不同浓度海水对菊芋幼苗生长及生理生化特性的影响

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摘要：种植抗盐耐海水植物是合理利用和开发海涂资源的有效措施之一。采用水培的方式，用 1/2 Hoagland 营养液培养菊芋幼苗至 6 叶完全展开时进行处理，设 0%（对照）、10%、25%和 50%海水 4 个处理。随后分别在第 4、8 和 12 天采样进行分析，研究不同浓度海水对菊芋幼苗生长、体内渗透物质的积累、保护性酶活性、膜透性及离子吸收分布的影响情况。结果表明：(1)在不同浓度海水处理下，菊芋地上部、地下部总鲜重及干物质重从 0%到 25%海水浓度没有明显变化，在 50%海水胁迫下显著下降，干物质百分比则为 50%海水处理的最高。随着时间延长，10%海水处理下，菊芋幼苗茎叶和根鲜重均增加，但与对照没有显著差异，25%海水处理生长速率较对照低，而 50%海水处理下根鲜重和干重都降低。(2)随着时间的延长、海水浓度的增加，菊芋幼苗叶片保护性酶系 SOD、POD、CAT 的活性呈上升趋势，在 10%海水处理下膜脂过氧化物 MDA 含量甚至低于对照，而 50%海水处理下的 MDA 含量较其他处理高，在 10%和 25%海水处理下膜透性较对照变化不显著，而 50%海水处理下膜透性增加明显，且随时间延长更显著。(3)菊芋幼苗叶片脯氨酸和可溶性糖含量随海水浓度增高而显著增加，随着时间的延长，10%和 25%海水处理下，脯氨酸含量先增加后降低，而 50%海水处理下，脯氨酸含量一直在升高，而 10%、25%和 50%海水处理下，可溶性糖含量先增加后降低。随海水浓度增高，菊芋幼苗地上部单位干重积累的 Na^+ 和 Cl^- 依次增大，且随着时间延长，10%、25%和 50%海水处理下地上部 Na^+ 和 Cl^- 含量均增大；而 K^+ 与 Na^+ 积累情况不同，K^+ 在 25%海水胁迫下地上部单位干重积累得最多，随着时间延长，25%和 50%海水处理下地上部 K^+ 含量均降低，且 50%海水处理下降低幅度更大；地下部单位干重积累的 Na^+、Cl^- 和 K^+ 情况与地上部单位干重积累的各离子趋势相似。由此可见，菊芋能够通过生理生化机制适应一定浓度海水的灌溉，即利用一定浓度海水灌溉菊芋是安全有效的。

关键词：海水胁迫；菊芋；渗透物质；酶活性；膜透性；离子

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Effects of seawater with different concentrations on growth and physiological and biochemical characteristics of *Helianthus tuberosus* seedlings

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Abstract: Growing plants that can tolerate seawater is one of the valid measures of reasonable use and exploitation of coastal beaches. Pot experiments were carried out to study effects of different concentrations seawater on growth, osmotica accumulation antioxidant enzyme, membrane leakage and ion distributions of *Helianthus tuberosus* seedlings. Four treatments with six replicates consisting of 0% (distilled water), 10%, 25% and 50% seawater were set up by a randomized complete block design. Plant aerial parts and roots were harvested 4, 8 and 12 days after treatment and assayed for fresh weight (FW), dry weight (DW), and contents of water, Na^+, K^+ and Cl^-. Meanwhile, leaves were assayed for activities of antioxidant enzymes, malondialdehyde (MDA) contents, electrolytic leakage percentage (ELP), proline and soluble-sugar contents. Results were as follows: (1) Compared with the control, there were slight changes of FW and DW in roots and aerial parts of

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Helianthus tuberosus seedlings treated with 10% and 25% seawater, whereas a significant ($p<0.05$) decrease in both FW and DW occurred under 50% seawater. But the water content in aerial parts and roots were the lowest under 50% seawater. (2) The activities of SOD (superoxide dismutase), POD (peroxidase) and CAT (catalase) in leaves of seawater-stressed plants were stimulated significantly compared with controlled plants, and increased with increasing seawater concentrations. Compared with the control, there was slight change of ELP in leaves of *Helianthus tuberosus* seedlings treated with 10% and 25% seawater, whereas ELP increased significantly with the treatment of 50% seawater. With time lasting, ELP increased more under 50% seawater. (3) Contents of proline and soluble-sugar increased with increasing seawater concentrations. Contents of proline increased on Day 8 and decreased on Day 12 under 10% and 25% seawater, but increased significantly on Day 8 and onward under 50% seawater. Contents of soluble-sugar on Day 8 increased compared with those on Day 4 and decreased on Day 12 under 10%, 25% and 50% seawater. Contents of Na^+ and Cl^- in the aerial parts increased with increasing seawater concentration. Compared with Na^+, K^+ contents were highest under 25% seawater. And the tendencies of Na^+, Cl^- and K^+ in roots were similar with those in the aerial parts. The results of the present study strongly suggest that *Helianthus tuberosus* could adapt appropriate concentration seawater irrigation through physiological and biochemical mechanisms, which was effective and secure.

Key words: seawater stress; *Helianthus tuberosus*; osmotica; antioxidant enzyme; membrane permeability; ions

1 INTRODUCTION

Salinity toxicity is a worldwide agricultural and eco-environmental problem. Approximately one-third of the world land surface is arid or semi-arid[1], in which fresh water shortage is the main limiting factor for sustainable development of agriculture[2~4]. It is estimated that there are approximately 18000 km coastline and 20779 km^2 salinised soils in coastal areas in China[4]. Seawater irrigating agriculture is a new branch of traditional agricultures and one of valid measures of reasonable use and exploitation of seawater, saline soils along the coast and salt-tolerant plants. Selection of seawater concentration is the most important for plant production of seawater irrigating agriculture because plasma membranes are injured by the accumulation of excess salts in plant tissues when grown under strong seawater irrigation[4].

Helianthus tuberosus is considered to be able to bear cold, drought, leanness and salinity. Its aerial part and tuber provide good quality feed and the tuber can be used as vegetable and for alcohol production[4~6]. We have carried out field experiments of seawater irrigating agriculture on *Helianthus tuberosus* in typical semi-arid coastal areas of north China since 1999[4].

The objective of this research was to investigate growth and the physiological and biochemical mechanisms of *Helianthus tuberosus* under seawater stress. Changes of the growth, inorganic and organic osmoticas, antioxidant enzymes and injury of membrane of seedlings on time-dependent were studied in the present work by water culture because *Helianthus tuberosus* is sensitive to seawater at seedling stage.

2 Materials and methods

2.1 Plant materials and treatments

Helianthus tuberosus tubers were provided by Experiment Base of "863" in Laizhou, Shandong Province. A slice of *Helianthus tuberosus* with a bud was surface sterilized with $HgCl_2$ (1.0 g/L) for 10 min, then rinsed thoroughly with distilled water, and germinated on moist sands for some days in an incubator at 25 ℃. Uniform germinated slices were selected and sown in plastic containers filled with quartz sand, grown in the glasshouse and watered with half strength Hoagland nutrient solution. When the sixth leaf fully spread, slices of plants with uniform size were planted into hydroponics plastic pots fitted with insulated covers. Each pot was covered with a polythene lid through which plants were supported over the nutrient solution. There was one plant in each pot. The pot contained 400 mL of half Hoagland nutrient solution, which was aerated for 2 h daily and renewed every other day extended for 12 d. Daily photoperiod was 12 h and maximum temperature was 25 ℃ while the daily minimum temperature at night was 15 ℃. The relative humidity was 60%~70%.

Seawater treatments were initiated by adding salt to the nutrient solution immediately after slices were transplanted to the nutrient solution. The salt was produced by evaporation from Laizhou bay seawater. The basic properties of Laizhou bay seawater contain HCO_3^- 0.132 g/L, Cl^- 17.515 g/L, SO_4^{2-} 3.867 g/L, Ca^{2+} 0.785 g/L, Mg^{2+} 1.027 g/L, K^+ 0.596 g/L, Na^+ 9.480 g/L. Four treatments were the concentrations of 0% (distilled water), 10%, 25% and 50% seawater. Every treatment had independent experiment with six replicates for measuring the growth, inorganic and organic osmoticas, antioxidant enzyme and membrance leakage.

2.2 Assays of fresh weight, dry weight, water and ion contents of aerial parts and roots

Fresh plant samples obtained 4, 8 and 12 days after seawater treatment were used for aerial parts and roots fresh weight analysis. The plants were oven-dried at 110 ℃ for 10 min and then dried to constant weight at 60 ℃. The dry samples were used for analysis of dry weight, content of water in aerial parts and roots. Content of water was determined using the following formula: $W = (1 - D/F) \times 100\%$, here W is content of water, D is dry weight and F is fresh weight.

Dry samples were milled before acid digestion in 1 mL 65% (V/V) ultra pure nitric acid at 500℃ for 6 h. Concentrations of Na^+ and K^+ were measured with flame absorption spectrophotometry[7]. Concentrations of Cl^- were determined by titration with standard silver nitrate after 50 mg dry samples were steamed in 20 mL distilled water at 100 ℃ for 45 min[8].

2.3 Lipid peroxidation assays

TBA (thiobarbituric acid) test was used for measuring lipid peroxidation in leaves. This determined concentrations of MDA (malondialdehyde) as an end product of lipid peroxidation. For this, leaf tissues (500 mg) were homogenised in 3mL 0.1% TCA (trichloroacetic acid) solution. The homogenate was centrifuged at 5000× g for 10 min and the supernatant was assayed for MDA concentration[9].

2.4 Enzyme activities assays

Fresh leaf samples of *Helianthus tuberosus* seedlings obtained 4, 8 and 12 days after seawater treatment were used for enzyme activities analysis. Leaves were frozen in liquid nitrogen immediately after harvesting and stored at −20 ℃ until enzyme assays. A portion of leaf material 1 g was mixed in 3mL of 0.05 mol/L phosphate buffer (pH 7.8) including 1 mmol/L EDTA and 2% (W/V) PVP. The homogenate were centrifuged at 10000×g for 15 min at 4 ℃. Supernatant was used for enzyme activity and protein content assays. All assays were done at 4 ℃. All spectrophotometric analyses were conducted on Shimadzu (UV-1600) spectrophotometer.

Superoxide dismutase (SOD) activity assay was based on the method of Giannopolitis and Ries[10], which measures the inhibition in the photochemical reduction of nitroblue tetrazolium (NBT) spectrophotometrically at 560 nm. One unit of enzyme activity was defined as the quantity of SOD required to produce a 50% inhibition of reduction of NBT and the specific enzyme activity was expressed as units mg^{-1} protein g FW. The reaction mixture contained 50 mmol phosphate buffer (pH 7.8), 750 μmol/L NBT, 130 mmol/L L-methionine, 0.1 mmol/L EDTA and 0.02 mmol/L riboflavin. Reactions were carried out at 25 ℃ under light intensity of about 300 μmol/(m · s) for 15 min.

Peroxidase activity (POD) was based upon the method as described by Herzog and Fahimi[11], which measures the increase in absorbance at 460 nm. The increase in A460 was followed for 3 min. To 0.1 mL of the enzyme extract, a substrate mixture containing acetate buffer (0.1mol/L, pH 5.4), ortho-dianisidine (0.25% in ethyl alcohol) and 0.1 mL 0.75 % H_2O_2 was added. Absorbance change of the brown guaiacol at 460 nm was recorded for calculating POD activity. Activity was expressed as units (μmol of H_2O_2 decomposed per minute) per mg of protein.

Catalase (CAT) activity was assayed using the method described by Aebi[12]. The reaction mixture contained 50 mmol/L phosphate buffer (pH 7.0), 45 mmol/L H_2O_2 and 100 μL of enzyme extract in a 3 mL volume. The activity was assayed by monitoring the decrease in absorbance at 240 nm as a consequence of H_2O_2 consumption. Activity was expressed as units (μmol of H_2O_2 decomposed per minute) per mg of protein.

Soluble protein concentration in the different extracts was measured at 660 nm by the method of Bradford[13] using the Folin-Ciocalteau reagent with bovine-serum albumin as a standard.

2.5 Osmotica substance assays

Proline was determined in nodule extracts using ninhydrin reagent. For the calculation of proline concentration, a standard curve was prepared with L-proline. Assay for soluble sugars followed the colorimetric method of Irigoyen *et al*[14].

2.6 Membrane permeability assays

The membrane permeability was measured by using electrical conductivity method described by Liu *et al*[15].

2.7 Statistical analysis

Analysis of variance was performed and statistical significance ($p < 0.05$) of differences among treatment means was judged by Duncan's New Multiple Range Test using SPSS software (10.0).

3 Results

FW of aerial parts and roots of *Helianthus Tuberosus* seedlings under 10% seawater increased with time and there were no significant difference compared with 0% seawater ($p<0.05$) (Table 1). While FW of aerial parts and roots treated with 25% seawater were lower than those treated with 0% seawater, and were 82% and 87% of those under 0% seawater on Day 12, respectively. Under 50% seawater, FW of aerial parts and roots significantly decreased on Day 12 compared to those on Day 4, and were only 33.8% and 27.0% of those on Day 4 (Table 1). The trends of DW of aerial parts and roots of *Helianthus Tuberosus* seedlings resembled those of FW. DW of aerial parts and roots all increased with the increasing level of seawater concentrations. For all harvest days, DW was significantly lower under 50% seawater treatment compared with other treatments. Like FW, DW of aerial parts and roots under 50% seawater all declined sharply over time. For example, DW of aerial parts and roots on Day 12 were only 45.8% and 70.0% of those on Day 4 (Table 1).

Table 1 Effects of seawater concentrations on fresh (FW) and dry biomass weight (DW) of aerial parts (AP) and roots (R) of *Helianthus tuberosus* seedlings (g)

Seawater concentration (%)	Day after treatment											
	4				8				12			
	FW		DW		FW		DW		FW		DW	
	AP	R	AP	R	AP	R	AP	R	AP	R	AP	R
0	3.48a[a)]	3.30a	0.42a	0.19a	4.26a	4.35a	0.49a	0.25a	5.58a	5.03a	0.59a	0.33a
10	3.56a	3.54a	0.43a	0.20a	4.58a	4.68a	0.53a	0.26a	5.59a	4.96a	0.58a	0.32a
25	3.36a	3.65a	0.38a	0.21a	4.18a	4.23a	0.46a	0.25a	4.59b	4.37b	0.53a	0.30a
50	1.42b	1.59b	0.24b	0.10b	1.28b	1.12b	0.22b	0.11b	0.48c	0.43c	0.11b	0.07b

Date are expressed as means ($n=3$), a) Means marked with the same letter in the same column are not significantly ($p<0.05$) different by Duncan's New Multiple Range Test

Contents of water in aerial parts and roots of *Helianthus tuberosus* seedlings with different treatments were shown in Table 2. Water contents of aerial parts and roots under 0%, 10% and 25% seawater didn't change significantly over time. While water contents changed greatly under 50% seawater over time. Water contents on Day 12 were only 92.8% and 89.3% of those on Day 4.

Table 2 Effects of seawater concentrations on contents of water in aerial parts (AP) and roots (R) of *Helianthus tuberosus* seedlings

Seawater concentration (%)	Day after treatment					
	4		8		12	
	AP	R	AP	R	AP	R
0	87.93a[a)]	94.24a	88.50a	94.25a	89.43a	93.44a
10	88.25a	94.36a	88.43a	94.44a	89.24a	93.42a
25	89.33a	94.25a	89.00a	94.09a	88.46a	93.14a
50	83.10b	93.71b	82.81b	90.18b	77.08b	83.72b

Date are expressed as means ($n=3$), a) Means marked with the same letter in the same column are not significantly ($p<0.05$) different by Duncan's New Multiple Range Test.

Membrane lipid peroxidation in *Helianthus tuberosus* seedling leaves was assessed by contents of MDA (Fig. 1). Compared to control, MDA contents under 10% seawater were lower than those of the control on Day 8 and Day 12. MDA contents under 25% seawater declined on Day 12 compared to those on Day 8. Under 50% seawater, MDA contents were higher than those of other treatments on Day 4, Day 8 and Day 12, and were 1.4, 1.7 and 5.3 times of those of the control, respectively. With prolonged treatment, contents of MDA on Day 12 increased and were 2.98 and 2.01 times of those on Day 4 and Day 8, respectively.

Table 3 showed that the effects of seawater concentrations on electrolytic leakage percentage (ELP) of *Helianthus tuberosus* seedlings leaves. Compared to the control, ELP under 50% seawater significantly increased on all sampling days. And over time the trends were more obvious. For example, ELP of leaves under 50% seawater reached 31.56% and 60.12% on Day 8 and Day 12, respectively. These results strongly suggest that leaves cells membrane permeability increased significantly under 50% seawater.

SOD activities of *Helianthus tuberosus* seedling leaves under different seawater concentrations were also shown in Fig. 1. SOD activities under 0%, 10% and 25% seawater all increased with time, and on Day 12 the activities were 1.1, 1.6 and 1.9 times of those on Day 4, respectively. Furthermore, SOD activities under 25% seawater were higher than those of 0% and

10% seawater. On Day 12, SOD activities under 25% seawater were 1.9 times for the control and 1.4 times for 10% seawater treatment. While SOD activities under 50% seawater on Day 8 were higher than those on Day 4 and Day 12. And they declined sharply on Day 12 and were only 41.6% of those on Day 8.

The trends of POD activities of *Helianthus tuberosus* seedling leaves were similar under 10%, 25% and 50% seawater. POD activities on Day 8 under 10%, 25% and 50% seawater were higher than those on Day 4 and Day 12. POD activities of the control on Day 12 were higher than those on Day 4 and Day 8. But they were lower than those under 10%, 25% and 50% seawater on Day 12. POD activities under 50% seawater were higher than those of other treatments on Day 4, Day 8 and Day 12. For instance, POD activities under 50% seawater were 50.0%, 90.3% and 59.0% higher than those under 25% seawater on Day 4, Day 8 and Day 12, respectively (Fig. 1).

Table 3　Effect of seawater concentrations on ELP of *Helianthus tuberosus* seedlings leaves (%)

Seawater concentration (%)	Day after treatment		
	4	8	12
0	4.99c[a)]	5.08b	5.20c
10	4.93c	5.01b	5.17c
25	5.44b	5.32b	6.03b
50	8.13a	31.56a	60.12a

Date are expressed as means ($n=3$), a) Means marked with the same letter in the same column are not significantly ($p<0.05$) different by Duncan's New Multiple Range Test

CAT activities of *Helianthus tuberosus* seedling leaves under 0%, 10%, 25% and 50% seawater all increased over time (Fig. 1). CAT activities under 0%, 10%, 25% and 50% seawater on Day 12 were 3.0, 5.8, 4.1 and 4.5 times of those on Day 4, respectively. CAT activities increased with increasing seawater concentration. CAT activities on day 4 under 50% seawater were 16.2%, 106.0%, 17.7% higher than 0%, 10% and 25% seawater, respectively. CAT activities under 10%, 25% and 50% seawater on Day 8 were 105.3%, 67.7%, 19.1% higher than those under 0% seawater, and on day 12 were 231.4%, 59.6% and 29.9% higher than those under 0% seawater.

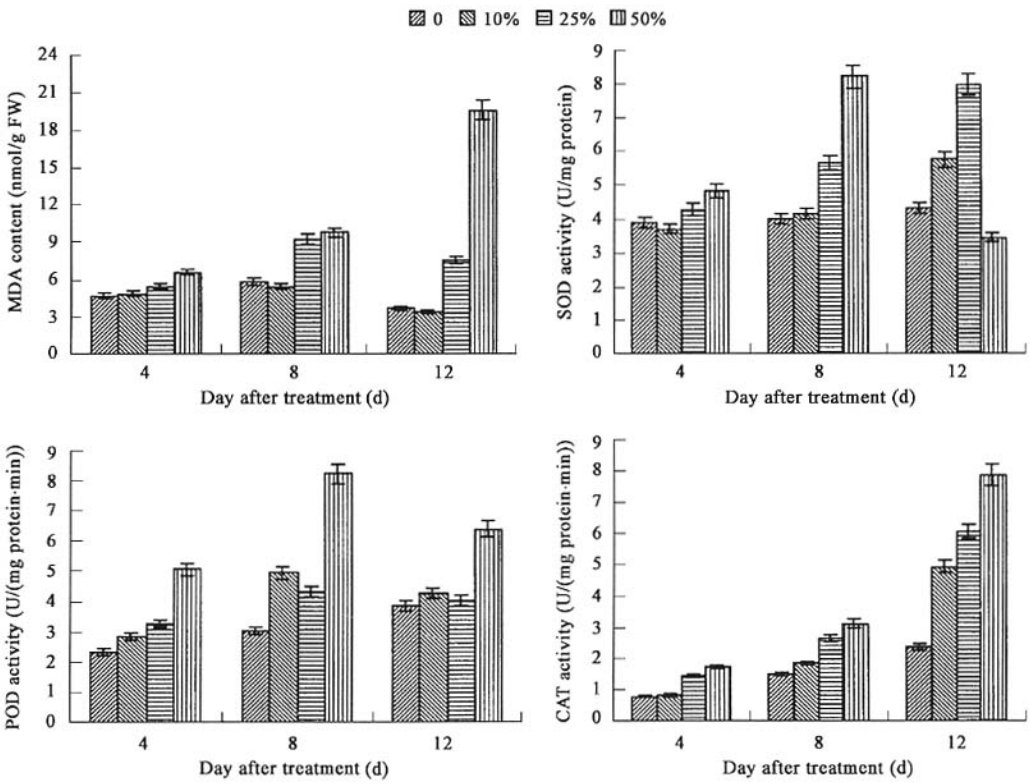

Fig. 1　MDA contents, SOD, POD and CAT activities in *Helianthus tuberosus* seedling leaves in response to seawater concentrations. Data represents the average of three replicates

Proline contents in *Helianthus tuberosus* seedling leaves under different treatments are shown in Table 4. proline contents under 10%, 25% and 50% seawater were all higher than those under 0% seawater on Day 4, Day 8 and Day 12 Furthermore, it showed a significant increase in proline contents with increasing seawater concentration. Proline contents under 50% seawater were 5.49, 121.76 and 421.84 times of those under 0% seawater respectively. Proline contents under 10% and 25% seawater on Day 8 were higher than those on Day 4 and Day 12. However, proline contents under 50% seawater increased sharply over time. Like the proline, soluble-sugar contents of *Helianthus tuberosus* seedling leaves rose with seawater concentrations increasing. And soluble-sugar contents under 10%, 25% and 50% seawater on Day8 were higher than those on Day 4 and Day 12, and those on Day 12 declined 60.4%, 42.5% and 39.4% of those on Day 8, respectively (Fig. 2).

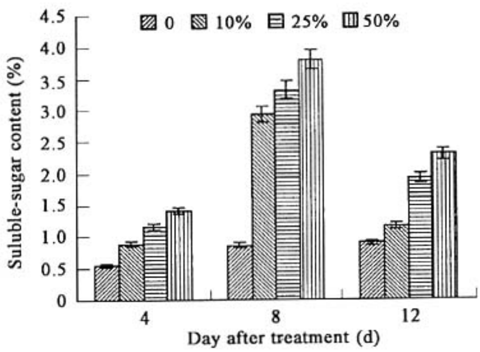

Fig. 2 Soluble-sugar contents of *Helianthus tuberosus* seedlings in response to seawater concentrations. Data represents the average of three replicates

Table 4 Effects of seawater concentrations on proline contents in leaves of *Helianthus tuberosus* seedling(μg/g FW)

Seawater concentration (%)	Day after treatment		
	4	8	12
0	17.12d[a)]	8.97d	5.20d
10	24.21c	41.64c	23.73c
25	41.72b	431.08b	233.68b
50	94.07a	1092.17a	2193.53a

Date are expressed as means($n=3$), a)Means marked with the same letter in the same column are not significantly ($p<0.05$) different by Duncan's New Multiple Range Test

Na^+ and K^+ contents in aerial parts and roots of *Helianthus tuberosus* seedlings with different treatments are shown in Table 5. Na^+ contents in aerial parts and roots rose significantly with increasing seawater concentration and time lasting. While K^+ contents in aerial parts and roots under 25% and 50% seawater declined over time. Under 0% and 10% seawater, the K^+ contents didn't change significantly.

Table 5 Effects of seawater concentrations on K^+ and Na^+ content of aerial parts (AP) **and roots** (R) **of *Helianthus tuberosus* seedlings**(mmol/g DW)

Seawater concentration (%)	Day after treatment											
	4				8				12			
	K^+		Na^+		K^+		Na^+		K^+		Na^+	
	AP	R	AP	R	AP	R	AP	R	AP	R	AP	R
0	1.24c[a)]	1.16c	0.05c	0.16d	1.12b	1.29b	0.06d	0.31d	1.04a	1.20b	0.07d	0.28d
10	1.44b	1.61a	0.17c	0.73c	1.20b	1.32b	0.41c	1.13c	1.05a	1.41a	0.55c	1.48c
25	1.61a	1.65a	0.71b	1.07b	1.33a	1.45a	1.05b	1.32b	1.12a	1.40a	1.29b	1.99b
50	1.28c	1.41b	0.96a	1.57a	0.91c	1.31b	1.27a	1.87a	0.45b	0.95c	1.69a	2.13a

Date are expressed as means($n=3$), a) Means marked with the same letter in the same column are not significantly ($p<0.05$) different by Duncan's New Multiple Range Test

Like Na^+, Cl^- contents in aerial parts and roots rose significantly with the seawater concentrations enhancing (Table 6). Cl^- contents in aerial parts and roots under 50% seawater were 9 and 8 times, 8 and 6 times, 9 and 7 times of those under 0% seawater on Day 4, Day 8 and Day 12, respectively. And with the time lasting, Cl^- contents in aerial parts and roots increased under 10%, 25% and 50% seawater.

4 Discussion

Salt tolerance of different plants and different growth stages of same plant is dissimilar because it is decided by their transmissibility of the plant. In the present study, there were distinct effects of 50% seawater on growth of *Helianthus tuberosus* seedlings. For example, the number of leaves, growth of stem, leaf and roots were restricted. It was possibly because the high salt concentration affected cell differentiation and the rate of cell extension. Solute infiltration increased with the augment of seawater concentration, which reduced the absorption of water and induced the fall of water content of *Helianthus tuberosus* seedlings.

One effect of free oxygen radicals accumulation in plant cells under stress is lipid peroxidation via oxidation of unsaturated fatty acids leading to membrane damage and electrolyte leakage. Membrane lipid peroxidation is thought to be one of the most important mechanisms of salt toxicity in higher plants[16]. SOD, CAT and POD are the major antioxidant enzymes associated with scavenging the active oxygen species (AOS) and SOD is likely to be central in the defence against toxic AOS[17]. However, SOD detoxifies superoxide anion free radicals accompanying the formation of H_2O_2, which is very damaging to the chloroplasts, nucleic acids and proteins[18] and can be eliminated by catalase and peroxidase[19,20].

Table 6 Effects of seawater concentrations on Cl^- contents of aerial parts (AP) **and roots** (R) **of *Helianthus tuberosus* seedlings** (mmol/g DW)

Seawater concentration (%)	Day after treatment					
	4		8		12	
	AP	R	AP	R	AP	R
0	0.21d[a)]	0.27d	0.26c	0.37d	0.27d	0.31c
10	0.87c	1.06c	1.16b	1.70c	0.93c	2.06b
25	1.19b	1.66b	1.93a	2.02b	1.60b	2.26a
50	1.87a	2.07a	2.00a	2.31a	2.35a	2.27a

Date are expressed as means ($n=3$), a) Means marked with the same letter in the same column are not significantly ($p<0.05$) different by Duncan's New Multiple Range Test

In the present study, under the stress of 50% seawater, contents of MDA and ELP were higher than those of other treatments on Day 4, Day 8 and Day 12, respectively, which indicated that the membrane configuration had been destroyed in a sort of way under 50% seawater. However, the lower contents of MDA under 25% seawater on Day 12 than those on Day 8 indicated that *Helianthus tuberosus* seedlings had adapted well past the initial shock period. Compared with control, there was no damage to the *Helianthus tuberosus* seedlings membrane under 10% seawater from Fig. 1 and Table 3. This indicates that *Helianthus tuberosus* seedlings may has a high hereditary and induced capability under seawater which provides it a better protection from oxidative damage caused by seawater. This protection might also have been due to significantly high constitutive activities of SOD as well as constitutive and induced activities of POX, APOX and CAT in the leaves of *Helianthus tuberosus* seedlings.

All antioxidant enzymes were stimulated in plants exposed to salt in the present study. This may be a general adaptive defence response of plants to toxic saline environments at early stages. It is noted that the induction of SOD activity coincided with an increase in the activity of the enzymes (POD and CAT) scavenging H_2O_2[21]. Bowler agrees that the cooperation between H_2O_2 scavenging enzymes and SOD plays an important role in resistance of plants to environmental stresses[22]. We believe that such coordination also plays a crucial role in preventing plants from salt injury. The results of this study show that SOD activities increased with the augment of seawater concentration, which may be a general adaptive defence mechanism of plants to seawater environments at early stages, although under 50% seawater SOD gradually lost activity on Day 12. POD is among the enzymes that scavenges H_2O_2 in chloroplasts which is produced through dismutation of O_2^- catalyzed by SOD[23]. Increased POD activity has also been reported in salt-tolerant and sensitive species of tomato[24] and rice cultivars[25]. POD activities in this study increased significantly with increased seawater concentration. The trends of POD activities over time were similar under 10%, 25%and 50% seawater. CAT activities together with SOD are the most effective antioxidant enzymes in preventing cellular damage[26]. Like POD and SOD activities in this study, CAT activities increased significantly with the augment of seawater concentration and the trends of CAT activities over time were similar under 0%, 10%, 25% and 50% seawater.

The accumulation of some organic solutes under saline conditions has been considered as an adaptation of plants against osmotic stress. Amino-acids such as proline and asparagines can play an important role in the osmotic adjustment of the plant under saline conditions[27]. Other organic compounds that accumulate in response to stress, such as soluble sugars, apparently play a role in the development of salt tolerance[28]. In addition, plant species adjust to high salt concentrations by lowering tissue osmotic potential with the accumulation of inorganic as well as organic solutes[29]. Cations Na^+ and K^+ and anion Cl^- are known to be the major inorganic components of osmotic potential[30,31]. In this study proline contents on Day 8 were higher than they on Day 12 with under 0%, 10% and 25% seawater, while proline contents under 50% seawater stress increased sharply during the experimental period. In function, proline is often regarded as a compatible osmolite associated with the salt-resistance mechanisms, and can also help lower the cell's osmotic potential. Our results revealed soluble sugar contents with different treatments increased earlier and declined later during the experimental period, suggesting that soluble sugar is an organic component of osmotic adjustment during the initial stages of stress. In the present study, Na^+ and Cl^- contents in aerial parts and roots increased with increased seawater concentration. Over time, Na^+ and Cl^- concentrations also increased. On the contrary, K^+ contents in aerial parts and roots declined with the time and also with increased seawater concentration. Thus high concentration of salts in seawater can affect the absorptions of ions and increases the accumulation of these ions and thus make them involved in osmotic adjustment.

The results of the present study strongly indicated that *Helianthus tuberosus* could adapt appropriate concentration seawater irrigation through physiological and biochemical mechanisms. In conclusion, our results indicate that low concentration seawater, such as 10% and 25% seawater didn't decrease *Helianthus tuberosus* seedling growth, even promoted growth during the experimental period. On the other hand, higher SOD, POD, CAT activities and MDA, proline, soluble sugar, Na^+, K^+ and Cl^- contents under 10% and 25% seawater, which probably come from an increased capacity for oxygen radical scavenging and maintenance of cellular membranes and osmotic adjustment, indicated the relationship between antioxidant defense and seawater tolerance. Further investigations are necessary to put forward the effect of seawater by means of subcellular compartmentation of antioxidative enzyme activities, which would be a useful tool for our understanding of seawater stress and explaining these phenomena. Also, the results of the present study coupled with the reports in the literature[4] strongly suggest that *Helianthus tuberosus* with appropriate concentration seawater irrigation is effective and secure.

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