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Accumulation capacity of ions in cabbage (*Brassica oleracea* L.) supplied with sea water

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ABSTRACT

Cabbage seedlings were grown hydroponically to study the effects of different concentrations of seawater on the seedling growth, ion content under one-fourth strength Hoagland's nutrient solution in the greenhouse. The biomass of various organs of cabbage seedlings as well as the whole plants was significantly higher in the treatments with 1 g and 2 g sea salt/L than the no-salt control, but the treatments with 4, 5 or 6 g sea salt/L caused a decrease in growth. Root/shoot ratio remained at the level of control regardless of the sea salt treatment. Na^+ and Cl^- concentration in different parts of cabbage seedlings increased significantly, whereas K^+ and Ca^{2+} concentration generally increased at low concentrations of sea salt and then decreased with increasing seawater concentration. Sodium and K^+ concentrations were significantly higher in the stems than roots and leaves regardless of the sea salt treatment. The sea salt treatment increased Mg^{2+} concentration in stems and leaves of cabbage seedlings. An increase in Na^+ and Cl^- concentration in roots, stems and leaves of cabbage seedlings was the main contributor to declining ratios of K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+. The obtained data suggest that cabbage seedlings have strong ability to sustain seawater stress by the regulation of transport and distribution of ions.

Keywords: salinity; *Brassica oleracea* L. var. *capitata*; salt tolerance; sea salt stress; ion uptake

Salinity is a major factor limiting plant growth and crop productivity (Allakhverdiev et al. 2000). Salt damage to plants is produced by a combination of several causes, including mainly osmotic injury and specific ion toxicity (Munns et al. 1995) that affect a wide variety of physiological and metabolic processes in plants. Significant areas of cultivated land are affected by salinity in more

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than one hundred countries. The increasing agricultural and environmental problems (including soil secondary salinization) on a worldwide scale have received much attention (Sun et al. 2009) and soil salinity poses a serious threat to crop yield and future food production (Moller and Tester 2007). High concentrations of salt in saline soils result in a root-cell membrane injury and loss of permeability (Meloni et al. 2004). Ionic homeostasis plays an important role in the physiology of all living cells; the regulation of ion fluxes is important for ensuring appropriately high concentration of essential ions and sufficiently low concentration of toxic ions (Abideen et al. 2014). These compatible solutes help maintain ion homeostasis and water relations; they alleviate the negative effects of high ion concentrations on the enzymes, stabilizing proteins, protein complexes, membranes and cellular function under salt stress conditions (Meloni et al. 2004).

Cultivating salt-tolerant plants and studying their mechanisms of tolerance is important for plant breeding, improvement of the environment and development of modern agriculture. Cabbage (*Brassica oleracea* L. var. *capitata*), a member of the mustard or cruciferous family (Brassicaceae), is derived from the wild mustard plant. It is native from the Mediterranean coast to the North Sea and is one of the important economical vegetables worldwide. In this paper, investigations of growth and specific physiological processes could provide comprehensive insights into the salt tolerance and the response mechanisms induced by salt stress in cabbage. Therefore, the effects of salt stress on plant growth characteristics, and ion absorption, transportation and allocation in cabbage were investigated.

MATERIAL AND METHODS

Plant material and growth conditions. Seeds of cabbage (*Brassica oleracea* L. var. *capitata*) were collected from Experimental Station 863 of Dafeng, Jiangsu, China. Seedlings cultivated hydroponically were grown 18 days before the commencement of treatments containing 400 mL of seawater solution. The crude salt was produced by evaporation of seawater in Laizhou Bay (118°32'–119°37'E, 36°25'–37°19'N). The ion composition in Laizhou Bay seawater included 0.13 g HCO_3^-/L, 3.87 g SO_4^{2-}/L, 17.33 g Cl^-/L, 0.79 g Ca^{2+}/L, 1.03 g Mg^{2+}/L, 0.60 g K^+/L, and 9.48 g Na^+/L. For the seawater treatments, crude sea salt was added to the growth medium and the concentrations were 0, 1, 2, 3, 4, 5 and 6 g/L, respectively (T0, T1, T2, T3, T4, T5 and T6, respectively). All treatments were set up in 12 plants in a completely randomized design. The nutrient solution was aerated continuously and replaced every 2 days. The seedlings were grown under the following conditions: 28°C/22°C day/night temperatures and relative humidity of 70–80% in a semi-controlled greenhouse in the Dafeng base of the Nanjing Agricultural University. After 21 days of seawater treatment, plant samples were collected for analysis.

Fresh and dry weight measurements. At harvest, roots, stems and leaves were separated, and the weight of fresh matter was recorded. For determination of dry weight, roots, stem and leaf were first oven-dried at 105°C for 15 min and then at 65–75°C until constant weight was obtained. Calculations were done as follows: whole-plant biomass (root biomass + stem biomass + leaf biomass), relative biomass (= average total biomass of a treatment/average total biomass of control × 100%) and root/shoot ratio (= root biomass/shoot biomass).

Determination of inorganic ion content. The samples were transferred to clean and dry digestion tubes and treated with 2 mL of concentrated HNO_3, and then, the tubes were cooled for 30 min. Digests were transferred to 50 mL volumetric flasks, left to stand for more than 1 h and the supernatant was decanted and analysed by an inductively-coupled plasma atomic emission spectrometer ICP-OES (inductively coupled plasma optical emission spectrometer, optima 2100DV, PekinElmer, Waltham, USA) to determine K^+, Na^+, Ca^{2+} and Mg^{2+} concentration in cabbage roots, stems and leaves. The following ratios were calculated: K^+/Na^+, Ca^{2+}/Na^+, and Mg^{2+}/Na^+. The content of Cl^- was measured by the silver nitrate titration method (Zhu et al. 2012).

Statistical analysis. The mean values of all parameters were derived from the measurements of six replicates, and the standard error of the means was calculated. The data were subjected to 1-way analysis of variance. The mean differences were compared by the Duncan's new multiple range test (SPSS 19.0, USA). Differences between individual means were tested using the least significant difference test at $\alpha = 0.05$.

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RESULTS

Sea salt stress effects on biomass accumulation and ion distribution in cabbage. Mild sea salt stress (up to T3) did not negatively influence whole-plant biomass accumulation in cabbage seedlings, but there was a downward trend from T4. Roots grew similarly in T0–T4 and declined at higher sea salt concentrations. The trends in leaf and stem biomass were the same, with unaltered growth at low salinities (T1 and T2) in comparison with the control, and declining significantly from T4 (stem) or T3 (leaves) and higher. The root:shoot ratio remained similar regardless of the sea salt treatment.

Effect of sea salt stress on ion content in tissues of cabbage. Under different seawater treatments, the Na^+ concentration in cabbage seedlings increased significantly (Figure 1a). There were significant differences between various organs of the same treatments. Na^+ concentration in different tissues followed the order of stem > leaf > root. The accumulation of Na^+ concentration in different tissues followed the order of stem > leaf > root regardless of the sea salt treatments. The change of Na^+ contents in cabbage seedlings was similar for all treatments as the experiment proceeded.

K^+ concentration in stems of cabbage seedlings was significantly higher than that in roots and leaves (Figure 1b). The content of K^+ in roots decreased more slowly with the increasing seawater concentration. The content of K^+ in stems decreased at seawater concentration significantly increased except control as the experiment proceeded. The contents of K^+ of T1 to T4 seawater treatments was more significant than those of T5 to T6 seawater treatments in stem. There were the highest contents of K^+ under T2 and T3 treatments in leaves.

Figure 1c showed that Ca^{2+} content in all parts of cabbage seedlings decreased under different seawater treatments. Ca^{2+} content in leaves was

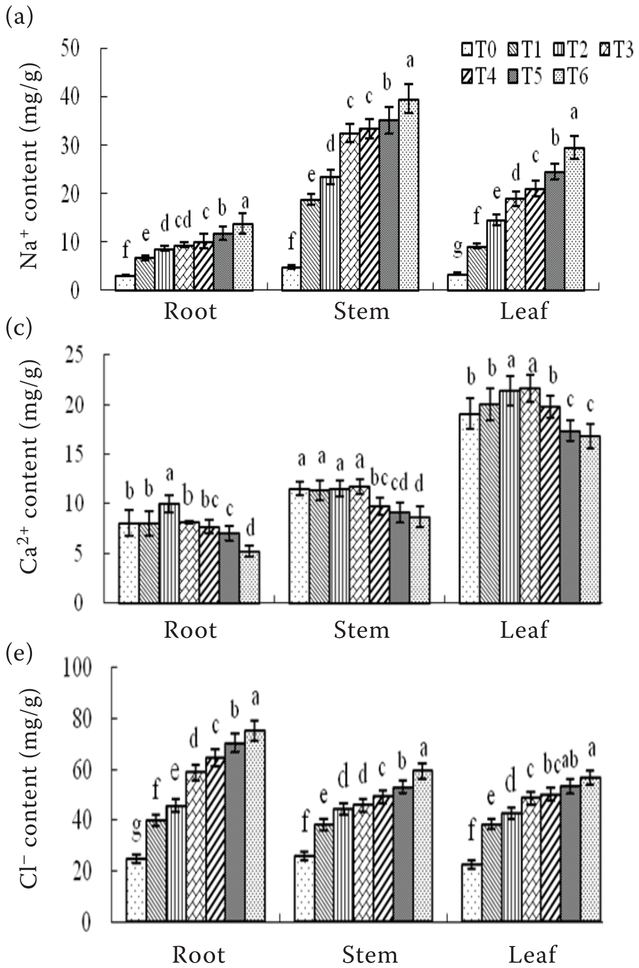

Figure 1. Effect of sea salt stress on ion content in different dry organs of cabbage seedlings (a) Na^+; (b) K^+; (c) Ca^{2+}; (d) Mg^{2+}; (e) Cl^-. T0 – 0, T1 – 1, T2 – 2, T3 – 3, T4 – 4, T5 –5, T6 – 6 g sea salt/L of the seawater

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higher than that in stems and roots, and the differences between each groups reached a significant level. Ca^{2+} content in stems showed no significant difference under different treatments.

Mg^{2+} concentration in roots, stems and leaves of cabbage was higher than under no-salt control (Figure 1d). With the increasing seawater concentration, the content of Mg^{2+} in the stems first increased significantly, then dropped from T3 treatment. Mg^{2+} content in leaves was higher than that in roots and stems after T3, T4, T5 and T6 seawater treatments.

In Figure 1e, the content of Cl^- in roots, stems and leaves showed an upward trend with the increasing seawater concentration. The concentration of Cl^- in roots was significantly higher than Cl^- content in stems and leaves.

Effects of sea salt stress on the ratio of ion content in cabbage. The data for the effects of salt stress on K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios in different tissues of cabbage under different treatments are presented in Table 1. The lower the ratio indicates the absorption inhibitory effect of Na^+ for K^+, Ca^{2+} and Mg^{2+}, the higher then the more severe salt damage. Compared with controls, K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios in roots, stems and leaves showed a downward trend in general with the increasing seawater concentration. K^+/Na^+, Ca^{2+}/Na^+, Mg^{2+}/Na^+ ratios in roots, leaves declined largely, and a significant difference between two different seawater treatments was observed, while Ca^{2+}/Na^+ declined more slightly. K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios in the stems were significantly lower than those in the roots and leaves. Under T1 treatment, ion content ratio in roots, stems and leaves fell to 55, 69, 61% (K^+/Na^+), 53, 74, 61% (Ca^{2+}/Na^+) , 44, 71, 59% (Mg^{2+}/Na^+) compared with no-salt control. These results indicated that Na^+ in roots, stems and leaves inhibit absorption of K^+, Na^+, and Mg^{2+}.

Ion distribution in various organs of cabbage. Na^+ content of various parts of the cabbage seedlings followed this order leaf > stem > root (Figure 2a). With the increasing sea salt concentration, Na^+ content of roots showed no significant difference between T0, T1, and T2 treatments, and no significant difference of stems was found between T3, T4, T5, T6 treatments. Na^+ content of leaves increased obviously from T0 to T6. Sea salt stress significantly affected Na^+ distribution of organs in cabbage seedlings. Roots are more susceptible to the effects of Na^+. Na^+ was transported to stems and leaves through the wood, and continued to accumulate in the shoot.

K^+ was mainly stored in the leaves of cabbage seedlings. Its content in roots was very low (Figure 2b). K^+ content of various organs decreased with the increasing seawater concentration. The comparison of results showed that there was no significant difference in K^+ distribution of roots, stems and leaves of cabbage seedling between different treatments.

From Figure 2c, Ca^{2+} content of various organs of cabbage seedlings followed the order leaf > stem > root. Ca^{2+} content of roots, stems, leaves accounted for 10, 19, 71; 10, 17, 73% of whole plants under T0, T1 treatments. In addition to T0, T2 treatments, Ca^{2+} content of the rest treatments gradually decline with increasing seawater concentration.

Mg^{2+} content of cabbage seedlings was reduced slightly after different seawater treatments (Figure 2d).

Table 1. Effects of different concentrations of sea salt stress on K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios of cabbage seedlings

	K^+/Na^+			Ca^{2+}/Na^+			Mg^{2+}/Na^+		
	root	stem	leaf	root	stem	leaf	root	stem	leaf
T0	7.66	8.91	8.34	2.54	2.34	5.58	0.99	0.56	0.93
T1	3.45	2.77	3.26	1.20	0.60	2.18	0.55	0.16	0.38
T2	2.35	2.06	2.09	1.16	0.49	1.48	0.44	0.14	0.24
T3	2.04	1.43	1.70	0.87	0.36	1.14	0.38	0.11	0.24
T4	1.86	1.36	1.39	0.75	0.29	0.94	0.34	0.11	0.20
T5	1.40	1.19	1.11	0.59	0.26	0.71	0.28	0.10	0.17
T6	1.17	1.06	0.88	0.38	0.22	0.57	0.23	0.08	0.14

T0 – 0, T1 – 1, T2 – 2, T3 – 3, T4 – 4, T5 –5, T6 – 6 g sea salt/L of the seawater

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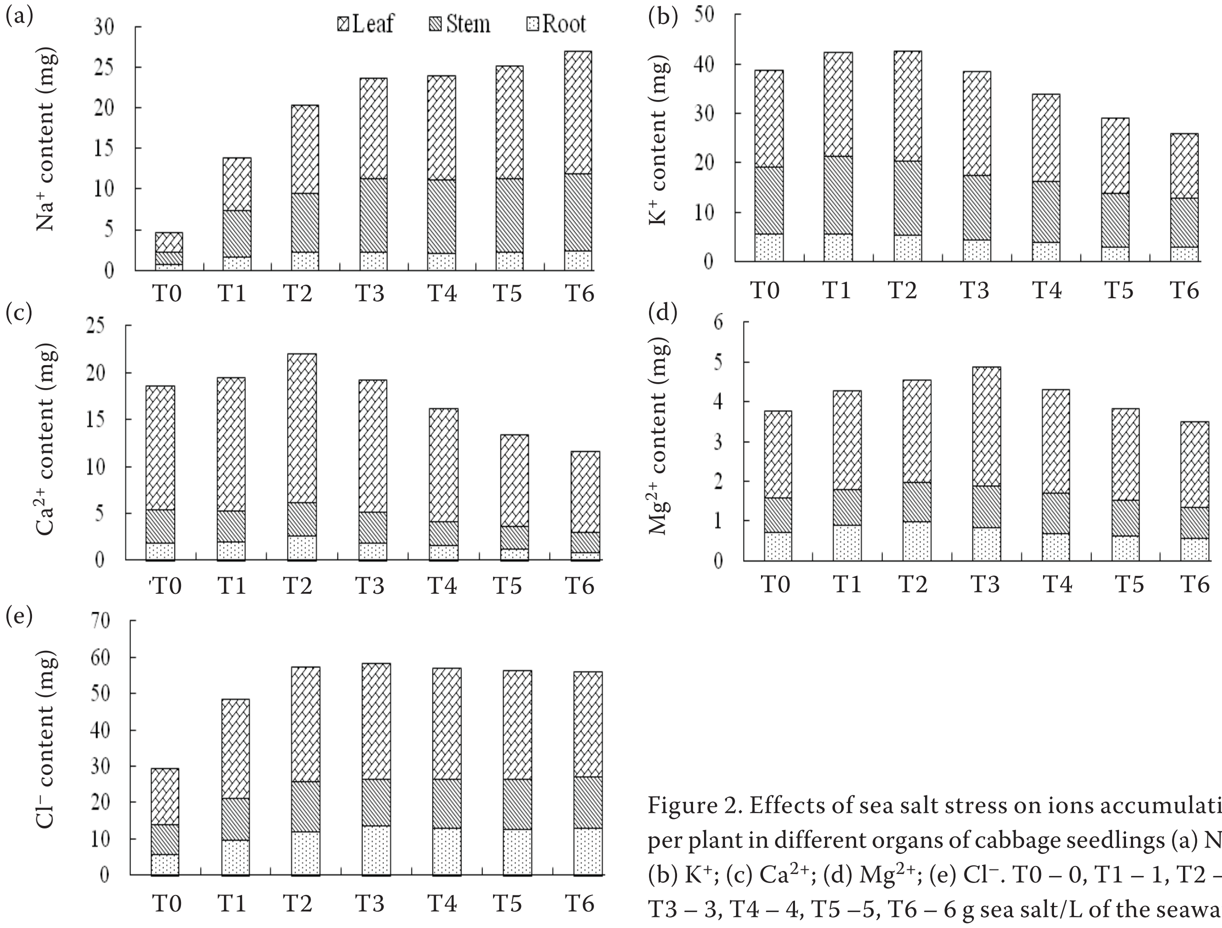

Figure 2. Effects of sea salt stress on ions accumulation per plant in different organs of cabbage seedlings (a) Na^+; (b) K^+; (c) Ca^{2+}; (d) Mg^{2+}; (e) Cl^-. T0 – 0, T1 – 1, T2 – 2, T3 – 3, T4 – 4, T5 –5, T6 – 6 g sea salt/L of the seawater

Mg^{2+} concentration in leaves of cabbage seedlings was significantly higher than that in the roots and stems. Mg^{2+} content in roots, stems and leaves were 22, 22, 57; 17, 21, 62% under T2, T3 treatments. Mg^{2+} content in the leaves was the highest under T3 treatment. The differences among all the sea salt treatments were not significant.

From Figure 2e, the influence of seawater treatments on the distribution of Cl^- in leaves was much greater than in stems and roots. With the increasing seawater concentration, Cl^- content of cabbage seedlings increased, there were significant differences between T0 and T2 treatments, but no significant differences were found between T3 to T6 treatments.

DISCUSSIONS

The treatments tested in the present study were chosen to reproduce conditions of some natural coastal zones, as characterized by infiltration of saline waters. Under these conditions, salinity is the major abiotic stress for plants (Long et al. 2010). An excess of salt in the culture medium can cause damage to plants mainly by ionic toxicity and osmotic stress. Growth inhibition is the most common physiological response of plants to salt adversity (Long et al. 2009, Wu et al. 2015). The treatments tested in the present study showed that with the increasing seawater concentration, the growth of roots, stems, leaves and the whole plants of cabbage seedlings were not significantly affected. An important indicator of the resistance of plants is the ratio of underground biomass and aboveground biomass, namely the ratio of root and stem. The greater the ratio of root and steam was, the higher was resistance of the plants. As it can be seen from Table 1, root/shoot ratio was higher than that in no-salt control under T1 to T3 treatments; under T4 to T6 treatments, root/shoot ratio remained at control levels. The evidence, therefore, suggested that cabbage seedlings have strong ability to sustain seawater stress.

Accumulation of inorganic solutes, such as cations Na^+ and K^+, can play a role independently or

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in combination with other mechanisms in maintaining the osmotic imbalance caused by the salt stress and influencing the osmotic potential adjustment of plant cells (Bayuelo-Jiménez et al. 2003). Regulation of transport and distribution of ions in different plant parts is an important feature of the mechanism of salt tolerance as well as that in the cell (Long et al. 2009). At the single cell level under seawater stress conditions, one of the most destructive consequences is a significant increase in Na^+ content and the reduction of K^+ content in plant tissue. K^+, Ca^{2+} and Mg^{2+} have important physiological functions, which are essential for plants to grow (Zhang et al. 2013). These ions at a relatively balanced state can play a normal physiological function. Na^+ is a key factor that limits the growth of plants in the case of seawater stress (Niu et al. 1995). Both Na^+ and Cl^- are the major ions that produce many physiological disorders in plants; Cl^- is the most dangerous (White et al. 2014). K^+ is necessary for a plant to maintain membrane integrity and functionality (Tang et al. 2015). Ca^{2+} can maintain membrane stability, help form cell walls and take part in signal transduction. Mg^{2+} is the key component of chlorophyll. Salinity not only caused high Na^+ accumulation in plants but also influenced the uptake of essential nutrients such as K^+ and Ca^{2+} through the effects of ion selectivity. The superior K^+ retention and efficient usage of compatible solutes are crucial components of osmotic adjustment for salt tolerance. The results of this experiment showed that with the increasing seawater concentration, Na^+ and Cl^- concentrations of roots, stems and leaves of cabbage seedlings increased significantly, while the contents of K^+, Ca^{2+} and Mg^{2+} decreased gradually, which led K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios to significantly decrease. Under T3 treatment, contents of Na^+ and Cl^- in cabbage seedlings were more than 2.79 g/kg and 2.94 g/kg compared to control, and the content of K was less than 7.92 g/kg than in control. It suggests that cabbage seedlings were under salt stress mainly by absorbing Na^+ and Cl^- to reduce cell osmotic potential, relieving salt stress damage. The balance of intracellular ions in living cells is an important for living cell. Proper regulation of ions is necessary for physiological activity of cells (Zhu 2003, Yan et al. 2013). It can be seen from the results of this experiment that the distribution of Na^+ content in various organs of cabbage seedlings followed the order of leaf > stem > root, and with the increasing seawater concentration, the Na^+ concentration in various organs found statistically significant increases. The experiment indicated that the Cl^- content order was leaf > stem > root. Distribution of K^+ and Ca^{2+} content in various organs of cabbage seedlings followed the order of leaf > stem > root. The content of Mg^{2+} in leaves was higher than that in stems and roots. K^+, Ca^{2+} and Mg^{2+} concentrations cut back in varying degrees with the increasing seawater concentration. Thus the roots of cabbage seedlings absorbed K^+, Ca^{2+} and Mg^{2+} then transported them to the leaves of cabbage ensuring the ion transportation, maintaining their physiological metabolism.

In conclusions, low sea salt concentration could promote biomass accumulation for cabbage seedlings, but high sea salt concentration significantly inhibited cabbage seedlings' growth. Both Na^+ and Cl^- concentrations increased with the increasing sea salt concentrations. But K^+, Ca^{2+} and Mg^{2+} concentrations decreased gradually when the sea salt concentrations reached high levels. The obtained data showed that cabbage seedlings had strong abilities to sustain seawater stress by regulating transportation and distribution of ions. Further, pyramiding regulatory genes controlling various aspects of salt tolerance (i.e., ionic and osmotic homeostasis) are expected to yield high tolerance to seawater and similar stresses. However, the effects of stress in relation to plant ontogeny should be assessed as realistic stress pressures that occur naturally in the field.

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