EFFECTS OF SEA WATER ON GERMINATION, GROWTH, ACCUMULATION OF ORGANIC COMPOUNDS AND INORGANIC IONS IN HALOPHYTIC GRASS *HELEOCHLOA SETULOSA* (TRIN) BLATT ET McCANN.

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SUMMARY

Eleven per cent seeds of *Heleochloa setulosa* germinated in 8 dS m^{-1} sea water and the process was totally impaired in 16 dS m^{-1} and higher salinity. Shoot and root length as well as fresh and dry weight of the 60-day-old plants of *H. setulosa* decreased in plants grown in 10 to 40 dS m^{-1} sea water salinity as compared to control. Protein content varying between 117 to 147 mg g^{-1} dry wt in shoots and roots was adversely affected beyond 20 dS m^{-1} salinity. Asparagine, aspartic acid, glycine, phenylalanine and proline increased in salt treated plants. Concentrations of total, reducing and major sugars in young plants decreased with increase in sea water salinity in growth medium. Accumulation of Na^+ and Cl^- in salt treated plants were greater than salt free plants, whereas Ca^{+2}, Mg^{+2}, and K^+ content did not show specific trend in relation to increased salinity.

Key words : **Amino acids, *Heleochloa setulosa*, minerals, proteins, sea water, sugars.**

INTRODUCTION

While practising an integrated coastal zone management, one needs to use halophytic species to the best of their potential (Clark 1996). Since *Heleochloa setulosa* is a good soil binder species growing in saline conditions in many parts of India, it can be used for preventing soil erosion in coastal areas. Understanding of salt tolerance mechanism during seed germination and at early growth stages is essential for introduction of salt tolerant species on saline soils (Leith 1999). Despite the occurrence of about 60 such salt tolerant species on 5700-km-long sea coast in India, little information is available about their role in coastal management and on biology of their salt tolerance. Therefore, the present study was undertaken to assess the effects of sea water dilutions on germination, growth and accumulation of osmoregulatory organic compounds and inorganic ions in vegetative organs of *H. setulosa*.

MATERIALS AND METHODS

Seeds of *H. setulosa* were randomly collected from plants growing at Ghogha (21° 45', 27° 14'). Hundred seeds of uniform size, shape and colour were germinated on Whatman No. 1 filter paper in petri dishes containing distilled water and sea water of 8 to 32 dS m^{-1} at room temperature (30-35°C). Four replications of each treatment were maintained and the data were statistically analyzed for the standard error of mean.

Plants were raised in perforated plastic pots containing sand thoroughly washed with distilled water. After 24 h of soaking, seeds were transferred to the pots, which were dipped in tap water every alternate day. After 7 days, the tap water was supplemented with half strength Hoagland's solution. Thirty day old plants were subjected to sea water of 10, 20, 30 and 40 dS m^{-1} salinity with daily inrement of 2 dS m^{-1}. Sixty days old plants were used for growth data and for estimation of organic compounds and mineral ions. Proteins were estimated by Folin-phenol reagent (Lowry *et al.* 1951) and amino acids by 2 dimensional paper chromatography (Joshi 1986) except proline, which was estimated by circular chromatography (Giri *et al.* 1952). Total and reducing sugars were measured by Folin and Malmrose (1929) method modified by Umbreit *et al.* (1959). Aqueous extract of the plant ash was used for estimation of Na^+ and K^+ by flame photometry, Ca^{+2} and Mg^{+2} by EDTA titration (Vogel 1978) and Cl^- on chloride meter (Elico EE-34 model).

RESULTS AND DISCUSSION

Only 11 per cent seeds germinated in 8 dS m^{-1} sea water (Table 1) with complete inhibition of the process beyond 16 dS m^{-1} treatment. However, even in distilled water only 77 per cent seeds germinated. Seeds, which failed to germinate at higher salinity sea water later on germinated in distilled water. This showed that seeds remained viable even after exposure to high sea water salinity for 25 days. Earlier studies on monocotyledons halophytes showing 75 per cent seed germination of *Juncus maritiimus* in sea water *per se* at 26° C (Rozema 1976); 92 per cent seed germination of the same species in 24 dS m^{-1} sea water (Joshi and Khairatkar 1995); 60 per cent seed germination of *Sporobolus wrightii* and *Leptochloa dubia* at 20 dS m^{-1} (Everitt 1983); 28 per cent seed germination of *Aeluropus litoralis* in 0.02 M NaCl (Pollak and Waisel 1971) and 48 per cent germination of *Sporobolus madraspatanus* in 16 dS m^{-1} sea water (Joshi and Misra 2000) indicate that *H. setulosa* seeds are highly salt sensitive despite the fact that the plants grow in higly saline conditions in nature.

Shoot and root length as well as fresh and dry weight of plants grown in sea water of different salinity decreased significantly with increasing salinity levels as compared to control. Moreover, plants performed quite well up to 30 dS m^{-1} sea water salinity (Table 2).

Table 1. Effects of sea water salinity levels on seed germination in *H. setulosa*.

Salinity (dS m^{-1})	Germination (25 D)	Per cent Recovery (25D)	Total (50 D)
0	76.5 ± 2.1	0.5 ± 0.3	77 ± 1.2
8	11.3 ± 1.4	60.5 ± 0.3	72 ± 1.7
16	0.3 ± 0.3	70.3 ±2.0	71 ± 2.2
24	0.0 ± 0.0	72 ± 1.3	72 ± 1.3
32	0.0 ± 0.0	74.5 ± 1.7	75 ± 1.7

± SEM

Daoud *et al.* (1999) have reported in case of *Sparitina alterniflora, Sporobolus spicatus, Salicornia europaea* that height of 45-day-old plants subjected to tap and 25 per cent sea water was greater than other saline treatments. On the other hand, maximum growth of *Artemisia maritima, Plantago maritima* and *Sporobolus madraspatanus* was noted in non-saline condition (Waisel 1972, Joshi and Misra 2000). Our data indicate that *H. setulosa* belongs to latter group of halophytes.

Amounts of water, ethanol and alkali soluble proteins varied between 8 to 147 mg g^{-1}dry wt in plants grown under non-saline and saline conditions (Table 3). There was initial increase in protein content in plants subjected to 10 and 20 dS m^{-1} sea-water, which later on declined in plants raised at higher salinity levels. These results, when compared with the protein content in young plants of other salt tolerant grasses namely, *Sporobolus*

Table 2. Effects of sea water salinity levels on growth of 60-day-old plants of *H. setulosa*.

	Salinity levels (dS m^{-1})				
	0	10	20	30	40
Root length (cm)	8.5 ± 0.4	6.9 ± 0.3	6.1 ± 0.3	5.7 ± 0.2	6.1 ± 0.3
Shoot length	10.6 ± 0.6	6 ± 0.4	5.2 ± 0.4	4.1 ± 0.3	4.6 ± 0.4
Fresh weight (mg $plant^{-1}$)	53.3 ± 5.8	16.2 ± 0.2	19.8 ± 1.2	12 ± 1.5	15.5 ± 0.9
Dry weight (mg $plant^{-1}$)	10.7 ± 0.9	4.1 ± 0.1	4.2 ± 0.3	3.2 ± 0.4	4.4 ± 0.3

± SEM

Table 3. Effects of sea water salinity levels on proteins, amino acids and sugars in 60-day-old plants of *H. Setulosa.*

	Salinity levels (dS m^{-1})				
	0	10	20	30	40
Proteins (mg g^{-1} dry wt.)					
		Shoots			
Water	20	21	30	20	18
Ethanol	32	46	60	37	37
Alkali	135	140	142	124	128
		Roots			
Water	9	9	11	8	8
Ethanol	14	17	15	16	16
Alkali	130	122	134	142	124
Amino acids (μg g^{-1} dry wt.)					
		Shoots			
Alanine	1394	1085	746	497	439
Asparagine	1298	2501	1750	3024	1961
Aspartic	4148	6217	5677	1131	4209
Glutamic acid	303	849	763	917	129
Glycine	804	859	625	554	443
Phenylalanine	1045	3882	1509	4211	3221
Proline	540	248	1786	1733	2283
Serine	705	1247	1041	1086	1102
Threonine	238	573	171	261	163
		Roots			
Alanine	743	415	130	270	361
Asparagine	888	1448	1176	1887	1807
Aspartic acid	2855	4259	4325	5119	3922
Glutamic acid	879	542	316	473	165
Glycine	866	1022	535	573	505
Phenylalanine	975	2303	1272	536	838
proline	383	147	1213	1327	2450
Serine	627	465	357	361	844
Threonine	173	223	217	197	209
Sugars (mg g^{-1} dry wt.)					
		Shoots			
Total sugars	7.7	6.7	6.5	4.7	5.2
Reducing sugars	2.6	1.9	1.8	1.6	1.7
Glucose	0.23	0.67	0/52	0.43	0.58
Galactose	1.1	0.53	0.89	0.41	0.16
Arabinose	1.07	0.49	0.7	0.23	0.3
		Roots			
Total sugars	7.9	7.5	6.7	4.6	3
Reducing sugars	2.2	2.1	2.1	1.8	1.2
Glucose	0.8	0.5	0.54	0.31	0.41
Galactose	1.4	0.64	0.64	0.5	0.63
Arabinose	0.7	0.74	0.69	0.36	0.41

madraspatanus (23 to 191 mg g^{-1}) and *Aeluropus lagopoides* (149 to 178 mg g^{-1}) show halophytic nature of *H. setulosa* (Joshi *et al.* 1996, Joshi and Misra 2000).

Accumulation of some free amino acids viz., asparagine, aspartic acid, phenylalanine, proline and serine in plants grown in 10 to 40 dS m^{-1} sea water was greater than those grown in control (Table 3). A reverse trend was observed for alanine, glutamic acid and glycine. Ahmad *et al.* (1981) observed an increase in concentrations of alanine, aspartic acid and to a lesser extent that of serine in *Puccinellia maritima* grown in 200-600 mM NaCl. Greater accumulation of the aforesaid amino acids was also observed in young plants of *A. lagopoides, J. mritimus, J. acutus,* and *S. madraspatanus* in response to increased sea water salinity in growth medium (Joshi *et al.* 1996, Joshi and Khairatkar 1995, Joshi and Misra 2000). Although osmoregulatory role of proline, glycine-betaine, sorbitol, pipeolique acid etc. have been elucidated (Popp *et al.* 1984), the physiological role of other amino acids occurring in quite high concentrations in salt tolerant plants including *H. setulosa* remains to be explained.

Amounts of total (4.7 to 7.7 mg g^{-1}), reducing (0.3 to 1.1 mg g^{-1}) and major sugars (Table 3) in shoots and roots of young plants generally decreased, excepting one or two cases in response to increasing sea water salinity. Rozema *et al.* (1978) and Gorham *et al.* (1981) observed a slight decrease in accumulation of sugars under salt stressed conditions in *Glaux maritima* and *Carex extensa.* Nevertheless, there are other halophytes, in which concentration of sugars increased under salt stressed condition (Joshi and Misra 2000). These and other contradictory reports calls for a need to classify sugars accumulating and non-accumulating halophytes so as to explain osmoregulatory role of sugars.

Accumulation of Na^+ varying between 0.61 to 1.17 meq g^{-1} and that of Cl^- between 0.7 to 1.69 meq g^{-1} (Table 4) in shoots and roots of young plants of *H. setulosa* was positively related with increase in salinity, whereas that of Ca^{2+}, Mg^{2+} and K^+ (Table 4) did not show any specific relationship with increase in salinity in growth medium. Furthermore, shoots accumulated more ions than roots. According to Joshi *et al.* (1996) and Joshi and Misra (2000), accumulation of Na^+ and Cl^- in vegetative organs of *A. lagopoides* and *S. madraspatamus* increased in response to increasing concentrations of sea water in

Table 4. Effects of sea water salinity levels on mineral ions (meq g^{-1} dry wt.) in 60-day-old plants of *H. setulosa.*

	Salinity levels (dS m^{-1})				
	0	**10**	**20**	**30**	**40**
			Shoots		
Na+	0.61	1.07	1.31	1.51	1.71
Cl^-	0.7	1.1	1.51	1.6	1.69
Ca^{2+}	0.35	0.55	0.49	0.45	0.39
Mg^{2}+	1	1.1	1.15	1.09	1.18
K^+	0.65	0.65	0.52	0.49	0.53
			Roots		
Na^+	0.45	0.75	0.99	1	1.32
Cl^-	0.5	0.72	0.91	1.09	1.23
Ca^{2+}	0.3	0.33	0.39	0.38	0.42
Mg^{2+}	0.89	0.8	0.83	0.88	0.95
K^+	0.35	0.27	0.3	0.25	0.32

growth medium. However, the Ca^{2+} and Mg^{2+} content were greater in control plants of *A. lagopoides* and K^+ concentrations did not reflect any specific relationship with salinity levels. Recently Abdelly *et al.* (1999) also reported high accumulation of Na^+ and Cl^- in *Spartina alternifolia.* Our findings on mineral composition also show such halophytic characteristics in *H. setulosa.*

From the present study it can be concluded that the seeds of *H. setulosa* failed to germinate even at moderate salinity of 8 dS m^{-1} sea water salinity but the young plants could withstand salinity up to 40 dS m^{-1} in growth medium. Therefore, if the species is to be introduced on saline wasteland, the young plants initially raised in nursery should be transplanted on saline soils with matching salinity levels.

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