BIOLOGIA PLANTARUM 46 (1): 137-140, 2003

BRIEF COMMUNICATION

Osmotically active compounds and their localization in the marine halophyte eelgrass

C.J. YE and K.F. ZHAO*

Institute of Plant Stress, Shandong Normal University, Jinan 250014, P. R. China

Abstract

Contents of various organic and inorganic osmotica in the leaves, shoots and roots of *Zostera marina* L. were determined and their contributions to the osmotic adjustment to seawater were calculated. The inorganic ions, especially Na^+, were the main osmotica. X-ray microanalysis indicated that vacuoles in the parenchymatous cells are the main sites in leaves accumulating toxic ions.

Additional key words: ion compartmentation, osmotica, X-ray microanalysis, *Zostera marina*.

Of all the angiosperms, only a few species can thrive in seawater submerged completely, which are usually called seagrasses for their grass-like appearance. Eelgrass (*Zostera marina* L.) is a widely distributed seagrass that usually forms large communities in the north hemispheres along the coastline (Den Hartog 1970). In recent years, the plant has attracted great attention of plant physiologists and ecologists for its rapidly degraded communities in the worldwide scope (Kemp *et al.* 1983, Stevenson *et al.* 1993). The plant can thrive in seawater within a salinity range of 0.5 - 3.3 %. There have been several studies on its salt-tolerance mechanisms including its invaginated plasma membrane of mature leaf epidermal cells and P-type H^+-ATPase on the plasma membrane (Arai *et al.* 1991, Pak *et al.* 1995, Fukuhara *et al.* 1996, Jose *et al.* 1999), but its osmotic adjustment, as one of the most important aspects for salt-tolerance, was not studied completely. In the present study, we determined contents of various organic and inorganic osmotica in the plant and calculated their contributions to the plant osmotic potential adjusted to balance the seawater osmotic stress. Ion X-ray microanalysis was carried out to determine the subcellular localization of inorganic ions in order to have a further understanding of its salt-tolerance mechanisms.

Eelgrass was collected off the coast at Qingdao and Changdao (Shandong Province, China) at a 6-m depth. The plants were transferred to laboratory in natural seawater within 6 h. Then they were frozen and stored in liquid nitrogen after being washed completely with tap water until further use for various assays.

Samples of dry leaves and/or roots and/or shoots were burnt to ash in a muffle furnace and a droplet of concentrated HNO_3 was added. The contents of Na^+, K^+, Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrometry (*Hitachi Z-8000*, Tokyo, Japan) (Li 1985), NO_3^- by colorimetry (Jing and Ding 1981) and Cl^- by titration (Jing and Ding 1981). Sugars were measured using anthrone colorimetry (Zhang 1985), free amino acids using ninhydrin method and organic acids using NaOH titration. The sap of the frozen leaves, shoots and roots was obtained with a syringe through squeezing the materials. Osmotic potentials of the sap were measured with an osmometer (*Osmomat 030*, *Gonotec*, Berlin, Germany). The fresh plant material was weighed before and after it was dried at 80 °C for 24 h and after that water content was determined. For each measurement, 4 replicates were carried out.

For subcellular localization of inorganic ions by X-ray microanalysis 5 × 5 mm^2 pieces of fresh material from the leaves, shoots and roots were mounted in the grooves of aluminum specimen holders which were plunged into liquid N_2-cooled isopentane and propane 1:3 (v/v) for rapid freezing and freeze-drying. Modified T-shape

---

Received 25 October 2001, *accepted* 25 February 2002.

Acknowledgements: Authors are grateful to the National Science Committee for financial assistance (G1999011700).

*Corresponding author; fax: (+86) 531 2966954, e-mail: zhaokefu@263.net

valves were used for infiltration with diethyl ether under high pressure at 27 °C for 24 h after freeze-drying. The samples were then cut into small pieces suitable for cutting with an ultramicrotome infiltrated with styrene-methacrylate, transferred into gelatine capsules and polymerized 7 d at 60 °C. Dry, semithin sections (1 μm) were cut on an ultramicrotone. Cross-sections of the roots were transferred to copper grid and coated with carbon. The sections were examined at a transmission electron microscope (*Hitachi-H800*) fitted with an *EDAX-9100* (*Hitachi*) energy-disperse X-ray analyzer at an accelerating voltage of 120 kV and take-off angle of 25'. The counting time for all analyses was 60 live second: the data were expressed as counts per second [s^{-1}] of an element peak after subtraction of the background. For each tissue compartment at least seven measurements were carried out and their average and SE were calculated (Fritz 1989, Qi and Fritz 1990). Each data were the mean ± SE of 7 replicates.

There was little difference in leaf water contents between the two ecotypes (Table 1). The difference in osmotic potentials among the different parts of the plant (leaf, shoot and root) was also insignificant for each ecotype.

The importance sequence of various organic osmotica in leaves of eelgrass from two areas to osmotic adjustment was sugars > free amino acids > organic acids (Fig. 1).

Na^+ was the most abundant ion in the plant (Fig. 2) whose concentration distribution in the plant (leaf, shoot and root) was similar to that of its osmotic potential. Na^+ contents in leaves and roots were higher than in shoots. The similar trend was found for Cl^-, but not for other ions. K^+ concentration was higher in leaf than in stem and lowest in roots. Cl^- content was much lower than $Na^+ + K^+$, although Cl^- was more abundant than K^+.

Table 1. Leaf water content [%], and osmotic potentials [MPa] of leaves, shoots and roots of eelgrass from two areas. Means of 4 replicates.

Ecotype	Water content	Osmotic potential		
		leaf	shoot	root
Qingdao	78	-2.05	-1.98	-2.17
Changdao	80	-2.50	-2.41	-2.62

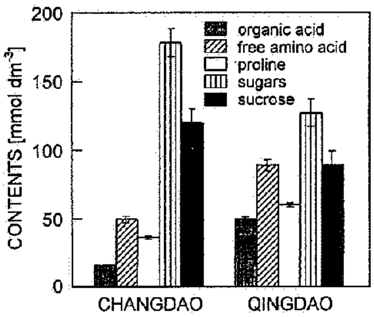

Fig. 1. Organic osmoticum contents in the leaves of eelgrass from two areas.

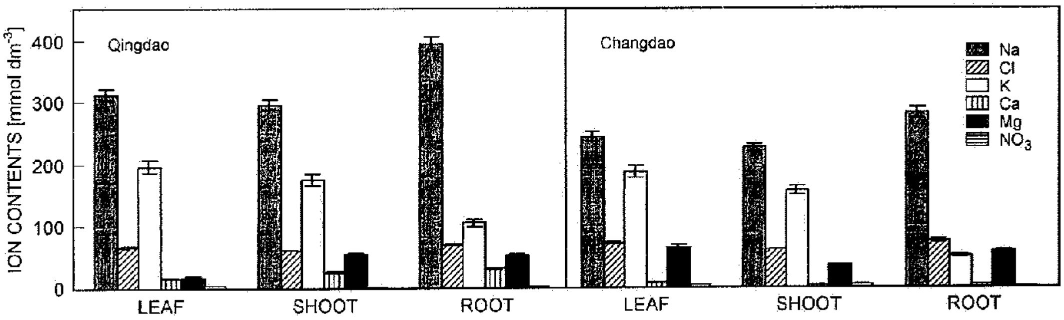

Fig. 2. Ion contents in leaves, shoots and roots of eelgrass from two areas.

Table 2. The contributions of organic and inorganic osmotica [%] to the osmotic potential. Measured osmotic potentials were -2.05 and -2.50 MPa in eelgrass leaves from Quindao and Changdao and corresponding calculated osmotic potentials were -1.85 and -2.36 MPa, respectively.

	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	NO_3^-	Σ inorg.	sugars	amino acids	organic acids	Σ org.
Quindao	32.8	18.2	1.1	8.8	8.9	0.51	70.3	10.8	13.6	4.2	29.7
Changdao	33.2	20.7	1.8	2.0	7.5	0.48	65.7	13.5	9.4	10.6	34.3

Table 3. Ions [s^{-1}] subcellular localization determined by X-ray microanalysis. Means ± SE, $n = 30$.

		Na^+	Cl^-	K^+
Leaf epidermis	cytoplasm	15.26 ± 1.17	15.30 ± 1.40	142.65 ± 8.45
	vacuole	77.24 ± 5.38	65.40 ± 5.92	64.65 ± 5.09
	apoplast	63.66 ± 8.01	92.03 ± 4.32	50.59 ± 2.80
Leaf mesophyll	cytoplasm	14.99 ± 1.50	15.17 ± 1.07	141.91 ± 7.33
	vacuole	70.58 ± 8.93	35.45 ± 1.67	40.29 ± 2.13
	apoplast	56.00 ± 5.86	30.70 ± 1.46	40.21 ± 2.94
Root epidermis	cytoplasm	35.10 ± 6.72	24.54 ± 4.15	138.35 ± 5.67
	vacuole	63.90 ± 10.90	35.07 ± 6.18	34.26 ± 1.47
	apoplast	61.33 ± 5.94	24.52 ± 5.32	31.23 ± 2.13
Shoot epidermis	cytoplasm	35.17 ± 8.25	9.77 ± 2.34	76.23 ± 14.45
	vacuole	62.08 ± 9.79	42.99 ± 7.59	15.88 ± 4.39
	apoplast	59.74 ± 15.77	11.55 ± 1.50	17.61 ± 5.79
Shoot parenchyma	cytoplasm	65.87 ± 5.70	23.90 ± 6.64	82.30 ± 13.53
	vacuole	45.07 ± 5.66	22.93 ± 4.87	26.15 ± 4.03
	apoplast	56.48 ± 3.41	20.75 ± 6.17	4.63 ± 1.11

For leaf epidermal and mesophyll cells, root and shoot epidermal cells, Na^+ and Cl^- concentrations in the vacuole were much higher than in the cytoplasm. But in the shoot parenhyma, Na^+ concentration in the cytoplasm and apoplast was much higher than in vacuole (Table 3).

Eelgrasses from the two areas (Qingdao and Changdao) were similar in their osmotic adjustment. Inorganic osmotica are more important than organic osmotica in osmotic adjustment. For eelgrass collected from Qingdao, osmotic potential caused by inorganic osmotica is 70.3 % of the calculated leaf sap potential and 65.7 % for eelgrass from Changdao (Table 2). Na^+ and K^+ are the most important inorganic ions which account for 32.8 % and 18.2 % of the calculated leaf sap potential for eelgrass from Qingdao and 33.2 % and 20 %, respectively, for eelgrass from Changdao (Table 2). There is a much larger gap between Na^+ and Cl^- contents in the plant than in the seawater for both the two ecotypes, and Cl^- content in the leaves was even much lower than K^+, whose content is very low in seawater (data not shown). So the imbalance can only be explained by the plant's selective absorption of inorganic ions (Na^+, K^+, Cl^-). Therefore, there must be certain substance(s) (organic or inorganic and un-measured in these experiments) with negative charge(s) to keep the cells electronically neutral. Of the organic osmotica, dissolved sugars and free amino acids are the most important organic osmotica, of which proline and sucrose are the most important amino acid and sugar, respectively (Fig. 1). Studies of the plant structure showed that the leaf epidermal cells (photosynthesizing cells) are very small with almost invisible vacuoles, but the mesophyll cells are very large with huge central vacuole; the cells in shoots are almost all parenchyma cells with vacuoles occupying more than 90 % of their volume (data not shown). Combined with the results of X-ray microanalysis, we can conclude that inorganic osmotica (Na^+, K^+) may accumulate mainly in the mesophyll cells but organic osmotica in the cytoplasm of the leaf epidermal cells. Considering the leaf epidermal cells' small volume, organic osmotica, although little in absolute amount, are enough for the cytoplasm osmotic adjustment to the seawater osmotic potential.

Considering the large volume of the vacuoles in the shoot parenchyma cells and small volume of cytoplasm of these cells, the phenomenon that Na^+ and Cl^- concentrations in the vacuoles are lower than those in the cytoplasm in shoot parenchyma tissues is not surprising (Table 3). At the same time the lowest Na^+ and Cl^- concentrations ware recorded in the cytoplasm of leaf epidermal cells (Table 3).

From the above, we can see that the common feature of eelgrass and the typical terrestrial halophytes is that they both synthesize limited amount of organic osmotica, which are compatible with the metabolic enzymes but cost more energy than to accumulate inorganic ions in the cytoplasm of the cells and accumulate a large amount of inorganic ions in the vacuoles to reach a lower osmotic potential. The difference lies in the compartmentation of inorganic ions (mainly Na^+, Cl^-) which is in the terrestrial halophytes within the cells (inorganic ions in vacuoles and organic osmotica in cytoplasm), but for eelgrass, also the transfer of inorganic ions from leaf epidermal cells to mesophyll cells, and further to the shoot parenchyma cells is included.

References

Arai, M., Pak, J.Y., Normura, K., Nitta, T.: Seawater-resistant, non-spherical protoplasts from seagrass leaves. - Physiol. Plant. **83**: 551-559, 1991.

Den Hartog, C.: The Sea-Grasses of the World. - North-Holland Publishing Co., Amsterdam 1970.

Fritz, E.: X-ray microanalysis of diffusible elements in plant cells after freeze-drying: pressure infiltration with ether and embedding in plastic. - Scanning Microscopy **3**: 517-526, 1989.

Fukuhara, T., Pak, J.Y., Ohwaki, Y., Tsujimura, H., Nitta, T.: Tissue-specific expression of gene for a putative plasma membrane H^+-ATPase in a seagrass. - Plant Physiol. **110**: 35-42, 1996.

Jing, J., Ding, Z.: [Analytical Methods of Plant Biochemistry.] - Science Press, Beijing 1981. [In Chinese.]

Jose, A.F., Maria, J.G., Huber, H.F.: Physiological evidence for a proton pump and sodium exclusion mechanisms at the plasma membrane of the marine angiosperm *Zostera marina* L. - J. exp. Bot. **50**: 1763-1768, 1999.

Kemp, W.M., Boynton, W.R., Twilley, R.R., Stevenson, J.C., Means, J.C.: The decline of submerged vascular plants in upper Chesapeake Bay: summary of results concerning possible causes. - Mar. Technol. Soc. J. **17**: 78-89, 1983.

Li, Q.: [Flame spectrometry and atomic absorption spectrometry.] - In: Handbook of Plant Physiological Experiments. Pp. 502-507. Shanghai Science and Technology Press, Shanghai 1985. [In Chinese.]

Pak, J.Y., Fukuhara, T., Nitta, T.: Discrete subcellular localization of a membrane-bound ATPase activity in marine angiosperms and marine algae. - Planta **196**: 15-22, 1995.

Qi, L., Fritz, E.: X-ray microanalysis of ion contents in stem tip meristem and leaves of *Populus* grown under potassium and phosphurs deficiency. - J. Plant Physiol. **136**: 61-65, 1990.

Stevenson, J.C., Staver, L.W., Staver, K.W.: Water quality associated with survival of submersed aquatic vegetation along an estuarine gradient. - Estuaries **16**: 346-361, 1993.

Zhang, Z.: Measurement of sugars in plant materials. - In: Handbook of Plant Physiological Experiments. Pp. 134-138. Shanghai Science and Technology Press, Shanghai 1985. [In Chinese.]