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THE EFFECT OF ISOSAKURANETIN (5,7-DIHYDROXY 4′-METHOXY FLAVANONE) ON POTASSIUM UPTAKE IN WHEAT ROOT SEGMENTS

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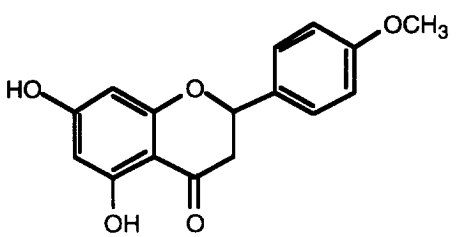
Key Word Index—*Triticum aestivum*; Gramineae; wheat; isosakuranetin (5,7-dihydroxy 4′-methoxy flavanone); root segments; potassium uptake; K^+-dependent acid extrusion.

Abstract—Isosakuranetin (ISK; 5,7-dihydroxy 4′-methoxy flavanone) is a plant exudate with known cytotoxic and fungicide properties. When tested on wheat root segments at a concentration of 70 μM it inhibited K^+-dependent H^+ extrusion and net uptake of K^+, while leaving the membrane potential (PD) unaltered. Fusicoccin (FC)+ISK treatment resulted in a slight membrane depolarization, while ISK alone did not alter O_2 consumption or alternative oxidase activity. ISK did not increase the pyruvate content in incubated root tissues or inhibit Fe^{2+} uptake. The observed drop in K^+ net uptake depended on a decrease in K^+ influx into the cell, leading to the suggestion that ISK may act on wheat root segments as an inhibitor of K^+ permeation. The lack of proton leakage and membrane disruption by ISK made this compound a weak candidate as a phytoalexin, and suggesting a major role as an allelopathic molecule. © 1997 Elsevier Science Ltd

INTRODUCTION

Phytoalexins have been shown to be produced by representatives of most of the plant families, in particular the Leguminosae [1]. One of the major effects of phytoalexins is the disruption of membrane integrity and the ensuing lack of ability to maintain a proton gradient [2]. A factor involved in the competition in plant communities is allelopathy, in which some classes of molecules, including phenolic compounds, act by inhibiting growth processes such as germination [3]. Even though no clear understanding of these phytoalexin/allelopathic properties has been achieved, many reports underline the ability of these compounds to alter the membrane transport processes of both low and high-M_r molecules [4]. Among these mechanisms, H^+/K^+ exchange has been widely studied with regard to the effect of some active molecules [5 and refs cited therein]. The aim of the present paper was to determine the effect of isosakuranetin (ISK; 5,7-dihydroxy 4′-methoxy flavanone) (**1**), a plant exudate with cytotoxic and antifungal properties which is present in the Rosaceae, the Myrtaceae, the Salicaceae, the Fabaceae and the Compositae [6–10] on K^+-dependent acid extrusion and potassium uptake in wheat root segments. The results of this study showed that ISK acts by affecting K^+ uptake and K^+-dependent acid extrusion.



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RESULTS AND DISCUSSION

The effect of ISK is to bring about a net decrease of both K^+-dependent acid extrusion and net K^+ uptake in wheat root segments. A series of experiments performed with several ISK concentrations indicated a significant positive correlation between ISK concentration and the observed inhibitory effects (Fig. 1). Table 1 shows that there was a significant difference in acid extrusion between controls and 70 μM ISK treated plants and that the inhibitory effect was maintained even on co-addition of 70 μM ISK and 10 μM fusicoccin (FC). However, the presence of FC lowered the percentage inhibition of acid extrusion

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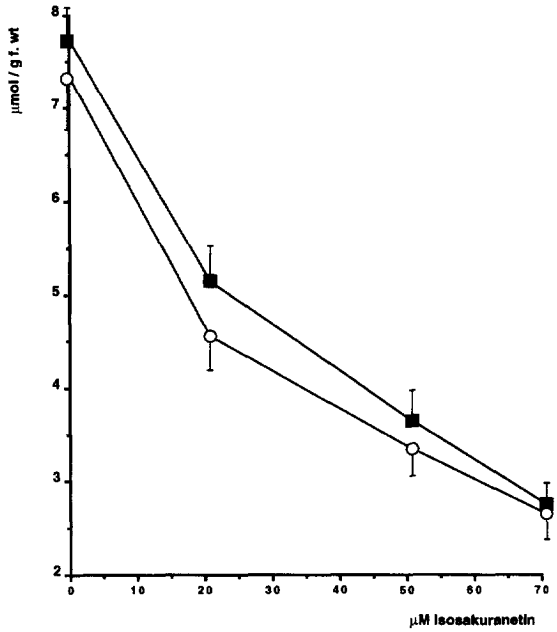

Fig. 1. Effect of isosakuranetin concentration on acid extrusion (circles) and K^+ uptake (squares). Vertical bars indicate standard deviation. Incubation conditions as described in Table 1.

by ISK. Potassium uptake showed the same trend with regard to ISK inhibition. However, the addition of FC had a lesser effect on ISK with respect to acid extrusion (Table 1). By contrast, ISK did not exert any significant effect on divalent cation uptake, and on the ensuing acid extrusion in the incubation medium, as demonstrated by the almost equal uptake of Fe^{2+} with respect to the control (Table 1). In order to evaluate if acid extrusion and K^+ uptake inhibition depended on a shortage of metabolic energy, root tissues were incubated in the presence of both ISK and HCN, and the O_2 consumption and alternative oxidase activity were evaluated. However, neither O_2 consumption nor alternative oxidase activity were significantly affected by ISK (Table 2). Furthermore, the unaltered pyruvate level (control: 60 (s.d. 1.9) nmol g^{-1} fresh weight; ISK: 55 (s.d. 2.6) nmol g^{-1} fresh weight), showed that normal catabolism was not shifted towards glycolysis. No leakage of K^+ was observed in wheat roots incubated with 70 μM ISK

Table 2. Inhibition of O_2 consumption of wheat root tissues incubated in the presence/absence of HCN by 70 μM isosakuranetin (ISK). Data expressed as $\mu l\ O_2\ g^{-1}$ fr. wt (s.d.)

Treatment	Control	ISK	% inhibition
No HCN	338 (42)	322 (50)	5
HCN	99 (14)	102 (14)	0

both in the presence and absence of FC (data not shown). The PD of wheat roots did not show any alteration when roots were incubated with ISK [Fig. 2(A)]. However, the addition of 70 μM ISK to root segments perfused with MES-Li and 10 μM FC showed a significant PD depolarization [Fig. 2(B)].

The results of this investigation indicated that ISK inhibits both K^+-dependent proton extrusion and K^+ uptake, without varying the buffering properties of the incubating medium. The inhibition of K^+-dependent proton extrusion and the lack of a proton leakage indicate a completely different effect of ISK when compared with known phytoalexins such as pterocarpans and rishitin used at almost the same concentration (100 μM) [2, 4]. The observed decrease in K^+ uptake may be caused by both a decrease in K^+ uptake and an increase in K^+ extrusion. However, the limited amount of K^+ extruded in the medium provided evidence for an inhibitory effect of ISK on K^+ uptake. ISK did not affect the amount of metabolic energy available to the cell since no effect on PD was observed. In fact, any possible effect on the plasma membrane ATPase would lead to a PD hyperpolarization/depolarization. The major effect of FC is the acidification of the incubation medium and the stimulation of K^+ uptake [11]. The use of FC lowered the PD of wheat root segments and the depolarization of the PD observed after ISK addition on FC perfused roots may indicate an inhibitory effect of ISK on FC-induced proton leakage. This could be explained by the probable effect of ISK on plasma membrane FC receptors [12]. Our data on O_2 consumption, alternative oxidase activity and pyruvate levels indicate that ISK does not affect the mitochondrial respiratory processes, and leaves unaltered the coupled reactions between glycolysis and oxidative processes. Fur-

Table 1. Inhibition in wheat roots of K^+-dependent acid extrusion ($-\Delta H^+$) and K^+ uptake (ΔK^+) in the presence/absence of 10 μM fusicoccin (FC), and of Fe^{2+} uptake (ΔFe^{2+}) by 70 μM isosakuranetin (ISK). Data are expressed as μmol g^{-1} fr. wt (s.d.)

Ions	Control	ISK	% inhibition	Control +FC	ISK +FC	% inhibition
$-\Delta H^+$	7.2 (0.55)	2.6 (0.27)	64	10.1 (0.56)	6.4 (0.27)	37
ΔK^+	7.6 (0.51)	2.7 (0.23)	65	10.9 (0.41)	5.9 (0.41)	46
ΔFe^{2+}	10.9 (0.33)	10.8 (0.62)	1	n.a.	n.a.	n.a.

n.a. = not applicable

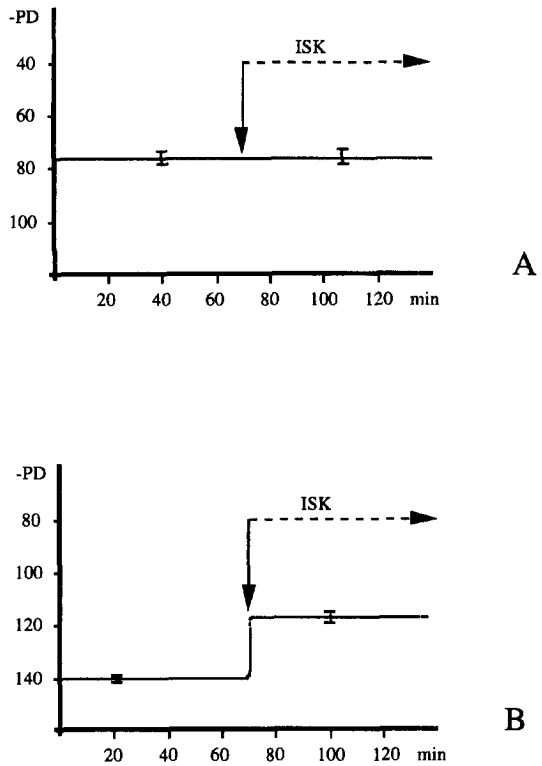

Fig. 2. A: Effect of 70 μM isosakuranetin (ISK) on the transmembrane potential difference (PD) in wheat roots incubated with 0.25 mM K_2SO_4 in 1.5 mM MES-Li buffer (pH 6.0). The addition of ISK (arrow) does not change the PD. B: Depolarization of the PD by 70 μM ISK (arrow) in wheat root segments perfused with 10 μM fusicoccin and 0.25 mM K_2SO_4 in a 1.5 mM MES-Li buffer (pH 6.0). Small bars indicate s.d.

thermore, insufficient metabolic energy did not seem to be a limiting factor since the extrusion of protons and the K^+ uptake was at the same rate in ISK treated tissues in the presence of FC. These results are in agreement with those obtained with xanthoxylin (2-oxy, 4-6-dimethoxyacetophenone), a compound produced by some Compositae in response to fungal infection, that also inhibits K^+ dependent acid extrusion and K^+ net uptake by wheat and maize root segments [5, 13]. The lack of inhibition of the uptake/extrusion of divalent cations suggested a specific ISK action on monovalent cations (Table 1) [5, 14].

In conclusion, it appears that ISK acts as an inhibitor of K^+ uptake. The lack of membrane disruption and proton leakage, a common primary function for many phytoalexins [2], makes this molecule a weak candidate as a phytoalexin. On the other hand, the occurrence of such a compound in several plant exudates [6–10] suggests a major role in the allelopathic mechanisms of inhibition of seed germination and seedling development, through the inhibition of monovalent cation uptake.

EXPERIMENTAL

Wheat seeds (cv Aquileia) were surface sterilized with 0.6% NaClO, rinsed with H_2O and dark germinated on filter paper moistured with 0.5 mM $CaSO_4$. Wheat seedlings were then transferred to an aerated 0.5 mM $CaSO_4$ soln for 10–12 days in a growth chamber (20° isothermal, 14/10 hr light/dark, PPD 60 $\mu E\ s^{-1}\ m^2$). Potassium uptake and proton extrusion were determined in root segments for 120 min. Acid extrusion was evaluated by titration of the incubation medium, whereas K^+ was supplied as K_2SO_4 and K^+ uptake was evaluated in the incubation medium by spectrophotometric atomic absorption at 766 nm. Divalent cation uptake (Fe^{2+}) was determined for 120 min in root tissues pre-incubated for 30 min in 0.4 nM lithium–succinate buffer (pH 5.5) by the colorimetric method described in ref. [15]. Pyruvate content was assayed according to ref. [16], whereas O_2 consumption and alternative oxidase activity were evaluated as reported in ref. [17]. The transmembrane potential difference (PD) was determined on the root elongating zone with glass micropipettes with a tip resistance of 5–10 MΩ and filled with 1 M KCl. Micropipettes were used as micro-salt bridges to Ag/AgCl electrodes and inserted vertically in the tissue by means of a Narishige micromanipulator. Wheat roots were equilibrated for 30 min in 1.5 mM MES-Li buffer (pH 6.0) containing 0.25 mM K_2SO_4 and after determination roots were perfused with the same buffer containing 70 μM ISK.

In all experiments ISK was dissolved in DMSO, which was added at the same concn in all controls. The results are reported as a mean of at least three replications.

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