Phytochemistry, Vol. 20, No. 7, pp. 1501–1504, 1981.
Printed in Great Britain.

0031-9422/81/071501-04 $02.00/0

THE ACCUMULATION OF Δ′-ACETYLORNITHINE AND OTHER SOLUTES IN THE SALT MARSH GRASS *PUCCINELLIA MARITIMA*

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(*Received* 29 *August* 1980)

Key Word Index—*Puccinellia maritima*; Gramineae; halophyte; Δ′-acetylornithine; proline, glutamine; compatible solute; salt tolerance.

Abstract—The salt marsh grass *Puccinellia maritima* was shown to accumulate several organic solutes when subject to low water potentials. These solutes include the non-protein amino acid Δ′-acetylornithine, the amide glutamine, the imino acid proline and soluble carbohydrates. The increase in organic solutes observed under saline conditions appears too large for all of them to be localized in the cytoplasm. It is suggested that solute synthesis may reduce the dependence of the plant on the absorption of sodium and chloride ions as sources of osmotically active solutes.

INTRODUCTION

Studies of the response of angiosperm halophytes to saline conditions suggest osmotic adjustment to the lowered water potentials occurring primarily via an accumulation of Na^+ and Cl^- ions [1]. High concentrations of these ions, however, are inhibitory to the metabolic processes of halophytes when tested in *in vitro* experimental systems [1]. It is suggested therefore that the bulk of the absorbed Na^+ and Cl^- are localized in the vacuole thereby circumventing a possible disruption of metabolism [1–3]. Intracellular osmotic adjustment is suggested to occur by a cytoplasmic accumulation of compatible solutes [4, 5] and among the compounds thought to function as such are proline [4, 6], methylated quaternary onium compounds [5, 7–9] and sugar alcohols [10, 11].

The salt marsh grass *Puccinellia maritima* has been shown to accumulate large amounts of proline when grown under saline conditions [4, 12]. In this paper we report that other solutes including the non-protein amino acid Δ′-acetylornithine are also accumulated in response to a lowering of external water potentials.

RESULTS

Changes in inorganic solutes in response to lowered external water potentials

The results in Fig. 1 show the changes which occur in the major cations and anions in leaf tissue of *P. maritima* when the external water potential was decreased by the addition of different NaCl concentrations. Over the range 50–200 mM NaCl, the concentration of Na^+ and Cl^- remained relatively constant but at higher external concentrations the tissue concentration of both ions increased markedly. It is striking that over the range 0–400 mM NaCl the tissue potassium content remains constant and even in plants grown at 600 mM NaCl tissue potassium (380 mM) and sodium (430 mM) concentrations are relatively similar. It appears from these results that in salt-grown plants of *P. maritima* K^+ remains a major solute. This contrasts with the behaviour of many other halophytic angiosperms in which there is an apparent substitution of K^+ by Na^+ [12–15]. The results in Fig. 1 show, however, that there was some replacement of tissue NO_3^- by Cl^- as the external salinity increased.

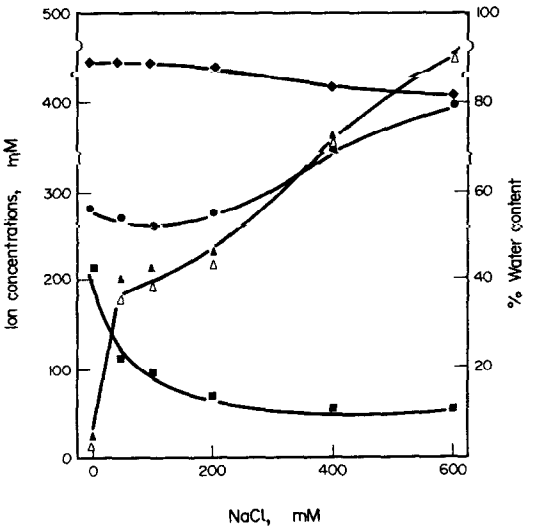

Fig. 1. Ionic status of leaves of salt-grown *Puccinellia maritima*. Sodium, ▲; potassium, ●; nitrate, ■; chloride, △; water content, ◆.

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Table 1. Ionic status of leaf tissue of *Puccinellia maritima* subject to osmotic stress

		μmol/g fr. wt				
	Π sol MPa	Na^+	Cl^-	K^+	NO_3	% H_2O
Control	−0.03	5	4	282	190	87.7
Mannitol (200 mM)	−0.48	4	10	391	194	79.7
PEG (15%)	−0.30	2	4	284	210	87.1

Plants were grown for a period of 7 days in the presence of either 200 mM mannitol or 15% PEG 6000 prior to harvest for analysis.

The results in Table 1 show that when the external water potential was decreased by the addition of non- or slowly penetrating osmotica such as PEG 6000 and mannitol, there was little change in tissue nitrate concentration but there was a large increase in potassium content of mannitol-treated plants. However, this increase appeared to be associated with a large decrease in tissue water content.

Changes in organic solute in response to lowered external water potentials

As reported previously, proline increased in plants grown in the presence of NaCl (Fig. 2). It is clear, however, from these results that the soluble amino fraction also increased and that there was an 8-fold increase in total soluble carbohydrates. TLC revealed the major components of the latter fraction to be glucose, fructose and sucrose.

Analysis of the soluble amino fraction showed that the increase in this fraction could be accounted for by accumulation of the amides glutamine and to a lesser extent asparagine, and the non-protein amino acid Δ′-acetylornithine (Fig. 3). Although glutamine is the major amino compound in the leaves of plants grown at 600 mM NaCl, an 8-fold increase (5–40 mM) occurred in the tissue concentration of Δ′-acetylornithine.

The tissue concentration of glutamate decreased in plants grown at salt concentrations in excess of 100 mM. There was, however, little change in aspartate at any of the salt concentrations tested. There were small increases in serine and alanine concentrations but the other amino acids (data not shown) remained constant over the range of salt concentrations tested.

When PEG 6000 and mannitol were used to lower the external water potential accumulation of proline, amides, Δ′-acetylornithine and soluble carbohydrates were observed in leaf tissue of plants subject to these osmotica (Table 2).

No simple relationship was apparent between solute accumulation and either the magnitude of the decrease in water potential of the rooting media or the extent of the osmotic stress (as measured by change in leaf water content) induced by different osmotica. Thus iso-osmotic solutions of mannitol (200 mM) and NaCl (100 mM) induced an increase in, for example, proline concentrations to 52 and 18 mM respectively. Mannitol did, however, induce an osmotic stress (79.7% leaf water content) comparable with that found in plants grown at 600 mM NaCl (80.4% leaf water content) but again the proline contents of such plants were markedly different, 52 mM in mannitol-treated ones and 103 mM in those in 600 mM NaCl.

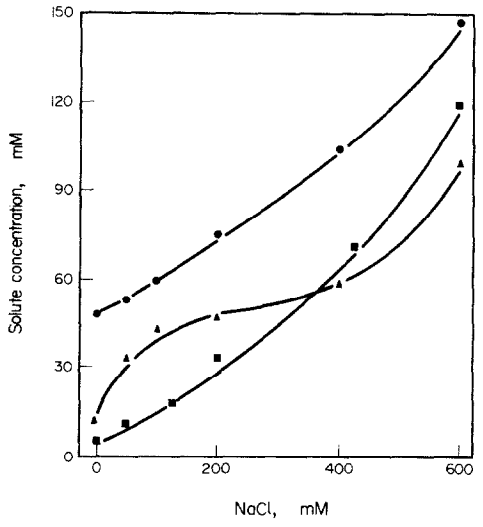

Fig. 2. Organic solute accumulation in the leaves of salt-grown *Puccinellia maritima*. Amino compounds, ●; proline, ■; soluble carbohydrates, ▲.

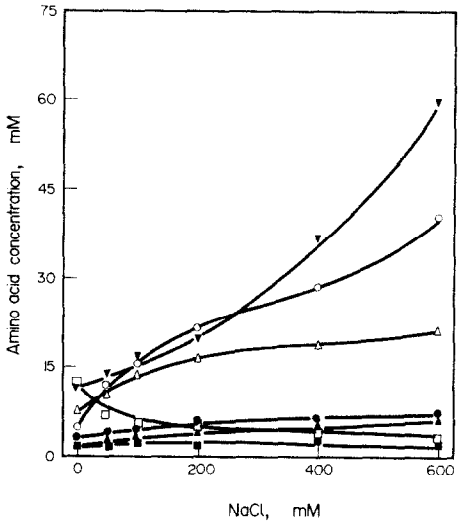

Fig. 3. Changes in amino compounds in the leaves of salt-grown *Puccinellia maritima*. Glutamine, ▼; Δ′-acetylornithine, ○; asparagine, △; glutamate, □; aspartate, ■; serine, ●; alanine, ▲.

Table 2. Organic solutes in leaf tissue of *Puccinellia maritima* subject to osmotic stress

		μmol/g fr. wt				
	Πsol MPa	Proline	Glutamine	Asparagine	Δ′-Acetyl-ornithine	Soluble carbo-hydrates
Control	−0.03	4	14	8	3	11
Mannitol (200 mM)	−0.48	58	36	12	30	50
PEG (15%)	−0.30	14	23	15	15	27

See Table 1 for details.

DISCUSSION

It is striking that when subject to low water potentials, leaf tissue of *Puccinellia maritima* accumulates a variety of organic solutes. The 8-fold increase in Δ′-acetylornithine is particularly interesting since this non-protein amino acid has not previously been reported to accumulate under stress conditions (see [16]). Fowden [17] has shown the presence of this compound in *P. maritima* and other festucoid grasses. Studies of its metabolism [18, 19] indicate that it is, like proline, synthesized from L-glutamate and that in *Onobrychis* there was considerably less metabolism of ^{14}C-labelled glutamate to proline compared with Δ′-acetylornithine. While the accumulation of Δ′-acetylornithine in stressed plants has not been investigated, this may be worthwhile, particularly in species not accumulating proline.

The present results show that there is in plants grown at 600 mM NaCl an increase of approximately 240 mM organic solutes. This compares with a $Na^+ + Cl^-$ concentration of 800 mM and suggests that not all of the accumulated organic solutes can be cytoplasmic. It is possible that the synthesis of organic solutes in *P. maritima* may reduce the plant's dependence upon the absorption of potentially disruptive inorganic ions as a source of solute for osmotic adjustment. Recent studies suggest, however, that current models of salt tolerance [1, 3] based on the vacuolar sequestration of NaCl may be too simplistic. Considerable variation in chloroplast and vacuolar concentrations of Na^+ and Cl^- have been reported in studies of *Suaeda maritima* [20, 21]. Heterogeneity in chloride distribution in leaf cells of the mangrove, *Aegiceras corniculatum*, has also been reported [22]. Variation in ion concentrations in different cells implies a similar variation in organic solute concentrations and clearly makes it difficult, if not impossible, to construct osmotic balance sheets from whole tissue analyses.

Although the present results do not permit an unequivocal role to be ascribed to the accumulation of Δ′-acetylornithine, they are consistent with it playing a role in the osmotic adjustment of *P. maritima* to low water potentials. They also prompt the speculation that other non-protein amino acids might accumulate in certain species when they are subject to stress conditions and therefore play a role in osmoregulation.

EXPERIMENTAL

Plant samples. Plants of *Puccinellia maritima* were collected from salt marshes at Pilling, Lancashire and Lancieux, France.

Growth conditions. Germinated seedlings were grown in water culture on one-fifth diluted complete nutrient soln of Aron and Hoagland [23]. The nutrient solution was aerated, topped up with deionized water each day and renewed every 7 days. After 8 weeks' growth, salt treatments were initiated by daily additions of 50 mM NaCl to give final concns of 0, 50, 100, 200, 400 and 600 mM. In the osmotic stress treatments daily additions of 50 mM mannitol and 2.5% (w/v) polyethylene glycol 6000 were made to give final concns of 200 mM and 15% respectively. The nutrient solns for these two treatments were renewed every 3 days. Plants were harvested 7 days after the saline and osmotic treatments were completed. Harvesting was carried out as described previously [10].

Analytical procedures. Inorganic ions: Dried samples were used for the determination of Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- (see [10]). Nitrate was determined on fresh samples by a cadmium reduction technique [24].

Soluble nitrogen: Soluble nitrogenous compounds were extracted and determined as described previously [10].

Identification of Δ′-acetylornithine: Descending PC was carried out on Whatman No. 3 paper using: *n*-BuOH–HOAc–H_2O (*12:3:5*); *PhOH–EtOH–H_2O (15:4:1)*; *PhOH–NH_3 (200:1)*. The R_{ala} values for Δ′-acetylornithine were 0.98, 1.48 and 1.42 respectively. The unknown compound co-chromatographed with authentic Δ′-acetylornithine.

High voltage electrophoresis (HVE) was carried out on Whatman No. 3 paper, in 0.75 M formic acid (pH 2.0) at 40 V/cm. The relative mobility of the unknown and Δ′-acetylornithine was 0.66 with respect to alanine.

After hydrolysis in 6 M HCl for 16 hr at 110°, the amino acid product was identified as ornithine using HVE and PC. Similarly on amino acid analysis of the hydrolysate on the short column of a Beckman analyser (using sodium citrate buffer, pH 4.25 at 32.5°) a single peak corresponding to ornithine, was obtained.

Routine determination of Δ′-acetylornithine was made on either a Technicon or LKB-BioCal amino acid analyser using lithium citrate buffers. The peak corresponding to Δ′-acetylornithine eluted immediately before proline.

Soluble carbohydrate: Soluble carbohydrates were extracted as for soluble nitrogen and were determined by an anthrone procedure [25].

Acknowledgements—We would like to thank Drs. L. Fowden and P. J. Lea for providing us with an authentic sample of Δ′-acetylornithine and Professor J. Hamelin for synthesizing a sample of the amino acid. We are grateful to Dr. D. Le Rudulier for helpful discussions and suggestions regarding amino acid analyses. The technical assistance of P. Hervochon in performing some of the amino acid analyses is acknowledged. Financial support from the Science Research Council (to G.R.S.) is acknowledged with thanks.

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