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RESEARCH ARTICLE

EFFECT OF NaCl STRESS ON GROWTH, YIELD AND SENNOSIDE CONTENT OF MEDICINAL PLANT *Cassia angustifolia* Vahl.

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ABSTRACT

A pot experiment was performed at the experimental research field, Department of Botany, University of Pune, to observe the response of *Cassia angustifolia* under different NaCl stress. The first NaCl (25mM NaCl concentration) treatment was given on 45 days after sowing (DAS) and the subsequent treatments were given in three stages at the interval of 15 days. All growth and yield parameters were measured at three phenological stages. The significance reduction in growth and yield parameters with the increase of salinity levels was observed. However, the content of sennoside content in leaves and pods increased with 25mM NaCl concentration.

INTRODUCTION

Salinity has considerable adverse impacts on productivity of plants. It adversely affects plant growth and development (Razmjoo *et al.*[1]). According to Pitman and Läuchli [2], 20% of cultivated lands are adversely affected by high salt concentration worldwide, which inhibits plant growth and yield. Removing salinity stress is a main issue in these regions to ensure agricultural sustainability. An excess of soluble salts in the soil leads to osmotic stress, specific ion toxicity and ionic imbalances (Munns[3]) and the consequences of these can be plant death or yield losses in both crop species and medicinal plants (Rout & Shaw[4]). Ashraf *et al.*[5] found that increasing salt concentrations caused a significant reduction in the fresh and dry masses of both shoots and roots as well as seed yield of *Ammolei majus*, while reduced plant fresh and dry yield in *Hyoscyamus niger*. Limited water supply is also another major environmental constraint in productivity of crop and medicinal plants. Moisture deficiency induces various physiological and metabolic responses like stomata closure and decline in growth rate and photosynthesis (Flexas and Medrano[6]). The results of Baher *et al.* [7] showed that greater soil water stress decreased plant height and total fresh and dry weight of *Satureja hortensis*. Colom and Vazzana [8] also showed that the number of stem per plant and plant dry weight was negatively related to water stress in *Eragrostis curvula*. *Cassia angustifolia* (Family: *Caesalpiniaceae*), popularly known as senna, is a valuable plant drug in ayurvedic and modern system of medicine for the treatment of constipation and is used to cure a large number of intestinal diseases (Aktar *et al.*[9]). Sennoside A and B are the two anthraquinone glycosides that are responsible for purgative action of senna. The production of sennoside not only depends upon the metabolic state of the source tissues, but also may be integrated with the stress factors. Senna has adaptability to a wide range of climates and soil conditions and its cultivation may be an alternative option in areas with drought and salinity problems. However, the performance of this plant in salinity stress environments, and the effect of these stresses on its sennoside production have not been studied well. The objective of this research was to evaluate the effect of salt stress on growth, yield and sennoside contents of senna.

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MATERIALS AND METHODS

Plant material

Authentic seeds of *Cassia angustifolia* Vahl were obtained from National Research Centre for Medicinal and Aromatic Plants, Boriavi, Gujarat. Pot culture experiments were conducted in the research field of Department of Botany, University of Pune, from October 2008 to May 2009 to determine the effect of NaCl stress on the growth, yield and secondary metabolites content i.e. sennoside a and b in senna at various phonological stages.

Experimental setup

A pot experiment was performed at the experimental research field, Department of Botany, University of Pune, during the period from October 2009 to May 2010 to observe the response of *Cassia angustifolia* under different NaCl stress

(control, 25mM, 50mM, and 100mM NaCl concentrations). The experiment was laid out following Randomized Complete Block Design where each treatment replicated three times. All growth and yield parameters were measured at three phenological stages i.e. pre-flowering (75 DAS), flowering (90 DAS) and post-flowering stage (120DAS). Seeds were sown in earthen pots (300 mm diameter) filled with 3 kg of a garden soil mixture containing farm yard manure (FYM) at 3:1 ratio. The electrical conductivity (EC) of the soil mixture was measured. Healthy, uniform size and pre-soaked six seeds of senna were sown in each pot, thinned to three per pot after germination. Each pot contain three seedlings were sufficiently watered every day. Twelve pots of uniformly growing seedlings were randomly divided into 4 sets, three pots per set. Each pot was considered a single replicates. One set was used as an untreated control. The remaining 3 sets were treated with the various stress treatments. Temperatures during the experiment were in the range of 28-30^{0}C during day and 19-21^{0}C at night.

Salinity treatments

Solution of 0, 25, 50, and 100mMwere prepared by dissolving calculated amount of NaCl (AR grade) with distilled water (DW). The treatment of NaCl was given through soil application at the age of 45 DAS. The first salt treatment (45th day) and the subsequent treatments were given in three stages (after 15 days of each treatment). The treatments were given to plants separately up to flowering stage (90 DAS).i.e. 45 DAS (1st treatment), 60 DAS (2nd treatment), 75 DAS (3rd treatment) and 90 DAS (4th treatment) by adding 500 ml of NaCl solution to the soil. The controls as well as NaCl-treated plants were watered uniformly. Care was taken to avoid drainage of the solution or water.

Plant growth and yield analysis

Growth parameters like plant height, number of branches, number of leaves/plant, leaf area measured at 75 DAS (preflowering stage), 90 DAS (flowering stage) and 120 DAS (postflowering stage). The total green leaf area per plant was measured with the help of a LICOR 3000 Leaf Area Meter and was expressed as cm^2 per plant. The total green leaf area per plant was measured with the help of a LICOR 3000 Leaf Area Meter and was expressed as cm^2 per plant. Yield attributes like number of flowers/plant, number of pods/plant, 100 seed weight were measured at 90 DAS and 120DAS respectively. Pod formation began at 90 DAS and therefore sampling of pods was done at 105 and 120 DAS. The whole plant and pods were taken for fresh weight in three replications from each treatment, and kept in oven at 60^{0}C for 48 h. Dry weight of the pods and whole plant biomass were recorded with the help of electronic top pan balance and expressed in gram per plant. Ten randomly selected third leaf from top of each plants from control and treatment was selected at 60 DAS and green pods at 120 DAS while, kept in oven at 60^{0}C for 48 h. for analysis of sennoside a and b content.

HPLC analysis of Sennoside content

HPLC analysis was done at 60DAS from leaves and 120DAS from pods. All the chemicals used were of AR grade. Methanol and water were obtained from Merck (Mumbai). The standard for the experiment was obtained from Mehta Pharmaceuticals Pvt. Ltd. (Mumbai).The leaves (1.0 gm.) were shade dried and finely powered and extracted with hexane (3 X 25 mL). The hexane extract was discarded and 25 ml of methanol: water (70:30, v/v) was added to mark, the suspension left overnight at room temperature (25^{0}C) and then extracted with the methanol: water mixture (2 X 25 mL). The extract was made up to 100mL with methanol: water and 10 µL sample was subjected to HPLC analysis. The extraction of pods was performed exactly as described above for leaf sample. The solution containing known concentration of range 10-100 µg/ml of sennoside was prepared in methanol, and used as standard for HPLC analysis. HPLC analysis was performed using a Water modular system consisting of two model 501 pumps, an automated gradient controller, a model U6K injector, an in-line solvent degasser, a model 996 photodiode array detector and Millennium 2010 chromatography management software. A Symmetry C_{18} column (150 X 406mm) was used for analysis, and spectral acquisition was performed at 285nm after scanning the standards. The solvent system consist of (A) methanol: water: acetic acid (80:20:0.1v/v/v; pH 4.0). The flow rate was maintained at 0.6ml/min for the first 20 min, while at 30 min it was 1.0ml/min. The HPLC data including height and area of the sennoside peak at particular retention time, of standard sennoside sample (with known concentration) was recorded. This data of standard sennoside sample was compared with the data obtained from plant samples and content of sennoside in the respective plant samples was calculated.

Statistical analysis

The data was presented as arithmetic means of three replicates ±standard deviation. The significance of the mean differences was explored through one-way-ANOVA statistics followed by DMRT (Duncan's multiple range test) at p=0.05 as a post hoc test. SPSS for Windows ver. 11.5 and Microsoft Excel 2003 were used to carry out statistical analyses and graphical data presentations.

RESULTS

Plant height

Plant height is an important growth index of plant. The plant height of senna was measured at different day after sowing (DAS) and the results are presented in Fig. 1. The differences of plant height due to salt stress were found distinctive significant (Fig. 1). At 75 DAS the highest plant height (8.47 cm) was found at control condition and the lowest (5.91 cm) was recorded from 100mM NaCl concentration. At 90 DAS, the highest plant height (24.51 cm) was found at control condition and the lowest (16.37 cm) was recorded from 100mM NaCl concentration. Similar decreasing trends were found with increasing salinity at 120 DAS (final harvest). Salinity had direct effect on plant height. A concentration-dependent decline in plant height was observed against application of NaCl at pre-flowering, flowering and post-flowering stages of plant. Results revealed highly significant differences in the plant height under various applied salt levels. The plant height was significantly greater in control, which closed followed by 25mM. The plant height decreased gradually with every increasing level in the salt concentration.

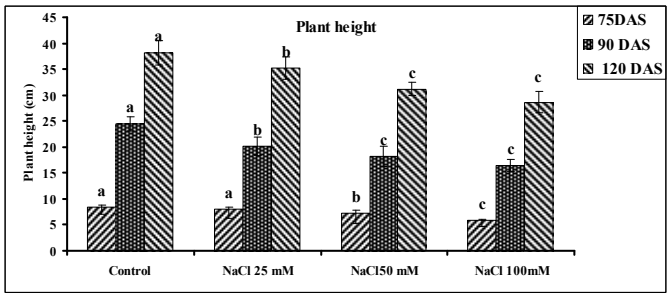

Fig. 1. Effect of NaCl with different concentrations on height of *Cassia angustifolia* at pre-flowering (75DAS), flowering (90DAS) and post-flowering stage (120DAS)

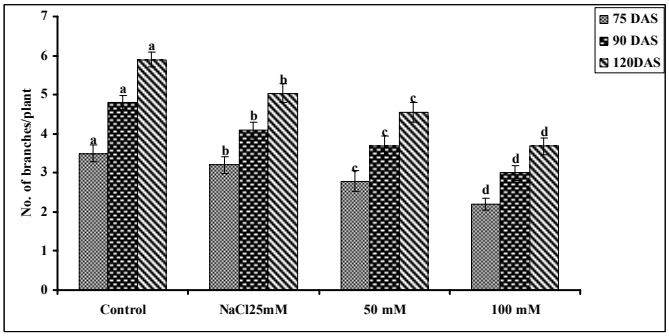

Fig. 1. Effect of NaCl with different concentrations on number of branches of Cassia *angustifolia* at pre-flowering (75DAS), flowering (90DAS) and post-flowering stage (120DAS)

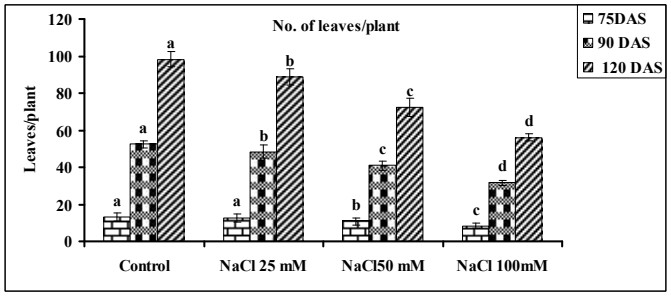

Fig. 2. Effect of NaCl with different concentrations on number of leaves/plant of *Cassia angustifolia* at pre-flowering (75DAS), flowering (90DAS) and post-flowering stage (120DAS)

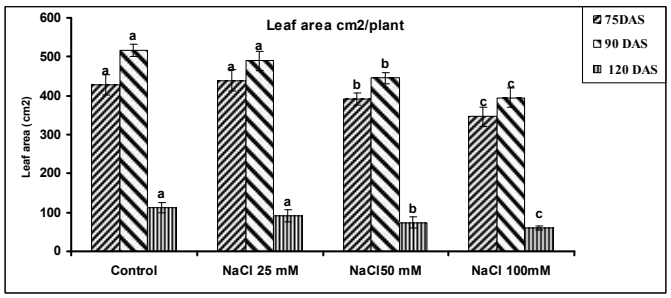

Fig. 3. Effect of NaCl with different concentrations on leaf area/plant of *Cassia angustifolia* at pre-flowering (75DAS), flowering (90DAS) and post-flowering stage (120DAS)

Number of branches per plant

Salinity also caused reduction in the number of branches. Results showed highly significant difference in the average number of branching (Fig.2). The number of branching gradually decreased with increasing salt concentration. They were maximum (5.89) in control and minimum (3.0) at 100mM NaCl treatment at 120 DAS.

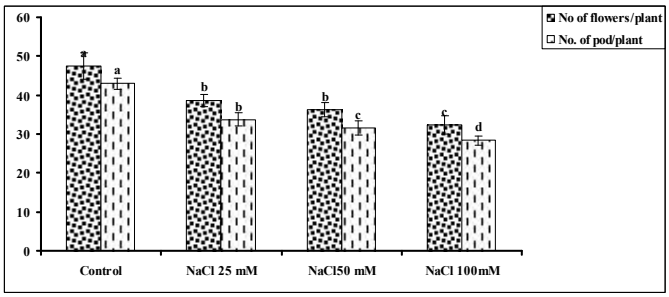

Fig. 4. Effect of NaCl with different concentrations on number of flowers and number of pods/plant of *Cassia angustifolia* plant

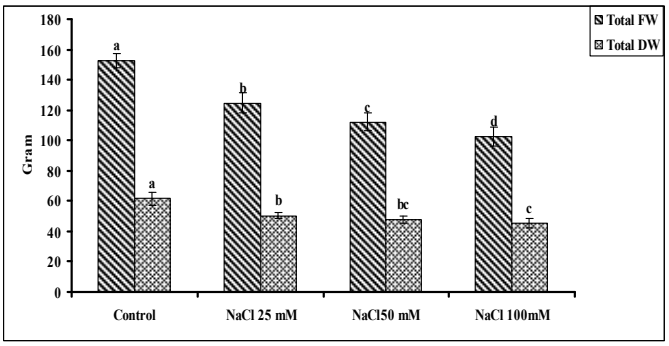

Fig. 5. Effect of NaCl with different concentrations on total fresh weight and total dry weight of *Cassia angustifolia* plant

Number of leaves per plant

The number of leaves varied significantly under various salt concentrations. Decline was more prominent at post-flowering stage with 100mM NaCl by 42.86 % over control. While at 25mM and 50 mM NaCl concentration number of flowers was decreased by 9.78% and 42.86% respectively over control (Fig. 3).

Leaf area

Leaf area showed the highest value in the control plants whereas; under salt conditions it decreased gradually with the increase in salinity (Fig.4). Highest reduction in leaf area was found at 120 DAS with 100 mM NaCl concentration by 23.56% over control. Whereas with 25 mM and 50mM NaCl concentrations, leaf area were reduced by 5.26 and 13.79% over control.

Number of flowers per plant

Highest number of flowers was found in control plants. At 25 mM NaCl concentration it was decreased by 18.56% while at 50mM and 100mM NaCl concentrations number of flowers was reduced by 23.47% and 31.76% over control (Fig. 5).

Number of pods per plant

Increasing NaCl concentrations, number of pods gradually decreased. Maximum reduction was found with 100 mM NaCl concentration by 33.82%. With 25mM and 50mM NaCl concentration number of pods decreased by 21.53% and 26.47% respectively over control (Fig. 5).

Seed weight

Highest weight of 100 seeds was found in control plants. Salt stress also caused a marked reduction in seed yield per plant

Table 1. Effect of NaCl treatments on pod fresh and dry weight, 100 seed weight and sennoside contents from leaf and pod of *Cassia angustifolia*

NaCl mM	Pod fresh weight (g plant^{-1})	Pod dry weight (g plant^{-1})	100 Seed weight (g plant^{-1})	Sennoside mg g^{-1} dry weight Leaf (60 Days after sowing)			Sennoside mg g^{-1} dry weight Pod (120 Days after sowing)		
				a	b	a+b	a	b	a+b
0 (Con)	31.1±3.9^{a}	6.5±0.2^{a}	2.6±0.6^{a}	26.1±2.7^{a}	8.0±0.7^{a}	34.2±2.4^{ab}	33.7±1.3^{a}	12.7±1.6^{ab}	46.4±2.9^{ab}
25	25.7±2.8^{b}	5.2±0.2^{b}	2.5±0.5^{ab}	28.1±1.4^{a}	8.3±0.4^{a}	36.4±1.9^{a}	34.0±1.3^{a}	13.6±1.6^{a}	47.7±2.6^{a}
50	24.1±2.4b^{c}	5.1±0.3b^{c}	2.3±0.1^{bc}	25.1±2.0^{a}	7.1±0.2^{a}	32.3±2.2^{b}	32.5±1.2^{a}	11.4±0.9^{b}	43.9±2.7^{b}
100	22.6±1.9^{c}	4.8±0.1^{c}	2.1±0.1^{d}	19.7±1.4^{b}	5.3±0.5^{a}	25.1±0.9^{c}	28.3±0.6^{b}	8.5±0.6^{c}	36.9±1.2^{c}
p-value	<0.001	<0.001	<0.001	0.00	0.19	<0.001	<0.001	<0.001	<0.001

(Table 1). 100 mM NaCl concentration caused maximum reduction in seed weight from *Cassia angustifolia* by 19.44%. While at 50mM and 100mM NaCl concentrations seed weight reduced by 6.17% and 12.36 % respectively over control.

Pod fresh and dry weight per plant

Reduction in pod fresh and dry weight due to salinity may be a cumulative effect of decline in the number of pods. The main reason for this reduction may be attributed to suppression of growth under salinity stress during the early developmental stages of the plants. At 25 mM, 50 mM and 100 mM NaCl concentration the reduction in pod fresh weight by 17.41%, 22.64%, 27.34% and dry weight by 19.17 %, 21.39% and 25.34% respectively over control (Table 1).

Fresh and dry weight of whole plant

Under 100 mM NaCl stress, the growth of the plants was highly affected and reduced the fresh and dry weight by 28.86 % and 27.49 % when compared with control plants (Fig.6). While at 25mM and 50mM NaCl concentrations reduction in fresh weight of whole plant by 18.27%, 22.48% and dry weight by 17.59% and 21.27% respectively over control.

Sinnoside content

Accumulation of secondary metabolites in the tissue under stress condition could have a bearing on the adaptability of the plant to stress condition. The sennoside (a+b) content in the leaves and pods increased under 25 mM NaCl concentration by 6.2% and 2.8% respectively over control. While at 50mM and 100mM NaCl concentrations sennoside content decreased by 6.2 % and 17.9% in leaves although 5.3% and 20.4% in pods respectively over control in *Cassia angustifolia* (Table 1).

DISCUSSION

Salt stress reduces plant growth and productivity by affecting morphological, anatomical, biochemical and physiological characteristics, processes and functions. Disturbed water and nutritional balance of plants may cause reduced crop yield in saline soil. Reduced plant height and other morphological characters are the most distinct and obvious effects of salt stress. Depressed growth due to high salinity is attributed to several factors such as, water stress, specific ion toxicity and ion imbalance stress or induced nutritional deficiency. In the present study, plant height of C*assia angustifolia* significantly decreased at higher salinity levels. Salinity stress results in a clear stunting of plants (Hernandez *et al.*[10] and Cherian *et al.*[11]). Plant height decreased by NaCl in tomato (Taffouo *et al.*[12] Hajer *et al.*[13]), rice (Alam *et al.* [14]), *Artemisia annua* (Arun *et al.*[15]), Green Bean (Yasar *et al.*[16]), chickpea (Mamo *et al.* [17]), soybean (Essa[18]) *Arachis hypogaea* (Mensah *et al.*[19]), wheat (Sadat-Noori [20]), *Catharanthus roseus* (Karadge and Gaikwad [21]) and *Hordeum vulgare* (Mehmet *et al.* [22]). Salinity caused reduction in all yield components of barley (Javed *et al.* [23]), *Ammi majus* (Hala *et al.*[24]), *Melissa officcinalis.* (Ahmet *et al.* [25]) under salt stress. Soil salinity affects various physiological and biochemical processes which result in reduced biomass production (Khalid *et al.* [26]).

Decreased branching due to salt stress in different crops (Singh *et al.*[27]; Narash *et al.*,[28] Mensah *et al.,*[19] Sadat-Noori,[20]). Salinity is accompanied by significant reductions in plant height and number of leaves per plant in tomato (Mohammad *et al.*[29]). Increased NaCl levels results in a significant decrease in leaf growth biomass in cotton (Meloni *et al.*[30]). The application of stress significantly affected plant growth components such as no. of branches, no. of leaves fresh weight and dry weight of five medicinal plants reported by Zahir and Farrukh[31]. Effect of water salinity has been reported for many crops including alfalfa (Keck *et al.*[32]), cotton (Meloni *et al.*[30]), pea (Lal,[33]), mentha (Ozturk,[34]) etc., showing drastic reduction in crop yield at higher salt concentrations. Kumar and Gill[35] showed that increasing salinity stresses caused a reduction, both in shoot and root yield of C*itronella*. Salinity stress resulted in the suppression of plant growth and a decline in essential oil concentration and yield in three *Cymbopogon* grasses (Ansari *et al.*[36]). Yagmur *et al.*[37] has also reported decreased fresh weight in wheat under salt stress. The number of leaves and leaf length got suppressed in all the test species under higher salinity regimes. Generally, leaf thickness increases under salt stress, which decreases leaf area. Singh and Singh[38] observed a considerable reduction in the leaf length of *Linum* under high NaCl stress. Leaf area is a good indicator of water and salinity stress, since leaf expansion generally requires a high turgor pressure for cell enlargement (Krieg,[39]). It is well accepted that osmotic adjustment plays a crucial role in plant adaptation to drought (Quisenberry,[40]). Salinity induced osmotic stress is considered responsible for the reduced leaf area in Canola and wild mustard (Huang & Redmann[41]). Hajar *et al.*[42] and Murillo-Amador & Trovo-Dieguez[43] also reported decreased leaf area under increasing salinity levels in *Nigella sativa* and cowpea, respectively. A similar decrease in leaf area was found in *Withania somnifera* under salt stress. Our findings agree with the previous workers in this respect.

Javed *et al.*[23] found that salinity caused reduction in grain yield and 100 grain weight in barley. Salt stress also caused a marked reduction in seed yield per plant. Such an adverse effect of salt stress on the growth and seed yield has earlier

been observed in a number of crops, *e.g.*, alfalfa (Serraj and Drevon[44], Esechie *et al*.[45]), carrot Gibberd *et al*.[46] and Hajar *et al*.[42] Secondary metabolite contents of senna i.e. sennoside (a and b) increased at low level of salinity. Similar results were found in some medicinal plants. Shain[47] reported increase alkaloid content in *Atropa belladonna, C. roseus* and *Solanum laciniatum* when subjected to low NaCl stress. Cheruth *et al*.[48] reported that soil salinity alters secondary metabolite accumulation in *Catharanthus roseus*. Accumulation of secondary metabolites like alkaloids in the tissue stress condition could have a bearing on the adaptability of the plant to stress condition. While at higher concentration of NaCl the secondary metabolites content decreased over control. Xanthotoxin (Cuomarins) content was decreased by high NaCl Stress in *Ammi majus* L. plant (Hala *et al*.[24])

Conclusion

It is, therefore, suggested that mild stress (25mM NaCl) can have a commercial application to obtain maximum amount of secondary metabolites of medicinal importance from both pods and leaves of *Cassia angustifolia* and it is concluded that *C. angustifolia* can be cultivated in salt-affected areas, which could increase its production of secondary metabolites at the plant level.

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