

BRIEF COMMUNICATION

The effects of NaCl on growth, water relations, osmolytes and ion content in *Kochia prostrata*

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Abstract

The effects of NaCl (0, 50, 100, 150 and 200 mM) on growth, water relations, glycinebetaine, free proline, ion contents, stomata number and size of *Kochia prostrata* (L.) Schard were determined. Shoot and root fresh and dry matter, root and shoot length, relative growth rate, net assimilation rate, relative water content, water use efficiency, soluble sugars and glycinebetaine contents were not changed at low NaCl concentrations, but they were significantly decreased at 200 mM NaCl. The K⁺, Mg²⁺ and Ca²⁺ contents, water potential, chlorophyll *a+b* and carotenoides contents, and stomata number and size were reduced already at low concentrations of NaCl. In contrast, the Na⁺, Cl⁻ and proline contents increased several times with increasing NaCl concentration. *Kochia prostrata* is a salt tolerant species, the optimal growth of this plant occurred up to 150 mM NaCl. The mechanisms of salt tolerance in the plant may be balance among ion accumulation and production of glycinebetaine, proline, soluble sugars for maintenance of pressure potential.

Additional key words: glycinebetaine, halophyte, proline, stomata number and size, salt stress, salt tolerance, water use efficiency.

Many arid and semi-arid regions in the world contain soils and water resources that are too saline for most of the common economic crops. The utilization of halophytic plants pasture and fodder production in saline soils is the only economic solution presently available (Yeo and Flowers 1980).

Salt stress causes inhibition of growth and development, reduction in photosynthesis, respiration, and protein synthesis and disturbs nucleic acid metabolism (Levine *et al.* 1990). Decrease in uptake of K⁺, Mg²⁺, Ca²⁺ and thereby decrease in growth at higher Na⁺ concentration have also been reported (Poonia and Virmani 1972).

In halophytes adaptation to salinity is associated with metabolic adjustments that lead to the accumulation of several organic solutes, such as sugars, polyols, betaines and free proline (Flowers *et al.* 1977, Gorham *et al.* 1981, Briens and Larher 1982). Accumulation of proline has been widely advocated for use as parameter of selection for salt stress tolerance (Storey and Wyn-Jones 1979). The proline shall be also used as energy and nitrogen

storing suppressed (Sudhakar *et al.* 1993). The content of soluble sugars may be a good criteria for choosing of resistance species to salinity and drought, furthermore, monosacharides obviously have a major role in the initial response to salinity and drought (Kerepesi 1998). It has been reported that in response to salinity stress, glycinebetaine is accumulated in the cytoplasm of barley and sugar beet (Stewart and Lee 1974, Wyn-Jones and Storey 1981).

Kochia prostrata is a major source of protein and carotene for the feeding of domestic herbivorous animals in arid and semi arid regions. Regarding this point, less information is available about salinity resistance of *Kochia prostrata* at different growth stages. To realize the response of this species to salinity, the following experiment was conducted to find mechanisms of salt resistance of the plants.

Seeds of *Kochia prostrata* (L.) Schrad were collected during autumn 2002 from Esfahan Province in Iran. Plants were grown in 15 cm diameter pots in a growth chamber at 25/16 °C (day/night), and a 16-h photoperiod

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Abbreviations: NAR - net assimilation rate, RGR - relative growth rate; RWC - relative water content, WUE - water use efficiency.

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(irradiance of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The plants were grown in half-strength Hoagland and Arnon solution (Moore 1960) until they reached about 10 cm in height. NaCl concentration was gradually increased by 50 mM NaCl increments at 2 d intervals to reach the maximum salinity of 200 mM NaCl after 10 d. The plants were grown in four replicates (20 plants per pot) in sand with 0, 50, 100, 150 and 200 mM NaCl. Fresh and dry mass of plant shoots and roots were measured at 30 d intervals after the highest salt concentration was reached. Dry mass was determined after drying for 48 h in a forced-draft oven at 70 °C. Relative growth rate (RGR), relative leaf growth rate (RLGR), net assimilation rate (NAR), leaf area ratio (LAR), and leaf area/mass (LMR) were calculated according to Beadle (1993).

Water potential was by measured with a pressure bomb (*Model 1900, Sky Instruments, Hertfordshire, UK*) on four randomly chosen shoots from each treatment (Richardson 1980). Leaf relative water content (RWC) estimation was done by incubating leaf samples (0.5 g) in 100 cm^3 of distilled water for 6 h and calculated according to Weatherley (1950). Stomata size and stomata number per unit leaf area were measured by microscope.

Chlorophyll *a*, *b* and carotenoid contents were estimated by Arnon method (1949) in leaf samples (0.25 g) homogenized in 5 cm^3 of acetone (80 %). Absorbance was recorded at 645, 633 and 470 nm (*Spectrophotometer CECIL Model 2000, Cambridge, UK*).

Free proline content in the leaves was determined following the method of Bates *et al.* (1973). Total soluble sugar was estimated by anthrone reagent (Yemm and Willis 1954). The K^+ , Na^+ , Cl^- , Mg^{2+} and Ca^{2+} contents were determined by ICP spectrometer (*Model 2001, GBC Ltd., Melbourne, Australia*). Before measurement, 1 g of plant tissue was dried in an oven at 500 °C for 2 h. 10 cm^3 of distilled water and 10 cm^3 HNO_3 (1 M) were added to the ash. The volume of supernatant was increased to 50 cm^3 and filtered. Glycinebetaine estimation was done in according to Greive (1983). The absorbance was measured at 365 nm with UV-visible spectrophotometer. Reference standards of GB (50 - 200 $\mu\text{g cm}^{-3}$) were prepared in 2 M sulfuric acid.

The experimental design was completely randomized design with 4 replications of 5 treatments. The data was subjected to an analysis of variance and the statistical significance of the results was analyzed by the Duncan test.

Statistical analysis showed significant impacts of NaCl on *Kochia prostrata*, shoot length and root, fresh and dry matter, RGR, RLGR, NAR, LAR and LMR. NaCl concentration up to 150 mM did not alter these parameters, but they declined at 200 mM NaCl. The exception was root length which increased at 200 mM NaCl (Table 1).

Water use efficiency (WUE) was significantly decreased at 200 mM NaCl, but it was constant at

Table 1. The effects of NaCl (0, 50, 100, 150 and 200 mM) on the growth and physiological parameters in roots and shoots of *Kochia prostrata*. Means \pm SE, $n = 4$, means in a row followed by a different letter are significantly different ($P < 0.05$) according to Duncan test.

Parameters	0	50	100	150	200
Shoot fresh matter [mg plant ⁻¹]	3500 \pm 108a	3200 \pm 115a	3100 \pm 125a	3000 \pm 232a	1400 \pm 327b
Root fresh matter [mg plant ⁻¹]	510 \pm 31a	530 \pm 40a	500 \pm 35a	520 \pm 21a	110 \pm 12b
Shoot dry matter [mg plant ⁻¹]	580 \pm 34a	540 \pm 24a	520 \pm 15a	512 \pm 38a	280 \pm 13b
Root dry matter [mg plant ⁻¹]	140 \pm 15a	120 \pm 12a	110 \pm 16a	112 \pm 12a	45 \pm 7b
Shoot length [cm]	21.7 \pm 2.6a	21 \pm 2.4a	19 \pm 2.5a	18.7 \pm 2.2a	10 \pm 2.4b
Root length [cm]	12.5 \pm 1.5a	14 \pm 2.4a	14.5 \pm 2.2a	15 \pm 1.8a	24 \pm 2.8b
RGR [g g ⁻¹ d ⁻¹]	0.29 \pm 0.06a	0.27 \pm 0.08a	0.27 \pm 0.07a	0.28 \pm 0.06a	0.11 \pm 0.04b
RLGR [m ² m ⁻² d ⁻¹]	0.32 \pm 0.02a	0.33 \pm 0.02a	0.31 \pm 0.03a	0.28 \pm 0.02a	0.14 \pm 0.03b
NAR [mg cm ² d ⁻¹]	0.49 \pm 0.01a	0.51 \pm 0.01a	0.49 \pm 0.01a	0.48 \pm 0.09a	0.22 \pm 0.01b
LAR [m ² kg ⁻¹]	56.5 \pm 1.4a	56.7 \pm 1.7a	54.5 \pm 1.2a	52.5 \pm 1.1a	36.5 \pm 1.2b
SLA [m ² kg ⁻¹]	64.2 \pm 1.3a	63.5 \pm 1.6a	64.5 \pm 1.2a	62.2 \pm 1.1a	28.5 \pm 1.5b
WUE [mg(d.m.) g ⁻¹ (H ₂ O)]	54 \pm 2.08a	50 \pm 1.58a	50 \pm 1.75a	48 \pm 1.12a	18 \pm 1.56b
Water potential [-MPa]	1.1 \pm 0.08a	1.7 \pm 0.11b	2.0 \pm 0.11c	2.2 \pm 0.12c	2.6 \pm 0.14d
RWC [%]	92 \pm 2.4a	91 \pm 2.3a	89 \pm 2.4a	88 \pm 2.6a	50 \pm 2.3b
Soluble sugars [mg g ⁻¹ (d.m.)]	27.5 \pm 2.0a	39.5 \pm 3ab	44.7 \pm 2.9ab	54.2 \pm 3.4b	146.7 \pm 7.6c
Proline [$\mu\text{g g}^{-1}$ (d.m.)]	150 \pm 7.0a	195 \pm 9b	252 \pm 9c	299 \pm 11d	396 \pm 20e
Glycinebetaine [$\mu\text{g g}^{-1}$ (d.m.)]	408 \pm 25a	507 \pm 26a	528 \pm 38a	539 \pm 64a	1015 \pm 73b
Stomata number	10 \pm 0.6a	11 \pm 0.4a	13 \pm 0.6a	14 \pm 0.8ab	15 \pm 0.6b
Stomata size [μm]	9.5 \pm 0.6a	6.2 \pm 0.6b	5.1 \pm 0.4bc	4.9 \pm 0.5bc	3.6 \pm 0.3c
Chlorophyll <i>a</i> [mg g ⁻¹ (d.m.)]	0.77 \pm 0.07a	0.55 \pm 0.05b	0.43 \pm 0.05bc	0.33 \pm 0.04c	0.16 \pm 0.03d
Chlorophyll <i>b</i> [mg g ⁻¹ (d.m.)]	0.73 \pm 0.02a	0.49 \pm 0.02b	0.42 \pm 0.02c	0.19 \pm 0.02d	0.09 \pm 0.02e
Carotenoids [mg g ⁻¹ (d.m.)]	0.69 \pm 0.02a	0.47 \pm 0.03b	0.31 \pm 0.02c	0.19 \pm 0.02d	0.19 \pm 0.03e

Table 2. The effects of NaCl (0, 50, 100, 150 and 200 mM) on ion concentrations [$\text{mg g}^{-1}(\text{d.m.})$] in roots and shoots of *Kochia prostrata*. Means \pm SE, $n = 4$, means in a column followed by a different letter are significantly different ($P < 0.05$) according to Duncan test.

Plant part	NaCl [mM]	Na ⁺	K ⁺	Cl ⁻	Mg ²⁺	Ca ²⁺	Na ⁺ /K ⁺
Root	0	8.6 \pm 1.34a	12.4 \pm 0.59a	4.2 \pm 0.68a	3.3 \pm 0.37a	3.2 \pm 0.59a	0.68 \pm 0.04a
	50	9.1 \pm 2.87b	11.6 \pm 0.51a	7.4 \pm 0.84b	2.4 \pm 0.49ab	5.3 \pm 0.03b	0.78 \pm 0.02a
	100	22.5 \pm 3.23b	9.9 \pm 0.87b	8.8 \pm 0.36bc	1.7 \pm 0.53bc	4.1 \pm 0.16a	2.31 \pm 0.09b
	150	25.1 \pm 1.96bc	8.1 \pm 0.30b	9.9 \pm 0.18c	1.1 \pm 0.09cd	3.4 \pm 0.27a	3.13 \pm 0.06c
	200	27.8 \pm 2.79c	2.9 \pm 0.30c	10.5 \pm 0.37c	0.5 \pm 0.12d	1.6 \pm 0.28c	9.62 \pm 0.07d
Shoot	0	12.3 \pm 0.55a	39.4 \pm 4.71a	5.3 \pm 0.56a	7.3 \pm 0.69a	11.6 \pm 0.57a	0.40 \pm 0.05a
	50	22.1 \pm 0.55a	22.1 \pm 2.57b	12.5 \pm 0.69b	4.8 \pm 0.35b	6.5 \pm 0.83b	1.20 \pm 0.12b
	100	44.3 \pm 4.91b	19.7 \pm 2.16b	13.8 \pm 0.63b	4.6 \pm 0.27bc	5.5 \pm 0.71bc	2.40 \pm 0.11c
	150	55.1 \pm 7.46b	16.2 \pm 1.78b	16.9 \pm 0.62c	3.4 \pm 0.17c	4.9 \pm 0.68bc	3.40 \pm 0.88c
	200	77.3 \pm 6.42c	12.2 \pm 2.63c	23.6 \pm 0.85d	2.8 \pm 0.42c	3.3 \pm 0.24c	6.80 \pm 0.68d

lower concentrations. The water potential decreased as NaCl concentration increased. No significant difference between 0, 50, 100 and 150 mM NaCl was observed for RWC, but it was significantly decreased at 200 mM NaCl (Table 1). The contents of proline, soluble sugars and glycinebetaine were raised by increasing NaCl concentrations. The content of the soluble sugars was significantly enhanced at 150 and 200 mM NaCl, but it was not significantly increased at lower concentration of the NaCl. The three fold increase in proline content was observed at 200 mM NaCl compared to the non saline control. The concentration of glycinebetaine was significantly increased only at 200 mM NaCl, it doubled in comparison with control treatment (Table 1).

The number of stomata per unit of leaf area in adaxial and abaxial epidermes was significantly decreased only by 200 mM NaCl concentrations. Stomata size was decreased as NaCl concentrations increased especially on adaxial epidermis (Table 1).

The maximum contents of chlorophyll *a*, *b* and carotenoides were in control treatment and the greatest reduction occurred at 200 mM NaCl (Table 1).

Na⁺ and Cl⁻ contents in shoot and root were increased due to increasing NaCl concentrations, while K⁺, Mg²⁺ and Ca²⁺ contents were decreased. The contents of Na⁺, Cl⁻, K⁺, Mg²⁺ and Ca²⁺ were higher in the shoots than in the roots. Ratios of sodium to potassium in the shoot and root were enhanced under 50, 100, 150 and 200 mM NaCl compared to the non saline control, but in root there was non significance difference between 0 and 50 mM NaCl (Table 2).

In *Kochia prostrata* low concentration of NaCl do not altered shoot and root fresh and dry matter, shoot and root length, RGR, RLGR, NAR, LAR, SLA, RWC, WUE, stomata number in leaf adaxial, stomata size in leaf abaxial and soluble sugars (up to 100 mM), glycinebetaine contents, K⁺ and Mg²⁺ contents in the root (up to 50 mM). Whereas affected water potential, chlorophyll

and carotenoides contents, stomata number in leaf abaxial, stomata size in leaf adaxial and ion accumulation (K⁺, Mg²⁺ and Ca²⁺). This is an accordance with the result of Khan *et al.* (2000) on *Suaeda fruticososa*, Ungar (1996) on *Atriplex patula*, Houle (2001) on *Aster laurentianus* and Sairam *et al.* (2002) on wheat. Many other halophytes have optimal growth in the presence of NaCl (Naidoo and Rughunanam 1990). High concentration of NaCl (200 mM) decreased biomass, K⁺, Mg²⁺ and Ca²⁺ contents, RWC, WUE, water potential, chlorophyll *a*, *b*, carotenoides content, stomata size and increased proline content, glycinebetaine, soluble sugar, root length, number of stomata per unit of leaf area, Na⁺ and Cl⁻ contents, ratio of sodium to potassium. These results are similar to those of Zhao *et al.* (1992) on *Suaeda salsa* and Atriplex *lentiformis*, Siddique *et al.* (2000) on wheat, Viliers *et al.* (1996) on *Atriplex semibaccata*, Pennypacker *et al.* (1990) on alfalfa, Karimi and Ungar (1984) on *Atriplex triangularis*, Khan *et al.* (2000) on *Atriplex griffithii*.

High concentration of NaCl inhibits growth of the plants due to toxicity of NaCl or low osmotic potential. To preserve sufficient water uptake osmotic adjustment occurred (production of proline, glycinebetaine and soluble sugars).

At high concentration of NaCl (200 mM) growth depression may originate from inhibited nutrient uptake, transport and utilization in the plants. In *Kochia prostrata* 200 mM NaCl induced Mg²⁺ deficiency. The concentration of Na⁺ and Cl⁻ by far exceeded their demand and lead to toxicity in the plants.

Our data indicated that, *Kochia prostrata* is a salt tolerant species, which the optimal growth occurred up to 150 mM NaCl. The mechanisms of salt tolerance in *Kochia prostrata* may be based mainly on the inclusion of the NaCl in vacuoles for pressure potential maintenance. Glycinebetaine, proline and soluble sugars are accumulated in the cytoplasm and its organelles in

order to maintain photosynthesis (Schroppe-Meier and Kaiser 1988). *Kochia prostrata* as salt accumulating

halophyte, could be planted successfully to improve low saline areas in arid and semi arid regions.

References

- Arnon, D.I.: Copper enzymes in isolated chloroplast, polyphenol oxidase in *Beta vulgaris*. - *Plant Physiol.* **24**: 1-15, 1949.
- Bates, L.S., Waldran, R.P., Tear, I.D.: Rapid determination of free proline for water studies. - *Plant Soil.* **39**: 205-208, 1973.
- Beadle, C.L.: Growth analysis. - In: Hall, D.O., Scurelock, J.M.O., Bolh ar-Nordenkamp, H.R., Leegood, R.C., Long, S.P. (ed.): *Photosynthesis and Production in a Changing Environment: a Field and Laboratory Manual*. Pp. 36-46. Chapman & Hall, London 1993.
- Briens, M., Larher, F.: Osmoregulation in halophytic higher plants: a comparative study of soluble carbohydrates, polyols, betaines and free proline. - *Plant Cell Environ.* **5**: 287-292, 1982.
- Flowers, T.J., Troke, P.F., Yeo, A.R.: The mechanism of salt tolerance in halophytes. - *Annu. Rev. Plant Physiol.* **28**: 89-121, 1977.
- Greive, C.M., Grattan, S.R.: Rapid assay for determination of water soluble quaternary-amino compounds. - *Plant Soil* **70**: 303-307, 1983.
- Gorham, J., Hughes, L.Y., Wyn Jones, R.G.: Low-molecular-weight carbohydrates in some salt stressed plants. - *Physiol. Plant.* **53**: 27-33, 1981.
- Houle, G., Morel, L., Reynolds, C.E., Siegel, J.: The effect of salinity on different developmental stages of an endemic annual plant, *Aster laurentianus*. - *Amer. J. Bot.* **88**: 62-67, 2001.
- Karimi, S.H., Ungar, I.A.: The effect of salinity on the ion content and water relation of *Atriplex triangularis*. - In: Tiedemann, A.R., McArthur, E.D., Stutz, H.C., Stevens, R., Johnson, K.L. (ed.): *Proceeding of the Symposium on the Biology of Atriplex and Related Chenopods*. Pp. 124-132. USDA Forest Service, Utah 1984.
- Kerepesi, I.: Osmotic and salt stresses induced differential alteration in water-soluble carbohydrate content in wheat seedling. - *J. agr. Food Chem.* **46**: 5347-5354, 1998.
- Khan, M.A., Ungar, I.A., Showalter, A.M.: The effect of salinity on the growth, water status, and ion content of a leaf succulent perennial halophyte, *Suaeda fruticosa* (L.). *Forssk.* - *J. arid Environ.* **45**: 73-84, 2000.
- Khan, M.A., Ungar, I.A., Showalters, A.M.: Effects of salinity on growth, water relations and ion accumulation of the subtropical perennial halophyte, *Atriplex griffithii* *Var. Stocksii*. - *Ann. Bot.* **85**: 225-232, 2000.
- Levine, R.L., Garland, D., Oliver, C., Amici, A., Clement, I., Lenz, A.G., Ahn, B.W., Shaltiel, S., Stadtman, E.R.: Determination of carboxyl content in oxidatively modified proteins. - *Methods Enzymol.* **186**: 464-478, 1990.
- Moore, R.H.: *Laboratory Guide for Elementary Plant Physiology*. - Burgess Publ., Minneapolis 1960.
- Naidoo, G.R., Rughunanan, R.: Salt tolerance in the succulent halophyte, *Salicornia natalensis*. - *J. exp. Bot.* **41**: 497-502, 1990.
- Pennypacker, B.W., Leath, K.L., Stout, W.L., Hill, R.R.: Techniques for stimulating field drought stress in greenhouse. - *Agron. J.* **82**: 951-957, 1990.
- Poonia, S.R., Virmani, S.M., Bhumla, D.R.: Effect of ESP (exchangeable sodium percentage) of soil on the yield, chemical composition and uptake of applied calcium by wheat. - *J. Indian Soc. Soil Sci.* **20**: 183-185, 1972.
- Richardson, S.G., McKell, C.M.: Water relations of *Atriplex canescens* as affected by the salinity and moisture percentages of processed oil shale. - *Agron. J.* **72**: 946-950, 1980.
- Sairam, R.K., Veerabhadra, R., Srivastava, G.C.: Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. - *Plant Sci.* **163**: 1037-1046, 2002.
- Schroppe-Meier, G., Kaiser, W.M.: Ion homeostasis in chloroplasts under salinity and mineral deficiency. I. Solute concentrations in leaves and chloroplasts from Spanish plants under NaCl or NaNO₃ salinity. - *Plant Physiol.* **87**: 822-827, 1988.
- Siddique, M.R.B., Hamid, A., Islam, M.S.: Drought stress effects on water relation of wheat. - *Bot. Bull. Acad. Sin.* **41**: 35-39, 2000.
- Stewart, G.R., Lee, J.A.: The role of proline accumulation in halophytes. - *Planta* **120**: 279-289, 1974.
- Storey, R., Wyn-Jones, R.G.: Responses of *Atriplex spongiosa* and *Suaeda monoica* to salinity. - *Plant Physiol.* **63**: 156-162, 1979.
- Sudhakaran, P.R., Reedy, M.P., Veeranjanyulu, K.: Effect of salt stress on the enzymes of proline synthesis and oxidation in Green gram seedling. - *Plant Physiol.* **141**: 621-623, 1993.
- Ungar, I.A.: Effect of salinity on seed germination, growth and ion accumulation of *Atriplex patula*. - *Amer. J. Bot.* **83**: 604-607, 1996.
- Viliers, A.J., Teichman, I., Rooyen, M.W., Theron, J.K.: Salinity induced changes on anatomy, stomatal counts and photosynthetic rate of *Atriplex semibaccata*. - *R. Br. South Africa J. Bot.* **62**: 270-276, 1996.
- Weatherley, P.E.: Studies in water relation of cotton plants, the field measurement of water deficit in leaves. - *New Phytol.* **49**: 81-87, 1950.
- Wyn-Jones, R.G., Storey, R.: Betaines. - In: Paleg, L.G., Aspinall, O. (ed.): *Physiology and Biochemistry of Drought Tolerance*. Pp.171-204. Academic Press, Sydney 1981.
- Yeo, A.R., Flowers, T.J.: Salt tolerance in the halophyte *Suaeda maritima* (L.) Dum. Evaluation of the effect of salinity upon growth. - *J. exp. Bot.* **31**: 1171-1183, 1980.
- Yemm, E.W., Willis, A.J.: The estimation of carbohydrates in plant extracts by anthrone. - *Biochem. J.* **57**: 508-514, 1954.
- Zhao, K.F., Harris, P.J.: The effect of iso-osmotic salt and water stresses on the growth of halophyte and non-halophyte. - *Plant Physiol.* **139**: 761-763, 1992.