

Responses of alfalfa to potassium, calcium, and nitrogen under stress induced by sodium chloride

M.G. KHAN*, M. SILBERBUSH and S.H. LIPS

Biostress Research Laboratory, Desert Agrobiological Research Center, The Jacob Blaustein Institute for Desert Research, Sede Boqer Campus, IL-84993, Israel

Abstract

The physiological responses of alfalfa (*Medicago sativa* L. cv. Gilboa) to salinity (100 mM NaCl) and some inorganic nutrients (K^+ , Ca^{2+} and N as NO_3^-) were investigated. Salinity caused a substantial reduction in biomass, carbon assimilation rate, stomatal conductance, water use efficiency, leaf area, relative growth rate, NO_3^- content and nitrate reductase activity, whereas, transpiration rate was slightly affected. Inclusion of K^+ , Ca^{2+} and N as NO_3^- in plant nutrient medium in combination or alone brought about a marked stimulation in control plants and moderated the salinity caused reductions in NaCl treated plants. In addition, plants also exhibited differences in these parameters at two growth stages.

Additional key words: CO_2 assimilation, dry mass, nitrate content, nitrate reductase activity, relative growth rate, stomatal conductance, water use efficiency.

Introduction

The demand to use brackish water is increasing in Israel and in other arid regions of the world. Increased salinity results in decreased growth and photosynthesis in non-halophyte species (Greenway and Munns 1980, Longstreth and Strain 1977, Ball and Farquhar 1984). Nitrate reductase (N.R.1.6.1.6.), the first enzyme in nitrate assimilation, can rapidly change its activity in response to various stress conditions. However, response to salinity stress are rather contradictory as both inhibition (Martinez and Cerda 1989, Rao and Gnanam, 1992) and stimulation (Misra and Dwivedi 1990) are reported. In some studies, the promotive action of nitrogen,

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Abbreviations: DMP - dry mass production; g_s - stomatal conductance; NRA - nitrate reductase activity; P_N - net photosynthetic rate, WUE - water use efficiency.

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* Corresponding author; present address: Department of Plant Science, Rohilkhand University, Bareilly, 243006, Uttar Pradesh, India

calcium and potassium on various physiological processes has been noticed (*e.g.*, Hawkins and Lewis 1993), however, information is still meagre on the role of these nutrients on carbon and nitrogen assimilation in crop species particularly under salinity stress.

In the present communication, we study the effects of N, Ca and K supplied either individually or in combination on growth parameters, gas exchange, nitrate content and nitrate reductase activity in alfalfa plants grown with or without NaCl.

Materials and methods

Plants: Alfalfa (*Medicago sativa* L. cv. Gilboa) seeds were disinfected in 1 % sodium hypochloride solution for 15 min and washed in running tap water and sown in plastic pots containing 5 kg of loamy sand soil. The soil was treated with Hewitt's nutrient solution and iron was provided as Fe-EDDHA. Following inorganic nutrients were added to the nutrient medium: N as KNO₃ (0.1 and 6 mM); K as KCl (0.1 and 2 mM); Ca as CaCl₂ (0.1 and 5 mM). NaCl was supplied in 0 and 100 mM concentrations. Salt treatment was given twice in a week followed by irrigation with de-ionized water. Six plants were raised in each pot and three identical sets were designed during the experiment. The whole experiment was conducted in a greenhouse with a temperature range of 32 - 12 °C during day and night, and a photoperiod of about 11 - 12 h. The first harvesting was done 80 d after planting, the second harvesting 30 d after regrowth.

Assays and determinations: Dry masses of root and shoots were determined after drying in a hot air oven at 80 °C for 48 h. Relative growth rate of the whole plant was calculated using dry masses of plants at two harvests (Hunt 1978):

$$\text{RGR} = \log_e W_2 - \log_e W_1 / t$$

where, W_2 and W_1 are dry masses at two growth stages, respectively, t is the time difference between two harvests.

Net photosynthetic rate, water use efficiency (WUE), stomatal conductance and transpiration rate of fully expanded young leaf were measured using an ADC-LCA-3 infra red gas analyzer (Analytical Development Corporation, Hoddesdon, England) connected to Parkinson's leaf chamber after assignment. The first observation was taken on day 80 after germination while the second one on day 30 after regrowth. All measurements were recorded at the midday point of the phytotron light cycle at temperature of 25 °C and 50 % relative humidity.

Nitrate content in roots and shoots was determined according to Cataldo *et al.* (1975). Nitrate reductase activity was determined in root and leaves at two growth stages. Fully expanded leaves and primary roots were taken for NR assays done according to Soares *et al.* (1985).

Statistical analysis: The statistical evaluation of data was done by factorial analysis using averages of three replicates. For significance, Fisher's PLSD test was adapted.

Results

Growth: Biomass accumulation was significantly lower in NaCl treated plants. However, the extent of reduction was more in shoot as compared with root. Shoot/root dry mass ratio was also declined. The inclusion of Ca^{2+} , K^+ and NO_3^- in nutrient medium resulted in higher accumulation of biomass in root and shoot and enhancement in root/shoot ratio at both growth stages. Moreover, plants showed even higher shoot and root dry mass when grown with supplemental Ca, K and N in combinations (Table 1).

Salinity depressed the relative growth rate (RGR) in plants in both N supplied or N deficient conditions. Conversely, RGR was markedly stimulated when nutrient medium contained additional Ca, K or N. Moreover, plants exhibited higher RGR values under combined application of these nutrients. The application of these nutrients was more effective in plants grown under salinity (Table 1).

Leaf area was reduced in NaCl-stressed plants as compared with control plants. The effect of NaCl was visible even from morphology of the plants. The leaf area was considerably increased by K or Ca, however, higher enhancement was recorded when nutrient medium contained Ca alone or in combinations than K alone (Table 1).

Gas exchange: Net photosynthetic rate (P_N) was slightly decreased under salinity stress, whereas, it was enhanced after inclusion of N or Ca in nutrient media (Fig. 1).

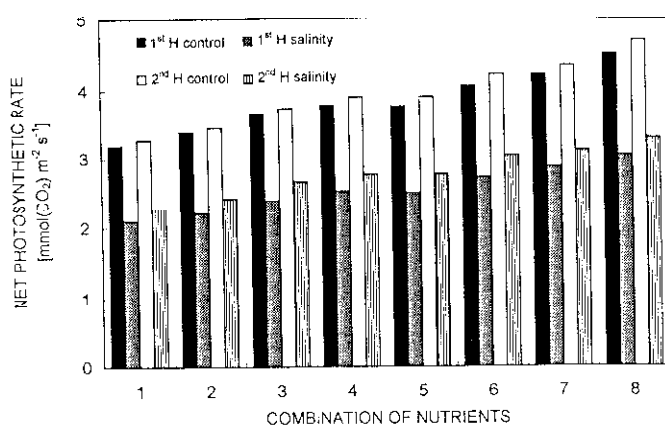


Fig. 1. Net photosynthetic rate in alfalfa plants as influenced by salinity (control, 1st harvest - *full columns*; control, 2nd harvest - *empty columns*; salinity, 1st harvest - *dotted column*; salinity, 2nd harvest - *stripped columns*) and inorganic nutrients in following combinations: 1 - 0 mM N + 0 mM K + 0 mM Ca; 2 - 0 mM N + 2 mM K + 0 mM Ca; 3 - 0 mM N + 2 mM K + 5 mM Ca; 4 - 0 mM N + 0 mM K + 5 mM Ca; 5 - 6 mM N + 0 mM K + 0 mM Ca; 6 - 6 mM N + 2 mM K + 0 mM Ca; 7 - 6 mM N + 0 mM K + 5 mM Ca; 8 - 6 mM N + 2 mM K + 5 mM Ca.

Water use efficiency (WUE, ratio between P_N and transpiration rate) reached lowest values in plants raised in saline medium at first growth stage. Whereas, it attained

Table 1. Effect of some inorganic nutrients (combinations) on biomass production in alfalfa plant grown with or without NaCl salinity.

Nutrient combinations	Root DM [g plant ⁻¹]		Shoot DM [g plant ⁻¹]		Leaf area [cm ²]		RGR	
	1 st harvest	2 nd harvest	1 st harvest	2 nd harvest	1 st harvest	2 nd harvest	1 st harvest	2 nd harvest
	control	salinity	control	salinity	control	salinity	control	salinity
N ₁ K ₁ C ₁	0.45	0.31	0.52	0.36	0.85	0.53	0.89	0.57
N ₁ K ₂ C ₁	0.48	0.34	0.54	0.39	0.88	0.56	0.92	0.62
N ₁ K ₁ C ₂	0.52	0.37	0.59	0.43	0.93	0.61	0.98	0.70
N ₁ K ₂ C ₁	0.56	0.41	0.65	0.50	0.95	0.64	1.01	0.74
N ₁ K ₂ C ₂	0.55	0.42	0.65	0.51	0.98	0.68	1.05	0.79
N ₂ K ₂ C ₁	0.56	0.43	0.67	0.53	1.01	0.72	1.09	0.82
N ₂ K ₁ C ₂	0.60	0.48	0.69	0.57	1.15	0.86	1.22	0.95
N ₂ K ₂ C ₂	0.63	0.54	0.72	0.54	1.24	1.01	1.36	1.10

N₁ - nitrogen (0.1 mM), N₂ - nitrogen (6 mM), K₁ - potassium (0.1 mM), K₂ - potassium (2 mM), C₁ - calcium (0.1 mM), C₂ - calcium (5 mM), control - 0 mM NaCl, salinity - 100 mM NaCl.

Table 2. Nitrate content in alfalfa plants as affected by salinity and some inorganic nutrients (Ca, N and K).

Treatments	Plant parts	Nitrate content [$\mu\text{mol}(\text{NO}_3)^- \text{g}^{-1}(\text{FM})$] 1 st harvest control	salinity	2 nd harvest control	salinity
$\text{N}_0\text{K}_0\text{C}_0$	Root	0.76 ± 0.07	0.42 ± 0.03	0.79 ± 0.07	0.50 ± 0.04
	Shoot	1.05 ± 0.15	0.62 ± 0.05	1.15 ± 0.13	0.72 ± 0.07
$\text{N}_0\text{K}_1\text{C}_0$	Root	0.95 ± 0.08	0.78 ± 0.07	0.98 ± 0.10	0.82 ± 0.08
	Shoot	1.85 ± 0.21	1.38 ± 0.25	2.05 ± 0.21	1.68 ± 0.18
$\text{N}_0\text{K}_1\text{C}_1$	Root	1.45 ± 0.20	1.28 ± 0.18	1.51 ± 0.19	1.35 ± 0.19
	Shoot	2.02 ± 0.21	1.80 ± 0.28	2.05 ± 0.21	1.84 ± 0.21
$\text{N}_0\text{K}_0\text{C}_1$	Root	0.81 ± 0.08	0.69 ± 0.05	0.85 ± 0.08	0.75 ± 0.07
	Shoot	1.15 ± 0.13	0.90 ± 0.08	1.36 ± 0.19	1.08 ± 0.11
$\text{N}_1\text{K}_0\text{C}_0$	Root	1.65 ± 0.18	1.32 ± 0.19	1.61 ± 0.20	0.33 ± 0.02
	Shoot	2.35 ± 0.28	1.80 ± 0.24	2.52 ± 0.26	2.02 ± 0.21
$\text{N}_1\text{K}_1\text{C}_0$	Root	1.85 ± 0.25	1.55 ± 0.16	1.90 ± 0.22	1.61 ± 0.18
	Shoot	2.67 ± 0.30	2.12 ± 0.22	2.70 ± 0.30	2.23 ± 0.25
$\text{N}_1\text{K}_0\text{C}_1$	Root	2.10 ± 0.22	1.84 ± 0.21	2.13 ± 0.24	1.87 ± 0.20
	Shoot	2.95 ± 0.33	2.51 ± 0.26	3.20 ± 0.41	2.78 ± 0.30
$\text{N}_1\text{K}_1\text{C}_1$	Root	2.90 ± 0.31	2.74 ± 0.30	2.98 ± 0.32	0.84 ± 0.08
	Shoot	3.45 ± 0.45	3.20 ± 0.41	3.60 ± 0.44	3.27 ± 0.41

Treatments as in Table 1.

maximum values in plants grown with additional N and Ca in combinations (Fig. 2). Moreover, the promotive action of these nutrients on WUE was also observed in plants grown with NaCl.

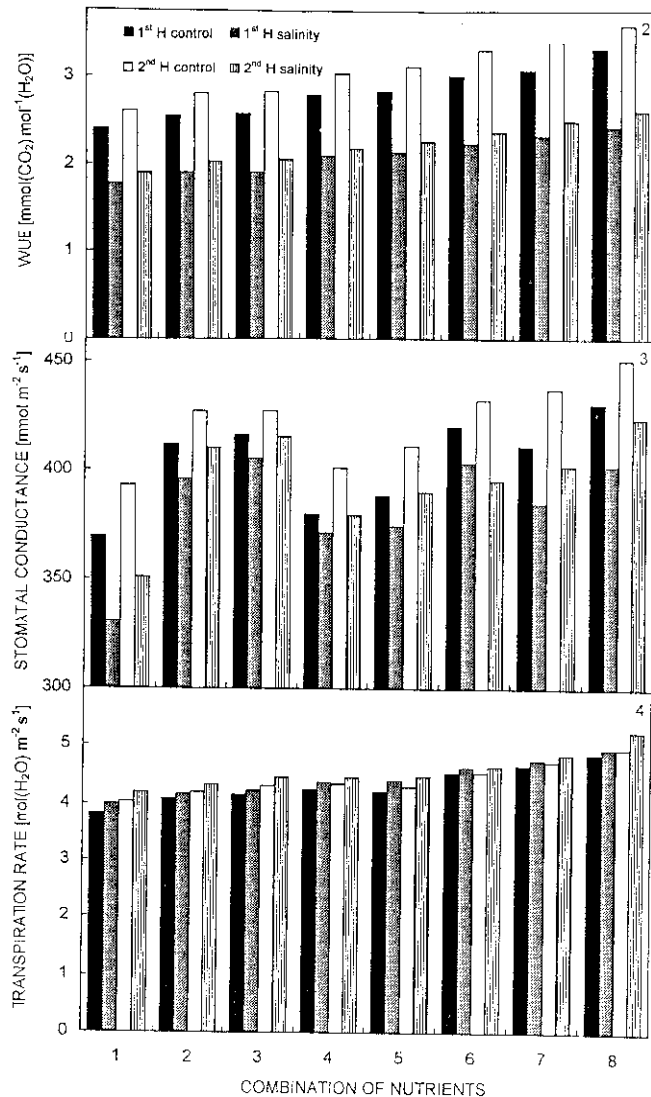


Fig. 2. Effect of salinity and inorganic nutrients on water use efficiency in alfalfa plants. Treatments as in Fig. 1.

Fig. 3. Stomatal conductance (g_s) in alfalfa as affected by salinity and inorganic nutrients. Treatments as in Fig. 1.

Fig. 4. Transpiration rate (E) in alfalfa plants as affected by salinity and inorganic nutrients. Treatments as in Fig. 1.

Stomatal conductance (g_s) was negatively affected by NaCl imposition in growth media (Fig. 3). However, a significant rise was observed in stomatal conductance in plants when supplied with K or Ca.

Salinity slightly stimulated transpiration rates (E) in alfalfa plants. E was further enhanced in plants under supplemental K or Ca (Fig. 4).

Nitrogen metabolism: Nitrate content in shoots and roots was substantially decreased by NaCl salinity at both growth stages. Inclusion of Ca, K and N in different combinations in growth medium had promotive effect on nitrate content in root and shoot as well (Table 2). The maximum nitrate was accumulated when plants were grown with combined Ca, K and N. Moreover, the promotive action of these nutrients was higher in salinized plants.

Nitrate reductase activity (NRA) was inhibited in leaves and roots of alfalfa plants in response to salinity and this inhibition was more in leaves (Fig. 5A,B).

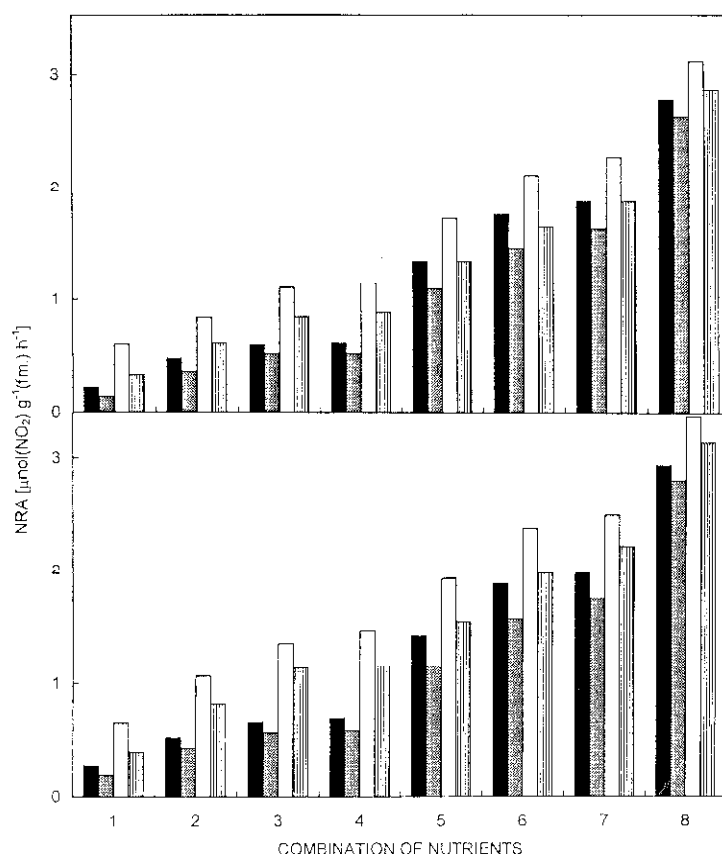


Fig. 5. Nitrate reductase activity in alfalfa plants (control, roots - *full columns*; control, leaves - *empty columns*; salinity, roots - *dotted column*; salinity, leaves - *stripped columns*) as affected by salinity and inorganic nutrients in combinations as in Fig. 1. A - 1st harvest, B - 2nd harvest.

Discussion

In the present investigation, biomass accumulation, relative growth rate and leaf area was reduced in alfalfa plants in response to NaCl salinity. This is in accordance with earlier reports on some other species (*e.g.*, Neumann *et al.* 1988, Plaut *et al.* 1989, Lips *et al.* 1990). Termaat and Munns (1986) concluded that the changes in leaf expansion would be determined by changes in cell wall properties. On the other hand, Neumann *et al.* (1988) indicated that salinity affects growth rate by changes in pressure potential. A steady supply of N to peanuts plants inhibited the reduction in growth caused by NaCl, since the uptake of N by the plants was renewed which allowed the full expansion of leaves as well as the production of new ones (Silberbush and Lips 1988). However, one should not exclude the possibility that factors limiting leaf expansion under salinity conditions also differ with plant species.

In alfalfa plants, P_N expressed on a unit area basis was substantially reduced due to salinity as outlined by several authors in some other species (*e.g.*, Gale *et al.* 1967, Downton 1977, Hawkins and Lewis 1993). In contrary, there are additional reports which indicated a non-significant effect of salinity on photosynthesis (Kaiser *et al.* 1983, Plaut *et al.* 1989). Salinity caused reduction in P_N is attributed to reduced g_s or due to reduction in capacity of photosynthetic machinery (Downton *et al.* 1985, Seeman and Critchley 1985, Seeman and Sharkey 1986). P_N and g_s declined in response to salinity, however, a linear relation cannot be established. In spite of the reduction in g_s , E was not significantly affected by NaCl.

Salinity decreased internal nitrate concentrations and NRA in various parts of alfalfa plants. Both nitrate content as well as NRA were higher in leaves in comparison to roots. Nevertheless, reduction in these parameters was also higher in leaves. In this way, our results are in consistence with some previous findings on several other species (Aslam *et al.* 1984, Martínez and Cerda 1989, Rao and Gnanam 1990, Ourry *et al.* 1992, Gouia *et al.* 1994).

Thus, our results indicated a substantial reduction in various growth, gas exchange and nitrogen assimilation parameters due to salinity, which could be overcome by additional supply of K, Ca and N dependent upon concentrations and combinations of nutrients applied.

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