Comparison of osmotic adjustment and ion balance strategies in nineteen alkali-tolerant halophyte species during adaptation to salt-alkalinized habitats in northeast China

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Abstract

Alkali stress is an important agricultural contaminant and has complex effects on plant metabolism. However, the underlying mechanism of alkali tolerance remains obscure. In the present study, 19 alkali resistant halophyte species grown on alkalinized grassland of northeast China were chosen as test species. The plants were harvested from the grassland, and the contents of inorganic ions, compatible solutes and organic acids (OAs) were determined. Finally, their osmotic adjustment and ion balance strategies to the salt-alkalinized habitats were compared. Results showed that all 19 halophytes accumulated betaine and soluble sugars as dominant organic osmolytes in protoplasm, and OAs played key roles not only in osmotic adjustment but also in ion balance. The halophytes had many osmotic adjustment and ion balance strategies in common, but also had a diversity of adaptative strategies to their salt-alkalinized habitats. For example, three species of Chenopodiaceae showed large accumulations of Na^+ and oxalate; however, the role of K^+ in osmotic adjustment was small. The five Poaceae halophytes mainly accumulated K^+, and Cl^- as their dominant contributor to negative charge (ion balance).

Keywords: Alkali stress, Osmolyte accumulation, Ion homeostasis, Semiarid grassland.
Abbreviations: EC–electrical conductivity, OA–organic acid, SS–soluble sugar, Pro–proline, AA–Amino acid, Ma–mannitol.

Introduction

The salinization of soil is a widespread environmental problem and an important factor in limiting agricultural productivity. Halophytes are remarkable plants that tolerate salt concentrations that kill 99% of other species (Flowers and Colmer, 2008). However, considerable studies have focused on sensitive species such as Arabidopsis and rice (Munns and Tester, 2008). These sensitive plants may only provide limited insights into mechanisms of salinity tolerance unless compared with halophytes (Flowers and Colmer, 2008). Although there have been numerous studies of halophytes, there is a lack of systemic and deeper research on their salinity tolerance. As salt tolerance is complex, various halophytes may have completely different tolerance mechanisms. Thus, we believe that a number of different species should be considered in this case. Important questions are: (i) whether all halophytes tolerate salt, fundamentally, in the same way; (ii) whether specific mechanisms can be identified and, if so, whether these are linked taxonomically; and (iii) whether specific mechanisms have evolved to deal with interactions between salinity and other environmental variables (Flowers and Colmer, 2008). To date, due to the lack of systemic comparisons on halophyte tolerance, these issues are unclear. Northeast China is rich in halophyte resources. However, in this area soil alkalization is a more severe problem than soil salinization where more than 70% of grassland is alkaline (Yang et al., 2007) and some with soil pH > 10. These salt-alkalinized soils are very complex, and in different soil patches the total component of salts and the proportion of neutral to alkaline salts can differ. Thus, the stresses imposed by these soil media on plants can be very complex, and involve osmotic stress, ion injury, high-pH stress, as well as interactions among various ions. Therefore, only a few alkali-tolerant halophytes can survive on these soils. The tolerance of these halophytes to their salt-alkalinized habitats relies on controlled uptake and compartmentalization of Na^+, K^+ and Cl^-, the synthesis of compatible solutes, maintaining intracellular ion balance or stable pH, and pH adjustment outside roots. It is possible that different species may have different adaptive strategies. Under salt stress, halophytes usually accumulate inorganic ions such as Na^+, K^+, NO_3^- and Cl^- in vacuoles to decrease cell water potential. They also synthesize low molecular-weight compatible solutes (e.g. betaine, proline, free sugars and polyalcohols) in the cytoplasm to prevent dehydration. In recent years, reports have showed that organic acids (OAs) may play important roles in the high-pH tolerance mechanism, and may participate in intracellular ion balance and pH adjustment outside roots (Yang et al., 2007; Yang et al., 2008). To date, most of salt stress research have still focused on NaCl stress (Munns and Tester, 2008; Charkazi et al., 2010; Jemâa et al., 2011; Ibraheem et al., 2011; Zhou et al., 2011) and some important points such as simple alkali stress or native alkaline stress, have been neglected (Yang et al., 2007; Yang et al., 2008). In the present study, 19 halophyte species growing on semi-arid alkalinized grassland of northeast China were chosen as test species.

Table 1. pH and electrical conductivity (EC) of the soils in habitats of various halophyte species.

Family	Species	Soil Electrical conductivity (μs/cm)	Soil pH
Chenopodiaceae	*Kochia Sieversiana*	301.3±7.8	10.10±0.16
	Suaeda glauca	775.3±30	9.70±0.02
	Suaeda salsa	561.0±4.6	10.23±0.02
Poaceae	*Calamagrostis macrolepis*	291.0±8.7	10.06±0.01
	Chloris virgata	119.7±1.8	9.46±0.05
	Puccinellia tenuiflora	102.0±6.5	9.28±0.02
	Leymus chinensis	70.7±1.2	8.55±0.14
	Phragmites hirsuta	448.7±5.7	10.14±0.02
Compositae	*Artemisia anethifolia*	136.3±33.3	8.76±0.05
	Artemisia scoparia	85.0±8.5	9.01±0.01
	Sonchus arvensis	116.0±1.5	9.15±0.07
	Kalimeris integrifolia	80.3±0.7	8.49±0.02
	Inula japonica	111.0±1.2	9.27±0.04
Leguminosae	*Melilotus officinalis*	86.3±5.4	8.92±0.03
	Lespedeza hedysaroides	78.7±0.9	8.82±0.05
	Astragalus complanatus	82.3±0.3	8.66±0.07
Asclepiadaceae	*Cynanchum chinense*	88.0±6.0	9.01±0.03
Cyperaceae	*Carex duriuscula*	130.0±6.4	9.26±0.01
Boraginaceae	*Messerschmidia sibirica*	198.0±15.0	9.78±0.02

The data of pH and electrical conductivity (EC) were the results of mensuration using a 1:5, (soil: water) suspension, and were the means ($\pm$S.E.) of three replicates.
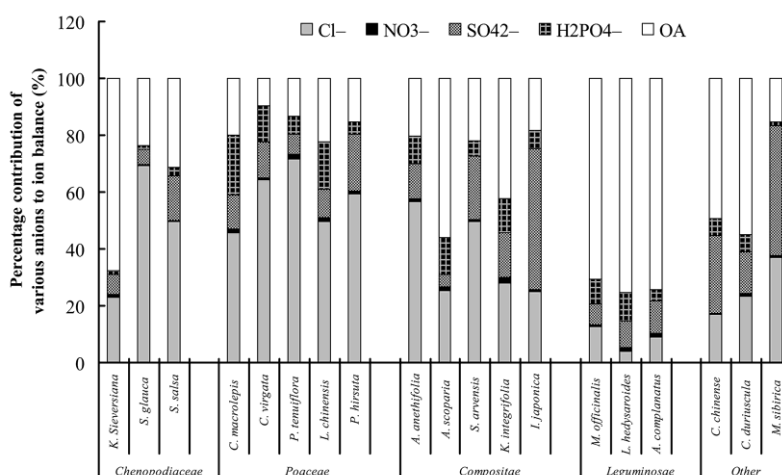

Fig 1. Percentage contribution of various anions to total negative charge of different halophytes growing in salt-alkalinized grassland. The percentages were calculated according to the means of each anion. OA, organic acid.

These plants were harvested from the grassland, and then the contents of inorganic ions, compatible solutes and OAs were determined. Finally, their physiological adaptive strategies to their salt-alkalinized habitats were analyzed and compared.

Results

Description of salt-alkalinized habitats of various halophyte specie.

The halophytes used in this study grow naturally on highly alkaline soils with pH 8.55-10.23 (Table 1), and even these halophytes can colonize bare alkaline patches as a pioneer species. Halophyte species are shown in Table 1. The tolerance of Chenopodiaceae to the natural salt-alkaline stress was strongest, and both EC and pH of soils in their habitats

were relatively high. The pH of soils in the habitat of *Suaeda salsa* exceptionally reached to 10.23 (Table 1).

Ion contents

Both Na$^+$ and Cl$^-$ contents in the three species of Chenopodiaceae were much higher than those for other species, and lowest for all species of Leguminosae (Tables 2 and 3). The sum of Na$^+$ and Cl$^-$ contents in *Suaeda glauca* reached 25.2% of dry weight (DW), but was only 0.12% of DW in *Lespedeza hedysaroides*. These values (25.2% and 0.12%) were calculated according to the molecular weights of Na$^+$ and Cl$^-$ and their molar contents. K$^+$ contents in most Compositae species were much higher than those for other species, and relatively low for two Chenopodiaceae species

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Table 2. Cation contents of different halophytes growing in salt-alkalinized grassland. The values are means (±SE) of three replicates.

Family	Species	Na^+ ($\mu mol\ g^{-1}$ DW)	K^+ ($\mu mol\ g^{-1}$ DW)	Na^+/K^+	Free Ca^{2+} ($\mu mol\ g^{-1}$ DW)	Free Mg^{2+} ($\mu mol\ g^{-1}$ DW)
Chenopodiaceae	*K. Sieversiana*	1863±51	195±7	9.57±0.15	2.3±0.1	77±3
	S. glauca	3720±54	275±7	13.52±0.14	4.0±0.4	151±2
	S. salsa	1698±710	468±11	3.57±1.48	3.8±0.6	144±3
Poaceae	*C. macrolepis*	43±1	367±2	0.12±0.00	11.9±0.4	38±0.2
	C. virgata	344±3	475±11	0.73±0.01	7.1±0.2	34±1
	P. tenuiflora	66±1	286±22	0.23±0.00	13.7±0.4	21±1
	L. chinensis	132±3	314±9	0.42±0.01	11.0±0.2	36±0.4
	P. hirsuta	54±2	241±9	0.22±0.01	23.6±0.2	92±2
Compositae	*A. anethifolia*	664±76	256±26	2.58±0.04	37.2±2.4	59±5
	A. scoparia	148±2	541±11	0.27±0.00	15.4±1.0	47±1
	S. arvensis	437±35	436±31	1.00±0.02	111±1.2	210±2
	K. integrifolia	64±0.5	592±5	0.11±0.00	26.0±0.4	46±0
	I. japonica	208±15	436±19	0.48±0.03	225±0.9	129±4
Leguminosae	*M. officinalis*	43±2	325±16	0.13±0.00	59.1±2.7	151±7
	L. hedysaroides	28±3	188±4	0.15±0.01	43.2±1.4	105±3
	A. complanatus	53±3	490±14	0.11±0.00	99.2±0.9	171±0.4
Asclepiadaceae	*C. chinense*	346±6	415±18	0.84±0.02	80.4±4.4	245±1
Cyperaceae	*C. duriuscula*	47±0.3	471±10	0.10±0.00	28.0±0.5	55±3
Boraginaceae	*M. sibirica*	768±35	236±4	3.26±0.10	30.6±4.1	135±9

Table 3. Anion contents of different halophytes growing in salt-alkalinized grassland. The values are means (± SE) of three replicates.

Family	Species	Cl^- ($\mu mol\ g^{-1}$ DW)	SO_4^{2-} ($\mu mol\ g^{-1}$ DW)	NO_3^- ($\mu mol\ g^{-1}$ DW)	$H_2PO_4^-$ ($\mu mol\ g^{-1}$ DW)
Chenopodiaceae	*K. Sieversiana*	589±7	89±2	26.8±1.9	34.7±1.0
	S. glauca	4691±1260	190±54	15.2±5..4	91.3±20.1
	S. salsa	1646±11	259±9	12.1±1.5	101±7.9
Poaceae	*C. macrolepis*	163±1	21±1	5.5±0.5	75.4±4.3
	C. virgata	428±109	42±11	5.2±1.3	83.9±23.5
	P. tenuiflora	200±3	10±1	5.0±0.1	18.0±0.1
	L. chinensis	167±3	17±0.4	4.8±0.2	55.6±0.6
	P. hirsuta	270±6	45±1	4.5±0.1	19.5±1.3
Compositae	*A. anethifolia*	449±7	50±1	6.0±0.8	75.6±1.8
	A. scoparia	114±2	10±1	6.7±0.5	57.7±1.8
	S. arvensis	840±7	189±1	10.3±3.1	88.4±5.4
	K. integrifolia	84±2	24±0.2	5.9±0.2	36.0±0.2
	I. japonica	259±11	256±14	7.6±1.4	63.7±5.4
Leguminosae	*M. officinalis*	80±1	24±1	4.9±0.3	54.8±0.4
	L. hedysaroides	16±2	18±1	5.7±0.9	39.2±5.9
	A. complanatus	83±6	51± 0.2	14.5±3.3	39.5±1.3
Asclepiadaceae	*C. chinense*	372±24	302±23	5.4±0.5	127±11.3
Cyperaceae	*C. duriuscula*	134±2	42±1	4.8±0.2	34.1±0.7
Boraginaceae	*M. sibirica*	351±27	215±15	5.7±0.5	13.6±0.6

Kochia Sieversiana and *S. glauca* (Table 2). The Ca^{2+} contents of the Chenopodiaceae were the lowest of all species (Table 2). The Mg^{2+} contents in the Poaceae were much lower than other species (Table 2). The Na^+/K^+ ratio in three species of Chenopodiaceae were much higher than those for other species (Table 2), and lowest for all species of Leguminosae. The SO_4^{2-} contents of Chenopodiaceae were also relatively high (Table 3). In addition, NO_3^- contents of the Chenopodiaceae were slightly higher than other species (Table 3).

Compatible solutes

Proline and free AA contents in all species were minimal, and SS and betaine were relatively high (Table 4). Comparison of different family or different species of same family showed that OA and betaine contents in the Chenopodiaceae were much higher than those in other species (Table 4). For other solutes, the difference between different species of same family was clear (Table 4).

Table 4. Organic solute contents of various halophytes growing in salt-alkalinized grasslands. The values are means (± SE) of three replicates.

Family	Species	Proline	Soluble sugar	Mannitol	Amino acid	Organic acid	Betaine
		(μmol g^{-1} DW)					
Chenopodiaceae	*K. Sieversiana*	2.9±0.2	83±4	18.5±2.6	10.2±0.03	864±17	496±22
	S. glauca	1.3±0.1	206±34	26.4±0.7	3.9±0.03	797±213	540±24
	S. salsa	1.1±0.1	158±3	31.1±2.1	5.3±0.04	528±14	353±15
Poaceae	*C. macrolepis*	1.0±0.0	188±6	40.8±0.2	4.5±0.2	35±1	180±5
	C. virgata	1.9±0.2	187±3	35.1±1.5	4.8±0.2	35±7	170±16
	P. tenuiflora	1.0±0.1	669±8	38.1±4.8	3.4±0.1	19±1	280±33
	L. chinensis	1.9±0.1	173±2	27.8±1.5	6.9±0.2	45±6	394±16
	P. hirsuta	0.9±0.0	238±0	27.4±0.8	5.4±0.2	35±0.5	324±40
Compositae	*A. anethifolia*	2.3±0.4	210±67	27.3±2.3	3.5±0.2	77±2	361±30
	A. scoparia	2.5±0.2	214±1	47.6±1.5	4.6±0.1	113±5	170±25
	S. arvensis	4.8±0.3	304±6	64.8±7.4	9.0±0.1	177±	183±7
	K. integrifolia	0.6±0.1	226±16	33.9±2.8	2.6±0.1	63±1	337±7
	I. japonica	3.1±0.2	452±12	37.3±0.8	4.4±0.3	90±4	437±43
Leguminosae	*M. officinalis*	4.6±0.1	291±12	66.0±5.1	11.0±0.2	198±33	238±6
	L. hedysaroides	1.8±0.3	113±3	51.2±1.5	5.0±0.1	136±3	150±18
	A. complanatus	1.6±0.0	161±7	56.9±2.0	7.6±0.1	311±11	353±28
Asclepiadaceae	*C. chinense*	4.3±0.2	276±8	35.8±0.5	12.8±0.2	438±11	169±7
Cyperaceae	*C. duriuscula*	0.7±0.0	331±6	29.7±0.2	4.4±0.03	145±14	131±47
Boraginaceae	*M. sibirica*	0.4±0.1	100±4	41.3±0.8	1.2±0.1	66±1	267±38

Table 5. Organic acid contents of various halophytes growing in salt-alkalinized grassland. The values are means (± SE) of three replicates.

Family	Species	Tartrate	Citrate	Malate	Formate	Lactate	Acetate	Succinate	Oxalate
		(μmol g^{-1} DW)							
Chenopodiaceae	*K. Sieversiana*	0.0	19.7±0.2	5.0±0.8	0.3±0.2	0.8±0.3	20.7±3.6	2.3±1	815±17
	S. glauca	31.8±1.5	2.9±0.2	4.3±0.4	0.4±0.1	0.2±0.1	4.7±0.5	0.0	753±214
	S. salsa	16.1±0.7	2.0±0.1	9.2±0.5	0.1±0.1	0.8±0.1	24.9±1.9	1.1±0.6	474±17
Poaceae	*C. macrolepis*	0.4±0.1	10.0±2.6	9.6±0.5	0.0	0.0	9.0±1.3	0.7±0.7	5.5±0.1
	C. virgata	0.0	6.7±0.3	3.5±0.5	1.5±0.1	1.5±1.5	8.9±4.7	3.2±	9.6±1.7
	P. tenuiflora	1.2±0.1	5.6±0.3	1.1±0.5	1.7±0.0	1.1±0.6	4.0±0.1	0.0	4.4±0.1
	L. chinensis	0.8±0.1	1.8±0.1	9.2±0.4	0.0	0.0	16.1±5.0	1.9±1	14.9±0.3
	P. hirsuta	1±0.05	7.9±0.2	8.6±0.6	0.0	0.0	8.0±0.5	2.3±0.2	7.4±0.3
Compositae	*A. anethifolia*	0.0	14.2±0.2	1.9±0.2	0.4±0.4	0.8±0.4	6.7±2.6	0.0	53.2±2.6
	A. scoparia	7.5±0.1	38.6±1.1	24.6±3.6	0.1±0.1	1.9±0.7	9.4±4.8	5.5±2.5	25.0±0.7
	S. arvensis	41.8±1	29.3±1.2	85.8±6	1.4±0.0	1.3±0.7	5.8±2.1	0.0	11.6±0.2
	K. integrifolia	0.0	11.0±0.3	8.4±0.9	0.0	2.9±1.8	7.7±0.5	3.4±0.2	29.8±0.4
	I. japonica	39.1±0.7	22.8±1.3	5.2±0.3	0.8±0.4	1.1±0.4	8.9±5.3	0.0	11.5±0.3
Leguminosae	*M. officinalis*	22.1±10	89.6±14	41.4±7.4	1.8±0.2	0.5±0.5	34.9±2.0	0.0	7.8±0.4
	L. hedysaroides	41.7±1.3	36.7±0.4	27.7±0.6	0.0	1.3±0.2	9.1±0.5	1.8±0.1	17.8± 0.3
	A. complanatus	0.0	166±3.8	45.4±3.6	0.8±0.8	1.4±0.7	92.7±4.1	0.0	7.0±0.1
Asclepiadaceae	*C. chinense*	54.4±1.6	216±6.3	151.2±2.5	0.4±0.0	0.8±0.2	5.4±0.8	0.0	10.0±1.3
Cyperaceae	*C. duriuscula*	4.1±0.5	46.6±20	63.6±9.2	0.4±0.4	1.0±0.1	21.5±2.5	0.0	7.5±0.6
Boraginaceae	*M. sibirica*	0.3±0.01	22.8±0.3	9.2±0.6	7.5±0.6	1.2±0.4	0.0	8.1±1.5	16.6±0.2

Organic acids

Organic acidcontents (OA) in the Chenopodiaceae were higher than in other species (Tables 4 and 5). Various halophytes differed in both contents and components of OAs (Tables 5 and 6). Tartrate, malate, citrate, succinate, acetate, oxalate, formate and lactate were all detected, in which malate, citrate and oxalate were the dominant components in most species. Total OA content of Poaceae were the lowest of all species (Table 4). In all species of Poaceae, acetate also was a dominant component of OA (Table 6). In addition, in *Sonchus arvensis* and *Inula japonica* in Compositae and *L. hedysaroides* of Leguminosae, tartrate was a dominant components of OA (Table 6). The oxalate content was particularly high in the Chenopodiaceae at 89.7–94.4% of total OAs in shoots (Tables 5 and 6); however, the contents of other OAs were low, especially formate and lactate.

Percentage contribution of each solute to osmotic adjustment

In this experiment, all halophytes accumulated betaine and SS as dominant organic osmolytes in protoplasm (Table 7), and accumulated Na^+, Cl^- and OA as the main osmolytes in vacuoles (Table 7). In all the species, the osmotic contributions of both proline and free AAs were very small (Table 7). Although Na^+, K^+, Cl^- and OAs were the main osmolytes in vacuoles for most species, their contributions to osmotic adjustment differed. The Na^+ and Cl^- contents in the Chenopodiaceae were much higher than in other species, and were lowest in the Leguminosae. In the Chenopodiaceae, Na^+, Cl^- and OA played crucial roles in osmotic adjustment, while the role of K^+ was small. The Chenopodiaceae had lower K^+ / Na^+ ratios in shoots (Table 2); however, K^+ played a greater osmotic role in the other halophytes (Tables 2 and 7). For example, all five Poaceae species mainly accumulated K^+ and maintained high K^+/Na^+ in shoots (Table 2). In addition, in all Leguminosae species and some others such as *K. Sieversiana*, *Cynanchum chinense* and *Carex duriuscula* the OAs also played a valuable osmotic role (Table 7).

Discussion

Natural salt-alkaline stress

In the present study, we selected 19 typical halophyte species growing on semi-arid alkalinized grassland of northeast China as test plants. In their natural salt-alkalinized habitats, the halophytes are subjected to the complex and severe abiotic stresses, which are more complex than single salt stresses. These halophytes live for extended periods on semi-arid alkalinized grassland, and they need to cope with not only salt and water stresses but also high-pH and nutrition stresses. High-pH stress can break the ionic balance and pH homeostasis in tissue (Yang et al., 2007; Yang et al., 2008). Thus, halophytes living in alkalinized grassland not only have to resist osmotic stress and maintain ionic balance, but must spend material and energy for regulating pH in the root environment. Our results indicated that 19 halophytes showed a diversity of osmotic adjustment and ion balance strategies (Table 7 and Fig. 1).

Osmotic adjustment and ion balance

In this study, all halophytes accumulated betaine and SS as

dominant organic osmolytes in protoplasm; Na^+, Cl^- and OA as the main osmolytes in vacuoles and osmotic contributions of both proline and free AAs in very small amounts (Table 7). Interestingly, in the Chenopodiaceae, Na^+, Cl^- and OA played crucial roles in osmotic adjustment, while the role of K^+ was small. However, K^+ played a greater osmotic role in the other halophytes such as the five Poaceae species (Tables 2 and 7). The pH homeostasis of the internal environment is related to all free ions and also all solutes with charge, and is a result of ion balance that includes organic and inorganic ions (Touraine et al., 1988; Yang et al., 2007; Yang et al., 2008). Na^+ is the main toxic ion in salinized soil (Blumwald, 2000; Zhu, 2003; Benderradji et al., 2011). In the present study, we found that most of halophytes accumulated larger Na^+ in shoots (Table 2). The influx of superfluous Na^+ may lead to *in vivo* ion imbalance and a stable pH of the halophytes. Our results also showed that, in salt-alkalinized habitats, the halophytes accumulate inorganic anions and synthesized organic acids, to neutralize massive positive charges and maintain intracellular ion balance (Fig. 1). However, inorganic anions and OAs may have different roles in various halophytes (Fig. 1). The dominant intracellular cations in all halophytes were Na^+ and K^+, while the donors of negative charge differed between species. For most species, OAs and Cl^- were the dominant components in maintaining ionic equilibrium (Fig. 1). In some species such as *S. glauca*, *S. salsa*, all Poaceae, and both Compositae species, the contribution of Cl^- to the total negative charge (ion balance) was higher than OAs (Fig. 1). However, in species such as *K. sieversiana*, all Leguminosae, *Artemisia scoparia* and *C. chinense*, OAs was the dominant factor in maintaining ionic equilibrium. For example, the contribution of OAs to the total negative charge was as high as 75.5% in *L. hedysaroides* (Fig. 1). In addition, in the species such as *Phragmites hirsuta, Sonchus arvensis, C. chinense, Messerschmidia sibirica and I. japonica,* SO_4^{2-} was also important in ion balance, and the dominant component in *I. japonica*. These results showed that OAs played key roles not only in osmotic adjustment but also in ion balance (Table 7 and Fig. 1). Particularly in the Chenopodiaceae, the oxalate content was the highest at 89.7–94.4% of total OAs in shoots (Tables 5 and 6), and reached to 7.33% of DW in *S. glauca*, in agreement with the results of our control experiment. We already conducted a control experiment on alkali tolerance of *S. glauca* (Yang et al., 2008) and *K. Sieversiana* (Yang et al., 2007). In this control experiment (sand culture method), we also observed both *S. glauca* and *K. Sieversiana* accumulated a larger oxalate. During the adaptation of halophytes to alkali stress or natural salt-alkaline stress, OA accumulation might be due to a massive influx of Na^+ and deficiency of inorganic anions, and OA metabolic regulation might be a key pathway for intracellular pH adjustment of these halophytes. These halophytes may also release OAs to regulate pH outside of root environment.

Comparison of adaptive strategies of nineteen halophytes

In these halophytes, the tolerance of Chenopodiaceae to natural salt-alkaline stress was stronger than in other species, and both EC and pH of soils in their habitats were relatively high (Table 1) in agreement with Zheng and Li (1999). Our results also revealed that the Leguminosae species were most susceptible to salt-alkaline stress, and the EC and pH of their soils were lowest (Table 1). Our results also suggest that

Table 6. Percentage contribution of various organic acids to total moles of organic acid in different halophytes growing in salt-alkalinized grassland. The percentages are calculated according to the means of each organic acid.

Family	Species	Tartrate (%)	Citrate (%)	Malate (%)	Formate (%)	Lactate (%)	Acetate (%)	Succinate (%)	Oxalate (%)
Chenopodiaceae	*K. Sieversiana*	0.0	2.3	0.6	0.0	0.1	2.4	0.3	94.3
	S. glauca	4.0	0.4	0.5	0.0	0.0	0.6	0.0	94.4
	S. salsa	3.0	0.4	1.7	0.0	0.2	4.7	0.2	89.7
Poaceae	*C. macrolepis*	1.0	28.4	27.3	0.0	0.0	25.6	1.9	15.7
	C. virgata	0.0	19.3	9.9	4.2	4.4	25.6	9.1	27.5
	P. tenuiflora	6.5	29.5	5.7	9.0	5.6	20.7	0.0	23.0
	L. chinensis	1.9	4.1	20.5	0.0	0.0	35.9	4.4	33.2
	P. hirsuta	2.8	22.5	24.4	0.0	0.0	22.8	6.6	20.9
Compositae	*A. anethifolia*	0.0	18.4	2.4	0.5	1.1	8.7	0.0	68.9
	A. scoparia	6.7	34.3	21.8	0.1	1.7	8.4	4.9	22.2
	S. arvensis	23.6	16.6	48.5	0.8	0.7	3.3	0.0	6.6
	K. integrifolia	0.0	17.5	13.2	0.0	4.6	12.1	5.4	47.2
	I. japonica	43.7	25.5	5.8	0.9	1.3	10.0	0.0	12.8
Leguminosae	*M. officinalis*	11.2	45.2	20.9	0.9	0.2	17.6	0.0	4.0
	L. hedysaroides	30.7	27.0	20.4	0.0	1.0	6.7	1.3	13.1
	A. complanatus	0.0	52.9	14.5	0.3	0.4	29.6	0.0	2.2
Asclepiadaceae	*C. chinense*	12.4	49.3	34.5	0.1	0.2	1.2	0.0	2.3
Cyperaceae	*C. duriuscula*	2.9	32.2	44.0	0.3	0.7	14.9	0.0	5.2
Boraginaceae	*M. sibirica*	0.4	34.7	14.1	11.4	1.8	0.0	12.4	25.2

higher resistance of Chenopodiaceae to the natural salt-alkaline stress might depend on their key physiological characters, namely the unique absorption mechanism of ions such as Na^+ and Cl^-, and higher accumulations of betaine and oxalate (Tables 2, 4 and 5). The phenomenon of high accumulations of ions and betaine has also been reported in other Chenopodiaceae, e.g. *Suaeda fruticosa* (Khan et al., 2000), and *Salicornia europaea* and *Suaeda maritime* (Moghaieb et al., 2004). A higher accumulation of Na^+, Cl^-, betaine and oxalate under salt or salt-alkaline stresses might be a common and genetic character of Chenopodiaceae halophytes, possibly related to their strong resistance to salt or salt-alkaline stresses, which should be further investigated.

Materials and methods

Plant materials

The experiment was conducted in the summer of 2009, in the semi-arid salt-alkalinized grasslands in the west of Jilin Province of China (44°40'–44°45' N, 123°44'–123°47' E). For this research, 19 halophyte species naturally grown in alkalinized grassland were chosen as test organisms including three Chenopodiaceae, five Poaceae, five Compositae, three Leguminosae, one Asclepiadaceae, one Cyperaceae, and one Boraginaceae species (Table 1). For a species, about 100 g of shoots were taken randomly from a clump, with three samplings from different clumps at a sampling site. The sampling site for each species was chosen in their most typical salt-alkalinized habitats. Each sample was considered a single replicate, and therefore there were three replicates per species. Plant materials were placed in liquid nitrogen, and then stored indoors in a cooler at –20°C. In addition, 200–300 g of the soil surrounding roots of each species was collected. Each soil sample was placed in a soil-sampling box,

and then stored indoors in a cooler at 4°C.

Measurement of physiological indices

The shoot samples were vacuum-dried at 40°C to a constant weight. Dry shoot samples (50 mg) were extracted with 10 ml of deionized water at 100°C for 30 min, and the extract used to determine the contents of free inorganic ions and OAs. The contents of NO_3^-, Cl^-, SO_4^{2-}, $H_2PO_4^-$ and oxalic acid were determined by ion chromatography (DX-300 ion chromatographic system; AS4A-SC ion-exchange column, CD M-II electrical conductivity detector, mobile phase: Na_2CO_3/$NaHCO_3$ = 1.7/1.8 mM; DIONEX, Sunnyvale, USA). Other OAs (malate, citrate, succinate, acetate, formate, malate, tartrate, glycolate and lactate) were also determined by ion chromatography (DX-300 ion chromatographic system; ICE-AS6 ion-exclusion column, CDM-II electrical conductivity detector, AMMS-ICE II suppressor, mobile phase: 0.4 mM heptafluorobutyric acid; DIONEX). An atomic absorption spectrophotometer (TAS-990, Purkinje General, Beijing) was used to determine contents of Na^+, K^+, and free Ca^{2+} and Mg^{2+}. Proline and soluble sugars (SS) were measured using ninhydrin and anthrone, respectively. Mannitol (Ma) content was measured using the method of Zhang and Li (2005). The betaine content was determined according to Grieve and Grattan (1983). Free amino acids (AA) were measured colorimetrically (Zhang 2002). The soil samples were air-dried and sieved to < 2 mm, their pH and electrical conductivity (EC) measured using a 1:5 (soil:water) suspension (Table 1). The EC of the suspensions were measured by an electrical conductivity apparatus (DDS-307, Shanghaileici corporation, Shanghai, China).

Statistical analyses

Analyses of data were performed using the statistical

program SPSS 13.0. All treatments had three replicates, and the means and standard errors (SE) were reported.

Table 7. Percentage contribution of each solute molarity to the total molarity in different halophyte species growing in the salt-alkalinized grassland. The percentages were calculated according to the means of each solute. OA–organic acid; SS–soluble sugar; Pro–proline; AA–Amino acid; Ma–Mannitol.

Family		Na^+ (%)	K^+ (%)	Ca^{2+} (%)	Mg^{2+} (%)	Cl^- (%)	NO_3^- (%)	SO_4^{2-} (%)	$H_2PO_4^-$ (%)	OA (%)	Pro (%)	SS (%)	Ma (%)	AA (%)	Betaine (%)
Chenopodiaceae	*K. Sieversiana*	42.8	4.5	0.05	1.8	13.5	0.62	2.0	0.8	19.9	0.07	1.9	0.4	0.2	11.4
	S. glauca	34.7	2.6	0.04	1.4	43.8	0.14	1.8	0.9	7.4	0.01	1.9	0.2	0.0	5.0
	S. salsa	31.4	8.6	0.07	2.7	30.4	0.22	4.8	1.9	9.8	0.02	2.9	0.6	0.1	6.5
Poaceae	*C. macrolepis*	3.7	31.2	1.02	3.3	13.8	0.47	1.8	6.4	3.0	0.09	16.0	3.5	0.4	15.3
	C. virgata	18.6	25.6	0.38	1.9	23.1	0.28	2.3	4.5	1.9	0.10	10.1	1.9	0.3	9.2
	P. tenuiflora	4.1	17.6	0.84	1.3	12.3	0.31	0.6	1.1	1.2	0.06	41.0	2.3	0.2	17.2
	L. chinensis	9.5	22.7	0.80	2.6	12.0	0.35	1.2	4.0	3.2	0.14	12.5	2.0	0.5	28.5
	P. hirsuta	3.9	17.5	1.71	6.7	19.6	0.33	3.3	1.4	2.5	0.06	17.2	2.0	0.4	23.5
Compositae	*A. anethifolia*	29.1	11.2	1.63	2.6	19.7	0.26	2.2	3.3	3.4	0.10	9.2	1.2	0.2	15.8
	A. scoparia	9.9	36.3	1.04	3.2	7.6	0.45	0.7	3.9	7.6	0.17	14.4	3.2	0.3	11.4
	S. arvensis	14.3	14.2	3.63	6.9	27.4	0.34	6.2	2.9	5.8	0.16	9.9	2.1	0.3	6.0
	K. integrifolia	4.1	38.5	1.69	3.0	5.4	0.38	1.5	2.3	4.1	0.04	14.7	2.2	0.2	21.9
	I. japonica	8.0	16.7	8.62	4.9	9.9	0.29	9.8	2.4	3.4	0.12	17.3	1.4	0.2	16.8
Leguminosae	*M. officinalis*	2.7	21.0	3.82	9.7	5.2	0.32	1.5	3.5	12.8	0.30	18.8	4.3	0.7	15.4
	L. hedysaroides	3.1	20.8	4.79	11.7	1.8	0.63	2.0	4.4	15.1	0.20	12.6	5.7	0.6	16.6
	A. complanatus	2.8	25.9	5.24	9.1	4.4	0.76	2.7	2.1	16.4	0.08	8.5	3.0	0.4	18.7
Asclepiadaceae	*C. chinense*	12.2	14.6	2.84	8.6	13.1	0.19	10.7	4.5	15.5	0.15	9.8	1.3	0.5	6.0
Cyperaceae	*C. duriuscula*	3.2	32.3	1.92	3.8	9.2	0.33	2.9	2.3	9.9	0.04	22.7	2.0	0.3	9.0
Boraginaceae	*M. sibirica*	34.4	10.6	1.37	6.0	15.7	0.26	9.6	0.6	2.9	0.02	4.5	1.9	0.1	12.0

Conclusion

The halophytes living in alkalinized grassland not only have to resist osmotic stress, but must maintain ionic balance. Our results indicated that 19 halophytes showed a diversity of osmotic adjustment and ion balance strategies, and also displayed a large amount in common. In these halophytes, the tolerance of Chenopodiaceae to natural salt-alkaline stress was stronger than in other species, and Leguminosae were most susceptible to salt-alkaline stress. Our results suggest that the strong resistance of Chenopodiaceae to natural salt-alkaline stress might be depended on their key physiological characters, namely the unique absorption mechanism of ions such as Na^+ and Cl^-, and higher accumulations of betaine and oxalate.

Acknowledgments

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