Botany Research International, 2 (1): 50-54, 2009
ISSN 2221-3635

Growth and Organic Constituent Variations with Salinity in *Excoecaria agallocha* L., an Important Halophyte

M. Jenci and S. Natarajan

Department of Botany, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India

Abstract: The present investigation was made to study the effect of different concentrations of sodium chloride and potassium chloride on growth and organic constituents of the seedlings of *Excoecaria agallocha*. The plant could survive a wide range of 100-500mM of salt concentrations. The upper limits for the survival of the seedlings were upto 500mM NaCl and 400mM KCl. The highest numbers of leaves, leaf area, fresh and dry weight were recorded at 300mM NaCl and 200mM KCl treatment. Beyond these levels, the growth parameters reduced drastically. Organic compounds such as amino acid and sugar decreased upto optimum level of 300mM NaCl and 200mM KCl and increased at higher concentrations. The starch and chlorophyll content increased upto 300mM NaCl and 200mM KCl, beyond these levels the contents decreased marginally. The proline content increased with the increasing salinity of both the salts.

Key words: Mangrove · Salinity · *Excoecaria agallocha* · Aminoacid · Proline

INTRODUCTION

Seven per cent of the land's surface and five per cent of cultivated lands are affected by salinity with the salt stress being one of the most serious environmental factors limiting the productivity of crop plants [1]. In India alone, about 30 million hectares of coastal land is lying barren and uncultivable because of soil affected by salinity.

Stresses associated with temperature, salinity and drought single or in combination are likely to enhance the severity of problems in the coming decades [2]. The concentration of the salt in the saline environment is normally measured as the chloride concentration or chlorinate and it is about 35 g per litre. Sodium is the dominant cation with concentration of 480 mM in the saline water and the soil.

Mangrove areas are highly productive and they are as good as agricultural land. In view of these, halophytes in general and mangroves in particular have been the subject of scientific investigations in the last three decades [3]. The effect of a variety of salts on the tolerance of the metabolism of several salt plants has been studied and several reviews on them have appeared in recent times [4].

The present study was made to investigate the salt tolerance of NaCl and KCl on growth and organic constituents of *E. agallocha* and optimum salt tolerance of this species was assessed.

MATERIALS AND METHODS

Excoecaria agallocha, an evergreen mangrove species belonging to the family Euphorbiaceae was used for the present investigation. This species was naturally growing in abundance in the salt marshes in the mangrove area of Pichavaram on the east coast of Tamil Nadu, India, about 10 km east of Annamalai University campus.

About one month old seedlings each with two leaves were uprooted without damaging the root system and they were planted individually in polythene bags (7”X5”) filled with the homogenous mixture of garden soil containing red earth, sand and farm yard manure (1:2:1). The planted seedlings were irrigated with tap water and maintained in the Botanical garden, Annamalai University. One month old well established seedlings were selected and treated with varying concentrations of NaCl and KCl (100-1000 mM). Above 500 mM NaCl and 300 mM KCl concentrations, seedlings were not survived. The experimental yard was rooted with transparent polythene sheet at a height of 3 m from the ground in order to protect the plants from rain. The samples were collected periodically at 60th and 120th day intervals and the data were analysed statistically with two-way Anova.

The leaf area was calculated manually. Total amino acid [5], total sugar [6], Starch [7], Chlorophyll [8] and Proline [9] were estimated.

Corresponding Author: Dr. S. Natarajan, Department of Botany, Annamalai University, Annamalainagar 608 002, Tamilnadu, India

RESULTS

The results on the effect of different concentrations of NaCl and KCl on the number of leaves, leaf area, shoot and root length, fresh weight and dry weight of leaf, stem and root of *E. agallocha* on the 60th and 120th day after saline treatment are present in Table 1.

Number of Leaves and Leaf Area: The effect of NaCl and KCl on number of leaves, leaf area, shoot length and root length is presented in Table 1. Both the salts had increased the number of leaves, leaf area, shoot length and root length with increasing concentrations. This was observed on the 60th and 120 th day at 300mM NaCl and 200mM KCl. At higher concentrations of both these salts, a gradual reduction in the number of leaves, shoot and root length leaf area was noticed. At the optimum concentration, the calculated leaf area was approximately equal in both the salts.

Fresh and Dry Weight: The fresh weight of leaf, stem and root had increased with increasing NaCl upto 300 mM in both the samples and there after it had decreased. The maximum increase in fresh weight was 84% on the 60th day sample and 49% on the 120th day sample when compared to those of control plants. The maximum increased of stem fresh weight was 80% on the 60th day and 40% on the 120th day than the control. The root fresh weight was always less than those of leaf and stem at all concentration of NaCl. A similar trend in fresh weight of leaf, stem and root was seen with KCl salinity at 200 mM. However, the effect of KCl on the root fresh weight was less pronounced when compared to that of NaCl.

The dry weight of leaf, stem and root had increased with increasing NaCl upto 300 mM in both the samples and there after it had decreased.The increase in dry weight of the leaf and stem at the optimum concentration was more than two fold. With KCl, the increasing trend in the dry weight of the three organs was noticed only upto 200 mM. In case of dry weight KCl had a greater effect on the dry weight of all organs when compared to that of NaCl treatment.

The result on the effect of NaCl and KCl salinities on the free amino acid, total sugar, starch, proline and chlorophyll contents are presented in Table 2. The F-values were significant at 5% level.

Aminoacid: The amino acid content of the leaf, stem and root decreased with increasing NaCl concentration upto 300 mM and at higher concentrations, it showed a gradual increase upto 500 mM NaCl. However, the highest value obtained in the leaf at 500 mM NaCl was 17% less than that of control. A similar trend was also seen in the stem and root tissues. KCl salinity showed a similar trend in the aminoacid content to that of NaCl salinity. However, the decrease in the aminoacid content was noticed only upto 200 mM KCl and thereafter, it registered an upward trend upto 400 mM.

Table 1: Effect of NaCl and KCl on growth parameters of *Excoecaria agallocha*

Parameters	Concentrations (mM)																			
	NaCl												Kcl							
	Control		100		200		300		400		500		100		200		300		400	
No. of Days	60	120	60	120	60	120	60	120	60	120	60	120	60	120	60	120	60	120	60	120
No. of. Leaves (plant $^{-1}$)	13	15	14	19	16	24	18	26	13	18	9	12	14	20	17	22	14	18	12	16
Leaf area (cm^2 plant $^{-1}$)	95.7	112.0	106.4	155.6	121.7	182.6	147.7	200.6	88.3	148.0	75.2	90.5	122.1	162.0	147.4	196.3	118.9	157.4	85.4	142.1
Shoot length (cm plant $^{-1}$)	17	32	18.2	33.3	18.7	36.8	21.8	38.7	16.5	24.8	15.1	22.6	19.1	34.0	21.1	36.0	16.8	30.2	16.2	28.6
Root length (cm plant $^{-1}$)	9.0	19.6	10.0	21.2	10.5	22.6	12.4	25.8	8.0	12.3	7.4	10.8	9.8	21.1	12.3	23.4	10.8	15.8	8.6	12.9
Fresh wt. (g plant $^{-1}$)																				
Leaf	2.47	3.81	2.73	4.12	3.26	4.47	4.54	5.68	2.31	3.49	1.96	3.26	2.86	4.11	3.54	4.53	2.74	3.68	2.18	3.33
Stem	2.64	4.21	2.97	4.38	3.48	4.90	4.75	5.89	2.73	4.36	2.68	4.17	2.97	4.44	3.62	4.68	2.68	3.96	2.51	3.73
Root	1.24	2.38	1.54	2.52	1.96	2.61	2.16	2.91	1.36	2.48	1.18	2.30	1.56	2.56	2.16	3.13	1.46	2.48	1.38	2.23
Dry wt. (g plant $^{-1}$)																				
Leaf	0.74	1.43	1.07	2.16	1.28	2.35	1.76	2.96	0.86	1.79	0.68	1.71	1.16	2.16	1.46	2.39	1.10	1.94	0.80	1.75
Stem	0.79	2.26	1.10	2.32	1.36	2.64	1.92	2.91	0.97	2.34	0.74	2.23	1.14	2.33	1.51	2.46	1.11	2.08	1.00	1.96
Root	0.55	0.98	0.78	1.05	0.93	1.21	1.04	1.32	0.69	1.03	0.56	0.96	0.81	1.06	1.08	1.30	0.74	1.03	0.70	0.92

					Fresh Weight			Dry Weight		
F-Values:	No. of leaves	Leaf area	Shoot length	Root length	Leaf	Stem	Root	Leaf	Stem	Root
	F_1=15.53[a]	F_1=12.36[a]	F_1=6.27[a]	F_1=7.46[a]	F_1=8.06[a]	F_1=4.73[a]	F_1=5.49[a]	F_1=8.09[a]	F_1=4.97[a]	F_1=7.06[a]
	F_2=6.79[a]	F_2=5.33[a]	F_2=11.36[a]	F_2=11.67[a]	F_2=6.83[a]	F_2=7.04[a]	F_2=8.23[a]	F_2=13.39[a]	F_2=16.72[a]	F_2=5.57[a]

[a] Significant at 1% and 5% level

Table 2: Effect of NaCl and KCl on organic compounds of *Excoecaria agallocha* (mg / g fr. wt.)

Parameters		Concentrations (mM) NaCl Control		100		200		300		400		500		Kcl 100		200		300		400	
No. of Days		60	120	60	120	60	120	60	120	60	120	60	120	60	120	60	120	60	120	60	120
Amino acid	Leaf	11.86	17.63	9.34	17.25	8.81	16.42	8.64	15.39	8.89	15.86	9.31	14.86	10.43	14.40	9.46	12.46	9.80	12.65	9.88	13.20
	Stem	9.27	12.22	8.63	12.00	8.52	11.63	8.27	11.24	8.38	10.27	8.43	9.77	8.82	11.10	8.68	10.80	8.92	11.23	8.98	11.58
	Root	7.23	9.63	6.93	9.27	5.53	8.76	5.43	7.16	5.81	7.35	6.01	7.59	6.82	8.96	6.68	8.25	6.92	8.36	7.18	8.67
Total sugar	Leaf	31.40	24.33	29.12	23.61	26.71	21.36	25.67	20.85	26.17	21.39	26.98	22.68	28.60	21.38	27.51	19.42	27.84	19.78	28.31	20.07
	Stem	19.96	17.71	17.90	16.87	17.64	16.53	16.63	15.18	16.78	16.08	17.06	16.85	18.31	16.11	17.86	15.66	18.43	16.07	18.92	17.05
	Root	18.36	16.43	16.61	14.86	15.62	14.33	15.36	13.96	15.92	14.76	16.08	15.37	17.64	15.28	17.21	14.45	17.80	15.83	18.01	16.03
Starch	Leaf	84.78	116.7	97.55	127.4	103.1	136.7	115.5	145.3	107.2	140.5	91.73	134.9	95.81	123.4	110.8	128.2	106.1	126.5	99.06	124.4
	Stem	71.85	92.04	84.13	95.23	98.78	108.9	104.7	106.9	96.04	114.2	85.20	111.3	86.05	98.66	97.73	114.8	89.67	105.2	87.15	100.2
	Root	55.08	75.02	61.45	86.15	67.16	91.42	70.34	98.91	57.31	86.19	56.06	76.01	65.26	96.62	74.01	110.9	64.86	95.27	58.77	91.52
Proline	Leaf	4.13	4.38	5.41	6.11	5.88	7.33	6.21	8.31	6.53	9.14	6.89	9.52	4.43	6.42	4.93	7.63	5.41	8.12	6.26	8.82
	Stem	3.06	3.86	4.46	6.62	4.74	7.80	5.28	8.18	6.04	8.47	6.45	9.03	3.82	4.81	4.31	5.68	4.94	6.51	5.03	7.21
	Root	1.14	2.16	1.79	2.26	1.86	3.59	1.94	4.47	2.35	4.73	2.73	5.38	1.63	2.63	1.96	2.86	2.54	3.45	3.38	4.26
Chloroph yll	Chl a	1.15	0.96	1.42	1.18	1.54	1.24	1.74	1.38	1.64	1.25	1.56	1.21	1.45	1.24	1.92	1.44	1.72	1.32	1.61	1.22
	Chl b	0.85	0.70	1.10	0.93	1.20	0.99	1.36	1.24	1.28	1.00	1.20	0.98	1.10	0.92	1.47	1.10	1.30	1.03	1.20	0.98
	Total Chl	2.00	1.66	2.52	2.11	2.74	2.23	3.10	2.62	2.92	2.25	2.76	2.19	2.55	2.16	3.39	2.54	3.02	2.35	2.81	2.20

F-Values:	Amino acid	Total Sugar	Starch	Proline	Total Chlorophyll
	F_1=66.10[a]	F_1=181.47[a]	F_1=77.56[a]	F_1=15.10[a]	F_1=24.0[a]
	F_2=5.97[a]	F_2=14.11[a]	F_2=17.26[a]	F_2=1.33[aa]	F_2=251.42[a]

[a] Significant at 1% and 5% level

[aa] Not Significant

Total Sugar: Total sugar content decreased in all the three organs with increasing NaCl upto 300 mM and with increasing KCl up to 200 mM. At higher concentrations of both the salts, a gradual increasing trend was recorded.

Starch: The starch content in all the three organs increased with increasing NaCl upto 300 mM and with increasing KCl upto 200 mM. At still higher concentrations, there was a gradual decrease in the starch content. The differences in the starch content at different levels of salinity followed the same trend as that of sugar.

Proline: There was a gradual rise in the level of proline in all the three organs on both the sampling days with increasing concentration of both NaCl and KCl. The leaf always had more proline than the stem and the root. There was 2-3 fold increase of proline on the 120th day at the highest salinity level.

Total Chlorophyll: An increasing trend in chlorophyll content of the leaf was noticed with increasing NaCl concentration upto 300 mM on the 60th day of salt treatment and thereafter, it steadily declined. There was a 55% increase in chlorophyll at 300 mM when compared to that of control plants. A similar increasing trend in the total chlorophyll content upto 200 mM KCl concentration was recorded and thereafter it gradually decreased.

DISCUSSION

In the present study, NaCl and KCl affected the growth of the seedlings of *E. agallocha* by increasing shoot and root length with increasing salinity (i.e.300 mM for NaCl and 200 mM for KCl). At the optimum concentration, both the salts had approximately similar effect on the growth of the seedlings. The data on growth response to revealed a depressed growth both in the absence of salts and at high salinities of two salts, NaCl appeared to be less damaging. A stimulation of growth in response to moderate levels of NaCl salinity has been reported for several halophytes. *Atriplex vesicaria* produced high yield in thr presence of 700 mM NaCl. The growth of *Sesuvium portulacastrum* showed positive effect to NaCl concentrations upto 600 mM and the upper limit for survival of this species was 900 mM [10]. On the other hand KCl was shown to be less effective in promoting growth and in some instance was toxic. There is evidence for synergism between NaCl and KCl [11-13]. Growth stimulation at low moderate external salinity has been reported for many hyalophytes [14-17].

The decline in leaf number at high salt concentrations was due to the leaf fall because of senescence. Salinity has been shown to be one of the external factors that influence the process of senescence and the consequent shedding of leaves [18-20]. The increase in the leaf area could be due to increase in water content of the leaves and increase in succulence.

Sodium chloride and potassium chloride salinity increased the fresh and dry weight of the leaf, stem and root with increasing salinity up to optimum concentrations. The increased fresh weight of the leaf tissue can be attributed to the increase in leaf thickness and the accumulation of ions and water in the tissues [14-19]. In the dicotyledonous halophytes it has been reported that Na^+ and Cl^- ions can amount for 30-50% of the dry weight. The dry weight increase could be attributed to the accumulation of inorganic salts and organic matter in the plant tissues.

Increasing concentrations of NaCl upto 300mM and Kcl upto 200mM had decreased the free amino acid and sugar content and at higher concentrations both salts, there was a gradual increase in the amino acid and sugar content. Accumulation of some amino acids viz, asparagin, aspartic acid and reversible trend was observed [18-21]. An increase in concentrations of alanine, aspartic acid and to a lesser extent that of serine in *Puccinella maritima* grown in 200- 600 mM NaCl. Greater accumulation of the amino acids was also observed in young plants in response to increased sea water salinity in growth medium [17-22]. Increased content of amino acids with increase in salinity was observed in many other crops [23-25].

The starch content had increased with increasing NaCl and KCl salinity upto optimum levels. The reverse situation was noticed at higher salinities. The increase in starch and decrease in total sugar had been attributed to the role of sodium on the stomatal opening. Salt stress induced reduction in sugar content has been reported [19-22]. Sucrose is considered to be the primary substrate for starch synthesis. It has been reported that the concentrations of sucrose and starch increase in excised and illuminated leaves plants [21-24]. It has also known that starch content increases under the conditions where sucrose content is increased [25-26].

Both NaCl and KCl salinities favoured chlorophyll synthesis in the leaves of *E.agallocha* up to the optimum concentrations of the respective salts. However, at higher concentrations, both the salts had decreased the chlorophyll content. A positive effect of NaCl salinity on chlorophyll synthesis in the halophyte, has been reported [21-23]. On the other hand, a decrease in the chlorophyll content under salinity has been reported in a number of halophytes [20-24].

Increasing NaCl and KCl salinity caused an increasing content of proline. A positive correlation exists between the proline content and salinity treatments. The accumulation of proline may be due to reduced turgor pressure and/or growth has hypothesized. Proline, which acts as an intracellular cytoplasmic osmoticum as it, accumulates to a higher degree under stress conditions, which may play an adaptive role for salt tolerance. The increase of free proline contents in some species [21-25]. It is concluded and recommended that the 300 mM NaCl and 200 mM KCl is a suitable soil salinity to utilize for this species.

ACKNOWLEDGEMENT

The authors are thankful to Professor Dr. K.P. Chellappan, Former, Head of the Department of Botany and Dean, Faculty of science, Annamalai University for having provided laboratory facilities and encouragement throughout the research work.

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