BIOLOGIA PLANTARUM 45 (2): 295-298, 2002

BRIEF COMMUNICATION

Growth and metabolism of senna as affected by salt stress

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Abstract

Pot culture experiments were conducted using different NaCl concentrations to assess their impact on the growth and metabolic changes in senna (*Cassia angustifolia* Vahl.). Five treatments (0, 40, 80, 120, and 160 mM NaCl) were given to the plants at three phenological stages, *i.e.* at pre-flowering, (45 days after sowing, DAS); flowering (75 DAS) and post-flowering (90 DAS) stages. A significant reduction in the biomass and length of the roots and shoots, photosynthetic rate, stomatal conductance, the total chlorophyll content, protein content, nitrate reductase activity, and reduced nitrogen content of the leaves was observed at each phenological stage with each salt concentration applied. Contrary to this, proline and nitrate contents of the leaves increased markedly. The post-flowering stage was most sensitive to NaCl treatment.

Additional key words: *Cassia angustifolia*, chlorophyll, NaCl, nitrate reductase activity, photosynthetic rate, proline, protein.

Salinity may affect various metabolic processes such as photosynthesis, protein synthesis, respiration, nitrogen assimilation and phytohormone turnover. It restricts the synthesis of cytokinins in roots and its transport to shoots, but promotes the synthesis of abscisic acid (ABA) (Mizrahi *et al.* 1970). Accumulation of organic solutes is an important process in plants undergoing salt adaptation. Compatible solutes enhance the intercellular osmotic potential and protect macromolecules against the deleterious effects of low water potential and high ion concentrations. The plants thus accumulate compatible solutes to adjust the osmotic potential. The present study investigates under field conditions the effect of NaCl stress on metabolic changes of senna, a leguminous plant with established medicinal utility.

Seeds of *Cassia angustifolia* Vahl. (*Caesalpiniaceae*) were procured from Gujarat Agricultural University, Anand, Gujarat. Pot culture experiments were conducted in the Hamdard University campus to determine the effect of salt stress on the growth and metabolic changes in senna at various phenological stages. Each pot contained 11 kg of sandy loam soil with pH 7.2 and electrical conductivity (EC) 0.21 mS cm^{-1}. Five concentrations of NaCl, *i.e.* 0 mM (T0), 40 mM (T1), 80 mM (T2), 120 mM (T3) and 160 mM (T4) were applied to the growing plants. The EC of these salt concentrations increased from 0.21 (T0) to 4.8, 8.5, 12.2, and 16.1 mS cm^{-1} in T1 - T4, respectively. The treatments were given to plants separately at three phenological stages, *i.e.* at pre-flowering (A1: 45 DAS), flowering (A2: 75 DAS) and post-flowering (A3: 90 DAS) stages, by adding 500 cm^3 of NaCl solutions to the soil. The control as well as NaCl-treated plants were watered uniformly. Care was taken to avoid drainage of the solution or water. The growth and physiological parameters were studied for each treatment at 15-d intervals until harvest. At every sampling, five plants were taken from each treatment and separated into leaves, stems and roots. The lengths of root and shoot were measured, and the plant materials were dried at 65 °C to analyse the dry matter production. Net

Received 6 February 2001, *accepted* 26 June 2001.

Abbreviations: ABA - abscisic acid; Chl - chlorophyll; DAS - days after sowing; EC - electrical conductivity; g_s - stomatal conductance; P_N - net photosynthetic rate.

Acknowledgements: Thanks are due to our laboratory colleagues for their unceasing help throughout this study. The first author is thankful to the Hamdard National Foundation, New Delhi for granting a research fellowship.

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photosynthetic rate (P_N) and stomatal conductance (g_s) of green leaves were recorded with a portable *LI-COR 6200* Photosynthesis System (Lincoln, USA) at temperature 37.9 °C, relative humidity 46.9 %, ambient CO_2 concentration 300 - 325 μmol mol^{-1}, and irradiance 1033 μmol m^{-2} s^{-1}. The total chlorophyll (Chl) content was estimated in the leaves according to the method of Hiscox and Israelstam (1979). The total soluble protein in the leaves was estimated according to the procedure of Bradford (1976), after precipitating the protein with 10 % trichloroacetic acid (TCA) solution, using bovine serum albumin as standard. The *in vivo* assay of nitrate reductase in leaves was carried out using the method of Kleeper *et al.* (1971) with a slight modification (Nair and Abrol 1973). The nitrate concentration of the leaves was estimated by the method of Grover *et al.* (1978). The reduced nitrogen in dried leaves was analysed using the Kjeldahl procedure (Linder 1944). Proline content of the leaves was estimated following the method of Bates *et al.* (1973). Fresh material (500 mg) was homogenised in 10 cm^3 of 3 % aqueous sulphosalicylic acid. The homogenate was centrifuged at 8832 *g* for 15 min. 2 cm^3 of aliquot of the supernatant was mixed with an equal volume of acetic acid and acid ninhydrin and incubated for 1 h at 100 °C. The reaction was terminated in ice, and 4 cm^3 of toluene was added. The chromatophore-containing toluene was then aspirated from the aqueous phase, and its absorbance determined at 520 nm with the help of a *Beckman DU 6408* Spectrophotometer (Fullerton, USA), using toluene for a blank.

Analysis of samples collected at harvest time (120 DAS), showed that the lengths and biomass of roots and shoots significantly decreased with increasing NaCl concentration. With 160 mM NaCl applied to the plants separately at pre-flowering, flowering and post-flowering stages, the root length was reduced by 30, 34, and 37 %, shoot length by 33, 39, and 40 %, the root biomass by 50, 54, and 55 % and shoot biomass by 53, 56, and 58 %, respectively, at the time of harvest as compared to the control (Fig. 1).

P_N and g_s of the leaves decreased in the treated plants compared with controls. At harvest time, P_N declined by about 45, 50, and 55 %, whereas the g_s by 43, 40, and 49 %, with 160 mM NaCl applied at the three phenological stages (Fig. 1). The Chl content declined by 39, 47, and 52 % with application of 160 mM NaCl solution (Table 1). The inhibition of photosynthesis in plants involves an interactive effect of decreased g_s, intercellular CO_2 concentration, content of photosynthetic pigments, and inactivation of photosynthetic enzymes including Rubisco. Sodium probably had a higher incidence than chloride in the decrease of P_N (Behboudian *et al.* 1986). Compared with controls, P_N increased significantly in alfalfa genotypes Anand-2, T-9 and IL-112, but remained unaltered in Anand-2 at a low salinity. Reduction in P_N at higher salinity was primarily due to the reduction of g_s (Anand *et al.* 2000) leading to a reduction in CO_2 uptake and hence, the decline in P_N. A decline in the Chl content is also correlated to the indirect effect of NaCl on the content of essential nutrients. Ouzounidou *et al.* (1997) have observed a decline in Mg^{2+}, Ca^{2+}, K^+ and Fe^{2+} in the above-ground parts of wheat plant.

Compared with the control, the soluble protein content declined at each stage with all the treatments. The decline at the harvest time was about 32, 38, and 43 % with 160 mM NaCl applied at the three growth stages, respectively (Table 1). Under salinity, a low water potential enhances the synthesis of ABA (Mizrahi *et al.* 1970), which plays

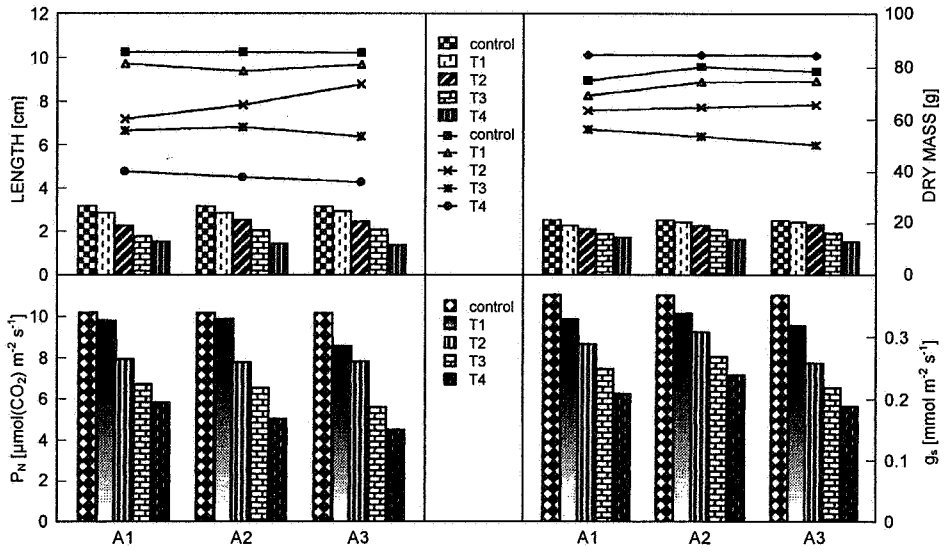

Fig. 1. Changes in the lengths of roots and shoots (*bar diagrams*), dry masses of roots and shoots (*line diagrams*), leaf photosynthetic rate, and leaf stomatal conductance of senna plant treated with NaCl (T1, T2, T3 and T4) given at pre-flowering (Al), flowering (A2), and post-flowering (A3) stages; and analysed at harvest time (120 DAS).

Table 1. Effect of NaCl treatments (T1, T2, T3, and T4) given at three phenological stages (A1, A2, A3) on chlorophyll content, NR activity, and contents of nitrate, reduced nitrogen, proteins, and proline in the leaves of senna collected at harvest time (120 DAS). Means ± SE, $n = 15$.

Stages/ treatments	Chl content [mg g^{-1}(f.m.)]	NR activity [μmol g^{-1}(f.m.) h^{-1}]	Nitrate [mg g^{-1}(f.m.)]	Reduced N [mg g^{-1}(d.m.)]	Proteins [mg g^{-1}(f.m.)]	Proline [mg g^{-1}(d.m.)]
Control	2.44 ± 0.10	2.13 ± 0.05	2.01 ± 0.17	31.85 ± 0.40	8.93 ± 0.29	119.7 ± 3.35
A1/T1	2.24 ± 0.06	1.97 ± 0.22	2.19 ± 0.09	28.61 ± 0.28	7.80 ± 0.14	144.3 ± 3.56
A1/T2	1.92 ± 0.02	1.69 ± 0.05	2.23 ± 0.13	24.90 ± 0.30	7.12 ± 0.18	242.6 ± 8.67
A1/T3	1.76 ± 0.06	1.44 ± 0.06	2.29 ± 0.10	20.48 ± 0.30	6.88 ± 0.14	422.9 ± 3.74
A1/T4	1.50 ± 0.08	1.25 ± 0.12	2.51 ± 0.15	17.81 ± 0.24	6.05 ± 0.09	583.5 ± 5.64
A2/T1	2.11 ± 0.05	1.95 ± 0.14	2.10 ± 0.09	27.42 ± 0.34	8.36 ± 0.08	223.7 ± 1.30
A2/T2	1.79 ± 0.05	1.61 ± 0.14	2.18 ± 0.09	24.92 ± 1.26	7.73 ± 0.16	431.3 ± 1.67
A2/T3	1.62 ± 0.06	1.33 ± 0.13	2.44 ± 0.12	19.85 ± 0.23	6.38 ± 0.08	556.7 ± 7.49
A2/T4	1.30 ± 0.04	1.17 ± 0.09	2.60 ± 0.15	16.77 ± 0.20	5.57 ± 0.09	635.3 ± 2.46
A3/T1	2.02 ± 0.08	1.96 ± 0.09	2.15 ± 0.10	26.05 ± 0.20	7.07 ± 0.04	225.6 ± 5.17
A3/T2	1.80 ± 0.07	1.61 ± 0.18	2.21 ± 0.09	23.21 ± 0.53	6.76 ± 0.06	429.7 ± 2.21
A3/T3	1.45 ± 0.11	1.21 ± 0.09	2.40 ± 0.08	18.54 ± 0.74	6.17 ± 0.10	541.3 ± 3.81
A3/T4	1.18 ± 0.05	1.01 ± 0.13	2.53 ± 0.16	13.89 ± 0.46	5.13 ± 0.42	661.0 ± 6.26

an essential role in plant-water relations by affecting the solute and water movement in the tissue (Davies and Mansfield I983). ABA also reduces protein synthesis and accelerates protein degradation (Trewaves 1972). Similarly, both osmotic and water stress enhance protein degradation and alter the pattern of protein synthesis (Dungey and Davies 1982, Vartanian *et al.* 1987).

The *in vivo* nitrate reductase (NR) activity and the reduced nitrogen content in the leaves declined with each treatment. At the final analysis, the decline in NR activity was up to 41, 45, and 53 % while in the N-content upto 44, 47, and 56 %, with the 160 mM NaCl used at the pre-flowering, flowering and post-flowering stages, respectively. The maximum reduction appeared with the application at post-flowering stage. The nitrate concentration in the leaves, on the other hand, increased in the treated plants, the extent of increase at the harvest time being 25, 29, and 26 % (Table 1).

Proline accumulation in the leaves was 4.9, 5.3 and 5.5 times higher than in controls at different phenological stages under 160 mM NaCl stress (Table 1). A strong correlation was observed between proline accumulation and the NaCl concentration applied. This may adjust the osmotic potential under salt stress, acting as a naturally occurring compatible solute (Yancey *et al.* 1982). In higher plants proline biosynthesis is generally by the glutamate pathway. Glutamate, the precursor of proline, may be controlled by pyrroline-5-carboxylate synthetase, which is regulated by proline via feed back inhibition (Delauney and Verma 1990). A loss of this feed back regulation has been observed under water stress (Delauney and Verma 1993). In the present study, changes in proline content due to NaCl stress suggest that permeability of membranes might be affected, as has been observed earlier (Ali *et al.* 1998, 1999). There exists a positive correlation between proline accumulation and the degree of plant tolerance to salinity (Lin and Kao 1996; Martinez *et al.* 1996, Mansour 2000).

Each concentration of NaCl affected all the parameters studied in senna, the losses being maximum at the highest concentration used. The post-flowering stage was the most affected stage, compared with pre-flowering and flowering stages. Finally, the tolerance might be linked to proline accumulation, which in turn increased its ability for water absorption under a moderate salinity stress.

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