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Growth and solute pattern of *Suaeda maritima* and *Suaeda asparagoides* in an abandoned salt field

Sung-Chul Choi[1], Sung-Hwan Lim[1], Sang-Hun Kim[1], Deok-Gyun Choi[1], Jong-Guk Kim[2] and Yeon-Sik Choo[1,*]

[1]Department of Biology, Kyungpook National University, Daegu 702-701, Korea
[2]Department of Life Science and Biotechnology, Kyungpook National University, Daegu 702-701, Korea

Abstract

To investigate the environmental adaptation and ecophysiological characteristics of *Suaeda maritima* and *S. asparagoides* under saline conditions, plant growth and density were analyzed according to environmental changes of habitats. The total ion content of soil decreased with time, which was caused by the predominance of exchangeable Na^+ and Cl^- in the upper layers. The population of *S. maritima* was more densely distributed in the region with higher ion contents of Cl^-, Mg^{2+}, K^+ and Na^+ than the population of *S. asparagoides.* Both species were showed a decreased population density according to increases in plant growth. Under the conditions of a salt field, *S. maritima* and *S. asparagoides* contained high inorganic ions to maintain low water potential, but low water soluble carbohydrate contents. In the case of free amino acid, *S. maritima* showed an especially high proline content, and contained rather large amounts of free amino acids, whereas *S. asparagoides* did not. Both species showed high inorganic ion contents in the leaves, which might be a mechanism of avoiding the ionic toxicity by diluting the accumulated ionic concentration with a high ratio of water content to dry weight. This result suggests that *S. maritima* seems to adapt to saline conditions by accumulating proline in addition to inorganic ions. *S. asparagoides* seems to adapt by osmoregulation processes, using inorganic ions rather than free amino acids.

Key words: inorganic ions, osmotic solutes, *Suaeda asparagoides*, *Suaeda maritima*

INTRODUCTION

The soil of regions with high salt concentrations support distinctive vegetation, and lower numbers of species are found in salt fields than in regions with either low or no salt concentration. Salinity is a major environmental stress and is a substantial constraint to crop production. Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within next 25 years and up to 50% by the middle of the 21st century (Wang et al. 2003). Therefore, new approaches are necessary to cope with these problems. One option is the use of more halophytic crop species, which can tolerate high levels of soil salinity.

Plants growing well under saline conditions necessarily have a greater salt tolerance, and this characteristic has influenced the ecological distribution of various plants (Flowers and Colmer 2008). By accumulating inorganic ions, halophytes absorb water by maintaining a high osmotic potential (Bradley and Morris 1991, Volkmar et al. 1998, Li et al. 2010). Betaine and proline are compatible solutes that accumulate in response to osmotic stress, and the accumulation of these osmolytes represents an important adaptive response to salt and drought

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*Corresponding Author
E-mail: yschoo@knu.ac.kr
Tel: +82-53-950-5346

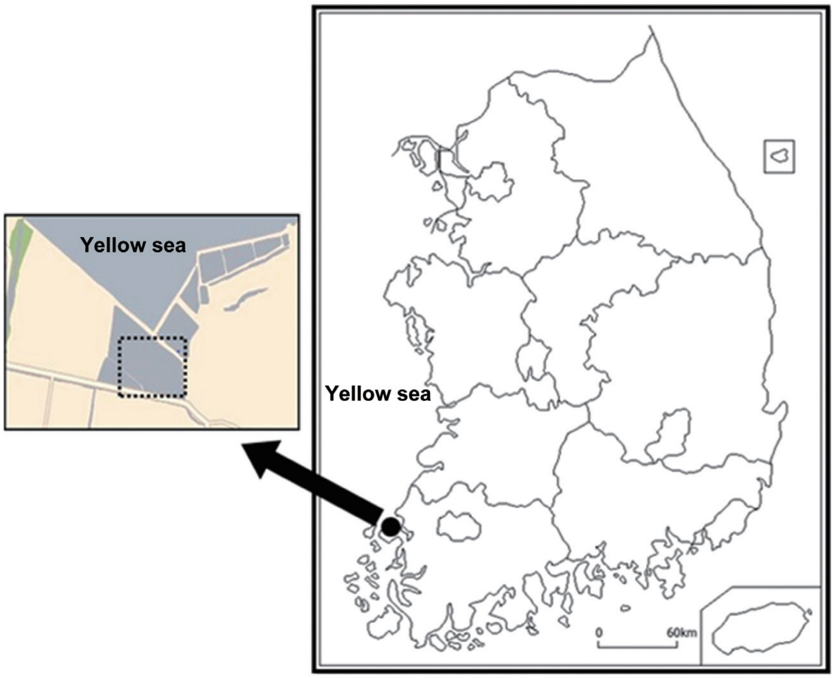

Fig. 1. A map showing the study site: Naeyang-ri, jido-eup, Sinaan-gun, Jeollanam-do, Korea (N 35°05' 52.4", E 126°12' 42.5" ; Tokyo datum).

stress (Rhodes and Hanson 1993, Di Martino et al. 2003, Moghaieb et al. 2004). Halophytes are being used not only as food crops and fodder, but also as ground cover to protect against soil erosion. Through the root development, soil porosity increases, and the physical condition of the soil is subsequently improved over time (Park et al. 1983, Rozema and Flowers 2008).

Chenopodiaceae are comprised of ~1,500 species in ~100 genera and are distributed worldwide. They are known to be a representative plant community with unique ecological habitats, such as deserts and salt marshes (Heywood 1993, Akhani et al. 1997). In Korea, 15 species (7 genera) have been reported, and are mostly distributed in coastal sand dunes and salt marshes (Lee 1988, Yang 1999).

From previous studies of genus *Suaeda*, their ecological distribution, germination, osmotic adjustment and morphological characteristics have been examined (Yokoishi and Tanimoto 1994, lhm et al. 2004, Lee et al. 2007, Yang et al. 2008). However, their adaptive mechanisms under dry and salty conditions have not yet been clearly clarified.

It is therefore essential to study plants with resistances to environmental stresses in order to create crops that can withstand stress conditions such as drought and increased salinity. Some plant species may possess unique physiological characteristics which critically influence environmental adaptation. It is therefore very necessary to identify these characteristics in order to investigate the environmental adaption of particular species (Choo and Albert 1997, Chimenti et al. 2002).

In this study, the changes of seasonal growth and plant density of *S. maritima* and *S. asparagoides* were analyzed, and ecophysiological characteristics of inorganic ions, soluble carbohydrates and osmolality were investigated in the examined species.

MATERIALS AND METHODS

Study site

The study site was located in Naeyang-ri, jido-eup, Sinaan-gun, Jeollanam-do, Korea (Fig. 1). The annual mean temperature and precipitation in the study area were 13.8°C and 1,125 mm, respectively. This study site is an abandoned farm where a community of *Suaeda maritima* Dum. predominate, whereas *Suaeda asparagoides* (Miq) Makino. is organized in patches, and small numbers of halophytes such as *Limonium tetragonum* appear. The area experiences no inflow of saltwater tides and is therefore supplied solely by rainfall.

Plant materials

S. maritima and *S. asparagoides* were sampled from July to September in 2011. After quadrats were placed at each site, the change in chemical characteristics of rhizospheric soil, plant density, height and osmotic solutes in leaves were examined during the growth period.

Soil sampling and measurement of chemical characteristics

Soil samples 15-20 cm below the surface of the plant were also collected to analyze the soil that the plants inhabit.

The collected soil samples were air dried. Soil samples (5 g) were added to distilled water (25 mL) and were shaken for 1 h. After the soil solution was filtered through filter paper (Whatman No. 40, 110 mm), soil pH, total ionic contents (calculated as NaCl equivalents) and chloride contents were measured using a pH meter (Orion US/710; Thermo Orion, Beverly, MA, USA), electronic conductivity meter (Mettler Check Mate 90; Mettler-Toledo Inc., Columbus, OH, USA) and chloride titrator (Titrators DL 50; Mettler Toledo Inc.).

Exchangeable cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) of soil solution extracted by 1 N ammonium acetate (CH_3COONH_4), were quantified using an inductively coupled plasma atomic emission spectroscopy (ICP-AES, Optima 7300 DV; Perkin Elmer, Waltham, MA, USA).

Plant sampling and plant water content

Leaves of *S. maritima* and *S. asparagoides* were collected from their natural habitats. After direct determination of fresh weight in habitats, leaf samples were dried in an oven (at 70°C for 3 days) to determine dry weight. Plant water (pw) content was measured as the difference between fresh weight and dry weight.

Measurement of inorganic ions

The dried plant material was ground to a homogeneous powder and was extracted with 95°C distilled water for 1 h, after which the sample was filtered with a GF/C filter (pore size 1.2 μm). Inorganic cations (K^+, Mg^{2+}, Ca^{2+}, Na^+) were determined by ICP-AES. The chloride content was measured using a chloride titrator (Titrators DL 50; Mettler Toledo Inc.).

Measurement of water-soluble carbohydrates, osmolality and amino acids

Total water-soluble carbohydrates of the plants were assayed using phenol-sulfuric acid method (Chaplin and Kennedy 1994). 20 μL of plant extract was mixed with 580 μL of distilled water, 400 μL of 5% phenol and 400 μL sulphuric acid, The solution was allowed to stand for 10 min before being shaken vigorously. Total carbohydrates were quantified through determining the absorbance at 490 nm using a spectrophotometer (UV mini 1240; Shimadzu, Kyoto, Japan) after a further 30 min. Glucese (2-40 μg in 200 μL) was used as standard solution.

Osmolality was measured by cryoscopy using an osmometer (Micro-Osmometer 3MO; Advanced Instruments, Needham Heights, MA, USA). Free amino acids were quantified using an amino acid analyzer (L-8900; Hitachi, Tokyo, Japan).

Statistical analyses

Data was analyzed by analysis of variance (ANOVA) using SPSS ver. 19.0 (SPSS Inc., Chicago, IL, USA). Graphs show means with standard error (s.e.). A Duncan's multiple range test was carried out to determine significant differences ($P < 0.05$) between the groups.

RESULTS AND DISCUSSION

The investigation area has a low water content, being dependent on rainfall alone, even though it is composed of clay. *S. maritima* are mainly distributed throughout the sample site, and *S. asparagoides* appears in patches along the roadside.

Chemical characteristics of soil

The soil was sampled once a month from July to September in 2011. Generally, the saline soil of the sample site showed pH values of 8.26-9.03 (Kim et al. 2006). However, the soil of investigation areas of *S. asparagoides* and *S. maritima* showed lower pH than the surrounding areas with the areas of *S. asparagoides* being restricted to pH 7.41-7.78 and *S. maritima* to pH 7.03-7.39 throughout the entire investigation area. The pH of *S. maritima* areas was somewhat lower than those of *S. asparagoides,* and soils of both species showed the lowest pH in August (Fig. 2).

The total ion and Cl^- content of soils in which both species appeared showed a gradual decrease throughout the survey period. The soil of *S. maritima* showed higher contents of total ions and Cl^- ions than that of *S. asparagoides*.

The soil of *S. asparagoides* showed the lowest total ion concentration of 183.5 μmol/g soil and Cl^- of 89.0 μmol/g soil in August, whereas *S. maritima* showed the lowest total ion concentration of 468.5 μmol/g soil and Cl^- of 275.5 μmol/g soil in September (Fig. 3). The reduction in the total ion content of soil was ascertained by the reduction in exchangeable $Na^+$and Cl^- content. It seems that this study area was desalinated by rainfall in August and plant uptake. As soil desalinization progressed, soil was classified as first saline-sodic soil, the next saline soil and then normal soil (Lee et al. 2003). The exchangeable soil Na^+ contents were 86.14-197.67 μmol/g soil for *S. asparagoides* and 193.63-278.12 μmol/g soil for *S. maritima,* showing a similar pattern to the total ion content. The salty soil and the salty land of Korea's West coast showed 160-200 μmol Na^+/g soil, which was relatively close to the ion content of the *S. maritima* community within the investigation area (Choo et al. 1999). The regions where *S. asparagoides* was found showed lower Na^+ contents.

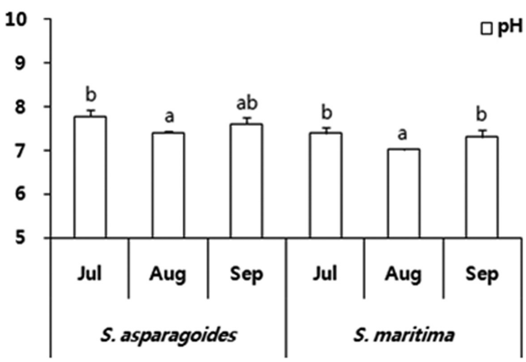

Fig. 2. Seasonal change of pH in soil of *Suaeda asparagoides* and *S. maritima*. Different letters indicate significant differences among three months from Duncan's test for response at sites separately ($P < 0.05$, $N = 3$).

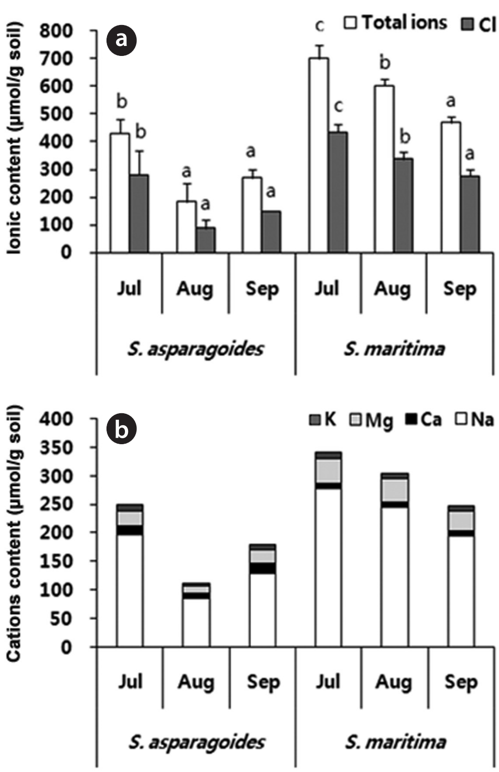

Fig. 3. Seasonal change of (a) total ions (μmol/g soil) and chloride (μmol/g soil) (b) exchangeable cations content (μmol/g soil) in soil of of *Suaeda asparagoides* and *S. maritima*. The different letters of (a) indicate significant differences among three months from Duncan's test for response at total ions and chloride separately ($P < 0.05$, $N = 3$).

Growth characteristics of plant

As part of the growth characteristics of *S. maritima* and *S. asparagoides*, the estimation of plant density and stem length monthly were identified by installing fixed quadrats within the sample site (Fig. 4).

The community of *S. asparagoides* showed a monthly gradual decrease of plant density, but plant length increased continuously. Plant length of *S. asparagoides* in reclaimed saline land was known to show a rapid increase from May to July (Kim 2009), however the results in this study showed continuous increasing of plant length after August.

The plant density of *S. maritima* decreased after July and the species maintained a regular plant density. There was a distinct increase in the growth rate of the plant after August. Most populations appeared to wither after September. Plant density is thought to be more of a limiting factor for the growth of *S. maritima* than environmental factors of the investigation area.

Physiological characteristics of plant

Leaves of *S. maritima* and *S. asparagoides* were collected once a month in their natural habitats from July to September in 2011. The ratio of water contents to dry weight in leaves of *S. asparagoides* and *S. maritima* is described in Fig. 5. *S. asparagoides* and *S. maritima* showed the ratio 8.01-9.86 and 7.42-8.44, respectively. Both plants contain a lot of water in their leaves, and they maintained a relatively regular water potential.

By maintaining a high ratio of water and tissue succulence, some chenopodiaceae and halopytes inhabiting in

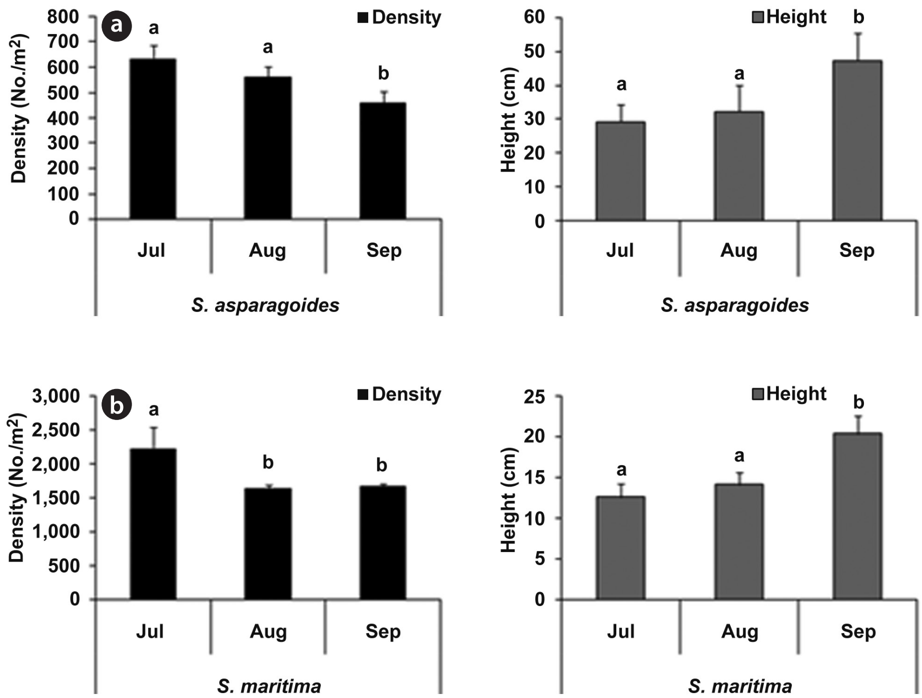

Fig. 4. Seasonal change of density (number/m^2) and shoot height (cm) of (a) *Suaeda asparagoides* and (b) *S. maritima*. The different letters indicate significant differences according to Duncan's test ($P < 0.05$, $N = 3$).

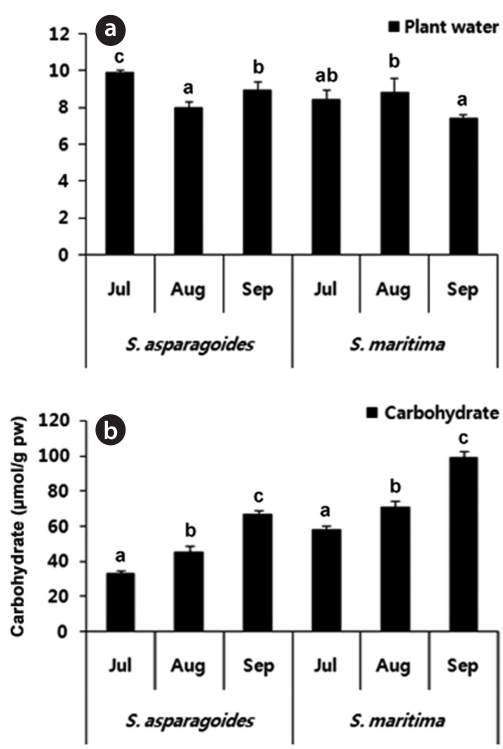

Fig. 5. Seasonal change of (a) the ratio of water contents to dry weight and (b) carbohydrate content (μmol/g plant water) in leaves of *Suaeda asparagoides* and *S. maritima*. The different letters indicate significant differences from Duncan's test for response of species separately ($P < 0.05$, $N = 3$).

saline habitats avoid ion toxicity by diluting accumulated Na^+ and Cl^-.

S. asparagoides and *S. maritima* contained very low soluble carbohydrate contents, which showed a tendency to increase throughout the study period (July-September) (Fig. 5). Soluble carbohydrates do not play a major role as an osmotic solute in either plant.

The osmolality of *S. asparagoides* slightly increased (903-1,013.91 μmol/g pw), but *S. maritima* showed constant osmolality in leaves during the study period (1,267.9-1,348 μmol/g pw). Osmolality of *S. asparagoides* and *S. maritima* were composed of inorganic ions (esp Na^+ and Cl^-) (Fig. 6).

Both plant species contain a small amount of soluble Ca^{2+} ions in their leaves. Generally, plants absorb Ca^{2+} ions in greater concentrations than those required for the maintenance of basic life activities, but the Ca^{2+} ion concentration in cytosol is extremely low because excessive internal Ca^{2+} ions can be harmful in certain conditions (Marschner 1995). Usually Ca^{2+} ions extracted by hot water originate from the vacuole, cytoplasm and organelles, and some fractions extracted by HNO_3 or HCl are known to contain oxalate bound Ca^{2+} ions (Kinzel 1989). Chenopodiaceous plants accumulated most incoming Ca^{2+} ions in the form of Ca-oxalate within vacuoles, therefore free Ca^{2+} ion exist in very small quantities in leaves of Cheno-

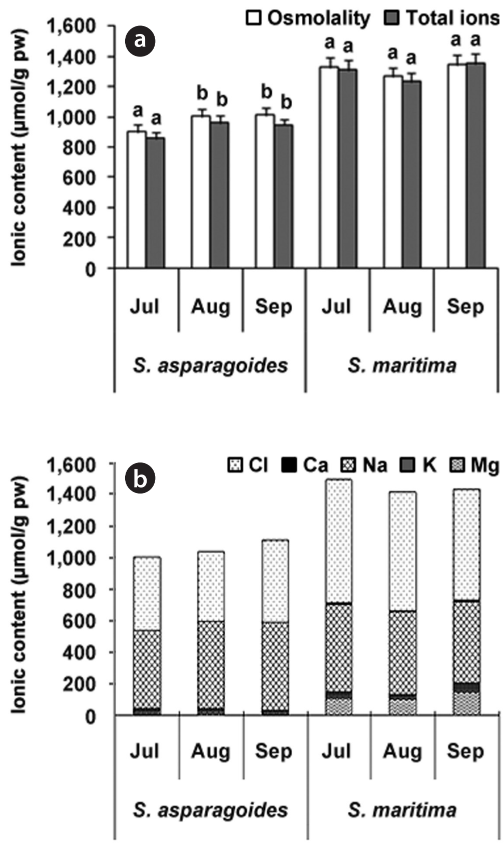

Fig. 6. Seasonal change of (a) osmolality and total ions content (μmol/g plant water), (b) chloride content (μmol/g plant water) in leaves of *Suaeda asparagoides* and *S. maritima*. The different letters indicate significant differences from Duncan's test for response of total ions and chloride separately ($P < 0.05$, $N = 3$).

Table 1. Free amino acid content (μmol/g plant water) in leaves of *Suaeda asparagoides* and *S. maritima*

	S. asparagoides	*S. maritima*
Asp	0.46	1.48
Thr	0.91	0.00
Ser	0.34	0.68
Glu	1.71	2.00
Gly	0.47	0.41
Ala	1.52	5.28
Cys	0.39	0.38
Val	1.65	2.59
Pro	2.56	9.34
Ile	0.83	1.67
Leu	0.58	1.10
Tyr	0.93	1.82
Phe	1.11	2.11
Lys	0.50	1.00
His	0.35	1.19
Arg	0.56	0.41
Met	0.07	0.11

podiaceous plant species (Kinzel 1989, Choo and Song 1998).

The content of free amino acids in the leaves of *S. asparagoides* and *S. maritima* was found to be very low. *S. maritima* contained a small amount of proline (Table 1). Plants stressed by low water potential are known to accumulate amino acids such as arginine, lysine, histidine, glycine and serine, as well as amide compounds such as glutamine and asparagine (Flores and Galston 1984, Pulich 1986).

The accumulation of proline, known as one of compatible solutes, is a common metabolic response of higher plants to water deficits, salinity stress and cold stress, and proline accumulation may play a major role in osmotic adjustment. Proline influences protects against biologically unfavorable consequences of dehydration (Binzel et al. 1987, Voetberg and Sharp 1991, Rhodes and Hanson 1993).

By identifying the low content of proline in the investigated *S. asparagoides,* proline does not seem to contribute to the osmotic regulation of this plant. However, *S. maritima* accumulates higher levels of proline in their leaves under salt conditions than *S. asparagoides,* and contained more proline than other chenopodiacous plant species. The change in proline content was previously determined to be increased when the plant was subjected to salt stress (Moghaieb et al. 2004). Generally, chenopodiacous plant species are known to accumulate glycine betaine for osmoregulation (McCue and Hanson 1990). In chenopodiacous species, such as those belonging to the genus *Atriplex*, the cytosol osmotic adjustment is thought to be mainly due to an accumulation of glycine betaine, which may also assume positive functions in relation to the maintenance of membrane integrity, and stability of other cellular structures under water-stress conditions (Shen et al. 2002, Wang and Showalter 2004). More studies on the interaction of proline, ion contents and glycine betaine in *S. maritima* are required to further elucidate these ecophysiological characteristics.

The habitats of *S. maritima* and *S. asparagoides* showed a gradual decrease in soil total ions, and it is caused by the changes content of exchangeable ion of Na^+ and Cl^- ions. The population of *S. maritima* was distributed in the region with higher ion contents of Cl^-, Mg^{2+}, K^+, and Na^+ than the population of *S. asparagoides*. Both species were assumed to show a decreased population density according to the increase of plant growth.

Under the saline conditions observed within the study area, *S. maritima* and *S. asparagoides* retained high concentrations of inorganic ions to maintain a low water potential, but maintained low water soluble carbohydrate contents. In the case of free amino acid, *S. maritima* contained rather larger amounts of free amino acids than *S. asparagoides,* and showed an especially high proline content. Both species showed high inorganic ion contents in the leaves, which may have been for the avoidance of ionic toxicity by diluting the accumulated ionic concentration with a high water content.

These results suggest that *S. maritima* seems to accumulate proline as a compatible solute for inorganic ions, whereas *S. asparagoides* seems to utilize osmoregulation process using inorganic ions rather than free amino acids. It is essential to study the ecophysiological characteristics of halophytes under various environments in order to select and engineer crops that are able to withstand salt stresses. An examination of the ecophysiological characteristics of halophytes is also required for the management of habitats and maintenance of species diversity in salt marshes.

ACKNOWLEDGMENTS

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