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ROOTING AND SHOOT GROWTH OF STEM CUTTINGS OF SALTCEDAR (*TAMARIX CHINENSIS* LOUR) UNDER SALT STRESS

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Abstract

A sand culture experiment was conducted to investigate the rooting of stem cuttings and growth of *Tamarix chinensis* irrigated with various dilutions of saline soil solutions extract (0 to 32 g L^{-1}) containing about 31.5% Na^+. Rooting of stem cuttings was higher at lower salinity, inhibited greatly when salinity was higher than 12 g L^{-1}, but still 43% of stem cuttings rooted at 32 g L^{-1} salinity treatment. Sapling growth was stimulated at 4 to 8 g L^{-1} salinity level and inhibited significantly by higher than 12 g L^{-1} salinity treatment although growth continued at 32 g L^{-1} salinity. Na^+ content in roots, stems and leaves increased with increase in salinity, while Ca^{2+} and K^+ content in leaves and stems decreased. The contents of Ca^{2+} and K^+ in roots remained unaffected by various treatments. With increase in salinity Mg^{2+} content increased in stems and leaves but decreased in roots. Proline contents of leaf, stem and root increased with increase in salinity which became more conspicuous at the salinity level of 12 g L^{-1} and above. Osmotic regulation was also formed an important mechanism for *Tamarix chinensis* while growing under salinity.

Introduction

Tamarix chinensis Lour (syn. *T. ramosissima*), a xero-halophyte shrub or small tree, is widely distributed in the drought-prone and saline lands in Central Asia and China. It is an economically important species used as fodder (dry young branches), as ornamental plant, in making farming tools, medium-density particle board, fire-proof plywood board and as fuel wood in the local area (Song *et al*., 2003; Gaskin & Kazmer, 2006; Zheng *et al*., 2006). Recent research has showed that *T. chinensis* can be used as host for the parasite *Cistanche tubulosa*,which yields expensive Chinese natural medicine (Ma *et al*., 2005). It is one of the most remarkable salt-tolerant species and the only native woody plant species for afforestation in saline soils of China using saline water irrigation (Song *et al*., 2003; Li *et al*., 2004). The species is widely distributed in Bohai coastal saline soil areas and has played an important role in dune stabilization and protection from strong winds. Recently, it has been over-cut during the construction. For rehabilitating this species, it is necessary to find some quick method for its propagation.

The seeds of *T. chinensis* species are minute (Young *et al*., 2004) with short, bristle like hairs, arranged spirally that aid in aerodynamics of the seed dispersal (Young *et al*., 2004). The seeds mature inconsistently and remain viable for less than 4 weeks at room temperature, although they may be stored for up to nearly one year if dried sufficiently in cold environment (Song *et al*., 2003; Young *et al*., 2004). The germination process is very sensitive to soil moisture, temperature and salinity. The seeds germinate only in summer when the salt content in soil is sufficiently decreased by rain (Song *et al*., 2003; Young *et*

al., 2004). The lack of viable seeds, sensitivity to salt stress and slow growth (40 day old seedling is 3 cm in height) have been the major constraints to reforestation with this species. Recently, progress has been made in developing vegetative propagation techniques in one *Tamarix* species; micro-propagation (tissue culture) and stem cutting provide alternative means of producing planting stock (Kleinkiopf & Wallace, 1974; Luccesini *et al*., 1993). However, the salinity prevailing in coastal areas may adversely affect establishment of young seedling. The effect of salinity on rooting of stem cuttings and seedling growth of *T. chinensis* has not been studied in the past (Glenn *et al*., 1998; Tomar *et al.*, 2003; Arndt *et al*., 2004).

Many physiological and biochemical processes are involved in plant response to salinity stress. Osmotic adjustment is the key mechanism, by which halophytes are able to adapt themselves to salinity and maintain good growth and development (Flowers *et al.*, 1977). Under salinity stress, plant cells actively accumulate certain types of solutes such as inorganic ions, free proline or its analogues and soluble sugar to improve their salinity-resistance by lowering water potential and maintaining turgor (Flowers *et al.*, 1977; Munns, 1993; Hasegawa, 2000; Munns, 2002; Arndt *et al.*, 2004; Jones *et al.*, 2006; Zhuang & Chen, 2006). Alternatively, excess of ions could prove toxic to the plant growth (Flowers *et al*., 1977; Munns, 1993, 2002). Salt excretion through salt glands is a mechanism to remove excess salt from tissues by several halophytes and it has been well documented in case of *Tamarix* species as well (Kleinkiopf & Wallace, 1974; Waisel, 1991; Shafroth *et al.*, 1995; Ramadan, 1998; Arndt *et al.*, 2004). Recent research on response of *Tamarix* to salinity stress focused on the growth, respiration, water use characteristics (Glenn *et al.*, 1998; Tomar *et al.*, 2003), activity of superoxide dismutase (Song *et al.*, 2006a) and expressed sequence tags (Wang, 2006, Li *et al.*, 2009). However, the effect of osmotica on the salinity tolerance of *T. chinensis* is rarely reported (Arndt *et al.*, 2004).

The object of the research was to investigate the physiological and biochemical responses of *T. chinensis* to salinity stress and evaluate stem cutting as an effective means for its vegetative propagation.

Materials and Methods

Stem cuttings of *T. chinensis* were harvested in mid November 2004 from plants growing in field along the coastal saline soils of Huanghua City, Heibei Province of China. One year old cuttings (7-9mm diameter) were selected and kept in moist sand at 4-8ºC for four months. Rooting and sapling growth studies in greenhouse of Nanpi Eco-agricultural Station of Chinese Academy of Science from 25th March to 25th May 2005. Cuttings 15cm in length were dipped up to 10cm in rooting solution (2µM IBA) for 24 hours and planted in containers at washed river sand keeping only 2 cm of the cutting protruded out of soil. There was a hole (3cm diameter) in container to drain the extra water. After the cuttings were inserted in the soil, different amounts of the extract from a saline soil (content of different ions in the extract is given in Table 1), corresponding to salinity level of 4, 8, 12, 16, 20, 24, 28 and 32 g L^{-1} salt were applied with irrigation every two days. Sufficient solution was applied to remove any remaining solution in sand to keep stable concentration of salinity. Air temperature in the greenhouse was maintained at 20-28ºC, with 70-90% relative humidity. Light intensity was reduced to 25% of ambient sunlight. Twenty cuttings were used for each treatment with three replications. Seedlings were harvested 2 months to measure growth.

Table 1. Composition of various ions and total salt in solution extracted from the soil (%).

Ions	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	K^+	Total Salt
Content	0.1	51.5	12.5	1.2	2.3	31.5	0.9	100

After shoots were removed, roots were washed with solutions of each corresponding treatment. Then shoots and roots were washed once with tap water and once with distilled water, blotted dry by tissue paper. Rooting percentage, longest root length, and root number were determined. Leaves, stems and roots were separated and oven-dried at 80ºC for 48 hours and dry weight (DW) was determined. The proline content in plant material was determined by ninhydrin colorimetry (Li, 2000). Total soluble sugars in plant material was determined by sulfuric acid anthrone colorimetry (Li, 2000) using an ultra violet spectrophotometer, Hitachi-V-520. The dried plant samples were ground and were wet ashed with HNO_3. The Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations in the plant tissue were determined using an atomic absorption spectrophotometer (Hitachi -170-10, Japan).

Statistical analysis was carried out using SPSS 11.0. A one-way ANOVA was carried out to determine differences among treatment groups for germination percentage and germination recovery percentage. A post-hoc Bonferroni test was used to determine if differences between individual treatment means were significant ($p< 0.05$).

Results

Effect of salt stress on rooting: The rooting percentage was 100% in non-saline control and in low salt stress (<8 g L^{-1} salt). The rooting percentage decreased significantly ($p<0.05$) as the level of salinity increased (Fig. 1). At the highest level of salinity (32 g L^{-1} salt, near sea water salinity), however, some 43% stem cuttings still rooted (Fig. 1). Cuttings had initiated root primorida and the roots emerged through the epidermis (Fig. 2). Callus formation was not observed in the end of the cuttings.

Effect of salt stress on growth plantlets from stem cuttings: At low level of salinity (4 g L^{-1} salt) the length of longest root length increased by 130% and root dry weight by 186% when compared to the 'no salinity' control (Fig. 3); they were decreased significantly ($p<0.05$) when the salinity level increased to 12 g L^{-1} level or more. However, the number of adventitious roots was not significantly affected by increase in salinity. Shoot length, shoot dry weight and branch number were not significantly affected by low level of salinity stress (<12 g L^{-1} salt) while they were decreased significantly ($p<0.05$) when the salinity level increased to 12g L^{-1} or more (Fig. 3). Shoot/root ratio decreased significantly under low salinity stress (4-12 g L^{-1} salt) comparing to the 'no salinity' control. However, the shoot/root ratio was not affected significantly by mild levels of salinity (16 and 20 g L^{-1} salt), and it decreased significantly under high level of salinity stress (>24 g L^{-1} salt).

Effect of salinity stress on osmoregulation: Most halophytes tend to accumulate proline and soluble sugar in their organs at high levels of salinity to resist stress. Our study showed that proline content in leaves and stems increased with an increase in salinity (Fig. 4). Leaves contained higher levels proline than stems in all treatments.

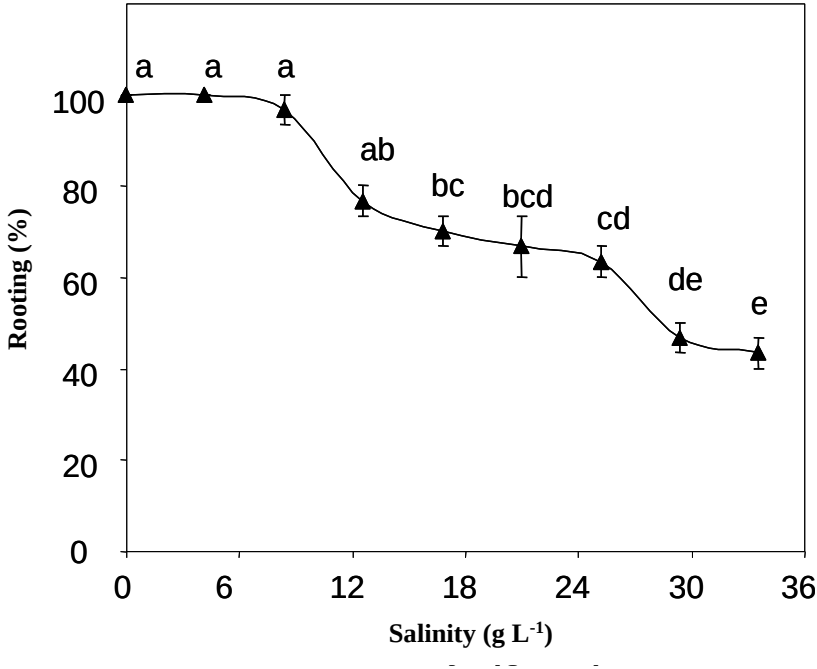

Fig. 1. Rooting of *Tamarix chinensis* cuttings under salt stress. Values are givens as means ± SE of three replicates. Different letters above the symbols indicate significant difference at $p<0.05$.

Fig. 2. *Tamarix chinensis* stem cuttings showing the pattern of adventitious roots.

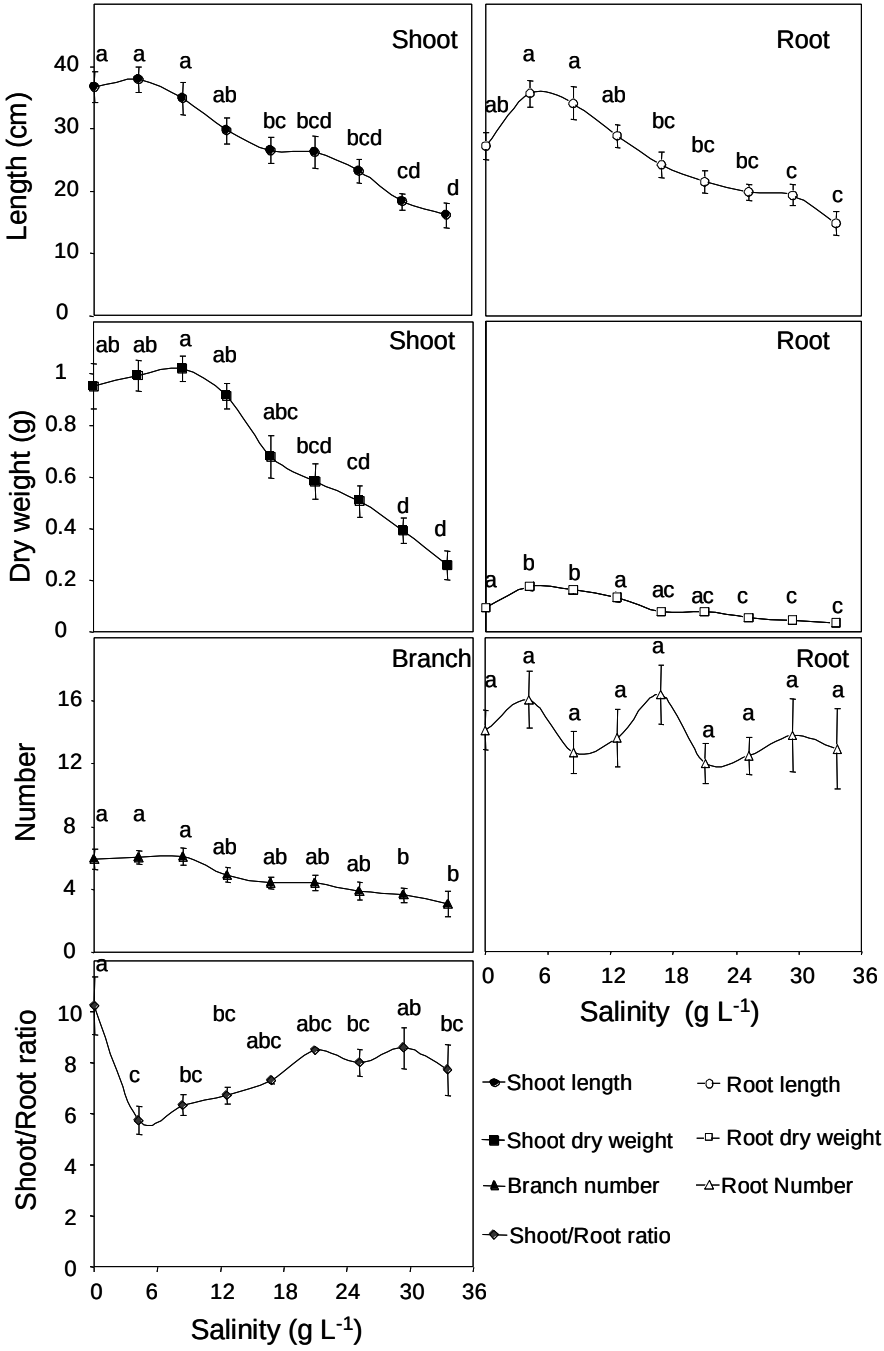

Fig. 3. Growth parameters (length, dry weight and number of shoot and root and shoot/root ratio) of *Tamarix chinensis* under salt stress. Values are givens as means ± SE of three replicates. Different letters above the symbols indicate significant difference at $p<0.05$.

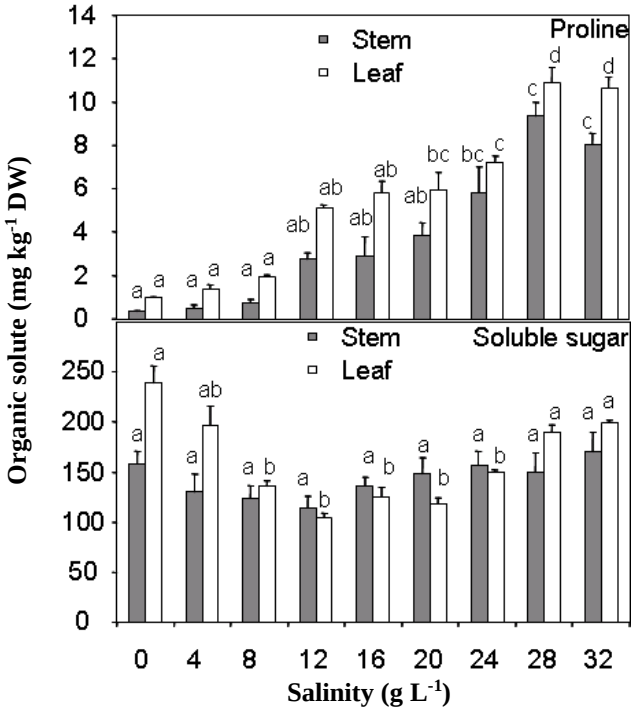

Fig. 4. Content of organic solutes (proline and soluble sugar) in stems and leaves of *Tamarix chinensis* under salinity stress. Values are givens as means ± SE of three replicates. Different letters above columns indicate significant difference at $p<0.05$.

Compared with non-saline control, leaf proline content at 20, 24, 28 and 32 g L^{-1} salt increased to 5.9, 7.0, 10.7 and 10.5 times respectively, and stem proline content at 20, 24, 28 and 32 g L^{-1} salt increased to 12.1, 18.1, 29.2 and 25.1 times respectively (Fig. 4). In contrast, the soluble sugar content in leaves and stems was high in low (4 g L^{-1} salt) and high (28 and 32 g L^{-1} salt) salinity stress, while mild salinity stress showed low soluble sugar content (Fig. 4).

The Na^+ content in the seedlings (roots, stems and leaves) increased with salinity, while K^+, and Ca^{2+}content in shoots (stems and leaves) decreased (Fig. 5). The contents of K^+ and Ca^{2+} in roots were not affected significantly by increase in the level of salinity. However, the Mg^{2+} content in leaves and roots increased with the increase of salinity and it decreased significantly in stems.

At 32 g L^{-1} salt solutions, Na^+ content in leaves, stems and roots was respectively 12.4, 6.2 and 2.8 times that in the control; and at 28 g L^{-1}, Mg^+ content in leaves and stems was respectively 3.1 and 1.6 times that in the control. The content of Ca^{2+} in leaves and stems decreased more sharply with salt stress than K^+ content and at 32 g L^{-1} the Ca^{2+} was respectively only 22.1 and 13.0% then in non-saline control.

Sodium and Mg^{2+} were the most dominant ions in the seedlings, especially under high salt stress. At 32 g L^{-1} salinity, total amount of Na^+ and Mg^{2+} accounted for 93.1% of the ions in leaves, 92.2% in stems and 85.5% in roots. Total ion concentration in the leaves was 5.33 mol kg^{-1} dry weight (DW), while it was no more than 2.61 mol kg^{-1} DW ions in roots (Fig. 6). The shoot biomass was over 86-92% of the total new growth biomass for this plant (Fig. 6).

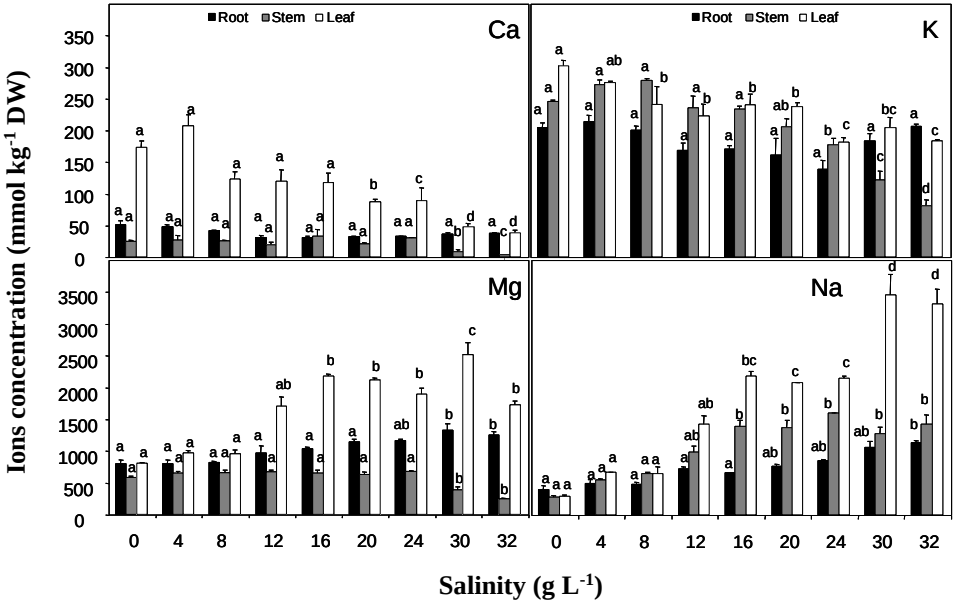

Fig. 5. Content of ions (Ca, Mg, K and Na) in roots, stems and leaves of *Tamarix chinensis* under salinity stress. Values are givens as means ± SE of three replicates. Different letters above columns indicate significant difference at $p<0.05$.

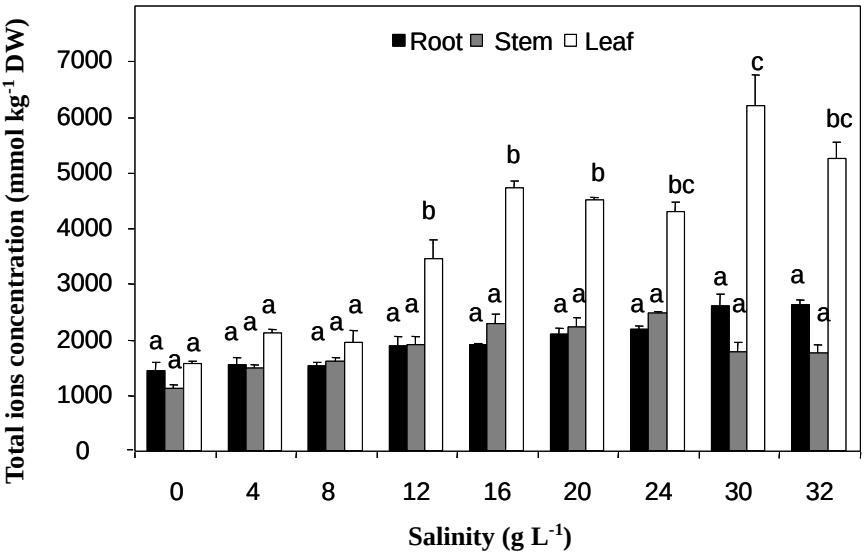

Fig. 6. Content of total ions (Ca + Mg + K + Na) in roots, stems and leaves of *Tamarix chinensis* under salinity stress. Values are givens as means ± SE of three replicates. Different letters above columns indicate significant difference at $p<0.05$.

Discussion

In coastal area of north China, salinity and fresh water shortage are the main edaphic factors limiting the vegetative establishment. Water table is shallow at 1 m and the water is highly saline (10-30 g L^{-1}). Utilizing the saline ground water, an economic use of abandoned arid lands is possible. *Tamarix chinensis* is the only native tree with high tolerance to salinity and drought that could be planted along the road in the area (Song *et al.*, 2006a). Our studies showed that *T. chinensis* cuttings might root quickly (in 7-14 days after incubation) and flourish in saline water (below 18 g L^{-1}). Thus, *T. chinensis* proved to be very suitable for propagation through cutting and a good species for afforesting in the area of north China having saline soil. Field experiments also showed that seedlings obtained from cuttings (70-100cm in length) were quicker to grow than from seeds (7-12 cm in length, unpublished data). *Tamarix chinensis* cuttings initiated their roots form root primorida and callus was not formed, which would imply that the species is easy to root. Stem cuttings is an efficient method to propagate this plant in salt and drought affected area.

Tamarix chinensis remained viable and grew well at seawater salinity like a true halophyte (Flowers *et al*., 1977). Low salinity (4-12 g L^{-1}) significantly stimulated seeding and root growth, which confirmed this hypothesis (Flowers *et al*., 1977). This observation corroborates previous studies on other halophytes, which showed sub-optimal growth in the media lacking salt (Flowers *et al*., 1977; Ohta *et al*., 1989; Short & Colmer, 1999; Mori *et al*., 2006; Koyro, 2006). It also implies that NaCl is an important "functional nutrient" for *T. chinensis* growth (Subbarao *et al*., 2003).

The salt-induced decrease in plant growth at high salinity might be due to energy losses through increased respiration and/salt pumping (Kleinkopf & Wallace, 1974), and biosynthesis of organic solutes for osmotic adjustment (Koyro, 2006) or because of nutritional imbalance, as shown in *Suaeda fruticosa* (Khan *et al*., 2000), *Cakile maritima* (Debez *et al*., 2004) and *Plantogo crassifolia* (Vicente *et al*., 2004; Koyro, 2006). The increase of Na^+ and Mg^{2+} in shoots with increase in salinity appears to help in osmoregulation and has also been reported in field investigations (Ramadan, 1998; Arndt *et al*., 2004). Similar results were observed in other halophytes (Khan *et al*., 2000; Debez *et al*., 2004; Koyro, 2006), where Na^+ played the main osmoregulation (Song *et al*., 2006b). Our results also indicate that Mg^{2+} is also important for osmoregulation.

Previous research on *Tamarix* sp. showed that salt rejection at the roots and excretion from shoots through salt glands (Ramadan, 1998) were the main mechanisms imparting salinity tolerance (Arndt *et al*., 2004). Our results (Fig. 4) also support the above observations as ion content in roots was not affected by increasing level of salinity in the growth medium (Ramadan, 1998; Vera-Estrella *et al*., 2005).

Proline and soluble sugar are known to be important for osmoregulation in plant. The proline content was promoted by salinity in our study. Similar results were obtained in some ecotypes of *Tamarix* (Jones *et al*., 2006) in field investigation. However, the content of proline was low. Hence, its contribution to osmoregulation might be limited as compared to that might caused by the accumulation of Na^+ and Mg^{2+}. It is also possible that some other compounds, as yet unidentified, that might be contributing to osmotic balance. Proline may have contributed to the protection of *T. chinensis* against high salinity in the soil by scavenging ROS generated by salt stress (Koyro, 2006). With increase in salinity, only modest accumulation of soluble sugar in stem and leaves of *T. chinensis* was observed. It implies that soluble sugars might not be the main

osmoregulation materials in this species. Modest accumulation of several other solutes might be possible, although we have not yet measured some important osmolytes, such as glycinebetain, sugar alcohols or proline analogues (Jones *et al*., 2006).

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