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Effect of salinity on seed germination, seedling growth, and inorganic and organic solutes accumulation in sunflower (*Helianthus annuus* L.)

G.-Q. Wu, Q. Jiao, Q.-Z. Shui

School of Life Science and Engineering, Lanzhou University of Technology, Lanzhou, P.R. China

ABSTRACT

To investigate the effects of saline stress on seed germination, ion distribution, and organic solutes changes of sunflower (*Helianthus annuus* L.), in the present study, seeds and 3-week-old seedlings were subjected to a series of external NaCl concentrations (5–200 mmol). The results showed that high salinity (200 mmol) remarkably inhibited the germination of seed and delayed germination time in sunflower. It was found that 25–200 mmol NaCl significantly reduced both stem and leaf dry weight. Concentrations of 100 and 200 mmol also caused a clear reduction in tissues water content. With the increase of NaCl concentration, Na^+ concentrations both in root and stem showed the increasing trend, whereas to a lesser degree in root than in stem. In leaf, Na^+ concentration remained unchanged when the external concentrations of NaCl were below 100 mmol, while significantly increased by 41-fold when plants were exposed to 200 mmol. By contrast, K^+ concentration in root displayed the decreasing trend with the increase of NaCl concentrations. Neither lower (5 and 10 mmol) nor higher (100 and 200 mmol) salinity significantly affected K^+ concentration both in stem and leaf, while moderate levels (25 and 50 mmol) significantly enhanced K^+ accumulation. High salinity significantly enhanced soluble sugar concentration in stem by 28% and proline in leaf by 166%. It was proposed that sunflower plants adapt to saline stress to some extent through regulating distribution of Na^+ and K^+, maintaining higher selective absorption capacity for K^+ over Na^+, and accumulating more osmoprotectants, such as soluble sugar and proline.

Keywords: soil salinization; sodium chloride; salinity tolerance; osmoregulation; osmotic adjustment; arid environment

Salinity is one of the major abiotic factors that limits plant growth and productivity in many regions of the world due to increasing use of poor quality of water for irrigation and soil salinization (Munns and Tester 2008, Rozema and Flowers 2008, Kronzucker and Britto 2011, Zhang and Shi 2013). One of the most detrimental effects of salinity is the accumulation of Na^+ and Cl^- ions in tissues of plants subjected to soils with high concentrations of NaCl (Maathuis et al. 2014). High concentration of Na^+ inhibits uptake of K^+ which is an essential macronutrient for plants growth and development that results in low productivity and may even cause death (Kronzucker et al. 2013, Gupta and Huang 2014). Plants have developed various physiological and biochemical mechanisms to maintain a relatively stable intracellular environment via accumulating various solutes under saline condition (Gupta and Huang 2014, Roy et al. 2014). The osmotic adjustment in plants can maintain the uptake of water and the turgor of cell, allowing regular physiological metabolisms (Radić et al. 2013). Proline, as an important osmoprotectant, contributes to osmotic adjustment, protecting enzymes from oxidative damage under saline condition (Ashraf and Harris 2004, Gupta and Huang 2014). In addition, under saline condition, the accumulation of other compounds such

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as soluble sugar, which at higher temperatures are non-toxic to cytoplasmic functions, allowing turgor maintenance and/or protection of macromolecular structure against the destabilizing effects of the decrease in water activity, has also been reported in many plants species (Juan et al. 2005).

Sunflower (*Helianthus annuus* L.), a species of the Asteraceae family, is the third most important source of edible vegetable oil worldwide (Talia et al. 2011). It is also thought to become an efficient source of biodiesel and widely cultivated in the arid and semi-arid regions of north China. Although the suppressing effects of salinity on sunflower plants were reported (Rauf et al. 2012), information regarding the effects of salinity on ion distribution and organic solutes accumulation in this species is yet incomplete.

The aim of the present study was to investigate seed germination, seedling growth, ion distribution, and organic solutes changes of sunflower to gradient salinity. For this purpose, the seeds and seedlings were subjected to various levels of NaCl, and an analysis of the parameters related to germination, growth, ion and organic solute accumulation was made.

MATERIAL AND METHODS

Seeds of sunflower (*Helianthus annuus* L. cv. Longkuiza3) were kindly provided by the Jingye Agriculture Science and Technology Ltd. Co., Gansu province, China, in mild December 2013. Seeds were surface-sterilized for 5 min in 75% (v/v) ethanol and rinsed 3 times with distilled water, soaked in distilled water for 12 h. For the germination tests, three 50-seed replicates were used with either distilled water (control) or solutions of NaCl (5, 10, 25, 50, 100, and 200 mmol). Seeds were placed on filter paper in 9 cm diameter Petri dishes containing 10 mL each solution. Seeds were considered as germinated when the radicle had protruded 2 mm through the seed coat.

The number of seed germinated was recorded daily for up to 7 days. From these germination counts, several germination attributes were calculated to characterize salinity tolerance, including coefficient of velocity of germination (CVG), germination rate index (GRI), and mean germination time (MGT) (Panuccio et al. 2014).

For morphological and physiological experiments, seeds were germinated in the Petri dishes. After 3 days from the beginning of germination, uniform seedlings were carefully transplanted to plugged holes in plastic pots (15 cm diameter × 20 cm height) filled with vermiculite irrigated with the modified Hoagland nutrient solution. Three-week-old plants were treated with the modified Hoagland nutrient solution supplemented with 0, 5, 10, 25, 50, 100, and 200 mmol NaCl for 7 days, respectively. Solution was renewed every 3 days. At the end of treatments, the roots of seedlings were washed twice for 8 min in ice-cold 20 mmol $CaCl_2$ to exchange cell wall-bound Na^+ (Wang et al. 2007) and the shoots were rinsed in deionized water to remove surface any salts. Shoot length was immediately measured by caliper. Roots, stems, and leaves were separated and blotted. Fresh weight (FW) was immediately determined and then samples were dried in an oven at 80°C for 48 h to obtain dry weight. Water content (WC) was estimated using the following formula as described by Wu et al. (2013):

$$WC\ (g/g\ DW) = (FW - DW)/DW.$$

Na^+ and K^+ concentrations were measured according to the method described by Wu et al. (2013). Na^+/K^+ ratios were calculated according to the method of Yue et al. (2012). The relative distribution of Na^+ (or K^+) in different tissues were estimated by the method as described by Wang et al. (2007). The selective absorption (SA) and selective transport (ST) for K^+ over Na^+ were estimated according to the method of Wang et al. (2009).

Soluble sugar was assayed by the method as described by Zhang et al. (2006) using the anthrone ethyl acetate reagent. Proline was measured according to the method of Bates et al. (1973) using the ninhydrin reagent.

The obtained data were examined by one-way analysis of variance (ANOVA) using statistical software (SPSS 19.0, Chicago, USA). Duncan's multiple range tests were performed to determine significant difference between means at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Compared with control (distilled water), NaCl solution of 10–200 mmol significantly reduced coefficient of velocity of germination (CVG) ($P < 0.05$), but increased mean germination time (MGT)

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Table 1. Effects of salinity on coefficient of velocity of germination (CVG), germination rate index (GRI) and mean germination time (MGT) in sunflower (*Helianthus annuus* L.) seeds

NaCl concentration (mmol)	CVG	GRI	MGT
	(%)		(days)
0	21.4 ± 0.2^{a}	19.2 ± 0.8^{a}	4.7 ± 0.02^{c}
5	21.3 ± 0.1^{ab}	19.3 ± 0.4^{a}	4.7 ± 0.01^{c}
10	20.9 ± 0.1^{c}	18.5 ± 0.5^{a}	4.8 ± 0.01^{b}
25	21.0 ± 0.1^{b}	18.3 ± 0.3^{a}	4.8 ± 0.01^{b}
50	20.8 ± 0.2^{c}	17.6 ± 0.8^{a}	4.8 ± 0.03^{b}
100	20.7 ± 0.1^{c}	17.8 ± 0.4^{a}	4.8 ± 0.01^{b}
200	19.6 ± 0.1^{d}	14.8 ± 0.9^{b}	5.1 ± 0.01^{a}

Fifty seeds were pooled in each replicate (n = 3). Within each column, means followed by different letters are significantly different at $P < 0.05$ (Duncan's test)

($P < 0.05$) (Table 1). High concentration (200 mmol) of NaCl also led to a significant reduction of germination rate index (GRI) by 23% ($P < 0.05$) (Table 1). CVG, GRI, and MGT are well known as three indeces that assess germination rate of seed (Panuccio et al. 2014). It was shown that higher CVG and GRI, and lower MGT represent higher and faster germination of seed (Panuccio et al. 2014). Our results indicated that high salinity remarkably inhibited the germination of seed and delayed germination time in sunflower. Similar results were recorded in other crops such as rice (*Oryza sativa*) (Xu et al. 2011), wheat (*Triticum aestivum*) (Akbarimoghaddam et al. 2011), and maize (*Zea mays*) (Khodarahmpour et al. 2012). The negative effects of high salinity may be due to ion toxicity on seed germination, as a consequence of a coincident increase in anion and cation (Panuccio et al. 2014).

One of the initial effects of salinity on plants was the reduction of growth rate (Gupta and Huang 2014). In the present study, 50, 100, and 200 mmol NaCl significantly deceased shoot length by 5, 6, and 20% ($P < 0.05$), respectively (Figure 1). These results are in agreement with the findings of Anuradha (2014) who reported a significant decline in shoot length at high salinity levels. It was observed that 25–200 mmol NaCl significantly reduced both stem and leaf dry weight ($P < 0.05$) (Figure 2a). Fresh weight of stem and leaf also showed the reducing trend at 25–200 mmol NaCl (data not shown). Reduction in FW at high salinity might be due to poor absorption of water from the growth medium due to physiological drought (Munns and Tester 2008). It was found that 10–200 mmol NaCl significantly deceased root FW ($P < 0.05$) (data not shown), while root DW significantly decreased only at 200 mmol NaCl ($P < 0.05$) (Figure 2a). 100 and 200 mmol NaCl also caused a clear reduction in tissues water content ($P < 0.05$) (Figure 2b).

With the increase of NaCl concentration, Na^+ concentrations both in root and stem showed the increasing trend, whereas to a lesser degree in root than in stem (Figure 3a). It was shown that Na^+ concentration was significantly higher in root than in stem and leaf in either the absence or the presence of salinity ($P < 0.05$) (Figure 3a). High levels of Na^+ in root can maintain the normal osmotic potential and prevent this ion from being transported to leaf, therefore, avoiding the accumulation of Na^+ into leaf (Xue et al. 2014). It was also found that Na^+ concentration in leaf remained unchanged when the concentrations of NaCl were below 100 mmol, while significantly increased by 41-fold when plants were exposed to 200 mmol NaCl ($P < 0.05$). High concentration of Na^+ in leaf could lead to osmotic damage and oxidative stress, affecting physiological and biochemical metabolisms, thus resulting in the suppression of plant growth (Gupta and Huang 2014).

K^+ participates in many cellular functions, such as activation of enzymatic reactions, charge balancing, and osmoregulation (Wakeel et al. 2011). Therefore, the control of K^+ homeostasis plays an

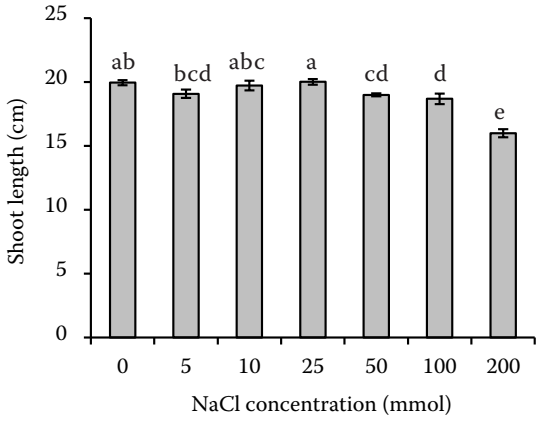

Figure 1. Effects of salinity on shoot length in sunflower (*Helianthus annuus* L.). Three-week-old seedlings were treated with 0, 5, 10, 25, 50, 100, and 200 mmol NaCl for 7 days. Three plants were pooled in each replicate (n = 8). Values are means ± standard error (SE) and bars indicate SE. Columns with different letters indicate significant differences at $P < 0.05$ (Duncan's test)

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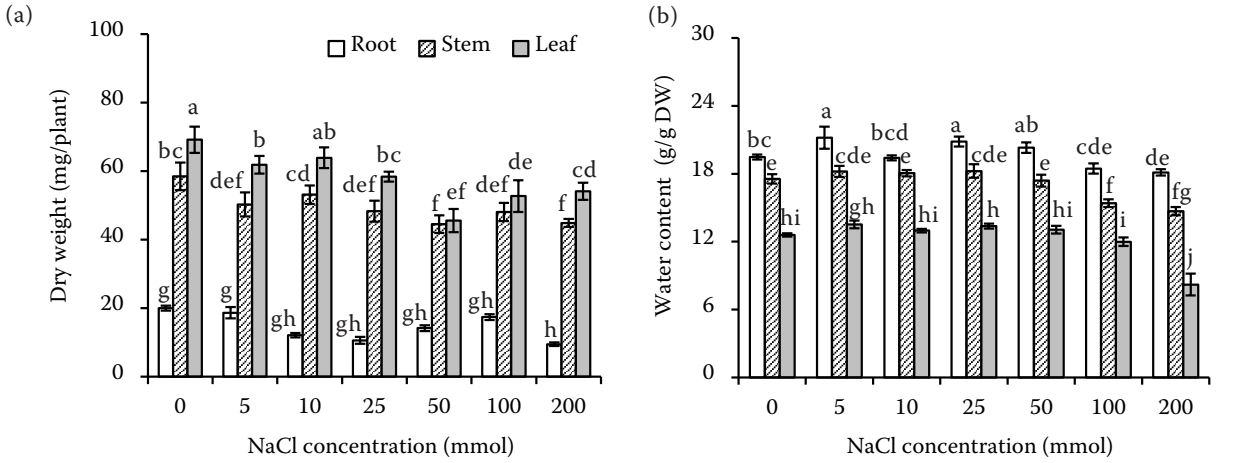

Figure 2. Effects of salinity on (a) dry weight and (b) water content in sunflower (*Helianthus annuus* L.). Three-week-old seedlings were treated with 0, 5, 10, 25, 50, 100, and 200 mmol NaCl for 7 days. Three plants were pooled in each replicate (n = 8). Values are means ± standard error (SE) and bars indicate SE. Columns with different letters indicate significant differences at $P < 0.05$ (Duncan's test)

important role in plants tolerant to saline stress. Salinity can induce plant nutritional disorders, such as the suppression of K^+ absorption (Shabala and Cuin 2008). In the present study, K^+ concentration in root displayed the decreasing trend with the increase of NaCl concentrations (Figure 3b). It was also observed that with the increase of NaCl concentration from 5–200 mmol, K^+ concentrations both in stem and leaf were maintained higher or stable compared to those under non-saline condition, except for K^+ concentration in leaf at 200 mmol. Compared to control, neither lower (5 and 10 mmol) nor higher (100 and 200 mmol) salinity significantly affected K^+ concentration, while moder-

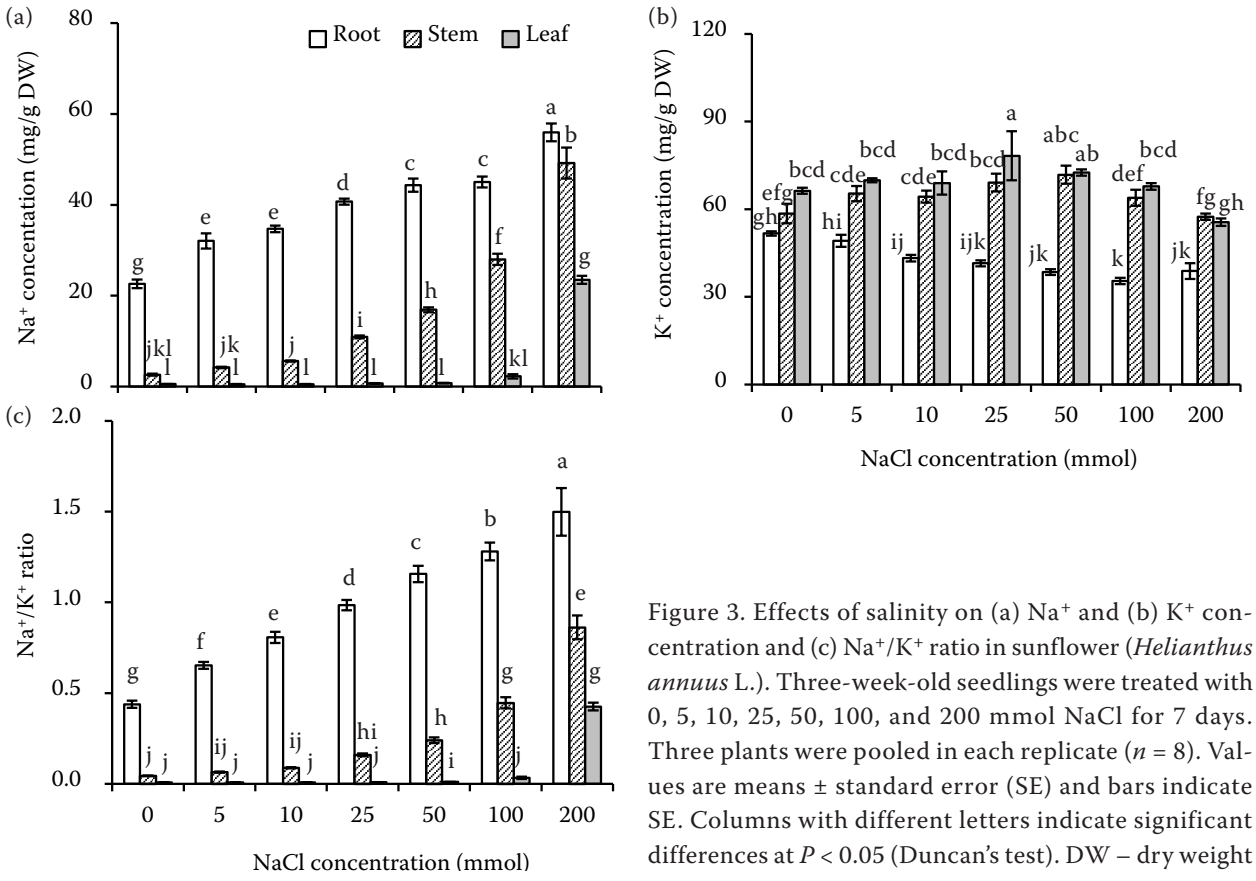

Figure 3. Effects of salinity on (a) Na^+ and (b) K^+ concentration and (c) Na^+/K^+ ratio in sunflower (*Helianthus annuus* L.). Three-week-old seedlings were treated with 0, 5, 10, 25, 50, 100, and 200 mmol NaCl for 7 days. Three plants were pooled in each replicate (n = 8). Values are means ± standard error (SE) and bars indicate SE. Columns with different letters indicate significant differences at $P < 0.05$ (Duncan's test). DW – dry weight

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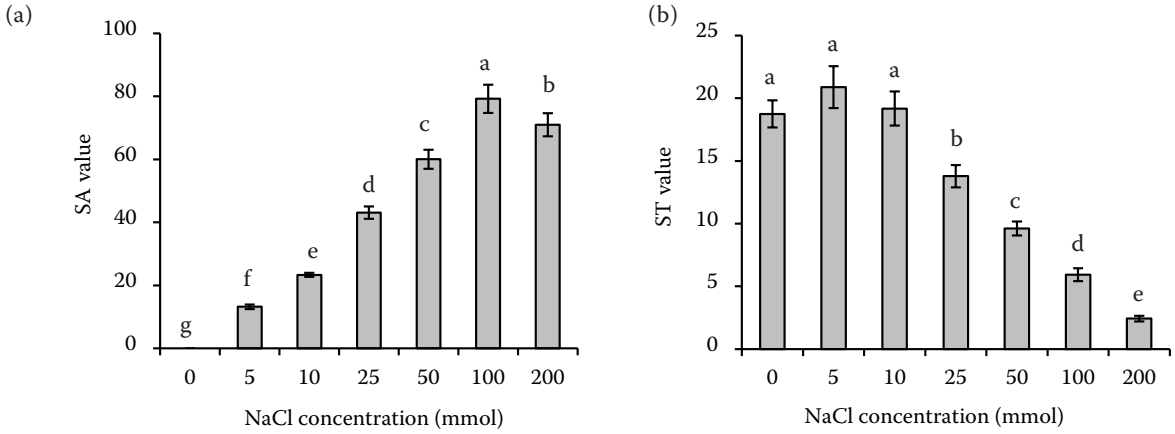

Figure 4. Effects of salinity on (a) the selective absorption (SA) and (b) selective transport (ST) for K^+ over Na^+ in sunflower (*Helianthus annuus* L.). Three-week-old seedlings were treated with 0, 5, 10, 25, 50, 100, and 200 mmol NaCl for 7 days. Three plants were pooled in each replicate (n = 8). Values are means ± standard error (SE) and bars indicate SE. Columns with different letters indicate significant differences at $P < 0.05$ (Duncan's test)

ate levels (25 and 50 mmol) significantly enhanced K^+ concentration both in stem and leaf ($P < 0.05$) (Figure 3b). These results suggested that sunflower plants can maintain K^+ homeostasis in shoot under saline stress.

It was shown that tissue Na^+/K^+ ratio showed the similar trend with Na^+ concentration in sunflower plants under either normal or saline conditions (Figure 3c). It is clear that high salinity caused a remarkable increase of Na^+/K^+ ratio in leaf ($P < 0.05$) (Figure 3c). 5–200 mmol NaCl also remarkably enhanced the capacity of selective absorption for K^+ over Na^+ ($P < 0.05$) (Figure 4a). When exposed to 5 and 10 mmol NaCl, plants showed the stronger capacity of selective transport for K^+ over Na^+, however, 25–200 mmol significantly reduced this value ($P < 0.05$) (Figure 4b). These results suggested that sunflower plants can maintain higher selectivity for K^+ over Na^+ under saline condition.

Further analysis of the data showed that Na^+ proportion significantly decreased in root by 10–58% while it increased in stem by 13–32% at 10–200 mmol NaCl ($P < 0.05$), respectively (Table 2). It is noteworthy that Na^+ proportion in leaf increased from 6% in plants under control condition to 32% in plants exposed to 200 mmol NaCl ($P < 0.05$) (Table 2). However, sunflower plants can maintain the stable Na^+ in leaf when the concentrations of NaCl were below 100 mmol (Table 2). Salinity significantly reduced K^+ proportion in root compared to control ($P < 0.05$) (Table 3). When exposed to concentrations of 50–200 mmol NaCl, K^+ proportion significantly increased in stem by 6–8% ($P < 0.05$) (Table 3). However, K^+ proportion in leaf remained unchanged by salinity (Table 3). These results indicated that sunflower plants adapted to salinity conditions by regulating distribution of Na^+ and K^+ in different tissues.

Table 2. Effects of salinity on Na^+ relative distribution in different plant parts

NaCl concentration (mmol)	Total quantities (mg/plant)	Na^+ proportion (%)		
		root	stem	leaf
0	0.66 ± 0.01^{e}	71.8 ± 1.3^{a}	22.4 ± 0.9^{e}	5.8 ± 0.5^{b}
5	0.76 ± 0.04^{e}	67.6 ± 2.0^{a}	28.1 ± 1.9^{e}	4.2 ± 0.5^{bcd}
10	0.85 ± 0.02^{de}	60.8 ± 2.2^{b}	35.4 ± 2.0^{d}	3.8 ± 0.3^{bcd}
25	1.16 ± 0.05^{c}	50.9 ± 2.4^{c}	45.6 ± 2.5^{c}	3.5 ± 0.2^{cde}
50	1.39 ± 0.07^{c}	43.2 ± 1.9^{d}	54.2 ± 2.0^{b}	2.5 ± 0.3^{de}
100	2.15 ± 0.09^{b}	32.1 ± 2.3^{e}	62.5 ± 2.7^{a}	5.4 ± 0.9^{bc}
200	4.01 ± 0.19^{a}	13.5 ± 1.0^{f}	54.6 ± 2.0^{b}	31.9 ± 1.6^{a}

Three plants were pooled in each replicate (n = 8). Within each column, means followed by different letters are significantly different at $P < 0.05$ (Duncan's test)

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Table 3. Effects of salinity on K^+ relative distribution in different plant parts

NaCl concentration (mmol)	Total quantities (mg/plant)	K^+ proportion (%)		
		root	stem	leaf
0	9.01 ± 0.29^{a}	12.2 ± 0.4^{a}	37.1 ± 0.7^{d}	50.7 ± 1.1^{ab}
5	8.34 ± 0.16^{a}	9.5 ± 0.6^{b}	38.8 ± 1.1^{d}	51.7 ± 1.2^{a}
10	8.37 ± 0.18^{a}	7.7 ± 0.3^{c}	40.5 ± 1.2^{bcd}	51.8 ± 1.3^{a}
25	8.44 ± 0.53^{a}	7.3 ± 0.6^{cd}	39.4 ± 1.6^{bcd}	53.3 ± 1.9^{a}
50	6.96 ± 0.25^{b}	7.5 ± 0.5^{cd}	45.5 ± 1.5^{a}	47.0 ± 1.6^{bcd}
100	7.12 ± 0.30^{b}	7.5 ± 0.3^{cd}	43.1 ± 2.2^{abc}	49.4 ± 2.3^{abcd}
200	5.94 ± 0.15^{c}	6.2 ± 0.4^{d}	43.4 ± 1.2^{ab}	50.4 ± 1.3^{abc}

Three plants were pooled in each replicate (n = 8). Within each column, means followed by different letters are significantly different at $P < 0.05$ (Duncan's test)

Soluble sugar and proline have been shown to be key osmolytes that contribute to osmotic adjustment (Farkhondeh et al. 2012, Radić et al. 2013). They can also enhance salt tolerance by protecting and stabilizing membranes and enzymes under saline stress (Ashraf and Harris 2004, Juan et al. 2005, Gupta and Huang 2014). In the present study, with the increase of NaCl concentrations, root soluble sugar concentration showed a gradually decreasing trend (Figure 5a). Salinity had no significant effect on soluble sugar concentration in leaf and proline concentration in root, while high level (200 mmol) significantly enhanced soluble sugar concentration in stem by 28% and proline concentration in leaf by 166% ($P < 0.05$), respectively (Figures 5a,b). These results suggested that sunflower plants can accumulate more soluble sugar in stem and more proline in leaf to protect photosynthetic apparatus from salinity damage under high salinity.

In conclusion, our results showed that high salinity remarkably inhibited the germination of seed and delayed germination time in sunflower. It is clear that 25–200 mmol NaCl significantly reduced both stem and leaf dry weight. Salinity remarkably reduced both K^+ and Na^+ proportions in root, while enhanced those in stem. High salinity also clearly enhanced soluble sugar accumulation in stem and proline accumulation in leaf. It was proposed that sunflower plants adapt to saline stress to some extent via regulating distribution of Na^+ and K^+, maintaining higher selective absorption capacity for K^+ over Na^+, and accumulating more osmoprotectants, such as soluble sugar and proline.

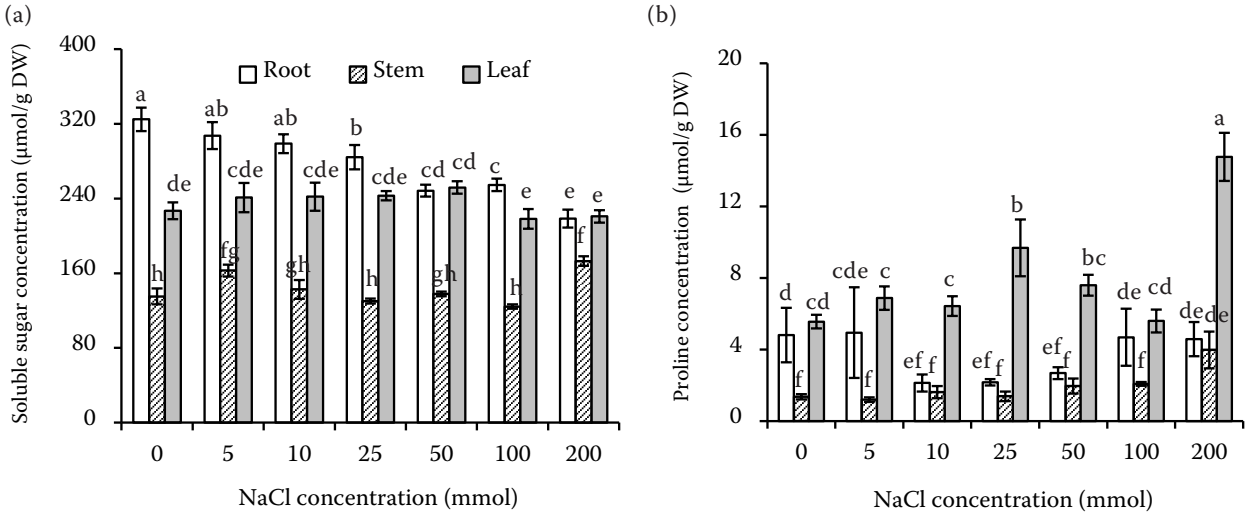

Figure 5. Effect of salinity on (a) soluble sugar and (b) proline concentration in sunflower (*Helianthus annuus* L.). Three-week-old seedlings were treated with 0, 5, 10, 25, 50, 100, and 200 mmol NaCl for 7 days. Three plants were pooled in each replicate (n = 8). Values are means ± standard error (SE) and bars indicate SE. Columns with different letters indicate significant differences at $P < 0.05$ (Duncan's test). DW – dry weight

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Corresponding author:
Dr. Guo-Qiang Wu, Lanzhou University of Technology, School of Life Science and Engineering, 287 Langongping Road, 730 050 Lanzhou, P.R. China; e-mail: wugq08@126.com