BRIEF COMMUNICATION

The effects of NaCl and $CaCl_2$ on photosynthesis and growth of alfalfa plants

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Abstract

The effects of 0, 30, 60, and 90 mM NaCl, and 0 and 5 mM $CaCl_2$ on certain parameters of photosynthesis and growth in alfalfa (*Medicago sativa* L. cv. Ghara yonjeh) plants were studied. The increasing NaCl concentration in the Hoagland nutrient solution decreased the contents of chlorophylls and the net photosynthetic rate, and increased the rate of respiration (R_D) and CO_2 compensation concentration in the leaves of treated plants. The contents of carotenoids (Car) were not significantly affected. The addition of 5 mM $CaCl_2$ enhanced the R_D and increased the Car contents in treated leaves. With the NaCl concentration in the culture medium increasing, the dry matter production in both root and shoot decreased, as well as the relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR). The addition of $CaCl_2$ caused a partial elimination of the NaCl effects on the root and shoot, RGR and NAR, and it decreased the LAR.

The salinity inhibits the growth of plants due to ionic toxicity lowering the water potential in the root medium, and/or an alteration in the plant ionic status (Greenway and Munns 1980). The effects of salinity depend upon the level of salinity, the ionic composition of the root medium, irradiance, air humidity, plant species, stage of plant growth, *etc.* An application of calcium reduces the NaCl salinity effects and results in a relatively increased growth of NaCl-treated plants (LaHaye and Epstein 1971, Hanson 1984, Cramer *et al.* 1985, 1986, 1987, Kent and Läuchli 1985, Kurth

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Abbreviations: Car - carotenoids; Chl - chlorophyll; LA - leaf area; LAR - leaf area ratio; LDM - leaf dry matter; LWCA - leaf water content per unit area; LWR - leaf dry matter per total plant dry matter; NAR - net assimilation rate; P_N - net photosynthetic rate; R_D - dark respiration rate; RDM - root dry matter; RFM - root fresh matter; RLGR - relative leaf growth rate; R/S - root-to-shoot ratio; SDM - stem dry matter; SE - standard error of mean; SHTI - shoot tolerance index; SLA - specific leaf area; TI - tolerance index; TOTTI - total tolerance index; Γ - CO_2 compensation concentration.

et al. 1986, Ward *et al.* 1986, Khavari-Nejad 1988b). In the present work, the effects of NaCl and $CaCl_2$ on photosynthesis, respiration, and growth of alfalfa (*Medicago sativa* L. cv. Ghara yonjeh) plants were investigated.

The seeds of alfalfa were germinated in the washed sand inside a growth cabinet with a dim light, 75 % air humidity, and an ambient temperature of 23 ± 1 °C. After the germination, young seedlings were transferred to a controlled environment with day/night temperatures of 28/17 (± 1) °C, 16 h photoperiod [irradiance of approximately 100 W m^{-2} (PAR) was supplied by natural irradiance with *Tungsten* lamps supplementing when desired]. At the 1st trifoliate leaf (8-d-old) stage, the seedlings of the same size and shape were transferred to 800 cm^3 plastic containers (5 plants per a container) with nutrient solutions comprising 5 mM KNO_3, 4 mM $Ca(NO_3)_2{\times}4$ H_2O, 2 mM $MgSO_4{\times}4$ H_2O, 1 mM KH_2PO_4, 0.09 mM $NH_4Fe(SO_4)$, and micronutrients. The plants were treated with 0 (controls), 30, 60, and 90 mM NaCl, either with or without adding 5 mM $CaCl_2$. All the solutions were kept at a pH of 6.5 and aerated throughout the whole experimental period. The amount of water was adjusted daily using distilled water, and renewed every 10 d.

The plants of 6 container sets (replicates), each container holding 5 plants, were harvested for growth analyses after 45 d of experimental growth period, using the equations introduced by Watson (1952), and Evans and Hughes (1962). The tolerance index (TI) of the root, shoot, and whole plant was calculated using the formula:

$$TI = \frac{\text{dry mass under stress condition}}{\text{dry mass under control condition}}.$$

Prior to the plants being harvested at the end of experimental growth period, the net photosynthetic rate (P_N), and R_D and CO_2 compensation concentration (Γ) were measured on a plant shoot using an infrared gas (CO_2) analyser (*225 MKS, Analytical Development Co.*, UK) (Khavari-Nejad 1980, 1986). The measurements were carried out under an ambient air temeprature of 25 ± 1 °C, 75 % air humidity, 350 $cm^3(CO_2)$ m^{-3} (for P_N and R_D), and 100 W m^{-2} PAR when desired. The contents of Chl and Car were measured spectrophotometrically using the method of Arnon (1949).

The NaCl salinity decreased the P_N, yet increased the R_D and Γ values (Table 1). The addition of $CaCl_2$ did not affect the P_N in NaCl-treated plants, but it increased the R_D and Γ values. In NaCl-treated alfalfa plants, the Chl contents of leaves were significantly lowered too, but the Car contents were not significantly affected. The addition of $CaCl_2$ caused an increase in the Car contents of the NaCl-treated leaves.

A similar P_N reduction in alfalfa was found by Shone and Gale (1983) and Khan *et al.* (1994b), and therefore the stomatal or non-stomatal limitations of photosynthesis might be involved (Seemann and Critchley 1985, Bethke and Drew 1992). An alteration of the source-sink relationship may also be the cause for the P_N reduction in NaCl-treated plants (Munns and Termaat 1986). The reduction in P_N of the NaCl-treated plants may also be due to a change in the content of manganese or magnesium (Cramer *et al.* 1991, Gouia *et al.* 1994, Gomez *et al.* 1996). The P_N reduction could as well be the result of a reduction in the Chl content of the NaCl-treated leaves. In the present work, the corellation between these two variables was highly significant (r = 0.98), similarly as in *Ipomoea pescaprae* (r = 0.97) (Venkatesan *et al.* 1995). Chl *a* is more susceptible to NaCl salinity than Chl *b* (Reddy and Vora 1986). The site of inhibitory action of NaCl salinity is probably in the photosystem 2 or nearby (Singh and Dubey 1995). In the present work, the Car contents were not affected by NaCl salinity. Other reports claim both the inhibitory (Reddy and Vora 1986, Singh and Dubey 1995) and promotory (Abd-El-Samad and Shaddad 1995) effects of NaCl salinity on the production of Car.

Table 1. The interaction of NaCl salinity and 5 mM $CaCl_2$ treatment on certain photosynthetic parameters in alfalfa plants (means ± SE). For abbreviations see their list.

Parameter	$CaCl_2$ [mM]	NaCl [mM] 0 (control)	30	60	90
P_N [μmol(CO_2) m^{-2} s^{-1}]	0	2.35±0.25	1.60±0.17	1.40±0.07	1.35±0.27
	5	1.32±0.16	1.62±0.18	1.52±0.04	1.42±0.13
Γ [μmol mol^{-1}]	0	83.25±2.71	124.25±1.88	138.00±7.51	138.00±4.81
	5	106.25±2.80	129.00±5.36	142.50±0.95	141.25±4.19
R_D [μmol(CO_2) m^{-2} s^{-1}]	0	1.90±0.11	2.50±0.14	2.87±0.70	2.95±0.71
	5	2.00±0.10	2.60±0.23	3.55±0.17	4.07±0.25
Chl *a* [g kg^{-1}(f.m.)]	0	2.30±0.15	1.60±0.16	1.25±0.13	1.20±0.04
	5	2.00±0.31	1.42±0.10	1.47±0.16	1.37±0.17
Chl *b* [g kg^{-1}(f.m.)]	0	1.40±0.10	1.10±0.07	0.85±0.09	0.80±0.05
	5	1.30±0.22	0.92±0.29	0.72±0.04	0.72±0.04
Chl (*a*+*b*) [g kg^{-1}(f.m.)]	0	3.70±0.26	2.57±0.20	2.07±0.16	2.00±0.07
	5	3.30±0.53	2.32±0.17	2.20±0.20	2.10±0.21
Xanthophylls [g kg^{-1}(f.m.)]	0	0.20±0.04	0.17±0.03	0.16±0.01	0.15±0.01
	5	0.18±0.03	0.25±0.04	0.26±0.02	0.30±0.01
Carotene [g kg^{-1}(f.m.)]	0	0.25±0.01	0.22±0.01	0.22±0.01	0.23±0.02
	5	0.25±0.02	0.30±0.02	0.29±0.04	0.34±0.02

The application of 5 mM $CaCl_2$ increased the contents of Car in the NaCl-treated plants (Table 1), which seems to be the result of Ca^{2+}/Na^+ interaction effect. The probability of photoinhibition in plants increases under NaCl salinity (Sharma and Hall 1991) and the Car efficiently protect against this effect.

NaCl salinity increased R_D, the rate of which was further accelerated in the presence of $CaCl_2$. This is in contrast with findings in *Tradescantia* (Khavari-Nejad 1988a,b) and sunflower plants (Khavari-Nejad 1988c). According to Rao and Rao (1981), the R_D increased under NaCl salinity in old wheat leaves, but it decreased in young ones. An increasing effect of NaCl salinity on R_D was reported by Schwarz and Gale (1981) in *Xanthium* plants. In the same report, NaCl salinity increased the Γ value. Similar results were found in *Tradescantia* (Khavari-Nejad 1988a), *Hordeum* (Rawson 1986), and *Capsicum* (Bethke and Drew 1992).

The rate of total growth was found lower under NaCl salinity as compared to that of controls. The production was smaller and succulent leaves were especially typical of alfalfa plants under NaCl salinity. In the absence of NaCl, the $CaCl_2$ treatment produced K^+ deficiency symptoms like necrosis on the margins of treated leaves, and an early senescence of old leaves. In the NaCl-treated plants, the leaf area production, and fresh and dry matter production decreased while the application of $CaCl_2$ lowered the NaCl effect (Table 2).

Table 2. The interaction of NaCl salinity and 5 mM $CaCl_2$ treatments on certain growth parameters in alfalfa plants (means ± SE). For abbreviations see their list.

Parameter	$CaCl_2$ [mM]	NaCl [mM] 0 (control)	30	60	90
LA [cm² plant⁻¹]	0	21.05 ±2.87	16.23 ±1.87	12.03 ±1.57	11.55 ±0.50
	5	19.41 ±1.00	20.55 ±1.60	12.80 ±1.07	12.20 ±1.04
LFM [g plant⁻¹]	0	0.241±0.028	0.202±0.024	0.152±0.022	0.190±0.012
	5	0.230±0.008	0.301±0.017	0.203±0.022	0.202±0.024
SFM [g plant⁻¹]	0	0.405±0.020	0.287±0.030	0.206±0.025	0.187±0.018
	5	0.380±0.019	0.303±0.026	0.215±0.023	0.198±0.028
RFM [g plant⁻¹]	0	0.215±0.038	0.180±0.029	0.163±0.034	0.189±0.020
	5	0.247±0.024	0.304±0.014	0.202±0.045	0.266±0.044
LDM [g plant⁻¹]	0	0.038±0.005	0.029±0.004	0.023±0.003	0.027±0.001
	5	0.040±0.003	0.044±0.003	0.028±0.003	0.029±0.003
SDM [g plant⁻¹]	0	0.066±0.006	0.048±0.006	0.039±0.007	0.032±0.003
	5	0.071±0.006	0.055±0.004	0.036±0.005	0.037±0.006
RDM [g plant⁻¹]	0	0.019±0.003	0.015±0.002	0.013±0.003	0.015±0.001
	5	0.027±0.002	0.033±0.004	0.018±0.004	0.020±0.003
R/S (d.m.)	0	0.180	0.198	0.213	0.258
	5	0.245	0.332	0.275	0.310
RTI	0	1.00	0.81	0.71	0.80
	5	1.40	1.75	0.94	1.10
SHTI	0	1.00	0.74	0.60	0.56
	5	1.06	0.94	0.61	0.64
TOTTI	0	1.00	0.75	0.61	0.59
	5	1.1	1.06	0.67	0.71
RGR [g kg⁻¹ d⁻¹]	0	86.68	80.97	77.59	77.99
	5	89.47	88.48	79.23	80.46
NAR [g m⁻² d⁻¹]	0	5.59	4.97	5.16	5.27
	5	6.60	6.43	5.31	5.92
RLGR [cm² m⁻² d⁻¹]	0	939.83	879.14	829.25	829.45
	5	923.56	1001.64	845.02	836.40
LAR [m² kg⁻¹(d.m.)]	0	15.50	16.29	15.03	14.79
	5	13.55	13.79	14.92	13.59
SLA [m² kg⁻¹(d.m.)]	0	54.76	55.86	53.81	43.03
	5	49.21	48.98	46.68	41.29
LWR [kg kg⁻¹(d.m.)]	0	0.283	0.291	0.279	0.343
	5	0.275	0.281	0.319	0.329
LWCA [g(H_2O) m⁻²]	0	97.60	106.66	106.05	141.97
	5	99.14	129.78	136.71	140.45

Increasing the NaCl concentration increased the root-to-shoot ratio which could be the result of a greater damage in the shoot than in root. Similar results were found by

Keck *et al.* (1984) and Khan *et al.* (1994a,b). With increasing NaCl salinity the TI decreased in both the root and shoot, but the application of 5 mM $CaCl_2$ lowered the effect of NaCl. The $CaCl_2$ effect on NaCl-treated plants was greater in the root than that in shoot (Table 2). The plant root growth is promoted under an osmotic stress (Subbarao *et al.* 1995), and the rate of root growth maximized in the culture medium containing 30 mM NaCl and 5 mM $CaCl_2$. NaCl salinity decreased the RGR, RLGR, NAR, SLA, and LAR, and increased the LWCA and LWR. Under NaCl salinity, the addition of $CaCl_2$ caused a partial increase in the RGR, RLGR, NAR, LWCA, and LWR, but also a partial decrease in the LAR and SLA.

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