Available online at www.sciencedirect.com
ScienceDirect
Scientia Horticulturae 111 (2006) 84–90
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

Sennoside content and yield attributes of *Cassia angustifolia* Vahl. as affected by NaCl and $CaCl_2$

A. Arshi [a,b], M.Z. Abdin [b], M. Iqbal [a,*]

[a] *Department of Botany, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India*
[b] *Department of Biotechnology, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India*

Received 15 June 2005; received in revised form 25 August 2006; accepted 30 August 2006

Abstract

Pot culture experiments were conducted to assess the extent of growth, photosynthetic capacity, sennoside concentration and yield attributes of Senna plant under the individual as well as combined influence of NaCl and $CaCl_2$. Six treatments, i.e. NaCl (80 and 160 mM), $CaCl_2$ (5 and 10 mM) alone and a combination of NaCl + $CaCl_2$ (80 + 10 and 160 + 10 mM) were given to the growing Senna plants at pre-flowering (45 DAS), flowering (75 DAS) and post-flowering (90 DAS) stages. Significant reductions were observed in pod biomass, leaf area, stomatal conductance, photosynthetic rate and sennoside concentration and yield, with each NaCl treatment. On the contrary, individual $CaCl_2$ treatments had a favourable effect. Under the effect of combination treatments, although these parameters were reduced, the extent of reduction was much less than one caused by NaCl treatments. The combined treatments thus mitigated the adverse effects caused by NaCl.

Keywords: *Cassia angustifolia* Vahl.; Photosynthetic rate; Salt stress; Stomatal conductance; Sennoside content; Sennoside yield

1. Introduction

Salinity stress has a negative impact on agricultural yield throughout the world. Salinity can seriously alter plant metabolic activities such as assimilation of mineral nutrients (Arshi et al., 2002, 2005; Munns et al., 2000; Papp et al., 1983) stomatal conductance (Ouerghi et al., 2000; Brugnoli and Lauteri, 1991), mesphyll conductance (Delfine et al., 1998), carbon metabolism, and/or efficiency of photosynthetic enzymes (Brugnoli and Bjorkman, 1992). High concentration of salts causes ion imbalance and hyper-osmotic stress in plants. These primary effects often lead to secondary stresses, such as oxidative stress, due to production of activated oxygen species, which can damage DNA, proteins, chlorophylls, and membrane functions (Zhu, 2001; Gomez et al., 1999; Hernandez et al., 1999).

Calcium is an essential plant nutrient and has a role in metabolic activities, like stabilization of membranes, signal transduction through second messenger, and control of enzyme activity (Helper and Wayne, 1995; Kirkby and Pilbeam, 1984). Ca^{2+} can help to remediate the adverse effect of salinity on plants. It helps in maintaining membrane integrity and ion-transport regulation and is essential for K^+/Na^+ selectivity (Hanson, 1984). Elevated Ca^{2+} concentration in nutrient solution mitigates the adverse effects of NaCl by inhibiting Na^+ uptake (Greenway and Munns, 1980; LaHaye and Epstein, 1969) and reducing membrane leakage (Leopold and Willing, 1984). Addition of calcium salts to a complete nutrient solution may alleviate suppression of root growth under high salinity level (Kent and Lauchli, 1985). K^+ concentrations in the root, reduced by salinity, can be restored to adequate levels by an additional supply of calcium, as it protects cell membranes from adverse effect of Na^+ and minimizes the leakage of cytosolic potassium. Calcium plays a vital role in the regulation of ionic relations in plants and in improving the soil physical conditions.

The present study evaluates the impact of NaCl and $CaCl_2$ on growth, leaf area, photosynthetic efficiency, stomatal conductance and sennoside content in Indian Senna.

2. Materials and methods

2.1. Experimental setup

Seeds of Indian Senna (*Cassia angustifolia* Vahl.) were procured from Gujarat Agricultural University, Anand. Pot

Abbreviations: DAS, days after sowing; EC, electrical conductivity; P_N, photosynthetic rate; g_s, stomatal conductance

* Corresponding author. Tel.: +91 11 26059688x5530; fax: +91 11 26059663.
E-mail address: iqbalg5@yahoo.co.in (M. Iqbal).

0304-4238/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2006.08.006

culture experiments were conducted at the experimental site of Botany Department in Hamdard University, New Delhi. The seeds were sown in earthen pots (12″ × 12″) in the last week of July and the crop lasted for 120–135 days. The pots were filled with 12 kg of sandy loam soil [pH 7.2, electrical conductivity (EC) 0.207 m mho/cm] and farmyard manure (FYM) in the ratio of 4:1. The basal dose of NPK was applied, as recommended for Senna crop, at the time of pot filling and 30 days after sowing. Ten seeds per pot were sown and one plant per pot was maintained from vegetative stage till harvest. NaCl (80 and 160 mM), $CaCl_2$ (5 and 10 mM) and combinations of $NaCl + CaCl_2$ (80 + 10 and 160 + 10 mM) were applied to the growing plants separately at three phenological stages, i.e. 45 DAS (at pre-flowering stage), 75 DAS (during flowering stage), and 90 DAS (at post-flowering stage), by adding 500 ml of the given solution to the soil. The control (without salt treatments) and the treated plants were kept at a uniform water supply.

2.2. Sampling and analysis of biomass

Sampling of leaves for dry matter, total leaf area, photosynthetic efficiency and stomatal conductance was started 15 days after the first treatment, i.e. at 60 DAS, and continued at 15 days interval till the harvest time, i.e. 120 DAS. Pod formation began at 90 DAS and therefore sampling of pods was done at 105 and 120 DAS.

The samples were taken in five replications from each treatment, and kept in oven at 65 ± 2 °C for 24 h. Dry weight of the pods were recorded with the help of electronic top pan balance (Eagle) and expressed in gram per plant.

2.3. Total leaf area

The total green leaf area per plant was measured in five replication with the help of a LICOR 3000 Leaf Area Meter (LICOR, Lincoln, USA) and was expressed as cm^2 per plant.

2.4. Photosynthetic efficiency and stomatal conductance

Net photosynthetic rate (P_N) and stomatal conductance (g_s) of middle leaflets of the compound leaf were recorded using a portable LICOR 6200 Photosynthesis System (LICOR, Lincoln, USA). The conditions during gas exchange measurements were: irradiance (I_R) 1493–1032 qn; temperature (T) 40.0–37.9 °C; relative humidity (RH) 46.9% and ambient carbon dioxide (CO_2)[a] 300–325 ppm.

2.5. Sennoside concentration

HPLC was employed for the quantitative analysis of sennosides *a*, *b*, *c* and total sennoside contents were measured in young and mature leaves and pods at 90 DAS (i.e. pre-flowering and flowering stage treatments).

The sennoside concentration of leaves was extracted using the method of Lemmli et al. (1985). One hundred milligram of dried (60 °C/72 h) leaf taken in vials was extracted three times with 20, 20 and 10 ml of double distilled water. The extract was pooled and vials re-kept in boiling water for 15 min. After cooling, the extract was filtered through Whatman's filter paper no. 1 and the final volume was made to 50 ml by adding double distilled water. Five milliliters of cooled extract was added with 10 ml of 15% ferric chloride, and incubated at 80 °C for 20 min. There after, 0.1 ml of concentrated HCl was added and extracted three times with 20, 20 and 10 ml of ether using a separating funnel. The aqueous layer was collected and its volume made up to 50 ml by adding double distilled water. High performance liquid chromatograph (HPLC Delta 600, Waters, USA) was employed for the quantitative analysis of sennosides *a*, *b* and *c*, using the method followed by Srivastava et al. (1983a,b). The solvent used in HPLC was a mixture of tetrahydrofurane with 2% glacial acetic acid and HPLC water (1:3) with the flow rate of 1 ml min^{-1} through the column C_{18} using UV detector (280 nm) set at the ambient temperature. Sennoside *a*, sennoside *b* and sennoside *c* are the main sennosides present in leaves and pods of Senna plant. Their concentration was calculated using the formula:

$$\text{Sennoside concentration (mg g}^{-1}\text{ dw)} = \frac{\text{Amount of sennoside in 10 µl} \times \text{peak area of sample}}{\text{Sample weight} \times \text{peak area of sennoside}}$$

2.6. Yield attributes

Sennoside yield was taken to be equal to [leaf dry weight × total sennoside content of leaves] + [pod dry weight × total sennoside content of pods] + [seed dry weight × total sennoside content of seeds] and expressed in milligram per plant (Srivastava et al., 1983b). The observations were recorded at 90 DAS.

2.7. Statistical analysis

The data collected were analyzed using the SPSS statistical package software version 10.0 (Chicago, USA). Two-way analysis of variance (ANOVA) was used to determine differences between treatments and plant growth stages. Mean separation was done by the Dunnett's test.

3. Results

3.1. Pod dry weight

Compared to the control, a significant decline in the dry weight of pods was observed in the last sampling (at 120 DAS) from plants treated with NaCl and with $NaCl + CaCl_2$. The reduction in dry weight due to NaCl treatments at pre-flowering (45 DAS), flowering (75 DAS) and post-flowering (90 DAS) stages was up to 36–69%, 40–71% and 33–60%, respectively. The decline was dose dependent. In contrast, corresponding treatments of $CaCl_2$ increased the pod dry weight by 6–16%, 7–17% and 5–13%, respectively, the effect being most pronounced at 10 mM $CaCl_2$. Combination

Table 1
Changes in pod dry weight (g/plant) of *C. angustifolia* affected by NaCl, $CaCl_2$ and NaCl + $CaCl_2$ stress given at pre-flowering, flowering and post-flowering stages

Treatments	Days after sowing (DAS)		
	90	105	120
Control	2.95[a]	3.35[a]	4.42[a]
Pre-flowering stage (A_1)			
80 mM NaCl	2.13 c	2.29 e	2.82 e
160 mM NaCl	1.41 d	1.38 b	1.38 f
5 mM $CaCl_2$	3.18 ab	3.51 b	4.69 b
10 mM $CaCl_2$	3.36 a	3.88 a	5.12 a
80 mM NaCl + 10 mM $CaCl_2$	2.39 c	2.61 d	3.12 d
160 mM NaCl + 10 mM $CaCl_2$	1.61 d	1.58 f	1.62 f
Flowering stage (A_2)			
80 mM NaCl	2.19 c	2.19 e	2.64 e
160 mM NaCl	1.51 d	1.29 f	1.27 b
5 mM $CaCl_2$	3.16 ab	3.61 b	4.71 b
10 mM $CaCl_2$	3.38 a	3.85 a	5.16 a
80 mM NaCl + 10 mM $CaCl_2$	2.41 c	2.65 d	3.34 d
160 mM NaCl + 10 mM $CaCl_2$	1.66 b	1.69 f	1.79 f
Post-flowering stage (A_3)			
80 mM NaCl		2.34 e	2.96 e
160 mM NaCl		1.55 d	1.77 b
5 mM $CaCl_2$		3.51 b	4.66 b
10 mM $CaCl_2$		3.75 a	4.98 a
80 mM NaCl + 10 mM $CaCl_2$		2.61 d	3.30 d
160 mM NaCl + 10 mM $CaCl_2$		1.71 f	1.89 f
	F-Value		
ANOVA			
Stage	0.46 ns	3.61*	24.54**
Salt stress	158.28**	4062.7**	5824.9**
Stage × salt stress	0.11 ns	8.05*	11.5*

ns, **, *, indicate non-significance or significance at $P \leq 0.01$ or 0.05, respectively. Values followed by a letter within a column for each stage of development were not significantly different using LSD at $P \leq 0.05$.
[a] These values were not different from the control using Dunnett's test at the 5% level.

Table 2
Changes in leaf area (cm^2/plant) of *C. angustifolia* affected by NaCl, $CaCl_2$ and NaCl + $CaCl_2$ stress given at pre-flowering, flowering and post-flowering stages

Treatments	Days after sowing (DAS)				
	60	75	90	105	120
Control	351.3[a]	423.5[a]	512.2[a]	289.8[a]	109.3[a]
Pre-flowering stage (A_1)					
80 mM NaCl	277.23 c	313.5 d	362.1 c	192.5 c	70.1 b
160 mM NaCl	202.6 d	225.9 e	231.4 d	116.7 d	37.1 c
5 mM $CaCl_2$	376.5 b	452.9 b	563.1 b	325.4 b	125.1 a
10 mM $CaCl_2$	412.8 a	499.9 a	628.5 a	362.9 a	139.6 a
80 mM NaCl + 10 mM $CaCl_2$	310.5 c	371.5 c	421.1 c	222.8 c	81.5 b
160 mM NaCl + 10 mM $CaCl_2$	226.8 d	260.2 e	271.8 d	140.3 d	44.7 c
Flowering stage (A_2)					
80 mM NaCl			377.9 d	201.5 d	74.1 c
160 mM NaCl			272.4 f	129.4 f	42.1 d
5 mM $CaCl_2$			541.6 b	318.5	125.1 a
10 mM $CaCl_2$			587.3 a	344.5 a	136.4 a
80 mM NaCl + 10 mM $CaCl_2$			433.9 c	231.1 c	85.9 b
160 mM NaCl + 10 mM $CaCl_2$			323.5 e	155.4 e	50.3 d
Post-flowering stage (A_3)					
80 mM NaCl				198.1 d	68.2 d
160 mM NaCl				133.8 f	34.1 f
5 mM $CaCl_2$				315.7 b	122.5 b
10 mM $CaCl_2$				339.6 a	130.9 a
80 mM NaCl + 10 mM $CaCl_2$				228.1 c	76.8 c
160 mM NaCl + 10 mM $CaCl_2$				153.6 e	41.7 e
			F-Value		
ANOVA					
Stage			4.88*	0.41 ns	8.44*
Salt stress			854.5**	394.9**	426.84**
Stage × salt stress			13.41*	1.09 ns	0.61 ns

ns, **, *, indicate non-significance or significance at $P \leq 0.01$ or 0.05, respectively. Values followed by a letter within a column for each stage of development were not significantly different using LSD at $P \leq 0.05$.
[a] These values were not different from the control using Dunnett's test at the 5% level.

treatment (NaCl + $CaCl_2$) given at the three stages, decreased the pod dry weight by 29–63%, 24–60% and 25–57%, respectively. This decrease was less than one caused by NaCl individually (Table 1).

3.2. Total leaf area

Leaf area increased gradually with age of the plant till 90 DAS. The extent of increase was, however, significantly low in NaCl-treated plants, the difference from the control showing a positive correlation with salt concentration. NaCl treatment given at post-flowering stage was most inhibitive at each corresponding concentration. The effect was maximum in the last sampling (at 120 DAS) with each single as well as combined treatment applied at any stage of plant development. The decline in leaf area due to three NaCl treatments was 36–66%, 32–62% and 38–69%, respectively. Application of $CaCl_2$ at three successive stages increased the leaf area by 14–28%, 14–25% and 12–20%, respectively, compared with the control. This effect was most pronounced at 10 mM $CaCl_2$ treatment. Combined treatment (NaCl + $CaCl_2$) caused a reduction of 25–59%, 21–54% and 30–62%, respectively. The reduction was markedly less than one caused by NaCl alone (Table 2).

3.3. Photosynthetic efficiency and stomatal conductance

The P_N declined with treatments of NaCl alone and NaCl + $CaCl_2$. Though NaCl treatments inhibited g_s and P_N at each stage of plant growth, greater damage was caused by treatments given at post-flowering stage (90 DAS) than those given at pre-flowering (45 DAS) and flowering (75 DAS) stages. The maximum decline was observed at 120 DAS with high concentrations of NaCl (160 mM) and NaCl + $CaCl_2$ (160 + 10 mM) applied at any stage of plant growth. Percent decline was 22–45%, 24–50% and 31–55% in P_N (Table 3) and 22–43%, 16–35% and 30–49% in g_s (Table 4) against NaCl

Table 3
Changes in photosynthetic rate (μmol CO_2 m^2 s^{-1}) in the leaves of *C. angustifolia* affected by NaCl, $CaCl_2$ and NaCl + $CaCl_2$ stress given at pre-flowering, flowering and post-flowering stages

Treatments	Days after sowing (DAS)				
	60	75	90	105	120
Control	13.42[a]	16.82[a]	19.17[a]	13.65[a]	10.20[a]
Pre-flowering stage (A_1)					
80 mM NaCl	11.40 bc	13.99 b	16.75 b	10.85 b	7.95 c
160 mM NaCl	8.91 d	11.61 c	12.14 c	7.97 c	5.84 e
5 mM $CaCl_2$	13.83 a	17.33 a	19.55 a	14.11 a	10.39 a
10 mM $CaCl_2$	13.97 a	17.58 a	19.86 a	14.45 a	10.56 a
80 mM NaCl + 10 mM $CaCl_2$	11.82 ab	14.16 b	17.48 b	10.93 b	8.23 b
160 mM NaCl + 10 mM $CaCl_2$	9.19 cd	11.89 c	12.51 c	8.21 c	6.69 d
Flowering stage (A_2)					
80 mM NaCl			16.12 c	10.66 c	7.80 c
160 mM NaCl			12.59 f	7.93 f	5.07 e
5 mM $CaCl_2$			19.61 b	14.13 b	10.41 b
10 mM $CaCl_2$			20.10 a	14.59 a	10.67 a
80 mM NaCl + 10 mM $CaCl_2$			14.82 d	10.24 d	7.63 c
160 mM NaCl + 10 mM $CaCl_2$			13.10 e	8.36 c	5.32 d
Post-flowering stage (A_3)					
80 mM NaCl				9.94 b	7.06 b
160 mM NaCl				7.42 b	4.56 c
5 mM $CaCl_2$				14.12 a	10.40 a
10 mM $CaCl_2$				14.32 a	10.64 a
80 mM NaCl + 10 mM $CaCl_2$				10.01 b	7.36 b
160 mM NaCl + 10 mM $CaCl_2$				7.60 b	4.82 c
			F-Value		
ANOVA					
Stage			1.05 ns	0.99 ns	7.14*
Salt stress			77.13**	59.85**	103.07**
Stage × salt stress			2.46*	0.14 ns	1.27 ns

ns, **, *, indicate non-significance or significance at $P \leq 0.01$ or 0.05, respectively. Values followed by a letter within a column for each stage of development were not significantly different using LSD at $P \leq 0.05$.
[a] These values were not different from the control using Dunnett's test at the 5% level.

Table 4
Changes in stomatal conductance (mol m^2 s^{-1}) in the leaves of *C. angustifolia* affected by NaCl, $CaCl_2$ and NaCl + $CaCl_2$ stress given at pre-flowering, flowering and post-flowering stages

Treatments	Days after sowing (DAS)				
	60	75	90	105	120
Control	0.62[a]	0.69[a]	0.77[a]	0.52[a]	0.37[a]
Pre-flowering stage (A_1)					
80 mM NaCl	0.51 b	0.55 b	0.65 b	0.41 b	0.29 b
160 mM NaCl	0.42 c	0.41 c	0.54 c	0.33 c	0.21 c
5 mM $CaCl_2$	0.63 a	0.71 a	0.79 a	0.54 a	0.39 a
10 mM $CaCl_2$	0.66 a	0.73 a	0.81 a	0.55 a	0.40 a
80 mM NaCl + 10 mM $CaCl_2$	0.53 b	0.57 b	0.67 b	0.43 b	0.30 b
160 mM NaCl + 10 mM $CaCl_2$	0.45 c	0.44 c	0.58 c	0.35 c	0.23 c
Flowering stage (A_2)					
80 mM NaCl			0.64 c	0.43 c	0.31 b
160 mM NaCl			0.57 d	0.38 d	0.24 c
5 mM $CaCl_2$			0.80 a	0.53 a	0.38 a
10 mM $CaCl_2$			0.82 a	0.56 a	0.39 a
80 mM NaCl + 10 mM $CaCl_2$			0.68 b	0.46 b	0.32 b
160 mM NaCl + 10 mM $CaCl_2$			0.60 cd	0.40 cd	0.26 c
Post-flowering stage (A_3)					
80 mM NaCl				0.41 b	0.26 bc
160 mM NaCl				0.29 c	0.19 d
5 mM $CaCl_2$				0.53 a	0.38 a
10 mM $CaCl_2$				0.54 a	0.40 a
80 mM NaCl + 10 mM $CaCl_2$				0.43 b	0.28 b
160 mM NaCl + 10 mM $CaCl_2$				0.32 c	0.22 cd
			F-Value		
ANOVA					
Stage			0.96 ns	12.44*	6.40*
Salt stress			117.5**	132.3**	92.25**
Stage × salt stress			0.98 ns	2.14*	1.16 ns

ns, **, *, indicate non-significance or significance at $P \leq 0.01$ or 0.05, respectively. Values followed by a letter within a column for each stage of development were not significantly different using LSD at $P \leq 0.05$.
[a] These values were not different from the control using Dunnett's test at the 5% level.

applied at the three stages, respectively. The NaCl + $CaCl_2$ treatments given at these stages, caused 19–44%, 23–48% and 28–53% decrease in the P_N, and 18–38%, 14–30% and 24–40% in g_s, with respect to the control. However, $CaCl_2$ treatments given at three times increased P_N by 2–4%, 2–5% and 2–5% and g_s by 5–8%, 3–4% and 4–8%, respectively (Tables 3 and 4).

3.4. Sennoside concentration in different plant parts

The maximum sennoside concentration was recorded in pods (46.59 mg/g) followed by those in immature leaves (33.96 mg/g) and mature leaves (22.53 mg/g). Amounts of *a*, *b* and *c* types of sennoside decreased at high concentrations of NaCl applied alone or in combination of $CaCl_2$. Nonetheless, $CaCl_2$ treatments had stimulatory effects (Table 5).

3.4.1. Immature leaves

Concentrations of sennosides *a*, *b* and *c* in young leaves decreased to the tune of 7–27%, 13–35% and 16–40% under the influence of NaCl stress applied at pre-flowering stage. Treatments at flowering stage also caused similar depressive effects. In contrast, $CaCl_2$ treatment given at pre-flowering stage, increased the concentration of sennosides *a*, *b* and *c* up to 11–16%, 5–8% and 7–9%. Treatments given at flowering stage increased the concentration of sennosides by 8–13%, 4–7% and 4–7%, respectively, with respect to the control. The NaCl + $CaCl_2$ treatments given at pre-flowering stage and flowering stage also caused a decline in sennoside concentrations, compared with the control, but the extent of decline was less than one caused by NaCl alone. The pre-flowering treatment was more effective than flowering time treatment (Table 5).

Table 5
Changes in concentration of sennosides *a*, *b*, and *c* (mg g^{-1} dw) in different plant parts of *C. angustifolia* affected by application of NaCl, $CaCl_2$ and NaCl + $CaCl_2$

Treatment	Young leaves			Mature leaves			Pods		
	Sa	Sb	Sc	Sa	Sb	Sc	Sa	Sb	Sc
Control	25.95[a]	7.23[a]	0.57[a]	18.20[a]	3.96[a]	0.22[a]	33.71[a]	11.82[a]	0.73[a]
Pre-flowering stage									
80 mM NaCl	24.10 b	6.31 c	0.48 b	14.77 bc	3.12 bc	0.17 dc	30.21 c	10.13 d	0.62 b
160 mM NaCl	18.83 c	4.71 e	0.34 c	12.05 c	2.44 d	0.12 d	25.71 d	8.73 f	0.55 c
5 mM $CaCl_2$	28.88 a	7.58 b	0.61 a	19.53 a	4.11 a	0.23 a	35.69 b	12.51 b	0.75 a
10 mM $CaCl_2$	30.03 a	7.81 a	0.62 a	20.11 a	4.36 a	0.24 a	37.59 a	13.27 a	0.78 a
80 mM NaCl + 10 mM $CaCl_2$	24.77 b	6.55 c	0.48 b	16.01 b	3.36 b	0.18 b	31.22 c	10.56 c	0.64 b
160 mM NaCl + 10 mM $CaCl_2$	20.55 c	5.12 d	0.37 c	13.44 bc	2.70 cd	0.14 cd	27.88 d	9.56 e	0.57 c
Flowering stage									
80 mM NaCl	23.66 b	6.48 b	0.47 b	14.81 c	3.22 ab	0.17 a	30.45 b	10.33 c	0.64 a
160 mM NaCl	20.44 c	4.93 c	0.33 d	12.34 e	2.54 ab	0.13 b	26.35 c	8.94 e	0.54 b
5 mM $CaCl_2$	28.10 a	7.50 d	0.59 e	19.22 a	4.05 b	0.23 c	35.13 d	12.12 f	0.75 c
10 mM $CaCl_2$	29.34 a	7.70 a	0.61 ab	19.86 a	4.25 ab	0.24 a	37.15 b	12.77 b	0.76 a
80 mM NaCl + 10 mM $CaCl_2$	24.95 b	7.02 a	0.52 a	16.23 b	3.41 a	0.21 a	31.33 a	10.69 a	0.66 a
160 mM NaCl + 10 mM $CaCl_2$	21.43 c	5.20 b	0.39 c	13.59 d	2.72 ab	0.15 a	27.92 c	9.66 d	0.59 b
Sa, sennoside *a*; Sb, sennoside *b*; Sc, sennoside *c*									
	F-Value								
ANOVA									
Stage	0.07 ns	95.36**	17.24**	0.00 ns	0.94 ns	9.72*	0.41 ns	0.04 ns	0.92 ns
Salt stress	47.64**	187.83**	68.90**	19.76**	3.80*	16.76**	120.04**	43.43**	107.78**
Stage × salt stress	0.68 ns	221.17**	77.18**	0.03 ns	3.24*	15.49**	0.20 ns	0.36 ns	1.62 ns

Observations were made at 90 DAS (i.e. after pre-flowering and flowering stage treatments). ns, **, *, indicate non-significance or significance at the $P \leq 0.01$ or 0.05, respectively. Values followed by a letter within a column for each stage of development were not significantly different using LSD at $P \leq 0.05$.
[a] Values followed by an were not different from the control using Dunnett's test at the 5% level.

3.4.2. Mature leaves

NaCl treatments reduced the sennoside concentration significantly in mature leaves. Concentrations of sennosides, *b* and *c* were reduced by 19–34%, 21–38% and 23–45%, respectively, against pre-flowering NaCl treatments, and by 19–32%, 19–36% and 23–41% with the flowering-stage treatments. On the contrary, $CaCl_2$ enhanced the sennoside contents, by 7–10%, 4–10% and 5–9% due to pre-flowering treatment and by 6–9%, 2–7% and 5–9% due to flowering-stage treatment, respectively. Combination treatments caused less reductions than NaCl treatments. The effect was maximum on sennoside *c* and pre-flowering treatment was more effective than the flowering stage treatment (Table 5).

3.4.3. Dry pods

The concentration of sennosides *a*, *b* and *c* declined by 10–24%, 14–26% and 15–25% under the influence of pre-flowering treatments of NaCl. Treatments at flowering stage reduced these sennosides by 10–22%, 13–24% and 12–26%, respectively. On the other hand, $CaCl_2$ treatments showed ameliorative effects. Concentration of sennosides *a*, *b* and *c* increased up to 6–11%, 6–12% and 3–7% with pre-flowering treatments and 4–10%, 3–8% and 3–4%, respectively, with flowering-stage treatments. The combined effect of salts (NaCl + $CaCl_2$) was depressive, but not as much as that of NaCl alone. The pre-flowering treatments of NaCl + $CaCl_2$ reduced the concentrations of sennosides *a*, *b* and *c* to the tune of 7–17%, 11–12% and 12–28%, whereas flowering-stage treatment reduced them by 7–17%, 10–18% and 10–19%, respectively, compared to the control. The effect of treatments given at pre-flowering and the flowering stages was almost comparable. Sennoside *c* was most sensitive (Table 5).

3.5. Yield attributes

3.5.1. Sennoside concentration

The sennoside yield per plant got reduced with each treatment of NaCl as well as of NaCl + $CaCl_2$ given at pre-flowering and flowering stages, but the decline caused by combination treatments was less (i.e. up to 56%) than one caused by NaCl (160 mM) treatments (i.e. up to 64%). On the contrary, sennoside yield increased by 27% with 10 mM $CaCl_2$ treatments. Of these, pre-flowering treatments were more effective than those given at flowering stage (Fig. 1).

4. Discussion

4.1. Plant growth

Plant biomass is inhibited by excess of solute taken up by plants from saline growth media (Munns et al., 2000). Salts may exert detrimental effects on plant growth through toxicity of one or more specific ions (Alam, 1999). NaCl application significantly reduces the total leaf area per plant, thereby causing a net reduction in photosynthate accumulation which consequently reduces plant growth. The reduction in the leaf area might suggest a role of NaCl in accelerating the leaf senescence. Effect of salinity on peach tree leaves included accumulation of Cl^-, leaf burning and a retarted leaf growth

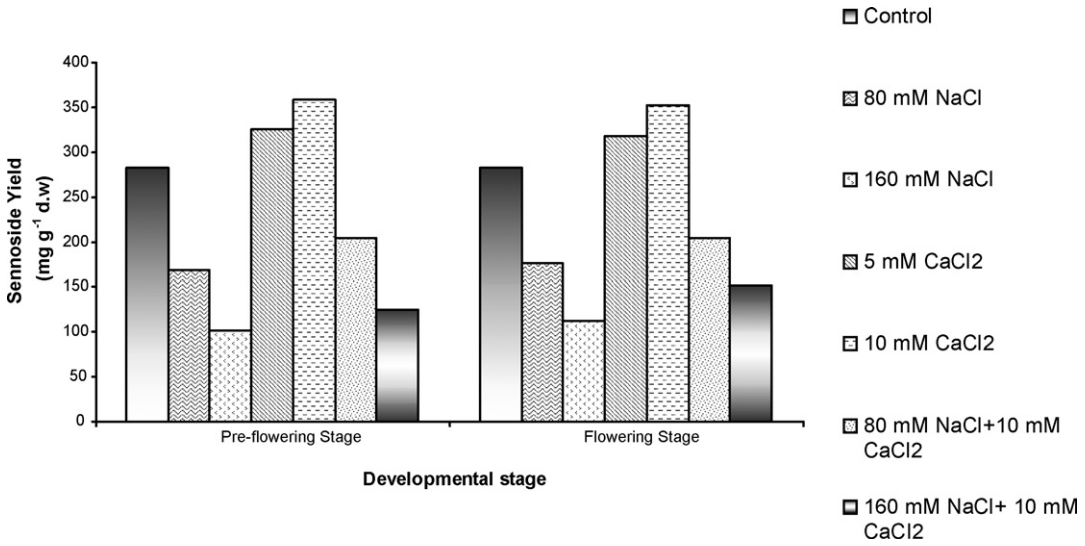

Fig. 1. Changes in sennoside yield (mg/plant dw) of *C. angustifolia* as a result of NaCl, $CaCl_2$ and NaCl + $CaCl_2$ treatments given at pre-flowering and flowering stages of plant development.

(Alam, 1999). Leaf area as well as leaf abundance is reduced under saline conditions (Pessarakli, 1999; Arnada et al., 1998). Calcium ions are well known to have a regulatory role in cell metabolism, and sodium ions may compete with calcium ions for membrane-binding sites. It has been hypothesized that a high calcium level can protect cell membrane from adverse effects of salinity (Cramer et al., 1986; Zidan et al., 1996). $CaCl_2$ promoted leaf growth and minimized adverse effect of NaCl in the present study.

4.2. Photosynthetic efficiency and stomatal conductance

P_N and g_s decreased against NaCl and NaCl + $CaCl_2$ stress in Senna plant. The lowering of P_N might be due to a possible relationship between photosynthetic rate and leaf Cl^- and/or Na^+ concentration (Gibberd et al., 2000). In the salt-affected plants, closing of stomata and a decrease in quantity and/or activity of Rubisco may cause a decline in P_N (Arshi et al., 2004, 2006; Heuer and Plaut, 1989).

In the present investigation, $CaCl_2$ slightly increased the P_N and g_s of *C. angustifolia* plants growing under saline conditions. By promoting water-holding capacity, physio-chemical activities, and stability and integrity of cytoplasmic membranes, calcium treatment has improved drought resistance in cotton plants (Chang et al., 1997). Ca^{2+} showed ameliorative effects, through reduced leaf abscission in citrus plants grown under salinity (Arnada et al., 1998).

4.3. Sennoside concentration

Sennosides *a* and *b* are stereoisomers, the former a dextrorotatory and the latter a meso form, both being glycosides of rhein-dianthrons and glucose. Sennoside *c* is a glycoside of heterodianthrons. Our observation that pods have the maximum sennoside content, followed by immature leaves and then mature leaves, substantiates some earlier studies (Gupta et al., 1977). The sennoside contents of leaves and pods decreased with each NaCl treatment. $CaCl_2$ caused stimulatory effect. Lohar et al. (1979) observed a decline in the leaf sennoside content with the onset of reproductive growth phase in *C. angustifolia* and *C. acutifolia*. It also declined during the rainy season, reportedly due to the leaching effect of rains. The newly sprouted leaves of the post-monsoon period were rich in sennoside content which again declined on maturation of leaves. The sennoside content of pods declined with growing age in both these species. The 2-day-old and 20-day-old pods contained the maximum and minimum amounts of sennoside, respectively (Sharma et al., 1982). A similar variation with season and age was exhibited by sennoside content of leaves in *C. fistula* (Cano et al., 1990). The highest percentage was detected on emergence of new leaves after the rainy season. In pods, the content was maximum at mid stage of fruit maturation. Older trees had a lower sennoside content than the younger ones (Cano et al., 1990).

The reduction in the sennoside contents could be due to the interference of Na^+ and Cl^- ions with enzymes associated with the sennoside biosynthesis pathway. The decreased photosynthetic rate due to salinity stress could also account for the decline in sennoside concentration.

An increase in the sennoside contents in different plant parts against $CaCl_2$ treatments indicates a diversion of primary metabolites to the synthesis of secondary metabolites (Bilia et al., 1992). Plants growing in the saline environment suffer injury due to osmotic stress, specific ion toxicity and ion imbalances. Osmotic stress results from lowering of soil water potential as the salt content of the soil leads to a water deficit or a state of dehydration in plants. The amounts of essential oils and alkaloids are enhanced under water deficit condition (Hoft et al., 1996; Saenz et al., 1993).

4.4. Yield attributes

The sennoside yield was reduced maximally (by 64% and 60%) with increasing NaCl levels and increased (by 27% and

25%) against $CaCl_2$ treatments; the reduction got minimized (by 56% and 46%) with the combined treatment. Sennoside yield depends on both economic yield and sennoside concentration. The decline in the alkaloidal output could be due to the reduced economic yield, which in turn is related to the reduced photosynthetic rate.

In conclusion, NaCl hampers growth parameters at each concentration. Calcium resists the stressful condition caused by salinity and minimizes its deleterious effects on plant performance. Normally, sennoside concentration was affected maximally by flowering treatments and sennoside *c* looks most sensitive to NaCl stress. Calcium treatment must therefore help in reclamation of the saline soil.

Acknowledgements

The authors thank their laboratory colleagues and Mr. Zakir A. Siddiqui for help during this study. The first author also thanks the CSIR, New Delhi, for granting her a Research Associateship.

References

Alam, S.M., 1999. Nutrient uptake by plants under stress conditions. In: Pessarakli, M. (Ed.), Handbook of Plant and Crop Stress. Marcel Dekker Inc., New York.

Arnada, B., Moya, J.L., Tadeo, F.R., Legaz, F., Prinomillo, E., 1998. Physiological and anatomical disturbances induced by chloride salts in sensitive and tolerant citrus: beneficial and detrimental effects of cations. Plant Cell Environ. 21, 1243–1253.

Arshi, A., Abdin, M.Z., Iqbal, M., 2002. Growth and metabolism of Senna as affected by salt stress. Biol. Plant. 45, 295–298.

Arshi, A., Abdin, M.Z., Iqbal, M., 2004. Changes in biochemical status and growth performace of Senna (*Cassia angustifolia* Vahl.) grown under salt stress. Phytomorphology 54, 109–124.

Arshi, A., Abdin, M.Z., Iqbal, M., 2005. Ameliorative effect of $CaCl_2$ on growth, ionic relations and proline content of Senna under salinity stress. J. Plant Nutr. 28, 101–125.

Arshi, A., Abdin, M.Z., Iqbal, M., 2006. Effect of $CaCl_2$ on growth performance, photosynthetic efficiency and nitrogen assimilation of *Cichorium intybus* L. grown under NaCl stress. Acta Physiol. Plant 28, 137–147.

Bilia, A.R., Cioni, P., Morelli, I., Coppi, A., Lippi, A., Tomei, P.E., 1992. Essential oil of *Satureja montana* L. composition and yield of plants grown under different environmental conditions. J. Essent. Oil Res. 4, 563–568.

Brugnoli, E., Lauteri, M., 1991. Effects of salinity on stomatal conductance, photosynthetic capacity and carbon isotope discrimination of salt-tolerant (*Gossypium hirsutum* L.) and salt sensitive (*Phaseolus vulgaris* L.) C_3 non-halophytes. Plant Physiol. 95, 628–635.

Brugnoli, E., Bjorkman, O., 1992. Growth of cotton under continuous salinity stress: influence of allocation pattern: stomatal and non-stomatal components of photosynthesis and dissipation of excess light energy. Planta 187, 335–347.

Cano, L.M., Odilia, A., Hernandez, H., Sanchez, J.R., 1990. Seasonal variations in the content of sennosides in leaves and pods of two *Cassia fistula* populations. Phytochem. 29, 3095–3099.

Chang, S.J., Doubler, C., Kilo, T.V., Suttner, R., Klein, J., Schmidt, M.E., Gibson, P.T., Lignot, D.A., 1997. Association of loci underlying field resistance to soybean sudden death syndrome (SDS) and cyst nematode (SCN) race 3. Crop Sci. 37, 965–971.

Cramer, G.R., Lauchli, A., Epstein, E., 1986. Effect of NaCl and $CaCl_2$ on ion activities in complex nutrient solution and root growth of cotton. Plant Physiol. 8, 792–797.

Delfine, S., Alvino, A., Zacchini, M., Lorets, F., 1998. Consequences of salt stress on conductance to CO_2 diffusion, rubisco characteristics and anatomy of spinach leaves. J. Plant Physiol. 25, 395–402.

Gibberd, G.R., Turner, M.C., Loveys, B.R., 2000. High vapour pressure deficit in a rapid decline of leaf water potential and photosynthesis of carrot grown on free-draining sandy soils. Aust. J. Agric. Res. 51, 839–847.

Gomez, J.M., Hernandez, J.A., Jimez, A.L., Del Rio, A., Sevilla, F., 1999. Differential response of antioxidant system of chloroplasts and mitochondria to long-term NaCl stress of pea plant. Free Rad. Res. 3, 11–18.

Greenway, H., Munns, R., 1980. Mechanism of salt tolerance in non-halophytes. Annu. Rev. Plant Physiol. 31, 149–190.

Gupta, R., Modi, J.N., Mehta, K.G., 1977. Studies on cultivation of Senna (*Cassia angustifolia* Vahl.) in north Gujarat. South Indian Hort. 25, 26–29.

Hanson, J.B., 1984. The function of calcium in plant nutrition. In: Tinker, P.B., Lauchli, A. (Eds.), Advances in Plant Nutrition, vol. 1. Praegue Publ., New York, pp. 159–208.

Helper, P.K., Wayne, R.O., 1995. Calcium and plant development. Annu. Rev. Plant Physiol. 36, 397–439.

Hernandez, J.A., Campillo, A., Jimez, A., Alarcon, J.J., Sevilla, F., 1999. Response of antioxidant systems and leaf water relations top NaCl stress in pea plant. New Phytol. 141, 241–251.

Heuer, B., Plaut, Z., 1989. Photosynthesis and osmotic adjustment of two sugarbeet cultivars grown under saline condition. J. Exp. Bot. 40, 437–440.

Hoft, M., Verpoorte, R., Beck, E., 1996. Growth and alkaloid contents in leaves of *Tabernaemontana* as influenced by light intensity, water stress and nutrient supply. Oecologia 107, 160–169.

Kent, L.M., Lauchli, A., 1985. Germination and seedling growth of cotton: salinity–calcium interaction. Plant Cell Environ. 8, 155–159.

Kirkby, E.A., Pilbeam, D.J., 1984. Calcium as a plant nutrient. Plant Cell Environ. 7, 397–405.

LaHaye, P.A., Epstein, E., 1969. Salt tolerance by plants: enhancement with calcium. Science 169, 395–396.

Leopold, A.C., Willing, R.P., 1984. Evidence for toxicity effects of salt on membranes. In: Tousivessen, R.C., Stales, G.H. (Eds.), Salinity Tolerance in Plants. Wiley, New York, pp. 67–76.

Lemmli, J., Verhaeren, E., Cuveele, J., 1985. Research on drugs containing anthraquinone principles. A note on alcoholic washing of Senna leaflets. Plant Med. Phytotherapie 19, 57–61.

Lohar, D.R., Bhatia, P.K., Gard, S.P., Chawan, D.D., 1979. Seasonal variation in the content of sennoside in Senna leaves. Pharm. World Sci. 1 (1), 206–208.

Munns, R., Guo, J., Passioura, J.B., Cramer, G.R., 2000. Leaf water status controls day-time but not daily rates of leaf expansion in salt-treated barley. Aust. J. Plant Physiol. 27, 949–957.

Ouerghi, A., Cornie, G., Roudani, M., Ayadi, A., Brulfert, J., 2000. Effect of NaCl on photosynthesis of two wheat species (*Triticum durum* and *Triticum aestivum*) differing in their sensitivity to salt stress. J. Plant Physiol. 15, 519–527.

Papp, J.C., Ball, M.C., Terry, N.A., 1983. Comparative study of the effect of NaCl salinity on respiration, photosynthesis and leaf extension growth in *Beta vulgaris* L. (Sugarbeet). Plant Cell Environ. 6, 675–677.

Pessarakli, M., 1999. In: Pessarakli, M. (Ed.), Handbook of Plant and Crop Stress. 2nd ed. Marcel Dekker Inc., New York.

Saenz, L., Santamaria, J.M., Villanueva, M.A., Loyala-Vargas, V.M., Oropeza, C., 1993. Changes in alkaloids contents of *Catharanthus roseus* L. (Don) as a result of water stress and treatment with abscisic acid. J. Plant Physiol. 142, 244–247.

Sharma, H.K., Daiya, K.S., Chawan, D.D., 1982. Sennosides and carbohydrates in developing pods of *Cassia* species. Pharma. World Sci. 4 (3), 83–84.

Srivastava, V.K., Maheshwari, M.L., Mandal, S., 1983a. A rapid HPLC method for analysis of sennosides in Senna. Indian J. Pharm. Sci. 45, 230–231.

Srivastava, V.K., Maheshwari, M.L., Mandal, S., 1983b. Investigation of chemical assay of sennoside in Senna (*Cassia angustifolia* Vahl.). Int. J. Trop. Agric. 1, 231–238.

Zhu, J.K., 2001. Plant salt tolerance. Trends Plant Sci. 6, 66–71.

Zidan, I., Azaizeh, H., Neumann, P.N., 1996. Does salinity reduce growth in maize root epidermal cells by inhibiting their capacity for cell wall acidification? Plant Physiol. 93, 7–11.