RESEARCH ARTICLE

Biochemical profiling for salinity tolerance in *Casuarina equisetifolia* L

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Salinity is an ecological factor of considerable importance, affecting a considerable area of irrigation projects in the agricultural fields on the order of 20% to 30%. One of the bioremediation approaches to reclaim saline soil is by employing saline resistant plant or tree accessions, which can adapt to the harsh environments and still be productive. One of the multipurpose trees which are being cultivated by farmers and wood based industries on large scale in Tamilnadu is *Casuarina equisetifolia*. This species has been a boon to tree cultivation as it has a wide range of ecological adaptability. The tree improvement program on this species has come up with a set of high productive clones of *C. equisetifolia* which now requires accession site matching so as to support appropriate utilization of waste and unproductive lands. In order to screen *Casuarina* clones at a nursery stage for saline tolerance is therefore highly significant in facilitating planting activities for saline affected areas. Hence identifying a suitable biochemical marker in combination with morphological and physiological studies conferring saline tolerance in *C. equisetifolia* was carried out. Six clones collected from Thiruchendur area was used for the study. Among them clone three and six tolerated salinity level up to 200 mM of sodium chloride for a period of 40 days.

Keywords: *C. equisetifolia*; Clones; Sodium chloride; Biochemical marker; Salinity tolerance

INTRODUCTION

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and the natural status of the environment. Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year 2050. Soil salinity is one of the complex abiotic phenomena adversely affecting agricultural production worldwide [1].

Salinity is one of the most severe environmental factors limiting the productivity of agricultural crops. Most crops are sensitive to salinity caused by high concentrations of salts in the soil. The cost of salinity to agriculture is estimated conservatively to be about $US 12 billion a year, and is expected to increase as soils are further affected [2].

The relative growth of plants in the presence of salinity is termed their salt tolerance. Biochemical adaptations to water logging and salinity are less well known, especially in woody plants. While salinity causes substantial damage to membranes, lesions in the plasma lemma and changes to the structure and permeability of the bimolecular lipid layer of root cells these changes have not been confirmed in waterlogged and salinised Australian trees like *Casuarina* [3].

Casuarina equisetifolia is an important multipurpose plant belonging to the family *Casuarinaceae*. It may be the only woody species growing over a ground cover of dune grasses and salt tolerant broadleaved herbs [4]. *C. equisetifolia* is used for the production of fuel, fiber and other valuable products like pulpwood for paper mils, tannin, timber, dye stuff, medicine etc. It is used to control erosion and its general tolerance to strong winds has encouraged its use in protective planning. Root nodules containing the actinorhizal symbiont *Frankia* enable *C. equisetifolia* to fix atmospheric nitrogen. It is remarkably suited for boundary planting as it does not intercept much of the incoming solar radiation and yields substantial quantities of green leaf manure on lopping besides other products. With high productivity and properties that enhance soil fertility, *C. equisetifolia* shows promises as an agro forestry species for arid and semi-arid areas. *Casuarina equisetifolia* consists of two subspecies, *C. equisetifolia spp. equisetifolia* L. Johnson, and the smaller *C. equisetifolia* spp. incana (Benth.) L. Johnson.

To reclaim the soil native qualities and to meet the demands of *C. equisetifolia* products various conventional and biotechnological approaches are being practiced. Identifying saline tolerant clones of *C. equisetifolia* to cultivate on saline soils is one such approach. Therefore it is essential for making marker assisted selection of *C. equisetifolia* plants at nursery stage In this approach molecular markers, biochemical or phytochemical markers, physiological markers and morphological markers play important role.

Phytochemicals are constitutive metabolites that enable plants to overcome temporary or continuous threats integral to their environment, while also controlling essential functions of growth and reproduction. All of these roles are generally advantageous to the producing organisms but the inherent biological activity of such constituents often causes dramatic adverse consequences in other organisms that may be exposed to them.

C. equisetifolia plants, which are highly tolerant to salt stress, primarily synthesize proline as a major compatible solute to adjust the osmotic pressure when Na accumulates in the cells, and maintain cell homeostasis under salt stress conditions. The changes in Na concentration in shoots and roots of seedlings treated with NaCl at various concentrations.

MATERIALS AND METHODS

The samples for the present study were obtained from the institute of forest genetics and tree breeding, Coimbatore. Six clones of *Casuarina equisetifolia* accessions collected from Thiruchendur were taken up for salinity tolerance study. Leaf/needle samples from the rooted clones were used for phytochemical variation study. The rooted clones of 11 months old clones were screened for various biochemicals/phytochemicals before and after sodium chloride treatment for 40 days. The photochemical concentrations of the different accessions along with morphological, anatomical and physiological parameters were compared against control to identify appropriate indices or markers for salinity tolerance in *C. equisetifolia* (Figures 1-3).

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Figure 1: Rooted *Casuarina* clones taken for study.

Figure 2: *Casuarina* clones before sodium chloride treatment.

Figure 3: *Casuarina* clones after sodium chloride treatment.

The study involved,

- Biometric analysis, morphological, physiological parameters and anatomical cross sectional studies.
- Analysis of phytochemicals using the spectrophotometric method [5].

Morphological study

The six clones of *Casuarina equisetifolia* L. is taken and treated with sodium chloride solution for salinity tolerance study. Parameters like root length, shoot length, collar thickness were measured using scale and vernier callipers.

Root length: Root length was measured from the collar region to the tip of the tap root and expressed in cm.

Shoot length: Shoot length was measured from the apex of the leaves to the collar region and expressed in cm.

Collar diameter: The clones were uprooted and root collar diameter was measured at the collar region of the plant and expressed in cm.

The cladodes of the clones before the salt treatment and after the salt treatment are studied. The cladodes are kept under Nikon Macroscope to study the morphological changes.

Thickness of the cladode: The cladode of the clone's thickness is measured using vernier caliper (Figure 4).

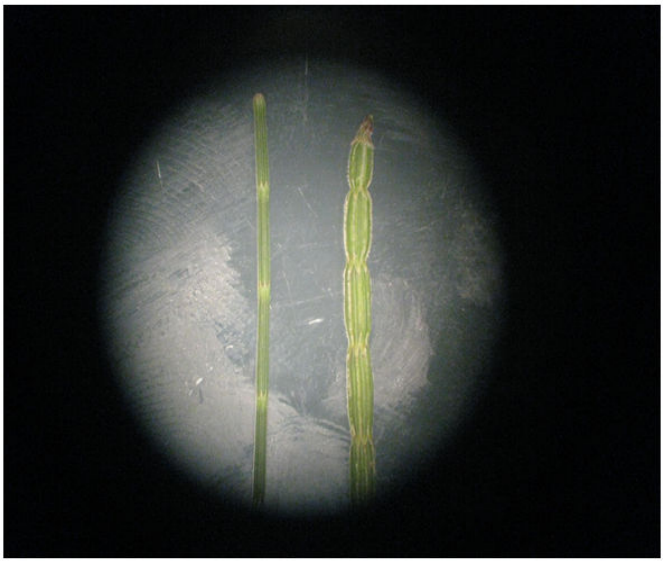

Figure 4: Macroscopic view of cladode. A). Control; B). After sodium chloride treatment.

Physiological study

Based on the biometric values obtained in morphological studies the physiological parameters were derived for the sturdiness coefficient and volume index.

Sturdiness coefficient (S.Q.):

S.Q.=Height (cm)/Diameter (cm)

Volume Index (V.I.):

V.I.=Diameter (cm^2)*height (cm) [6,7].

Anatomical study

To understand the internal structure of the cladodes of control and sodium chloride treated clones, a cross section was taken, stained with saffranin and viewed under Nikon fluorescent microscope.

Statistical analysis

Experiments were carried out in completely randomized design and the data obtained were subjected to Analysis of Variance (ANOVA) using standard statistical package, Genstat 5, to test the significance at 5% level of confidence [8].

Biochemical analysis

Phytochemical markers are extensively being used in forestry and horticulture for estimation of phytochemical analysis in breeding populations, controlled crosses, heterozygosity, gene flow etc. Ten phytochemicals systems were selected for distinguishing the clones which includes total carbohydrates-anthrone method, reducing sugar dinitrosalicylic acid method, protein spectrophotometric method, free amino acid spectrophotometric method proline spectophotometric method. Nitrate reductase spectrophotometric method, chlorophyll spectrophotometric method, phenol spectrophotometric method, tannin vanillin hydrochloride method, anthocyanin-spectrophotometric method [9-17].

RESULTS

Morphological parameters

Morphometric data were obtained for parameters such as root length, shoot length total plant height and collar thickness. It was clear from the study that there was no effect of the duration of treatments (days) on all the four morphometric parameters while significant difference was seen due to influence of clones and concentration of sodium chloride on the parameters such as root length, total plant height and collar thickness. However clone effect was significant only on shoot length but not on sodium chloride concentration.

Factorial effect suggest that combined effect of (i) Days × sodium chloride concentration, (ii) Days × clones and (iii) Days × clone × sodium chloride concentration had nil effect on all the four parameters. Whereas, significant effect was recorded by interaction of clones and sodium chloride concentration on parameters such as root length, total plant height and collar thickness. Only shoot length was found to be uninfluenced by clone × sodium chloride interaction (Tables 1-8).

TABLE 1

Effect of NaCl concentrations on root length of *C. equisetifolia* clones

Clones	Treatments	Before NaCl treatment	After Nacl treatment
C1-TCR 090202	Control	25.5	26.67
	100 mM NaCl	20.47	20.47
	200 mM NaCl	34	34
	300 mM NaCl	23.9	23.9
	400 mM NaCl	26.5	26.5
C2-TCR 080201	Control	22.9	25.23
	100 mM NaCl	18.1	18.1
	200 mM NaCl	26.47	27.27
	300 mM NaCl	18.13	18.13
	400 mM NaCl	16.97	16.97
C3-TCR 040104	Control	25.17	25.33

	100 mM NaCl	25.5	27.5
	200 mM NaCl	21.83	20.67
	300 mM NaCl	20.33	20.33
	400 mM NaCl	14.17	14.17
C4-TCR 070102	Control	30.33	32.2
	100 mM NaCl	27.57	27.57
	200 mM NaCl	30.9	30.9
	300 mM NaCl	22.17	22.17
	400 mM NaCl	33.53	33.53
C5-TCR 020105	Control	19.83	22.33
	100 mM NaCl	14.67	14.67
	200 mM NaCl	18.67	18.67
	300 mM NaCl	18.67	18.67
	400 mM NaCl	18.5	18.5
C6-TCR 100102	Control	28.57	27
	100 mM NaCl	31.53	31.8
	200 mM NaCl	28.7	29.07
	300 mM NaCl	25.63	25.63
	400 mM NaCl	27.47	27.47

TABLE 2
ANOVA for root length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	1	3.81	3.81	0.09	0.76
Clone	5	2929.77	585.95	13.76	<.001
NaCl	4	709.77	177.44	4.17	0.003
Days.Clone	5	3.02	0.6	0.01	1
Days.NaCl	4	7.93	1.98	0.05	0.996
Clone.NaCl	20	1623.91	81.2	1.91	0.018
Days.Clone.NaCl	2	23.08	1.15	0.03	1
Residual	120	5109.69	42.58		
Total	179	10410.99			

TABLE 3
Effect of NaCl concentrations on shoot length of C. *equisetifolia* clones

Clones	Treatments	Before NaCl treatment	After NaCl treatment
C1-TCR 090202	Control	9.17	10.5
	100 mM NaCl	8.83	8.83
	200 mM NaCl	5.17	5.17
	300 mM NaCl	7	7
	400 mM NaCl	6.7	6.7
C2-TCR 080201	Control	10.63	10.63

	100 mM NaCl	10.17	10.17
	200 mM NaCl	9.2	9.3
	300 mM NaCl	9.27	9.27
	400 mM NaCl	11.37	11.37
C3-TCR 040104	Control	7.83	7.83
	100 mM NaCl	7.67	7.83
	200 mM NaCl	8.33	8.5
	300 mM NaCl	9.83	9.83
	400 mM NaCl	9.17	9.17
C4-TCR 070102	Control	11.57	12.53
	100 mM NaCl	10.43	10.43
	200 mM NaCl	13.33	13.33
	300 mM NaCl	10.07	10.07
	400 mM NaCl	9.7	9.7
C5-TCR 020105	Control	5.17	6.33
	100 mM NaCl	6.33	6.33
	200 mM NaCl	5.67	5.67
	300 mM NaCl	8.17	8.17
	400 mM NaCl	9.43	9.43
C6-TCR 100102	Control	12.93	13.5
	100 mM NaCl	9.83	10.8
	200 mM NaCl	9.67	10.13
	300 mM NaCl	10.07	10.07
	400 mM NaCl	9.67	9.67

TABLE 4
ANOVA for shoot length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	1	1.74	1.74	0.16	0.693
Clone	5	431	86.2	7.73	<.001
NaCl	4	32.07	8.02	0.72	0.58
Days.Clone	5	0.72	0.14	0.01	1
Days.NaCl	4	2.78	0.7	0.06	0.993
Clone.NaCl	20	262.03	13.1	1.18	0.287
Days.Clone.NaCl	20	3.18	0.16	0.01	1
Residual	120	1337.72	11.15		
Total	179	2071.24			

TABLE 5
Effect of NaCl concentrations on total height of C. *equisetifolia* clones

Clones	Treatments	Before NaCl treatment	After NaCl treatment
C1-TCR 090202	Control	34.67	37.17

	100 mM NaCl	29.3	29.3
	200 mM NaCl	39.17	39.17
	300 mM NaCl	30.9	30.9
	400 mM NaCl	33.2	33.2
C2-TCR 080201	Control	33.53	35.87
	100 mM NaCl	28.27	28.27
	200 mM NaCl	35.67	36.57
	300 mM NaCl	27.4	27.4
	400 mM NaCl	28.33	28.33
C3-TCR 040104	Control	33	33.17
	100 mM NaCl	33.17	35.33
	200 mM NaCl	30.17	29.17
	300 mM NaCl	30.17	30.17
	400 mM NaCl	23.33	23.33
C4-TCR 070102	Control	41.9	44.73
	100 mM NaCl	38	38
	200 mM NaCl	44.23	44.23
	300 mM NaCl	32.23	32.23
	400 mM NaCl	43.23	40.7
C5-TCR 020105	Control	25	28.67
	100 mM NaCl	21	21
	200 mM NaCl	24.33	24.33
	300 mM NaCl	26.83	26.83
	400 mM NaCl	27.93	27.93
C6-TCR 100102	Control	41.5	40.5
	100 mM Na Cl	41.37	42.6
	200 mM NaCl	38.37	39.2
	300 mM NaCl	35.7	35.7
	400 mM NaCl	37.13	37.13

TABLE 6
ANOVA for total height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	1	7.32	7.32	0.18	0.672
Clone	5	4625.48	925.1	22.8	<.001
NaCl	4	765.55	191.39	4.72	0.001
Days.Clone	5	2.63	0.53	0.01	1
Days.NaCl	4	24.87	6.22	0.15	0.961
Clone.NaCl	20	1377.46	68.87	1.7	0.043
Days.Clone.NaCl	20	39.18	1.96	0.05	1
Residual	120	4869.13	40.58		
Total	179	11711.62			

TABLE 7
Effect of NaCl concentrations on collar thickness of C. *equisetifolia* clones

Clones	Treatments	Before NaCl treatment	After NaCl treatment
C1-TCR 090202	Control	4.84	4.59
	100 mM NaCl	4.56	4.31
	200 mM NaCl	4.54	4.46
	300 mM NaCl	4.19	4.1
	400 mM NaCl	3.17	3.1
C2-TCR 080201	Control	2.82	2.8
	100 mM NaCl	4.94	4.76
	200 mM NaCl	3.45	3.46
	300 mM NaCl	3.45	3.41
	400 mM NaCl	3.45	3.41
C3-TCR 040104	Control	4.23	4.24
	100 mM NaCl	3.85	3.86
	200 mM NaCl	3.75	3.75
	300 mM NaCl	3.42	3.33
	400 mM NaCl	3.28	3.24
C4-TCR 070102	Control	2.9	2.91
	100 mM NaCl	3.07	3
	200 mM NaCl	2.8	2.76
	300 mM NaCl	3.21	3.03
	400 mM NaCl	3.26	3.21
C5-TCR 020105	Control	2.76	2.78
	100 mM NaCl	3.31	3.27
	200 mM NaCl	3.02	2.97
	300 mM NaCl	4	3.97
	400 mM NaCl	3.58	3.55
C6-TCR 100102	Control	3.26	3.29
	100 mM NaCl	3.21	3.22
	200 mM NaCl	3.54	3.53
	300 mM NaCl	3.37	3.35
	400 mM NaCl	3.51	3.49

TABLE 8
ANOVA for collar thickness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	1	0.001239	0.001239	0.38	0.537
Clone	5	0.238711	0.047742	14.74	<.001
NaCl	4	0.036677	0.009169	2.83	0.028

Days.Clone	5	0.001065	0.000213	0.07	0.997
Days.NaCl	4	0.000245	0.000061	0.02	0.999
Clone.NaCl	20	0.296165	0.014808	4.57	<.001
Days.Clone.NaCl	20	0.001092	0.000055	0.02	1
Residual	120	0.388612	0.003238		
Total	179	0.963806			

Clones ranked for morphological parameters

Root length: C1, C4, C6>C2, C3, C5

Shoot length: C2, C4, C6>C1, C3, C5

Total height: C4, C6>C1>C2, C3>C5

Collar thickness: C1>C3>C2, C5, C6>C4

Macroscopic image also clearly showed the swelling of cladode son sodium chloride treatment as evident from the Tables 9-13.

Physiological parameters

TABLE 9

Thickness of the cladode

Clones	Treatments	Before NaCl treatment	After NaCl treatment
C3-TCR 040104	100 mM NaCl	0.65	1.54
	200 mM NaCl	0.66	1.29
C6-TCR 100102	100 mM NaCl	0.67	1.22
	200 mM NaCl	0.68	1.42

TABLE 10

Effect of NaCl concentrations on sturdiness quotient of C. *equisetifolia* clones

Clones	Treatments	Before NaCl treatment	After NaCl treatment
C1-TCR 090202	Control	75.53	89.74
	100 mM NaCl	64.2	68.34
	200 mM NaCl	86.52	88.07
	300 mM NaCl	74.22	76.08
	400 mM NaCl	105.49	107.94
C2-TCR 080201	Control	121.9	131.99
	100 mM NaCl	57.57	59.91
	200 mM NaCl	104.92	107.09
	300 mM NaCl	82.54	83.77
	400 mM NaCl	82.45	83.58
C3-TCR 040104	Control	79.21	79.23
	100 mM NaCl	84.92	90.47
	200 mM NaCl	81.08	79.01
	300 mM NaCl	88.26	90.89
	400 mM NaCl	72.17	72.94
C4-TCR 070102	Control	144.32	154.11
	100 mM NaCl	143.63	146.51
	200 mM NaCl	162.23	164.8
	300 mM NaCl	109.71	118.44
	400 mM NaCl	135.92	132.16
C5-TCR 020105	Control	92.32	104.86

	100 mM NaCl	64.65	65.59
	200 mM NaCl	88.41	96.64
	300 mM NaCl	67.09	67.6
	400 mM NaCl	80.7	81.27
C6-TCR 100102	Control	127.01	123
	100 mM NaCl	131.93	134.86
	200 mM NaCl	108.99	111.99
	300 mM NaCl	107.39	107.83
	400 mM NaCl	106.83	107.45

TABLE 11
ANOVA for sturdiness quotient

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	1	441.8	441.8	0.46	0.501
Clone	5	90227.6	18045.5	18.62	<.001
NaCl	4	11433.9	2858.5	2.95	0.023
Days. Clone	5	115.1	23	0.02	1
Days. NaCl	4	220.2	55.1	0.06	0.994
Clone. NaCl	20	28154.6	1407.7	1.45	0.112
Days. Clone. NaCl	20	497.8	24.9	0.03	1
Residual	120	116301.3	969.2		
Total	179	247392.3			

TABLE 12
Effect of NaCl concentrations on volume index of C. *equisetifolia* clones

Clones	Treatments	Before NaCl treatment	After Nacl treatment
C1-TCR 090202	Control	7.82	7.81
	100 mM NaCl	6.12	5.41
	200 mM NaCl	8.1	7.81
	300 mM NaCl	5.4	5.16
	400 mM NaCl	3.31	3.18
C2-TCR 080201	Control	2.69	2.81
	100 mM NaCl	6.84	6.36
	200 mM NaCl	4.32	4.41
	300 mM NaCl	3.15	3.07
	400 mM NaCl	3.36	3.27
C3-TCR 040104	Control	6.23	6.3
	100 mM NaCl	5.22	5.59
	200 mM NaCl	4.48	4.33
	300 mM NaCl	3.53	3.33
	400 mM NaCl	2.51	2.45
C4-TCR 070102	Control	3.54	3.79

	100 mM NaCl	3.25	3.1
	200 mM NaCl	3.44	3.33
	300 mM NaCl	3.32	2.97
	400 mM NaCl	4.93	4.33
C5-TCR 020105	Control	1.89	2.2
	100 mM NaCl	2.29	2.23
	200 mM NaCl	2.26	2.2
	300 mM NaCl	4.29	4.23
	400 mM NaCl	3.6	3.56
C6-TCR 100102	Control	4.61	4.57
	100 mM NaCl	4.31	4.53
	200 mM NaCl	4.91	4.94
	300 mM NaCl	4.11	4.08
	400 mM NaCl	4.58	4.53

TABLE 13
ANOVA for volume index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Days	1	0.328	0.328	0.15	0.698
Clone	5	166.774	33.355	15.41	<.001
NaCl	4	28.664	7.166	3.31	0.013
Days. Clone	5	0.599	0.12	0.06	0.998
Days. NaCl	4	0.497	0.124	0.06	0.994
Clone. NaCl	20	210.492	10.525	4.86	<.001
Days. Clone. NaCl	20	1.396	0.07	0.03	1
Residual	120	259.814	2.165		
Total	179	668.564			

Clones ranked for physiological parameters

Sturdiness quotient: C4>C6>C1, C2, C3, C5

Volume index: C1>C2, C3, C6>C4, C5

Anatomical study

From the anatomical study it was seen that the leaves were modified into tiny structures and found attached to the stem thereby referred to as 'cladode'. The vascular tissues under 10X magnification were found to be intact and clear in control (NaCl untreated) whereas the tissues were found distorted and expanded in sodium chloride treated cladode (Figures 5 and 6).

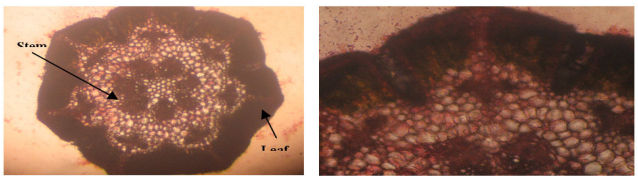

Figure 5: Cross section of control cladode at 5X and 10X magnification.

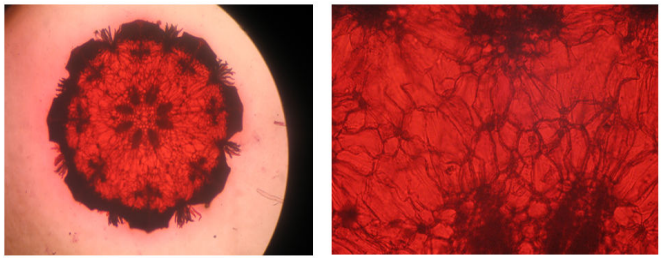

Figure 6: Cross section of sodium chloride treated cladode at 5X and 10X magnification.

Biochemical analysis

Phytochemical analysis is used to distinguish the cultivars of crabapple include proteins, aminoacids, reducingsugar, carbohydrates, proline, chlorophyll, anthocyanin, nitrate reductase, phenol and tannin [18]. The results obtained in the present study for ten parameters have been tabulated (Tables 14-23).

TABLE 14
Effect of NaCl concentrations on protein content of C. *equisetifolia* cladodes

Clones	Before NaCl treatment					After NaCl treatment				
	Control	100 m M	200 mM	300 mM	400 mM	Control	100 mM	200 mM	300 mM	400 mM
Clone 1	126.99	128.01	129.02	130.79	132.31	124.9	0	0	0	0
Clone 2	114.08	119.14	125.73	128.26	138.14	110.02	0	0	0	0
Clone 3	121.42	125.98	129.27	133.07	136.87	102.36	64.18	58.61	0	0
Clone 4	120.15	121.67	124.46	128.51	130.54	102.1	0	0	0	0
Clone 5	130.54	132.06	134.34	136.87	138.14	128.05	0	0	0	0
Clone 6	121.67	124.97	126.74	129.27	131.04	120.17	88.75	67.22	0	0

TABLE 15
Effect of NaCl concentrations on total free amino acid content of C. *equisetifolia* cladodes

Clones	Before NaCl treatment					After NaCl treatment				
	Control	100 mM	200 mM	300 mM	400 mM	Control	100 mM	200 mM	300 mM	400 mM
Clone 1	2.09	3.75	4.58	5	5.83	1.96	0	0	0	0
Clone 2	0.8	0.85	2.09	5.83	8.74	0.75	0	0	0	0
Clone 3	2.09	8.32	9.98	12.89	14.55	2.08	86.8	113.37	0	0
Clone 4	0.02	2.09	4.58	6.66	8.32	0.02	0	0	0	0
Clone 5	4.58	5.41	8.74	10.4	13.72	4.32	0	0	0	0
Clone 6	19.95	4.17	5.41	10.4	12.89	18.95	144.51	1174.79	0	0

TABLE 16
Effect of NaCl concentrations on chlorophyll content (mg/g cladode) of C. *equisetifolia* cladodes

Clones	Before NaCl treatment					After NaCl treatment				
	Control	100 mM	200 mM	300 mM	400 mM	Control	100 mM	200 mM	300 mM	400 mM
Clone 1	0.2	0.22	0.26	0.28	0.31	0.19	0	0	0	0
Clone 2	0.27	0.31	0.33	0.26	0.26	0.26	0	0	0	0
Clone 3	0.23	0.25	0.26	0.21	0.26	0.22	0.18	0.21	0	0
Clone 4	0.27	0.28	0.29	0.28	0.26	0.29	0	0	0	0
Clone 5	0.21	0.24	0.26	0.24	0.4	0.17	0	0	0	0
Clone 6	0.27	0.28	0.29	0.25	0.21	0.23	0.02	0.25	0	0

TABLE 17
Effect of NaCl concentrations on anthocyanin content (mg/g cladode) of C. *equisetifolia* cladodes

Clones	Before NaCl treatment					After NaCl treatment				
	Control	100 mM	200 mM	300 mM	400 mM	Control	100 mM	200 mM	300 mM	400 mM
Clone 1	0.0072	0.006	0.004	0.042	0.035	0.01	0	0	0	0
Clone 2	0.0072	0.012	0.0033	0.074	0.12	0.01	0	0	0	0
Clone 3	0.027	0.0033	0.052	0.016	0.035	0.02	8.6E-06	2.1E-06	0	0

Clone 4	0.013	0.04	0.021	0.014	0.032	0.02	0	0	0	0
Clone 5	0.081	0.0039	0.0053	0.0013	0.0026	0	0	0	0	0
Clone 6	0.0086	0.015	0.0073	0.055	0.02	0.01	6.5E-06	2.1E-06	0	0

TABLE 18

Effect of NaCl concentrations on tannin content (mg/g cladode) of C. *equisetifolia* cladodes

Clones	Before NaCl treatment					After NaCl treatment				
	Control	100 mM	200 mM	300 mM	400 mM	Control	100 mM	200 mM	300 mM	400 mM
Clone 1	17.55	18.17	18.71	19.25	21.94	17.6	0	0	0	0
Clone 2	16.25	16.94	17.17	20.4	21.25	16.33	0	0	0	0
Clone 3	21.94	22.25	22.79	23.02	24.32	20.85	3.01	1.48	0	0
Clone 4	21.79	22.48	24.32	24.86	26.56	21.45	0	0	0	0
Clone 5	23.86	25.09	26.56	27.09	27.94	22.66	0	0	0	0
Clone 6	21.79	22.79	23.56	27.32	28.94	20.59	14.09	15.32	0	0

TABLE 19

Effect of NaCl concentrations on nitrate reductase content (mg/g cladode) of C. *equisetifolia* cladodes

Clones	Before NaCl treatment					After NaCl treatment				
	Control	100 mM	200 mM	300 mM	400 mM	Control	100 mM	200 mM	300 mM	400 mM
Clone 1	5.37	5.27	5.46	5.05	4.5	4.98	0	0	0	0
Clone 2	5.47	5.59	5.41	6.16	5.48	5.38	0	0	0	0
Clone 3	5.31	4.45	5.31	5.47	5.58	5.25	0.32	0.5	0	0
Clone 4	5.72	6	6.34	5.31	5.4	4.96	0	0	0	0
Clone 5	6.87	6.77	7.5	6.76	6.49	5.96	0	0	0	0
Clone 6	7.31	6.76	7.03	6.31	5.6	6.75	0.68	0.72	0	0

TABLE 20

Effect of NaCl concentrations on phenol content (mg/g cladode) of C. *equisetifolia* cladodes

Clones	Before NaCl treatment					After NaCl treatment				
	Control	100 mM	200 mM	300 mM	400 mM	Control	100 mM	200 mM	300 mM	400 mM
Clone 1	0.52	0.6	0.01	0.005	0.14	0.52	0	0	0	0
Clone 2	0.63	0.81	0.53	0.19	0.12	0.59	0	0	0	0
Clone 3	0.92	0.51	0.03	0.38	0.52	0.83	2.22	2.84	0	0
Clone 4	1.92	1.99	1.9	1.97	1.8	1.52	0	0	0	0
Clone 5	0.76	2.66	1.97	2.03	1.91	0.69	0	0	0	0
Clone 6	2.83	2.53	2.43	2.4	2.43	2.76	4.67	4.5	0	0

TABLE 21

Effect of NaCl concentrations on proline content (mg/g cladode) of C. *equisetifolia* cladodes

Clones	Before NaCl treatment					After NaCl treatment				
	Control	100 mM	200 mM	300 mM	400 mM	Control	100 mM	200 mM	300 mM	400 mM
Clone 1	4.86	4.86	3.69	3.39	2.74	4.08	0	0	0	0
Clone 2	4.18	3.78	3.69	3.52	3.23	3.98	0	0	0	0
Clone 3	1.34	1.27	0.75	0.26	0.02	1.29	8.55	11.16	0	0
Clone 4	1.3	1.49	1.85	1.95	2.21	1.03	0	0	0	0
Clone 5	1	1.23	1.33	1.98	2.18	0.9	0	0	0	0
Clone 6	2.31	2.63	2.83	2.96	3.32	1.3	17.1	26.04	0	0

TABLE 22

Effect of NaCl concentrations on total carbohydrates content (mg/g cladode) of C. *equisetifolia* cladodes

Clones	Before NaCl treatment					After NaCl treatment				
	Control	100 mM	200 mM	300 mM	400 mM	Control	100 mM	200 mM	300 mM	400 mM
Clone 1	34.04	30.97	31.11	28.73	29.05	33.09	0	0	0	0
Clone 2	33.93	33.28	31.25	28.63	27.06	32.17	0	0	0	0
Clone 3	33.33	33	31.82	31.69	31.71	31.13	13.07	10.78	0	0
Clone 4	32.43	31.04	27.04	28.4	26.41	30.15	0	0	0	0
Clone 5	32.26	31.82	32.03	31.36	30.97	31.09	0	0	0	0
Clone 6	32.17	31.96	30.97	30.69	28.63	31.16	15.69	12.75	0	0

TABLE 23

Effect of NaCl concentrations on reducing sugar content (mg/g cladode) of C. *equisetifolia* cladodes

Clones	Before NaCl treatment					After NaCl treatment				
	Control	100 mM	200 mM	300 mM	400 mM	Control	100 mM	200 mM	300 mM	400 mM
Clone 1	1.03	1.27	1.52	1.64	1.88	1	0	0	0	0
Clone 2	3.56	1.56	0.34	2.28	3.59	3.32	0	0	0	0
Clone 3	0.12	0.36	0.79	1.12	1.33	0.11	15.35	13.6	0	0
Clone 4	2.95	3.04	3.16	3.43	3.71	2.85	0	0	0	0
Clone 5	1.06	1.82	2.25	4.04	4.98	1.05	0	0	0	0
Clone 6	1	1.94	2.22	2.86	2.92	1	25.62	41.81	0	0

DISCUSSION

From the experiment it was clear that clone 3 and clone 6 were able to survive high saline conditions upto 200 mM concentration. Others clones showed mortality at the end of 40 days of salt treatment. Salinity adversely affects plant by inducing injury, inhibiting growth, altering in plants morphology and anatomy, often being a prelude to mortality [19]. It was supported by significant variations in root length, shoot length, total plant height and collar diameter. However the response on clone three was different compared to that of clone 6. Salinity inhibits vegetative growth of non-halophytes, with reduction of shoot growth more than root growth [20]. Through macroscopic observations, the cladode thickness was found to increase in a remarkable manner between the saline treated and non-treated clones. Clone 3 recorded an increase in thickness by an average of 0.77 mm when compared to the untreated while clone 6 to showed an increment in thickness by an average of 0.64 mm, thereby conferring modifications in plant morphology to adverse conditions. Leaves become thicker and more succulent. The great leaf thickness may reflect more layers of mesophyll cells, larger cells or both [21].

With regard to physiological parameters, the clone 4 ranked highest for sturdiness quotient and clone 1 ranked highest for Volume Index. Both clones 3 and 6 recorded only intermediate values for these physiological parameters supporting prevalence of growth constraints [22].

Anatomical study revealed distorted changes in cladode parenchyma emphasizing pressure exertion on the cells which could be due to increase in water accumulation to regulate osmosis [23-25].

Among the most cited studies related to anatomical modifications induced by salinity stress which could not detect differences in root diameter after 4 weeks of growth under saline conditions, but this author reported that salinity was associated with a greater number of small diameter xylem

vessels. In contrast, Robert E found an increase in root diameter produced by salinity and suggested that a reduction in cell size, an increase in root diameter and a smaller plant size could be adaptive advantages for prolonged survival in saline or dry soils. Other workers increased suberization and thickening of the endodermis, which in turn resulted in an increase in the diameter of both the root and the vascular cylinder. With regard to the effect of salinity on stems, Plaza BM, et al., found that salinity retarded the differentiation of xylem and phloem elements while stimulating excessive growth of the cortex parenchyma cells. Unfortunately there are fewer studies on the effect of salinity on stems than on leaves and roots.

CONCLUSION

Biochemical study showed increasing trend for parameters such as free amino acids, phenols, praline content and reducing sugars. Whereas, there was a noticeable decline in proteins, anthocyanins, tannins, carbohydrates and nitrate reductase activity. However, it was observed that chlorophyll content did not face a drastic changes within the 40 days period of saline exposure. Remarkable variations for free aminoacid content, proline content and reducing sugars suggest them as dependable markers for screening saline tolerance in *Casuarina equisetifolia*.

In non-halophytes, salt induced inhibition of plant growth is accompanied by metabolic dysfunction, including decreased photosynthetic rate and changes in enzyme activity. In halophytes physiological activities may be stimulated or not altered by salt concentrations that are inhibitory in non-halophytes. Salinity decreases carbohydrates or growth hormones thereby inhibiting growth. High salt concentration inhibit enzymes by impeding the balance of forces controlling the protein structure. Salinity affects negatively the nutritional balance of the on Dalbergia sissoo tree indicated that the use of saline irrigation water decreased the contents of chlorophyll and carotenoids while a pronounced increase was noticed for praline, phenols and indole contents.

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