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Responses of Halophytes to High Salinities and Low Water Potentials[1]

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ABSTRACT

The effects of nonsaline polyethylene glycol (PEG)-6000 and saline seawater solutions of comparable osmotic potential on the concentrations of organic solutes and inorganic ions in the tissues of halophytes (*Plantago maritima L., Triglochin maritima L., Limonium vulgare Mill., Halimione portulacoides (L.) Aell*) have been investigated. Studies were made to determine whether high salinities induce specific ion effects that are absent in plants grown in nonsaline solutions of comparable osmotic potential. Over-all, the responses of each species to the two different treatments (seawater or PEG) are similar; the accumulation of organic solutes (compatible osmotica) in tissues is primarily correlated with a decrease in the osmotic potential of culture solutions. Depending on the species, sorbitol, proline, reducing sugars, quaternary ammonium compounds, and α-amino nitrogen accumulate in tissues as the water potential of the tissues falls. Within a species there are differences in the concentrations of inorganic ions and organic solutes between roots and shoots of plants grown at high salinities or at high concentrations of PEG.

Halophytes accumulate low mol wt organic solutes when grown under saline conditions (16, 22-26). The solutes include proline, pipecolic acid, and 5-hydroxy pipecolic acid and methylated QAC,[2] such as glycine-betaine, and homobetaine. In addition, some halophytes such as *Plantago maritima* L., accumulate reducing sugars and sorbitol at high salinities (12, 22). These compounds are thought to act as nontoxic osmotica and to be preferentially accumulated in the cytoplasm, where they are largely responsible for maintenance of osmotic potential, since cytoplasmic concentrations of inorganic ions are low (7, 8, 24, 25). Critical evidence of localization of these substances within plant cells is lacking. Few direct measurements of cytoplasmic electrolyte concentrations in higher plants exist, although there is evidence that ions, such as Na^+, may be excluded from the cytoplasm (7, 11, 13).

High concentrations of organic solutes such as glycine-betaine were found in some halophytes, even when grown at low salinity, whereas other organic solutes, such as proline, increased in concentration only when the external salinity was high (25).

Changes in the concentrations of these solutes have been examined in relation to different external salinities and, with two exceptions, no attempts have been made to separate osmotic effects from those associated with salinity. Kluge (15) and Rozema (17) found little difference in the effects of mannitol and salt solutions at the same osmotic potential on proline accumulation for a number of species, and concluded that proline accumulated in response to changes in the external osmotic potential. In this study effects of nonsaline (PEG-6000) and saline solutions of comparable osmotic potential on tissue concentrations of organic solutes and inorganic ions of halophytes have been investigated. The purpose was to examine whether elevated concentrations of these organic solutes were produced by halophytes in response to low external osmotic potentials or whether high salinities induced specific ion effects absent in plants fed with nonsaline solutions of comparable osmotic potential.

Four perennial halophytes, which show contrasting responses to salinity, have been studied. *P. maritima* L. accumulates sorbitol (12, 22); *Triglochin maritima* L. produces proline; *Limonium vulgare* Mill. produces both proline and QAC, and *Halimione portulacoides* (L.) Aell. accumulates QAC but not proline (22, 25). Changes in the shoot and root levels of the major inorganic cations, reducing sugars, sorbitol, proline, QAC, and α-amino acids were examined in these species when they were grown at different external osmotic potentials. Data on water potentials and water contents of the tissues also were obtained.

MATERIALS AND METHODS

Culture of Plants. Seeds of *L. vulgare, P. maritima*, and *T. maritima*, and seedlings of *H. portulacoides* were collected from the upper level of a salt marsh at Stiffkey, Norfolk, England in August, 1976. These were sown or planted in September, 1976 in peat trays containing a garden loam soil to which was added dilute seawater (1 part seawater: 19 parts water). Seawater solutions were prepared from Rila Marine Mix (Rila Products Inc., N. J.). Seedlings were transplanted in October, 1976 into river-washed sand in 15.25-cm-diameter pots. Until February 1, 1978, plants were grown in a heated greenhouse without supplementary lighting, were watered with tap water as required, and weekly were fed with 100 ml of a 0.1-strength nutrient solution (14). During February, 1978, additions of the nutrient solution were made three times a week to all cultures.

Daily additions of either 100 ml of seawater or a solution of PEG-6000 commenced on March 1, 1978. In those cultures designated to receive solutions of low osmotic potential, there was a gradual increase in the concentration of the solutions to the required level, so that by mid-March all cultures were receiving appropriate treatments. Different solutions of artificial seawater (0, 5, 20, 50, 100%) or solutions of PEG-6000 which gave comparable osmotic potentials (−0.05, −0.02, −0.5, −1.2, −2.4 MPa) were used. Tap water was added to controls. Solution osmotic potentials were determined with a Wescor 5100 series vapor pressure osmometer. After 2 weeks of treatment plants were harvested. Plants did not show adverse effects associated with different treatments, except that the oldest leaves of *Triglochin* plants turned yellow. These were discarded.

Tissue Water Potentials. Under greenhouse conditions it was impossible to control soil and leaf water potentials fully. In order to avoid differences in tissue water potential associated with

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[2] Abbreviations: QAC: quaternary ammonium compounds; MPa: megaPascal; 1 MPa = 10 bars.

changes in diurnal conditions, plants were harvested between 0830 and 0900 hours. Immediately before harvest, leaves were washed in distilled H_2O for 30 s to remove excreted salts and blotted dry. Four leaf segments (<1 cm long) were cut away from the midrib and placed in a Wescor C-52 sample chamber for water potential measurements. Roots were treated in a similar manner, except that they were washed for 1 min to remove sand. There was a delay of not more than 5 min between harvesting and placing tissue samples in the Wescor chamber. After 3-h equilibration the water potentials of three replicate samples for each treatment were measured with a Wescor HR 33T dewpoint microvoltmeter.

Leaf and Root Water Content. Duplicate 2.5-g samples of leaves or roots were washed, blotted, weighed, and dried at 80 C for 48 h, then reweighed.

Tissue Ion Concentrations. After wet digestion of 1.5 g of oven-dried tissue (1) which had been washed in distilled H_2O before being dried, concentrations of K^+, Na^+, Ca^{2+}, and Mg^{2+} were determined with a Pye-Unicam SP.190 atomic absorption spectrophotometer. From water contents and internal ion contents of tissues, estimates were made of the mean ion concentrations.

Estimation of Nitrogen Compounds and Total Nitrogen. A 2.5-g (fresh weight) tissue sample was cut into segments, frozen in liquid H_2, and transferred to 50 ml of 60% (v/v) ethanol at 4 C for 48 h. The sample was filtered and the filtrate evaporated to dryness, redissolved in 2 ml of 0.8 M potassium acetate, buffered to pH 4.85 with acetic acid. Total α-amino acids and proline were determined using the ninhydrin and acid-ninhydrin methods, respectively (5). The micro-Kjeldahl technique was used to determine both soluble and total nitrogen in tissues (20). QAC were measured colorimetrically (25).

Sorbitol and Reducing Sugars. Tissue (5 g) was extracted with 80% (v/v) ethanol and sorbitol was measured (3, 4) after the ethanol extract was extracted with chloroform to remove pigments. A portion of the aqueous fraction was subjected to preparative paper chromatography with triple development in propanol-ethyl acetate-water (7:1:2, v/v/v). The hexitol band was detected by test strips and cut out. Hexitols were eluted with boiling 80% ethanol and the eluate evaporated to dryness. The polyols were acetylated in an acetic anhydride-pyridine mixture (1:1, v/v). GLC was used for the quantitative determination of sorbitol using a column operated isothermally at 220 C (18). After initial verification of the presence of sorbitol, quantitative estimations of the amounts of sorbitol in aliquots of the aqueous fraction were obtained from enzymic assays using sorbitol dehydrogenase (EC 1.1.1.14). The dinitro-salicylic acid method was used to measure amounts of reducing sugars in another aliquot of the aqueous extract. Glucose was used as a standard.

RESULTS

Mean concentrations of major cations in shoots and roots of the four species in response to the different treatments are given in Tables I through IV. The values expressed on the basis of tissue water content assume no binding of cations. Estimates of K and Na have been used to calculate the approximate contribution which salts of these two elements make to the osmotic potential. Values calculated assume chloride as the dominant anion, and an osmotic coefficient of 0.9 (25) (Tables I–IV).

Data on ionic concentrations indicate differences between species and between treatments. Under both saline and nonsaline conditions, over-all ionic concentrations and sodium concentrations in particular are higher in the shoot than the root. The shoot acts as a sink for Na ions when plants are grown at high salinities. Under saline conditions shoots of *P. maritima*, *T. maritima*, and to a lesser extent *H. portulacoides* contain high levels of Na and relatively low levels of K, unlike roots, where concentrations of

Table I. *Ionic Concentrations (mM), Based on Tissue Water Content, in Roots and Shoots of Plantago maritima Grown in Sand Culture*

Cultural solutions contained either different dilutions of seawater or different concentrations of PEG at similar osmotic pressures (Π_{sol}:MPa). Calculated Π of tissues based on $Na^+ + K^+$ concentrations, assume Cl^- as anion and an osmotic coefficient of 0.9.

	Π_{sol}	Saline Treatments					PEG Treatments				
		Na^+	K^+	Ca^{2+}	Mg^{2+}	Π_{cal}	Na^+	K^+	Ca^{2+}	Mg^{2+}	Π_{cal}
Shoot	0.05	78	74	55	14	0.7					
Root	(Control)	22	53	24	22	0.3					
Shoot	0.2	136	76	41	17	0.9	47	135	67	16	0.8
Root		39	47	16	19	0.4	40	92	7	23	0.6
Shoot	0.5	204	77	41	22	1.3	112	141	93	20	1.1
Root		112	140	27	25	1.1	47	74	23	21	0.5
Shoot	1.2	300	80	38	26	1.7	176	138	119	32	1.4
Root		136	150	34	39	1.3	59	118	41	41	0.8
Shoot	2.4	472	136	63	42	2.7	130	239	104	33	1.6
Root		200	166	47	47	1.6	65	160	35	22	1.0

Table II. *Ionic Concentrations (mM), Based on Tissue Water Content, in Roots and Shoots of Triglochin maritima Grown in Sand Culture*

Cultural solutions contained either different dilutions of seawater or different concentrations of PEG at similar osmotic pressures (Π_{sol}:MPa). Calculated Π of tissues based on $Na^+ + K^+$ concentrations, assume Cl^- as anion and an osmotic coefficient of 0.9.

	Π_{sol}	Saline Treatments					PEG Treatments				
		Na^+	K^+	Ca^{2+}	Mg^{2+}	Π_{cal}	Na^+	K^+	Ca^{2+}	Mg^{2+}	Π_{cal}
Shoot	0.05	141	131	36	24	1.2					
Root	(Control)	76	95	32	21	0.8					
Shoot	0.2	97	125	25	16	1.0	140	134	55	18	1.1
Root		65	45	40	20	0.5	86	103	41	21	0.8
Shoot	0.5	200	99	41	19	1.3	153	159	71	25	1.4
Root		85	64	28	17	0.7	122	133	52	23	1.1
Shoot	1.2	407	117	43	38	2.3	174	146	65	24	1.4
Root		209	175	42	37	1.7	99	84	58	37	0.8
Shoot	2.4	347	152	46	36	2.2	257	242	138	49	2.2
Root		250	167	32	48	1.9	109	158	76	44	1.2

Table III. *Ionic Concentrations (mM), Based on Tissue Water Content, in Roots and Shoots of Limonium vulgare Grown in Sand Culture*

Cultural solutions contained either different dilutions of seawater or different concentrations of PEG at similar osmotic pressures (Π_{sol}:MPa). Calculated Π of tissues based on $Na^+ + K^+$ concentrations, assume Cl^- as an anion and an osmotic coefficient of 0.9.

	Π_{sol}	Saline Treatments					PEG Treatments				
		Na^+	K^+	Ca^{2+}	Mg^{2+}	Π_{cal}	Na^+	K^+	Ca^{2+}	Mg^{2+}	Π_{cal}
Shoot	0.05	95	202	63	45	1.3					
Root	(Control)	59	94	89	18	0.7					
Shoot	0.2	261	314	74	56	2.6	100	208	20	28	1.4
Root		72	151	59	28	1.0	43	87	44	28	0.6
Shoot	0.5	238	151	36	47	1.7	141	227	44	73	1.6
Root		80	59	33	23	0.6	138	169	115	54	1.4
Shoot	1.2	286	228	36	54	2.3	315	200	70	65	2.3
Root		231	268	179	115	2.2	101	164	78	43	1.2
Shoot	2.4	400	298	130	153	3.1	320	292	86	137	2.7
Root		288	199	91	76	2.2	90	173	90	45	1.2

Table IV. *Ionic Concentrations (mM), Based on Tissue Water Content, in Roots and Shoots of Halimione portulacoides Grown in Sand Culture*

Culture solutions contained different dilutions of seawater (Π_{sol}:MPa). Calculated Π of tissues based on $Na^+ + K^+$ concentrations, assume Cl^- as an anion and an osmotic coefficient 0.9.

	Π_{sol}	Na^+	K^+	Ca^{2+}	Mg^{2+}	Π_{cal}
Shoot	0.05	116	69	82	34	0.8
Root	(Control)	78	101	73	48	0.8
Shoot	0.2	413	258	73	59	3.0
Root		153	163	71	77	1.4
Shoot	1.2	468	250	107	65	3.2
Root		318	245	263	127	2.5
Shoot	2.4	584	297	122	96	3.9
Root		360	250	220	72	2.7

these ions are more nearly equal. In *L. vulgare*, in contrast, Na/K ratios are less, indicating the presence of high concentrations of K in tissues (Table III). In all four species a decrease in the osmotic potential of the culture solutions below −0.5 MPa results in only a moderate increase in the internal ion content of roots. This is especially evident in roots of plants treated with PEG, where K frequently replaces Na as the dominant ion. Therefore, over a wide range of external osmotic potentials, the internal ion content of roots does not change substantially. At high salinities or high concentrations of PEG, values of calculated osmotic potentials of the tissues based on Na and K contents are frequently less than or only slightly more negative than values of the osmotic potentials of the culture solutions. As some of the Na is unlikely to contribute to the osmotic potential of the tissues, the estimates indicate that other solutes must be acting as compatible osmotica in tissues of plants treated with solutions of low osmotic potential.

In Table V water contents of shoots and roots of plants subject to different treatments are given. Although a fall in the ratio of water content to dry weight is associated with decreasing external osmotic potential, the differences largely reflect the accumulation of salts in tissues. Over a wide range of external osmotic potentials only small adjustments in water contents of shoots and roots of the species are evident. Woody roots of *Halimione* and *Limonium* have a relatively low water content.

The water potential of tissues of all species falls as the external salinity or concentration of PEG increases (Table VI). The lowering of the water potentials of shoots and roots in excess of the potentials of the culture solutions is most marked in *L. vulgare* and *H. portulacoides* and least evident in *P. maritima* and *T. maritima*. Water potentials of the shoots and roots of *Limonium* and *Halimione* were as much as 1.0 to 2.0 MPa below that of the solution in which the plants were cultured. In addition, water potentials of plants treated with PEG frequently were lower than corresponding values for plants grown in saline cultures. Likewise at low salinities and low concentrations of PEG, root water potentials were considerably lower than osmotic potentials of the corresponding culture solutions. Although sand cultures were periodically flushed with water, sand culture water potentials are probably lower than those of the culture solutions because of evapotranspiration and accumulation of salts in the sand.

Although tissue water potentials were measured and not osmotic potentials, the difference between these potentials is unlikely to be more than a few bars (6, 21). As a check, in a separate experiment, values of osmotic potential of cell sap of plants receiving certain treatments were compared with values of water potential of corresponding tissues (Table VII). A Wescor 5100 series vapor pressure osmometer was used to measure the osmotic potential of an extract of cell sap obtained from crushed plant tissue. The small differences between these respective potentials implies that under these experimental conditions, turgor pressure is likely to be only a few bars.

Accumulation of sorbitol in response to low external osmotic potentials is restricted to *P. maritima* (Table VIII). A linear relationship exists between a fall in external osmotic potential and the accumulation of sorbitol in roots and shoots. Where the osmotic potential of the culture solution is −2.4 MPa, the concentration of sorbitol in shoots is from 56 to 83 mM and in roots between 84 and 118 mM, compared with values of between 17 and 19 mM for this polyol in plants grown in the absence of seawater or PEG. Under conditions of low external osmotic potential a greater accumulation of sorbitol occurs in roots than in shoots. Concentrations of the polyol in both roots and shoots of plants treated with PEG are higher than corresponding values for saline-treated plants. The higher concentration is consistent with lower water potentials in the roots and shoots of plants treated with the highest concentration of PEG (Table IV).

Associated with the increase in sorbitol concentrations is a rise in the level of soluble organic nitrogen in roots of *Plantago*, particularly in cultures where the osmotic potential of the culture solution is lower than −0.5 MPa (Table VIII). The rise which is not a linear function but a step function is almost entirely accounted for by the accumulation of α-amino nitrogen in tissues. The highest concentrations of soluble nitrogen occur in roots of plants treated with PEG. Concentrations of α-amino nitrogen in shoots and concentrations of reducing sugars in both shoots and roots show little response to the perturbations.

Proline accumulates in both shoots and roots of *T. maritima* in response to low external osmotic potentials (Table IX). The concentration of the amino acid exceeds 27 mM in both shoots and roots of plants treated with solutions of low osmotic potential (−2.4 MPa). Corresponding increases in the concentrations of α-amino nitrogen, total soluble nitrogen, and reducing sugars are also evident in both roots and shoots.

As in *P. maritima* much of the increase in total soluble nitrogen reflects a rise in the level of α-amino nitrogen in the tissues in response to low water potentials. High concentrations of reducing

Table V. *Water Content of Shoots and Roots as a Ratio of Water Content to Dry Weight of Halophytes Grown at Different Salinities or at Different Concentrations of PEG*

Solutions of PEG had the same osmotic potentials (MPa) as saline solutions (ψ_s). The data are the means of two determinations.

Species	ψ_s	% of Seawater					PEG: g/l				
		0 −0.05	5 −0.2	20 −0.5	50 −1.2	100 −2.4	0 −0.05	70 −0.2	200 −0.5	330 −1.2	490 −2.4
Plantago	Shoot	13.1	13.1	9.2	10.8	6.1	13.1	9.4	7.3	5.9	5.0
maritima	Root	7.5	4.0	4.8	3.0	3.0	7.5	5.9	4.0	3.1	2.7
Triglochin	Shoot	8.3	9.2	10.2	8.0	5.5	8.3	8.4	8.9	7.3	3.9
maritima	Root	5.3	6.2	4.1	3.8	3.4	5.3	4.4	2.8	2.8	2.2
Limonium	Shoot	4.1	4.6	5.3	4.4	2.4	4.1	5.8	4.8	3.3	2.4
vulgare	Root	2.5	3.3	4.5	1.5	2.1	2.5	2.8	2.3	2.2	2.1
Halimione	Shoot	6.4	7.8		3.8	3.4					
portulacoides	Root	3.0	2.0		1.1	1.4					

Table VI. *Water Potential (MPa) of Shoots and Roots of Halophytes Grown at Different Salinities or at Different Concentrations of PEG*

Solutions of PEG had the same osmotic potentials as saline solutions (ψ_s). The data are the means of three determinations.

Species	ψ_s	% of Seawater					PEG: g/l				
		0 −0.05	5 −0.2	20 −0.5	50 −1.2	100 −2.4	0 −0.05	70 −0.2	200 −0.5	330 −1.2	490 −2.4
Plantago	Shoot	−0.7	−0.8	−1.3	−1.9	−2.6	−0.7	−1.1	−1.3	−1.9	−3.7
maritima	Root	−0.6	−0.7	−1.3	−1.7	−2.5	−0.6	−0.7	−1.3	−1.7	−3.7
Triglochin	Shoot	−0.6	−0.8	−1.6	−1.8	−2.5	−0.6	−0.8	−1.8	−2.7	−3.4
maritima	Root	−0.6	−0.8	−1.5	−1.6	−2.4	−0.6	−0.7	−1.8	−2.3	−2.6
Limonium	Shoot	−0.8	−1.5	−2.6	−3.5	−4.8	−0.8	−1.7	−2.6	−3.5	−4.6
vulgare	Root	−0.6	−0.7	−1.7	−2.4	−3.3	−0.6	−1.2	−2.2	−3.1	−3.5
Halimione	Shoot	−1.5	−1.6		−2.5	−3.6					
portulacoides	Root										

Table VII. *Values of Osmotic Potential (MPa) of Leaf Cell Sap (Ψ_s) and Water Potential (Ψ_w) of Leaf Tissue of Halophytes Grown at Different Salinities or Different Concentrations of PEG-6000 (S = Seawater; PEG = Polyethylene glycol)*

Species	Culture Solution	Cell Sap	Leaf Tissue
	Ψ_{sol}	Ψ_s	Ψ_w
Limonium	−0.2 (S)	−2.6	−2.6
vulgare	−0.2 (PEG)	−3.5	−3.5
	−0.5 (S)	−2.8	−2.7
	−0.5 (PEG)	−4.9	−4.8
Triglochin maritima	−2.4 (S)	−4.1	−4.0
Plantago maritima	−2.4 (S)	−4.2	−4.0
Halimione portulacoides	−0.5 (S)	−2.6	−2.4

sugars in shoots of plants treated with PEG have been observed in three separate experiments.

The responses of shoots and roots of *L. vulgare* to low external osmotic potentials are different. In shoots an increase in concentrations of soluble nitrogen, α-amino nitrogen, proline, and reducing sugars occurs as the concentration of PEG or salinity is increased (Table X). Methylated QAC show only a modest rise in concentration in shoots of plants grown in cultures where the osmotic potential of the culture solution is at or above −0.5 MPa. In roots quaternary nitrogen compounds also are at low concentrations, irrespective of the treatment, but α-amino nitrogen, reducing sugars, and total soluble nitrogen are maintained at high concentrations over a wide range of external osmotic potentials.

Methylated QAC increase in shoots and roots of *H. portulacoides* to 58 and 80 mM, respectively, when plants are subject to a decrease in external osmotic potential to −2.4 MPa (Table XI). Much of the increase in the concentrations of these compounds in tissues occurs at comparatively low salinities. Unlike *Limonium* and *Triglochin*, plants of *Halimione* contain very low concentrations of proline. Amounts of α-amino nitrogen, total soluble nitrogen, and reducing sugars increase in response to salinity, particularly in the roots.

DISCUSSION

An apparent decrease in the ratio of water content to dry weight of roots and shoots of plants is associated with a decrease in external osmotic potential (Table V). The apparent fall largely reflects accumulation of inorganic ions in tissues of low water potential. Plants of *Limonium* grown at low salinities or low concentrations of PEG show a slight increase in succulence, but over-all succulence does not increase in the four species under conditions of low osmotic potential.

Changes in shoot water potentials of different species in relation to a fall in external osmotic potential may be classified into two groups (Table VI). *P. maritima* and *T. maritima* maintain only a small water potential gradient between leaves, roots, and the external solution, particularly at high salinities, whereas in *Halimione* and *Limonium* large differences in water potential exist between shoots and external solutions.

In *P. maritima* increases in sorbitol concentrations in both shoots and roots of plants grown under saline conditions or in the presence of PEG are directly related to the fall in water potential of the tissues (Table VIII). Aside from studies of Stewart *et al.* (22) and Jefferies *et al.* (12), there appears to be no additional evidence that this polyol is a compatible osmotic solute in higher plants grown under saline conditions. Hill and Ahmadjan (9) and Brown and Hellebust (4) have shown that sorbitol is an intracellular osmotic solute in the green alga, *Stichococcus bacillaris*. The concentration of sorbitol at saturation (4.6 M) in an aqueous solution is higher than that of mannitol and it forms a solution of pH 7 and requires no counter ion. Enzymic inhibition in the presence of sorbitol appears to be low (19). If it is assumed that the cytoplasm occupies 10% of the cell volume and that all of the sorbitol is located in the cytoplasmic compartment, a concentration of the polyol of 1.2 M (0.12 M was the highest concentration recorded) might be expected to generate an osmolality of at least 1.3 osmol/kg (−3.17 MPa). In addition to the accumulation of

Table VIII. *Concentrations (mM) of Soluble Nitrogen, α-Amino Nitrogen, Reducing Sugars and Sorbitol Based on Tissue Water Content in the Roots and Shoots of Plantago maritima Grown in Sand Culture*

Culture solutions contained either different dilutions of seawater or different concentrations of PEG at similar osmotic pressures (Π_{sol}:MPa).

		Saline Treatments				PEG Treatments			
	Π_{sol}	Sol. N	α-NH_2-N	Sorbitol	Reducing Sugars	Sol. N	α-NH_2-N	Sorbitol	Reducing Sugars
Shoot	0.05	9	9	16	20				
Root	(Control)	10	11	19	20				
Shoot	0.2	10	13	17	12	10	13	14	7
Root		46	21	25	14	39	37	26	29
Shoot	0.5	6	7	23	10	15	9	32	15
Root		18	21	21	28	37	45	40	31
Shoot	1.2	7	7	27	7	23	19	47	23
Root		104	96	40	19	212	196	72	46
Shoot	2.4	8	9	56	10	39	42	83	21
Root		83	80	84	51	191	185	118	33

Table IX. *Concentrations (mM) of Soluble Nitrogen, α-Amino Nitrogen, Proline and Reducing Sugars Based on Tissue Water Content in the Roots and Shoots of Triglochin maritima Grown in Sand Culture*

Culture solutions contained either different dilutions of seawater or different concentrations of PEG at similar osmotic pressures (Π_{sol}:MPa).

		Saline Treatments				PEG Treatments			
	Π_{sol}	Sol. N	α-NH_2-N	Proline	Reducing Sugars	Sol. N	α-NH_2-N	Proline	Reducing Sugars
Shoot	0.05	22	14	8	29				
Root	(Control)	8	4	6	19				
Shoot	0.2	27	20	8	16	28	26	7	17
Root		24	21	2	23	42	33	1	18
Shoot	0.5	42	38	26	30	58	39	27	47
Root		20	15	3	16	64	73	8	31
Shoot	1.5	50	40	15	19	82	47	32	85
Root		30	26	8	53	83	82	14	38
Shoot	2.4	77	46	40	42	108	78	38	220
Root		79	66	27	76	168	130	45	41

Table X. *Concentrations (mM) of Soluble Nitrogen, α-Amino Nitrogen, Proline, QAC, and Reducing Sugars Based on Tissue Water Content in Roots and Shoots of Limonium vulgare Grown in Sand Culture*

Culture solutions contained either different dilutions of seawater or different concentrations of PEG at similar osmotic pressures (Π_{sol}:MPa).

		Saline Treatments					PEG Treatments				
	Π_{sol}	Sol. N	α-NH_2-N	Proline	QAC	Reducing Sugars	Sol. N	α-NH_2-N	Proline	QAC	Reducing Sugars
Shoot	0.05	38	28	2	3	33					
Root	(Control)	90	62	3	3	22					
Shoot	0.2	71	55	4	5	38	37	35	2	4	38
Root		127	82	1	9	183	60	57	3	2	129
Shoot	0.5	63	54	12	10	34	57	36	5	7	47
Root		120	87	5	6	117	64	52	3	4	150
Shoot	1.2	84	58	17	16	21	73	46	9	11	79
Root		157	90	17	7	136	65	43	5	5	127
Shoot	2.4	101	65	33	15	50	141	75	65	10	110
Root		168	123	24	5	104	123	84	11	37	37

Table XI. *Concentrations (mM) of Soluble Nitrogen, α-Amino Nitrogen, QAC, and Reducing Sugars Based on Tissue Water Content in Roots and Shoots of Halimione portulacoides Grown in Sand Culture at Different Salinities (Π_{sol}:MPa)*

	Π_{sol}	Sol. N	α-NH_2-N	QAC	Reducing Sugars
Shoot	0.05	27	3	20	4
Root	(Control)	89	63	23	2
Shoot	0.2	86	32	47	16
Root		127	52	58	28
Shoot	1.2	88	31	78	13
Root		288	87	83	114
Shoot	2.4	74	27	58	9
Root		294	136	80	116

sorbitol, large quantities of α-amino nitrogen accumulate in roots, but not in shoots of plants, treated with solutions of PEG or seawater. The accumulation in roots is consistent with a reduced synthetic capacity of plants or increased protein degradation or a combination of these, when plants are grown at low osmotic potentials. It is well known that the growth of many halophytes is reduced at high salinities (7). In plants subject to drought stress, soluble amino acids increase in concentration. These act as a carbon and nitrogen reserve during rehydration (2, 10). In this study the origin of this α-amino nitrogen is uncertain as levels of proteolytic enzymes have not been measured.

A number of laboratory and field studies have demonstrated that *T. maritima* accumulates proline (12, 22–26) under saline conditions. Results of this study show that at comparatively high salinities and high concentrations of PEG the proline content of

tissues is low (Table IX). These findings confirm those reported in the literature which indicate that if plants are subject only to hyposaline conditions proline content is low (23, 24). Under drought conditions the concentration of proline in tissues is also insensitive to mild stress (10). As in *P. maritima* there is an increase in total α-amino nitrogen in plants treated with seawater or solutions of PEG, although in *Triglochin* the increase is evident in both shoots and roots. The accumulation of reducing sugars in shoots of plants of this species treated with PEG, which has been observed by the authors in other experiments, was not evident in plants grown under saline conditions. An assumption underlying the use of PEG-6000 is that the compound is not absorbed and metabolized by plants. It is possible that either the breakdown products of this compound produced by bacterial action or traces of low mol wt impurities in the PEG are absorbed and metabolized by the plants.

The response of *L. vulgare* to low osmotic potentials is of interest because in this species different organic compounds are produced which may act as compatible osmotica. In shoots concentrations of QAC show a modest rise as salinity or concentration of PEG in the external solution is increased (Table X), although the rate of increase is not sustained in plants treated with solutions of low water potential (−2.4 MPa). These results are consistent with those of Storey and Wyn Jones (25), Storey *et al.* (24), and Stewart *et al.* (22), which indicate that these compounds occur at relatively high concentrations, even in the absence of salinity, and that under highly saline conditions the concentration of proline rather than QAC increases in the tissues.

H. portulacoides maintains a high internal salt level even at low salinities, however, amounts of inorganic ions in tissues build up as salinity rises (Table XI). A rise, particularly in roots, in concentrations of reducing sugars, proline, α-amino nitrogen, methylated QAC, and total soluble nitrogen is associated with increased salinity. It is known that in this species QAC may act as compatible osmotica (22, 24). Two uncertainties not resolved by this study or those discussed above, are localization of these compounds within the different cellular compartments and amounts of these different substances which contribute to the lowering of the osmotic potential. For example, all of the inorganic ions will not be in aqueous solution. Some Na is likely to be present in the cell wall and the activity coefficients of the ions in the cytoplasm and in cell organelles may depart considerably from 1.0. In a previous study (11) estimated concentrations of Na and K in the cytoplasm and vacuole of root cells of seedlings of *Triglochin maritima* grown at different salinities did not exceed 0.148 M. Yeo (27) has shown that in leaf cells of *Suaeda maritima* the cytoplasmic concentration of Na is about 0.165 M, when plants are grown in solutions of NaCl. These results taken together indicate that calculations of ion concentrations based on analyses of total ion content of tissues may overestimate the contribution different ions make to the maintenance of the osmotic potential.

In spite of these uncertainties, the results show that in the four species studied the responses of plants treated with seawater or solutions of PEG are similar indicating that most of the effects can be ascribed to low osmotic potentials and not salinity *per se*. These conclusions are similar to those of Rozema (17) who used NaCl and mannitol to study the effects of salt and low osmotic potentials on *Juncus gerardii*, a rush of salt marsh communities. The results also indicate that secondary effects on plants associated with the use of PEG appear minimal, at least over short periods of 14 days or less.

Under natural conditions such as in estuaries, where rapid changes in salinity occur, the rates of production of these organic solutes in halophytes may be inadequate for these compounds to serve as compatible osmotica. High concentrations of organic solutes, such as proline, may be characteristic of plants growing in saline environments in which large changes in salinity are seasonal and not diurnal. Dainty (6) has discussed turgor regulation in plants subject to rapid fluctuations in salinity. There is also evidence (12) that under natural conditions the over-all concentration of these substances in plants is not directly related to the water potential of tissues, because these solutes appear to fulfill a dual role as compatible organic solutes as well as carbon and nitrogen sources for growth and development.

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