Journal of Experimental Botany, Vol. 52, No. 354, pp. 99–104, January 2001

Journal of Experimental Botany

Glucose-induced activation of rubidium transport and water flux in sunflower root systems

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Received 5 June 2000; Accepted 11 August 2000

Abstract

Excised 20-d-old sunflower roots (*Helianthus annuus* L. cv. Sun-Gro 393) were used to study the effect of different sugars on rubidium and water fluxes. The roots sensed and absorbed glucose from the external medium inducing the activation of rubidium accumulated in the root (Rb^+ root), the flux of exuded rubidium (J_{Rb}) and, to a lesser degree, the exudation rate (J_v). These effects were also triggered by fructose, but not by 6-deoxyglucose (6-dG), a glucose analogue which is not a substrate for hexokinase (HXK). The effect of 2-deoxyglucose (2-dG), an analogue that is phosphorylated but not further metabolized, was complex, suggesting an inhibitory effect on solute transport to the xylem. The amounts of glucose required to activate rubidium and water fluxes were similar to those previously reported to regulate different processes in other plants (0.5–10 mM). When sorbitol was used instead of glucose, neither rubidium uptake (Rb^+ root plus J_{Rb}) nor J_v was activated. It is proposed that glucose present in the root plays an important signalling role in the regulation of Rb^+ (K^+) and water transport in plant roots.

Key words: Exudation rate, glucose, *Helianthus annuus*, rubidium transport, sunflower.

Introduction

Sugars mediate regulatory processes and trigger signal transduction pathways in very different living cells such as prokaryotes or animal cells. An important amount of information dealing with the regulatory processes triggered by glucose and fermentable sugars is available in the yeast *Saccharomyces cerevisiae*, which serves as a model of cell-walled eukaryotic organisms. The RAS-cAMP pathway (Thevelein, 1991) and the main glucose repression/derepression pathway (Johnston and Carlson, 1992; Trumbly, 1992) are the best studied ones, and components of both pathways and the genes coding for the corresponding proteins have been identified. The many glucose-induced regulatory phenomena raise the question of how the presence of glucose is sensed. For most glucose-induced regulatory effects, transport and phosphorylation, but not further metabolism of the sugar, are required. Therefore, glucose transport and phosphorylation are considered to be key components in the glucose-sensing system although it is unclear how the cell senses the sugar and transduces the information (Blázquez *et al*., 1993; Thevelein and Hohmann, 1995).

One of the different effects that are induced by glucose or other fermentable sugars in *Saccharomyces cerevisiae*, is the activation of K^+ (Rb^+) influx (Alijo and Ramos, 1993; Ramos *et al*., 1992). This process requires glucose influx and phosphorylation, but not further metabolism, and it is independent of the glucose-induced activation of the plasma membrane ATPase (Serrano, 1983; Ramos *et al*., 1992).

Sugar-mediated gene regulation in plants is receiving increasing attention in recent years (Koch, 1996). Three different sugar-sensing systems have been proposed to exist in higher plants: a sucrose pathway, sensing sucrose as a regulatory molecule; a pathway directly associated to the transport of hexoses; and an HXK-sensing system similar to the one in yeasts and animal cells (Smeekens and Rook, 1997, and references therein). The HXK-sensing

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system is the best studied one and it controls many important processes related to photosynthesis (von Schaewen *et al*., 1990; Krapp *et al*., 1993; Sheen, 1994) or germination (Pego *et al*., 1999). In the regulation of many of these processes, addition of glucose analogues that cannot be phosphorylated were ineffective but 2-deoxyglucose, which is a substrate for HXK, was an effective inhibitor of genes encoding photosynthetic enzymes (Jang and Sheen, 1994). Recently it has been shown that, when expressed in *Arabidopsis*, yeast HXK2 is enzymatically active but its signalling function is lost (Jang *et al*., 1997). In relation to these regulatory processes, many questions are still open.

Previous reports have shown that the translocation of sugars from shoot to root is related to K^+ uptake by the root (Bowling, 1968; Pitman and Cram, 1973). More recently it has been reported that the presence of carbohydrate such as glucose in the external medium increased the exudation rate in *Plantago* roots (De Boer *et al*., 1983), and stimulated Rb^+, K^+ and water fluxes in sunflower roots (Fournier *et al*., 1987). The existence of different hexose transporters, expressed in a tissue-specific manner, and in response to external or internal signals, has been demonstrated (Caspari *et al*., 1994; Truernit *et al*., 1996). However, most of the information on sugar transport in plants relates to the movements within the phloem or it has been obtained using single cell organisms (algae or transgenic yeasts expressing plant genes) (Sauer *et al*., 1994). Moreover, there is no direct information showing that roots can sense and transport glucose from the external medium.

The objective of the present work was to study the effect of different sugars on Rb^+ uptake (Rb^+ accumulated in the roots and exuded into the xylem) and water flow in sunflower root systems. This study reports that sunflower roots take glucose from the external medium and that this substrate can induce regulatory processes leading to the activation of Rb^+ and water transport.

Materials and methods

Plant material and growth conditions

Sunflower seeds (*Helianthus annuus* L. cv. Sun-Gro 393, Eurosemillas S.A., Córdoba, Spain) were surface-sterilized in 0.5% (v/v) sodium hypochlorite for 1 min, and germinated in the dark for 4 d at 28 °C in perlite moistened with 5 mM $CaCl_2$. On the fourth day, the seedlings were put in a plant growth chamber with a relative humidity between 60–80%, a temperature of 22/18 °C day/night, a photoperiod of 14 h of light and a photosynthetic photon flux density of 350 μmol m^{-2} s^{-1} (fluorescent tubes, Sylvania cool white VHO). The next day, the 5-d-old seedlings were transferred individually to glass flasks wrapped in aluminium foil. The flasks contained 790 ml of a nutrient solution with the following composition: 2.5 mM $Ca(NO_3)_2$, 0.5 mM KCl, 1.0 mM $MgSO_4$, 0.25 mM $Ca(H_2PO_4)_2$, 12.5 μM H_3BO_3, 1.0 μM $MnSO_4$, 1.0 μM $ZnSO_4$, 0.25 μM $CuSO_4$, 0.2 μM $(NH_4)_6Mo_7O_{24}$, and 10 μM Fe-ethylenediamine-di-*o*-hydroxyphenylacetic acid. The pH of the nutrient solution was adjusted to 5.5 with NaOH.

The plants were grown in the growth chamber for 15 d. The nutrient solution was continuously aerated using an air pump. The volume was adjusted to 790 ml daily. The nutrient solution was renewed on day 7 and the day before the exudation assays.

Exudate collection

The exudation experiments were performed with 20-d-old plants and were started 30 min after switching on the lights of the growth chamber. In the same flasks in which the plants had grown, the nutrient solution was changed for a new solution according to the type of assay. In the assays with different sugars (glucose, sorbitol, fructose, 6-deoxyglucose, and 2-deoxyglucose), the new solution had the same basic composition as the growing solution except that RbCl (1 mM) replaced KCl (0.5 mM), plus different concentrations of each sugar. In these experiments, rubidium (RbCl) was used in the solution as a tracer for K^+. In the assays with radioactivity, the new solution contained 2 mM $CaSO_4$, with [^{14}C]glucose or [^{14}C]sorbitol (10 mM each). Glucose or sorbitol in the root medium were labelled by adding radioactive glucose (Glucose ^{14}C (7.4 MBq ml^{-1}), Amersham) or sorbitol (Sorbitol ^{14}C (7.4 MBq ml^{-1}), Amersham), to reach a final concentration of 10^4 cpm μmol^{-1}.

Immediately after the change to the new solution, the plants were de-topped 1 cm above the transition zone and pieces of tightly fitting latex tubing were affixed to the cut stumps. The fluid freely exuding into xylem vessels was collected in test tubes for 6 h; during this period, the root external medium was continuously aerated using an air pump and kept at 25 °C. The collected volume of sap was determined by measuring the difference in weight of the test tube, before and after collection of the exuding sap. Afterwards, the roots were individually washed in 150 ml of a cold 5 mM $CaSO_4$ solution for 5 min to allow the exchange of the cell wall contents. Finally, the roots and their exuding saps were individually weighted, frozen and stored at −20 °C.

Intact plants assays

Similar to the assays with root systems, other experiments were performed with intact plants. Instead of the plants being de-topped, they were kept in the growth chamber, changing the nutrient solution for a new solution where RbCl (1 mM) replaced KCl (0.5 mM), plus glucose (10 mM). After 3 h and 6 h at conditions of transpiration, the plants were collected, weighed, frozen and stored at −20 °C.

Other analysis

Rb^+ was determined by atomic absorption spectrophotometry (Perkin Elmer 1100 B), either directly in the exudate or after extraction from the roots or shoots with a 10% acetic acid solution (Benlloch *et al*., 1989). Total solutes of the xylem sap were determined by means of a vapour pressure osmometer (Wescor Vapro™ 5520).

In the assays with radioactive glucose or sorbitol, the radioactivity (cpm) present both in the xylem sap and in the root was determined. In the first case, a sample (100 μl) of the exudate was taken, introduced in a vial containing 5 ml of liquid scintillation cocktail and radioactivity was counted (Beckman LS 6000 TA). In the second case, after the exudation, the roots were washed, weighed, dried at 60 °C for 48 h and combusted in an oxidizer (Packard 307). The $^{14}CO_2$ released was trapped

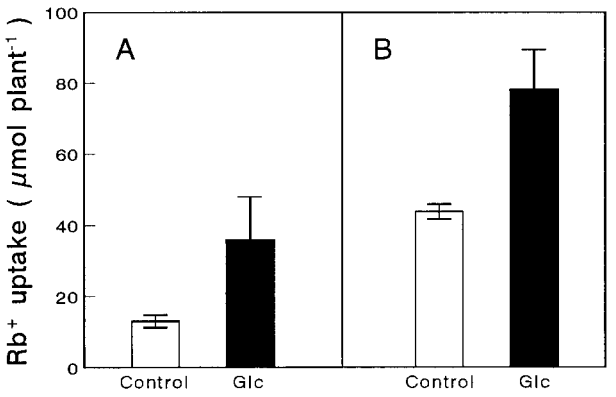

Fig. 1. Effect of glucose in the root medium on the Rb^+ uptake by intact plants. Plants were kept for 3 h (A) and 6 h (B) in a solution, which had the same basic composition as the growing solution except that RbCl (1 mM) replaced KCl (0.5 mM). Glucose (10 mM) was added at time zero. Control (□), glucose (■). Values are means of four plants ±SE.

and counted in 15 ml of Permafluor V : Carbosorb (2 : 1, v/v) (Menéndez *et al.*, 1994).

The concentration of glucose in the xylem sap was determined using a commercial kit of D-Glucose (D-Glucose/D-Fructose, Boehringer Mannheim) using the protocol suggested by the manufacturer.

Experimental design and statistical analysis

A random experimental design was utilized in all cases. Four replicates for each treatment were used, and the results shown are means ±SE.

Results

Glucose activates Rb^+ uptake and exudation rate

When sunflower root systems were kept in a medium containing RbCl (1 mM), the presence of glucose (10 mM) in the root medium increased exudation rate (J_v) (120.4±4.7 versus 103.7±3.1 µl h^{-1} g^{-1} FW), the flux of Rb^+ into the xylem (J_{Rb}) (2.80±0.55 versus 0.40±0.02 µmol h^{-1} g^{-1} FW) and Rb^+ accumulated in the root (Rb^+ root) (29.85±0.59 versus 8.33±0.30 µmol g^{-1} FW). The effect on Rb^+ uptake (Rb^+ root plus J_{Rb}) was much more pronounced than the effect on water flow: J_{Rb} and Rb^+ root increased 7 and 3.5 times, respectively, and J_v increased 1.2 times. In intact plants, after 3 h and 6 h under conditions that allowed transpiration, glucose also increased Rb^+ uptake, although this effect was lower than in root systems (Fig. 1).

The flux of total solutes in the xylem sap (J_s) was also measured in order to determine whether the increase of Rb^+ in the exudate was due to a specific activation of glucose on J_{Rb}. The presence of sugar in the medium increased J_s (6.33±0.24 versus 3.04±0.09 µmol h^{-1} g^{-1} FW), finding that from the total stimulatory effect of glucose on J_s, 73% was due to the stimulatory effect on J_{Rb}.

The activation of J_{Rb} and J_v by glucose may suggest that the root could absorb external glucose. In order to confirm this idea, sorbitol was used instead of glucose in some experiments. Sorbitol is usually used as a negative control to study sugar-induced regulatory phenomena in fungi because is not transported by the cells and does not induce any regulatory effect (Thevelein and Hohmann, 1995). Two different experiments were performed.

Table 1. *Effect of the type of radioactive sugar (glucose or sorbitol) in the root medium, on the radioactivity present both in the xylem sap (^{14}C xylem sap) and in the root (^{14}C root)*

Roots were exuding for 6 h in a solution containing $CaSO_4$ (2 mM), with [^{14}C]glucose or [^{14}C]sorbitol (10 mM each). Values are means of four roots ±SE of the mean.

Treatment	^{14}C xylem sap (cpm ml^{-1})	^{14}C root (cpm g^{-1} FW)
Glucose	12 382±300	124 599±2533
Sorbitol	4 161±229	27 039±1059

(i) In a first set of experiments, the presence of sorbitol (10 mM) in the external medium did not affect J_v, J_{Rb} or Rb^+ root (data not shown).

(ii) In a second set of experiments, labelled sorbitol or glucose (10^4 cpm µmol^{-1}) was used to obtain information about their possible absorption. Sunflower root systems were immersed for 6 h in the presence of radioactive glucose or sorbitol and radioactivity was determined both inside the root and in the xylem sap (Table 1). In the case of glucose, high amounts of ^{14}C were found inside the root showing that external glucose entered and reached root cells. In addition, in the xylem sap, lower values of radioactivity were present (Table 1). When the concentration of glucose was measured in the xylem sap by using a chemical method, it was found that the concentration was 70 µM. This value is much lower than expected from the radioactivity present in the xylem sap, indicating that most of the glucose was already metabolized by the root. In the case of sorbitol, the amounts of radioactivity measured were significant but much lower, showing that the roots absorbed some amounts of sorbitol although with much lower efficiency than in the case of glucose.

Because all the previous experiments were performed in the presence of 10 mM glucose, it was decided to determine the effect of the presence of different glucose concentrations in the external medium on Rb^+ uptake. It was found that at 1 mM glucose, the activation of Rb^+ uptake (Fig. 2) and the stimulation of J_v (Fig. 3) reached similar values to those observed at 10 mM. From the data in Fig. 2, it was possible to deduce that half-maximal activation of Rb^+ uptake occurred at 0.1–0.2 mM glucose.

Effect of other monosaccharides

It is well known that the addition of glucose or other fermentable sugars to a yeast cell suspension triggers many regulatory processes that require phosphorylation,

but not further metabolism of the sugar (Alijo and Ramos, 1993; Thevelein and Hohmann, 1995). There is some evidence that this may also be the case in plants (Smeekens and Rook, 1997) and some experiments were performed to study the effect of several monosaccharides (glucose, fructose, 6-deoxyglucose, and 2-deoxyglucose) on J_v, J_{Rb} and Rb^+ root. The sugars (1 mM each) were added to the external medium at the onset of the exudation process. The results presented in Fig. 3 show that the presence of fructose stimulated J_v, J_{Rb} and Rb^+ root, although with a lower efficiency than glucose. The stimulatory effect exerted by fructose was, as in the case of glucose, much higher on Rb^+ transport than on water transport. On the other hand, 6-deoxyglucose (6-dG), a glucose analogue that cannot be phosphorylated, did not activate any of these parameters. Finally, 2-deoxyglucose (2-dG), which is a substrate for HXK but is not further metabolized, showed a complex effect because its presence inhibited J_v and J_{Rb} but did not affect the Rb^+ accumulation by the root (Fig. 3).

In order to obtain a clearer picture of the effects of sugars, the time-course of J_v and J_{Rb} after glucose or 2-dG addition was followed. Three different results are remarkable (Fig. 4): (i) Control roots maintained similar values of water and Rb^+ fluxes during the 6 h of exudation, suggesting that these roots were in a stable physiological state. (ii) The presence of glucose significantly increased J_v and J_{Rb} through time (Fig. 4A, B). This increase was more pronounced on J_{Rb}. (iii) The inhibition of water and Rb^+ fluxes due to 2-dG was more evident with time, suggesting an inhibitory effect inside the root cells (Fig. 4A, C).

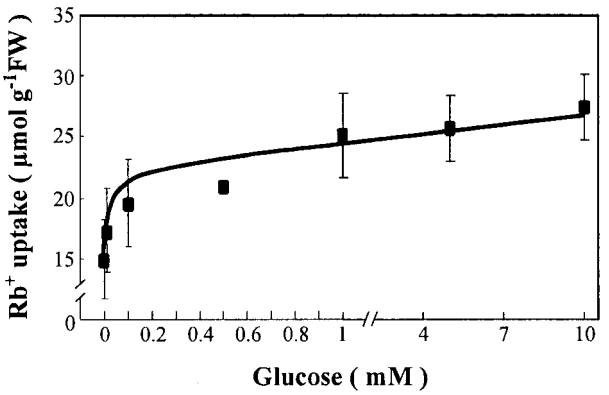

Fig. 2. Effect of glucose in the root medium on the Rb^+ uptake by the root. Roots were exuding for 6 h in a solution, which had the same basic composition as the growing solution except that RbCl (1 mM) replaced KCl (0.5 mM). Glucose was added at the onset of the exudation. Values are means of four roots ±SE.

Discussion

The presence of glucose in the external medium activated Rb^+ accumulation by the root, its release to the xylem sap and the exudation rate in sunflower root systems (Fig. 3). It has previously been reported that ion transport into the xylem favoured the exudation rate (Glinka, 1980). This is probably the case in this system where the observed stimulation of water flux in the root could be a consequence of the increase in Rb^+ uptake. The possibility that the stimulation of J_v could be produced by an osmotic effect of the glucose, might be excluded due

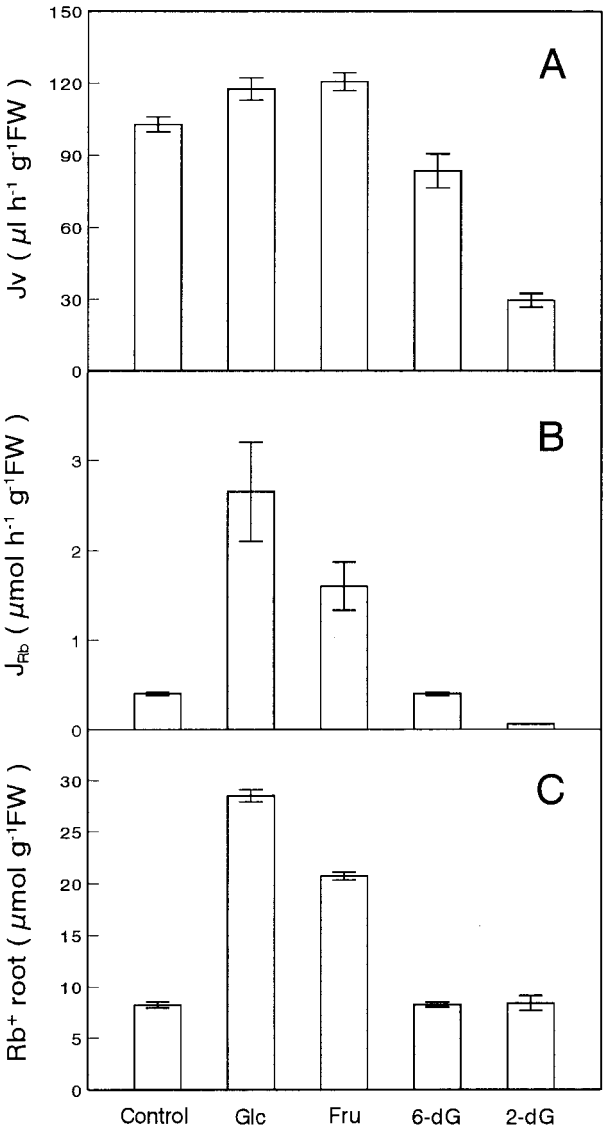

Fig. 3. Effect of different sugars in the root medium on the exudation rate (A), the Rb^+ flux into the xylem (B), and the Rb^+ accumulation in the root (C). Roots were exuding for 6 h in a solution containing the same basic composition as the growing solution except that RbCl (1 mM) replaced KCl (0.5 mM). Each sugar (1 mM) was added at the onset of the exudation. Control (without sugar), Glc (glucose), Fru (fructose), 6-dG (6-deoxyglucose), 2-dG (2-deoxyglucose). Values are means of four roots ±SE.

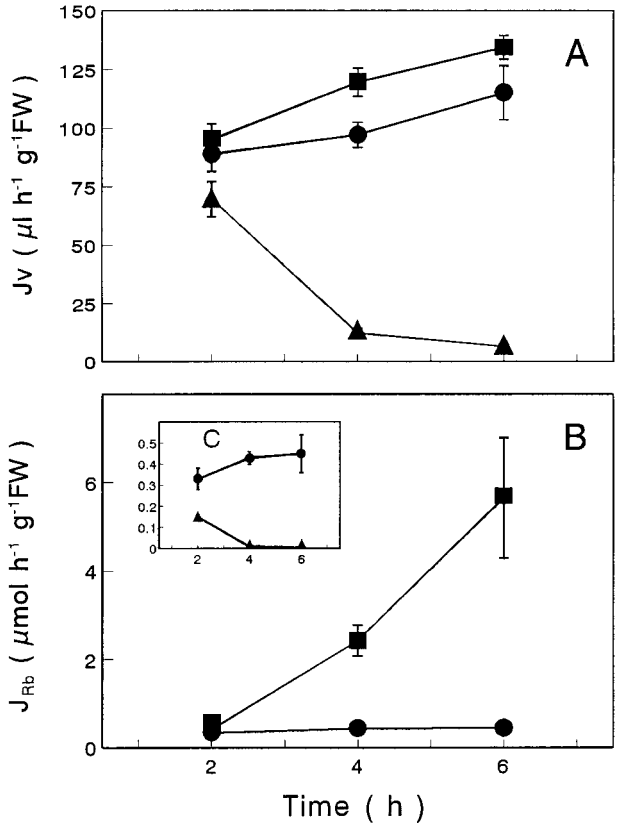

Fig. 4. Time-course of the exudation processs, expressed as: (A) exudation rate (J_v), (B, C) Rb^+ flux into the xylem (J_{Rb}). Roots were exuding for 6 h in a solution containing the same basic composition as the growing solution except that RbCl (1 mM) replaced KCl (0.5 mM). Each sugar (1 mM) was added in the root medium at the onset of the exudation. Without sugar (●), glucose (■), 2-deoxyglucose (▲). Note that the values without sugar are the same in (B) and (C, inset). Values are means of four roots ±SE.

to the low concentration of glucose measured in the xylem sap.

The presence of fructose in the external medium induced a similar effect to the one triggered by glucose (Fig. 3). This fact fits with the results usually obtained in yeasts: the presence of fermentable sugars, but not other substrates such as sorbitol, regulates different metabolic pathways in *Saccharomyces cerevisiae* (Pernambuco *et al*., 1996). In these experiments, sorbitol did not affect either J_{Rb} or J_v and the amount of this sugar that was able to enter the root was dramatically lower than in the case of glucose (Table 1).

It is well known that glucose is a widely used energy source. However, these results suggest an additional effect of glucose probably related to a signalling and regulatory phenomenon: (i) the glucose-induced activation of ion uptake mainly affected Rb^+ transport and not other solutes present in the xylem sap, as previously mentioned in the Results; (ii) in transpiring intact plants, in conditions at which there are not energetic deficiencies, the presence of glucose also increased Rb^+ uptake in the plant (Fig. 1).

One possible explanation for the glucose-induced stimulatory effect on Rb^+ transport would be the existence of a sugar/Rb^+ cotransport. The lack of activation obtained using 6-dG (Fig. 3), indicates that this may not be the case and that absorption and phosphorylation are two basic steps in the activation of Rb^+ transport. Increasing evidence supports the existence of an HXK-sensing system in plants requiring phosphorylation but not further metabolism to trigger a regulatory cascade (Smeekens and Rook, 1997). In yeast, it has been shown that glucose activates K^+ (Rb^+) transport. This process requires glucose transport to the inside of the cell, and phosphorylation of the sugar is necessary and sufficient to trigger the activation (mutants lacking the three kinases able to phosphorylate glucose do not activate Rb^+ transport). In addition, it was also proposed that this effect was specific for Rb^+ transport and independent of other regulatory processes triggered by glucose such as the activation of the plasma membrane ATPase (Ramos *et al*., 1992; Alijo and Ramos, 1993).

The glucose analogue 2-dG inhibited water and Rb^+ fluxes to the xylem over time (Fig. 4). Working with yeasts the activation of Rb^+ transport by 2-dG was observed only in very special conditions: in cells with low capacity of Rb^+ transport and with limited capacity to phosphorylate glucose (mutants with only one active sugar kinase) (Alijo and Ramos, 1993). In addition, in a wild type yeast, a clear inhibition of Rb^+ transport was observed in the presence of 2-dG. These results were explained on the basis of the detrimental effect of 2-dG on the ATP level, and subsequently on the ATPase activity and on Rb^+ influx. The balance between the negative and positive effects of 2-dG would determine the final observed effect (Alijo and Ramos, 1993). A similar phenomenon may be expected in plant roots: 2-dG would compete for ATP and could inhibit fluxes from roots to the xylem.

It is worth noting that, in this work, the amounts of glucose required to activate Rb^+ and water fluxes (0.5 to 10 mM) were similar to the concentrations of sugar that are needed to repress genes coding for photosynthetic enzymes through the HXK-sensing pathway (Jang and Sheen, 1994).

Most of the available information on sugar transport has been obtained by using single cell organisms (algae or transgenic yeasts) or by studying sugar movements in the phloem (Sauer *et al*., 1994). It is proposed now that sunflower roots absorb glucose from the external medium, and that the sugar absorption and subsequent phosphorylation induce a regulatory process leading to the stimulation of Rb^+ and water fluxes. Although significant amounts of glucose are not usually found in soil, these data indicate that the internal level of glucose

in the root plays an important role in the regulation of Rb^+ (K^+) transport in plant roots. Because of the important role of K^+ in cell growth, it is possible that the glucose-induced activation of K^+ transport from root to shoot participates in the regulation of shoot growth. From this point of view, it seems reasonable to propose that sugar transport from shoot to root would activate the mechanism of K^+ transport and, as a consequence, would promote plant growth.

Acknowledgements

This work was supported by the Ministerio de Educación y Cultura (Spain) under grants numbered PB98-1026 (MB) and PB98-1036 (JR).

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