

EXTRACELLULAR FERRICYANIDE REDUCTION IN AEROPONICALLY AND HYDROPONICALLY GROWN CUCUMBER ROOTS: EFFECT OF EXCISION

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Abstract

Exogenous ferricyanide reduction by the roots of cucumber (*Cucumis sativus* L.) seedlings grown aeroponically (AP), in glass pots on moistened filter paper and hydroponically (HP), in a culture solution was measured for 7 days after germination.

In case of short term incubation (2 h) there was generally no difference in transplasmalemma electron transport of AP and HP roots, even if they had been excised, but after long term incubation (6 h) AP roots exhibited higher capacity for ferricyanide reduction.

Soluble sugar content of roots under AP condition increased parallel with the ferric-reducing activity.

Assuming that in vivo, NADH and NADPH provided from the cytoplasm are the main substrates of the ferricyanide reductase reaction, in long term experiments, the reduction of ferricyanide at the root surface reflects the energy reserves of plant part in question. The difference in ferricyanide reduction of AP and HP roots in short term incubation coincided with the maximal difference in soluble sugar content.

Comparing the earlier results of K^+ uptake of AP and HP cucumber seedlings with the ferricyanide reduction of roots potassium uptake doesn't seem directly coupled to the ferric-reducing activity of roots.

Key words: cucumber seedling, aeroponic and hydroponic cultures, plasma membrane electron transfer

Abbreviations: AP, aeroponic; DCMU, 3-(3,4-dichlorophenyl)1,1-dimethylurea; HP, hydroponic; FeCN, $K_3Fe(CN)_6$

Introduction

The early concept, that redox reactions at the surface of plant roots were involved in the ion uptake, was developed by LUNDEGÅRDH (1955) into the hypothesis of anion respiration: redox processes of the plasmalemma ensure the driving force for anion uptake.

The experiments on the interdependence between redox processes of plasmalemma, light induced changes in membrane potential and ion uptake was revived by NOVAK and IVANKINA (1977; 1978). They found correspondence between time-courses of membrane potential, K^+ transport and ferricyanide reductase activity of the plasmalemma of *Elodea* cells which had analogous responses to light turning on and off (NOVAK et al., 1988).

Redox reactions at the plasmalemma of higher plants can be investigated by two main method of approach. In vivo, the electrons of a cytoplasmic donor — which proved to be NAD(P)H in the known cases (SIMONS et al. 1984a; QIU et al.,

1985) — reduce an external, impermeable oxidant, such as $K_3Fe(CN)_6$ (NOVAK and IVANKINA, 1978), hexachloroiridate IV (LÜTHEN and BÖTTGER, 1988) or O_2 (KOMOR et al., 1987).

Redox components and reactions, e.g. NAD(P)H-ferricyanide oxidoreductase (BARR et al., 1986), are well characterised in highly purified plasmalemma preparations, too (ASKERLUND et al, 1988; LIN, 1982; MØLLER and BÉRCZI, 1985).

Two main types of plasmalemma redox activities have been characterized recently. The first, — so-called „Turbo reductase” — has been identified in the roots of Fe-deficient plants (except: Gramineae), and exhibits an inducible character (BIENFAIT, 1985). The second, the „Standard reductase” which doesn't depend on Fe-status of plants, is a constitutive one at the plasmalemma of all plants investigated (QIU et al., 1985; BIENFAIT, 1985).

Other possible physiological functions of plasmalemma electron transfer in plant cells have been excellently reviewed by DAHSE et al. (1989).

Earlier results at our department demonstrated striking differences in the growth and K^+ ($^{86}Rb^+$) uptake of wheat and cucumber plants grown under hydroponic (HP) or aeroponic (AP) conditions (ZSOLDOS et al., 1987). AP-grown seedlings exhibited a very low uptake rate of K^+ , phosphate, sulphate and nitrate which seemed to be a passive process. This phenomenon developed about three days after soaking the seeds.

The possibility of the involvement of plasmalemma redox systems in ion uptake derived from the evidence for the electrogenicity of the redox pumps (SIMONS et al., 1984b). Adding external NADH to the incubation medium of maize protoplasts LIN (1984) found an increase in membrane potential, with simultaneous increase of K^+ influx.

In the present paper we investigated the *in vivo* FeCN reductase activity in HP and AP cucumber roots in order to compare the tendencies with earlier findings in K^+ uptake. Furthermore, experiments were carried out to estimate the effect of energy reserves of roots on FeCN reduction in connection with root excision.

Materials and Methods

Plant cultures: Seeds of cucumber (*Cucumis sativus* L. cv. Budai csemege) were surface-sterilised for 20 min in commercial bleach) 1 part 5% NaOCl to 3 parts H_2O). After rinsing with distilled water they were germinated for 24 hours in the dark at 25 °C on filter paper moistened with nutrient solution (TARI and SZABÓ, 1990), in 500 ml glass pots. From the second day the seedlings were grown under controlled conditions (25/20 °C day/night temperature, 12 h light period, 70% relative humidity) either in pots, on filter paper (aeroponic conditions) or in hydroponic cultures.

AP seedlings were sprayed every day with nutrient solution. One AP plant received one-thirtieth part of the total nutrient quantity for a HP plant. Plants in HP were floated on hydrophobic plastic discs.

Redox assay: Generally 10 excised or intact roots of cucumber seedlings were rinsed in $5 \cdot 10^{-4}$ M $CaCl_2$ solution and placed into 100 ml beakers containing the assay solution in an appropriate volume. The fresh mass: volume ratio was kept constant (0.01 g/ml) every day. The incubation medium contained 1 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol-2-(N-morpholino)ethanesulphonic acid (TRIS-MES), pH = 6.5, 1 mM $CaCl_2$ and 1 mM $K_3Fe(CN)_6$. The beakers were shaken and tissues were incubated

in the dark during the 6 hours of experiments. The optimum parameters of FeCN reduction were determined by 3-day-old AP plants. The rate of $K_3Fe(CN)_6$ reduction was measured as a decrease in A_{420} after correction for A_{500} at 23 °C. We couldn't find any difference between FeCN reduction performed under nonsterile or aseptic conditions.

Analysis of soluble sugars: 0.3 g of homogenized plant material was extracted with 80% ethanol. Extracts were centrifuged at 12,000 g for 15 min and resulting pellet reextracted in 80% ethanol. The combined supernatants were evaporated to dryness and resuspended in 2 ml of distilled water. Soluble sugars of roots were estimated by the phenol-sulfuric acid method (DUBOIS et al., 1956) and expressed as glucose equivalent. Reducing sugars were measured in an aliquot by the method of NELSON (1944).

Oxygen uptake of roots: According to VAN DER WERF et al. (1988) oxygen consumption of one root cut into 1 cm long segments was measured in $5 \cdot 10^{-4}$ M $CaCl_2$ solution with a Clark type oxygen electrode (Hansatech) at 23 °C in the dark for 15–30 min (fresh mass: volume ratio was 20 mg/ml), and repeated 6 times in two independent experiments.

Statistics: The results were subjected to statistical evaluations with F- and Student-t tests. Numerical data of FeCN reduction are given as means \pm SE, $n = 5$.

Results and Discussion

FeCN reduction at the root surface can be measured on intact plants (BÖTTGER and HILGENDORF, 1988; VAN BAUSICHEM, 1988; CSEH, 1988), excised roots (IVANKINA and NOVAK, 1988) and root segments (SIJMONS et al., 1984a; QIU et al., 1985). In these later cases the excision, as a wounding and as the cessation of sugar and hormone transport from the shoots, can directly and indirectly influence the transmembrane electron transport, e.g. effect of auxins on plasmalemma redox system are well documented (BRIGHTMAN, 1988; BÖTTGER and HILGENDORF, 1988).

The optimum conditions for FeCN reduction of cucumber roots were determined in preliminary experiments (Fig. 1). (Optimum pH and root number see in Materials and Methods). Fe-efficiency reactions in Fe-free nutrient solution, among

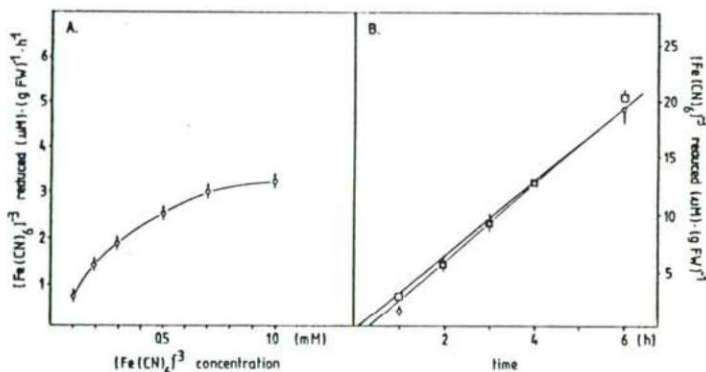


Fig. 1. Ferricyanide reduction by roots of aeroponically grown cucumber seedlings on the third day of germination. A: Effect of ferricyanide concentration on ferricyanide reductase activity of intact plants. Incubation period was 6 hours. B: Time-course of ferricyanide reduction in intact (\circ) and excised (\square) AP cucumber roots. Incubation solution contained 1 mM Tris-Mes (pH=6.5), 1 mM $CaCl_2$ and in B. 1 mM $K_3Fe(CN)_6$. Data are expressed as a mean \pm SE ($n = 5$)

others „Turbo” reductase activity in cucumber roots developed on the 7th day under HP. (Data not shown). So we suppose that the reduction of ferricyanide at least at early stages of seedling growth reflects the standard reductase activity.

FeCN reduction of AP and HP cucumber roots was determined for the first 7 days of the development (Fig. 2).

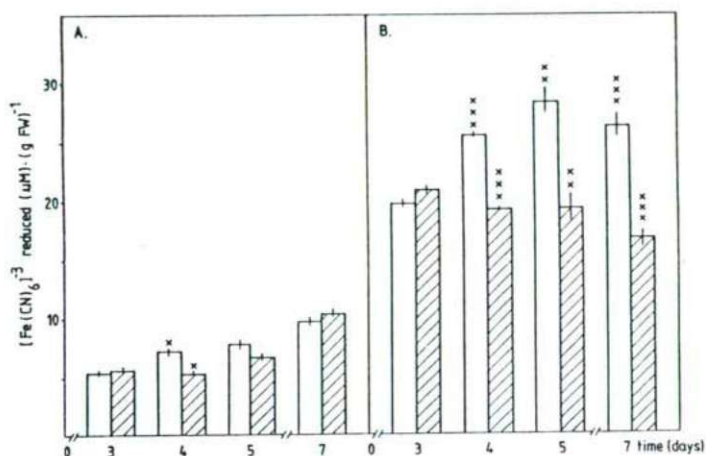


Fig. 2. Time-course of ferricyanide reduction at the root surface of AP (empty columns) and HP (hatched columns) cucumber plants. Incubation periods were 2 (A) and 6 (B) hours. Data are expressed as a mean \pm SE ($n=5$). Significant at $P=0,05$ (single asterics), $P=0,01$ (double asterics) and $P=0,001$ level (triple asterics).

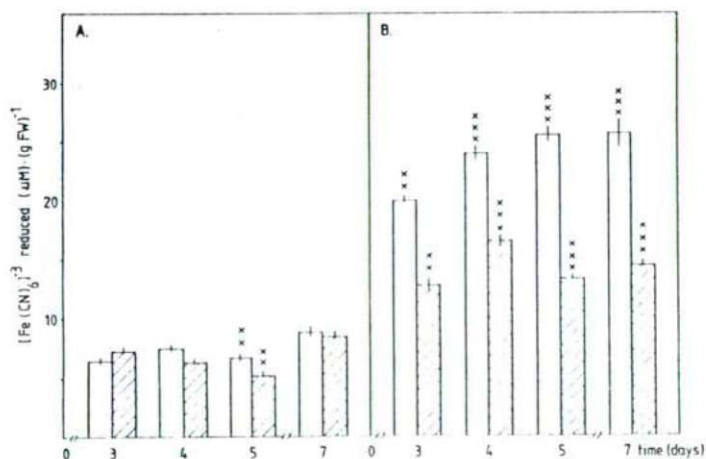


Fig. 3. Time-course of ferricyanide reduction at the root surface of excised AP (empty columns) and HP (hatched columns) cucumber roots. Incubation periods were 2 (A) and 6 (B) hours. Otherwise as in Fig. 2.

The effect of root excision has also been analysed (Fig. 3). In short term experiments (2 h), except one case, there was no significant difference between AP and HP roots, but in longer incubation (6 hours) AP tissues exhibited higher capacity for FeCN reduction both in intact and in excised roots. Excision did not affect the FeCN reduction of AP roots but resulted in lower values in HP ones.

In photosynthetic tissues such as *Elodea* cells transplasmalemma electron transport was inhibited by DCMU, an inhibitor of noncyclic electron transport in chloroplast in the light, and at the same time glucose, a substrate for respiration, resulted in an increase of FeCN reductase activity in the dark (NOVAK *et al.*, 1988).

Carbohydrate content of roots can be modulated by several external or internal factors. Light-induced changes in abscisic acid level (WILKINS *et al.*, 1974; DURING *et al.*, 1960) or in cell wall-bound invertase activity of wheat roots (KRISHNAN *et al.*, 1985) have been known to increase the sugar content of roots.

AP roots in Petri dishes or in glass pots on filter paper are exposed to several conditions which affect the substrate pool for respiration or respiration itself: they are growing in the light, in a relatively closed room where the concentrations of CO₂ and O₂ and water potential differ from hydroponics.

The effect of AP conditions on soluble and reducing sugar content and oxygen consumption of roots are summarized in Figs. 4 and 5.

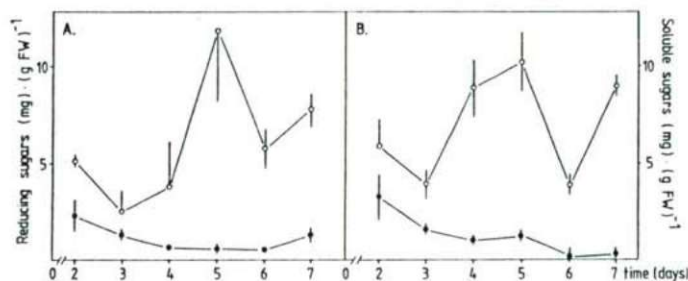


Fig. 4. Changes in the level of reducing (A) and soluble (B) sugar content of AP (○) and HP (●) cucumber roots. Vertical bars denote SD.

Changes in soluble carbohydrate level of the roots of intact plants correspond well with the FeCN reductase activity in long term incubation, but a peak in O₂ consumption of AP roots follows the increase in the substrate level for respiration. This suggests that the conventional respiratory control (ADP availability for mitochondrial electron transport chain, control of glycolysis and Krebs tricarboxylic acid cycle by ATP) took place before the increase in O₂ consumption.

On the contrary, the oxygen consumption of excised root segments depended on the endogenous soluble sugar content of the tissue during 20 hours incubation period of maize root segments (SAGLIO and PRADET, 1980). Exogenous glucose could restore the respiratory O₂ consumption to the initial value, indicating a control of respiration by the substrate when the tissue was in shortage of energy reserves.

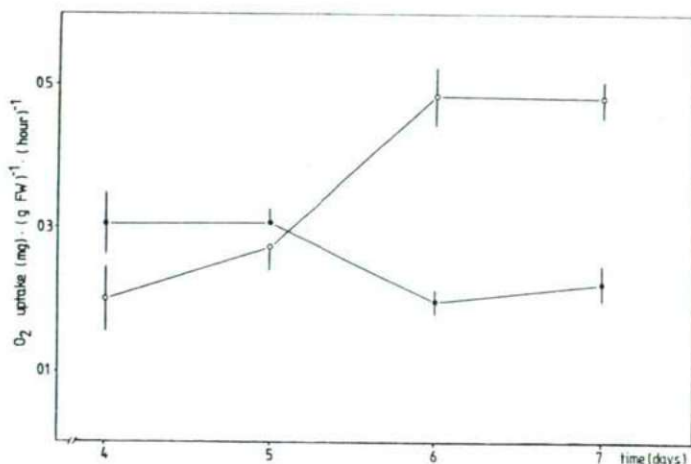


Fig. 5. Changes in total O₂ uptake of AP (○) and HP (●) cucumber roots. Vertical bars represent \pm SD (n=6).

Root segments with high soluble carbohydrate reserves can maintain higher respiration rate and reduced coenzyme level which result in higher capacity for the reduction of exogenous FeCN in long term experiments.

The data presented here demonstrate that there is no difference in FeCN reducing activity of intact and excised roots of AP and HP plants if the incubation period is short enough. Results of longer incubation and of excision, which decreased FeCN reduction of HP but did not that of AP roots, reflect the differences in carbohydrate reserves of roots.

In short term incubations we found differences only at the peak of soluble sugar content in AP roots.

These data support the view of ERDEI et al. (1989) who suggest that K⁺ uptake need not be directly coupled to the redox activity at the plasmalemma of the Fe-deficient and sufficient sunflower and wheat roots. The low K⁺ uptake rate in AP cucumber roots can be explained by the increase in osmotically active sugars which can substitute K⁺ under osmotic stress (CUTLER et al., 1977).

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