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Influence of Abscisic Acid on K^+ Absorption by Leaf Discs of *Solanum tuberosum*

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Abstract. It is shown that, contrary to what is generally found, treatment with abscisic acid (ABA) of potato leaf tissues resulted in an increase of K^+ uptake. Comparison with other hormones was made: BAP induced an inhibition and GA_3 a stimulation of K^+ uptake. The uptake was sensitive to several metabolic inhibitors, external pH and ATPase inhibitors while *p*-chloromercuribenzenesulfonic acid (PCMBS) had no effect. Uptake kinetics revealed the presence of both saturable and linear components which were both stimulated by ABA treatment. Our data are consistent with an effect of ABA on the active and passive components of K^+ uptake. These results are discussed in relation to the action of ABA on foliar senescence and the action on ion partitioning in the whole plant.

It is well known that abscisic acid (ABA) plays an important role in many physiological processes in the whole plant (Zeevaart and Creelman 1988). It regulates senescence, dormancy, seed development and water and ionic balance; for instance it is well known that ABA induces the closure of stomata which is explained as an active efflux of K^+ (Mc Robbies 1981) linked to a H^+ flux to the vacuole (see Suleiman *et al.* 1989, for more details). It also was found, like other phytohormones, to control the partitioning of nutrients between source and sink organs, but the results appear contradictory (Porter 1981, Schussler *et al.* 1984, Clifford *et al.* 1986). The divergence of results probably originates in the endogenous level of ABA (and other hormones) that controls the attractive strength of sinks.

In potato plants, it has been shown that ABA and GA have antagonist effects in the tuberization phenomenon: ABA promotes while GA_3 inhibits it (Ewing 1987). Translocation and accumulation of nutrients may not be dissociated from this process. If applied exogenously to the leaves, GA_3 inhibits and ABA stimulates the fluxes of ^{32}P (Poder *et al.* 1988) sucrose (Lowell and Booth 1967) and K^+ (unpublished results) towards the tuber.

On the other hand, we have demonstrated with isolated leaves of potato plant that

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ABA increases the transport and accumulation of various ions towards the ABA – treated leaflet (Suleiman *et al.* 1990).

The fact that ABA may regulate the long distance transport of K^+ gives rise to some expectation that it may also be involved in a change of cell permeability.

The aim of the present paper was to examine the effect of treatment with ABA on K^+ uptake by foliar tissue.

MATERIAL AND METHODS

Plant material

All experiments were performed with potato tubers (*Solanum tuberosum* L. cv. Bintje) provided by the INRA Station of Ploudaniel (Finistère, France).

Tubers were stored at 4 °C until use. Leaves and discs were obtained from plants grown in vermiculite in growth chambers irrigated with a complete nutrient solution. A photoperiod of 16 h was provided and the irradiance was 25 W m^{-2} The average day and night temperatures were 22 and 18 °C.

To measure the effect of hormone on cell permeability, discs 10 mm in diameter cut outside the main veins were used. Incubation with hormone (10^{-4} M) was always performed for 24 h in the dark at 24 °C. Then, the discs were washed and used for absorption experiments. In recent work (Suleiman *et al.* 1990), we have shown that this technique can be used to study the effect of hormones in long distance transport because the absorption of K^+ by leaf discs does not involve any wounding effect.

Uptake

^{86}Rb (IRE, Fleurus, Belgium) was used as a marker of K^+; 2.77 10^{11} Bq l^{-1} were added to a 1 mM MES solution (pH 6.0) containing 1 mM KCl. After the appropriate uptake period, discs were collected and washed with cold KCl (10 mM) for 5 min. The amount of radioactivity taken up by the discs was measured with a Geiger-Müller counter (Philips PW 4003).

Number of experiments

Each value is the mean of 12 individually measured discs and each experiment was repeated three or more times. Statistical treatment was made each time it was

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Abbreviations: ABA, abscisic acid; BAP, benzylaminopurine; CCCP, carbonylcyanide -*m*-chlorophenylhydrazone; CK, cytokinin; DCCD, N, N'-dicyclohexylcarbodiimide; DNP, 2,4-dinitrophenol; GA, gibberellin; HEPES, N-2-hydroxy-ethylpiperazine-N'-2-ethanesulphonic acid; MES, 2(-N-morpholino) ethane-sulphonic acid; PCMBS, *p*-chloromercuribenzenesulfonic acid; Tris, tris (hydroxymethyl) aminomethane.

possible (see Tables 1 and 4 for instance). Moreover the stimulation of K^+ uptake after ABA treatment has been found in more than one hundred experiments.

RESULTS

Influence of ABA on K^+ uptake. Comparison with BAP and GA_3

Fig. 1 shows that after 24 h treatment with ABA (10^{-4} M), the stimulation of K^+ uptake was 66 %. Indeed, this stimulation varied greatly from one experiment to another: ranging from 18 % to 80 %. This result was quite unexpected and opposite to most data concerning other tissues: leaves and coleoptiles (Horton and Bruce 1972; Reed and Bonner 1974) or roots (Pitman and Wellfare 1978; Behl and Jeschke 1979). The influence of ABA concentration was also conducted between 10^{-6} and 10^{-4} M; 10^{-4} M presents the optimal dose (results not given).

If the discs were treated with other hormones, the rate of uptake was inhibited by 29 % with BAP (10^{-4} M) and increased by 31 % with GA_3 (10^{-4} M). Thus, the effect is specific to the used hormone.

Stimulation by ABA of K^+ uptake

1. Influence of external pH

The undissociated molecules of ABA readily penetrate cell membranes and this uptake is favoured by a low pH (Everat-Bourdouloux *et al.* 1984). Consequently,

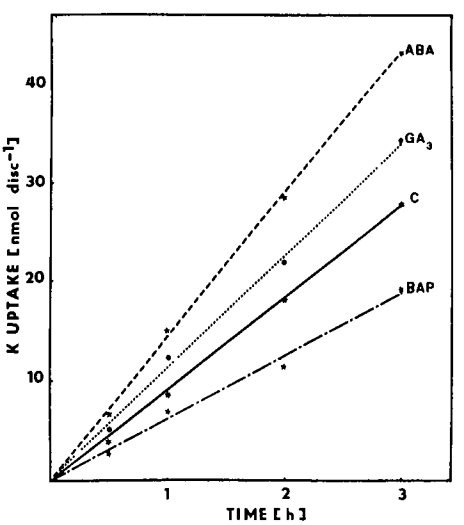

Fig. 1. Comparative action of ABA (10^{-4} M), BAP (10^{-4} m) and GA_3 (10^{-4} M), present during a 24 h treatment, on the absorption of ^{86}Rb (KCl, 1 mM) in potato leaf discs. Each point is the average of 12 measurements. C: control.

TABLE 1

Effect of external pH on uptake of ^{86}Rb (KCl, 1 mM). Measurements after a 24 h treatment with or without ABA (10^{-4} M). The buffers (10 mM) used were sodium citrate (pH 4–5). MES (pH 6) and HEPES (pH 7–8). Results expressed in nmol h^{-1} $disc^{-1}$ ± SE and in per cent of controls (C). Means of 5 experiments

	pH 4	pH 5	pH 6	pH 7	pH 8
C	4.71 ± 0.36	5.01 ± 0.36	7.0 ± 0.96	7.62 ± 1.52	6.22 ± 1.39
ABA	6.3 ± 0.49	7.9 ± 0.61	10.71 ± 1.44	9.83 ± 2.03	8.50 ± 0.4
% of control	134	158	153	129	137

the incubation with ABA was done at pH 6. Below this value, the plant tissue could undergo damage during the treatment. On the other hand, we have studied the influence of the pH of the uptake medium. As shown in Table 1, the uptake and the stimulation reached a maximum at pH 5–6.

2. Influence of K^+ concentration

Fig. 2 shows the concentration dependence (0–50 mM) of K^+ uptake in potato leaf discs. Uptake below 10 mM displayed apparent saturation kinetics. At higher concentrations, a linear component appeared to be superimposed on the saturable system. The treatment with ABA induced a stimulation of both components.

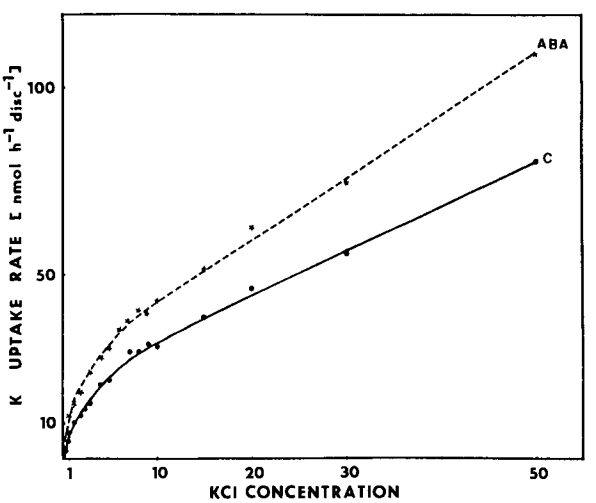

Fig. 2. Effect of ABA (10^{-4} M) on K^+ uptake kinetics in potato leaf discs. Measurements after a 24 hrs treatment with or without ABA.

TABLE 2

Influence of ABA on the saturable and linear components of K^+ uptake in potato leaf discs. Eadie-Hofstee transformation of K^+ influx data yielded the K_m and V_{max} values for saturable curves. Linear regression performed ovar the concentration range of 9 to 50 mM yielded the slope of the linear component

	K_m [mM]	V_{max} [nM $disc^{-1}$ h^{-1}]	Slope of the linear component [nM $disc^{-1}$ h^{-1} mM^{-1}]
Control	3.97	37.10	1.19
ABA	3.76	53.61	1.63

In Table 2, the values of the kinetic constants Km and V_{max} for the saturable system are reported. It shows that the ABA-treatment induced an increase in the value of V_{max} with no change of the value of apparent Km. Moreover, ABA produced a stimulation in the slope of the linear component.

3. Effect of calcium

Calcium is known to maintain membrane integrity (Jensen *et al.* 1987) though Ca^{2+}/K^+ interactions are complicated and dependent upon tissue (Hourmant and Penot 1971).

The influence of Ca^{2+} on K^+ uptake is shown in Table 3. Ca^{2+} (0.5 mM) added to the absorption medium, decreased K^+ uptake by 43–47 % but did not cancel the stimulation due to ABA.

The interaction $Ca^{2+}K^+$ is more complex than it seems and for the following

TABLE 3

Effect of calcium ($CaCl_2$ 0.5 mM) on uptake of ^{86}Rb (KCl, 1 mM) by potato leaf discs. Measurements after a 24 h treatment with or without ABA (10^{-4} M). Results expressed in nmol h^{-1} $disc^{-4}$ ± SE and in percent of controls (in brackets the percentage of inhibition induced by calcium)

	$-Ca^{2+}$		$+Ca^{2+}$	
	Control	ABA	Control	ABA
	7.8 ± 0.5	11.6 ± 0.5	3.8 ± 0.6 (51 %)	5.3 ± 0.7 (54 %)
% of stimulation by ABA	–	149 %	–	139 %

TABLE 4

Effect of metabolic inhibitors on the rates of ^{86}Rb (KCl, 1 mM) absorption by potato leaf discs pretreated or not with ABA (10^{-4} M). The inhibitors were added to the uptake medium (except for PCMBS, used in a 30 min. pretreatment). The results are expressed in nmol h^{-1} $disc^{-1}$ ± SE and in percent of controls (in brackets)

Inhibitor	0	DNP (100 µM)	CCCP (25 µM)	DCCD (100 µM)	Orthovanadate(500 µM)	PCMBS (1 mM)
Control	9.8 ± 0.6	3.2 ± 0.86 (53 %)	3.8 ± 0.2 (39 %)	5.5 ± 1.4 (56 %)	6.5 ± 0.9 (66 %)	8.9 ± 0.5 (91 %)
ABA	13.6 ± 0.6	6.8 ± 0.99 (50 %)	4.6 ± 0.7 (34 %)	7.2 ± 1.3 (53 %)	9.4 ± 0.2 (69 %)	13.0 ± 0.6 (96 %)

experiments, Ca^{2+} was not added. Analysis of the interaction Ca^{2+}/K^+ and Ca^{2+}A-BA is now in progress (Penot *et al.* 1990).

4. Action of ABA on passive and active components

The effect of specific inhibitors was studied. DNP and CCCP are both uncouplers of oxidative phosphorylation. DCCD and Na-orthovanadate are ATPase inhibitors and PCMBS a non-penetrating sulfhydryl reagent.

The data presented in Table 4 demonstrate that K^+ uptake was for the control and the ABA-treated discs dependent on cellular metabolism as shown by the effect of CCCP and DNP. The inhibition by DCCD and especially by orthovanadate (specific inhibitor of plasmalemma ATPase) indicated an involvement of the plasmalemma ATPase. The same inhibitory effect was obtained for the control and treated discs.

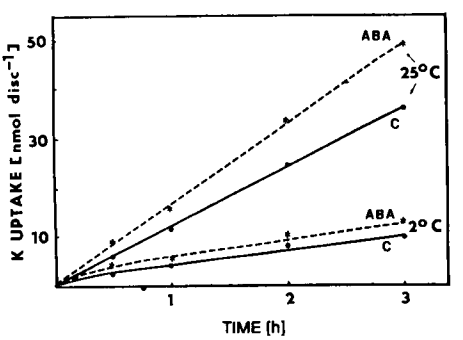

Fig. 3. Effect of temperature on the uptake of ^{86}Rb (KCl, 1 mM) by potato leaf discs. Measurements after a 24 h treatment with or without ABA (10^{-4} M). Each point is the average of measurements of 12 discs. C: control.

The lack of effect of PCMBS suggests that SH groups were not implicated in K^+ transport.

If the absorption was carried out at 2 and 25 °C, the rates of absorption were respectively inhibited by 68 % and 73 % in the control and ABA-treated discs, but ABA seems also to slightly increase the passive component (Fig. 3).

DISCUSSION

The present results show a stimulatory effect of ABA on K^+ uptake. Most of the data reported an inhibitory action of ABA on ion and specially k absorption by coleoptiles (Reed and Bonner 1974), root (Shaner *et al.* 1975; Pitman and Wellfare (1978), and stomata (Horton and Moran 1972).

When potato leaf discs were treated with ABA, this hormone produced an increase in the rate of K^+ absorption, ranging between 18 and 80 %. This effect does not seem to be a permeabilization of the membrane while ABA did not change K^+ efflux (data not shown), as has been shown for other solutes (Harkers *et al.* 1986; Pustovoitova 1987).

When various metabolic inhibitors were added to the uptake medium (Table 4), the stimulation induced by ABA was partially maintained, indicating that ABA acts at the same time on the active component of K^+ uptake linked to the metabolism and on the passive component. This is confirmed by the action of temperature (Fig. 3).

Kochian and Lucas (1982) and Kochian *et al.* (1985) propose that K^+ influx in corn roots could be resolved into a saturable component (involving ATPase participation) and a linear component (passive process, implicating K^+ channels). The K^+ kinetics in potato leaf tissue showed the same pattern and ABA stimulated both components (Fig. 2 and Table 2). DCCD and orthovanadate (specific plasmalemma ATPase inhibitor) inhibited K^+ uptake (Table 4) showing an ATPase implication at low K concentration. Thus, the effect of ABA could result from an action on K^+ channels, in agreement with Satter and Moran's hypothesis (1988) and from an action on available metabolic energy resulting in a stimulation of the active component, Indeed, ABA increases the ATP level (results to be published).

The question is then raised as to the relationship between the ABA-induced K^+ stimulation and a physiological process at the cellular level or in the whole plant. In the first approach, the stimulated K^+ uptake could be connected to an effect on senescence. As a matter of fact, BAP, known to be an antisenescence hormone, induced an inhibition of K^+ uptake (Fig. 1). Nevertheless, this interpretation is not probable because GA_3, which is also an antisenescence hormone, increased K^+ uptake as ABA did (Fig. 1). In the second approach this stimulated K^+ uptake cannot be related to an effect on the stomatal movement because it is well-known that ABA decreases the K^+ fluxes to the guard-cells (see introduction).

In the context of the long distance transport, the action of a hormone on the

cellular permeability may explain the mobilizing or exporting capacity of an organ (sink or source). The role of ABA in hormone-directed transport is not yet sufficiently explored; this hormone can have a positive (Karmoker and van Steveninck 1979, Schüssler *et al.* 1984, Clifford *et al.* 1986) negative (Porter 1981) or null effect (King and Patrick 1982). In a previous work, we have shown that ABA induces an oriented transport of ions to the treated zone of isolated potato leaves (Suleiman *et al.* 1990). The present results demonstrate that ABA increases K^+ uptake by leaf tissue. This suggests an association between the physiological activities of ABA at the cellular level and the long distance transport of K^+, but the nature of ABA effects is still unclear (action on the membrane, growth effect, protein synthesis, production of energy?).

Nevertheless, the experiments described here demonstrate that ABA does not necessarily act as an inhibitor of the ATPase-carrier complexes.

On the other hand, it is well known that hormone action may be expressed by means of second messengers: Ca^{2+}, calmodulin (Elliott 1986), Poovaiah and Veluthambi 1986, Owen 1988). Work in progress seems to indicate that this hypothesis could be retained to explain part of the ABA action (Penot *et al.* 1990).

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