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Studies on the Mechanism of Salt Tolerance in *Salicornia europaea* L.

II. High osmosis of epidermal cells in stem*

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Abstract : To understand how cells sense and respond to salt stress, we investigate changes in plasmolitic concentration to NaCl of epidermal cells in *Salicornia europaea* during the growth period. The inorganic ion concentration in tissues and plasmolitic responses to NaCl of epidermal cells in wild *Salicornia* were compared with other halophytes. Cell responses to high salt concentration were compared with plants grown in MS medium as a low salinity.

The incipient plasmolytic NaCl concentration in epidermal cells in *Salicornia* stems rose from 1.6% to 2.2% during the growth period. Main inorganic ions in *Salicornia* at the end of growth stage were 63 mM Na^+ and 107 mM Cl^- per 100 g fresh weight. When plants were grown in MS medium, the size of epidermal cells was about 2 fold large, and osmotic pressure in plants was 35–50% low compared with wild plant cells. Incipient plasmolytic NaCl concentration in epidermal cells of plants grown in wild and MS medium was almost same. However, most of the epidermal cells grown in MS medium showed severe plasmolysis under hypertonic NaCl solutions. The results suggest that the cells of *Salicornia* can accumulate NaCl during the growth period and accumulated NaCl may make adjustment of osmotic pressure in the cells.

By contrast with other halophytes, *Salicornia* showed high Na^+ and Cl^- concentrations in tissues and high plasmolitic concentration to NaCl in epidermal cells.

Key words : Epidermal cell, Halophyte, Inorganic ions, Plasmolysis, *Salicornia*.

***Salicornia europaea* L. の耐塩機構に関する研究** II. 茎の表皮細胞における高浸透性：桃木芳枝・加藤茂**・上村英雄（東京農業大学生物産業学部・**東京農業大学総合研究所）

要 旨：植物細胞がどのように塩ストレスに反応するかを理解するため，自生アッケシソウ（*Salicornia europaea* L.）の生育期間における表皮細胞の塩化ナトリウム溶液に対する原型質分離濃度の推移を検討した．また，アッケシソウの無機イオン濃度と表皮細胞の塩化ナトリウム溶液に対する反応を，他の塩生植物と比較した．さらに，高濃度の塩化ナトリウム溶液に対する表皮細胞の反応をMS培地（低塩濃度）で生育したアッケシソウと比較検討した．自生アッケシソウの茎における表皮細胞の塩化ナトリウム溶液に対する限界原形質分離濃度は，生育期間中に1.6%から2.2%に上昇した．生育終期のアッケシソウ体内における主な無機イオンは，63 mM Na^+ イオンと107 mM Cl^- イオンであった．MS培地で生育した植物の表皮細胞は，自生アッケシソウの表皮細胞の約2倍の大きさとなり，植物体の浸透圧は35–50%低かった．また，MS培地で生育したアッケシソウの表皮細胞における塩化ナトリウム溶液に対する限界原形質分離濃度は，自生のものとほぼ同じであった．しかし，高濃度の塩化ナトリウム溶液に浸漬した場合，同処理の自生アッケシソウの表皮細胞に比べ，過度の原形質分離を起こした．これらの結果から，自生アッケシソウの表皮細胞は，生育過程においてNaClを蓄積し，蓄積したNaClを細胞内の浸透調節に役立てていることが示唆された．

なお，アッケシソウの植物体内のNaCl濃度および表皮細胞の塩化ナトリウム溶液に対耐する原型質分離濃度は，他の塩生植物よりも高いことが認められた．

キーワード：アッケシソウ，塩生植物，原形質分離，表皮細胞，無機イオン．

The detrimental effects of salinity are due to the influence of ions on the water activity of the external solution and/or to the direct effects of the ions on the physiological and biochemical functions of the cell[6,7,9,11,19]. In a previous paper[18], both the increasing osmotic pressure and pH in *Salicornia* plants during their growth period were remarkable. And then, glycinebetaine was found in tissues of aerial and root portions in *Salicornia* plants. The osmotic control also may be regulated by accumulation of ions in the cells.

The present paper investigated changes in

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Table 1 Salinity, EC, pH and osmotic pressure in sand and seawater of Lake Notoro-ko and MS medium.

Sample	Salinity	EC	pH	Osmotic pressure
(Sample : H_2O)	(1 : 5)	(1 : 5)	(1 : 2.5)	(1 : 5)
	‰	mS • cm^{-1}		mOsm • Kg^{-1}
Sand	1.3±0.1	1.64±0.02	7.2±0.0	33±5
Seawater	5.0±0.4	8.86±0.11	8.1±0.3	139±14
MS medium	0.1±0.0	1.16±0.03	5.1±0.0	37±2
Sample	Salinity	EC	pH	Osmotic pressure
(sample : H_2O)	(1 : 0)	(1 : 0)	(1 : 0)	(1 : 0)
	‰	mS • cm^{-1}		mOsm • Kg^{-1}
Seawater	29.1±2.7	37.00±3.44	8.4±0.2	931±8
MS medium*	0.6±0.0	5.03±0.34	5.6±0.1	199±2

Values are means of salinity, EC, Ph and Osmotic pressure in three determinations with standard errors.

* MS medium without agar.

Table 2 Changes in isotonic and incipient plasmolytic NaCl concentrations in epidermal cells of wild *Salicornia* plants during the growth period.

Month after germination (date of sampling)	Region of stem	Isotonic NaCl conc.		Incipient plasmolytic NaCl conc.
		Lower limit conc.	Upper limit conc.	
		%		%
2 (5/24)	Upper	1.0±0.1	1.5±0.15	1.6±0.15
3 (6/28)	Upper	1.1±0.2	1.7±0.1	1.8±0.2
	Middle	1.1±0.1	1.7±0.2	1.8±0.1
	Lower	1.1±0.1	1.7±0.1	1.8±0.15
4 (7/31)	Upper	1.3±0.2	1.8±0.25	1.9±0.2
	Middle	1.3±0.1	1.8±0.1	1.9±0.1
	Lower	1.3±0.1	1.8±0.2	1.9±0.1
5 (8/28)	Upper	1.4±0.1	1.9±0.1	2.0±0.1
	Middle	1.5±0.15	2.0±0.2	2.1±0.1
	Lower	1.5±0.2	2.0±0.1	2.1±0.2
6 (9/24)	Upper	1.5±0.1	2.0±0.1	2.1±0.15
	Middle	1.6±0.1	2.1±0.15	2.2±0.1
	Lower	(−)	(−)	(−)

Values are means of 10 plants with standard errors.

(−) Stems were lignified.

isotonic and incipient plasmolytic NaCl concentrations in epidermal cells of wild *Salicornia* plants from the early stage of development to the end of growth stage. Also, isotonic and incipient plasmolytic NaCl concentrations in epidermal cells and inorganic ion concentrations in tissues of *Salicornia* were compared to other halophytes at the their end of growth phase. Further, cell responses to high salt concentration were compared between plants cultivated in MS medium[20] as condition of a low salinity.

Materials and Methods

Salicornia europaea L., and other species *Glaux maritima* L., *Atriplex gmelini* C. A. Mey, *Merten-*

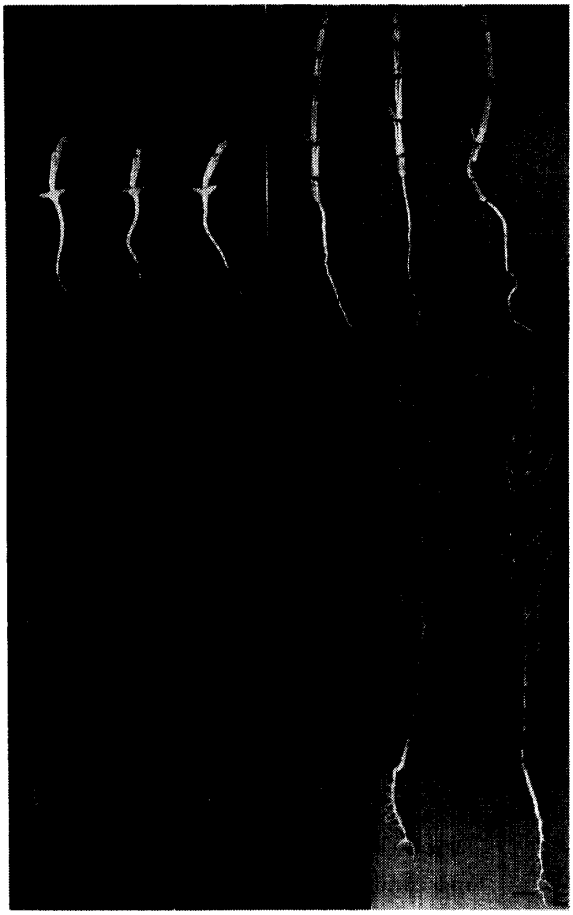

Fig. 1. Plant shapes in *Salicornia europaea* L. during the growth period. Bars are 1 cm.
A; the early stage of development, 2 months after germination (end of May),
B; the middle of June,
C; the middle of July,
D; the middle of August,
E; the end of growth stage (end of September).

sia asiatica Macbr. which were growing around *Salicornia* colonies, were collected in the Misaki area around Lake Notoro-ko[18] in the eastern region of Hokkaido, Japan. *Salicornia* plants were collected every month from the end of May, 1993, i.e. at the early stage of plants development, to the end of September, 1993, i. e. at the end of the growth stage. The other species were collected at the end of September. Murashige-Skoog (MS)[20] medium was used to provide a condition of a low salinity. Plants were germinated in MS medium and grown in the same medium. The medium was refreshed every 2 weeks. Composition of seawater and MS medium is shown in Table 1.

For determination of NaCl tolerance, the epidermal cells in main stems at upper, middle and lower regions in *Saliconia* plants and leaves at middle region of the other plants were used for all experiments. Epidermal cells were then incubated in NaCl solutions for 15 h and isotonic and plasmolytic conditions in cells were examined by a microscope. Ten plants were used for each experiment. Size of epidermal cells were measured by a micrometer.

Osmotic pressure and pH in seawater or MS medium were determined by a micro-osmometer[22] (Advance Model 3MO) and pH-meter (Horiba, M-8L), respectively. Osmotic pressure was measured using the same procedure as described in a previous paper[18]. For measurement of electrical conductivity, sands or MS medium was mixed with distilled water and shaken for 1 h. The conductivity of the supernatant was measured by a conductive meter (TOA, CM-20S). The chloride concentration (chlorinity) of seawater of Lake Notoro-ko or MS medium was measured by titration with $AgNO_3$[14].

For inorganic ion analysis, plants of *Salicornia* and *Glaux* were washed carefully with deionized water. Each sample was cut and homogenized for 5 min in a blender with deionized water. The homogenate was filtered through 0.45 μm filter (Toyo Roshi Kaisha Ltd., DISMIC-25) and was analyzed for inorganic ions content. Inorganic ions (cations and anions) of the homogenate were analyzed by ion chromatography (Shimazu, IC-6A). The eluent for monovalent cations (Na^+, NH^{4+} and K^+) analysis was 5 mM HNO_3 solution and IC-C1 was used as column. The eluent for divalent cations (Mg^{2+} and Ca^{2+}) was 40 mM tartaric acid and 20 mM ethylenediamine solution, and IC-C1 was used as column. The eluent for anions (F^-, Cl^-, NO_2^-, PO_4^{3-}, Br^-, NO_3^- and SO_4^{2-}) was 1 mM p-hydroxybenzoic acid and 1.1 mM N, N-diethylethanol amine solution, and IC-A1 was used as column. Twenty grams of fresh plants were used for each experiment.

Results

Changes in isotonic and incipient plasmolytic concentrations in *Salicornia* plants during the growth period are shown in Table 2. Growth conditions in wild *Salicornia* plants from 2 months after germination (end of May) to the end of growth stage (end of september) are shown in Fig. 1. The range of

Table 3. Isotonic and incipient plasmolytic NaCl concentrations, and size of epidermal cells in wild *Salicornia* and other plants growing around Lake Notoro-ko at the end of growth stage.

Plant	Isotonic NaCl conc.		Incipient plasmolytic NaCl conc.	Size of epidermal cell
	Lower limit conc.	Upper limit conc.		
	%		%	μm
Salicornib europaea	1.6±0.2	2.1±0.15	2.2±0.1	44.1±0.8×26.5±1.3
Glaux maritima	1.4±0.15	1.8±0.1	1.9±0.15	44.3±1.0×26.6±1.2
Atriplex gmelini	1.0±0.1	1.5±0.2	1.6±0.2	50.5±0.5×31.9±1.4
Mertensia asiatica	0.5±0.05	0.9±0.1	1.0±0.1	42.6±0.9×28.8±1.3

Values are means of 10 plants with standard errors.

a) Measurements are made on 10 plants in the middle region of the stems for *Salicornia* and leaves of other plants.

b) Size of 10 epidermal cells are measured for each of 10 plants.

Table 4. Isotonic and incipient plasmolytic NaCl concentrations, and size of epidermal cells in *Salicornia* plants under different growth conditions.

Growth condition	Growth period (Month)	Osmotic pressure	Isotonic NaCl conc.		Incipient plasmolytic NaCl conc.	Size of epidermal cell
			Lower limit conc.	Upper limit conc.		
		$mOsm \cdot Kg^{-1}$	%		%	μm
Wild	1	658±15	1.0±0.1	1.5±0.1	1.6±0.1	54.7±2.2×40.4±1.4
	2	741±10	1.1±0.2	1.7±0.1	1.8±0.15	50.1±1.8×38.7±1.7
MS	1	312±9	1.0±0.2	1.7±0.1	1.8±0.15	94.1±4.2×53.2±2.6
medium	2	479±11	1.1±0.1	1.9±0.2	2.0±0.1	101.1±4.2×51.8±2.3

Values are means of 10 plants with standard errors.

Size of 10 epidermal cells are measured for each of 10 plants.

isotonic NaCl concentration in epidermal cells of stems in *Salicornia* plants changed from 1.0 -1.5% at the early stage of development to 1. 6-2.1% at the end of growth stage. Incipient plasmolytic NaCl concentration also increased about 0.1% per month and ranged from 1.6% at the early stage of development to 2.2% at the end of their growth. Isotonic and plasmolytic NaCl concentrations of epidermal cells in other plants are shown in Table 3. Incipient plasmolytic NaCl concentrations were 2.2% in *Salicornia*, 1.9% in *Glaux*, 1.6% in *Atriplex* and 1.0% in *Mertensia* plants. The range of isotonic NaCl concentration of *Salicornia* plants was also higher by 0.2-1.1% at the lower limit and by 0.3-1.2% at the upper limit, respectively. Isotonic and plasmolytic NaCl concentrations and plasmolytic conditions in hypertonic NaCl of plants grown in wild and MS medium are shown in Table 4 and Fig. 2, respectively. When plants were grown in MS medium, isotonic and incipient plasmolytic NaCl concentrations were almost same with wild plant cells (Table 4). The size of the cells was about twice as large as those of cells from wild plant cells and osmotic pressure in plants was 35-50% lower than wild ones. However, epidermal cells of plants grown in MS medium showed severe plasmolysis if epidermal tissues were incubated in 5% and 10% NaCl solutions (Fig. 2). On the other hand, some cells of wild *Salicornia* plants were still maintained turgor even though epidermal tissues were exposed to hypertonic NaCl solutions (Fig. 2).

The results of inorganic ion analysis of *Salicornia* plants and *Glaux* plants grown

Fig. 2. Plasmolysis of epidermal cells in *Salicornia* plants grown in wild and MS medium conditions.
Left : epidermal cells in wild *Salicornia* plants (1 month after germination). (×400)
Right : epidermal cells in *Salicornia* plants cultivated in MS medium (1 month after germination). (×200)
A ; epidermal cells in isotonic 1.5% NaCl solution,
B ; incipient plasmolysis in 1.6% NaCl,
C ; plasmolysis in 5% NaCl,
D ; plasmolysis in 10% NaCl,
E ; epidermal cells in isotonic 1.7% NaCl solution,
F ; incipient plasmolysis in 1.8% NaCl,
G ; plasmolysis in 5% NaCl,
H ; plasmolysis in 10% NaCl.

around the Lake Notoro-ko is shown in Table 5. The main inorganic ions contained in both plant tissues were sodium by 25-26% and chloride by 63-67%. The NaCl concentrations is *Salicornia* plants was higher than that of *Glaux* plants.

Discussion

When plants are exposed to salinity, osmotic adjustment is a fundamental adaptive response of plant cells for survival and growth under saline conditions[7,9,24]. Levitt[17] suggests that salt tolerance in succulent stem of halophytes appeared to be due to the ability of exclusion system of salt ions from organelles, such as vacuoles. Thus, plasmolysis of the epidermal cells in succulent stem of *Salicornia* plants would indicate salt tolerance.

Isotonic NaCl concentrations in epidermal cells of *Salicornia* plants rose about 0.6% during their growth period. Incipient plasmolytic NaCl concentration also rose 0.1% per month and ranged from 1.6 to 2.2% during the growth period. Concentrations of Na^+ and Cl^-

Table 5 Inorganic ion components in *Salicornia europaea* L. and *Glaux maritima* L. et the end of growth stage.

Ion	Plant	
	Salicornia europaea	*Glaux maritima*
	mM $100g^{-1}$ fresh weight	
Na	63.2±2.1	52.2±1.7
NH_4	0.2±0.0	0.3±0.1
K	3.5±0.7	4.8±1.7
Mg	4.4±0.7	0.4±0.0
Ca	0.4±0.1	0.4±0.0
Cl	106.5±0.8	84.1±0.6
NO_3	trace	trace
PO_4	0.3±0.0	trace
SO_4	1.1±0.4	4.1±2.8

Values are means of two experiments with standard errors. Twenty grams of fresh plants were used for each experiment.

in tissues of *Salicornia* plants at the end of growth stage were about 63 mM and 107 mM per 100 g fresh weight, respectively. The results suggest accumulation of NaCl in *Salicornia* plant cells during the growth period. In contrast with other halophytes grown around *Salicornia* colonies, incipient plasmolytic NaCl concentration of epidermal cells and the Na^+ and Cl^- concentrations of tissues in wild *Salicornia* were high. Further, the Cl^- concentration in *Salicornia* plants was 1.7 fold higher than that of the Na^+ concentration. An exceeding concentration of Cl^- in halophytes was also found in mangrove plants[15]. It suggested that physiological function of Cl^- in mangrove plants can be indicates oxygen evolving system in photosynthesis[5,13,15]. On the other hand, Bernstein[2] reported that Cl^- increases the elongation of the palisade cells, causing increased succulence. The physiological function of Cl^- in *Salicornia* plants is necessary to further investigations.

Osmotic adjustment of salt-adapted cells was due to in large part to the accumulation of Na^+ and Cl^-[4]. A number of papers reported that Na^+ and Cl^- accumulated intracellularly in the vacuole of salt-adapted cells, and organic solutes, such as sugars, free amino acid, betaine and proline accumulated to significant levels in the cytoplasm during adaptation to salt stress or water deficits[1,7,8,10,12,16,17,21,23,24]. As cells become tolerant to NaCl, numerous physological and biochemical mechanisms function as significant adaptive processes in salinity tolerance[4,11]. Further, Singh et al.[21] concluded that numerous gene products increased as cells adapted to NaCl.

When *Salicornia* plants were grown in MS medium as a low salinity, the size of epidermal cells was about twice as large as the wild plant cells and osmotic pressure was 35-50% lower than wild ones. However, most of the cells of plants grown in MS medium showed severe plasmolysis under NaCl solutions compared with wild plant cells. Binzel et al.[4] demonstrated that the contribution of Na^+ and Cl^- to cell dry weight in tobacco plants did not increase proportionately to the level of NaCl to which the cells were adapted. Further, the water content of cells declines during adaptation to salinity, resulting in as much as 5-fold reduction in cell volume[3,11]. They suggest that smaller cells would inherently have an increased capacity to accumulate ions, perhaps mediated by enhanced transport capabilities as a function of the plasma membrane and tonoplast surface area to volume ratios. Then, the accumulation of high intracellular concentrations of Na^+ and Cl^- may be facilitated by the limited expansion of salt adapted cells.

It is conceivable that the small cells of wild *Samicornia* grown under a salinity would have ability to accumulate high concentration of NaCl by the limited expansion of salt adapted cells. And then, these results indicates that accumulated NaCl also may make osmotic adjustment.

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