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EFFECTS OF EXOGENOUS GLYCINEBETAINE ON SALT TOLERANCE AND Na^+ TRANSPORT IN BARLEY. I. WHOLE SEEDLINGS

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Studies on the influence of exogenous glycinebetaine on salt tolerance and ion transport were carried out using barley (*Hordeum vulgare*) seedlings grown in exenic Hoagland's medium under laboratory conditions. Exogenous glycinebetaine reduced Na^+ content of shoot and root of barley seedlings exposed to 25mM NaCl for 24hours, while K^+ content was not affected. The time course of the influence of glycinebetaine on ^{22}Na influx revealed complex interactions with enhanced initial Na uptake by glycinebetaine. Involvement of glycinebetaine in the ion fluxes and salt tolerance is discussed.

INTRODUCTION

Following the reports that choline, probably after metabolism to glycinebetaine, enhanced the halotolerance of the respiratory apparatus of a moderately halophilic bacterium (Rafaeli-Eshkol, 1968; Rafaeli-Eshkol and Avi-Dor, 1968), the influence of exogenous applications of these compounds on the salt tolerance of cereal seedlings was tested (Wyn Jones, 1972). Choline chloride (1 mM), added to the root medium, promoted the growth of 3 day-old maize coleoptiles subjected to the NaCl shock. Glycinebetaine and other related compounds also had small, but statistically dubious, promotive effects. However, under such conditions the ability of added choline and glycinebetaine to ameliorate salt stress in maize seedlings was later confirmed (Wyn Jones *et al.*, 1974). The basis for this phenomenon was initially examined by determining the effect of glycinebetaine on K^+ and Na^+ levels in barley grown at relatively low salinity (25 mM NaCl). In this and the following paper we report further investigations into these phenomena; in particular into the effects of exogenous

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glycinebetaine on Na^+ transport and compartmentation. Preliminary reports on some of these data have been presented previously (Wyn Jones & Ahmad, 1978; Wyn Jones, 1980).

MATERIALS AND METHODS

Seeds of *Hordeum vulgare* (cv. California Mariout) were surface sterilized with 1% sodium hypochlorite and germinated in sterile petri dishes at 20°C. After 4 days, the seedlings were transferred to sterilized Hoagland's No. 1 solution. The pH of the final solution was maintained between 5-6. Six day old plants were placed in sterilized Hoagland's medium containing 25 mM NaCl with or without 1 mM glycinebetaine for 24 hours. In experiments involving isotope uptake by seedlings, sodium was labelled with ^{22}Na (10μc/l). After suitable time intervals, the plants were removed from the labelled solution, allowed to exchange with unlabelled cold solution of identical composition for 10 min and then blotted and weighed. ^{22}Na was determined by counting β-radiation using Philips Liquid Scintillation Analyser (Philips Ltd., Eindhoven, Holland). Liquid Scintillator Unisol (Koch-Light Labs. Ltd.) was used both as a solvent and scintillator. Methods for chemical determination of Na^+ and K^+ were the same as those used by Storey and Wyn Jones (1977).

RESULTS AND DISCUSSION

After a 24 hour exposure to 25 mM NaCl, the seedlings also exposed to glycinebetaine had significantly lower root Na^+ contents and a slightly lower shoot Na^+ level than those not treated with glycinebetaine (Table 1). K^+ levels were unaffected by the glycinebetaine treatment. Calculation of the net uptake of Na^+ over the 24hour experimental period also showed that uptake was significantly decreased by exogenous glycinebetaine (0.59 μmole/g FW/hour to 0.33 μmole/g FW/hour). In the time course of the influence of glycinebetaine on Na, ^{22}Na influx was followed over the 24 hour experimental period and this revealed that the interaction (s) were unexpectedly complex. Over the first 6 hours Na^+ uptake was enhanced by glycinebetaine while uptake into shoot at 2 hours was slightly lower (data not presented). Later, the rate of uptake in the root receiving the betaine decreased while that of the untreated root was maintained so that after 24 hours the total uptake into the glycinebetaine-treated roots was lower than the control as repor-

ted in Table 1. After 12 hours, the glycinebetaine treatment increased Na^+ uptake into the shoot but by the end of 24 hours there was no significant difference. An experimentation of the influence of exogenous glycinepetaine on ^{22}Na uptake into the roots of whole barley seedlings over a range of salt concentration again showed a complex pattern, with a stimulation between 0.3 and 3.0mM Na^+ and inhibition between 10-25 mM salt (data not presented). Clearly, the interpretation of such data is extremely difficult, if not impossible, and the flux interactions are examined in greater detail in a simpler, excised root system in the paper following this one.

Table 1. *Effect of glycinebetaine on the uptake of K^+ and Na^+ by barley plants subjected to 25mM NaCl for 24 hours*

	Zero time (before addition of NaCl)	Treatments[1]			
		NaCl (25mM)	NaCl (25mM) + Glycinebetaine (1mM)	Effect[2] of gb.(%)	Significance
Shoot K^+ (μmol/g fr. wt.)	165	176	183	104	—
Na^+ ,,	7.7	19	16	85	$p<0.05$
,,	8.6	102	103	101	—
Na^+ ,,	11 7	26	20	76	$p<0.01$

1. Mean values of 16 replicates for each treatment, 2. gb. = glycinebetaine.

The results presented in this paper, nevertheless, strongly indicate that the simple hypothesis that glycinebetaine is accumulated as a metabolically-inert, cytoplasmic solute (Wyn Jones *et al.*, 1977), is inadequate, and the accumulation, or a metabolic consequence thereof, clearly influence both ion fluxes and the ability of tissue to withstand a particular salt load. The data on the effect of glycinebetaine on the stabilization of enzymes and membranes were noted earlier (Pollard and Wyn Jones, 1979). However, this evidence does not allow more rigorous analysis of these phenomena and the tedious demands of rigorously or even partially exenic culture do not lend themselves to detailed experimentation.

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