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Compartmentation of ions and organic compounds in *Salicornia brachiata* Roxb.

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Abstract

The actively growing stems of *Salicornia brachiata* (60-d old) were dissected into three major tissue layers: vascular, spongy mesophyll and palisade. Each layer was analysed for chlorophyll, protein, amino acids, and sugar contents and the activities of ATPase and phosphatase (alkaline and acid). The differences in organic compounds and enzyme activities in these different tissues have been correlated with the ion content of the corresponding tissues.

Introduction

The halophytes are able to maintain the water potential gradient (osmoregulation) by accumulation of inorganic ions and/or low molecular mass organic compounds in their tissues (*e.g.* Flowers *et al.* 1977, Cavalieri 1983, Wyn Jones 1984, Flowers 1985, Weretilnyk *et al.* 1989). As a result of the accumulation of ions in the shoots these plants are also supposed to develop higher ion fluxes in their tissues than glycophytes (Flowers 1985).

It has ben shown that salinity does not modulate the properties of a number of enzymes (Flowers 1972a, Von Willert 1974) and there is no specific difference between glycophytes and halophytes (Flowers 1972b, Greenway and Osmond 1972). A few enzymes such as ATPase (Kim and Weber 1980 and Iyengar *et al.* 1991), malate dehydrogenase (Yopp 1974) and peroxisomal glycolate oxidase (Austenfeld 1976) are tolerant to salt in *Salicornia* species. Compartmentation of ions and certain metabolites in different tissues of *Salicornia* species have been reported (Weber *et al.* 1977, Stumpe and O' Leary 1985, Reddy *et al.* 1992). The occurrence of enzymes and accumulation of metabolites and ions in different tissues of *S. brachiata* are not clearly studied. In this paper an effort is made in this direction.

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Materials and methods

Salicornia brachiata Roxb. plants (approx. 60 d old) were collected from salt marshes of Bhavnagar (21°75'N; 72°14'E). The developing shoots were separated and frozen in liquid nitrogen. Using a stereomicroscope and dispersable seaples each of the three major tissues from the individual internodes was dissected away from the surrounding tissue layers. The tissue layers of palisade, spongy mesophyll and vascular tissues were analysed separately.

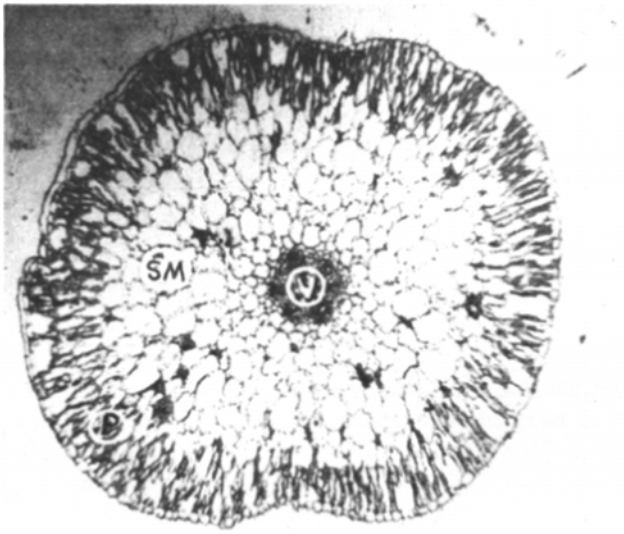

Fig. 1. Scanning electron micrograph of *Salicornia brachiata* stem cross section. P - palisade, SM - spongy mesophyll, V - vascular tissue.

Each type of the dissected tissue was analysed (*i*) for chlorophyll by quickly crushing in 80 % acetone, (*ii*) for enzyme analysis by freezing in liquid nitrogen followed by extraction in tris buffer (pH 7.5) at 0 - 4 °C, and (*iii*) for mineral ions and metabolites by drying at 60 °C for 48 h. Chlorophyll content was determined spectrophotometrically using the method of Arnon (1949). ATPase and phosphatase were assayed using the procedures of Kim and Weber (1980) and Yamaya and Matsumato (1981), respectively. Proline was determined by the procedure used by Bates *et al.* (1973). The oven-dried samples were ashed and then analysed for sodium, potassium, zinc, copper and iron by using atomic absorption spectrophotometer (*Perkin Elmer 703*), calcium and magnesium by the versinate method (Vogel 1978) and chloride by the method of Volhard (1956). Soluble-sugar contents were determined by the method of MacReady *et al.* (1951) and amino acids according to Moore and Stein (1948). Protein contents were estimated following the procedure of Lowry *et al.* (1951). Electrical conductivity of the soil saturation paste was measured according to Richards (1956).

Frozen tissues (liquid nitrogen) were cut at low temperature and mounted on aluminium mounts for scanning electron microscopy (SEM) and for energy dispersive X-ray micro analysis (EDAX) studies. The samples were coated with gold palladium and dried at critical point-drying (using a vacuum drier) and observed in a *S4-10 Cambridge* stereo scan microscope. For EDAX analysis the electron microprobe detectors were standardized using KCl and NaCl. Elemental data were recorded as a line scan and quantitative content as a print out in X-ray with counts per second using an electron microprobe.

Results and discussion

Fig. 1 clearly shows that the palisade, mesophyll and vascular tissue layers are distinct. Sodium concentration is higher (66.52 %) in spongy mesophyll than in palisade (26.89 %) and vascular (16.58 %) tissues (Table 1). Similarly calcium, magnesium and zinc concentrations were higher in spongy mesophyll cells, whereas

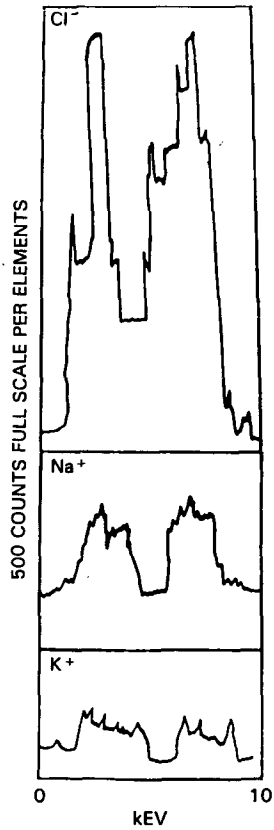

Fig. 2. EDAX analysis for Na, K^+ and Cl^- across the centre of the young shoot of *Salicornia brachiata*.

Table 1. Concentration of ions [mg g^{-1}(dry mass)] in different tissue layers of *Salicornia brachiata* (mean of three independent replications).

Tissue layer	Na^+	K^+	Na^+/K^+	Ca^{2+}	Mg^{2+}	Zn^{2+}	Mn^{2+}	Fe^{2+}	Cu^{2+}	Cl^-
Palisade	12.34	11.39	1.08	15.11	13.70	3.76	0.13	0.023	Trace	43.08
	±2.83	±1.60		±0.81	±0.67	±0.33	±0.03	±0.003		±13.79
Spongy mesophyll	30.54	9.74	3.13	17.82	18.43	4.67	0.057	0.145	Trace	50.81
	±1.00	±2.35		±0.67	±0.20	±1.31	±0.008	±0.012		± 4.67
Vascular	3.02	4.65	0.64	8.52	5.67	0.22	0.027	0.267	Trace	18.08
	±0.19	±1.32		±0.33	±0.58	±0.05	±0.008	±0.04		± 0.84

Table 2. Distribution of chlorophyll, protein, amino acids, soluble sugar contents and ATPas and phosphatase (acidic + alkaline) activities in different tissue layers of *Salicornia brachiata* (mean ± SD of three independent replications).

	Palisade	Spongy mesophyll	Vascular
Chlorophyll [mg g^{-1}(d.m.)]	15.89 ± 0.75	4.92 ± 0.37	1.30 ± 0.05
Protein [mg g^{-1}(d.m.)]	50.52 ± 4.31	36.32 ± 5.32	33.49 ± 3.05
Proline [μg g^{-1}(d.m.)]	46.00 ± 3.25	51.32 ± 1.05	48.43 ± 2.48
Amino acids [mg g^{-1}(d.m.)]	1.26 ± 0.31	1.29 ± 0.43	1.25 ± 0.09
Soluble sugars [mg g^{-1}(d.m.)]	33.74 ± 3.45	41.39 ± 5.43	46.70 ± 1.32
ATPase [mg(phosphorus released) g^{-1}(f.m.)]	3.65 ± 0.49	4.49 ± 0.34	4.25 ± 0.91
ATPase [μg(phosphorus released) mg^{-1}(protein)]	273.66 ± 22.14	523.00 ± 68.11	515.33 ± 77.58
Acid phosphatase [μg(PNP released) g^{-1}(f.m.)]	950.29 ± 53.52	903.29 ± 12.48	1104.99 ± 37.06
Acid phosphatase [μg(PNP released) mg^{-1}(protein)]	72.30 ± 12.65	105.38 ± 5.35	243.05 ± 29.45
Alkaline phosphatase [μg(PNP released) g^{-1}(f.m.)]	401.60 ± 37.90	299.00 ± 3.82	409.60 ± 11.32
Alkaline phosphatase [μg(PNP released) mg^{-1}(protein)]	30.33 ± 4.36	20.88 ± 3.82	27.00 ± 1.28

the manganese concentration was high in palisade and the iron concentration high in vascular tissues. Potassium and chloride were more or less equally distributed over mesophyll and palisade cells. Each of the tissues has a distinctive Na^+/K^+ ratio (Table 1). SEM and EDAX analysis of different tissues (Fig. 2) confirm the results of the chemical analysis of the tissues.

The large and highly vacuolated spongy tissue cells (Fig. 1) are morphologically suited to store salt and water. Gorham and Wyn Jones (1983), Storey *et al.* (1983b) and Weber *et al.* (1977) have also reported higher accumulation of Na^+ in highly vacuolated mesophyll cells. The highest total ion concentration observed in the spongy mesophyll cells is similar to the findings of Khan *et al.* (1986) for *S. pacifica.* On the other hand in *S. biglovii* the amount of Na^+ present in the palisade tissue was not significantly different from that of the spongy mesophyll cells (Stumpf and O'Leary 1985). An even distribution of Cl^- is seen in all the tissue layers and this is in conformity with the results of Eshel and Waisel (1979). The high concentration of Zn^{2+} found in the mesophyll cells may be eseential to maintain the membrane integrity (Welch *et al.* 1982).

Further analysis of each tissue layer confirmed that the majority of chlorophyll is present in palisade tissue (Table 2). The protein content also showed a similar trend to that of chlorophylls, whereas soluble sugars, free proline and total amino acids concentrations were more in spongy mesophyll cells (Table 2).

Lower quantity of protein observed in the spongy mesophyll cells can be due to poor synthesis or increased hydrolysis of protein caused by the high salt concentration. Hall and Flowers (1973) and Stumpf and O'Leary (1985) have reported inhibition of protein synthesis due to salinity. The decline in protein percentage in spongy mesophyll cells can be in agrement with the increased size of the vacuoles and the elongation of the cell walls (Fig. 1). The trend of the accumulation of amino acids with salt concentration is similar to the reports of Joshi and Iyengar (1982), Cavalieri (1983) and Joshi (1986).

The activity of ATPase was higher in spongy mesophyll cells than in palisade and vascular tissues. The phosphatases activities (acid and alkaline) also showed significant differences in different tissues (Table 2).

The increase in ATPase activity observed in the spongy mesophyll can be related to the increased influx of ions into the vacuoles and cytoplasm under saline conditions (*e.g.* Kim and Weber 1980, Iyengar *et al.* 1991).

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