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RESEARCH ARTICLE

K^+ and Na^+ fluxes in roots of two Chinese Iris populations

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Abstract Maintenance of ion homeostasis, particularly the regulation of K^+ and Na^+ uptake, is important for all plants to adapt to salinity. Observations on ionic response to salinity and net fluxes of K^+, Na^+ in the root exhibited by plants during salt stress have highlighted the need for further investigation. The objectives of this study were to compare salt adaptation of two Chinese Iris (*Iris lactea* Pall. var. *chinensis* (Fisch.) Koidz.) populations, and to improve understanding of adaptation to salinity exhibited by plants. Plants used in this study were grown from seeds collected in the Xinjiang Uygur Autonomous Region (Xj) and Beijing Municipality (Bj), China. Hydroponically-grown seedlings of the two populations were supplied with nutrient solutions containing 0.1 (control) and 140 $mmol \cdot L^{-1}$ NaCl. After 12 days, plants were harvested for determination of relative growth rate and K^+, Na^+ concentrations. Net fluxes of K^+, Na^+ from the apex and along the root axis to 10.8 mm were measured using non-invasive micro-test technique. With 140 $mmol \cdot L^{-1}$ NaCl treatment, shoots for population Xj had larger relative growth rate and higher K^+ concentration than shoots for population Bj. However, the Na^+ concentrations in both shoots and roots were lower for Xj than those for Bj. There was a lower net efflux of K^+ found in population Xj than by Bj in the mature zone (approximately 2.4 – 10.8 mm from root tip). However, no difference in the efflux of Na^+ between the populations was obtained. Population Xj of *I. lactea* continued to grow normally under NaCl stress, and maintained a higher K^+/Na^+ ratio in the shoots. These traits, which were associated with lower K^+ leakage, help population Xj adapt to saline environments.

Keywords *Iris lactea* Pall. var. *chinensis* (Fisch.) Koidz, population, K^+ and Na^+, ion flux, non-invasive micro-test technique

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1 Introduction

NaCl stress in plants can cause inhibition of essential ion uptake. When plants are transferred to a saline culture solution, there will be a reduction in plant K^+ content with an increased in external Na^+/K^+ ratio. Therefore, the tolerance of many plants to NaCl stress may rely on controlled uptake of these ions (i.e. capacity for Na^+ exclusion or K^+ inclusion) [1].

The study of K^+ and Na^+ fluxes along roots in plants could be important for understanding the strategies plants use to survive in a saline environment. Fluxes of ions can be measured using the non-invasive micro-test technique (NMT), a promising method to calculate fluxes from the measured difference in electrochemical potential. Using this technology, differences in net ion fluxes has been obtained from several plant species. For example, a net K^+ efflux was observed in salt-sensitive wheat, showing that the ability to retain K^+ is relevant to their salt-tolerance [2]. Similar results have been found in studies on barley, in which the reduction in dry mass was attributed to higher K^+ leakage [3]. This net Na^+ efflux in roots contributed to adaptation of barley in a saline environment [4] and the K^+/Na^+ ratio could be an indicator of salt tolerance. Additionally, a higher ability for Na^+ exclusion was retained by *Poplar euphratica* roots, especially under long-term salinity stress [5].

Chinese Iris (*Iris lactea* Pall. var. *chinensis* (Fisch.) Koidz., F. Iridaceae) (Fig. 1), a monocotyledonous halophyte, is considered an important salt-tolerant species in areas where salinity is increasingly problematic [6–8]. In China, it is grown in arid deserts or saline areas, and widely distributed in the northern and western provinces, suggesting that the species has great potential for cultivation in areas where salinity and drought is becoming a serious problem. It can be used for animal feed, landscaping, water and soil conservation, papermaking etc. The cultivation of this species can be of considerable ecological and economical value. However, *I. lactea* populations may exhibit different adaptation to salinity or drought, as ionic responses to salinity vary between plant populations. For example, plant accessions from the inland zone of China (e.g. western provinces) have often been found to be relatively drought-resistant because the environments from which they come are significantly more adverse than conditions in the eastern provinces [9].

There has been limited research on the salt tolerance of different *I. lactea* populations, so ionic responses to salinity and net fluxes of K^+, Na^+ in the root exhibited by *I. lactea* during salt stress warrant further investigation. Thus, the relative growth rate of, ion content in, and net K^+ and Na^+ efflux from, roots of two *I. lactea* populations was determined in this study, with the aim of enhancing understanding of ionic relation in this species.

Fig. 1 Photos of wild Chinese Iris (*Iris lactea* Pall. var. *chinensis* (Fisch.) Koidz.)

2 Materials and methods

Seed of *I. lactea var. chinensis* were collected randomly from the arid zone (E93°30′, N42°48′, 740 m) in the Xinjiang Uygur Autonomous Region (Xj) and from the semi-arid zone (N40°34′, E116°10′ 540 m) in northern Beijing Municipality (Bj). Xingjiang has an average annual precipitation and evaporation of 34.9 mm and 2800 mm, respectively, whereas Beijing has 580 and 1830 mm, respectively. In Xingjiang and Beijing the average temperature are 10.0 and 8.5°C, respectively. After collection, the seeds were stored at 4°C to minimise loss of viability.

Seedlings of two populations (Bj and Xj) were supplied with the following nutrient solutions: 0.75 $mmol \cdot L^{-1}$ K_2SO_4, 0.65 $mmol \cdot L^{-1}$ $MgSO_4$, 0.1 $mmol \cdot L^{-1}$ KCl, 2.0 $mmol \cdot L^{-1}$ $Ca(NO_3)_2$, 0.25 $mmol \cdot L^{-1}$ KH_2PO_4, 0.01 $mmol \cdot L^{-1}$ H_3BO_3, 0.001 $mmol \cdot L^{-1}$ $MnSO_4$, 0.0001 $mmol \cdot L^{-1}$ $CuSO_4$, 0.001 $mmol \cdot L^{-1}$ $ZnSO_4$, 5×10^{-6} $mmol \cdot L^{-1}$ $(NH_4)_6Mo_2O_4$, 0.1 $mmol \cdot L^{-1}$ Fe-EDTA. The pH was adjusted to 6.0±0.1 with KOH and H_2SO_4. Nutrient solutions were renewed every 2 d. Day/night temperatures and average relative humidity were (24±2)°C /(21±1)°C and 60%/85%, respectively.

When the hydroponically-grown seedlings were about 75 mm, they were treated with the nutrient solutions containing either 0.1 (control) or 140 $mmol \cdot L^{-1}$ NaCl, which was applied gradually by adding 70 $mmol \cdot L^{-1}$ d^{-1}. Shoots and roots of the plants were sampled at the commencement of treatment to allow for determination of relative growth rate. After final salinity concentrations were reached, stress treatment was started and applied for 12 days. At the end of stress periods, one seedling from each treatment was collected. Relative growth rate (RGR) was calculated using the equation:

$$RGR = (\ln W_2 - \ln W_1)/(T_2 - T_1),$$

where W_1 and W_2 represent shoot dry weight at the beginning of treatments and at the end of the experiment, respectively, and T_1 (first day of treatment) and T_2 (final day of treatment), time in days.

Seedlings were harvest for determination of K^+ and Na^+ content. K^+ and Na^+ content in shoots and roots was measured using the inductively coupled plasma (ICP) method. In this study, ion concentrations were calculated as mole per dm^3 tissue water to provide a more physiologically relevant interpretation in halophytes such as *I. lacteal* [8].

Net fluxes of K^+ and Na^+ were measured with a non-invasive micro-test technique (NMT; BIO-IM system; Younger USA Sci. and Tech. Corp., Amherst, MA, USA) as described by Sun et al. [5] and Chen et al. [10], at Xuyue Sci. and Tech. Corp., Ltd..

One hour prior to measurement, three or four seedlings were taken from the growth chamber, the roots were immediately rinsed with redistilled water and equilibrated for 30 min in the measuring solution for ion flux measurements. K^+ or Na^+ were monitored in the following solutions: 0.1 $mmol \cdot L^{-1}$ KCl, 0.1 $mmol \cdot L^{-1}$ $CaCl_2$, 0.1 $mmol \cdot L^{-1}$ $MgCl_2$, 0.5 $mmol \cdot L^{-1}$ NaCl, 0.3 $mmol \cdot L^{-1}$ MES, 0.2 $mmol \cdot L^{-1}$ Na_2SO_4, pH 6.0. First, 0.05–0.50 $mmol \cdot L^{-1}$ KCl and 0.5–5.0 $mmol \cdot L^{-1}$ NaCl were used to calibrate the ion-selective electrode. Liquid ion exchangers were XY-SJ-K (Sigma #60031, Xuyue Sci. and Tech. Corp., Ltd.) and XY-SJ-Na (Sigma #71178, Xuyue Sci. and Tech. Corp., Ltd.), respectively. The roots were placed in a perspex measuring chamber containing 15 mL of a fresh measuring solution and restrained at the bottom of the chamber. After restraining the roots, scanning at 0.6 mm increments commencing from the apex continued along the root axis until 10.8 mm (where ionic flux responses become less variable). The measuring zone was 0–10.8 mm from the root apex with the root cap zone (< 0.6 mm from root tip), the elongation zone (0.6–1.8 mm from root tip), and the mature zone (> 1.8 mm from root tip). The ion-selective microelectrodes were moved between two positions (30 μm amplitude) close to the plant material in a pre-set excursion at an adjustable programmable frequency of 0.2 Hz by a computerized micromanipulator (Fig. 2).

From the measured difference in electrochemical potential of these ions between two positions, ion fluxes were calculated using MageFlux software (Xuyue Sci. and Tech. Corp., Ltd., http://xuyue.net/mageflux/make_new.php) by Fick's law of diffusion as described [5].

Statistical analysis was performed with the SPSS statistical program (Version11.5, SPSS Inc., 2003), and treatment means were compared by least significant differences at $P = 0.05$.

3 Results

3.1 Plant growth status

Figure 3 shows that the shoots of *I. lactea* under controlled conditions had a higher RGR than the roots, but no difference between the two populations was observed. When the NaCl concentration in the medium increased to 140 mmol·L^{-1}, there was a 1.2-fold larger RGR for shoots of population Xj than for population Bj, whereas RGR of roots in two populations was similar (Fig. 3).

3.2 Concentrations of K^+ and Na^+

Table 1 shows the concentrations of K^+ and Na^+, and the K^+/Na^+ ratio in shoots and roots of two *I. lactea* populations. The results indicated that *I. lactea* populations did not differ in K^+ concentration either in the shoots or the roots under controlled conditions. The same trend in Na^+ concentration and K^+/Na^+ ratio was observed. The NaCl concentration of 140 mmol·L^{-1} caused the K^+ concentration in the two *I. lactea* populations to decline, especially in roots. Also, the K^+ concentration was slightly higher in shoots of population Xj than in those of Bj, and lower in roots of population Xj than in those of Bj. It can be seen from Table 1 that roots of population Xj transport K^+ more than roots of Bj. In NaCl treatment, population Bj had a higher Na^+ concentration than was recorded in Xj, and the effect (1.4 times) was more pronounced in roots. More-

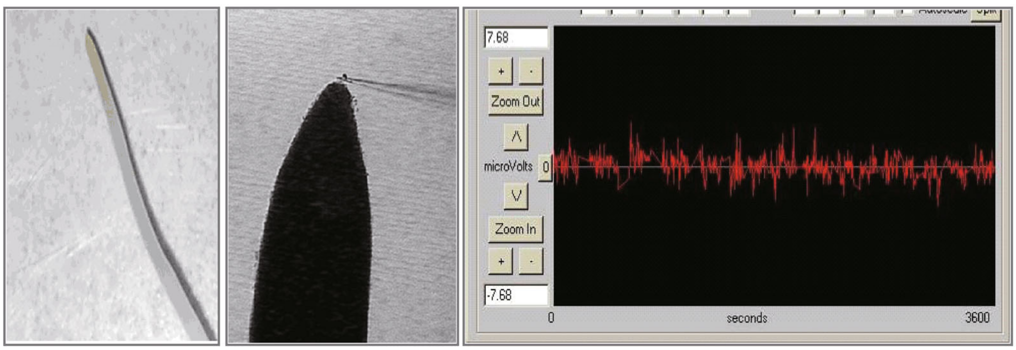

Fig. 2 Microelectrode ion flux estimation technique (MIFE) in root of *I. lacteal*

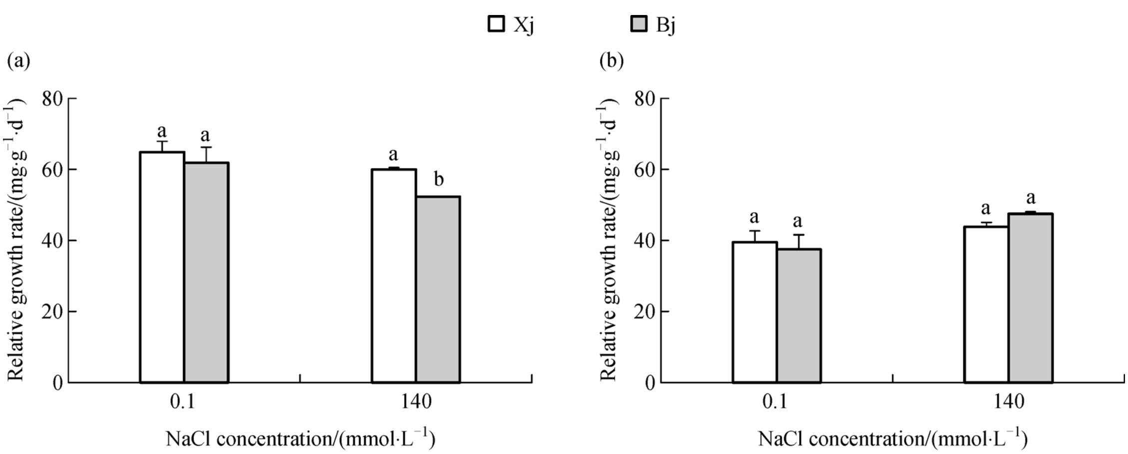

Fig. 3 Relative growth rate in shoots (a) and roots (b) of two *Iris lactea* populations. Seedlings were subjected for 12 days to 0.1 and 140 mmol·L^{-1} NaCl. For each group of two bars, means with different letters are significantly different at $P < 0.05$ using least significant differences (LSD) test.

Table 1 Concentrations ($mmol \cdot g^{-1}$DW) of K^+, Na^+ and K^+/Na^+ ratio in shoots and roots of two *Iris lactea* populations

NaCl/($mmol \cdot L^{-1}$)	Provenance	Shoot			Root		
		K^+	Na^+	K^+/Na^+	K^+	Na^+	K^+/Na^+
0.1	Xj	0.81a	0.11a	7.39	1.03a	0.19a	5.35
0.1	Bj	0.82a	0.15a	5.31	1.19a	0.19a	6.30
140	Xj	0.64a	0.62b	1.03	0.27b	0.74b	0.36
140	Bj	0.44b	0.70a	0.63	0.48a	1.03a	0.46

Note: Seedlings were subjected for 12 days to 0.1 and 140 $mmol \cdot L^{-1}$ NaCl. Values marked with different letter represent significant difference at $P < 0.05$ between two populations at a given NaCl level.

over, there was a higher K^+/Na^+ ratio in shoots of population Xj than in shoots of Bj, which showed population Xj had a higher ability to maintain K^+/Na^+ ratio.

3.3 Net fluxes of K^+ and Na^+ in roots

Figure 4 illustrates the effect of NaCl on net K^+ flux in roots of two *I. lactea* populations. Under controlled conditions, a higher K^+ efflux in population Xj was recorded in the elongation zone, which was less than 3.2 mm from the tip. However, K^+ efflux in the mature zone was the same for the two populations. For plants grown at 140 $mmol \cdot L^{-1}$ NaCl, a lower K^+ efflux in population Xj was recorded in the mature zone. These results indicate that population Xj retained more K^+ under NaCl stress.

Figure 5 indicates the effect of NaCl on net Na^+ flux in

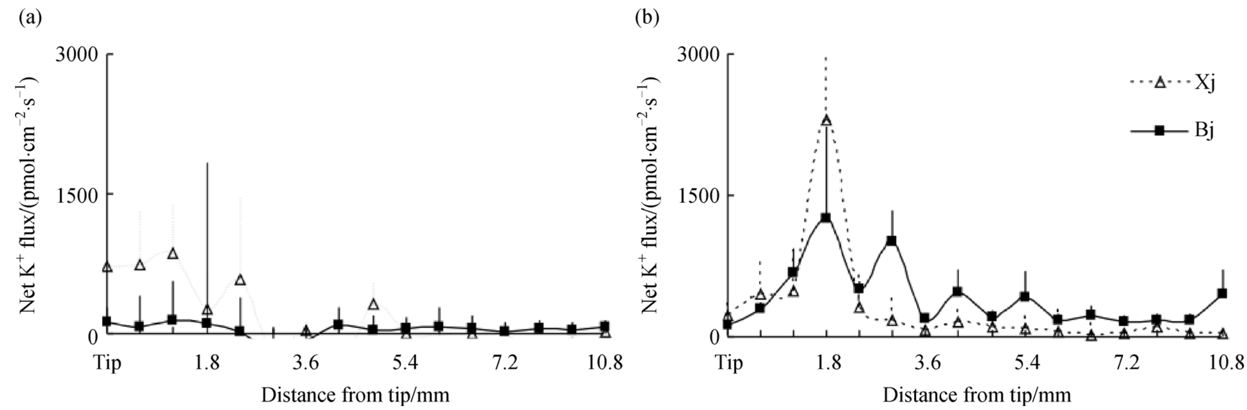

Fig. 4 Net K^+ flux in roots of two *Iris lactea* populations. Seedlings were subjected for 12 days to 0.1 and 140 $mmol \cdot L^{-1}$ NaCl. K^+ fluxes were measured at 0.6 mm intervals, starting from the root tip. Each point is the mean of 3–4 individual plants. Vertical bars represent standard error. (a) Control; (b) salt.

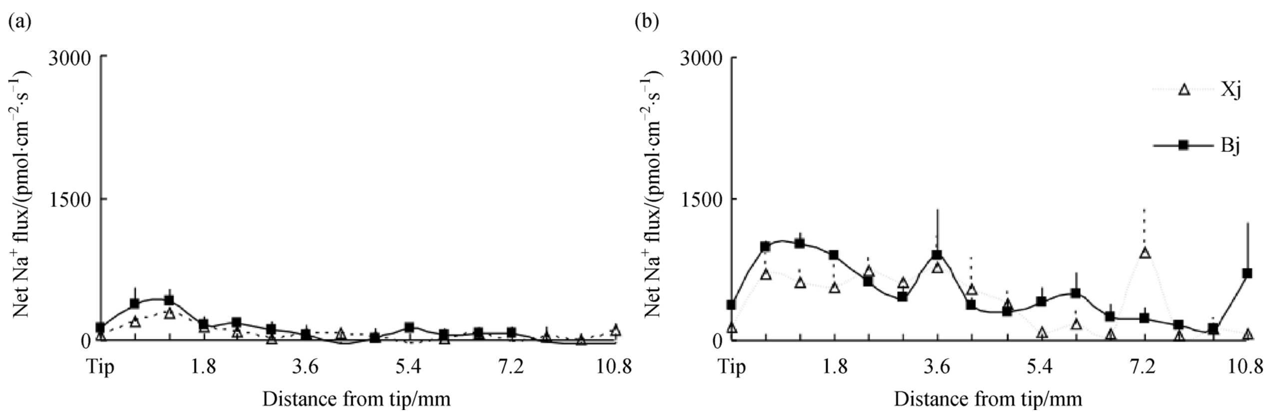

Fig. 5 Net Na^+ flux in roots of two *Iris lactea* populations. Seedlings were subjected for 12 days to 0.1 and 140 $mmol \cdot L^{-1}$ NaCl. Na^+ fluxes were measured at 0.6 mm intervals, starting from the root tip. Each point is the mean of 3–4 individual plants. Vertical bars represent standard error. (a) Control; (b) salt.

roots of two *I. lactea* populations. There was a low Na^+ efflux in the two populations, which was due to the very low Na^+ concentration in the control. Na^+ efflux increased as Na^+ concentration in the medium was raised to 140 mmol·L^{-1}. Notably, no difference between the two populations was detected, with the Na^+ effluxes both about 700 pmol·cm^{-2}·s^{-1}. The two populations had the same capacity for Na^+ exclusion at 140 mmol·L^{-1} NaCl.

4 Discussion

In population Xj, the proportion of dry mass from shoots was high, which resulted in a slight reduction in shoot RGRs. In contrast, population Bj, had a relative low shoot RGR, indicating NaCl had an adverse effect on its growth. This was consistent with the result obtained for sorghum [11]. The above result shows that Xj is better adapted for saline environments.

Potassium is an essential nutrient for higher plants, but K^+ leakage can be induced by salinity. Thus, the ability to retain K^+ is essential for plant to be adapted to salinity. The K^+ leakage for *I. lactea* was highest in the elongation zone, and declined in the mature zone. However, the rate of decline was less than in barley as found in earlier work, which showed a 90% reduction in K^+ leakage in the mature zone [12]. This difference may depend on genotype and the intensity of NaCl stress. At 140 mmol·L^{-1} NaCl, a lower K^+ efflux in population Xj was found in the mature zone, suggesting that this root zone is important for retaining K^+. Therefore, the greater ability to retain K^+ appears to contribute to the adaptation of population Xj to salinity.

In addition, capacity for Na^+ exclusion contributes to salt-tolerance, especially in Na^+ excluders, as was found for *I. lactea*. Na^+ efflux increased as external Na^+ concentration was raised to 140 mmol·L^{-1}, indicating that both populations adapted to salinity by Na^+ exclusion. However, there was no difference between the two populations, and it was evident that Na^+ concentration in the shoots (0.13 – 0.17 mmol·L^{-1}) had not accumulated to toxic level. Therefore, Na^+ exclusion may not help population Xj adapt to 140 mmol·L^{-1} NaCl stress. From the result of K^+ leakage in roots of *I. lactea*, we can infer that better adaptation to salinity in populations (i.e. population Xj) was associated with the process of K^+ inclusion rather than Na^+ exclusion.

The two populations did not differ in K^+ concentration under controlled conditions. However, under NaCl stress, population Xj retained K^+ in the shoots. Nevertheless, there was no difference in Na^+ concentration between the two populations, indicating population of Xj preferentially retained K^+ in the shoots, when the two populations were under the same ionic stress induced by 140 mmol·L^{-1} NaCl. Flowers and Colmer suggested that the net $S_{K:Na}$ by monocotyledonous halophytes was high at low salinity [13], which was why the monocotyledonous halophyte, *I. lactea*, was used in this study. Numerous studies have revealed that the K^+/Na^+ ratio rather than Na^+ concentration could be a good indicator of plant stress. This study demonstrated that the higher growth in saline environments exhibited by population Xj may be attributed to its ability to maintain the K^+/Na^+ ratio.

5 Conclusions

Population Xj of *I. lactea* continued to grow normally under 140 mmol·L^{-1} NaCl stress, which exhibited a better adaptation compared with population Bj. Its greater ability to maintain the K^+/Na^+ ratio in shoots could be associated with lower K^+ leakage in this population. These traits help population Xj adapt to saline environments. The findings in this paper, however, are based only on the experiments involving 140 mmol·L^{-1} NaCl stress. The ion flux and absorption characteristics of ions by Chinese Iris with higher salt levels and the relationship of various influencing factors need further studies.

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Compliance with ethics guidelines Pinfang Li and Biao Zhang declare that they have no conflict of interest or financial conflicts to disclose.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Ashraf M. Some important physiological selection criteria for salt tolerance in plants. *Flora-Morphology, Distribution, Functional Ecology of Plants*, 2004, 199(5): 361–376
2. Cuin T A, Betts S A, Chalmandrier R, Shabala S. A root's ability to retain K^+ correlates with salt tolerance in wheat. *Journal of Experimental Botany*, 2008, 59(10): 2697–2706
3. Chen Z, Shabala S, Mendham N, Newman I, Zhang G, Zhou M. Combining ability of salinity tolerance on the basis of NaCl-induced K^+ flux from roots of barley. *Crop Science*, 2008, 48(4): 1382–1388
4. Chen Z, Pottosin I I, Cuin T A, Fuglsang A T, Tester M, Jha D, Zepeda-Jazo I, Zhou M, Palmgren M G, Newman I A, Shabala S. Root plasma membrane transporters controlling K^+/Na^+ homeostasis in salt-stressed barley. *Plant Physiology*, 2007, 145(4): 1714–1725
5. Sun J, Chen S, Dai S, Wang R, Li N, Shen X, Zhou X, Lu C, Zheng X, Hu Z, Zhang Z, Song J, Xu Y. NaCl-induced alternations of cellular and tissue ion fluxes in roots of salt-resistant and salt-sensitive poplar species. *Plant Physiology*, 2009, 149(2): 1141–1153

6. Zhao K F, Li F Z, Zhang F S, eds. *Halophyte in China*. Beijing: Science Press, 2013, 450 (in Chinese)
7. Bai W B, Li P F, Li B G, Fujiyama H, Fan F C. Some physiological responses of Chinese Iris to salt stress. *Pedosphere*, 2008, 18(4): 454–463
8. Zhang B, Li P F, Fan F C. Ionic relations and proline accumulation in the shoots of two Chinese Iris germplasms during NaCl stress and subsequent relief. *Plant Growth Regulation*, 2012, 68(1): 49–56
9. Shi X X, Mao P C, Zhang G F, Meng L. The comparison of drought resistance on 15 Chinese Iris germplasm at seedling stage. *Acta Agrestia Sinica*, 2007, 15: 352–358 (in Chinese)
10. Chen J, Xiao Q, Wu F, Dong X, He J, Pei Z, Zheng H. Nitric oxide enhances salt secretion and Na^+ sequestration in a mangrove plant, *Avicennia marina*, through increasing the expression of H^+-ATPase and Na^+/H^+ antiporter under high salinity. *Tree Physiology*, 2010, 30(12): 1570–1585
11. de Lacerda C F, Cambraia J, Oliva M A, Ruiz H A. Changes in growth and in solute concentrations in sorghum leaves and roots during salt stress recovery. *Environmental and Experimental Botany*, 2005, 54(1): 69–76
12. Chen Z, Newman I, Zhou M, Mendham N, Zhang G, Shabala S. Screening plants for salt tolerance by measuring K^+ flux: a case study for barley. *Plant, Cell & Environment*, 2005, 28(10): 1230–1246
13. Flowers T J, Colmer T D. Salinity tolerance in halophytes. *New Phytologist*, 2008, 179(4): 945–963