CHAPTER 9

A COMPARATIVE STUDY ON RESPONSES OF GROWTH AND SOLUTE COMPOSITION IN HALOPHYTES SUAEDA SALSA AND LIMONIUM BICOLOR TO SALINITY

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Abstract. *Suaeda salsa* (leaf succulence) and *Limonium bicolor* (secreting) are common halophytic species grown in coastal saline soil area in China. They possess different physiological adaptations which help them to avoid salt stress. Their mechanism of salt tolerance is varied and not properly understood. Therefore, the proposed plan to grow them in highly saline conditions could be hampered. The present study was designed to study the effect of salinity on growth and various solute compositions. Growth of *S. salsa* showed a 94% and 48% increases in comparison to control in 50 and 100 mM NaCl respectively in both shoot and root while at high salinity (400 mM NaCl) shoot and root dry weight were not significantly different from control. However, in *L. bicolor* root showed little promotion of shoot growth at 150 and 100 mM NaCl respectively and growth was substantially inhibited at 400 mM NaCl. *Suaeda salsa* accumulated more Na^+ and Cl^- ions in comparison to *L. bicolor*. These ions accumulated more in shoots of *S. salsa* whereas distributions of ions were similar in both shoots and roots of *L. bicolor*. Shoot soluble sugar decreased and proline increased with increase of external salinities of both species but shoots of *L. bicolor* contained relatively higher amount of sugar and proline at high salinity levels.

1. INTRODUCTION

Salinisation of soils and groundwater is a serious land-degradation problem in arid and semi-arid areas, and is increasing steadily in many parts of the world due to poor irrigation and drainage practices, which cause a great reduction for crop productivity (Lambers, 2003). As an alternative method to restore saline land, the utilization of halophytes attracted more attention due to their salt tolerance characteristics and potential economic values (Flowers, 1977; Glenn et al., 1999; Lieth, 1999; Barrett-Lennard, 2002; Zhao et al., 2002).

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The principal mechanism of halophytic adaptation may be the high Na^+ and Cl^- absorption. The halophytes which are able to grow at high salinity can generate a high turgor in their cells by the high internal Na^+ and Cl^- concentrations. They also have some adaptive features like salt secretion (salt glands & bladders) or increase succulence to deal with high concentration of ions in the cell (Greenway & Munns, 1980). Difference of growth and physiological response between closed related plants to salinity is particularly interesting and may result to the identification of a number of factors that influence salt tolerance (Tester & Davenport, 2003). These differences can be used for the management practices of different halophytic species even for screen and selection of salt tolerant crops.

Suaeda salsa (Chenopodiaceae) a leaf succulent annual and *L. bicolor* (Plumbaginaceae) a perennial secreting halophyte has been reported to be widely distributed in saline areas of China. Both species could be used cash crops in high saline soils of China. Young seedling of *S. salsa* can be used as vegetable and the seeds contain approximately 25% of high quality oil. *Limonium bicolor* is a traditional Chinese herb, which has a haemostatic function (Zhao & Li, 1999; Zhao et al., 2002). The objective of this study was to compare the salt tolerance and mechanism of osmoregulation of the halophytes with different physiological adaptations.

2. MATERIALS AND METHOD

2.1. Plant materials and growth conditions

Seeds of *S. salsa* and *L. bicolor* were collected from coastal saline soils area in Hebei Province, China and were sown in plastic containers filled with clean sands and kept in a greenhouse at Nanpi Eco-Agricultural Experimental Station, Chinese Academy of Sciences under an approximately 28°C maximum and 23°C minimum temperature. After seed emergence the plants were irrigated daily with a half-strength Hoagland solution (Hoagland & Arnon, 1950). One week later, seedlings with a uniform size were selected and transferred to 8 L plastic tubs containing aerated Hoagland solution. Five plants were transplanted in each tub. Salinity treatments included 0 (CK), 50, 100, 200 and 500 mM NaCl. The NaCl concentrations were gradually increased by 100 mM NaCl increments at one day interval until to reach the maximum level of each treatment. For each treatment there were 3 replications, and the solutions were changed weekly to avoid ion accumulation. The duration of treatments was 30 days.

2.2. Growth measurements

At the end of experiments, plants of each tub were harvested and divided into shoots and roots. The shoots and roots were washed with cold deionized water, blotted dry, and fresh weights were measured. Then they were oven dried at 70°C for 48 hours and the dry weights were measured.

2.3. Chemical analysis

The dried shoot and root samples were ground separately and were wet ashed with HNO_3 digestion. The Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations were analyzed by Hitachi 170-10 Atomic Absorption Spectrophotometer. Shoot soluble sugar was determined by the phenol-sulfuric acid method (Dubois et al., 1956) and proline by the Ninhydrin method of Bates et al., 1973.

2.4. Statistical analysis

Data were analyzed by using the SPSS 11.0 software and means were tested using Duncan's multiple range tests ($p < 0.05$).

3. RESULTS

3.1. Growth

Shoot and root dry weight of *S. salsa* was highest in 50 mM NaCl, and no significant growth inhibition was recorded at highest salinity treatments (Figure 1). There was small but significant root growth promotion with increase in salinity but root growth at highest salinity was not significantly different from control. Shoot growth in *L. bicolor* showed a little promotion at low salinities (Figure 1) and some inhibition at higher salinities. There was no effect of salinity on the roots growth of *L. bicolor* except at higher concentrations.

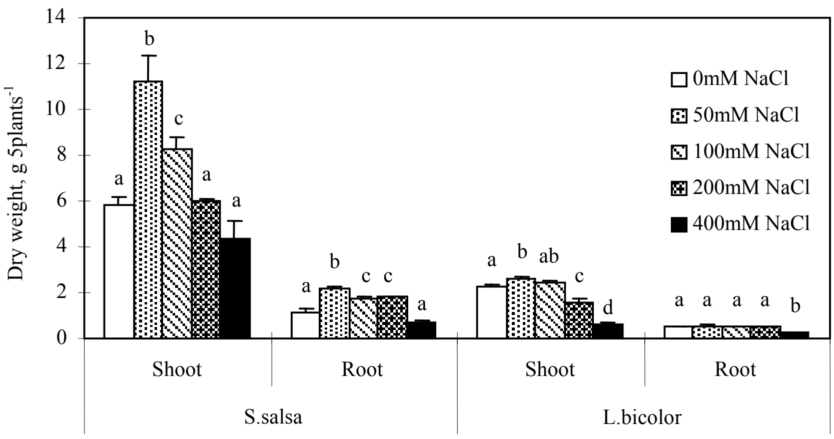

***Figure 1.** Effect of salinity on shoot and root dry weight of two plant species. Same letters within each species indicate no significant difference ($p < 0.05$) according to Duncan's multiple range tests. Bars indicate s.e. of means (n=3)*

3.2. Ions

Sodium content of *Suaeda salsa* shoot increased rapidly with the increase in salinity reaching to about 19000 µmol.g.dw^{-1} (Table 1). However, in comparison root ion concentration could only reach about 2000 µmol.g.dw^{-1}. While Na concentration in *Limonium bicolor* was even less than 1500 µmol.g.dw^{-1} and there was not significant difference between root and shoot. Potassium concentration decreased with increase in salinity (Table 1). The decrease was more pronounced in the leaf of both plants particularly in the case of *S. salsa* (Table 1). Calcium concentration decreased with the increase in salinity and the decrease was higher in the roots of both plants in comparison to shoots (Table 1). Magnesium concentration of shoots of *S. salsa* decreased substantially with increase in salinity while small reduction is recorded in *Suaeda salsa* roots and for *L. bicolor* for both roots and shoots (Table 1).

Table 1. Effect of salinity on Cl, Na, K, Ca, and Mg (µmol/g DW) accumulation in the shoots and roots of S. salsa *and* L. bicolor.

			NaCl concentration, mM				
			0	*50*	*100*	*200*	*400*
Cl	*S. salsa*	Shoot	1939.4a*	2858.3b	3120.7b	3608.4c	5798.5d
		Root	229.3a	1524.1b	1538.1b	2039.1c	3192.4d
	L. bicolor	Shoot	970.4a	1651.0b	2263.4c	2334.4c	2703.9d
		Root	367.9a	630.4b	1281.0c	1730.3d	3334.7e
Na	*S. salsa*	Shoot	383.6a	3558.2b	9139.9c	17638.7d	19315.7e
		Root	347.3a	1267.2b	1444.6b	1730.0b	2740.4c
	L. bicolor	Shoot	27.4a	698.9b	1010.4c	1374.2d	2170.4e
		Root	103.9a	501.4b	1107.4c	1455.1d	1504.8d
K	*S. salsa*	Shoot	2180.9a	1652.1a	719.1b	603.7b	320.4c
		Root	1286.6a	1103.3a	920.8ab	887.3ab	797.2b
	L. bicolor	Shoot	585.8a	581.1a	496.6a	338.9b	355.3b
		Root	361a	341.4a	290.2a	249.6ab	103.9b
Ca	*S. salsa*	Shoot	46.7a	26.6b	21.3c	14.7d	12e
		Root	53.1a	46.6a	35.2ab	25.8ab	14.4b
	L. bicolor	Shoot	38.1a	34.5a	21.1b	16.3b	11.7b
		Root	26.2a	21.8a	20.1a	19.7a	16.7a
Mg	*S. salsa*	Shoot	273.4a	220.9a	138.1b	82.9b	56.2b
		Root	160.5a	149.9a	143.6a	133.8a	88.7a
	L. bicolor	Shoot	140.0a	103.7b	101.9b	84.9bc	78.2c
		Root	139.2a	131.7a	102.6b	100.4b	87.4b

** Means in a row followed by the same letter are not significantly different ($p<0.05$).*

The shoots of *S. salsa* accumulated higher Na^+ than that of roots which was increased with the increase in NaCl concentrations until 200 mM then decreased (Figure 2). In *L. bicolor*, shoots Na^+ and roots Na^+ was almost at the same level. Shoots K^+ of *S. salsa* were higher than that of roots K^+ in 0 and 50 mM NaCl and lower in the higher NaCl concentrations. However in *L. bicolor* shoots K^+ were higher than roots K^+ in all the treatments. *S. salsa* shoots accumulated lower Ca^{2+} than that of roots in all treatments, and *L. bicolor* shoots accumulated lower Ca^{2+} only in higher concentrations. With the present of 100 mM and 50 mM or more NaCl, shoot Mg^{2+} was lower than roots Mg^{2+}.

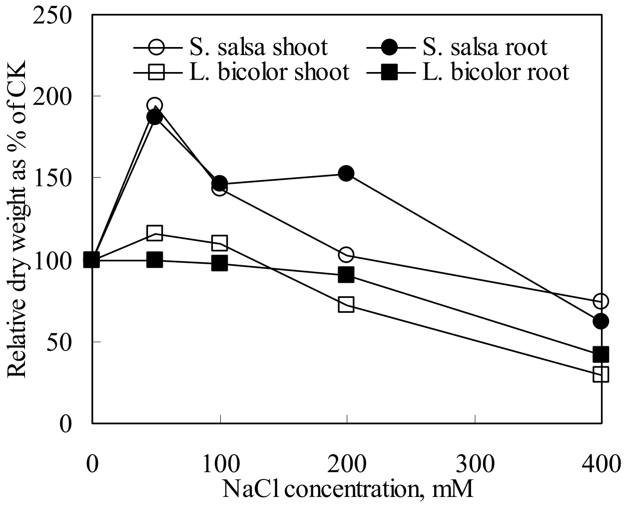

Figure 2. *Comparison of salinity effect on relative shoot and root dry weight of* S. salsa *and* L. bicolor

3.3. Soluble sugar and Proline

In both species, shoot soluble sugar contents were decreased with the increase of NaCl concentrations (Figure 3).

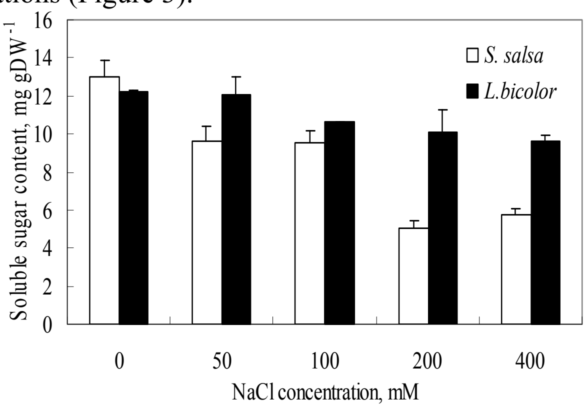

Figure 3. *Effect of salinity on leaf soluble sugar content of* S. salsa *and L.* bicolor. *Bars indicate s.e. of means (n=3).*

The shoot soluble sugar declined in 400 mM NaCl to 39% and 78% in comparison to control in *S. salsa* and *L. bicolor* respectively. The total shoot soluble sugar contents of *S. salsa* were lower than that of *L. bicolor* under saline conditions. Shoot proline contents were increased with the increase in NaCl concentrations in both species, and the increase of proline in *L. bicolor* was greater at higher salinity levels (Figure 4).

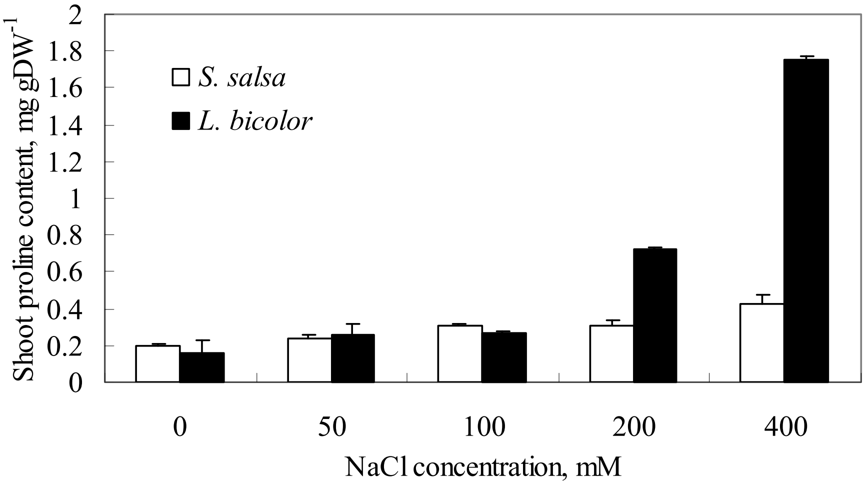

Figure 4. *Effect of salinity on leaf proline content of* S. salsa *and* L. bicolor. *Bars indicate s.e. of means (n=3).*

The proline content of *S. salsa* and *L. bicolor* shoots grown in 400 mM was increased 1-fold and 9-fold respectively.

4. DISCUSSION

Suaeda salsa showed optimal growth at 100 mol m^{-3} NaCl. Comparative results have been reported for *S. depressa* (Williams & Ungar, 1972), *S. maritima* var. *flexils* and var. *marcrocarpa* (Boucaud & Ungar, 1976), *S. australis* (Robinson & Downtown, 1985), *S. maritima* (Yeo & Flowers, 1980), *S. ussuriensis* (Kef-Fu et al., 1986), *S. salsa* (Ke-Fu et al., 1995). In fact, many dicotyledonous halophytes show optimal growth in the presence of salt (Naidoo & Raghunan, 1990; Khan et al., 2001). Secreting halophyte, *L. bicolor,* shoot growth was significantly inhibited above 100 mol m^{-3} NaCl but leaf succulent, *S. salsa*, showed no effect of salinity. Though the lower concentration of NaCl increased root growth in *S. salsa*, however, in comparison root growth of *L. bicolor* showed some inhibition at 400 mM NaCl. At moderate NaCl concentrations, the increase in weight was greater in *S. salsa* in comparison to *L. bicolor*. This may be due to the rise in inorganic ion content in *S. salsa* (Flowers et al., 1977) to absorb more water to maintain high succulence. It was considered that the increase in growth was the possible compensatory mechanisms for regulating salt concentration (Albert, 1975).

Suaeda salsa and *L. bicolor*, as most of halophytes can accumulate higher Na^+

and the accumulation was increased with the increase of NaCl levels. However comparing the two species, there are some differences in the ion accumulation and partitioning. The Na^+ accumulation in *S. salsa* plant was higher than that of *L. bicolor*. *Suaeda salsa*, as leaf succulent halophyte, accumulated most of Na^+ in shoots which was ranged from 74% to 94% of total Na^+ through all NaCl treatments, similar to the result that more than 90% of Na^+ in halophytes is in the shoot (Flowers, 1975). However *L. bicolor* as halophyte with salt glands, Na^+ accumulations was almost similar between shoots and roots. The difference between two species in the internal ion regulation may be due to the different mechanisms of salt tolerance, especially with the leaf succulence and salt glands. Though both species had the highest shoot water content in 100 mM NaCl (for *S. salsa* 93%, for *L. bicolor* 87.5%), *S. salsa* maintained the higher water content (>92%) in all the NaCl concentrations and the water content in shoots of *L. bicolor* varied greatly (from 87.5% in 100 mM NaCl to 81% in 400 mM NaCl) (Figure 5).

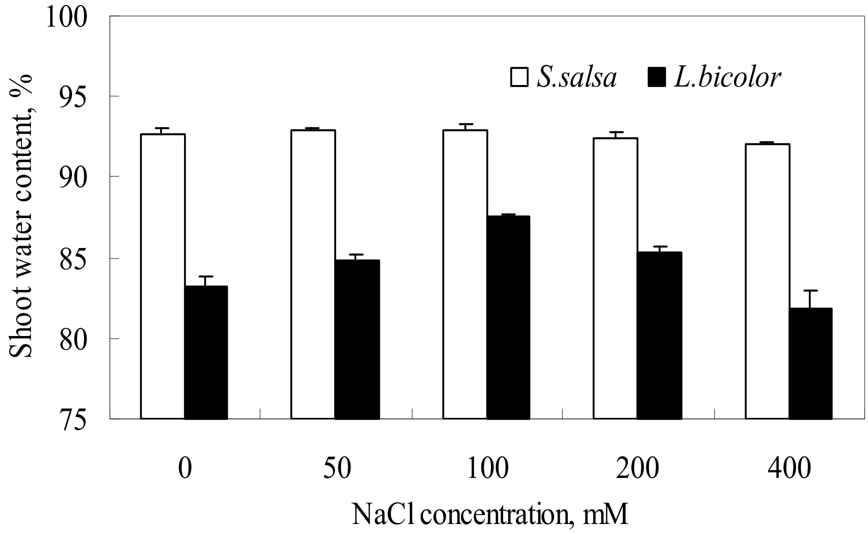

Figure 5. *Effect of salinity on shoot water content of* S. salsa *and* L. bicolor. *Bars indicate s.e. of means (n=3).*

The higher shoot water content avoids the higher ion accumulation in *S. salsa*. Nevertheless, in *L. bicolor*, salt glands removed salt from leaf surface that has reached to the shoot, so salt content in shoots and roots was similar, and also the glands remove water from leaf which results in lower shoot water content (Tester & Davenport, 2003).

The accumulations of K^+, Ca^{2+} and Mg^{2+} in shoots and roots of both species were decreased with the increase of NaCl in solutions. Similar results have been reported for *Salvadora persica* (Maggio et al., 2000) and *Atriplex canescens* (Richardson & McKell, 1980). However, if comparing the species, K^+, Ca^{2+} and

Mg^{2+} in *L. bicolor* was more stable than in *S. salsa*, this may be due to removal of salt through salt glands to keep the ion balance between shoots and roots. *Suaeda salsa* accumulated more K^+ in roots than in shoots under higher salinity levels. The higher K^+ in roots may continuously transport K^+ to shoots to avoid K^+ deficiency in shoots (Zhang, 2002).

Sugars, proline and other organic solutes are considered to improve salt tolerance by contributing to osmotic balance and preserving enzyme activity in the presence of toxin ions (Shannon, 1997; Greenway & Munns, 1980). In this study it was shown that in both species the shoot soluble sugar content was declined and shoot proline content was increased with the increase of NaCl concentration. However comparing the two species, the shoots of *L. bicolor* contained relatively higher soluble sugar and proline than that of *S. salsa* especially at higher salinity levels. Rathert (1984) reported that salinity causes greater sucrose increases in salt-sensitive species than in tolerant species. Greenway and Munns (1980) indicated that adaptive role of proline is related to survival rather than to maintenance of growth. Based on these reports, it is assumed that the higher soluble sugar and proline contents in shoots of *L. bicolor* than that of *S. salsa* indicate that *S. salsa* is more salt tolerance than *L. bicolor*.

The results of present study indicated that leaf succulent halophyte *S. salsa*, and secreting halophyte *L. bicolor*, showed increase in growth at moderate salinities. The higher salinity inhibited the growth of *L. bicolor* more than *S. salsa*. The ion accumulations and partitioning were different. *Suaeda salsa* accumulated a large amount of ions than *L. bicolor* and most of the ions were in the shoots, but for *L. bicolor* the ions in shoots and roots were approximately similar. Under higher salinity conditions, the shoot soluble sugar was decreased and proline increased. The shoot of *L. bicolor* contained relatively higher soluble sugar and proline than that of *S. salsa* especially at higher salinity levels. Based on the context, it was considered that *S. salsa* was more salt tolerant than *L. bicolor*. The abovementioned observations suggest that *S. salsa* is more salt tolerant, however, exact mechanism of other salt tolerance needs to studied.

5. ACKNOWLEDGEMENT

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