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Effects of NaCl Stress on the Growth and Physiological Changes in Oat (*Avena sativa*) Seedlings

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Abstract

The oat (*Avena sativa*) is a kind of cereal grain, which has high saline-alkali tolerance. This experiment was carried out to investigate and compare the growth and physiological changes of oat seedling. Oat was grown under five concentrations of NaCl stress (48, 72, 96, 120 and 144 mmolL^{-1}). The results showed that NaCl stress had no effect on the survival rate and organic acids. With the increasing of the NaCl concentration, tiller number, the chlorophyll, K^+, Ca^{2+}, NO^{3-}, H^2PO^{4-} contents, shoot length, the shoot biomass, and shoot water content were decreased significantly. However, the Cl^-, Na^+, Na^+/K^+, SO_4^{2-} and proline contents were extremely increased. K^+, Ca^{2+}, dry weight, and water content of shoots changed greater than that of roots. While Na^+ and Na^+/K^+ of shoots changed less than that of roots. When NaCl concentration was less than 96 mmolL^{-1}, the length, dry weight, and water content of roots had no significant changes. Based on this investigation, it can be concluded that oat seedlings accumulated more proline, Cl^- and SO_4^{2-} to maintaining osmotic and ion balance. In addition, NaCl stress had no significant effect on the growth of roots, and the roots can play the interceptive and protective role with a stronger salt tolerance. The roots can change the distribution of Na^+, then it decreased the harm on the shoots and increased the tolerance of oat seedling.

Keywords: adaptation strategies, salt stress, oat, ion distribution, proline

Introduction

Saline-alkali stress is the most serious factors in limiting crop production, and has brought severe harm to ecological environment and animal husbandry development, which has been clearly demonstrated by a number of studies (Manivannan *et al*., 2007; Shi and Sheng, 2005; Shi and Wang, 2005). Around the world, saline-alkali land is widely distributed in more than 100 countries, covered 4.34×10^8 hm^2 of the arable area (Wang *et al*., 2009). In addition, soil salinity has adversely affected about 30% of the irrigated and 6% of the total land area, resulted in a monetary loss of $12 billion in agricultural production (Fahad *et al*., 2015).

NaCl and Na_2SO_4 are the main virulence salts in salinized soils of northern China (Kawanabe and Zhu, 1991). Salt stress can greatly influence plant osmotic adjustment and ion injury (Munns, 2002). To resist the harm of salt stress, plants usually accumulate organic acids, soluble sugar (Kerepesi and Galiba, 2000), proline (Ashraf *et al*., 2007), some other smaller molecular organic compounds in the cytoplasm, and inorganic ions as an adaptation strategy. For example, under NaCl stress, seashore Paspalum (Lee *et al*., 2008; Marcum and Murdoch, 1994) can mainly synthesize the soluble sugars and other organic matters whereas in *Cynara cardunculus* and wheat (Benlloch-González *et al*., 2005; Li *et al*., 2009), the inorganic ions such as Cl^- played a major role in osmotic adjustment to resist salt stress.

Over the years, most studies about the effects of salt stresses on plants have mainly focused on the leaves of adult seedlings (Lourenco *et al*., 2013; Zhang and Mu, 2009). However, little information exists concerning the growth and physiological effects on young seedling's shoot and root. Previous researches showed that the effects of salt stress on different plant parts varied greatly among different types of plant species. For example, fresh and dry weight of *Haloxylon recurvum* increased with the increasing NaCl concentrations (Khan *et al*., 2000). Under the NaCl stress, the biomass of young umbu stems and leafs decreased significantly while the biomass of roots did not significantly decrease, and even at low concentrations, the biomass drastically increased (Da-Silva *et al*., 2008). However, under salt stress, the damage of sunflower roots is significantly

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larger than that of the stems and leaves (Hussain and Rehman, 1995).

Oat is an annual graminaceous plant. As an early maturation traditional crop, it can mature in live culms and can provide both grain and straw. It has a high grain yield, which can increase to over 2300 kg/hm^2, and its protein and fat contents are 16.6% and 5.6%, respectively (Wei *et al.*, 2007). Oat is extremely tolerant of drought, coldness, and mineral deficiency, which can grow in many types of soil and have a high salt resistance. So it is widely grown in the arid and semi-arid areas. However, very few previous studies focused on the growth and physiological changes of oat under salt stress. It is not clearly that how the physiological mechanisms of oat resisted salt stress. Therefore, this paper applies the neutral salt NaCl as stress treatment to oat seedlings, and the purpose is to discuss: (1) the salt tolerance mechanism and adaptation strategy under NaCl stress; (2) the differences of the growth and physiological mechanisms between shoots and roots of oat under NaCl stress. A better understanding of the early seedling processes under salt stress should facilitate the effective utilization of this species in salinization soil in Northeast China.

Materials and Methods

Plant materials and its cultivation

The experiment was conducted in 2012 at an experimental field of Northeast Normal University. The naked oat cultivar No. 2 White oat was used as the experimental material, with a growth period of 80-85 d and the thousand-seed weight is 30 g.

Homogeneous and full seeds were sown in 20-cm diameter plastic pots containing washed sand. All pots were placed outdoors and protected from the rain. The plastic pots were watered with sufficient Hoagland nutrient solution every afternoon after the emergence of seedlings commenced. Each pot contained 20 seedlings after thinning. The Hoagland solution consisted of 5.00 mmol L^{-1} Ca^{2+}, 2.00 mmol L^{-1} Mg^{2+}, 6.04 mmol L^{-1} K^+, 22.2 μmol L^{-1} EDTA-Fe^{2+}, 6.72 μmol L^{-1} Mn^{2+}, 3.16 μmol L^{-1} Cu^{2+}, 0.765μmol L^{-1} Zn^{2+}, 2.10 mmol L^{-1} SO_4^{2-}, 1.00 mmol L^{-1} $H_2PO_4^-$, 46.3 μmol L^{-1} H_3BO_3, 0.556 μmol L^{-1} H_2MoO_4, and 15.04 mmol L^{-1} NO_3^-.

Stress conditions and treatment

The neutral salt NaCl was used as a salt stress in this experiment. NaCl mixed with distilled water was set at five different concentrations: 48, 72, 96, 120 and 144 mmol L^{-1}.

When oat seedlings were four weeks old, 48 pots with uniform seedlings were selected and randomly divided into 16 sets, with three pots per set. One set was used as a control, and the other 15 sets were treated with various stress treatments. The stress treatments were carried out every day from 16:00 to 18:00 and treated with 500 mL nutrient solutions containing the appropriate stress salts per pot, by watering three times thoroughly. The control group was thoroughly watered with only Hoagland nutrient solution. The duration of the treatment was 9 days.

Pretreatment and determination

Investigation and pretreatment of oat seedlings: On the day after the final treatment, the survival rate, tiller number and plant height of each treatment were investigated. After that, all oat seedlings in each pot were harvested, and washed with tap water followed by distilled water. The roots and shoots were separated, and then weighed. Then the shoots and roots were oven-dried at 105 °C for 15 minutes and then dried at 70 °C for 48 hours to a constant weight. The dry weight of shoot and root samples were taken, respectively, and the water content of shoots and roots were equal to the differences between fresh weight and dry weight.

Determination of chlorophyll content: After 9 days of stress treatments, fresh functional leaves were collected from the plants and cut into small segments in order to determine the concentration of chlorophyll *a* and *b*. Chlorophyll was extracted from 0.5 g fresh samples with 80% acetone solution. The extractant was centrifuged. The optical density (OD value) of the diluted solution was determined at 663 and 645 nm. The concentrations of chlorophyll a (C_a) and b (C_b) were calculated using formulas (1) and (2), respectively.

$$C_a = 0.0127OD_{663} - 0.00269OD_{645} \quad (1)$$
$$C_b = 0.0229OD_{645} - 0.00468OD_{663} \quad (2)$$

Determination of the organic solutes and the inorganic ions: Dry shoot and root samples (0.1 g) were treated with 10 mL deionized water at 100 °C for 1 h, and the filtrate of shoots and roots was used to determine the contents of the organic solutes and the inorganic ions.

The organic solutes were determined by ion chromatography-DX-300 ion chromatographic system (DIONEX, Sunnyvale, USA) determination conditions: ICE-AS6 ion-exclusion column; CDM-II electrical conductivity detector; AMMS-ICE II interference suppressor; mobile phase of 0.4 mmol L^{-1} perfluorobutyric acid; a flow rate of 1.0 mL/min; the column temperature set at 20 °C; and the injection volume of 50 μL. The content of the organic acids was calculated with all components that could be determined in the dry samples (Li *et al.*, 2009).

The inorganic cations (Na^+, K^+, free Ca^{2+}) were determined by an atomic absorption spectrophotometer (TAS-990; Purkinje General, Beijing, China). Anions (NO_3^-, Cl^-, SO_4^{2-}) were determined by ion chromatography – DX-300 ion chromatographic system (DIONEX, Sunnyvale, USA), determination conditions: AS4A-SC ion exchange column, CD M-II conductivity detector, mobile phase was: Na_2CO_3/$NaHCO_3$=1.7/1.8 mmolL^{-1}. $H_2PO_4^-$ content was determined by molybdenum blue method (Li *et al.*, 2009; Zhang *et al.*, 2009).

The dried samples of shoots and roots were homogenized by powdering in order to determine the free proline content. Free proline was assayed by the acid-ninhydrin method. The dried tissue samples (0.1 g) were mixed with 3% sulfosalicylic acid. The extracted solution was treated in boiling water for 10 minutes and then centrifuged. The upper clear liquid of the tubes was extracted and spectrophotometrically determined at 520 nm.

Statistical analysis of data

All parameters were analyzed by one-way ANOVA using the statistical Software SPSS 13.0 (SPSS Inc, Chicago, IL, USA). The means and standard errors (SE) were reported. The level of statistical significance was $P<0.05$.

Results

Effects of NaCl stress on survival rate and tiller number of oat seedlings

Under NaCl stresses, different salt concentration had no effect on the survival rate of oat seedlings (Fig. 1A). When NaCl

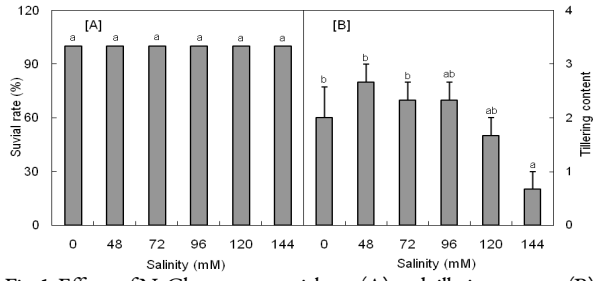

Fig. 1. Effects of NaCl stress on survial rate (A) and tillering content (B) of oat seedlings, the values are means (±SE) of triplicate samples

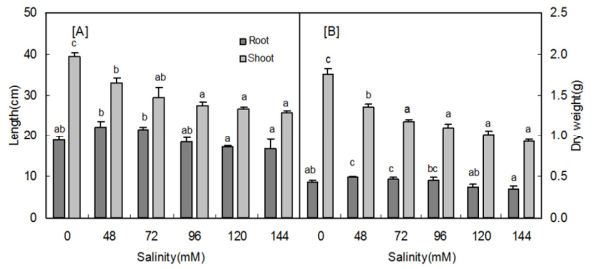

Fig. 2. Effects of NaCl stress on length (A) and dry weight (B) of shoots and roots, the values are means (±SE) of triplicate samples

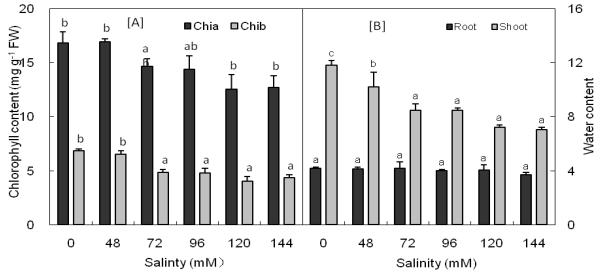

Fig. 3. Effects of NaCl stress on the chlorophyll (A) content and water content (B) in shoots and roots of oat seedlings, the values are means (±SE) of triplicate samples

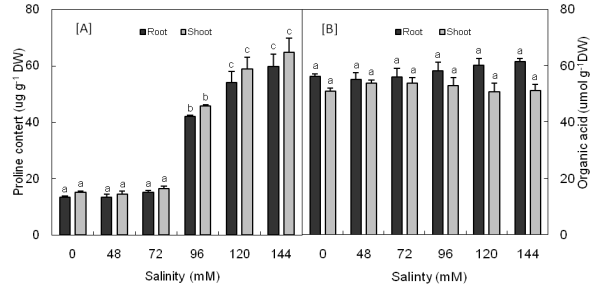

Fig. 4. Effects of NaCl stress on proline (A) and total organic acids (B) of shoot and root, the values are means (±SE) of triplicate samples

concentration was higher than 144 mmolL^{-1}, the tiller number significantly decreased (P<0.05) and was lower than the controls (Fig. 1B).

Effects of NaCl stress on growth of shoots and roots

With the increasing salinity, there was a significant decrease in the shoot length of oat seedlings (P<0.05). Under 144 mmolL^{-1}NaCl stress, shoot length decreased to 0.75 times compared with that of the controls. The root length had no significant changed under NaCl stress. The effect of NaCl stress on shoot length was more significant than on root length (Fig. 2A).

Under NaCl stresses, the shoot biomass also significantly decreased with the increasing stress concentration (P<0.05). At 144 mmolL^{-1} NaCl, shoot biomass decreased to 0.53 times compared with the shoot biomass observed in the controls. The root biomass of oat seedlings was also significantly decreased at 144 mmolL^{-1} NaCl, with the decrease to 0.82 times compared with the controls (P<0.05), but had no significant changed when NaCl was less than 96 mmolL^{-1}. Similarly, the effect of NaCl stress on the shoot biomass was much greater than that of the root biomass (Fig. 2B).

Effects of NaCl stress on chlorophyll and water content of oat seedlings

Under NaCl stress, the contents of chlorophyll *a* and *b* were both significantly decreased with the increasing stress concentration. At 96 mmolL^{-1} NaCl, the content of chlorophyll *a* decreased to 0.86 times, while the content of chlorophyll *b* decreased to 0.7 times compared with the controls. Generally, the effect on chlorophyll *a* is less than that of chlorophyll *b* under salt stress (Fig. 3A).

With the increasing salinity of NaCl stress, the water content of shoots extremely significantly decreased (P<0.05), which at 144 mmolL^{-1} NaCl dropped to 0.6 times. The water content of roots under NaCl stress was also decreased (P<0.05), but had no significant changes, which decreased to 0.89 times compared with the water content in the controls. Generally, the effect of NaCl stress on the shoot water content of oat seedlings is greater than that on root water content (Fig. 3B).

Effects of NaCl stress on organic solute contents of shoots and roots

There was an extremely significant increase in proline content of oat shoots and roots when NaCl was greater than 96 mmolL^{-1} (P<0.01). The proline content in shoots was increased by 3.26 times under NaCl stress, and the proline content in roots by 3.41 times compared with the control (Fig. 4A). The effect on organic acids of the shoots and roots did not show a significant change (P<0.05).

Effects of NaCl stress on inorganic ion contents of shoots and roots

Inorganic cations: With the increasing stress concentrations, the Na^+ content in shoots and roots of oat seedlings was extremely significantly increased (P<0.01). Compared with the controls, the Na^+ content in shoots increased to 6.5 times, while increased to 7.4 times in roots. The increasing content of Na^+ in oat roots was greater than that in the shoots (Fig. 5A).

Under NaCl stress, the K^+ content in shoots and roots of oat seedlings were significantly decreased (P<0.01) with the increasing stress concentrations. Compared with the controls, the K^+ contents in shoots decreased 0.74 times, and decreased to 0.64 times in roots. Overall, the decreasing content of K^+ in roots was greater than that in shoots (Fig. 5B).

The Ca^{2+} content in shoots and roots were also significantly decreased (P<0.05) with the increasing stress concentration, and the decreased content of Ca^{2+} in oat roots was greater than that in the shoots (Fig. 5C). There was a significant increase of the Na^+/K^+ in shoots and roots with the increasing stress concentrations under NaCl stress. The Na^+/K^+ in shoots increased to 9.4 times under salt stress while increased to 11.2 times in roots compared with the controls. The increasing Na^+/K^+ in roots was greater than that in shoots (Fig. 5D).

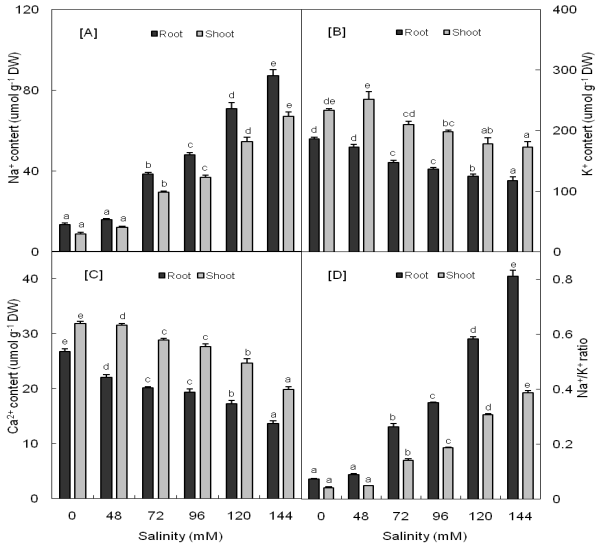

Fig. 5. Effects of NaCl stress on Na^+(A), K^+(B), Ca^{2+}(C), Na^+/K^+(D) in shoot and roots, the values are means (±SE) of triplicate samples

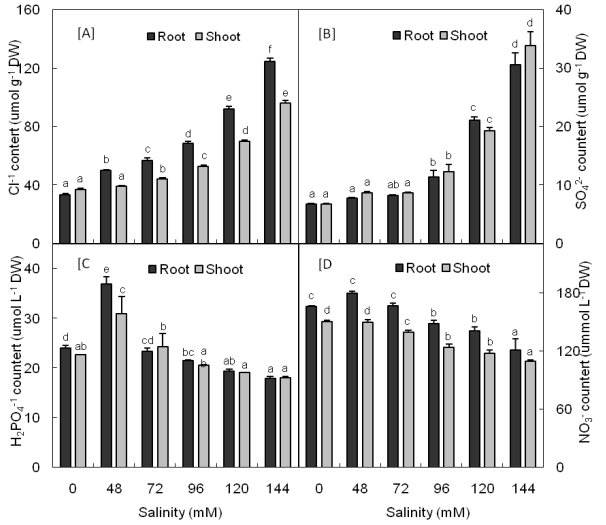

Fig. 6. Effects of NaCl stress on Cl^-(A), SO_4^{2-}(B), $H_2PO_4^-$(C), NO_3^-(D) in shoot and roots, the values are means (±SE) of triplicate samples

Inorganic anions: The Cl^- contents in shoots and roots had a very significant increase under NaCl stress (P<0.01). At 48 mmolL^{-1} NaCl, the Cl^- content increased 1.06 times, and at 144 mmolL^{-1} NaCl increased 2.59 times compared with the controls (Fig. 6A). The changes of Cl^- content in roots were greater than that in shoots under NaCl stress. The SO_4^{2-} content in shoots and roots were also significantly increased under salt stress (P<0.01). The increased content of SO_4^{2-} in shoots was much greater than that of roots (Fig. 6B). The NO_3^-, $H_2PO_4^-$ contents in shoots and roots were also extremely significantly decreased (P<0.01) (Fig. 6C, D).

Discussion

Effects of NaCl stress on roots and on shoots in oat seedlings

The harmful effect of NaCl stress was mainly led to Na^+ toxicity and the physiological drought caused by high osmotic stress, resulting from the declined water potential of plants under high concentration salt stress (Khan, 2000; Munns, 2002). Due to a variety of plant species, different plant sections also have different responses on the growth and physiological changes to salt stress. Plants usually comparted the salt ion in plant roots or stored the salt ion in the stems, thereby reduced the transportation and harmful effects to other parts. In addition, plants could improve salt tolerance by excreting the salt ions out of the body through special ion channels (Munns and Tester, 2008). In the previously available reports on the comparison of salt tolerance between the aboveground and underground plant sections under salt stress, the results were varied greatly (Khan *et al*., 2000; Da-Silva *et al*., 2008; Hussain and Rehman, 1995). Some experts thought that the plant shoots were more sensitive to salinity than the roots, such as *Areca catechu* (Da-Silva *et al*., 2008) while others thought that the plant roots were more sensitive to salinity than shoots, such as corn (Hajlaoui *et al*., 2009) and wheat (Li *et al*., 2009).

In this study, the growth and physiological responses of oat seedlings showed different change tendencies under NaCl stress. The root length of oat seedlings, especially the root biomass decreased less than that of the shoots under salt stress, while the Na^+ content in roots increased higher than that in shoots. However, the K^+ content in roots showed a greater decrease than that of the shoots. As a result, the Na^+/K^+ ratio in roots is much higher than that in the shoots. Lower Na^+ and higher K^+ contents in the cytoplasm are very important for the maintenance of the enzymatic vigour (Munns and Tester, 2008). The Na^+ entered the plant cells through the high-affinity K^+ transporter (HKT) and non-selective cation channels (Shi and Wang, 2005). Our results also showed these changes. This may be attributable to an inhibitory effect of salinity on K^+ absorption, which relies on the transmembrane proton gradient (Munns, 2002). As a absorbing water organs, mineral nutrition and compound variety of physiological active substances, the number, size, biomass, and physiological conditions of roots directly affect the strength of crop tolerance (Yousfi *et al*., 2010; Zhang *et al*., 2011). Under salt stress, the roots could play a interceptive and protective role, have a stronger salt tolerance than that of the shoots. The results of this experiment indicated that the different sections of various plants really had different sensitive tendencies to the growth and physiological responses under salt stress. Therefore, this study provides and supplements previous research results on the saline-alkaline tolerance of various plants.

Adaptation strategies under NaCl stress

Most Halophytes have a different ion balance adjustment mechanism and osmoregulation mechanism under salt stress. Inorganic ions and proline also play an important role in ion balance regulation (Khan, 2000; Ashraf *et al*., 2007). The results of this study showed that in order to maintaining osmotic and ion balance, oats mainly took the physiological response mechanisms and adaptation strategies by massively accumulating Cl^-, SO_4^{2-}, and proline, and had nothing to do with the organic acids. This also implies that salt stress such as NaCl is different from the alkaline salt stress such as $NaHCO_3$ andNa_2CO_3, based on previous studies (Li *et al*., 2009; Lin et al., 2011).

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