt concentrations determined by PIXE [10] in root hairs and roots of soybean seedlings (*Glycine max* cv. Maple Arrow) and wheat seedlings (*Triticum aestivum* cv. Kolibri), 8 days old, germinated on mineral agar with one half concentrated Hoaglands solution. Concentrations in μg per g dry matter (ppm) ± standard deviation.

method [10, 11]. The potassium and the sulfur content in root hairs of soybean is very similar to the other parts of the root system (Table I). This is also observed in the comparison of root hairs and roots from wheat, although the absolute concentrations are lower by a factor of three. The standard deviations for the macro-nutrients determined by the PIXE method are comparatively large. However, especially the quantitative ratio between the various elements is very accurate, due to a continuous counting of the samples for 20 min with monitoring all elements at the same time [10]. The phosphorus concentration in the root hairs of soybean is only about half the figure from the total root system. Apparently root hairs do not serve as storage cells for phosphate. The most remarkable difference in mineral accumulation in root hairs of soybean was found with iron, cobalt and calcium (Table I). The iron concentration of about 400 ppm is about ten times as large as in the other parts of the root system in 8 days old seedlings. The accumulation factor for cobalt and calcium is between 6 and 8 in root hairs compared to roots. It is interesting to note that molybdenum a component of nitrate reductase and of nitrogenase component II (Mo-Fe-protein) is present in similar quantities in root hairs and roots. The enrichment factors for calcium, iron and cobalt and also the absolute concentrations in root hairs of wheat are significantly smaller compared to the soybean. From these results we conclude that *Rhizobium japonicum* can find after infection of a soybean root hair not only a supply of carbon and energy source, but also a supply of calcium, iron and cobalt, for which a high requirement by this bacterium is established [8, 9].

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