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Influence of ageing and abscisic acid on potassium uptake by potato tuber tissues

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Abstract

The potassium uptake by potato tuber discs tissues freshly cut and after 24 h of ageing in the presence or not of abscisic acid was investigated. Uptake kinetics revealed a biphasic dependence on external K^+ concentrations. At concentration less than 10 mM, uptake was mediated by a saturable component and a linear component became apparent at higher concentrations. At low K^+ concentrations (1mM), the capacity of K^+ uptake diminished by 2 times after ageing. Treatment of tissues with ABA increased the rate of K^+ uptake. In both fresh and aged tissues the uptake was strongly enhanced by fusicoccin and decreased by several metabolic inhibitors and ATPase inhibitors, underlying the active nature of uptake and suggesting the involvement of a plasmalemma H^+-ATPase in K^+ transport system.

Additional key words: H^+-ATPase, phytohormones, *Solanum tuberosum* L.

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Abbreviations: ABA - abscisic acid, CaM - calmodulin, DCCD - N,N'-dicyclohexyl-carbodiimide, DNP - 2,4-dinitrophenol, FAP - furfurylaminopurine, FC - fusicoccin, f.m. - fresh mass, GA_3 - gibberellic acid, HEPES - N-2-hydroxy-ethylpiperazine-N'-2-ethane sulphonic acid, IAA - indole-3-acetic acid, MES - 2(-N-morpholino)ethane-sulphonic acid, NEM - N-ethylmaleimide, SHAM - salicyl hydroxamic acid, W_7 - N-(6-aminohexyl)-5-chloro-1-naphthalene-sulphonamide.

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Introduction

Depending on potassium concentration in the extracellular medium, K^+ transport and its regulation are accomplished via two distinct pathways. The first, characterized as a channel-mediated K^+ uniport is a low-affinity system which operates at high external K^+ concentrations (> 1 mM; Sentenac *et al.* 1992, Schroeder *et al.* 1994, Michelet and Boutry 1995). The second is a high-affinity K^+ transport system, characterized as a ion-coupled antiport or symport. The proton-motive force created by the H^+-ATPase provides the energy required for these transports. However, the mechanism and the regulation of this high-affinity pathway that could account for K^+ accumulation at low K^+ concentrations remains controversed.

It has been proposed that the plant plasma membrane H^+-ATPase might directly mediate an H^+/K^+ exchange that could contribute to K^+ influx into the plant cell in addition to H^+ efflux (Lucas and Kochian 1988, for review see Briskin and Hanson 1992), however, several observations have been postulated an other system which was characterized as a plasma membrane H^+/K^+ symporter (Maathuis and Sanders 1994, Schachtman and Schroeder 1994, Michelet and Boutry 1995, Briskin and Gawienowski 1996). According to Briskin and Gawienowski (1996), the plant plasma membrane H^+-ATPase does not mediate direct K^+ transport, rather this enzyme provides a driving force for cellular K^+ uptake by secondary mechanisms, such as K^+ channels or H^+/K^+ symporters. Nevertheless, the possibility of direct K^+ transport by other ATPases (K^+-ATPase) cannot be ruled out (Kochian *et al.* 1989, Briskin and Gawienowski 1996).

On the other hand, it is well known that the H^+-ATPase has a major role in cellular responses to plant growth regulators. Indeed, like other phytohormones, ABA plays an important role in the regulation of nutrient fluxes by acting at the cellular level. However, although recent advances made in the understanding of its regulatory role in ions (especially K^+) and sugar uptake (Schüssler *et al.* 1984, Clifford *et al.* 1986, Suleiman *et al.* 1990, 1991, Penot *et al.* 1991, Feray *et al.* 1992, Penot *et al.* 1993), the molecular mechanism of ABA action at "sink" tissues is not yet sufficiently known. It has been postulated that the hormone may act presumably on the membrane ATPase-carrier complexes and could influence the membrane integrity and act on a H^+-pump coupled to K^+ transport (Van Steveninck and Van Steveninck 1983).

In potato, an ATPase activity has been characterised in the tuber cells (Oparka 1986, Ladyzhenskaya *et al.* 1991), but its role in nutrients uptake and its regulation by phytohormones are not yet fully resolved. In a recent work, Penot *et al.* (1993) reported that a 24 h treatment of the tissues with ABA prevented the increase of sucrose uptake. The aim of the present paper was to study general properties of K^+ uptake in potato tuber and to determine whether ABA inhibited sucrose uptake is linked to the activity of a primary translocation system (ATPase).

Materials and methods

Preparation of tuber discs: Potato (*Solanum tuberosum* L. cv. Spunta) tubers from plants grown in the field were used. After harvest tubers were stored at 4 °C in the

dark until use, without any conservation reagent. Discs of 10 mm diameter and 1 mm thick, cut in the central parenchyma were used immediately (fresh tissues) or after a 24-h period (aged tissues) in medium with or without ABA at temperature of 22 ± 2 °C in the dark (100 discs dm^{-3} of ageing solution). This medium contained chloramphenicol (50 mg dm^{-3}) to avoid proliferation of bacteria (Hourmant *et al.* 1979, Penot *et al.* 1993) and $CaSO_4$ (0.5 mM). Calcium is believed to be important for preservation of membrane integrity (Yan *et al.* 1992) and able to activate K^+-channels (for review see Bentrup 1990). The pH of the medium was adjusted at 6.0 and the ageing solution was continuously stirred on a reciprocal shaker (200 shakes min^{-1}). Then the discs were washed and used for uptake experiments.

Potassium uptake: ^{86}Rubidium, provided by *IAEA*, was used as a marker of K^+. The incubation medium contained 100 mM mannitol, 5 mM buffer MES (pH 6.0), 0.5 mM $CaSO_4$, 1 mM (or more) KCl and ^{86}Rb (4.6 kBq cm^{-3} or more). After appropriate uptake period (3 h), the tissues were collected and rinsed 3 times (10 min each) with cold 100 mM mannitol. The samples were then brought to the boil in 10 cm^3 80 % ethanol. Afterward 0.5 cm^3 of distilled water and 5 cm^3 of liquid scintillation cocktail (*Instagel*) were added to the tissues residues. The amount of the radioactivity taken up by the samples was determined with a *Tricarb 1000* (*Packard*, Austria) scintillation counter.

Results and discussion

Influence of K^+ concentration: Potassium uptake showed a biphasic dependence on external KCl concentrations. A saturable component dominated uptake at concentrations below 10 mM and a linear component became apparent at higher concentrations (Fig. 1, see Eadie-Hofstee plot of K^+ influx data on inset). Similar characteristics were shown in corn roots (Kochian and Lucas 1982, Kochian *et al.* 1985) and in potato leaf (Suleiman *et al.* 1991). According to Kochian *et al.* (1985), the saturable component of K^+ influx involves an ATPase participation, whereas the linear component implies K^+ channels.

Influence of ABA and FC: In order to check the participation of ATPase in K^+ uptake at low K^+ concentrations (1 mM), the effects of ABA, FC and specific inhibitors were investigated. ABA (20 μM) in the uptake medium induced a stimulation of both saturable and linear components of K^+ uptake (Tables 1, 2) by fresh tissue. Likewise fusicoccin (FC) which stimulates the plasmalemma H^+-ATPase (Marra *et al.* 1992), increased K^+ uptake by 50 % (Table 2), whereas 100 μM furfurylaminopurine (FAP) stimulated slightly the K^+uptake, IAA and GA_3 had no effect (results not shown).

Ageing of tissues (24 h) decreased the K^+ uptake rate (Table 2). This result opposite to most data concerning uptake of K^+ and other solutes (Gronewald *et al.* 1979, Lin 1979, Penot *et al.* 1993), however, the ageing is very complex and seem to differ from one plant to another. The decrease observed would be explained by either an important passive influx of K^+ in fresh tissues caused by the excision and/or a decrease of the carrier affinity in the course of ageing. The presence of 20 or 100 μM

ABA in the ageing medium stimulated markedly K^+ uptake, more than in fresh tissue (Fig. 2, Table 2). In the same conditions, FC added only to the uptake medium, also increased K^+ uptake (Table 2) more than in fresh tissue.

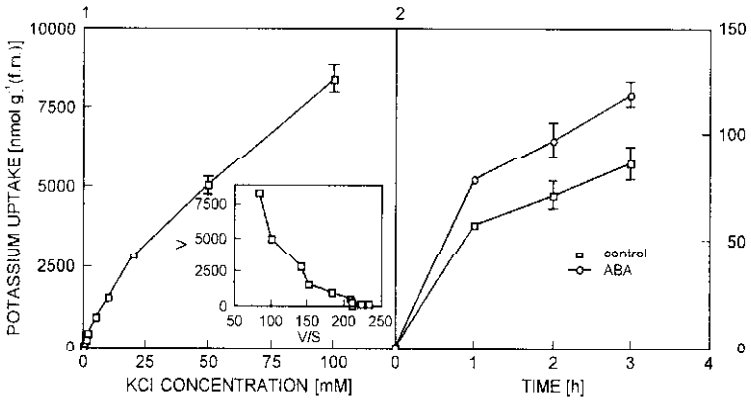

Fig. 1. Effect of potassium concentration on the absorption of ^{86}Rb by fresh potato tuber discs. The tissues were incubated for 3 h. Inset: a representative Eadie-Hofstee plot of K^+ uptake (V - K^+ uptake, S - K^+ concentration).

Fig. 2. Influence of 20 μM ABA in ageing medium (24 h) on the absorption of ^{86}Rb by potato tuber discs in the presence of 1 mM KCl. The tissues were incubated for 3 h.

Table 1. Effect of 20 μM ABA on ^{86}Rb uptake by fresh potato tuber discs in the presence of KCl at four concentrations. The tissues were incubated for 3 h. Means ± SE, $n = 3$; percentage of control in brackets.

KCl [mM]	^{86}Rb uptake [nmol g^{-1}(f.m.)] 0.1	1	10	100
Control	23.3 ± 2.0	210.3 ± 11.2	1510.8 ± 76.3	8342.5 ± 419.9
ABA 20 μM	24.2 ± 1.2 (104)	245.0 ± 17.0 (116)	1842.2 ± 170.9 (122)	9475.2 ± 496.6 (114)

Such a positive effect of ABA was in agreement with some data concerning ion (especially K^+) or sugar uptake by other tissues (Van Steveninck 1972, Hartung *et al.* 1980, Saftner and Wyse 1984, Suleiman *et al.* 1990, 1991, Penot *et al.*1991) but opposite to other data which reported an inhibitory effect of ABA (Shaner *et al.* 1975, Pitman and Wellfare 1978, Penot *et al.* 1993).

Sensitivity to specific inhibitors: K^+ uptake was dependent on cellular metabolism as shown by the inhibitory effect of KCN, SHAM and DNP, added to the uptake medium, however K^+ uptake by fresh tissues was more sensitive to the presence of KCN than that by aged tissues (Table 2), indicating the participation of the cyanide-resistant respiration pathway during ageing (Solomos 1977) which was, however, sensitive to SHAM (Table 2). Short-term NEM exposure (5 min) reduced K^+ uptake in both control and ABA-treated tissues (Table 3) indicating the involvement of thiol groups. The effect of DCCD and the specific inhibitor of plasmalemma ATPase

orthovanadate (Table 3), argued in favour of the involvement of a specific plasmalemma H^+-ATPase in K^+ uptake. Also FC which activates proton pump-ATPase, thus increasing the potential difference across the plasma membrane and the uptake of K^+(Kurkdjian and Guern 1989, Hager *et al.* 1991) increased greatly

Table 2. Effect of KCN, DNP, SHAM, ABA, FAP and FC on ^{86}Rb uptake [nmol g^{-1}(f.m.)] by fresh and aged potato tuber discs incubated for 3 h in the presence of 1 mM KCl. For aged tissues, ABA was present during ageing. Other compounds were added to the uptake medium. Means ± SE, $n = 4$; percentage of control in brackets.

	Fresh tissues	Aged tissues
Control	163.9 ± 9.0 (100 %)	82.8 ± 4.1 (100 %)
1 mM KCN	122.1 ± 8.0 (74.5 %)	71.3 ± 2.0 (86.1 %)
0.5 mM DNP	120.3 ± 8.1 (73.4 %)	59.3 ± 3.4 (71.6 %)
1 mM SHAM	-	69.2 ± 2.0 (83.6 %)
20 μM ABA	204.2 ± 2.8 (124.6 %)	111.2 ± 4.0 (134.3 %)
100 μM ABA	-	122.9 ± 2.7 (148.4 %)
100 μM FAP	206.2 ± 10.8 (125.8 %)	-
20 μM FC	247.2 ± 5.3 (150.8 %)	264.3 ± 30.9 (319.2 %)

K^+ uptake, especially by aged tissues (Table 2). This difference in the response of fresh and aged tissues to FC (and to ABA) might indicate a contribution of the passive component to the uptake of K^+ by fresh tissues. Moreover, all inhibitors used decreased K^+ uptake by tissues treated or not with ABA, indicating that the hormone acts likely at the same time on the active and the passive components of K^+ uptake (Tables 2, 3).

Table 3. Effect of various inhibitors on ^{86}Rb uptake [nmol g^{-1}(f.m.)] by potato tuber discs. The tissues were pretreated for 24 h or not with 100 μM ABA and incubated for 3 h in the presence of 1 mM KCl. The inhibitors were added to the uptake medium (except for NEM used in a 5-min pretreatment). Means ± SE, $n = 3$; percentage of uptake without inhibitors in brackets.

Inhibitor	Control	100 mM ABA
0	82.4 ± 2.0 (100 %)	111.6 ± 4.3 (100 %)
0.5 mM NEM	62.8 ± 4.2 (76.2 %)	73.8 ± 1.8 (66.1 %)
100 μM DCCD	55.6 ± 6.3 (67.6 %)	-
500 μM orthovanadate	67.3 ± 0.9 (81.7 %)	95.3 ± 1.3 (85.4 %)
100 μM erythrosine B	89.2 ± 1.0 (108.2 %)	114.2 ± 2.7 (102.3 %)

With regard to the mechaism of ABA action, it has been shown in the same material that this hormone enhanced the long-distance transport of K^+ into the growing tuber and increased K^+ uptake by leaf tissue (Suleiman *et al.* 1991, Penot *et al.* 1991, Feray *et al.* 1992). These data together suggested a relation between the action of ABA at the cellular level and the long distance transport of K^+ but the mechanism of action remains unclear. Van Steveninck and Van Steveninck (1983) suggested that ABA could act, at membrane level, on a H^+-pump coupled to

K^+ transport. Nevertheless, our results demonstrate that the positive action of ABA seems to be connected with the numerous metabolic changes which normally occur during the ageing process (hyperpolarisation of the membrane, stimulation of ATPase-H^+-pump) but probably not to active synthesis of nucleic acids, proteins and lipids, and increase in cyanide-resistant respiration (Van Steveninck 1975, Solomos 1977, Hourmant and Penot 1979, Penot *et al.* 1993, Pierce and Hendrix 1981).

In conclusion, the observed stimulation of K^+ uptake by ABA could result presumably from both an action on the plasmalemma H^+-ATPase and likely, as suggested by Satter and Moran (1988), on the configuration and number of K^+ channels. However, the relationship between increased K^+ uptake and Ca^{2+} and H^+ fluxes should not be exclued (Reddahi *et al.* 1999).

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