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Sodium plays a more important role than potassium and chloride in growth of *Salicornia europaea*

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Abstract *Salicornia europaea* is a succulent euhalophyte that belongs to the Chenopodiaceae family. It is found that moderate concentration of NaCl can dramatically stimulate the growth of *S. europaea* plants. To elucidate the mechanism underlying the phenomenon, morphological and physiological changes of *S. europaea* in response to different ions, including cations (Na^+, K^+, Li^+, Cs^+) and anions (Cl^-, NO_3^-, CH_3COO^-) were investigated, and the effects of Na^+, Cl^- and K^+ on the growth of *S. europaea* were also studied. Na^+ was more effective than K^+ and Cl^- in stimulating shoot succulence, cell expansion, and stomatal opening. Plants treated with Na^+ (including NaCl, Na^+, $NaNO_3$) showed better plant growth, increased photosynthesis and less cell membrane damage than those untreated and treated with 200 mM of Cl^- and K^+ (including KCl and KNO_3). Both SEM-X-Ray microanalysis and flame emission results revealed that well developed *S. europaea* plants had a higher content of sodium but lower potassium and chlorine. It is concluded that sodium plays a more important role in the growth and development of *S. europaea* than potassium and chloride.

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Introduction

Salinity is one of the major environmental factors limiting plant growth and productivity. Although Na^+ has been considered a micronutrient, excess Na^+ level is obviously toxic to plants (Blumwald 2000). High concentration of NaCl in soil imposes ion imbalance and hyperosmotic stress upon plant, leading to membrane disorganization, ion toxicity, and oxidative damage (Zhu 2001; Shabala and Cuin 2007). To cope with high salinity, both glycophytes and halophytes have evolved adaptation mechanisms, among which maintenance of ionic homeostasis has emerged as a crucial input in plant salt-stress response (Flowers and Colmer 2008). Particularly, a few plants can utilize ions for osmotic adjustment by compartmentalizing ions into cell vacuole (Apse et al. 1999; Blumwald 2000).

Potassium is traditionally considered as an important macronutrient and a major solute contributing to osmotic pressure and ionic strength (Maathuis and Amtmann 1999; Tester and Davenport 2003). Both Na^+ and K^+ have a similarity of the hydrated ionic radii, thus making it difficult to discriminate between them, and this is the basis of Na^+ toxicity (Blumwald 2000). In most plants, Na^+ does not act strictly as a necessary nutrient required for growth, but only if K^+ supply is limited, the addition of Na^+ may promote the growth of some plants, particularly for many salt tolerant and halophytic plants by contributing to turgor formation (Maathuis and Amtmann 1999; Xiong and Zhu 2002; Tester and Davenport 2003). For many glycophytes, salt tolerance

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is linked with maintenance of high cytoplasmic K^+ concentration in leaf cells (Kronzucker et al. 2006; Shabala and Cuin 2007; Flowers and Colmer 2008). Does the same mechanism play effectively in halophytes? As reviewed by Flowers and Colmer (2008), halophytes show a diversity of growth responses to increasing salinity, from a dramatic stimulation to inhibition. To what extent there is also a diversity of mechanisms remains to be resolved. There is a clear difference in the balance of Na^+ and K^+ used by different halophytes. Therefore, there is clearly still much to be learned about halophytes and the diversity of mechanisms that they employ to cope with salinity.

S. europaea, a succulent euhalophyte in Chenopodiaceae, is widely distributed in coastal and inland salt marshes. Without salt glands or salt bladders, *S. europaea* plants can accumulate up to 50% NaCl of dry weight in shoot, thus conferring high salt tolerance (Davy et al. 2001; Ushakova et al. 2005). In previous experiments, we observed that moderate concentration of NaCl could dramatically stimulate *S. europaea* plants growth (Wang et al. 2009). Whether this stimulation is Na^+ and/or Cl^- specific or not? What's the mechanism underlying it? To answer these questions, we investigated morphological and physiological changes of *S. europaea* in response to different ions, including cations (Na^+, K^+, Li^+, Cs^+) and anions (Cl^-, NO_3^-, CH_3COO^-) in the present study. The respective contribution of Na^+, Cl^- and K^+ to the growth of *S. europaea* was studied on and some possible mechanisms were discussed.

Materials and methods

Plant growth conditions and treatments

S. europaea seeds were collected from coastal area of Jiangsu Province, China. The experiments were conducted from April to August in Beijing Botanical Garden. The plants were grown in a greenhouse at 25/20°C (day/night) with a photon flux density (PFD) of 1000 μmol m^{-2} s^{-1}, a relative humidity of 50–60%, and a photoperiod of 16/8 h (light/dark). About 30 seeds were sown on vermiculite damped with tapped water in the plastic pots (8 × 8 × 8 cm). After germination, seedlings were irrigated weekly with half-strength modified Hoagland nutrient solution. 30 days after sowing, plants were thinned to 5 per pot to be treated. In the previous study, we observed that *S. europaea* produced greatest biomass with the supply of 200 mM NaCl (Wang et al. 2009). Therefore, in this study, 200 mM of NaCl, Na-Hoagland, Hoagland-Cl, $NaNO_3$, KNO_3, KCl, LiCl, CsCl, CH_3COONa and CH_3COOK were used. All treatments were performed by adding salinities to the half-strength modified Hoagland nutrient solution in 50 mM increments every 24 h, until the final concentrations of 200 mM were reached. The plants were then irrigated with these solutions with 5-day intervals and all morphological and physiological assays were performed after 50 days of treatment. 25 plants in 5 pots were treated with each solution as 1 replicate and 3 replications were performed.

Plants grown in the half-strength modified Hoagland nutrient solution served as the control, which was still at the vegetative stage under the conditions of 25/20°C (day/night), with a photon flux density (PFD) of 1,000 μmol m^{-2} s^{-1}, a relative humidity of 50–60%, and a photoperiod of 16/8 h (light/dark). Na-Hoagland and Hoagland-Cl solution was made up in accordance with Kingsbury and Epstein (1986). To make up the Na-Hoagland solution, 150 mM of $NaNO_3$, 20 mM of Na_2SO_4 and 10 mM of NaH_2PO_4 were supplied to half-strength modified Hoagland nutrient solution. Hoagland-Cl solution was made up of 51 mM of KCl, 36 mM of $MgCl_2$, 34 mM of $CaCl_2$ and 9 mM of NH_4Cl with half-strength modified Hoagland nutrient solution. The final pH of all the solutions was adjusted to 7.0. The electrical conductivity (EC), osmotic pressure (OP) and ionic composition of the treatment solutions were listed in Table 1.

Measurement of plant height, shoot diameter, fresh weight, dry weight and shoot succulence

The shoot height and fresh weight (FW) were determined immediately after plant harvesting. Dry weight (DW) was determined after drying for 72 h in an oven at 60°C. Total water content (TWC) was calculated as following: TWC = [(FW − DW)/FW] × 100. The shoot succulence ratio was calculated as the following formula: succulence ratio = (shoot diameter × FW × TWC)/(shoot height × DW).

Light and scanning electron microscope analysis

Middle sections of the fifth shoot segment from bottom were used for morphological analysis. The epidermis strips were examined under a Leica DMLN light microscope (Leica, Wetzlar, Germany) as described (Zhang et al. 2001). The diameter of the shoot epidermal cells and the ratio of open to total stomata (opened stoma/total stoma × 100%) were determined in the morning. A 0.5-cm-long section of shoots was fixed in 4% glutaraldehyde for 24 h at 4°C, dehydrated in ethanol and then embedded in paraffin. The microtome sections (10 μm) were stained with mercury–bromophenol blue combined with fast green as described (Tian et al. 2007). And then, the samples were examined under light microscope or Laser Scanning Confocal Microscopy (LSM 510 META, ZAISS, Germany) as described (Zhang et al. 2001) with the following settings: ex = 458 nm, em = 514 nm, power 5%, zoom 4, and mild

Table 1 Electrical conductivity (EC), osmotic pressure (OP) and ionic composition of the treatment solutions

Solution feature	Treatment										
	Control	NaCl	Na-Hoagland	Hoagland-Cl	$NaNO_3$	KNO_3	KCl	LiCl	CsCl	CH_3COONa	CH_3COOK
EC (dS/m)	1.4	18.6	17.1	19.2	18.2	21.4	23.5	16.8	13.2	13.3	16.5
OP (mOs/kg)	40	448	391	327	403	387	480	428	242	359	363
Ions (mM)											
Na^+	0	200	200	0	200	0	0	0	0	200	0
K^+	6	6	6	57	6	206	206	6	6	6	200
Ca^{2+}	5	5	5	39	5	5	5	5	5	5	5
Mg^{2+}	1	1	1	37	1	1	1	1	1	1	1
Fe^{2+}	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Li^+	0	0	0	0	0	0	0	200	0	0	0
Cs^+	0	0	0	0	0	0	0	0	200	0	0
NH_4^+	0	0	0	9	0	0	0	0	0	0	0
NO_3^-	15	15	165	15	215	215	15	15	15	15	15
SO_4^{2-}	1	1	21	1	1	1	1	1	1	1	1
$H_2PO_4^-$	1	1	11	1	1	1	1	1	1	1	1
Cl^-	0	200	0	200	0	0	200	200	200	0	0
CH_3COO^-	0	0	0	0	0	0	0	0	0	200	200

scanning. Then scanning electron microscope (SEM) was carried out as described by Peng et al. (2004).

Gas exchange and chlorophyll fluorescence measurements

Net photosynthesis, stomatal conductance, and transpiration rate were measured using a portable infrared gas analyzer-based photosynthesis system (LI-6400, Li-Cor Inc., USA). The photosynthetic photon flux density was maintained at 1,000 μmol m^{-2} s^{-1} by an internal 6400-02BLED source. All the measurements were carried out from 09:00 to 11:00 a.m. During collection of the measurements, the air relative humidity was about 60% and the ambient CO_2 concentration about 400 μmol CO_2 mol^{-1}.

Chlorophyll fluorescence was determined in intact plants using a fluorescence attachment (6400-40, Li-Cor Inc., USA). After plant were kept in darkness for 30 min, the potential maximum photochemical efficiency of photosystem II (Fv/Fm) was measured by application of a 1 s saturation flash.

Measurement of shoots cell membrane damage

Shoots cell membrane damage was measured as the leakage of electrolytes from shoots cells using a conductivity meter, according to the method described by Gibon et al. (1997). The cell membrane damage represented as electrolytes leakage (%) was calculated as: electrolytes leakage (%) = S1/S2 × 100, where S1 is the conductivity of leaves detached from plants, and S2 is that of boiled leaves (Rizhsky et al. 2002).

Quantification of Na^+ and K^+ content

Fresh tissues were washed with distilled water immediately after harvest, dried at 60°C for 72 h in an oven, and subsequently ground into fine powders with a mortar and pestle. About 300 mg of powders were added in 10 mL of 500 mM HNO_3 and then incubated in 80°C for 1 h. After filtering the extracts, the contents of both Na^+ and K^+ were measured by an atomic absorption spectroscopy (PE-5100ZL, USA).

X-ray microanalysis

In order to determine diffusible elements in situ, X-ray microanalysis coupled with SEM was performed in accordance with Peng et al. (2004). Shoots of different ions-treated *S. europaea* were washed with distilled water for three times. The middle sections of the fifth segment from shoots were dipped in 5% agar, inserted to a depth of 1.0 cm in a copper holder, and immediately sliced free-hand with a razor blade to get transverse sections, and then frozen in liquid nitrogen. The samples were freeze-dried in vacuum, carbon coated with a vacuum sputter coater, and stored in a desiccator. Samples were analyzed in an X-650

scanning electron microscope equipped with an energy-dispersive X-ray detector (EDX-9100, Hitachi, Japan). Map-scan model was performed. Counts per second (CPS) of the main elements were determined. The counting time for analysis was about 100 s, making full scale intensity reached 5,049. Then, these spectra were transformed to normalized data by the professional software in the computer of JSM-6300. The data were expressed as CPS of an element peak after subtraction of the background and all the detectable elements were transformed into the relative element weight. Five replications were applied for each treatment.

Statistical analysis

All data were presented as mean ± standard deviation (SD). The significance was tested using least significant difference (LSD) at 5% level. The SPSS 12.0 statistical package was used for all statistical analysis.

Results

High concentration of Na^+ rather than K^+ and Cl^- stimulates *S. europaea* growth

After exposure to different ions for 50 days, most plants grew well. *S. europaea* grew better in the presence of 200 mM Na^+ (including NaCl, Na-Hoagland solution, and $NaNO_3$) than potassium ions (including KCl and KNO_3) or the control (Fig. 1a, b). By contrast, plants treated with 200 mM CsCl, LiCl, CH_3COOK and CH_3COONa died before the 6th, 15th, 20th, and 25th days, respectively (Fig. S1).

As shown in Fig. 1c–e, plants treated with Na^+ (including NaCl, Na^+, $NaNO_3$) showed significantly higher shoot height, fresh and dry weight than those in the control ones. There were no significant differences in shoot height and fresh weight between plants treated with 200 mM of Cl^-, KNO_3 and the control plants. However, 200 mM of KCl inhibited the plant growth significantly.

A significant difference between Na^+ and Cl^--treated plants was observed (Fig. 1). Even though a similar level of shoot water content was obtained (Fig. 1f), Na^+ treatment could significantly increase shoot height, fresh weight, and dry matter compared to Cl^- treatment (Fig. 1c–e). These results provided straightforward

Fig. 2 Morphological changes of *S. europaea* shoots under different ▶ treatments. **A–G** Epidermal cells and the stomata from epidermis strips under a light microscope, **H–N** shoots morphological changes and proteins accumulation in different ions treated plants, **O–U** total proteins in shoot examined under Confocal. After treatment, the shoot succulence was also observed under SEM on both cellular and subcellular levels (from *a* to *g*). *EC* epidermal cell, *EP* epidermis, *PR* proteins, *ST* stoma, *VB* vascular bundle

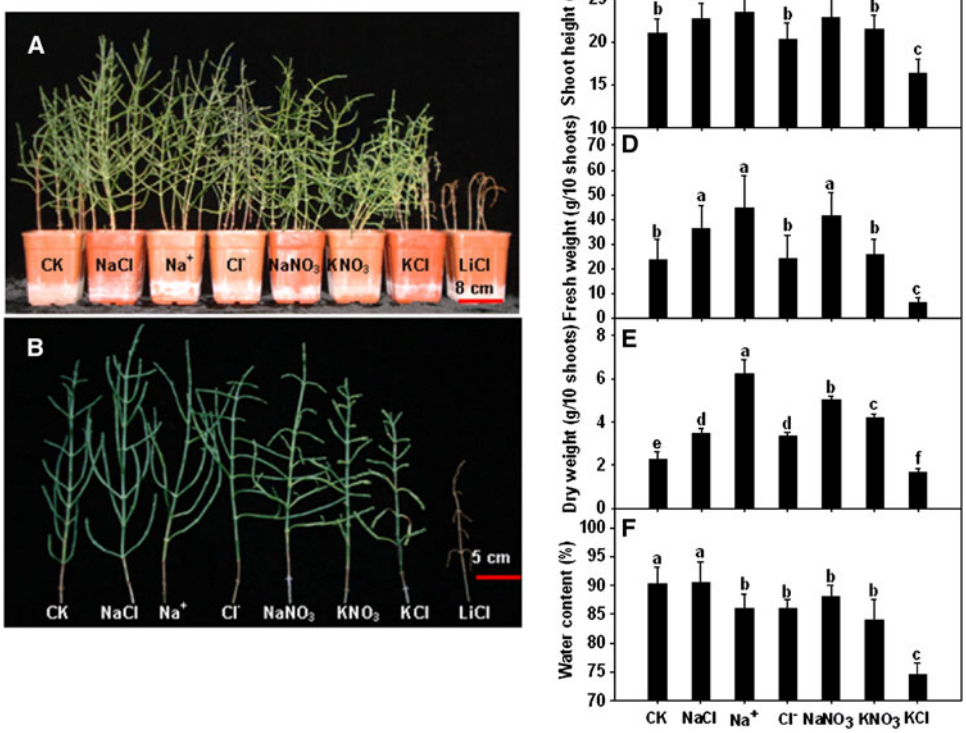
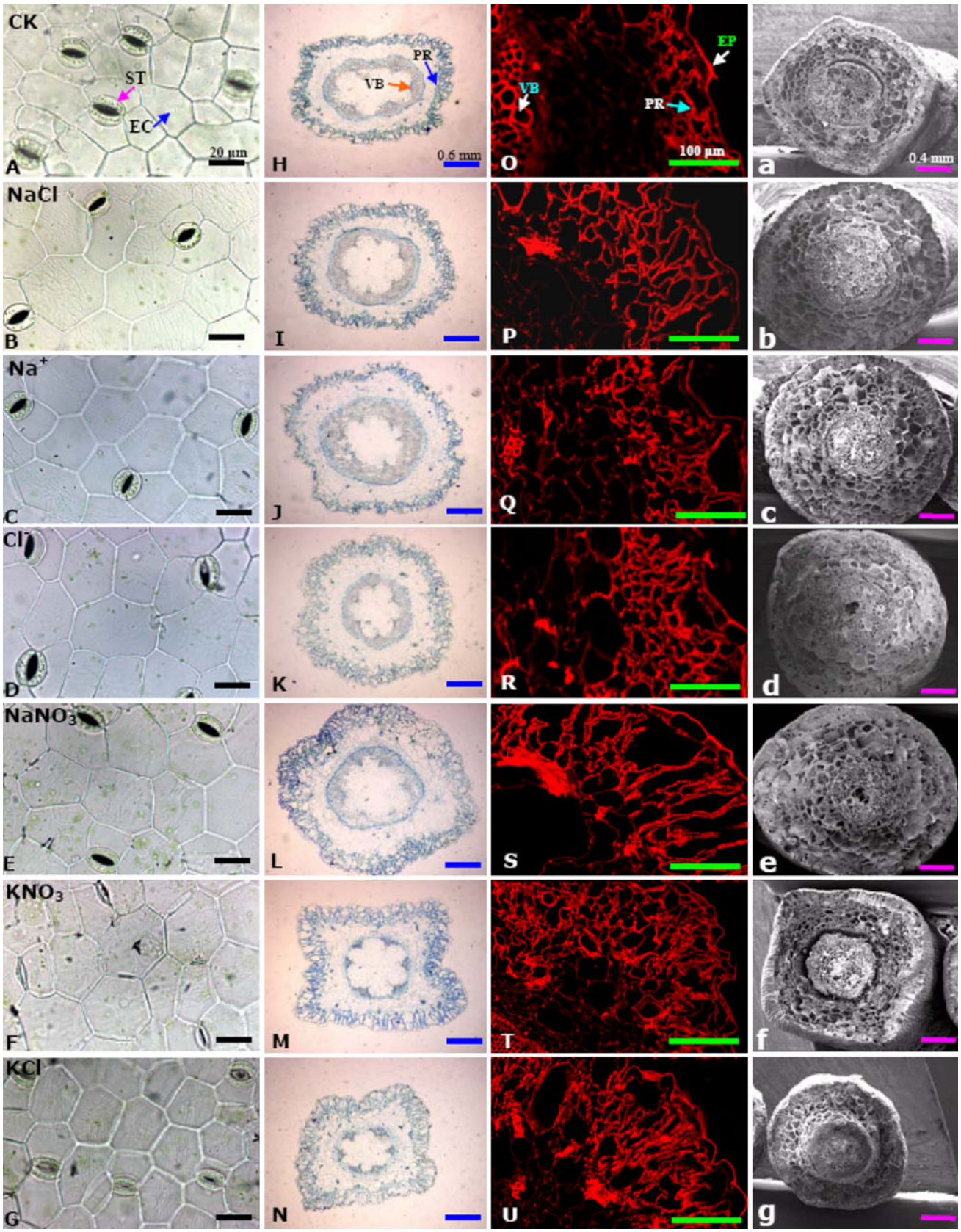


Fig. 1 Growth of *S. europaea* under different treatments. **a** the whole plants, **b** typical shoots from different treatments, **c** plant height, **d** shoot fresh weight, **e** shoot dry weight, **f** water content. *CK*, *S. europaea* plants irrigated by half-strength modified Hoagland nutrient solution. The statistical values are presented as mean ± SD ($n = 25$ for **c** and **f**; $n = 3$ for **d** and **e**). Values with *different letters* are significantly different at $P \leq 0.05$


CK
ST
EC
20 μm
A
PR
VB
0.6 mm
H
EP
VB
PR
100 μm
O
0.4 mm
a
NaCl
B
I
P
b
Na+
C
J
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c
Cl-
D
K
R
d
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evidences that Na^+ is more important than Cl^- for *S. europaea* under salinity.

The results also demonstrated that Na^+ was more important than K^+ for *S. europaea* plant growth under salinity (Fig. 1). Plants supplied with 200 mM of NaCl or $NaNO_3$ grew well (Fig. 1a, b), whereas the increased growth suppression and the abnormal expansion of terminal shoots in KNO_3-treated plants were observed (Fig. 1b). Plants treated with 200 mM KCl was weaker, and some of them wilted after a long time (more than 1 month) treatment (Fig. 1a, b). Compared with NaCl, plants treated with KCl or KNO_3 showed a significant decrease in all the examined parameters. For example, the shoot height, fresh weight, dry weight and water content of plants treated with KCl was only about 72, 18, 48 and 82% of those of plants treated with NaCl, respectively (Fig. 1c–f).

It was remarkable that although no significant difference for shoot water content between NaCl-treated plants and the control was observed, plants treated with other ions showed decreased water content as compared to the control (Fig. 1f).

Na^+ stimulated cell expansion, stomatal opening, and shoot succulence more significantly than K^+

The results of morphological studies revealed that diameters of both epidermal cells (Figs. 2A–G, 3a) and parenchyma cells (Fig. 2O–U, a–g) increased obviously after sodium ions treatments. Under treatments with 200 mM of NaCl, Na^+, and $NaNO_3$, the diameters of epidermal cell were 30.43, 29.79, and 34.37 μm, respectively (Fig. 3a). These values were significantly greater than those in both the control (25.88 μm) and the potassium ions-treated ones (for KNO_3, 28.01 μm, and for KCl, 26.28 μm). Accordingly, larger shoot diameters were observed in sodium ions-treated plants (2.71, 2.46, and 2.57 mm in the NaCl, Na^+, and $NaNO_3$-treated plants, respectively) as compared to the control (2.24 mm) and KCl-treated ones (2.15 mm) (Fig. 3b). These observations were consistent with the SEM results (Fig. 2a–g). Compared with Cl^- treatment, no significant increase of both shoot and epidermal cell diameters was found in plants treated with 200 mM NaCl, Na^+ and $NaNO_3$ (Fig. 3a, b).

We also observed that the ratio of open to total stomata increased significantly after exposure to 200 mM of NaCl, Na^+, Cl^-, and $NaNO_3$ (Figs. 2A–E, 3c). Compared to the control, both KNO_3 and KCl-treated plants showed a dramatic increase of stomata closure (Figs. 2F–G, 3c).

After Na^+ treatments, *S. europaea* developed pronounced shoot succulence compared to other treatments (Fig. 3d). The shoot succulence ratios were 2.25, 3.93, 4.02, 2.54, 4.12, 2.52, and 0.64 in the control, 200 mM NaCl, Na^+, Cl^-, $NaNO_3$, KNO_3, and KCl-treated plants, respectively. Correspondingly, the relative succulence ratios were 1.00, 1.75, 1.79, 1.13, 1.82, 1.12, and 0.28 for these plants as compared to the control plants, respectively (Fig. 3d). In contrast to KNO_3 and KCl treatments, $NaNO_3$ and NaCl-treated plants had a significant increase of succulence ratio. Particularly, relative succulence ratio in

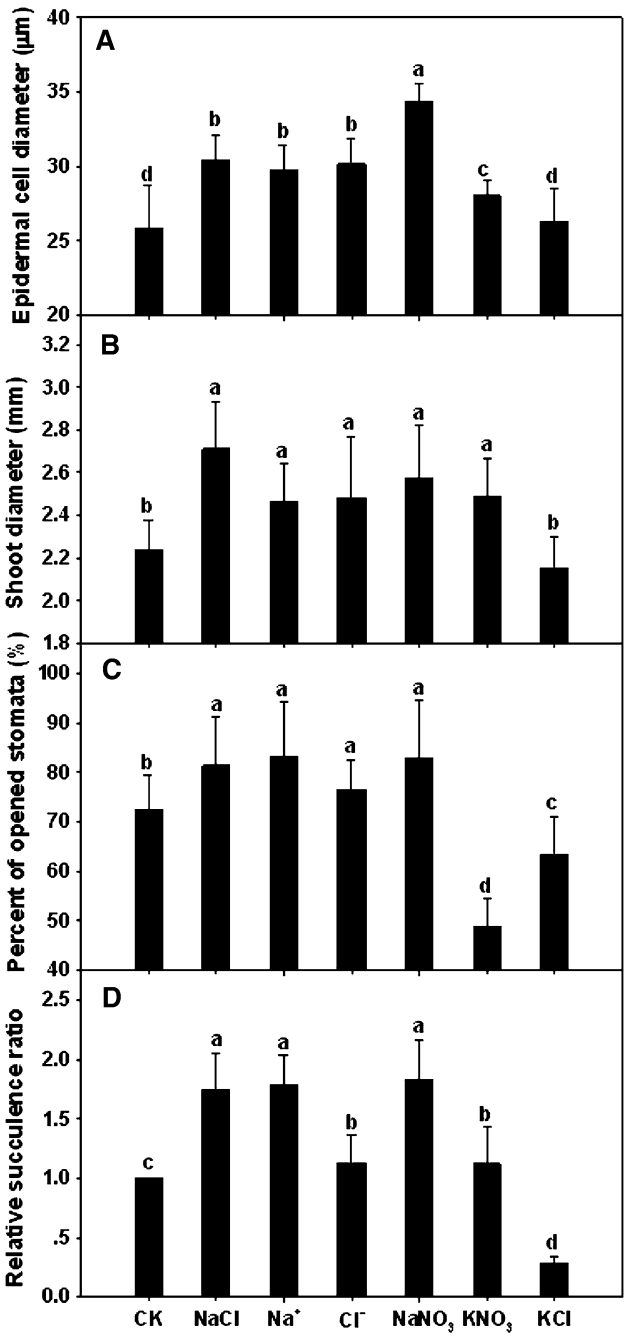

Fig. 3 Effect of different treatments on epidermal cell diameter (**a**), shoot diameter (**b**), ratio of open stomata (**c**) and relative succulence ratio (**d**) for *S. europaea* shoots. The statistical values are presented as mean ± SD ($n = 500$ for **a** and **c**; $n = 25$ for **b** and **d**). Values with *different letters* are significantly different at $P \leq 0.05$

Na-Hoagland treated plants (1.79) also was significantly higher than that in Hoagland-Cl treatment (1.13) (Fig. 3d).

Plants treated with Na^+ displayed better photosynthetic performance and less cell membrane damage than those treated with K^+ and Cl^-

Net photosynthesis rate (*A*), transpiration rate (Tr), stomatal conductance (gs) and the potential maximum photochemical efficiency of photosystem II (Fv/Fm) were measured on plants treated with different ions for 50 days. As shown in Fig. 4a–c, in contrast to the control ones, plants treated with Na^+ (including NaCl, Na^+, $NaNO_3$) showed significantly higher *A*, Tr and gs. Plants treated with 200 mM of Cl^- showed no significant differences with the control ones. However, *A*, Tr and gs in plants treated with KNO_3 and KCl were significantly lower than the control. The Fv/Fm showed similar trends, except that of plants treated with Cl^- was also significantly higher than the control (Fig. 4d).

To further study the effects of different ions on *S. europaea* plants, shoots cell membrane damage, estimated by the percent of ion leakage, was determined. The results indicated that there were no obvious differences in ion leakage between the control plants and plants treated by NaCl, $NaNO_3$ and Cl^-, which were between 39 and 47%. Na^+-treated plants showed the lowest value of ion leakage (33%). However, more ion leakage occurred in plants treated by KNO_3 and KCl (59 and 72%, respectively) (Fig. 4e).

Well developed *S. europaea* plants has a higher content of Na but lower K and Cl

To investigate the relative contributions of Na^+ and K^+ to the growth of *S. europaea*, the Na^+ and K^+ contents in shoots were firstly assayed by flame emission. The results indicated that Na^+ content increased significantly with additional sodium ions, with K^+ level decreasing dramatically, whereas plants treated with 200 mM K^+ (including KCl and KNO_3) exhibited much higher content of K^+ but lower content of Na^+ in their shoots (Fig. 5I). It was noteworthy that *S. europaea* contained a much higher K^+ than Na^+ in shoots under both Cl^- and LiCl treatments (Fig. 5I).

We further performed X-ray microanalysis to determine the ion in situ distributions at tissular level in different ions-treated *S. europaea*. In order to confirm whether *S. europaea* plants could compartmentalize large amount of ions, particularly sodium, potassium, and chloride, in endodermis tissues of shoots under salinity, the transverse section of endodermis tissues in shoots were scanned by X-ray microanalysis (Fig. 5a, b). The typical spectra of SEM-X-ray

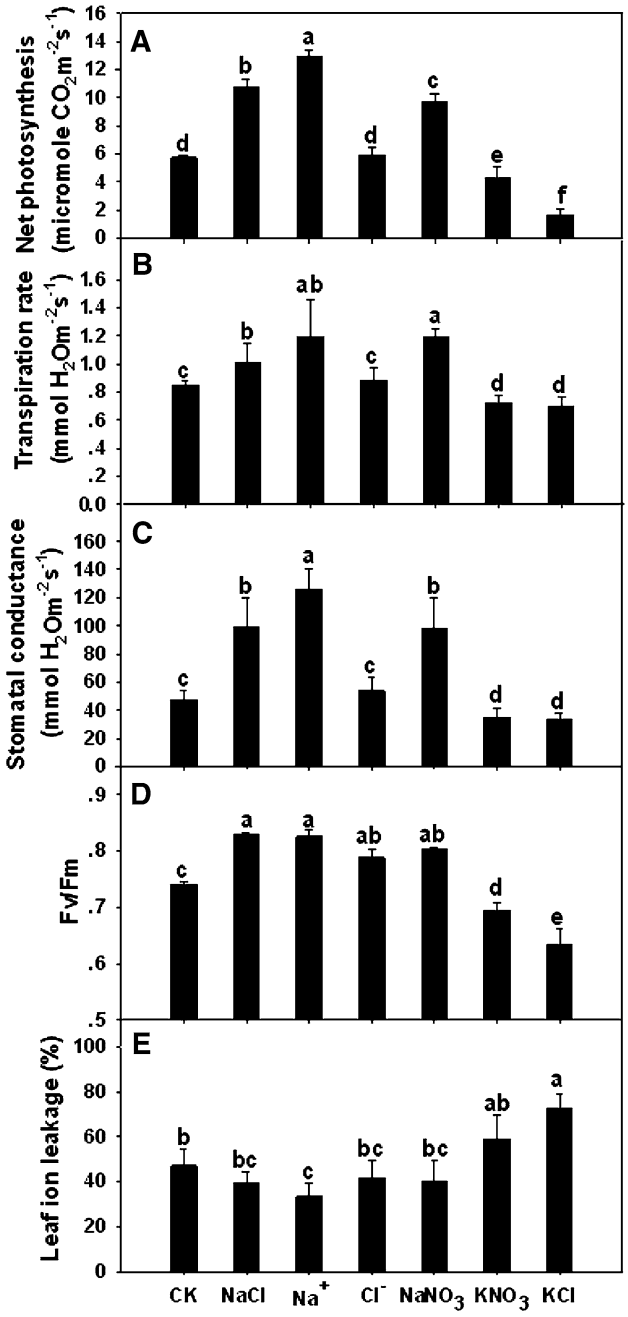

Fig. 4 Effect of different treatments on plant photosynthesis performance and cell membrane ion leakage. **a** Net photosynthesis rate, **b** transpiration rate, **c** stomatal conductance, **d** Fv/Fm, **e** leaf ion leakage. Values are mean ± SD ($n = 9$ for **a**, **b**, **c** and **d**; $n = 3$ for **e**). Values with *different letters* are significantly different at $P \leq 0.05$

microanalysis collected from different ions-treated plants were presented (Fig. 5A–H). Relative content of the detected elements was very different in different ions-treated plants (Fig. 5). In all tested plants, the relative content of Na element was highest in Na-Hoagland treated plants (31.16 ± 2.25), followed by the $NaNO_3$ and NaCl-treated ones (19.25 ± 3.58, and 19.23 ± 1.15, respectively), then

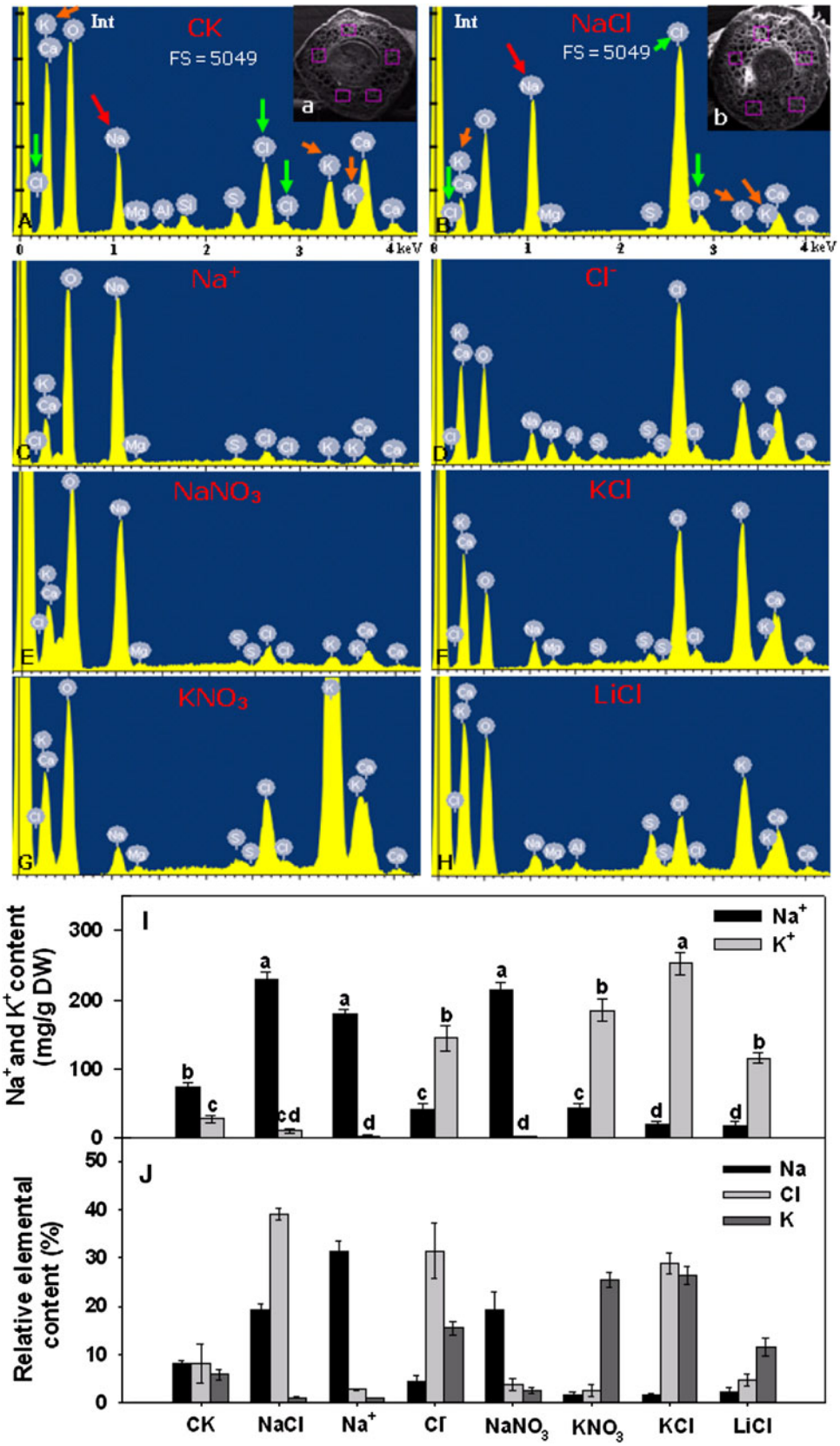

Fig. 5 Energy-dispersive X-ray microanalysis of element compositions in *S. europaea* shoots treated by different ions. **a**–**h** Typical spectra of SEM-X-ray microanalysis from endodermal tissues of plant shoots under different treatments. Panels *a* and *b*, the typical SEM maps of transverse sections of plant shoots. The *squares* in *a* and *b* represent the sample collected position in the shoot endodermal tissues. **j** Na, K, and Cl element relative content the shoot endodermal tissues, **i** Na^+ and K^+ content in plant shoots measured by flame emission. *FS* full-length scales, *Int* intensity. *Arrows* indicated the Na, K, and Cl position in the energy spectra. Values are mean ± SD ($n = 5$). Values with *different letters* are significantly different at $P \leq 0.05$

the control (8.08 ± 0.70). In addition, the lowest was obtained in 200 mM KCl-treated plants, which contained only 1.5% of Na element (Fig. 5J). This is due to the accumulation of high relative content of both K and Cl elements in shoots of KCl-treated plants, which were 26.24 ± 1.88, and 28.89 ± 2.15, respectively (Fig. 5J).

Different from Na, K relative content was highest in KCl-treated plants (26.24 ± 1.88), followed by KNO_3-treated plants (25.44 ± 1.47), and then, Cl^--treated ones (15.48 ± 1.42). Compared to potassium ions, the sodium ions-treated plants contained far less K element in their shoots (Fig. 5J). In addition, the lowest K content was obtained from Na^+-treated plants, which was only about 1.0% (Fig. 5J). After treated with chloride ions (including Cl^-, NaCl, and KCl), much higher contents of Cl element were examined. On the contrary, the Cl relative content decreased dramatically in 200 mM of Na^+, $NaNO_3$, and KNO_3-treated plants, which were 2.77 ± 0.26, 3.78 ± 1.35, and 2.47 ± 1.23, respectively (Fig. 5J). It was noteworthy that LiCl-treated plants contained far less Cl element than other chloride ions treated neither plants nor the control ones (Fig. 5J).

Discussion

It was reported that *Suaeda maritima* mature leaf cells contained relatively lower cytoplasmic K^+ concentration than Na^+, and halophytes in the Chenopodiaceae family had evolved to adjust to these concentrations (Flowers and Colmer 2008). Our results clearly demonstrated that well developed *S. europaea* (Fig. 1) (including plants treated with 200 mM NaCl, Na^+ and $NaNO_3$) contained much higher content of sodium than potassium (Fig. 5). These results were also consistent with the observations in many euhalophytes, such as *S. maritima* (Moghaieb et al. 2004), *Suaeda aegyptiaca* (Askari et al. 2006), *Climacoptera lanata* and *Atriplex micrantha* (Balnokin et al. 2005). In fact, there have been many reports that KCl is more toxic to the plant growth than the equivalent concentration of NaCl (Yeo and Flowers 1980; Wang et al. 2001; Ramos et al. 2004). For example, 340 mM KCl produced only 28% of the dry mass of plants growing in an equivalent concentration of NaCl in *S. maritima* (Yeo and Flowers 1980). Why this should be so is not known, although a number of years ago Yeo (1981) concluded, based on data from flux analysis, that halophytes such as *S. maritima* were able to retain Na^+, but not K^+ in vacuoles (Flowers and Colmer 2008).

Succulence is an important adaptation to salt tolerance contributing to the regulation of internal ion concentrations for many halophytes, especially in the family of Chenopodiaceae (Short and Colmer 1999). Increased succulence can stimulate the plant development because of both the dilution effects of the increased water content for sodium ions and the specific stimulation of sodium for growth in many halophytes (Greenway and Munns 1980; Short and Colmer 1999). In the ice plant *Mesembryanthemum crystallinum,* the greater leaf and stem succulence can ameliorate the ionic and osmotic stress effects, and provide a long-term reservoir of water storage to facilitate improved reproductive capacity under high salinity conditions (Agarie et al. 2007). Cell expansion, as well as shoot succulence, can provide more cellular space for the ion regulation and osmotic adjustment (Apse and Blumwald 2002; Ottow et al. 2005). This might also be an important salt tolerance mechanism in *S. europaea.* After Na^+ treatments, *S. europaea* developed pronounced shoot succulence compared to other treatments (Fig. 3d). In contrast to KNO_3 and KCl treatments, $NaNO_3$ and NaCl-treated plants had a significant increase of succulence ratio, indicating sodium is more effective than potassium for shoot succulence in *S. europaea.* Particularly, relative succulence ratio in Na-Hoagland treated plants was 1.79 (Fig. 3d), which was statistically significant higher than that in Hoagland-Cl treatment (1.13), revealing sodium is more effective than chloride for shoot succulence in *S. europaea.* As for cell expansion, the diameters of both epidermal cells (Figs. 2A–G, 3a) and parenchyma cells (Fig. 2O–U, a–g) also increased obviously after sodium ions treatments. These values were significantly greater than those in both the control and the potassium ions-treated ones. However, compared with Cl^- treatment, no significant increase of both shoot and epidermal cell diameters was found in plants treated with 200 mM NaCl, Na^+ and $NaNO_3$, indicating Cl^-, as well as Na^+ and NO_3^- (Fig. 3a, b), could somewhat stimulate cell expansion and shoot development for *S. europaea.*

Salt may affect plant growth indirectly by decreasing the rate of photosynthesis (Meloni et al. 2003). In the study, instead of decrease, the net photosynthesis rate in plants treated with 200 mM Na^+ (including NaCl, Na^+, $NaNO_3$) increased significantly compared with the control (Fig. 4a). Accordingly, the transpiration rate and stomatal conductance in these plants were higher than those in the control plants (Fig. 4b, c). These results indicated that these treatments were not proposed as a stress for euhalophyte *S. europaea*, instead they could stimulate the plant growth. Maintenance of greater leaf turgor can lead to the maintenance of greater photosynthetic capacity and growth in plants exposed to at low soil water potential (Gupta and Berkowitz 1987; Plaut and Federman 1991). High concentration of Na^+ in cell of *S. europaea* may act as an effective osmotic adjuster to maintain cell turgor, thus to promote photosynthetic capacity and plant growth. On the other hand, there perhaps exist specific sodium catalyzed enzymes in *S. europaea*. Flowers

and Dalmond (1992) found from in vitro studies using polysomes of three halophytes (*Atriplex isatidea*, *Inula crithmoides* and *S. maritima*) that incorporation of ^{35}S-methionine into protein was stimulated by Na^+ (100 mM) in the presence of a suboptimal concentration (25 mM) of K^+. There are evidences indicating some cytosolic enzymes of halophytes have slightly modified forms to provide unequivocal tolerance to high Na^+ (Tester and Davenport 2003; Agarie et al. 2007), and some enzymes may also have evolved to be dependent on high concentrations of Na^+ for activities (Flowers and Dalmond 1992; Dastidar et al. 2006). Unfortunately, little attentions have been paid to investigate such a potential mechanism till now.

However, 200 mM of K^+ (including KCl and KNO_3) inhibited plant photosynthesis significantly comparing with the control (Fig. 4a). Salt stress decreases photosynthesis through stomatal and non-stomatal (direct effect on the photosynthetic apparatus) factors. Stomatal factors are generally more significant at medium salinities and non-stomatal limitations are more relevant at high salinity (Everard et al. 1994). The much lower ratio of open to total stomata, stomatal conductance and Fv/Fm ratio in plants treated with K^+ (including KCl and KNO_3) suggested that the decrease in photosynthesis rate of these plants was due to both stomatal closure and the decreased PSII activity (Figs.3c, 4c, d). These results suggested that 200 mM of K^+ (including KCl and KNO_3) could adversely affect the photosynthetic apparatus and be toxic to plant growth, which was consistent with the more cell membrane damage (Fig. 4e).

The Na^+/K^+ ratio is an important determinant for salt tolerance in glycophytes where cellular K^+ concentration is higher than that of Na^+. In halophytes, this ratio can be reversed and higher cellular Na^+ concentrations were reported. Riehl and Ungar (1982) investigated the ion accumulation in *S. europaea* under different saline field conditions. The results showed more Na^+ was accumulated in shoots of *S. europaea* and the Na^+/K^+ ratio was 1.5–3.5 under different saline field conditions. Moghaieb et al. (2004) reported that the Na^+/K^+ ratio in shoots of *S. europaea* was 1.3–1.75 under 85–510 mM NaCl. Higher Na^+/K^+ ratios in *S. europaea* were also reported, which were about 5.5 and 7.8 under 3 and 6% NaCl conditions, respectively (Ushakova et al. 2005). In this study, based on the results from X-ray microanalysis, the Na^+/K^+ ratios were 16.7, 30.5 and 7.6 in plants treated by 200 mM of NaCl, Na-Hoagland and $NaNO_3$, respectively, which were much higher than those reported before (Fig. 5J). These discrepancies may be due to the different plant developmental stage and different treatment duration. For example, Na^+ and K^+ content was determined after 21 and 30 days of NaCl treatment in the study of Moghaieb et al. (2004) and Ushakova et al. (2005), respectively. While in this study, *S. europaea* plants were treated for 50 days before the ion content being measured. In addition, NaCl was used in all above reports, while Na-Hoagland and $NaNO_3$ were also used in this study. It is that the much higher Na^+/K^+ ratio in Na-Hoagland treated plants than that in NaCl treated plants suggested Cl^- may somewhat promote the cell absorption of K^+. The results that more K^+ were detected in Cl^- and LiCl-treated plants than that in the control further confirmed the above surmise (Fig. 5I, J), which should be further studied in the future.

In addition, *S. europaea* is a euhalophyte that can withstand more than 1000 mM NaCl salinity (Ushakova et al. 2005). Its leaf is degenerated, making the plant height and shoot diameter easy to be quantified (Fig. 1). Besides, the epidermis strips can be easily peeled off from shoot segments, thus making the determination of epidermal cell diameter and stomata behavior convenient (Fig. 2). Furthermore, the shoot water content, total biomass, and even the cortical cell diameter are easy to be determined. These characters facilitate the quantification of cell expansion and shoot succulence, indicating the possibility of *S. europaea* to be used to investigate cell expansion, succulence and salinity adaptation mechanism in extremophilic euhalophytes.

In conclusion, our results demonstrated that Na^+ could dramatically increase the growth and development of *S. europaea* as compared to K^+. In addition, we provided strong evidences that Na^+ is more effective than both K^+ and Cl^- in stimulating cell expansion and shoot succulence. Based on our observations in *S. europaea* and the reported results in many other euhalophytes, we bring forward the opinion for the first time that sodium plays a more important role than potassium and chloride in the growth and development of some euhalophytes. Our results indicated that *S. europaea* might be used to investigate cell expansion, succulence, and salinity adaptation mechanism in extremophilic euhalophytes.

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