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Variation of Sodium Uptake Rate in *Suaeda glauca* and Its Relation to Plant Size and Salt Acclimation

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Abstract: To characterize the pattern of sodium uptake by *Suaeda glauca* plants at mild salinity, this study investigated the temporal variation of sodium uptake rate (mmol Na⁺/plant/day), and its relation to plant size (i.e., main stem length) and salt acclimation, for *S. glauca* grown in nutrient solutions with 6, 8, or 10 mM NaCl. During the 23-day salt treatment, sodium uptake rate in *S. glauca* increased gradually at 5- to 7-day intervals with time advancement and showed a positive exponential relationship with the development of plant size. During the first week (0–7d) of salt treatment, weekly average sodium uptake rate was higher in larger than in smaller plants. During the last week (18–23 d) of salt treatment, non-salt-acclimated plants showed a similar sodium uptake rate (on a weekly average) to salt-acclimated plants when their plant sizes were not significantly different. These results suggest that at the largest plant size (i.e. one week before harvesting as a leafy vegetable), *S. glauca* reached the maximum level of sodium uptake rate, which was affected to a larger degree by plant size than by salt acclimation on a weekly scale.

Key words: *Suaeda glauca*, mild salinity, sodium uptake rate, plant size, salt acclimation

Nutrient solutions of mild-salinity (containing 6–10 mM Na⁺) discharged from greenhouse production can be used for hydroponic production of *Suaeda glauca* as a leafy vegetable. As a halophyte, *Suaeda glauca* is not only salt-tolerant, but can also remove large amount amounts of sodium from the environment (Kong and Zheng 2015). Reducing the absolute amount of Na⁺ in discharges plays an important role in the prevention of soil salinization, since salt ions in most saline soils are dominated by Na⁺ (Flowers et al. 1977).

By characterizing the temporal variation of sodium uptake by individual *S. glauca* plants grown in a mild salinity solution at weekly intervals, it can provide information for applying this halophyte for use in greenhouse discharged nutrient solutions for a short period (e.g. one week) when its sodium uptake rate (mmol Na⁺/plant /d) is at the peak. In this case, the sodium removing efficiency would be maximized, while avoiding too large of an increase in Na⁺ concentration in discharged solutions caused by evapotranspiration (Kong and Zheng 2015).

In the literature, information about the temporal variation of sodium uptake rate in hydroponic *S. glauca* is not available. A previous study with purslane, hydroponically-grown at mild salinity (i.e., 6–10 mM Na⁺) indicated that each plant removed a two- to four-fold greater amount (mmol) of Na⁺ from the solution per day during the early vs. late post-transplant phase (i.e., 0–7 d vs. 7–26 d after transplanting), despite a gradual increase in Na⁺ uptake rate with plant growth during the later three-week growth stages (Kong and Zheng 2014). The late-phase increase in Na⁺ uptake rate is understandable, since a tight coupling has been identified between Na⁺ uptake and plant

growth as well as plant size (Cheeseman 1988; Lv et al. 2012). The peak of sodium uptake rate during the early phase might be due to salt acclimation (Kong and Zheng 2014). When a plant is exposed to a saline solution for the first time, a large amount of Na^+ absorption could provide a rapid means of turgor adjustment for plants to match the abrupt decrease in osmotic potential of the medium (Bernstein 1975; Raven 1985; Essah et al. 2003). Afterwards, to avoid the toxicity of excessive Na^+ accumulation, a plant could initiate many other osmotic adjustments such as synthesis of low-molecular-weight organic compounds in the form of osmolytes (Yazici et al. 2007; Slama et al. 2015) which functionally reduce Na^+ uptake. From these previous studies, variation in sodium uptake rate is likely associated with plant size as well as salt acclimation.

If most halophytes follow the same variation pattern of sodium uptake rate as hydroponic purslane and the corresponding explanation is reasonable, we could suggest the following hypotheses: 1) At 6–10 mM NaCl, hydroponic *S. glauca* would show a temporal variation pattern of Na^+ uptake rate similar to purslane; 2) during the first week after the start of salt treatment, Na^+ uptake rate would be higher in large vs. small *S. glauca* plants; 3) for similar-sized large *S. glauca* plants, Na^+ uptake rate would be higher in non-salt- vs. salt-acclimated plants during the last week of a salt treatment (i.e., the week immediately before plant harvest).

The objective of this study was to explore the variation of Na^+ uptake rate in hydroponically-grown *S. glauca* at 6–10 mM NaCl and its relation to plant size and salt acclimation by testing the above hypotheses.

MATERIALS AND METHODS

Plant materials, experimental design, and growing conditions

The experiment was conducted in a greenhouse adjacent to the Edmund C. Bovey Building at the University of Guelph, Guelph, Ontario, Canada (lat. 43°33'N, long. 80°15'W). Seeds of *S. glauca* (Yancheng Green Garden Saline Soil Agricultural Science Company, Jiangsu, China) were sown in a peat-perlite mix of 1:1 by volume on 10 and 25 April 2013. Twenty days after sowing, 36 early-sowed and 72 late-sowed seedlings with three pairs of true leaves were transplanted into rockwool cubes (1.5" Starter Plugs; Grodan Inc., Milton, Ontario, Canada), which were embedded in Styrofoam disks and placed to float on a nutrient solution in a plastic pot (14-cm top and 12-cm bottom diameters × 15 cm high). Each pot had one Styrofoam disk with four seedlings grown in individual rockwool cubes, except for the blank control pot which had one disk and four un-planted rockwool cubes. Each pot contained ≈1.5 L of solution with the following nutrients (mg·L⁻¹): 278.3 N, 36.7 P, 420 K, 97.6 Ca, 1.58 Mg, 100.8 S, 0.53 Cu, 0.21 B, 0.53 Mn, 0.53 Zn, 0.16 Mo, and 1.05 Fe. On 22 May 2013, i.e., seven and twenty-two days after the transplanting of the small (late-sowed) and large (early-sowed) plants, respectively, all of the large plants and one half of the small plants were transferred to nutrient solutions with 6, 8, and 10 mM NaCl, indicating the start of the salt treatment. The large plants were treated in salt for 7 days, whereas the small plants (salt-acclimated) were treated for 23 days. Eighteen days later, another half of the smaller plants (non-salt-acclimated) were also transferred to the above NaCl-containing solutions for five

days. Details of the experimental design are illustrated in Fig. 1. The initial Na^+ concentrations of the above three NaCl-containing nutrient solutions were measured as 6.9 ± 0.1 , 8.8 ± 0.2 , and 10.8 ± 0.3 mM, with electrical conductivities (ECs) of 3.34 ± 0.03 , 3.54 ± 0.05 , and 3.81 ± 0.06 $\text{dS}\cdot\text{m}^{-1}$, respectively. The nutrient solutions were changed every 5–7 d to reduce nutrient and/or NaCl concentration variability. During the experiment, the nutrient solution pH ranged between 5.5 and 7.0. Pots were arranged in a randomized block design with three replicates.

The greenhouse conditions were set at 18-h light/ 6-h dark by supplementing natural sunlight with high pressure sodium lamps to achieve a photosynthetic active radiation at canopy level averaging no less than 397 ± 34 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and 22 to 28 °C light/20 to 22 °C dark with a relative humidity between 50% and 60%.

Measurements of plant growth and sodium uptake

Two plants were randomly chosen from each pot within each block for growth measurements. Every week the main stem length was measured both at the beginning and end of the week until 23 d after the start of salt treatment (Fig.1), and the means of the above two stem length measurements were considered as the weekly plant size. Every week, and every 1–3 d during the first and last week, nutrient solutions were sampled to measure Na^+ uptake rate ($\text{mmol}/\text{plant}/\text{d}$; Fig.1), following the same method used by Kong and Zheng (2014). Specifically, a sample of fresh and 1-, 2-, 5-, or 7-d-old nutrient solution was analyzed for Na^+ concentration using a portable pH/ISE meter and sodium electrode (Thermo Orion model 290A; Waltham, MA, USA). Na^+ uptake rate by the plants

in each pot during an interval period of solution sampling was calculated as follows:

$$\text{Na}^+ \text{ uptake rate (mmol/plant/d)} = \frac{(V_i \times C_i - V_f \times C_f) - (V_i' \times C_i' - V_f' \times C_f')}{4 \times t}$$

Where V_i and V_f are the initial and final volumes (L) of solution in each pot during each two solution samplings, respectively; and C_i and C_f are the initial and final Na^+ concentrations (mM) in the solution in each pot during each two solution samplings, respectively. V_i' , V_f' , C_i' , and C_f' are the initial and final volumes (L) and the initial and final Na^+ concentrations (mM) of solution in the blank control pot during each two solution samplings, respectively. In the denominator, t is the treatment time (d) of an interval period of two solution samplings, and 4 is the number of seedlings in each pot.

Statistical analysis

Data were subjected to analysis of variance using the DPS (Data Processing System) Software (version 7.05; Refine Information Tech. Co., Hangzhou, China) and were presented as mean $\pm$ SE (standard error); separation of means was performed using Duncan's new multiple range test at the $P \leq 0.05$ level. Regression analyses were used to determine the relationship between variation of Na^+ uptake rate and plant size in the small, salt-acclimated plants.

RESULTS

Variation of sodium uptake rate and plant size

Temporal variation of sodium uptake rate showed a similar pattern among the plants at 6, 8, and 10 mM NaCl (Fig. 2). Specifically, sodium uptake rate increased gradually

after a 13-d salt treatment and peaked during the last week (i.e., 18–23 d after the start of treatment). However, stem length increased gradually after a 7-d salt treatment. Throughout the whole growth process, sodium uptake rate had a significantly positive exponential relationship with stem length.

Effect of plant size on variation of sodium uptake rate

During the first week (7 d) of salt treatment, large plants had a significantly higher sodium uptake rate (based on a weekly average) than small plants, regardless of NaCl concentrations. However, large and small plants showed a similar temporal variation pattern at 1- to 3-d intervals, with a peak sodium uptake rate during the first day of salt treatment (Fig. 3).

Effect of salt acclimation on variation of sodium uptake

During the 5 days of salt treatment, the sodium uptake rate (on 5-d average) was not significantly different between salt- and non-salt-acclimated plants with similar stem length (Fig. 4). However, the temporal variation pattern of the sodium uptake rate was different between salt- and non-salt-acclimated plants. Non-salt-acclimated plants showed a significantly higher sodium uptake rate during the early 1-d vs. late 4-d salt treatment. To contrast, salt-acclimated plants showed a significantly higher sodium uptake rate during the late 3-d vs. early 2-d salt treatment.

DISCUSSION

Effect of plant size on sodium uptake

In the present study, the variation of sodium uptake rate in *S. glauca* was tightly associated with plant size. During the first week of salt treatment, the large plants had a higher weekly average sodium uptake rate than the small plants (Fig. 3). Throughout the entire salt treatment period, the sodium uptake rate increased exponentially with the extension of the main stem at 5- to 7-d intervals (Fig. 2), indicating that plant size plays an important role in determining sodium uptake rate in *S. glauca*. Therefore, the results prove that the second hypothesis is true, which implies that it is better to incorporate large-sized plants into discharge disposal systems to maximize the sodium removal efficiency of *S. glauca* when planting density does not vary with growth stage.

Previous studies have also confirmed that rate of Na^+ uptake and delivery to the shoot is related to plant growth rate as well as plant size (Flowers et al. 1977; Cheeseman 1988). In *Suaeda marina*, with the change in growth, Na^+ uptake and transport in the plant was altered accordingly; there was a close linkage between Na uptake and plant growth, which resulted in a rather constant ion concentrations in the shoot (Yeo and Flowers 1986). The integration between sodium uptake and plant growth could be explained through transport mechanisms within the plant. In halophytes, during the initial adaption to a sudden increase in external Na^+ concentration, large quantities of sodium are absorbed into plant, retained away from the salt-sensitive cytoplasm, and localized within the vacuole (Harvey et al. 1981; Yeo 1981), which could result in more negative shoot water potentials than those in the external medium, thereby maintaining the movement of water into the plant

(Clipson et al. 1985; Clipson et al. 1988). Afterward, with plant growth, to maintain the sodium concentration in plant tissue an increased rate of sodium uptake by individual plants must thereby be associated with an increase in plant size due to increased cell number and size (Flowers et al. 1977).

From the above explanation it is possible that for the same plant the tight relationship between plant size and Na^+ uptake could be observed only after the end of salt acclimation, not during the initial stages of adaptation. Plants exposed to increased salinity levels where excessive Na^+ uptake is unparallel to plant size, could provide a way for the re-establishment of a water potential gradient into the plant following a sudden increase in external Na^+ concentration (Yeo and Flowers 1980). This was also supported, in the present study, by the unparallel temporal variation of Na^+ uptake rate and stem length of *suaeda glauca* during the first two weeks, which resulted in an exponential, not a linear, relationship between Na^+ uptake rate and stem length. More obviously in hydroponic purslane growing at mild salinity, the sodium uptake rate was associated with plant size only during the later three weeks, and showed the highest value during the first week when the plants were freshly transferred from a non-saline to a saline environment (Kong and Zheng 2014). The above information suggests that salt acclimation is possibly another important factor affecting the rate of sodium uptake.

Effect of salt acclimation on sodium uptake

A previous study on purslane reported a higher sodium uptake rate in each plant during the first seven days of growth vs. later growth stages, and the authors attributed the

higher sodium uptake rate to osmotic adjustment of plants in response to salt step-up (Kong and Zheng 2014). It suggests the possibility that, due to using sodium uptake for osmotic adjustment, non-salt-acclimated plants may have a higher sodium uptake rate than salt-acclimated plants during a one-week period (approximately), if the plant sizes are similar.

Unexpectedly, non-salt-acclimated *S. glauca* plants had a higher sodium uptake rate than salt-acclimated plants only during the first day of salt treatment, but showed no differences in the 5-d average sodium uptake rate from salt-acclimated plants (Fig. 4). This suggests that the truth of the third hypothesis is dependent on time scale.

It should be noted that like purslane, salt acclimation is also a factor which can affect sodium uptake rate for *S. glauca*, but the effect is easily covered by the larger time scale (e.g., 5 days), and can be observed at a smaller time scale (e.g., 1 day). The difference between the two plant species possibly indicates that it may take *S. glauca* vs. purslane a shorter time to uptake sodium for plant osmotic adjustment in response to external salinity increase, which requires further research for verification. Studies in *Suaeda maritima* confirmed that readjustment of the osmotic potential proceeds sufficiently and rapidly, and salt adaptation takes the form of a rapid increase in sodium transport to the shoot, which is maximal after 6–12 h in response to an increase in external salt concentration (Clipson et al. 1988). The osmotic potential in the majority of leaves examined had virtually reached a new steady-state level within 24 h, suggesting that readjustment would largely have taken place within the initial 24 h period (Clipson 1987).

Temporal variation pattern of sodium uptake rate

Unlike purslane, the weekly variation of sodium uptake rate in *S. glauca* did not show a peak during the first week of salt treatment, but exhibited a gradual increase trend with time advancement at 5- to 7-d intervals (Fig. 2). However, when studied at a smaller time scale (i.e., at 1- to 3-d intervals during a week), the temporal variation pattern of sodium uptake rate in non-salt-acclimated, but not salt-acclimated, *S. glauca* plants was similar to that in purslane at 7-d intervals (Figs. 3 and 4). Our results indicated that the truth of the first hypothesis varied with time scale as well as salt acclimation.

The difference in temporal variation pattern between purslane and *S. glauca* is possibly due to different time-lengths for osmotic adjustment by sodium uptake and different amounts of sodium uptake during the first week for osmotic adjustment in the two plant species. On one hand, as mentioned previously, like *Suaeda maritima*, it may take only 24 h for *S. glauca* to finish osmotic adjustment by sodium uptake in response to an external salinity increase (Clipson 1987; Clipson et al. 1988), but the process may take a longer time for purslane. On the other hand, during the first week of salt treatment, when the main stems were around 10 cm long, *S. glauca* showed a lower sodium uptake rate (≈ 0.01 vs. 0.02 mmol/plant/d) than purslane (Kong and Zheng 2014). A previous study indicated that *S. glauca* accumulated a large amount of organic osmolytes, such as total soluble sugars and proline, aside from Na^+ (Jia et al. 2011). However, during the first 24 h of salinity, high Na^+ accumulation in plant tissues plays an important role in osmoregulation and maintenance of water absorption (Mori et al. 2011). Therefore, it is possible that purslane, rather than *S. glauca*, might perform osmotic adjustments mainly

by Na⁺ accumulation during the first week of salt treatment.

In summary, sodium uptake rate in *S. glauca* is associated with plant size to a large degree, with relation to salt acclimation only at a small time scale, and showed a gradual increase trend at 5- to 7-d intervals peaking around one week before harvest (when the plant size is largest).

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REFERENCES

- Bernstein, L. 1975.** Effects of salinity and sodicity on plant growth. Annu. Rev. Phytopathol. **13**: 295–312.
- Cheeseman, J. M. 1988.** Mechanisms of salinity tolerance in plants. Plant Physiol. **87**: 547–550.
- Clipson, N. J. W. 1987.** Salt tolerance in the halophyte *Suaeda maritima* L. Dum. growth, ion and water relations and gas exchange in response to altered salinity. J. Exp. Bot. **38**: 1996–2004.
- Clipson, N. J. W., Lachno, D. R. and Flowers, T. J. 1988.** Salt tolerance in the halophyte *Suaeda maritima* L. Dum.: abscisic acid concentrations in response to constant and altered salinity. J. Exp. Bot. **39**: 1381–1388.

- Clipson, N. J. W., Tomos, A. D., Flowers, T. J. and Jones, R. G. W. 1985.** Salt tolerance in the halophyte *Suaeda maritima* L. Dum. Planta. **165**: 392–396.
- Essah, P. A., Davenport, R. and Tester, M. 2003.** Sodium influx and accumulation in *Arabidopsis*. Plant Physiol. **133**: 307–318.
- Flowers, T. J., Troke, P. F. and Yeo, A. R. 1977.** The mechanism of salt tolerance in halophytes. Annu. Rev. Plant Physiol. **28**: 89–121.
- Harvey, D. M. R., Hall, J. L., Flowers, T. J. and Kent, B. 1981.** Quantitative ion localization within *Suaeda maritima* leaf mesophyll cells. Planta. **151**: 555–560.
- Jia, J., Cui, X., Wu, J., Wang, J. and Wang, G. 2011.** Physiological and biochemical responses of halophyte *Kalidium foliatum* to salt stress. Afr. J. Biotechnol. **10**: 11468–11476.
- Kong, Y. and Zheng, Y. 2014.** Hydroponic production of purslane as a sodium-removing vegetable in NaCl-rich nutrient solution. HortScience. **49**: 201–206.
- Kong, Y. and Zheng, Y. 2015.** *Suaeda glauca* can be produced hydroponically at moderate NaCl salinity. HortScience. **50**: 847–850.
- Lv, S., Jiang, P., Chen, X., Fan, P., Wang, X. and Li, Y. 2012.** Multiple compartmentalization of sodium conferred salt tolerance in *Salicornia europaea*. Plant Physiol. Biochem. **51**: 47–52.
- Mori, S., Suzuki, K., Oda, R., Higuchi, K., Maeda, Y., Yoshida, M. and Tadano, T. 2011.** Characteristics of Na⁺ and K⁺ absorption in *Suaeda salsa* (L.) Pall. Soil Sci. Plant Nutr. **57**: 377–386.
- Raven, J. A. 1985.** Regulation of pH and generation of osmolarity in vascular plants: a

cost–benefit analysis in relation to efficiency of use of energy, nitrogen and water. *New Phytol.* **101**: 25–77.

Slama, I., Abdelly, C., Bouchereau, A., Flowers, T. and Saviouré, A. 2015. Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Ann. Bot.* **115**: 433-447.

Yazici, I., Turkan, I., Sekmen, A. H. and Demiral, T. 2007. Salinity tolerance of purslane (*Portulaca oleracea* L.) is achieved by enhanced antioxidative system, lower level of lipid peroxidation and proline accumulation. *Environ. Exp. Bot.* **61**: 49–57.

Yeo, A. R. 1981. Salt tolerance in the halophyte *Suaeda maritima* L. Dum.: Intracellular compartmentation of ions. *J. Exp. Bot.* **32**: 487–497.

Yeo, A. R. and Flowers, T. J. 1980. Salt tolerance in the halophyte *Suaeda maritima* L. Dum.: evaluation of the effect of salinity upon growth. *J. Exp. Bot.* **31**: 1171-1183.

Yeo, A. R. and Flowers, T. J. 1986. Ion transport in *Suaeda maritima*: its relation to growth and implications for the pathway of radial transport of ions across the root. *J. Exp. Bot.* **37**: 143-159.

Fig. 1. Schematic experimental design and sampling date.

→ Time axis; — period of no salt treatment; period of salt treatment [vertical line(s) inside the frames indicate the date of changing solution]; Δ solution sampling for determining of sodium uptake rate; $\odot$ plant sampling for measurement of main stem length. Seeds of large and small plants were sown on 10 and 25 April, 2013, respectively.

Fig. 2. Variation of Na⁺ uptake rate and main stem length of individual *S. glauca* plants grown in hydroponic solutions with additions of 6 (A), 8 (B), and 10 (C) mM NaCl at different growth stages and their relationship (D). Data are means $\pm$ SE ($n = 3$). Bars or dots marked with the same letter are not significantly different at $P \leq 0.05$, according to Duncan's new multiple range test. ** Behind the value of R^2 indicates that the calculated regression is significant at $P \leq 0.01$.

Fig.3. Comparison of Na⁺ uptake (A–C) by *S. glauca* in hydroponic solutions with additions of 6, 8, and 10 mM NaCl between large (L) and small (S) plants, characterized by different main stem lengths (D). Data are means ± SE (n = 3). Bars bearing the same letter are not significantly different at $P \leq 0.05$, according to Duncan's new multiple range test. Legends followed by *** denote the differences in average Na⁺ uptake rate during 7-d salt treatment between large and small plants are significant at $P \leq 0.001$.

Fig.4. Comparison of Na^+ uptake (A–C) by *S. glauca* in hydroponic solutions with additions of 6, 8, and 10 mM NaCl between non-salt- and salt-acclimated plants, of similar plant size, characterized by main stem length (D). Data are means $\pm$ SE ($n = 3$). S0 and S indicate the plants 0- and 18-d acclimated to 6–10 mM NaCl, respectively. The upper and lower numbers on the x-axis of A–C is time after the start of salt treatment for S and S0, respectively. Bars bearing the same letter are not significantly different at $P \leq 0.05$, according to Duncan's new multiple range test. Legends followed by NS denote that the differences in average Na^+ uptake rate during 5-d salt treatment between S0 and S are not significant at $P \leq 0.05$.