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Abscisic acid stimulates sucrose uptake into tobacco leaf discs

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SUMMARY

The effects of abscisic acid (ABA) on the uptake of sucrose into discs from tobacco (*Nicotiana tabacum*) source leaves were studied. ABA stimulated an increase in sucrose uptake up to three-fold, depending on the osmotic potential of the medium. Maximal stimulation was observed around 200 mOsm, and at 1 μM ABA. A tentative explanation for the discrepancy between these results and the previously described inhibition by ABA of sucrose uptake into leaf discs from other plant species is presented.

Key-words: abscisic acid, cell turgor, *Nicotiana tabacum*, phloem loading, sucrose uptake.

INTRODUCTION

Abscisic acid (ABA) influences sugar uptake in source and sink tissues from various plant species. It stimulates sugar uptake by sink tissues of beet, apple and strawberry (Beruter 1983; Saftner & Wyse 1984; Archbold 1988). In source tissues, inhibition of sucrose uptake by ABA was described for *Ricinus communis, Beta vulgaris, Petunia hybrida,* and *Phaseolus vulgaris* (Vreugdenhil, 1983). It was assumed that in source tissue this uptake represented phloem loading. Estruch *et al.* (1989), using isolated veins of *Pisum sativum* leaves, also reported inhibition of sucrose uptake by ABA. This effect depended on the turgor of the tissue, the highest degree of inhibition occurring at 300 mOsm of the incubation medium.

In this paper, we describe a stimulating effect of ABA on the uptake of sucrose by discs from tobacco source leaves. A tentative explanation for the differential effects of ABA on the uptake of sucrose in leaf discs from various plants species is presented.

MATERIALS AND METHODS

Plant material

Tobacco (*Nicotiana tabacum*, cv Samsun NN) plants were grown in a greenhouse at 16 h PAR. Only mature non-senescing leaves were used in the experiments.

Sucrose uptake

The abaxial epidermis of the leaves was stripped off and discs (diameter 8 mm) were cut with a cork borer. Pretreatment of the discs with the desired amount of ABA, and uptake of sucrose were essentially as described before (Vreugdenhil, 1983): discs were floated,

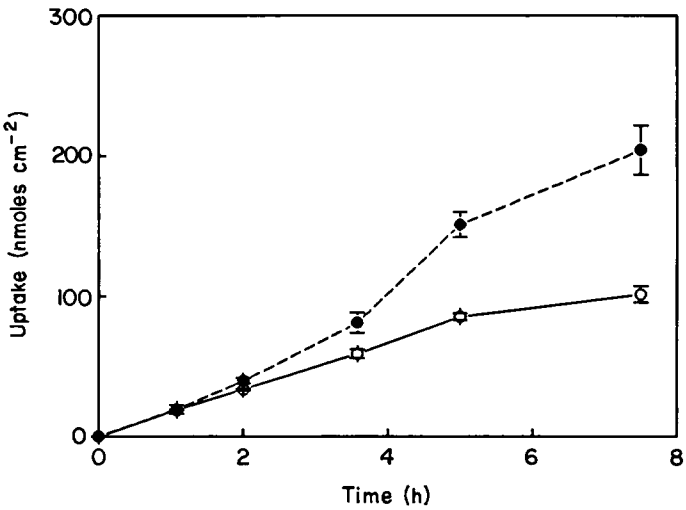

Fig. 1. Time course of the effect ABA on the uptake of sucrose into tobacco leaves discs. The discs were incubated on standard medium containing 200 mM mannitol and 1 mM ^{14}C-sucrose, in the presence (●) or absence (○) of 10 μM ABA. Data are the averages ± SE of 5 or 6 discs.

abaxial surface down, on 5 ml incubation medium (10 mM 4-morpholinoethanesulphonic acid, 10 mM KCl, 1 mM $CaCl_2$, pH 5·0, with NaOH) with the desired amount of ABA. The osmolarity of the medium was varied by the addition of either mannitol or poly-ethylene glycol (PEG 6000, MW 6000–7500). Osmolarity of the media was determined with a vapour pressure osmometer (Wescor 5500). The discs were incubated at 30°C in darkness with gentle shaking. After the desired time, the incubation medium was replaced by the same medium, containing 1 mM sucrose, supplemented with ^{14}C-sucrose (specific activity 21·6 GBq.mmol^{-1}). The discs were allowed to take up sucrose for 30 min, unless indicated otherwise. They were rinsed two times with incubation medium, without sucrose, and transferred to scintillation vials. After bleaching according to Porter (1980), the radioactivity was determined with a liquid scintillation counter.

In one experiment, the effect of potassium was tested by addition of a series of KCl concentrations ranging from 1 to 30 mM, both during pre-incubation and sucrose uptake.

RESULTS

Uptake of sucrose by tobacco leaf discs from a 1 mM sucrose solution was linear with time for *c.* 5 h and then levelled off. In the presence of 10 μM ABA, uptake of sucrose was stimulated, the effect becoming more pronounced with prolonged incubation. A significant increase in sucrose uptake due to ABA was attained between 3·5 and 5 h after the start of incubation. Thereafter, the rate of uptake returned to its initial value (Fig. 1). In further experiments the discs were routinely pre-incubated with or without ABA for 4 h prior to sucrose uptake.

Figure 2 shows the concentration-dependence of the stimulation of sucrose uptake by ABA. At 1 μM ABA the maximal stimulation was reached, whereas half maximal stimulation occurred between 0·1 and 1 μM ABA.

The uptake of sucrose was strongly dependent on the osmolarity of the medium. Uptake rates increased three- to fourfold by increasing the osmolarity of the medium from

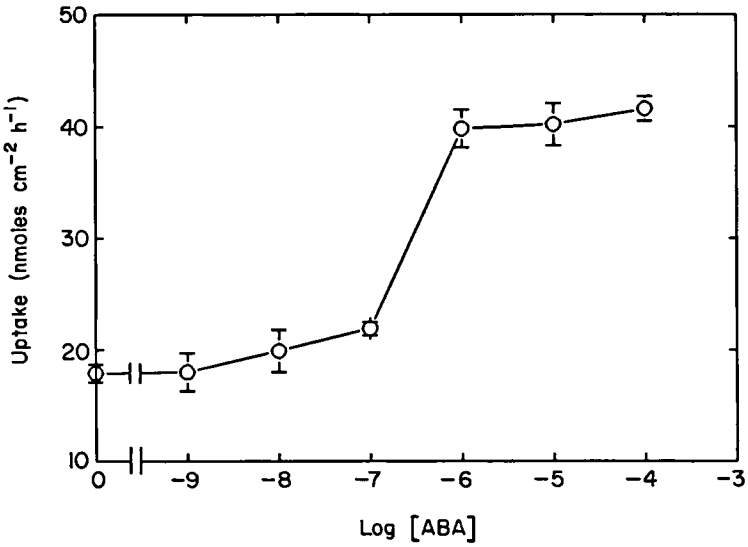

Fig. 2. Effect of a series of ABA concentrations on the rate of uptake of sucrose into tobacco leaf discs. Discs were pre-incubated on standard medium containing 200 mM mannitol and the indicated concentration of ABA for 4 h, and then incubated for 30 min on the same media supplemented with 1 mM ^{14}C-sucrose. Data are the averages ± SE of 6 or 7 discs.

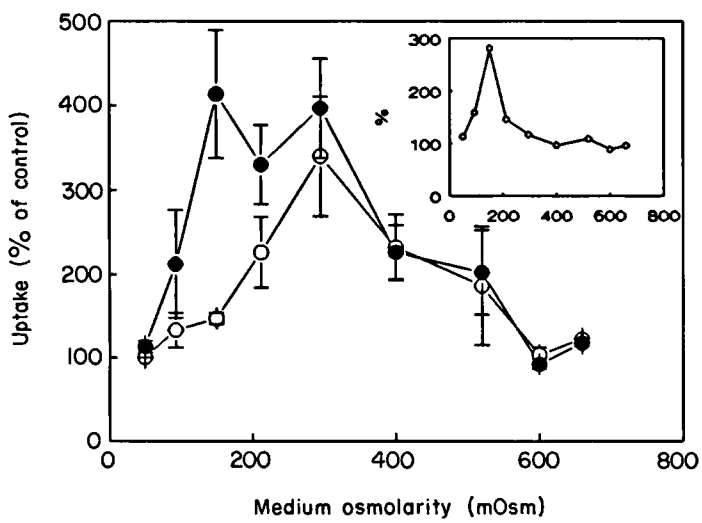

Fig. 3. Effect of ABA on the rate of uptake of sucrose by tobacco leaf discs determined at various osmolarities of the incubation medium. Discs were pre-incubated for 4 h on standard medium containing various amounts of PEG-6000, in the presence (●) or absence (○) of 10 μM ABA, and then incubated for 30 min on the same media supplemented with 1 mM ^{14}C-sucrose. Data are expressed as a percentage of the control (50 mOsm, no ABA) and are the averages ± SE of 2–10 experiments with 10 discs each. The inset shows the relative effects of ABA at the various osmolarities of the media.

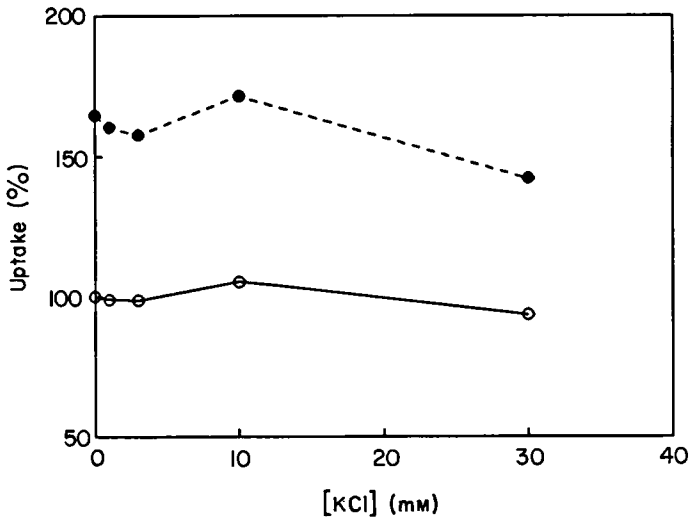

Fig. 4. Effect of KCl on the rate of uptake of sucrose into tobacco leaf discs. Discs were pre-incubated for 4 h on standard medium containing 200 mM mannitol, in the presence (●) or absence (○) of 10 μM ABA, and then incubated for 30 min on the same media supplemented with 1 mM ^{14}C-sucrose. Data are expressed as a percentage of the control (no KCl, no ABA) and are the averages of two experiments with 6–8 replicates per treatment.

100 to 300 mOsm. In the absence of ABA the highest uptake rate was observed at *c.* 300 mOsm. The optimal osmolarity for the uptake in the presence of 1 μM ABA was around 200 mOsm (Fig. 3). As a result, ABA significantly stimulated sucrose uptake at medium osmolarities ranging from 100 to 300 mOsm. Above 300 mOsm no effect of ABA was observed.

Potassium, in the range between 1 and 30 mM, had no significant effect on sucrose uptake, neither in the presence nor in the absence of ABA (Fig. 4).

DISCUSSION

The data presented in this paper are in contrast with our previous findings that ABA inhibits uptake of sucrose into source leaves from various species (Vreugdenhil 1983). They also contradict the findings of Estruch *et al.* (1989), who reported inhibition of sucrose uptake into isolated veins of pea leaves. According to Turgeon (1986), full grown tobacco leaves as used in the present studies, export photo-assimilates and are thus, by definition, source leaves. Therefore, the differences between our findings and previous reports cannot be explained by a different physiological status of the used material. This discrepancy can be explained by the difference in plant tissue used for the uptake studies: isolated veins versus leaf discs. In isolated veins, uptake most likely represents loading into the phloem (Daie, 1986). Sucrose uptake by leaf discs can either be by direct loading into the phloem, or uptake into the mesophyll followed by translocating towards the phloem, depending on the species (Gamalei & Pakhomova 1981; Turgeon & Wimmers 1988). In isolated veins, the uptake of sucrose is stimulated by low concentrations of potassium (Van Bel & Koops 1985; Estruch *et al.* 1989). In the tobacco discs no significant effect of potassium on sucrose uptake was observed. This indicates that sucrose uptake in this system represents uptake into the mesophyll rather than phloem loading. The conflicting

data on the effects of ABA on sucrose uptake might be explained by assuming that ABA inhibits direct loading of sucrose into the phloem, and stimulates uptake into the mesophyll. Further research is needed to validate this hypothesis.

Sucrose uptake by tobacco leaf discs exhibited a clear optimum at an osmolarity of the medium around 300 mOsm. The same phenomenon was described by others for uptake by isolated veins of celery (Daie 1987) and pea (Estruch *et al.* 1989) and by potato tuber tissue (Oparka & Wright 1988). In the presence of ABA the optimal osmolarity of the medium was shifted to *c.* 200 mOsm. Assuming that sucrose uptake is controlled by the turgor of the tissue, this might indicate that ABA influenced the water relation of the tissue in such a way that the optimal turgor for sucrose uptake was reached at a lower osmolarity of the incubation medium. However, the literature on the effects of ABA on cell turgor is contradictory and confusing: Jones *et al.* (1987) reported that ABA could either increase or decrease the cell turgor of wheat roots, depending on the tissue and the localization in the root. Eamus & Tomos (1983) reported that ABA raised the mean hydraulic conductivity of the membranes of *Rhoeo discolor* leaf epidermal cells, without changing the turgor pressure.

In conclusion, the present data, combined with previous findings (Vreugdenhil 1983; Estruch *et al.* 1989) indicate that the regulation of sucrose uptake by ABA is a complex phenomenon, depending on the plant species and the type of tissue.

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