

Physiological and Biochemical Responses of Jerusalem Artichoke Seedlings to Mixed Salt-Alkali Stress Conditions

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Abstract

Soil salinization and alkalization frequently co-occur in the grassland, but little information exists concerning the mixed effects of salt-alkaline stress on plant. Jerusalem artichoke is an economically and ecologically important energy crop and also considered as a salt-tolerant species. In this study, we investigated the effects of 12 mixed salt-alkaline conditions on the seedling growth and responses of Jerusalem artichoke to such conditions. The results showed that the seedling growth decreased with the increasing salinity and pH, and the destructive effects were more markedly under the interactions of highest salinity and pH. The Na^+ , Mg^{2+} and Ca^{2+} concentrations were all increased with the increasing salinity and pH, but the K^+ kept stable. The Cl^- concentration increased when the treatment without alkali salts, and the NO_3^- and H_2PO_4^- concentrations were decreased with the increasing salinity. Jerusalem artichoke seedlings enhanced organic acids and proline to supply the shortage of inorganic anions and cope with osmotic stress from the high Na^+ concentration. Above results show that the toxicity effects of the interactions of salt stress and alkali stress on plant is much greater than that only salt or alkali stress. A better understanding of the seedlings of Jerusalem artichoke under mixed salt-alkali stress conditions should facilitate the effective utilization of this species under such complex environment in Northeast China.

Keywords: Jerusalem artichoke, mixed salt-alkali stress, physiological changes, organic acids

Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) is a tuberous plant belongs to the sunflower family. It was introduced into Europe in the late 1500s from North America, and then cultivated across Europe and Asia over the centuries, both for human consumption and as a feed for grazing livestock (Baldini *et al.*, 2004). Currently, Jerusalem artichoke is cultivated for many application areas such as source of sugars, green forage, crude material for production of ethanol, and pharmaceuticals applications (Baldini *et al.*, 2004; Saengthongpinit and Sajjaanantakul, 2005). In addition, it grows very rapidly and can reach a height of 2-4 m. It is also has highly tolerant to salt soil due to the deep root system (Zhao *et al.*, 2008). In conclusion, Jerusalem artichoke is an economically and ecologically important energy crop species for humans.

Soil salinization and alkalization is an important environmental problem all over the world, especially in China. Salinity-alkalinity soil decreases the plant growth and production and also lead to large area of land degradation. For example, arable land acreage of the world is 1.5×10^9 ha, but 23% (0.34×10^9 ha) of the area is saline, and 37% (0.56×10^9 ha) is sodic (Tanzi, 1990; Lin *et al.*, 2014). In the Northeast of China, almost 70% of the grassland has been serious degraded due to

salt-alkali soil, and the area is expanding all the time (Deng *et al.*, 2006).

In addition, soil salinization and alkalization are co-occur, and the stress effect on the plant is always much greater than that only salt or alkali stress. Previous studies have been proved that salt stress and alkali stress are great differed due to the high pH, and the alkali stress is more severe than salt stress (Shi and Yin, 1993; Guo *et al.*, 2009; Zhang and Mu, 2009). Under salt stress, plants always maintain high K^+ and Ca^{2+} levels and exclude Na^+ from the shoots, synthesize many organic solutes such as proline in the cytoplasm to resist the osmotic stress, and also accumulate inorganic ions such as Cl^- in order to keep ion balance (Kerepesi and Galiba, 2000; Ashraf *et al.*, 2007). While under alkali stress, accumulation of organic acids is the main way to resist the high pH stress. However, the conditions of plant surviving in the salt-alkali soil are very complex, contain both the salt and alkali stresses (Shi and Yin, 1993; Shi and Wang, 2005; Li *et al.*, 2010). Thus, the effect of mixed salt-alkali stress should be distinguished from salt stress or alkali stress, and the physiological and biochemical responses to such conditions maybe also unique.

There are a few reports on the effects of mixed salt-alkali stress on plant seedlings (Shi and Wang, 2005; Peng *et al.*, 2008; Yang *et al.*, 2011), and also some reports on seed germination (Li *et al.*, 2010; Lin *et al.*, 2014). However, most

Table 1. Salt composition and molar ratio of various treatments

Treatments	Salt composition and molar proportions			
	NaCl	Na ₂ SO ₄	NaHCO ₃	Na ₂ CO ₃
A	1	1	0	0
B	1	2	1	0
C	1	1	1	1
D	9	1	1	9

Table 2. Stress factors of various treatments

Treatment	Stress factors						
	pH	Salinity/mM	Na ⁺ /mM	Cl ⁻ /mM	SO ₄ ²⁻ /mM	HCO ₃ ⁻ /mM	CO ₃ ²⁻ /mM
A0	7.02	0	0	0	0	0	0
A1	7.13	20	30	10	10	0	0
A2	7.14	40	60	20	20	0	0
A3	7.22	60	90	30	30	0	0
B0	7.02	0	0	0	0	0	0
B1	7.95	20	30	5	10	5	0
B2	8.05	40	60	10	20	10	0
B3	8.10	60	90	15	30	15	0
C0	7.02	0	0	0	0	0	0
C1	9.32	20	30	5	5	5	5
C2	9.42	40	60	10	10	10	10
C3	9.49	60	90	15	15	15	15
D0	7.02	0	0	0	0	0	0
D1	10.05	20	30	9	1	1	9
D2	10.09	40	60	18	2	2	18
D3	10.18	60	90	27	3	3	27

studies described how the plant changed under such condition. To our knowledge, the physiological adaptation mechanism of plant seedlings especially the Jerusalem artichoke is still unclear. A better understanding of the seedlings of Jerusalem artichoke under mixed salt-alkali stress conditions should facilitate the effective utilization of this species under such complex environment in the Northeast of China.

In this study, mixtures of two neutral salts (NaCl and Na₂SO₄), two alkaline salts (NaHCO₃ and Na₂CO₃) were used in different proportions to simulate 12 treatments of mixed salt-alkaline conditions. The aims were (1) to test the effects of mixed salt-alkali stresses on the seedling growth of Jerusalem artichoke, (2) to explore the physiological adaptation mechanism of Jerusalem artichoke under such conditions.

Materials and Methods

Design of the mixed salt-alkali stress conditions

In order to simulate the mixed salt-alkali conditions, NaCl, Na₂SO₄, NaHCO₃ and Na₂CO₃ were chose based on the salt composition of the salt-alkali soil in the Northeast of China (Peng et al., 2008). Four salts were mixed in different proportions according to the salinity and pH in the Songnen grassland. Four treatment groups (A-D) were set with the increasing alkalinity. Salt composition and molar ratio of various treatments are shown in Table 1. Within each group, three concentrations were used (20, 40 and 60 mM). Total 12 mixed stress treatments (A₁-D₃, pH 7.02-10.18, and salinity 20-60 mM) are shown in Table 2.

Plant material

Experiments were conducted in Northeast Forestry University (126°38'E, 45°45'N, Harbin, China) during May and June. Jerusalem artichoke (*Helianthus tuberosus* L.) tubers were

sown in 15-cm-diameter plastic pots filled with 2.5 kg washed sand. Each pot was taken as a single replicate, and contained three tubers and watered until emergence. All the plants were irrigated with Hoagland nutrient solution once a day from emergence to 40 d after sowing. The Hoagland nutrition solution in our research contained 5.00 mM Ca²⁺, 2.00 mM Mg²⁺, 6.04 mM K⁺, 22.2 μM EDTA-Fe²⁺, 6.72 μM Mn²⁺, 3.16 μM Cu²⁺, 0.765 μM Zn²⁺, 2.10 mM SO₄²⁻, 1.00 mM H₂PO₄⁻, 46.3 μM H₃BO₃, 0.556 μM H₂MoO₄, and 15.04 mM NO₃⁻ (Lin et al., 2014). Plants were cultivated in a greenhouse with natural light.

Stress treatment and seedling harvest

The Jerusalem artichoke seedlings were subjected to stress treatments after 40 d, and 52 pots with uniform seedlings were divided randomly into 13 sets, four pots per set. Each set contained four replicates. The pots were watered daily with nutrient solution containing the appropriate stress salts as above stated. The control seedlings were watered with only the nutrient solution. The total treatment duration was 8 d. Harvest seedlings were washed with distilled water twice, and then the shoots were retained, the fresh weight was determined for each plant. The shoots were oven-dried at 80 °C for 15 min and then at 65 °C to a constant weight. The water content (WC) was calculated using the formula (FW-DW)/DW. FW is short for fresh weight, DW is short for dry weight.

Determination of inorganic ions and organic solutes

Dry samples of plant material (50 mg) were treated with 10 mL deionized water at 100 °C for 1 h and the extract was taken to determine free inorganic ions, organic acids. An atomic absorption spectrophotometer (TAS-990, Purkinje General, Beijing) was used to determine the Na⁺, K⁺, Mg²⁺ and Ca²⁺ contents. The NO₃⁻, Cl⁻ and H₂PO₄⁻ contents were determined by ion chromatography (DX-300 ion chromatographic system, USA), and the

Table 3. Two-way ANOVA of effects of salinity, pH and their interactions on the growth and physiological index of Jerusalem artichoke

Index	Item	Group	Salinity	Group×Salinity
Fresh weight	F-ratio ^p	74.93***	146.33***	0.63 ^{ns}
Dry weigh	F-ratio ^p	124.0***	81.1***	0.8 ^{ns}
Water content	F-ratio ^p	65.1***	248.4***	14.5***
Na ⁺ content	F-ratio ^p	259.0***	1038.8***	33.98***
K ⁺ content	F-ratio ^p	2.5 ^{ns}	0.7 ^{ns}	6.8***
Ca ²⁺ content	F-ratio ^p	125.7***	495.5***	14.8***
Mg ²⁺ content	F-ratio ^p	252.5***	1027.6***	28.7***
Cl ⁻ content	F-ratio ^p	190.4***	43.7***	19.0***
NO ₃ ⁻ content	F-ratio ^p	211.4***	79.9***	2.5*
H ₂ PO ₄ ⁻ content	F-ratio ^p	111.0***	1099.6***	13.3***
Citrate acid	F-ratio ^p	176.5***	899.2***	28.5***
Oxalate acid	F-ratio ^p	192.3***	764.1***	32.6***
Maliate acid	F-ratio ^p	215.8***	785.5***	40.4***
Proline	F-ratio ^p	1351.0***	1116.8***	530.4***

*** $P < 0.001$; ns means not significantly

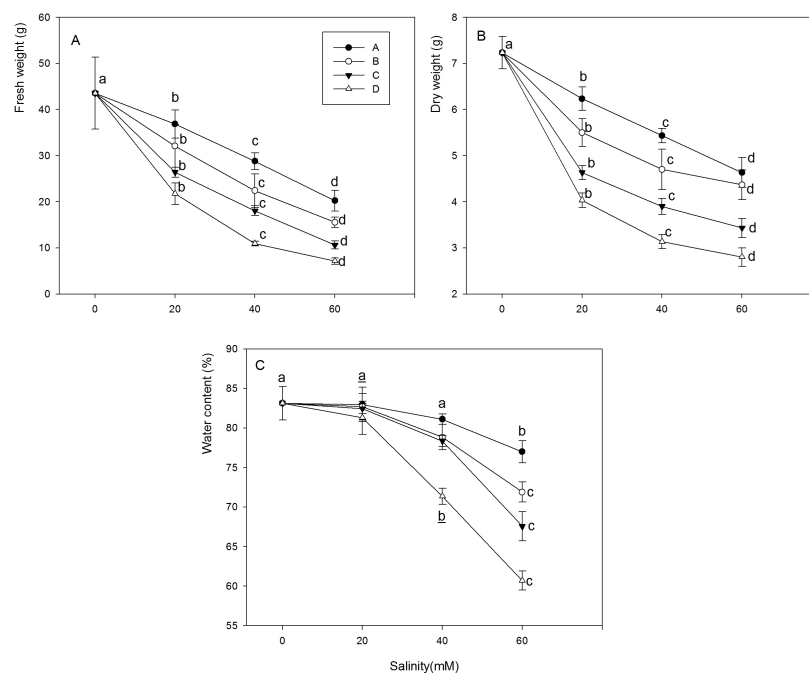


Fig. 1. Effects of mixed salt-alkaline stresses on fresh weight (A), dry weight (B) and water content (C) of Jerusalem artichoke

The values are the means of four replicates. Means followed by different letters are significantly different at $P < 0.05$ according to a least significant difference test

measurement conditions were as follows: AS4A-SCion exchange column, CD M-II electric conductivity detector, mobile phase was $\text{Na}_2\text{CO}_3/\text{NaHCO}_3 = 1.7/1.8$ mM. For the analysis of organic acids, the measurements were also undertaken using ion chromatography (DX-300 ion chromatographic system, USA), and the measurement conditions were as follows: ICE-AS6 analytical column, CDM-II electric conductivity detector, AMMS-ICE II interference suppressor, mobile phase was 0.4 mM perfluorobutyric acid, flow speed was 1.0 mL/min, column temperature was 20 °C, sample size was 50 μL . The content of proline was measured using ninhydrin, according to Zhu *et al.* (1983).

Data analysis

All data were analyzed using SPSS 13.0. Tukey's tests were performed for multiple comparisons to determine significant ($P < 0.05$) differences between individual treatments. A two-way

ANOVA was used to test the effects of the factors (pH and salinity) and their interactions on seedling growth and physiological change index. The data were expressed as mean \pm S.E.

Results

Effects on the seedling growth and water content

The fresh and dry weights of Jerusalem artichoke seedlings were significantly affected by pH and salinity ($P < 0.001$, Table 3), but not significantly by the interaction of the two factors. Maximum seedling weight (fresh and dry) were obtained both in the distilled water, which were 43.6 g and 7.2 g, respectively. Seedling weight decreased with the increasing salinity in all the treatment groups, and more markedly in group D (Fig.1). At the highest salinity (60 mM), the dry weight in group A was 4.6 g, while 4.3 g, 3.4 g and 2.8 g in the other 3 groups, respectively.

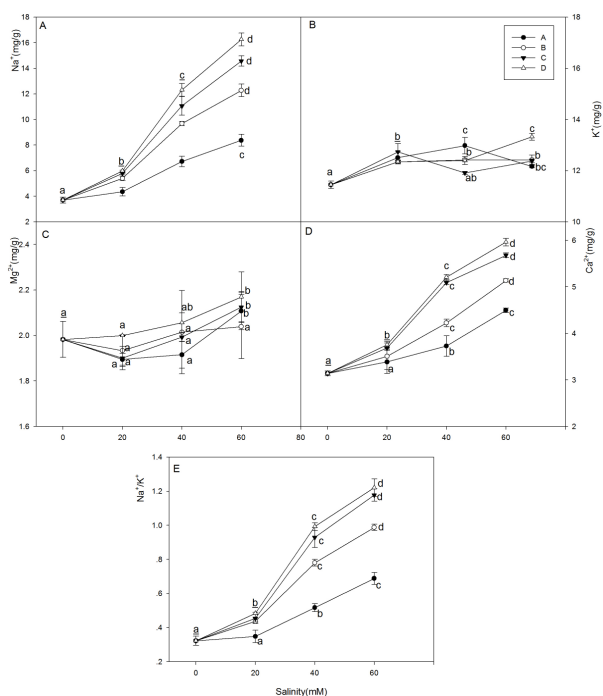


Fig. 2. Effects of mixed salt-alkaline stresses on Na⁺ content (A), K⁺ content (B), Mg²⁺ content (C) Ca²⁺ content (D) and Na⁺/K⁺ (E) of Jerusalem artichoke

The values are the means of four replicates. Means followed by different letters are significantly different at $P < 0.05$ according to a least significant difference test

Water content in the shoot was significantly affected by pH, salinity and the interaction of the two factors ($P < 0.001$, Table 3). Maximum water content was also obtained in distilled water. In group A, the water content significantly decreased only when the salinity reached 60 mM. In group D, when the salinity reached highest (60 mM), the water content decreased of 25% compared to the control (Fig. 1).

Effects on inorganic ions contents

The Na⁺, Mg²⁺ and Ca²⁺ contents were significantly affected by the pH, salinity and the interaction of the two factors ($P < 0.001$, Table 3). However, the K⁺ content was not significantly affected. Within each group, the Na⁺ contents were increased with the increasing salinity, and reached the highest value at 60 mM. In addition, the ratio of the alkali salts were also affected the Na⁺ content. The Na⁺ content in group D is 16.3 mg/g but only 8.4 mg/g in the group A at 60 mM salinity (Fig. 2A).

The K⁺ content changed irregularly in all the treatments, but compared with the control, the K⁺ content did not change significantly (Fig. 2B). In addition, due to the Na⁺ content increased sharply with the increasing alkali salts, the Na⁺/K⁺ had the similar change trends with the Na⁺ content. The maximum value (1.2) was appeared at 60 mM in the group D (Fig. 2E). In addition, the Mg²⁺ and Ca²⁺ contents were also showed a increasing trends with the increasing salinity and the ratio of alkali salts. The maximum values were both appeared at 60 mM in the group D (Fig. 2CD).

The NO₃⁻, Cl⁻ and H₂PO₄⁻ contents were all significantly affected by the pH, salinity and the interaction of the two factors ($P < 0.001$, Table 3). The Cl⁻ content significantly increased with

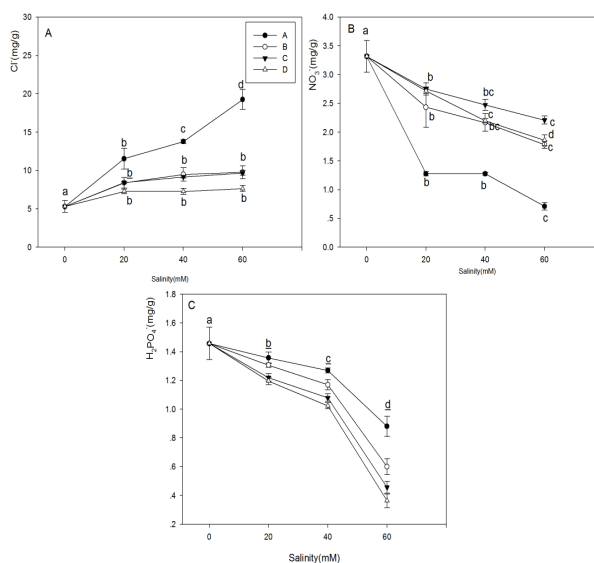


Fig. 3. Effects of mixed salt-alkaline stresses on Cl⁻ content (A), NO₃⁻ content (B) and H₂PO₄⁻ (C) of Jerusalem artichoke

The values are the means of four replicates. Means followed by different letters are significantly different at $P < 0.05$ according to a least significant difference test

the increasing salinity at A group ($P < 0.05$), and was 3.7 times than that in the control group at the highest salinity. However, the Cl⁻ content significantly increased when the salinity reached 20 mM, and kept unchanged when the salinity was much higher. The changing trends were similar between NO₃⁻ and H₂PO₄⁻ contents, both of the values were decreased with the increasing salinity and reached the lowest at 60 mM salinity in the final (Fig. 3).

Effects on organic solutes contents

Maliatic acid, citric acid and oxalic acid were detected in the shoot of Jerusalem artichoke under mixed salt-alkali stress conditions. The three organic acids were significantly affected by the pH, salinity and the interaction of the two factors ($P < 0.001$, Table 3). In addition, the three organic acids had the similar change trends in each stress group, they were all increased when the alkali salt ratio was higher. There was no significant difference in the maliatic acid and citric acid contents compared with the control in group A except oxalic acid. At the highest salinity stress, the organic acids contents were 1.8 times, 3.6 times and 2.2 times than that of the control, respectively (Fig. 4).

The proline content was significantly affected by the pH, salinity and the interaction of the two factors ($P < 0.001$, Table 3). Although the proline content increased with the salinity in the group A and B, the rangeability was not large. However, in the group D, when the salinity reached 60 mM, the proline content was 13.5 times than that of the control (Fig. 5).

Discussion

In this study, the 12 treatments covered various salt-alkali stress conditions in a range of pH from 7.02 to 10.18, which is approximate to the Songnen grassland in the Northeast of China. In the salt-alkali soil of Northern China, the stress factors are very difficult to simulate. However, the mixed stress treatments used in our research made the research of complex natural environment conditions possible. Most previous studies

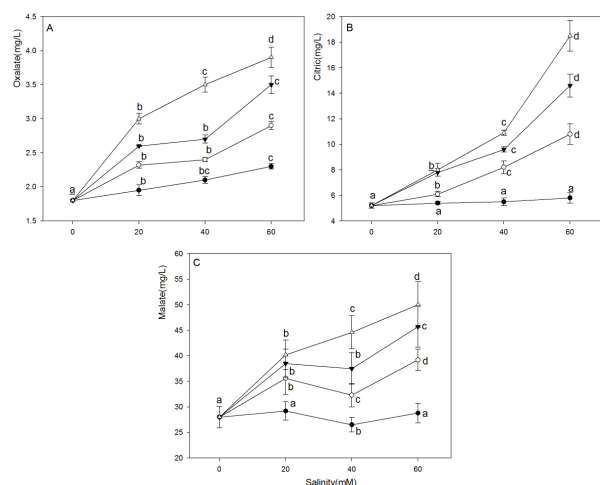


Fig. 4. Effects of mixed salt-alkaline stresses on oxalate acid content (A), citric acid (B) and maliate acid (C) of Jerusalem artichoke

The values are the means of four replicates. Means followed by different letters are significantly different at $P < 0.05$ according to a least significant difference test

only concerned salt stress of alkali stress (Munns, 2002; Debez et al., 2004; Ashraf and Foolad, 2007; Yang et al., 2007, 2009, 2010). However, little information exist concerning the mixed salt-alkali stress conditions.

Seedling establishment is the most important stage for plant surviving in the salt-alkali conditions. This stage is also very sensitive to salinity and alkalinity in the soil. Understanding the physiological responses of Jerusalem artichoke to mixed salt-alkali stress conditions during seedling stage has an important exploring significance. Our results showed that the more ratio of the alkali salts in the treatment, the lower of the fresh weight and dry weight. This indicated that the high pH played important roles in seedling growth, especially at higher salt concentration. The negative effects of salt stress on the plant are mainly contains osmotic stress and toxic ion, but the alkali stress exerts the high pH impacts. Once the salt stress and alkali stress are mixed, the destructive effects on the plant is much greater due to the mixed effects of salinity, pH and toxic ions. Losing water is also a quick way to osmotic adjustment in response to the osmotic stress for most plants (Lissner et al., 1999). With the increasing salt stress, the water content in the shoots of Jerusalem artichoke were decreased. The reasons maybe that the root was damaged under stress condions and then the ability of water absorption was decreased. In addition, many organic solutes were synthesis under such conditons and the water content in the shoots were reduced.

In general, the Na^+ entered the plant cells through the high-affinity K^+ transporter (HKT) and non-selective cation channels (Zhu, 2003). Most plants in the salt environment accumulated a large number of Na^+ and simultaneously inhibited the K^+ absorption (Munns, 2002). In this research, although the Na^+ concentration increased, the K^+ concentration was not affected. Similar results were also found in some other halophytes in Songnen grassland in China, such as *Kochia sieversiana* and *Suaeda glauca* (Yang et al., 2007, 2008). This showed that the increased Na^+ concentrations did not induce the decreased K^+ concentrations in shoot of Jerusalem artichoke, indicating that a specific ion transport mechanism may exit between Na^+ and K^+

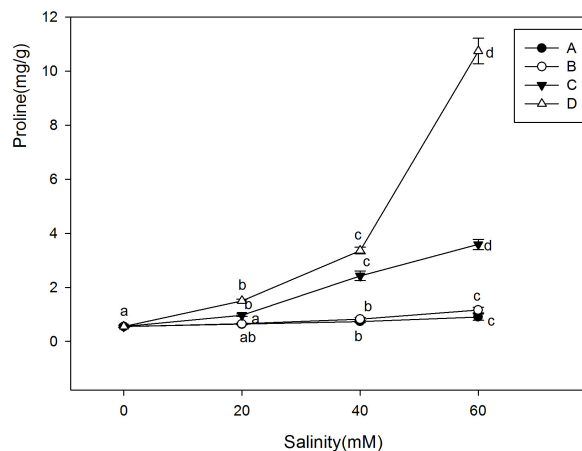


Fig. 5. Effects of mixed salt-alkaline stresses on proline content of Jerusalem artichoke

The values are the means of four replicates. Means followed by different letters are significantly different at $P < 0.05$ according to a least significant difference test

absorptions in this species. However, due to the great number of the Na^+ , the Na^+/K^+ increased sharply at higher salinity in group D. High Na^+/K^+ ratio can affect ions regionalization, lead to ions imbalance, and also reduce enzyme activities. This is why the fresh and dry weights were lowest in this treatment.

Under the circumstance of higher Na^+ concentration, most plants accumulate the inorganic anions, such as Cl^- and NO_3^- in order to keep the ionic balance. In our study, when the treatment was without alkali salts, Jerusalem artichoke accumulated much Cl^- to balance the influx of Na^+ (Fig. 3A). However, once the treatment contained the alkali salts, all the anion contents decreased, indicating that high pH inhibited absorptions of Cl^- . Similar results were also reported for other plants, like *Chloris virgata* (Yang et al., 2010). Seedlings accumulated much organic acids to compensate the anions deficit caused by high pH. Accumulation of organic acids not only played an important osmotic role but also buffered excess toxic cations and kept ionic balance, such as maliate acid, citric acid and oxalate acid in this species. Although a great number of inorganic ions accumulate in vacuoles, plants can also synthesize low molecular mass organic solutes in the cytoplasm, such as proline to prevent dehydration and protect biomacromolecules (Parida and Das, 2005, Long et al., 2010). In the present study, proline was accumulated under stress conditions, especially at higher salinity in group D, indicating that changes of proline content in seedlings may be a special physiological response to high intensity pH stress.

Acknowledgements

The research was supported by the National Natural Science Foundation of China (31470467, 31502013) and the Natural Science Foundation of Heilongjiang Province (C2015060).

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