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SALT TOLERANCE OF *SALICORNIA UTAHENSIS* FROM THE GREAT BASIN DESERT

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Abstract

Seed germination of *Salicornia utahensis* (Tiderstorm) Munz (Chenopodiaceae), a stem-succulent halophyte from the playas of Great Basin Desert, Utah, was reduced to 50% at 300 mM NaCl and few seeds germinated at 900 mM NaCl. Plants grown for 60 days at 0 to 1000 mM NaCl, showed better growth at 400-600 mM NaCl and was similar to control at higher salinities up to 1000 mM. Tissue water content (g g^{-1} dry mass) of shoots in 200 to 400 mM NaCl treatments was higher than in nutrient solution controls and equal to control in 1000 mM NaCl. Water potential was higher than control at 200-600 mM NaCl and there was no significant difference at higher salinity concentrations. With increasing salinity, Na^+, Cl^-, NO_3^- generally increased, K^+ decreased while Ca^+, Mg^+ remained almost unchanged. Stem succulent individuals of *S. utahensis* in this study showed higher degree of salt tolerance through accumulating large quantities of Na^+ and Cl^- when treated with 200 to1000 mM NaCl.

Introduction

Saline soils and brackish water are widespread worldwide but the problem is particularly acute in arid and semi arid regions (Kafi & Khan, 2008). This renders conventional agriculture impractical due to ubiquitous salt sensitivity of our field crops. Vast tracts of lands along the seacoast and inland lie barren as a consequence because the only underground water available there for irrigation is inadequate and/or are of poor quality (Khan *et al*., 2009). Identifying suitable halophytic plants and making commercial use of their attributes can go a long way in not only meeting our various requirements but has other benefits like easing pressure on arable lands and good quality irrigation water and stabilizing the fragile ecosystem (Khan & Weber, 2006).

Salicornia species from the family Chenopodiaceae are obligate halophytes found generally in sea coast marshes as well as in salt playas (Ungar, 1978). Several species occur in Europe where some of them, *S. europaea* for instance, are known as vegetable delicacy (Lieth *et al*., 2006). Species of *Salicornia* are reported as good source of oil but *S. bigelovii* has received particular attention in this context (Glenn *et al*., 1991); the venture has not so far been a commercial success though. Nonetheless, it is expected that other member of the family may possess similar attributes. It is however imperative that seed germination and subsequent growth of especially the young seedling of the candidate species be examined as these stages determine the future plant population which is crucial in evolving any cash crop halophyte. The present study reports the germination and seedling growth responses of *Salicornia utahensis*, a perennial halophytic plant endemic to central Utah, USA to salinity.

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Material and Methods

Seeds of *S. utahensis* (Tiderstorm) Munz were collected from inland salt playas of Goshen, Utah, USA (39:57:06N 111:54:03W, 1360 m above sea level), separated from the inflorescence, surface sterilized using the fungicide Phygon (Hopkins Agricultural Chemical Co., Madison, Wis.) and stored at 4^{o}C. Germination was carried out in 50 mm x 9 mm tight-fitting plastic Petri plates with 5 ml of 0, 300, 600 or 900 mM NaCl solution. Each plate was placed in a 10 cm diameter plastic Petri plate as an added precaution against loss of water by evaporation. Four replicates of 25 seeds each were used for each treatment. Seeds were germinated in a growth chamber at an alternating temperature regime of 20-30^{o}C where the higher temperature coincided with the 12-h light period (Sylvania cool white fluorescent lamps 25 µmol-m-^{2}.s-^{1}, 400-750 nm) and the lower temperature coincided with the 12-h dark period. Seeds were considered germinated at the emergence of radicle which was recorded for 20 days on every alternate day.

For seedling growth under salinity, cores along with soil of young seedlings about 3 cm size were planted in sand filled plastic pots of 12.7 cm diameter x 12.7 cm height and thinned after one week to leave five equal sized seedlings in each pot. Five salinities (200, 400, 600, 800, 1000 mM NaCl) and a control without salts with four replicates were used and these pots were sub-irrigated after placing them in plastic trays containing half strength Hoagland solution. Water level in the trays was adjusted daily and the solutions were completely replaced every seven days. The salinity was increased gradually by 200 mM at alternate days till the required concentration was achieved. The temperature and light/dark regimes during growth were same as described above for seed germination. The plants were harvested 60 days after attaining desired salinity and dried at 80^{o}C for 48 h in a forced draft oven after recording their fresh weight.

For determination of ions concentration, 0.5 g dried plant material was boiled in 25 ml distilled water for two hr at 100^{o}C in a dry heat bath, cooled and filtered. Cl^-, NO_3^- and SO_4^{2-} were measured with a DX-100 ion chromatograph while Na^+, K^+, Ca^{2+} and Mg^{2+} were analyzed using a Perkin Elmer model 360 Atomic Absorption spectrometer. Water potential at different salinities was determined with a plant moisture stress meter (PMS Instruments). The results were subjected to one-way ANOVA to determine significant differences among means and Bonferroni test was carried out for significance between individual treatments (Anon., 1999).

Results

Germination of *S. utahensis* seeds was drastically reduced by substrate salinity and only a few seeds germinated at the highest salinity (900 mM NaCl) with very poor germination at the lower salt levels (Table 1). However, the growth of plants as evidenced in their shoot and root weights (both fresh and dry, Fig. 1) remained unaffected by salinity up to 1000 mM NaCl. The growth was rather stimulated at 200-400 mM and reduced with further increase in salts but was still comparable with control (minus-salt) treatment. The foliage as well as the roots contained high amounts of water as compared to control, increased at 200 and 400 mM while plants at higher salt concentrations had water contents similar to control (Fig. 2). The values of water potential in the foliage had similar trend i.e. higher (more negative compared to control, Fig. 3) up to 600 mM and comparable with control at higher salinity levels.

Table 1. Original and extrapolated values of seed germination (%) of *Salicornia utahensis*.

NaCl (mM)	0	300	600	900
Original values	78	38	25	5
Extrapolated	100	49	33	7

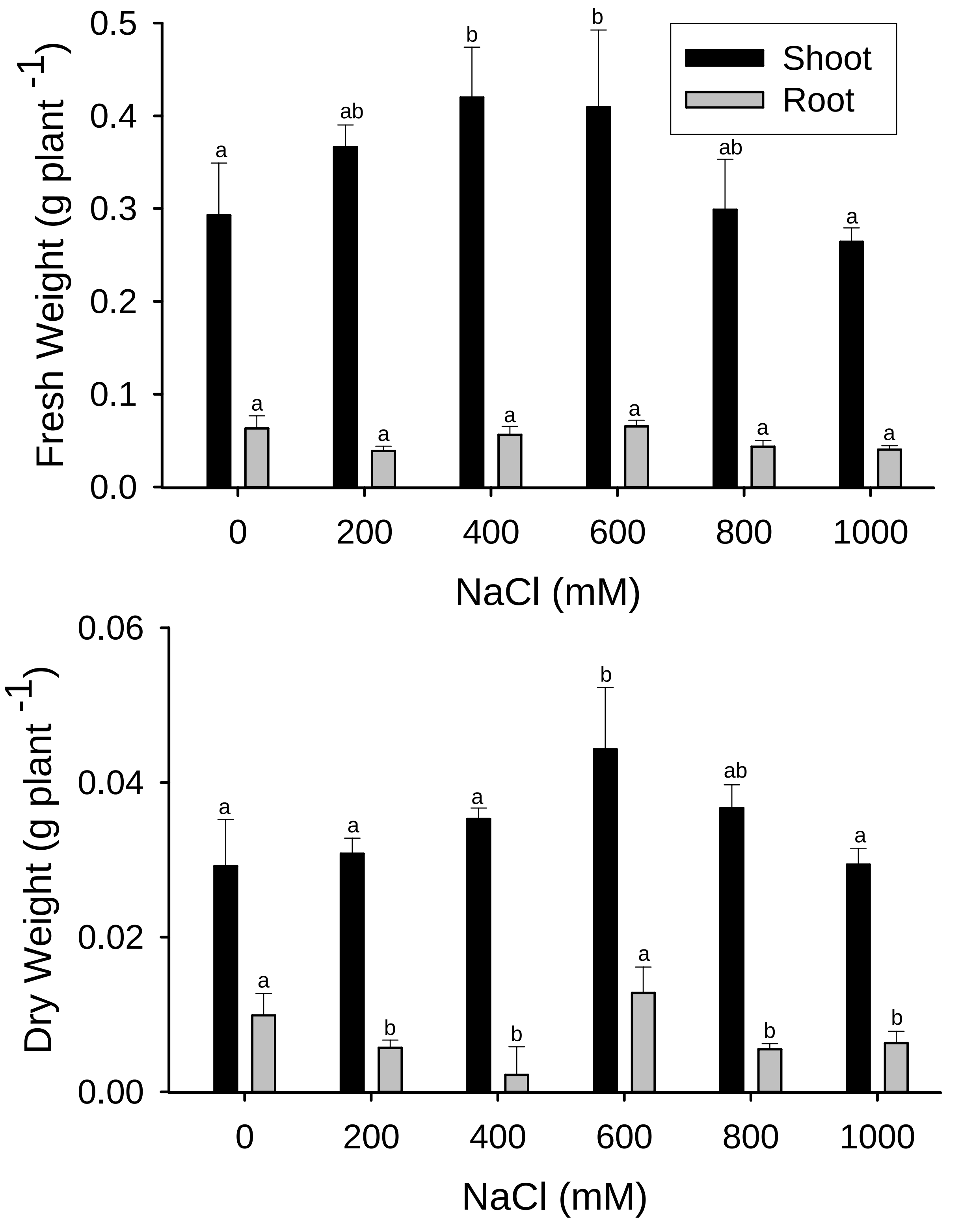

Fig. 1. Effect on NaCl on fresh weight and dry weight of *Salicornia utahensis* shoots and roots. Bar represents means with standard errors. Different letters above bars represents a significant difference ($p<0.05$) between salinity treatments.

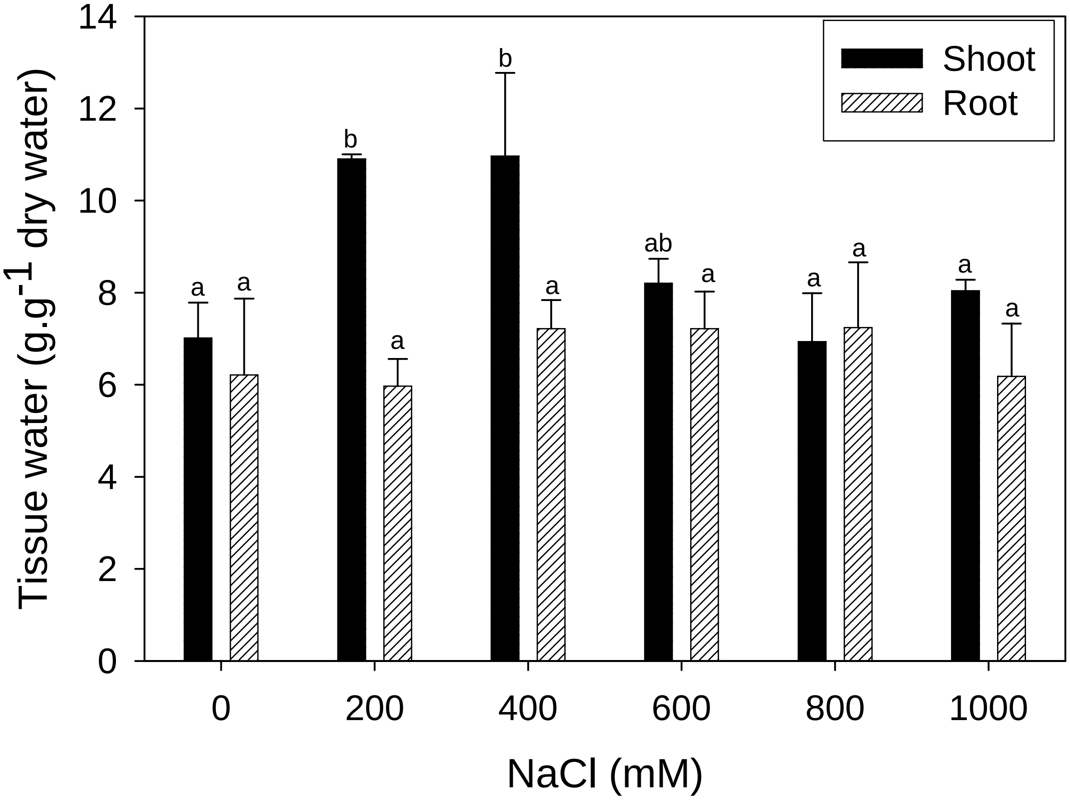

Fig. 2. Effect on NaCl on tissue water content of *Salicornia utahensis* shoots and roots. Bar represents means with standard errors. Different letters above bars represents a significant difference ($p<0.05$) between salinity treatments.

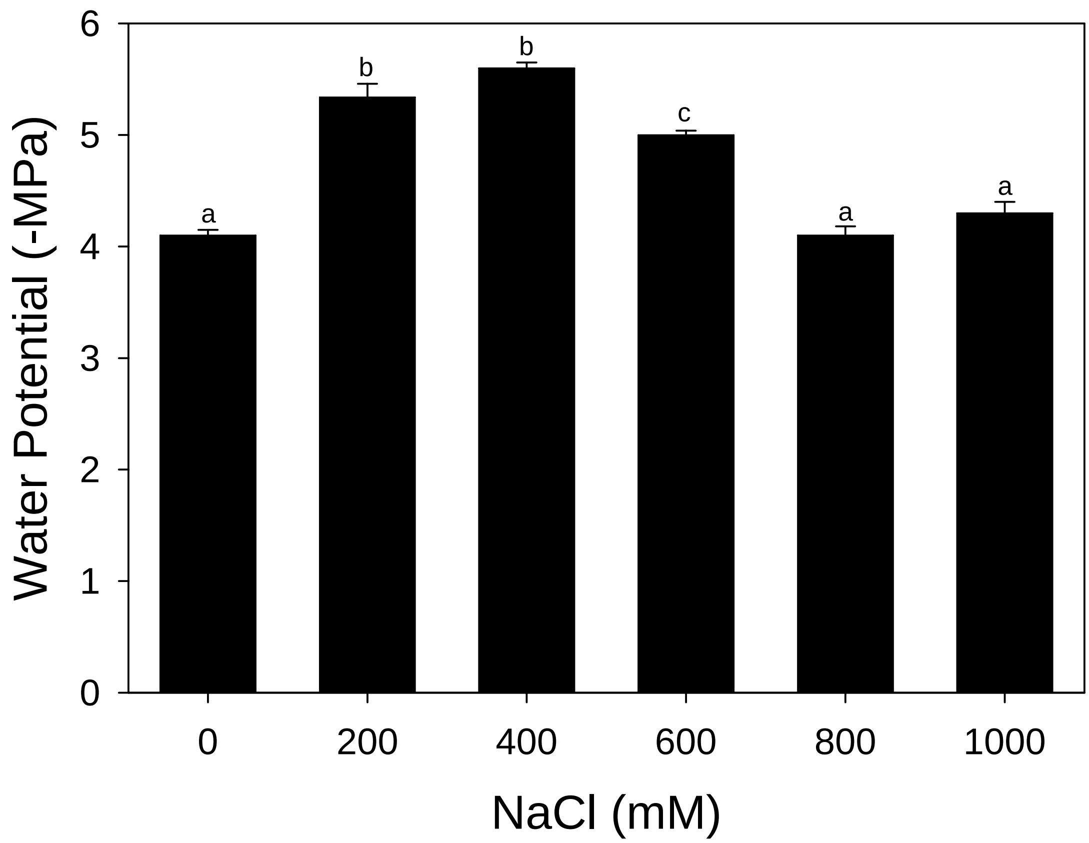

Fig. 3. Effect of NaCl (0, 200, 400, 600, 800 and 1000mM) on water potential of *Salicornia utahensis*. Bar represents means with standard errors. Different letters above bars represents a significant difference ($p<0.05$) between treatments.

Table 2. The effect of salinity on the concentration of cations and anions in shoot of *Salicornia utahensis*. Values represent means ± standard error.

NaCl (mM)	Na^+ (mM)	K^+ (mM)	Ca^{++} (mM)	Mg^{++} (mM)	Cl^- (mM)	SO_4 (mM)	NO_3 (mM)
0	414.7	18.4	0.6	12.2	339.5	3.2	0.4
	± 49.2	± 2.9	± 0.1	± 0.2	± 21.2	± 0.5	± 0.02
200	563.9	10.1	0.8	16.4	374.3	7.7	1.4
	± 55.4	± 0.4	± 0.3	± 2.0	± 33.8	± 0.4	± 0.11
400	757.7	5.9	0.6	12.2	741.1	5.1	9.1
	± 48.8	± 0.6	± 0.2	± 2.7	± 38.3	± 0.3	± 1.1
600	1849.2	7.3	0.6	12.7	1872.9	6.2	2.3
	± 10.2	± 0.5	± 0.5	± 2.7	± 157.9	± 0.5	± 0.5
800	2035.3	5.4	0.6	13.1	2146.3	3.9	5.2
	± 32.1	± 1.5	± 0.02	± 0.33	± 314.9	± 0.9	± 0.3
1000	2573.2	7.1	0.5	10.7	2382.7	4.5	3.1
	± 41.5	± 0.9	± 0.2	± 1.9	± 377.5	± 0.4	± 0.4

Table 3. The effect of salinity on the concentration of cations and anions in roots of *Salicornia utahensis*. Values represent means ± standard error.

NaCl (mM)	Na^+ (mM)	K^+ (mM)	Ca^{++} (mM)	Mg^{++} (mM)	Cl^- (mM)	SO_4 (mM)	NO_3 (mM)
0	554.7	21.2	1.4	28.2	197.8	1.2	0.7
	± 38.2	± 1.5	± 0.4	± 0.8	± 42.3	± 0.2	± 0.02
200	589.9	15.1	0.4	1.9	243.8	1.6	1.4
	± 35.4	± 3.4	± 0.3	± 0.7	± 67.7	± 0.4	± 0.11
400	858.7	12.3	0.8	1.6	356.6	1.8	1.9
	± 48.8	± 0.6	± 0.2	± 0.4	± 78.5	± 0.3	± 1.1
600	880.6	8.0	0.6	1.3	776.5	0.2	2.6
	± 39.2	± 0.9	± 0.2	± 0.4	± 38.2	± 0.02	± 0.5
800	989.3	10.9	0.6	1.2	860.3	0.3	2.5
	± 35.1	± 0.7	± 0.02	± 0.3	± 70.7	± 0.02	± 0.3
1000	1064.2	10.3	0.6	1.3	647.9	0.1	3.2
	± 63.5	± 0.9	± 0.1	± 0.1	± 98.1	± 0.02	± 0.4

Ion concentration in both root and shoot (Tables 2, 3) showed a general trend of increase in Na, Cl and NO_3 with increasing salinity. It was also noted that Ca and Mg remained almost unaffected in shoot but decreased in roots while SO_4 increased at low salts (generally in up to 600 mM) but decreased or remained unaffected at higher salinities used in this trial.

Discussion

Salicornia utahensis is a highly salt tolerant plant distributed in playas of Utah. Its seed germination was however, observed to be highly susceptible to salts in the present study. Even if the low seed germination under control (about 78%, which could be due to dormancy or seed damage) is discounted and considered as 100%, the extrapolated values under salinity do not show any significant improvement (Table 1).

Seed germination, being a determinant of population survival and perpetuation of progeny, attains significance in the life cycle of plant but it is also a very sensitive stage and minor disturbance in for instance moisture, temperature etc can be harmful (Khan & Gul, 2006). Ungar (1999) has also reported that even the extreme halophytes have better seed germination in non-saline conditions. Similarly, the early seedling growth is also susceptible to stresses as the plant has not yet adapted to face the adversities. The dormancy adapted by halophytic seeds plays an important role in overcoming these difficulties whereby the seed just lies in the soil waiting for favorable circumstances to germinate and complete the early growth stage in lesser hostile environment (Khan & Ungar, 2006). This is an adaptive mechanism and a necessity for the halophytes for their survival. The seeds of the present study showed only about 50% germination at 300 mM with drastic reduction at higher salinities, the un-germinated seeds were mostly dormant (data not shown). In nature, they probably germinate during the favorable window of opportunity and develop into plants which tolerate fairly higher salinities than those damaging germination.

Salicornia utahensis showed the typical response of a true halophyte where the seedling growth at 60 days from imposition of 1000 mM NaCl was as good as under control while stimulation in growth was observed up to 600 mM NaCl compared to no-salt treatment. A true halophyte is considered to be capable of remaining viable and complete its life cycle at seawater salinity (Flowers *et al*., 1977; Pasternak & Nerd, 1996; Rengasamy *et al*., 2003, Flowers & Colmer, 2008). In our study, the salinity threshold of *S. utahensis* was reached at around 3% (about 500 mM) salts whereas it survived >5% salinity which is much higher than most seawaters.

Physiological drought, created by low osmotic potential of substrate compared to that inside the plant and specific ion toxicities are two main hazards for plants growing in salinity (Khan *et al*., 2006; Flowers & Colmer, 2008). Halophytes are generally salt accumulators – a strategy that they adopt to cope with the low osmotic potential of root zone, but if enough tissue moisture is available, as in the present case, and with compartmentation of ions in vacuoles or plant parts (older leaves for instance which drop off and reduce salt load, not studied here), the overall damage due to salts is reduced considerably (Koyro, 2006). Many plants growing in salinity produce organic compounds which act as compatible osmotic solutes (Ungar, 1991). Members of Chenopodiaceae generally accumulate glycinebetaine in shoot for this purpose. This avoids ions toxicity but is a more energy intensive process compared to osmotic adjustment with inorganic ions (Ashraf & Harris, 2004; Khan *et al*., 1998).

Salinity in the present study was made with NaCl and these ions were readily absorbed by the plants according to the substrate concentration. High level of succulence may have diluted the hazards of specific ion effect and helped in sustained growth. Further more selective sequestration in vacuoles as reported for *Plantago* species (Muhling & Lauchli, 2002; Wong *et al*., 2007; Song *et al*., 2008) which was not studied here, may have been operative. It was also evident (Tables 2, 3) that more potassium was being retained in roots allowing less accumulation in shoots while magnesium was freely transported and evenly distributed in shoots under all salinity levels. In independent studies in our labs (Shaikh, 2008, Zehra, 2008) potassium salts (both chloride and sulfate) were found more toxic than magnesium for *Dicanthium annulatum, Eragrostis ciliaris*, and *Phragmites karka*. This also gets support from unpublished work from our lab on *Haloxylon stocksii* and *Suaeda fruticosa* seed germination.

World population continues to increase while resources of good quality water are not enough and arable lands are constantly lost to salinity at alarming pace. Incidentally, these problems generally occur with more severity in already resource-poor third world countries which are predominantly located in arid region. This necessitates search for suitable alternatives to meet the demand and has consequently brought research on halophytes in limelight. These plants of saline habitats can be put to an array of uses for mankind along with opportunities related to reforestation or replanting and ecological recovery of saline areas, coastal development and protection, production of cheap biomass for renewable energy and environmental conservation through carbon sequestration (Khan *et al*., 2006).

Species of *Salicornia*, known to occur on locations like salt playas and sea coasts, are capable of tolerating high salinities. Unlike *S. bigelovii*, publicized as a source of oil, *S. utahensis* has not received much attention as an oilseed crop. If this potential usage is established, this will make it particularly attractive for cultivation in saline lands using brackish water irrigation which are present in abundance in most arid regions. The growth of *S. utahensis* in the present study was stimulated up to 600 mM NaCl and was not reduced even at 1000 mM. With various cultural practices and soil amendments i.e. application of farmyard manure/fertilizers, optimum spacing, suitable irrigation regimes etc., the production could be improved. Further more, being a perennial, it can be grown for several years without reseeding making the planting more economical if adopted commercially.

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