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ARTICLE *in* JOURNAL OF PLANT NUTRITION AND SOIL SCIENCE · JANUARY 1993

Impact Factor: 1.46 · DOI: 10.1002/jpln.19931560210

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Role of Plasmalemma H^+ ATPase in Sugar Retention by Roots of Intact Maize and Field Bean Plants

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Angenommen: 27. Juli 1992

Summary - Zusammenfassung

Net release and net uptake of sugars by roots of intact maize (*Zea mays* cv. Blizzard) and field bean (*Vicia faba* L. cv. Alfred) were studied at micromolar external sugar concentrations that are relevant to the rhizosphere. Besides various sugars not further characterized there was net release of glucose, fructose, sucrose, arabinose, ribose, and galactose. The net release of these sugars into the root medium (0.1 mM $CaSO_4$) was stimulated by the protonophore CCCP (10 µM), the sulfhydryl reagent NEM (300 µM), the specific inhibitor of plasmalemma H^+ ATPase vanadate (0.5 mM), and by the inhibitor of the glucose carrier phlorizin (2 mM). Net uptake of glucose, fructose, and arabinose from 10 µM external concentrations was inhibited by these substances. Stimulation of net release and inhibition of net uptake was most pronounced for glucose. Sucrose added to the root medium was hydrolyzed by invertase activity leading to glucose and fructose uptake by roots. It is concluded that the retention of sugars by plant roots is not only determined by plasmalemma permeability but is also controlled by the H^+ electrochemical gradient established by ATPase activity (retrieval mechanism). The proton gradient drives a sugar/H^+ cotransport system that is selective for glucose but may also transport other sugars, particularly in the absence of glucose.

Die Bedeutung der Plasmalemma-H^+-ATPase für die Zuckerretention von Wurzeln intakter Mais- und Ackerbohnenpflanzen

Nettoabgabe und Nettoaufnahme von Zuckern durch Wurzeln intakter Mais- (*Zea mays* L. cv. Blizzard) und Ackerbohnenpflanzen (*Vicia faba* L. cv. Alfred) wurden bei mikromolaren Zuckerkonzentrationen im Außenmedium, die für Rhizosphärenbedingungen von Bedeutung sind, untersucht. Neben verschiedenen, nicht näher charakterisierten Zuckern wurden Glucose, Fructose, Saccharose, Arabinose, Ribose und Galactose abgegeben. Die Nettoabgabe dieser Zucker in das Wurzelmedium (0,1 mM $CaSO_4$) wurde durch das Protonophor CCCP (10 µM), das Sulfhydrylreagenz NEM (300 µM), den spezifischen Inhibitor der plasmalemmagebundenen H^+-ATPase Vanadat (0.5 mM) und durch den Inhibitor des Glucosecarriers Phlorizin (2 mM) gefördert. Die Nettoaufnahme von Glucose, Fructose und Arabinose bei je 10 µM Außenkonzentration wurde durch diese Substanzen gehemmt. Die Stimulierung der Nettoabgabe und die Hemmung der Nettoaufnahme waren am deutlichsten bei Glucose. Dem Wurzelmedium zugegebene Saccharose wurde durch Invertaseaktivität hydrolysiert, Glucose und Fructose wurden von den Wurzeln aufgenommen. Es wird die Schlußfolgerung gezogen, daß die Retention von Zuckern durch Pflanzenwurzeln nicht nur von der Permeabilität des Plasmalemmas abhängt, sondern auch vom elektrochemischen Protonengradienten kontrolliert wird, der durch ATPase-Aktivität aufgebaut wird (Retrieval-Mechanismus). Der Protonengradient treibt einen Zucker/H^+-Cotransport an, der selektiv für Glucose ist, aber auch andere Zucker, besonders in Abwesenheit von Glucose, transportieren kann.

1 Introduction

Numerous investigations have shown that a significant amount of assimilates (up to 20 %) produced by photosynthesis is released into the rhizosphere as root exudates (*Mc Dougall*, 1970; *Barber* and *Martin*, 1976; *Sauerbeck* and *Johnen*, 1976; *Martin*, 1977; *Haller* and *Stolp*, 1985). These root exudates are important for the nutrition of microorganisms and, thus, the microbial activity in the rhizosphere is higher than in the bulk soil. Root exudates also affect the nutrient acquisition of plants (*Marschner*, 1986; *Mengel* and *Kirkby*, 1987).

Insoluble and soluble organic compounds may contribute equally to root exudation (*Lambers*, 1987). Since sugars, organic acids, and amino acids diffuse out of roots in response to concentration gradients they are also designated as diffusates (*Uren* and *Reisenauer*, 1988). The diffusion rate not only depends on the concentration gradient but, according to Fick's law, also on the plasmalemma permeability of root cells. Consequently, sugar efflux from roots

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is determined by the sugar concentrations of both cytosol and rhizosphere as well as by the plasmalemma permeability.

Besides passive diffusion of sugars out of root cells there is evidence of active sugar uptake into root cells. *Zea mays*, *Picea abies*, and *Beta vulgaris* root cells selectively take up glucose (and to a minor extent fructose and other sugars) after invertase-catalyzed hydrolysis of sucrose (*Lin* et al., 1984; *Getz* et al., 1987; *Xia* and *Saglio*, 1988; *Salzer* and *Hager*, 1991).

Although there is some uptake of sucrose into maize root tips (*Xia* and *Saglio*, 1988), significant plasmalemma transport of sucrose into roots has only been demonstrated for sugar beet (*Lemoine* et al., 1988). The kinetics of sugar uptake can be resolved into a linear (low affinity) and a saturable (high affinity) component. The latter appears to be carrier-mediated (*Lin* et al., 1984; *Getz* et al., 1987; *Lemoine* et al., 1988; *Xia* and *Saglio*, 1988).

Active sugar uptake is probably energized by an electrochemical proton gradient, established by H^+ ATPase activity which drives sugar/H^+ cotransport (*Reinhold* and *Kaplan*, 1984). This may be concluded from inhibition of active sugar uptake by protonophores, ATPase inhibitors and high medium pH (*Lin* et al., 1984; *Getz* et al., 1987; *Xia* and *Saglio*, 1988). Also transient alkalinization of the test medium by *Chlorella vulgaris* during sugar uptake indicates H^+/sugar cotransport (*Komor* et al., 1989).

Re-uptake of sugars once released into the rhizosphere (retrieval mechanism) could represent an important tool of roots to minimize sugar release into the rhizosphere. Since all investigations so far have been carried out at millimolar concentrations, too high to be relevant to rhizosphere conditions, we tested the effect of various inhibitors on net release and net uptake of sugars by intact maize and field bean plants at micromolar sugar concentrations in the test medium.

2 Materials and Methods

2.1 Plant cultivation

Maize (*Zea mays* L. cv. Blizzard) and field bean (*Vicia faba* L. cv. Alfred) seeds were imbibed in aerated 0.5 mM $CaSO_4$ for 1 day and then germinated at 20 - 25°C in the dark on filter paper moistened with 0.5 mM $CaSO_4$. After 4 days maize seedlings were transferred to 5 l plastic containers (4 plants per container) with 1/5 concentrated nutrient solution. this was substituted by 1/2 concentrated solution after 4 days. After an additional 4 days full strength nutrient solution was given and subsequently replaced every 4 days. The full strength nutrient solution had the following composition: 3.0 mM NH_4NO_3, 0.3 mM NaH_2PO_4, 2.0 mM K_2SO_4, 4.0 mM $CaCl_2$, 2.0 mM $MgSO_4$, 0.2 µm H_3BO_3, 0.1 µM $CuSO_4$, 0.01 µM $(NH_4)_6MO_7O_{24}$, 0.2 µM $MnSO_4$, 0.1 µM $ZnSO_4$, 100 µM Fe-EDTA.

After 1 week of germination field bean seedlings were illuminated (80 W m^{-2}) and after another week transferred to a 100 l plastic container (64 plants per container) with 1/10 concentrated nutrient solution which was substituted by 1/5 and 1/2 concentrated solutions after 5 days and 10 days, respectively. Full strength nutrient solution was given after an additional 5 days and subsequently replaced every 5 days. The full strength nutrient solution had the following composition: 1.0 mM NH_4NO_3, 0.6 mM Na_2HPO_4, 2.0 mM K_2SO_4, 4.0 mM $CaCl_2$, 1.0 mM $MgSO_4$, 4.0 µM H_3BO_3, 1.0 µM $CuSO_4$, 0.2 µM $(NH_4)_6Mo_7O_{24}$, 6.0 µM $MnSO_4$, 1.0 µM $ZnSO_4$, 50 µM Fe-EDTA, 0.2 µM $CoCl_2$. The pH of the nutrient solution was kept constant at pH 6.2 by continuous titration with 0.1 M NaOH using a Schott pH stat system.

Maize and field bean plants were cultivated under controlled conditions in a growth chamber. Illumination (80 W m^{-2}) followed a day/night cycle of 15 h/9 h with temperatures of 25°C and 18°C (maize) or a day/night cycle of 13 h/11 h with temperatures of 18°C and 12°C (field beans).

2.2 Experimental procedures

As compounds were not labelled but measured chemically, it was not possible to determine true sugar influx or efflux rates. Instead experimental conditions were chosen which allowed to measure net release (efflux > influx) or net uptake (efflux < influx) of sugars.

2.2.1 Sugar net release experiments

Plants were used for the experiments when the 6th leaf (maize) or 8th leaf (field beans) had developed. Roots were thoroughly washed with 0.1 mM $CaSO_4$ in order to exchange nutrients from the root apoplast. Entire plants were transferred to 5 l pots containing 0.1 mM $CaSO_4$ solution. Plants remained there for about 5 minutes. This procedure was carried out three times and plants were then transferred to test solutions.

Each experiment comprised eight 1 l pots containing 0.1 mM $CaSO_4$ solution with or without inhibitors (see Results), so that each treatment was made up of four replicates (4 pots, each supporting 4 plants). Plants remained in the solutions for 1 hour and after that the pH change of the solutions and the net release of sugars were determined. For this purpose solutions were vacuum-filtered through 0.2 µm membrane filters (Schleicher and Schüll), concentrated 40 fold by means of a vacuum rotary evaporator at 35 - 40°C, and purified using 3 ml reversed-phase octadecylsilane (C 18) or on-guard-P columns (Dionex).

2.2.2 Sugar net uptake experiments

The experiments were carried out as described above. However, at the beginning, 10 µM glucose, 10 µM fructose, and 10 µM arabinose were added to the uptake solutions. During the experiment (5 hours) aliquot samples of 10 ml solution were collected after 15 and 30 minutes and after 1, 2, 3, 4, and 5 hours. The solutions were vacuum-filtered through 0.2 µm membrane filters and then purified as described.

2.3 Analytical procedures

2.3.1 Proton net release

Proton net release by the roots of intact plants was determined using a pH Ross electrode (model 81 - 02, Orion) and a microprocessor ionalyzer (model 901, Orion). Acidification of the root medium (initial pH about 5.5) was linear with time for the first hour and reached a minimum pH level between 4.7 and 5.0 in control treatments within three hours. Proton net release was determined by measuring the pH values before and after the one-hour experiments. From the difference in H^+ activities before and after the experiments net H^+ release was calculated. This procedure is justified because the activity coefficients of the test solutions used were close to 1 (unless otherwise stated).

2.3.2 Sugar analysis

Sugar analysis was achieved by means of pulsed amperometric detection after separation of sugars by anion exchange chromatography (ion chromatograph type 4000i, Dionex). Sugars were separated on an HPIC AS6 column using a basic eluant (100 mM NaOH). Since it was not possible to separate the hexoses glucose, galactose, mannose, and the pentose xylose with the 100 mM NaOH eluant the samples were measured also after separating with a 5 mM NaOH eluant.

Due to their pK values (between 12 and 14) hydroxyls of sugars dissociate in the basic eluant and retention times of the various sugars depend on the number and the position of hydroxyl groups. The electrochemically active sugars were detected by means of a gold electrode using pulsed amperometric detection with a repeated sequence of 3 applied potentials (E_1, E_2, E_3). E_1 (+ 0.1 V) was applied for 300 milliseconds to oxidize the electrochemically active sugars. To clean the electrode surface a more positive potential (E_2 = 0.6 V) was applied for 120 milliseconds, followed by E_3 (- 0.8 V) for 300 milliseconds to reduce gold oxide back to gold. The most important potential is E_1 at which the sugar oxidation current is measured which is proportional to sugar concentration. The pulsed amperometric detection of sugars is characterized by high sensitivity which allows the measurement of 50 nM sugar concentrations (Dionex technical note, 1987).

2.3.3 ATP analysis

The nucleotide adenosinetriphosphate (ATP) was determined enzymatically after extraction with 0.6 M perchloric acid according to *Schubert* and *Mengel* (1986). Briefly, 1 g of root fresh matter was rapidly frozen in liquid N_2 and stored at -20°C. In order to avoid incomplete extraction 100 mg EDTA were added to the roots during homogenization in 5 ml 0.6 M perchloric acid at 0°C. The homogenate was then centrifuged for 10 min at 0°C and 10000 g. In the supernatant the nucleotide was determined with a UV-test method (Boehringer Mannheim, Germany).

2.3.4 Statistical treatment

Variation is indicated by standard error. Significant differences between treatments were calculated using the t-test.

2.3.5 Abbreviations

CCCP, carbonylcyanide-3-chlorophenylhydrazone; NEM, N-ethylmaleimide.

3 Results and Discussion

3.1 Effect of inhibitors on net H^+ release

The H^+ electrochemical gradient across the plasmalemma of root cells is established primarily by H^+ ATPase activity (*Spanswick*, 1981; *Mengel* and *Schubert*, 1985; *Schubert* and *Mengel*, 1989). Consequently net H^+ release by ATPase activity may be used to describe the effect of various inhibitors on the H^+ gradient.

Table 1 summarizes the effect of inhibitors on net H^+ release within 1 h treatment. The variation of control values may be explained by the fact that experiments were started at differing daytime (i.e. variable duration of illumination at the beginning of experiments).

Table 1: Effect of CCCP, NEM, vanadate, and phlorizin on net H^+ release by roots of intact maize and field bean plants in 0.1 mM $CaSO_4$ within 1 h treatment

Tabelle 1: Einfluß von CCCP, NEM, Vanadat und Phlorizin auf die H^+-Nettoabgabe von Wurzeln intakter Mais- und Ackerbohnenpflanzen in 0,1 mM $CaSO_4$ innerhalb einer einstündigen Versuchsdauer

Treatment	Maize	Field bean
	µmoles H^+ l^{-1}	
Control	6.7	1.6
10 µM CCCP	- 1.0***	- 1.0**
Control	12.2	n.d.
300 µM NEM	2.1***	n.d.
Control	10.5	6.8
500 µM vanadate	1.5**	1.8***
Control	36.1	4.3
2 mM phlorizin	4.1***	- 1.6***

Significant differences at * P = 5 %, ** P = 1 %, or *** P = 0.1 % level, respectively

The protonophore and uncoupler of oxidative phosphorylation carbonylcyanide-3-chlorophenyl-hydrazone (CCCP) changed net H^+ release into net H^+ uptake (Tab. 1, negative sign). This is in line with the effect of CCCP on ATP supply to the H^+ ATPase (Tab. 2) and on the H^+ permeability of the plasmalemma (*Felle* and *Bentrup*, 1977). Both these effects contribute to the collapse of the H^+ electrochemical gradient (*Felle*, 1987; *Schubert*, 1989). A similar result was obtained with the sulfhydryl reagent N-ethylmaleimide (NEM, Tab. 1) which decreases ATP concentrations (Tab. 2) and interferes with proteins that bear SH groups (*Schöberl*, 1958).

Vanadate was the most selective inhibitor used in this study. This may be concluded from the observation that, as a specific inhibitor of plasmalemma-bound H^+ ATPase (*O'Neill* et al., 1983; *Mengel* and *Schubert*, 1985,) vanadate did not affect ATP supply (Tab. 2). Unchanged ATP concentration is a strong argument against side effects of vanadate. The significant influence on apparent net H^+ release may be partly ascribed to H^+ buffering but a direct effect on the ATPase activity *in vivo* can be inferred from the depolarization by vanadate (*Thibaud* et al., 1986) and from

Table 2: Effect of CCCP, NEM, vanadate, and phlorizin on ATP concentrations of roots of intact maize plants after 1 h treatment

Tabelle 2: Einfluß von CCCP, NEM, Vanadat und Phlorizin auf die ATP-Konzentrationen von Wurzeln intakter Maispflanzen nach einstündiger Versuchsdauer

Treatment	- inhibitor	+ inhibitor	% of control
	µmoles ATP/kg root fresh weight		
10 µM CCCP	856 (± 113)	339 (± 86)	40
300 µM NEM	1023 (± 125)	690 (± 60)	67
500 µM vanadate	789 (± 42)	789 (± 73)	100
2 mM phlorizin	1129 (± 59)	1045 (± 80)	93

increased inhibition during cation-stimulated net H^+ release (*Schubert* and *Läuchli*, 1986). Phlorizin, an inhibitor of glucose transport and ATP synthesis in isolated mitochondria (*Felle* et al., 1983) did not affect ATP levels in maize roots (Tab. 2) but, markedly, net H^+ release (Tab. 1). This result suggests that phlorizin does not only inhibit the glucose carrier but may somehow interfere with net H^+ release.

In conclusion, all tested inhibitors strongly inhibited net H^+ release and therefore reduced the H^+ electrochemical gradient at the plasmalemma of maize and field bean root cells. Because of vanadate specificity any effect of this inhibitor on membrane permeability is improbable. Side effects of the other inhibitors that partly decreased ATP concentrations were minimized by choosing short experimental procedures.

3.2 Effect of inhibitors on sugar transport

During a 1 h experimental period there was net release of various sugars by maize and field bean roots into 0.1 mM $CaSO_4$ test solution. Besides many sugars not further characterized we identified glucose, fructose, sucrose, arabinose, ribose, and galactose (Tab. 3).

Even though nothing is known about the compartmentation of these sugars in the root cells, millimolar tissue concentrations indicate passive efflux into the external medium (Tab. 4). The low amount and the diversity of sugars exuded into the medium supports the concept that phloem unloading in maize roots occurs via the symplastic pathway (*Giaquinta* et al., 1983) and that exudation represents passive diffusion of sugars out of root cells. Net release of various sugars by plant roots was demonstrated before (e.g. *Schönwitz* and *Ziegler*, 1982).

Net release of some of the sugars identified was stimulated by inhibitors. This was especially the case for glucose (Tab. 3). Since sugar concentrations of roots were not significantly increased (except for sucrose the concentration of which was doubled by CCCP in maize roots, Tab. 4) it is unlikely that the stimulation of net release of sugars was due to an increase of sugar concentrations within root cells.

Table 3: Effect of CCCP, NEM, vanadate, and phlorizin on sugar net release rate of roots of intact maize and field bean plants in 0.1 mM $CaSO_4$ within 1 h treatment

Tabelle 3: Einfluß von CCCP, NEM, Vanadat und Phlorizin auf die Nettoabgaberaten von Zuckern aus Wurzeln intakter Mais- und Ackerbohnenpflanzen in 0,1 mM $CaSO_4$ innerhalb einer einstündigen Versuchsdauer

Treatment	Glucose		Fructose		Sucrose		Arabinose		Ribose		Galactose	
	µmoles (kg root fresh weight · h)$^{-1}$											
	-	+	-	+	-	+	-	+	-	+	-	+
Maize												
10 µM CCCP	21.4	77.9***	3.7	12.1**	3.3	3.3	57.4	77.9	0.0	0.0	1.1	2.4
300 µM NEM	2.1	27.1***	1.7	7.0	0.6	2.2	1.2	1.9	0.1	5.6***	0.0	0.0
500 µM vanadate	3.1	5.8*	2.1	2.8	1.1	0.8	6.0	7.6	5.9	2.7	0.8	0.6
2 mM phlorizin	21.3	125.8***	7.0	22.4	2.3	9.9	5.9	6.0	1.0	5.8**	0.0	0.0
Field bean												
10 µM CCCP	2.2	23.2**	0.8	12.3**	1.4	14.0*	0.3	4.2**	1.6	13.0*	0.9	6.3**
300 µM NEM	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
500 µM vanadate	1.6	2.4	0.9	0.8	0.9	0.9	1.0	1.1	1.2	0.9	0.0	0.0
2 mM phlorizin	10.1	51.6**	2.8	13.5**	4.8	8.1	4.6	16.4*	4.9	11.3*	0.0	3.2*

Significant differences between control (-) and inhibitor treatment (+) at *P = 5 %, **P = 1 %, or ***P = 0.1 % level, respectively

Table 4: Effect of CCCP, NEM, vanadate, and phlorizin on sugar concentrations of roots of intact maize plants after 1 h treatment

Tabelle 4: Einfluß von CCCP, NEM, Vanadat und Phlorizin auf die Zuckerkonzentrationen von Wurzeln intakter Maispflanzen nach einstündiger Versuchsdauer

Treatment	Glucose	Fructose	Sucrose	Arabinose	Ribose	Galactose
	mmoles/kg root fresh weight					
Control	4.50 (±1.37)	1.76 (±0.60)	0.07 (±0.02)	0.58 (±0.28)	0.60 (±0.20)	n.d.
10 µM CCCP	3.90 (±0.74)	1.38 (±0.30)	0.16 (±0.03)	0.12 (±0.02)	0.43 (±0.09)	n.d.
Control	1.52 (±0.30)	1.46 (±0.26)	0.63 (±0.06)	0.92 (±0.21)	0.71 (±0.08)	0.41 (±0.13)
300 µM NEM	1.12 (±0.11)	1.37 (±0.18)	0.50 (±0.11)	1.04 (±0.02)	0.48 (±0.07)	0.57 (±0.05)
Control	1.94 (±0.10)	0.71 (±0.09)	0.17 (±0.03)	0.32 (±0.23)	0.43 (±0.22)	n.d.
500 µM vanadate	2.73 (±0.41)	0.83 (±0.12)	0.20 (±0.03)	0.08 (±0.03)	0.29 (±0.04)	n.d.
Control	0.96 (±0.12)	0.52 (±0.09)	0.09 (±0.01)	0.33 (±0.10)	0.25 (±0.04)	0.36 (±0.06)
2 mM phlorizin	0.84 (±0.24)	0.39 (±0.05)	0.15 (±0.03)	0.34 (±0.01)	0.36 (±0.04)	0.27 (±0.03)

Also an increase of sugar net release from an inner compartment such as the vacuole can be excluded since vanadate does not inhibit the tonoplast ATPase (*Leonard*, 1988).

We therefore postulate that net sugar release by the roots of maize and field bean plants was increased by inhibitors because the re-uptake of sugars was inhibited. This is supported by the finding that net sugar uptake by roots of intact maize (Figs. 1-3) and field bean (not shown) plants was significantly decreased by inhibitors. Transport from the external medium (micromolar concentrations) into the root cells (millimolar concentrations) occurs actively (i.e. against a concentration gradient).

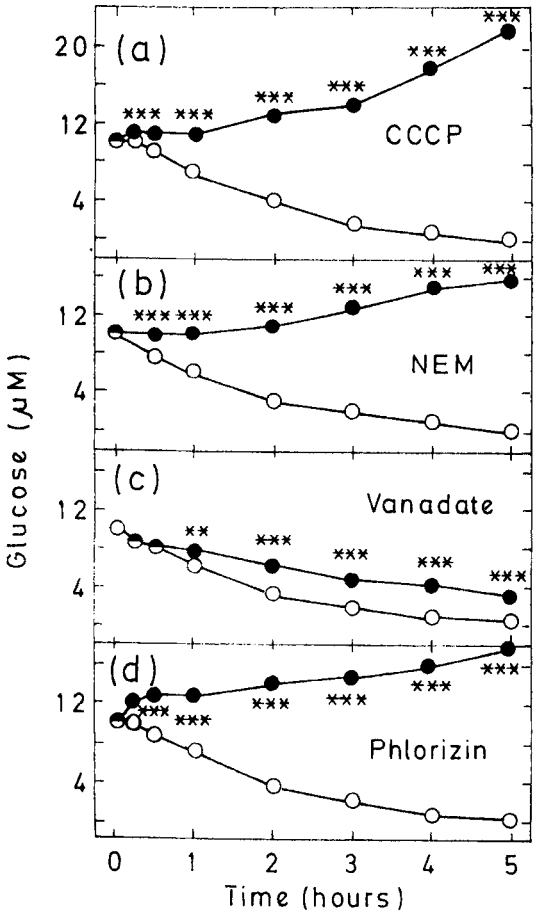

Figure 1: Effect of CCCP (10 µM, a) NEM (300 µM, b), vanadate (500 µM, c), and phlorizin (2 mM, d) on the time course of glucose concentration in the root medium of intact maize plants. Root medium at time '0' was 10 µM glucose + 10 µM fructose + 10 µM arabinose + 0.1 mM $CaSO_4$; (○) control, (●) inhibitor treatment. Significant differences at *P = 5 %, **P = 1 % or ***P = 0.1 % level, respectively.

Abbildung 1: Einfluß von CCCP (10 µM, a), NEM (300 µM, b), Vanadat (500 µM, c) und Phlorizin (2 mM, d) auf den Zeitverlauf der Glucosekonzentration im Wurzelmedium intakter Maispflanzen. Das Wurzelmedium bestand zum Zeitpunkt '0' aus 10 µM Glucose + 10 µM Fructose + 10 µM Arabinose + 0,1 mM $CaSO_4$; (○) Kontrolle, (●) Hemmstoff. Signifikante Unterschiede mit einer Irrtumswahrscheinlichkeit von *P = 5 %, **P = 1 % bzw. ***P = 0,1 %.

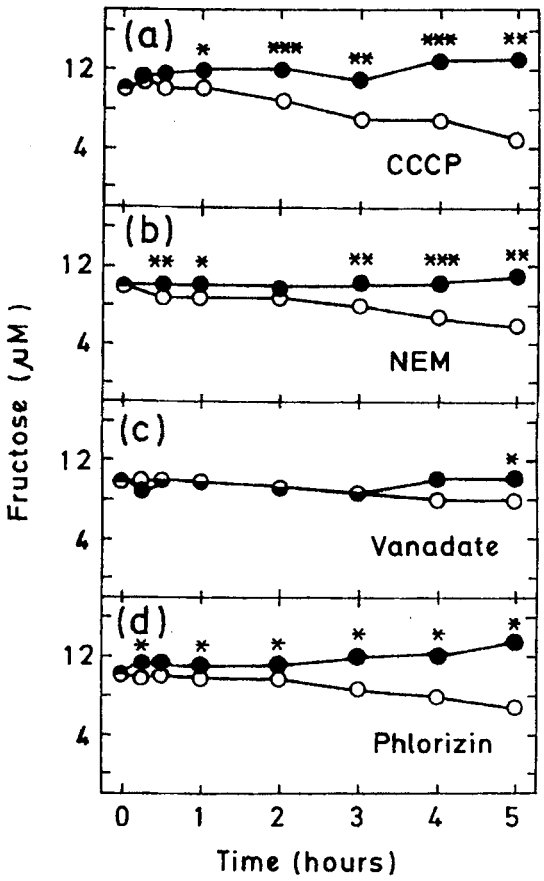

Figure 2: Effect of CCCP (10 µM, a), NEM (300 µM, b), vanadate (500 µM, c), and phlorizin (2 mM, d) on the time course of fructose concentration in the root medium of intact maize plants. Experimental conditions were as described for Fig. 1.

Abbildung 2: Einfluß von CCCP (10 µM, a), NEM (300 µM, b), Vanadat (500 µM, c) und Phlorizin (2 mM, d) auf den Zeitverlauf der Fructosekonzentration im Wurzelmedium intakter Maispflanzen. Die Versuchsbedingungen waren wie für Abb. 1 beschrieben.

The specificity of vanadate seems to rule out the involvement of an active sugar transport mechanism that is not related to ATPase activity. Also since bacteria do not possess E_1E_2 ATPase activity (*Nelson*, 1988), that is specifically inhibited by vanadate, possible bacterial contamination is unlikely to have affected the results. The same applies to possible fungal effects. Five hours preincubation of roots in 0.1 mM $CaSO_4$ + 0.1 mM glucose (a treatment that should have stimulated fungal activity at the root surface) did not change net glucose uptake (not shown).

The strongest effect of inhibitors was not only found for net glucose release (Tab. 3) but net uptake of fructose and arabinose only occurred when the test medium was depleted of glucose (compare Fig. 1 with Figs. 2 and 3). This is underlined by another experiment. Addition of sucrose to the test medium revealed that, after invertase-catalyzed sucrose hydrolysis (*Salzer* and *Hager*, 1991), glucose was preferentially taken up by maize roots (Fig. 4). Fructose uptake was observed when the test medium was depleted of

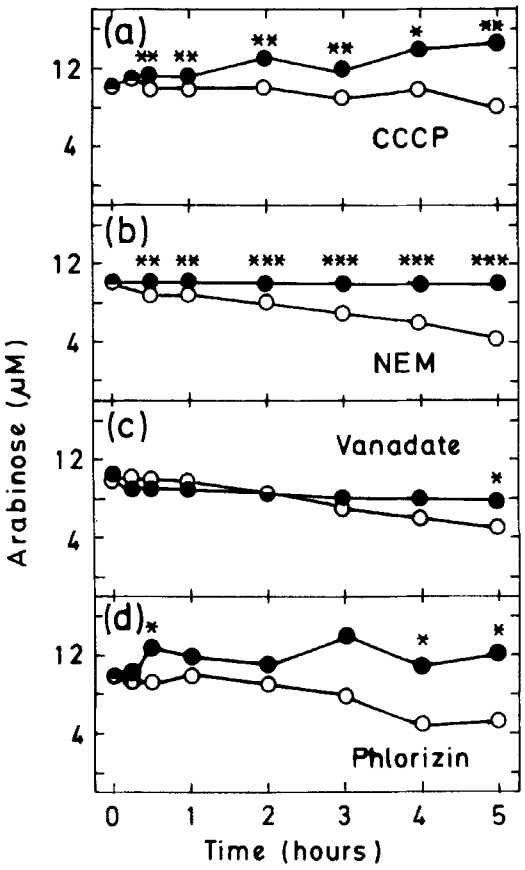

Figure 3: Effect of CCCP (10 µM, a), NEM (300 µM, b), vanadate (500 µM, c), and phlorizin (2 mM, d) on the time course of arabinose concentration in the root medium of intact maize plants. Experimental conditions were as described for Fig. 1.

Abbildung 3: Einfluß von CCCP (10 µM, a), NEM (300 µM, b), Vanadat (500 µM, c) und Phlorizin (2 mM, d) auf den Zeitverlauf der Arabinosekonzentration im Wurzelmedium intakter Maispflanzen. Die Versuchsbedingungen waren wie für Abb. 1 beschrieben.

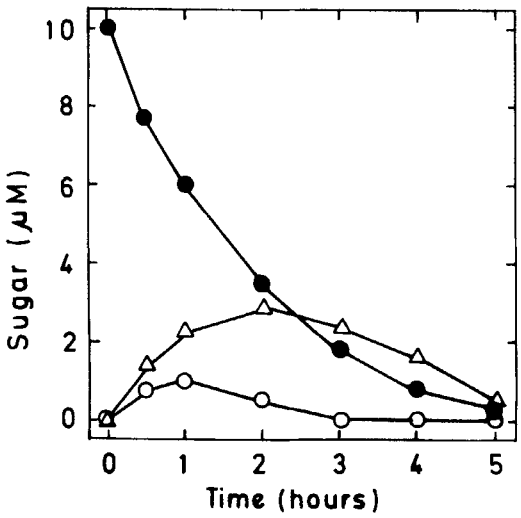

Figure 4: Time course of sucrose (•), fructose (Δ), and glucose (°) concentrations in the root medium of intact maize plants. Root medium at time '0' was 10 µM sucrose + 100 µM $CaSO_4$.

Abbildung 4: Zeitverlauf der Konzentrationen von Saccharose (•), Fructose (Δ) and Glucose (°) im Wurzelmedium intakter Maispflanzen. Das Wurzelmedium bestand zum Zeitpunkt '0' aus 10 µM Saccharose + 100 µM $CaSO_4$.

glucose. This is in agreement with the finding that the hexose carrier in the plasmalemma of root cells is not only specific for glucose (*Lin* et al., 1984; *Getz* et al., 1987) but that binding of glucose to the carrier allosterically inhibits fructose transport (*Xia* and *Saglio*, 1988).

4 Conclusions

Net sugar release and net sugar uptake experiments with roots of intact maize and field bean plants suggest that sugar retention by root cells is essentially controlled by the H^+ electrochemical gradient established by H^+ ATPase activity. Degradation of the H^+ gradient stimulates net sugar release because of disturbed re-uptake of sugars (in particular glucose) via a proton/sugar cotransport system. Thus the retention of sugars by root cells not only depends on the plasmalemma permeability but also on the H^+ electrochemical gradient. *Matzke* (1988) found an analogous retrieval mechanism for organic anions and amino acids. In this context it is of particular interest that heterotrophic microorganisms have developed mechanisms to tap the plant for carbohydrates by selectively inhibiting the plasmalemma H^+ ATPase (*Patrick*, 1989). *Cercospora beticola* is such an example which produces an ATPase-specific toxin and thereby obviates sugar retrieval by plant cells (*Macri* et al., 1980; *Blein* et al., 1988).

Acknowledgement

We are grateful for financial support by the *Deutsche Forschungsgemeinschaft (DFG).*

References

Barber, D. A. and *J. K. Martin* (1976): The release of organic substances by cereal roots into the soil. New Phytol. 76, 69-80.

Blein, J.-P., I. Bourdi, M. Rossignol and *R. Scalla* (1988): *Cercospora beticola* toxin inhibits vanadate-sensitive H^+ transport in corn root membrane vesicles. Plant Physiol. 88, 429-434.

Felle, H. (1987): Proton transport and pH control in *Sinapis alba* root hairs: A study carried out with double-barrelled pH micro-electrodes. J. Exp. Bot. 38, 340-354.

Felle, H. and *F.-W. Bentrup* (1977): A study of the primary effect of the uncoupler carbonyl cyanide m-chlorophenylhydrazone on membrane potential and conductance in *Riccia fluitans*. Biochim. Biophys. Acta 464, 179-187.

Felle, H., J. P. Gogarten and *F.-W. Bentrup* (1983): Phlorizin inhibits hexose transport across the plasmalemma of *Riccia fluitans*. Planta 157, 267-270.

Getz, H.-P., D. Knauer and *J. Willenbrink* (1987): Transport of sugars across the plasma membrane of beetroot protoplasts. Planta 171, 185-196.

Giaquinta, R. T., W. Lin, N. L. Sadler and *V. R. Franceschi* (1983): Pathway of phloem unloading of sucrose in corn roots. Plant Physiol. 71, 362-367.

Haller, T. and *H. Stolp* (1985): Quantitive estimation of root exudation of maize plants. Plant Soil 86, 207-216.

Komor, E., B.-H. Cho, S. Schricker and *C. Schobert* (1989): Charge and acidity compensation during proton - sugar symport in *Chlorella* (1989): The H^+-ATPase does not fully compensate for the sugar-coupled proton influx. Planta 177, 9-17.

Lambers, H. (1987): Growth, respiration, exudation, and symbiotic associations: The fate of carbon translocated to the roots, in P.J. Gregory, J.V. Lake and D.A. Rose: Root Development and Function. Cambridge University Press, Cambridge, p. 124-145.

Lemoine, R., J. Daie and *R. Wyse* (1988): Evidence for the presence of a sucrose carrier in immature sugar beet tap roots. Plant Physiol. 86, 575-580.

Leonard, R. T. (1988): Plasma membrane H^+-ATPase, in J.L. Harwood and T.J. Walton: Plant Membranes - Structure, Assembly and Function. The Biochemical Society, London, p. 179-188.

Lin, W., M. R. Schmitt, W. D. Hitz and *R. T. Giaquinta* (1984): Sugar transport in isolated corn root protoplasts. Plant Physiol. 76, 894-897.

Macri, F., A. Vianello, R. Cerana and *F. Rasi-Caldogno* (1980): Effects of *Cercospora beticola* toxin on ATP level of maize roots and on the phosphorylating activity of isolated pea mitochondria. Plant Sci. Lett. 18, 207-214.

Marschner, H. (1986): Mineral Nutrition of Higher Plants. Academic Press, London.

Martin, J. K. (1977): Factors influencing the loss of organic carbon from wheat roots. Soil Biol. Biochem. 9, 1-7.

Matzke, H. (1988): Anionenabscheidung der Wurzel bei symbiotisch ernährtem *Trifolium pratense*. Ph. D. Thesis Justus Liebig University, Giessen.

McDougall, B. M. (1970): Movement of ^{14}C-photosynthate into the roots of wheat seedlings and exudation of ^{14}C from intact roots. New Phytol. 69, 37-46.

Mengel, K. and *E. A. Kirkby* (1987): Principles of Plant Nutrition, 4th edition, International Potash Institute, Bern, Switzerland.

Mengel, K. and *S. Schubert* (1985): Active extrusion of protons into deionized water by roots of intact maize plants. Plant Physiol. 79, 344-348.

Nelson, N. (1988): Structure, function, and evolution of proton ATPases. Plant Physiol. 86, 1-3.

O'Neill, S. D., A. B. Bennett and *R. M. Spanswick* (1983): Characterization of a NO_3-sensitive H^+-ATPase from corn roots. Plant Physiol. 72, 837-846.

Patrick, J. W. (1989): Solute efflux from the host at plant-microorganism interfaces. Aust. J. Plant Physiol. 16, 53-67.

Reinhold, L. and *A. Kaplan* (1984): Membrane transport of sugars and amino acids. Annu. Rev. Plant Physiol. 35, 45-83.

Salzer, P. and *A. Hager* (1991): Sucrose utilization of the ectomycorrhizal fungi *Amanita muscaria* and *Hebeloma crustuliniforme* depends on the cell wall-bound invertase activity of their host *Picea abies*. Bot. Acta 104, 439-445.

Sauerbeck, D. and *B. Johnen* (1976): Der Umsatz von Pflanzenwurzeln im Laufe der Vegetationsperiode und dessen Beitrag zur "Bodenatmung". Z. Pflanzenernähr. Bodenk. 139, 315-328.

Schöberl, A. (1958): Thiophile Substanzen in der Eiweiß- und Enzymchemie. Angew. Chem. 70, 646-650.

Schönwitz, R. and *H. Ziegler* (1982): Exudation of water-soluble vitamins and of some carbohydrates by intact roots of maize seedlings (*Zea mays* L.) into a mineral nutrient solution. Z. Pflanzenphysiol. 107, 7-14.

Schubert, S. (1989): Measurement of electro-chemical gradients in roots, in Methods of K-Research in Plants. Proc. 21st Colloqu. Intern. Potash Institute, Bern, Switzerland, p. 37-47.

Schubert, S. and *A. Läuchli* (1986): Na^+ exclusion, H^+ release, and growth of two different maize cultivars under NaCl salinity. J. Plant Physiol. 126, 145-154.

Schubert, S. and *K. Mengel* (1986): Effect of light intensity on proton extrusion by roots of intact maize plants. Physiol. Plant 67, 614-619.

Schubert, S. and *K. Mengel* (1989): Important factors in nutrient availability: root morphology and physiology. Z. Pflanzenernähr. Bodenk. 152, 169-174.

Spanswick, R. M. (1981): Electrogenic ion pumps. Annu. Rev. Plant Physiol. 32, 267-289.

Thibaud, J.-B., A. Soler and *C. Grignon* (1986): H^+ and K^+ electrogenic exchanges in corn roots. Plant Physiol. 81, 847-853.

Uren, N. C. and *H. M. Reisenauer* (1988): The role of root exudates in nutrient acquisation, in B. Tinker and A. Läuchli: Advances in Plant Nutrition, Vol. 3, Praeger, New York, p. 79-114.

Xia, J.-H. and *P. H. Saglio* (1988): Characterization of the hexose transport system in maize root tips. Plant Physiol. 88, 1015-1020.

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