

Original Research Article

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Effect of Chloride and Sulphate Dominated Salinity on Growth and Chlorophyll Content of Senna (*Cassia angustifolia* Vahl.)

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ABSTRACT

The present study was aimed at evaluating chloride and sulphate dominated salinity stress tolerance status of the Senna (*Cassia angustifolia* Vahl.) at vegetative, flowering and pod maturity stage, a pot factorial experiment based on randomized complete design with three replicates was conducted in screen house. Four varying EC levels viz. control (without salt), 4, 8 and 12 dSm⁻¹ of each salinity types along with nutrients supplemented in sand filled polythene bags. The present study revealed that various growth attributes such as plant height, number of leaves per plant, leaf dry weight per plant and stem and branches dry weight per plant progressively reduced with the successive increase of EC levels in the growing medium at different stages. Although the two salinity types did not differ significantly in their response on plant height, number of leaves per plant and leaf dry weight per plant at the vegetative stage but their differential effect became more pronounced at the flowering and pod maturity stages. Significant differential response of the two salinity types on stem and branches dry weight per plant was recorded only at the pod maturity stage. Significant decline in chlorophyll 'a', chlorophyll 'b' and total chlorophyll content of Senna with concomitant increase of EC level at the vegetative stage. More conspicuous reduction in growth attributes and contents of above pigments was observed under sulphate dominated salinity as compared to chloride dominated salinity. Senna appeared to be highly salt tolerant medicinal herb as its plant survived and reproduced at highest salinity level (12 dSm⁻¹).

Keywords

Salinity stress,
Growth,
Chlorophyll,
Sulphate, Chloride
and senna

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Introduction

Cassia angustifolia Vahl. is commonly known as Indian senna in English, Senai in Hindi and sonamukhi in Rajasthani and it is an important medicinal plant species belonging to the family caesalpiaceae. It is a native to Sudan and Arabia and cultivated mainly in India and Pakistan. It is now also grown on a small scale in Andhra Pradesh, Karantaka, Kerala,

Madhya Pradesh, Maharashtra, Rajasthan and Haryana. The total export of senna leaves from India is of about Rs. 20 million. The leaves and pods of this species produce crude drug senna. *C. angustifolia* grows well in warm arid regions. It can be grown in a wide range of soil. Sandy loam soil of pH range 6 to 8.5 is suitable for its cultivation. For economic yield as rainfed crop, it requires on average rainfall of 25 to 40 cm. The leaves and pods of

Cassia angustifolia are cathartic, contains sennosides A, B, C, D, rhein and aloe-emodin. It is useful in loss of appetite, hepatomegaly, splenomegaly, indigestion, malaria, skin diseases, jaundice and anaemia. It is also used as expectorant, wound dresser, carminative and laxative.

Soil salinity is one of the major abiotic stresses which restricts the distribution and productivity of crops. No climatic zone in the world is free from salinization although the general perception is focused on arid and semi-arid region.

The total global area of salt affected soils including saline and sodic soils is 831 m ha (Martinez-Beltran and Manzur, 2005), mainly restricted to arid and semi-arid regions, where land degradation, water shortage and population growth are a major concern (Geissler *et al.*, 2010). Agricultural production in future will increasingly rely on our ability to grow plants on salt affected and marginal lands using saline water (Roezma and Flowers, 2008). In India about 6744968 hectares of land is affected with salinity, whereas, it accounts for 232556 hectares in state of Haryana (CSSRI, ICAR, Govt. of India, 2012). The excessive presence of these salts in the soils adversely affects several plant processes that in turn affect crop yields. Osmotic adjustment, i.e. reduction of osmotic potential due to net solute accumulation, is an important mechanism of salt tolerance in plants (Sairam and Tyagi, 2004). The reduction in osmotic potential in salt-stressed plants may stem from accumulation of inorganic ions (Na^+ , Cl^- , Ca^{2+} and K^+) and compatible solutes (soluble carbohydrates, amino acids, proline) and soluble sugars (Ahmad *et al.*, 2006), allow the plant to overcome such failure and to re-establish a water potential gradient which gives it the possibility to absorb water and restore plant turgor (Xiong and Zhu, 2002).

Amelioration and utilization of these saline soils have been the focus of research for the last few decades. Technical measures like physical, chemical and mechanical have been developed to reclaim the soil, however, due to economic viability and ecological concerns, these technologies are less attractive to the farmers. With the diversification of agriculture, medicinal and aromatic plants are gaining importance in the national scenario. So, there is growing global demand for medicinal plants. With increasing demand of food for an ever increasing population in India, it is not possible to bring lands under cultivation for aromatic and medicinal plants. Salt affected lands due to high soluble salt content, excessive exchangeable Na^+ and poor physical properties are not suitable for raising conventional food crops. So, marginal and salt lands could be exploited for the cultivation of medicinal plants such as Senna. The present experiment was planned to study growth parameter and chlorophyll content in Senna in response to salt stress.

Materials and Methods

The experimental site was in the screen house, Department of Botany and plant physiology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana. Seeds of Senna var. sona for these experiments were obtained from the Institute of Herbal Heritage (A unit of Asian Medicinal plants and Health care trust) Sonamukhi Nagar, Sangaria Fanta, Salawas Road, Jodhpur-342005 (Rajasthan), India.

Culture conditions

The plants were raised in polythene bags (18" X 15"), each containing 12 kg of dune sand. The sand filled polythene bags were saturated with the solution of respective salinity treatment along with the nutrient (Hogland and Arnon, 1950) before sowing. Two types

of salinity i.e. chloride [Cl⁻:SO₄²⁻(7:3); Na⁺:Ca²⁺+Mg²⁺ (1:1); Ca²⁺:Mg²⁺ (1:3)] and sulphate (SO₄²⁻:Cl⁻ (7:3); Na⁺:Ca²⁺+Mg²⁺ (1:1); Ca²⁺:Mg²⁺ (1:3) dominated salinity with three replication was given at 4 different salinity level such as 0 (control), 4, 8 and 12 dSm⁻¹. 15 seeds of Senna were sown on variously treated sand beds in polythene bags. The moisture in the bags was maintained at field capacity by adding water as and when required. After establishment of seedlings thinning was done to maintain 3 plants of uniform size in each bag.

Plant height

Height of plants (cm) was measured from the soil surface to the terminal bud with the help of a ruler at vegetative, flowering and pod maturity stages (Table 1).

Number of leaves per plant

All the leaves of each plant were counted and recorded at vegetative, flowering and maturity stages (Table 2).

Leaf dry weight per plant

At the time of harvesting plants were taken out carefully from the bags and after washing away the sand particles adhering to the roots with copious tap water, root and shoot portions were separated with the help of scissors. Leaves were separated and dried in labelled paper envelopes in an oven at 60°C for 48 h and then their dry weights (g) were determined after bringing them to room temperature with the help of an electronic balance (Table 3).

Stem and branches dry weight per plant

Shoot portions excluding leaves were dried in labelled paper envelopes in an oven at 60°C for 48 h and then their dry weights (g) were

determined after bringing them to room temperature with the help of an electronic balance (Table 4).

Estimation of chlorophyll content

The Chlorophyll pigments were estimated according to the method of Hiscox and Israeltdam (1979) using Dimethyl sulphoxide (DMSO).

Procedure

One hundred mg of fresh leaf portion was kept into a test tube containing 5 ml of Dimethyl sulphoxide (DMSO). The test tube was then placed in an oven at 60° C for about 2 h or more (if required) to facilitate the extraction of the pigments. After 2 h and at attaining the room temperature, absorbance was read at 645 and 665 nm on a computer aided spectrophotometer (spectrophotometer – 119) running a multiple wavelengths programme. DMSO was used as blank. Calculations for different pigments were made according to Welburn (1994).

$$\text{Chlorophyll 'a' (mg g}^{-1}\text{)} = ((12.3 \times A_{665} - 2.86 A_{645}) \times V \times 1000) \times W$$

$$\text{Chlorophyll 'b' (mg g}^{-1}\text{)} = ((19.3 \times A_{645} - 3.6 A_{665}) \times V \times 1000) \times W$$

V = Volume of DMSO (ml)

W = Weight of tissue (g)

$$\text{Total chlorophyll content (mg g}^{-1}\text{)} = \text{Chl 'a' + Chl 'b'}$$

Results and Discussion

The growth of senna plants adversely affected at the vegetative stage, maximum flowering and pod maturity stages with the build-up of salinity in the growing medium. The various

growth attributes such as plant height, number of leaves per plant, leaf dry weight per plant and stem and branches dry weight per plant progressively reduced with the successive increase of EC levels in the growing medium at different stages. The more reduction in plant height (10.54 cm), in number of leaves per plant (7.50) and in stem and branches dry weight per plant (1.378 g) observed at flowering stage and in leaf dry weight per plant (1.027 g) at maturity stage.

Although the two salinity types did not differ significantly in their response on plant height, number of leaves per plant and leaf dry weight per plant at the vegetative stage but their differential effect became more pronounced at the flowering and pod maturity stages. Significant differential response of the two salinity types on stem and branches dry weight per plant was recorded only at the pod maturity stage. Sulphate dominated salinity was found more depressive than chloride dominated salinity in this regard.

Heidari and Sarani, 2012 studied the effect of salinity on growth in Chamomile (*Matricaria chamomilla* L.) in controlled environment. Salinity treatment was 0, 50, 100 and 150 mM NaCl in nutrient solution. Results indicated that increasing salinity from 0 to 150 mM, decreased fresh weight of shoot (76.3%) and increased of root fresh weight (53.8%). Reductions in various growth attributes with the rise of salt stress were also recorded by many workers in *Catharanthus roseus* (Jaleel *et al.*, 2008), *Atriplex hortensis* Var. (Kachout *et al.*, 2009) and Morocco (Belaqziz *et al.*, 2009). Greater toxicity of sulphate relative to chloride on growth has been reported by Deepika (2015) in German chamomile. Contrastingly, chloride salinity was found more detrimental to growth than sulphate salinity in chandrashura by Singh (2004), Varshney and Singh (2006). These results demonstrate that it is not only the level of

salinity whose magnitude is harmful to growth and development but also the kind and nature of salt responsible for salinization are of crucial importance in affecting the plant growth and development. Stunting of plants and reduced dry matter production under saline environment are ascribed to both the availability of soil water and increased toxicity of sodium and chloride or sulphate ions (Gorham *et al.*, 1985, Munns, 2009).

Present research findings have revealed significant decline in chlorophyll 'a', chlorophyll 'b' and total chlorophyll content of 4th leaf (from top of the plant) of Senna with concomitant increase of EC level at the vegetative stage. More conspicuous reduction in the contents of above pigments was observed under sulphate dominated salinity as compared to chloride dominated salinity. Contrastingly, more reduction in above chlorophyll pigments observed under salt stress in isabgol (Nehru, 2003), chandrashura (Singh, 2004) and German chamomile (Deepika *et al.*, 2015).

The decline in chl 'a' content with rise of salinity was more pronounced than chl 'b'. The chl 'a' has been reported to breakdown more rapidly than chl 'b' in the presence of salts indicating its more instability. The stability of chl 'b' in salt poisoned plants must be due to the firm bond between this pigment and chloroplastic stroma. This bond may be provided by aldehyde group of pigment or by a different protein (Mercado, 1973). The reduction in leaf chlorophyll content under salt is attributes to the interference of salt ions with the *de novo* synthesis of proteins that constitute the structural components of chloroplast, rather than breakdown of chlorophyll (Sudhakar *et al.*, 1991). The decrease in the content of chlorophyll was demonstrated to be associated with the increased activity of chlorophyllase under salinity (Rao *et al.*, 1980) (Fig. 1–3).

Table.1 Plant height (cm) of Senna at different growth stages under varying salinity

EC level (dSm ⁻¹)	Vegetative Stage		Mean	Flowering Stage		Mean	Maturity Stage		Mean
	Salinity type(ST)			Salinity type(ST)			Salinity type(ST)		
	Chloride dominated	Sulphate dominated		Chloride dominated	Sulphate dominated		Chloride dominated	Sulphate dominated	
0(control)	7.08	7.08	7.08	24.21	24.21	24.21	30.23	30.23	30.23
4	6.70	6.67	6.69	21.99	18.69	20.34	27.91	22.02	24.97
8	5.97	5.60	5.78	19.17	16.12	17.65	25.41	21.20	23.31
12	4.23	4.21	4.22	12.50	8.58	10.54	19.73	17.28	18.51
Mean	5.99	5.89		19.47	16.90		25.82	22.68	
CD at 5%,	ST = N.S., EC = 0.28, ST×EC = 15.21			ST = 1.01, EC = 0.18, ST×EC = 15.21			ST = 0.75, EC = 1.07, ST×EC = 1.5		

Table.2 Number of leaves per plant of Senna at different growth stages under varying salinity

EC level (dSm ⁻¹)	Vegetative Stage		Mean	Flowering Stage		Mean	Maturity Stage		Mean
	Salinity type(ST)			Salinity type(ST)			Salinity type(ST)		
	Chloride dominated	Sulphate dominated		Chloride dominated	Sulphate dominated		Chloride dominated	Sulphate dominated	
0(control)	8.33	8.33	8.33	16.52	16.56	16.56	22.22	22.22	22.22
4	7.78	7.78	7.78	15.22	12.33	17.78	19.45	16.22	17.83
8	6.11	5.45	5.78	13.33	10.55	11.94	17.11	14.67	15.89
12	4.89	4.44	4.67	8.56	6.44	7.50	13.55	11.11	12.33
Mean	6.78	6.50		13.42	11.47		18.08	16.06	
CD at 5%	ST = N.S., EC = 0.58, ST×EC = N.S			ST = 0.59, EC = 0.83, ST×EC = 1.17			ST = 0.52, EC = 0.74, ST×EC = 1.05		

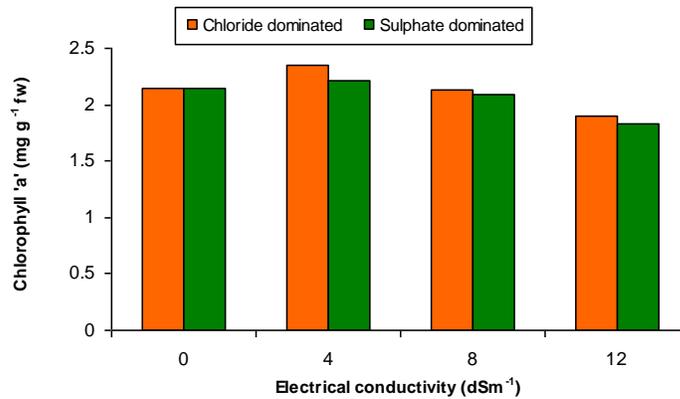
Table.3 Leaf dry weight per plant (g) of Senna at different growth stages under varying salinity

EC level (dSm ⁻¹)	Vegetative Stage		Mean	Flowering Stage		Mean	Maturity Stage		Mean
	Salinity type(ST)			Salinity type(ST)			Salinity type(ST)		
	Chloride dominated	Sulphate dominated		Chloride dominated	Sulphate dominated		Chloride dominated	Sulphate dominated	
0(control)	0.158	0.158	0.158	1.327	1.327	1.327	1.615	1.615	1.615
4	0.141	0.140	0.141	1.262	1.118	1.190	1.596	1.451	1.524
8	0.124	0.120	0.122	1.085	0.919	1.002	1.433	1.256	1.344
12	0.098	0.090	0.094	0.799	0.701	0.750	1.089	0.965	1.027
Mean	0.130	0.127		1.118	1.016		1.433	1.322	
CD at 5%	ST = N.S., EC = 0.009, ST×EC = N.S			ST = 0.049, EC = 0.069, ST×EC = N.S.			ST = 0.04, EC = 0.061, ST×EC = 0.065, ST×EC = N.S.		

Table.4 Stem and branches dry weight per plant (g) of Senna at different growth stages under varying salinity

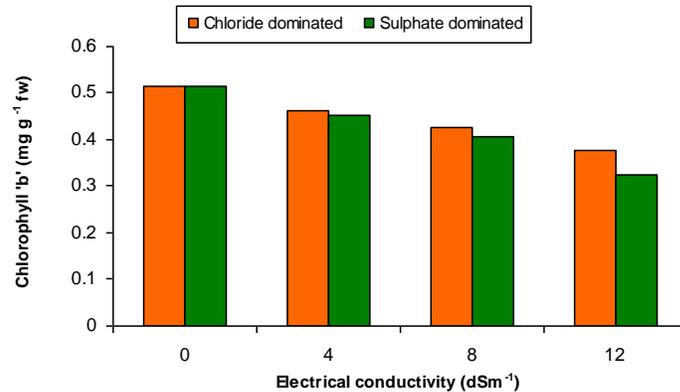
EC level (dSm ⁻¹)	Vegetative Stage		Mean	Flowering Stage		Mean	Maturity Stage		Mean
	Salinity type(ST)			Salinity type(ST)			Salinity type(ST)		
	Chloride dominated	Sulphate dominated		Chloride dominated	Sulphate dominated		Chloride dominated	Sulphate dominated	
0(control)	0.587	0.587	0.587	1.913	1.913	1.913	2.163	2.163	2.163
4	0.584	0.557	0.571	1.780	1.663	1.722	2.110	1.950	2.030
8	0.484	0.477	0.481	1.563	1.470	1.517	2.010	1.893	1.952
12	0.390	0.315	0.353	1.423	1.333	1.378	1.820	1.717	1.768
Mean	0.511	0.484		1.670	1.595		2.026	1.931	
CD at 5%	ST = 0.026, EC = 0.037, ST×EC = N.S			ST = 0.049, EC = 0.070, ST×EC = N.S.			ST = 0.067, EC = 0.095, ST×EC = N.S.		

Fig.1 Chlorophyll 'a' (mg g⁻¹ fresh weight) content of leaves of senna at vegetative stage under varying salinity



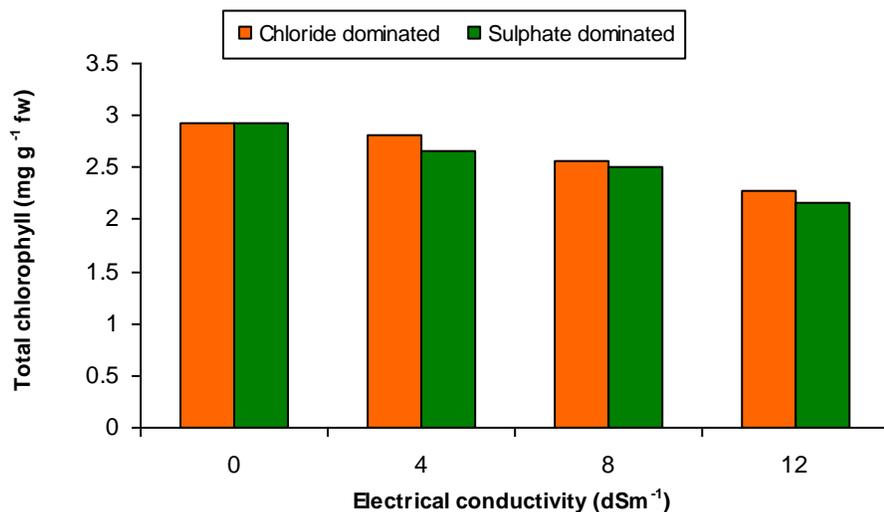
CD at 5%, ST = 0.014 EC = 0.020 ST×EC = 0.028

Fig.2 Chlorophyll 'b' (mg g⁻¹ fresh weight) content of leaves of senna at vegetative stage under varying salinity



CD at 5%, ST = 0.006 EC = 0.008 ST×EC = 0.012

Fig.3 Total chlorophyll (mg g^{-1} fresh weight) content of leaves of senna at vegetative stage under varying salinity



CD at 5%, ST = 0.018 EC = 0.025 ST \times EC = 0.036

Reduction of chlorophyll pigments under salt stress were also reported in sorghum cultivars by Azooz *et al.*, (2004), senna by Arshi *et al.*, (2004), *Cantharanthus roseus* by Elfeky *et al.*, (2007) and Jaleel *et al.*, (2008), German chamomile by Deepika *et al.*, (2015), tomato by Tantawy *et al.*, (2009) and *Atriplex hortensis* var. by Kachout *et al.*, (2009) which support the present findings.

Survival of plants and more than 50% yield of economically important parts i.e. leaves dry weight per plant upto 12 dSm^{-1} EC level demonstrates that Senna can be grown successfully in both chloride or sulphate dominated lands upto salinity of 12 dSm^{-1} EC level.

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