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Comparative effects of salts and seawater on seed germination in halophytic grass *Halopyrum mucronatum*, Stapf

Sudhakar S. Khot* and Arvind J. Joshi

Department of Life Sciences, Bhavnagar University, Bhavnagar, Gujarat, India

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ABSTRACT

Halopyrum mucronatum, Stapf. is a coastal sand dune grass, growing along seacoast of India and Pakistan. It serves as strong sand binder and usually spread by stolons within established populations, but is capable of invading new areas through the dispersal of seeds. Seed germination is one of the important stages in the life cycle of halophytes as it determines establishment and existence of these species in saline conditions. Attempts were made to determine effects of 0 to 3 percent concentrations of NaCl, KCl, $CaCl_2$, $MgCl_2$, Na_2SO_4, $MgSO_4$ and seawater on seed germination behavior of the species. As a general trend, germination percentage as well as rate of germination decreased with increase in salinity. More than 99 % seeds germinated in non-saline control and the process was almost completely inhibited beyond 1 % NaCl, KCl, Na_2SO_4 and seawater. NaCl was most inhibitory salt while $MgSO_4$ exhibited least inhibitory effects among the studied salts. With few exceptions, recovery germination varied from 60 to 97.7 % irrespective of the salts used for pretreatment when ungerminated seeds in salt solutions were transferred to distilled water.

Key words: Seed germination, halophyte, *Halopyrum*, seawater, salt stress

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INTRODUCTION

Physicochemical factors such as salinity and flooding often are considered to be the determining factors controlling the establishment and zonation patterns of species in salt marsh and salt desert environment [1]. The halophytes are recognizably plants that survive high concentrations of electrolyte in their environments. These environments are normally dominated by NaCl, but may contain a variety of other salts, Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCl and Na_2CO_3 [2]. Germination is one of the critical stages in the life cycle of halophytes because the process decides their perpetuation in adverse ecological conditions [3, 4].

Although halophytes grow in highly saline conditions their seeds exhibit different levels of upper limit of salt tolerance during germination and the source from which seeds were obtained may be critical in determining their germination response when exposed to salt concentrations [5, 6]. Usually, higher salinities reduce the germination percentage and rate of germination [7, 8 and 9], however halophyte seeds does not lose viability and exhibit recovery germination when the stress conditions are alleviated.

Halopyrum mucronatum, Stapf., is a stoloniferous, perennial, coastal sand dune grass. It grows about 1 to 1.5 m in height and occupies the rarely inundated dune steppe having low salinities. It is a perennial grass which is the second most common species along the Arabian Sea coast after *Arthrocnemum macrostachyum* [10]. Within established populations, *H. mucronatum* can usually spread by stolons but it is capable of invading new areas through the dispersal of caryopses [8]. The fresh seeds showed more than 85 % germination in distilled water at room temperature (unpublished data). Surprisingly, seeds became dormant after 8-months-storage at room temperature. The dormancy was alleviated best at alternative temperature of 10^0 / 30^0 C (night / day). Khan and

Ungar observed that the optimum temperature for the germination of winter seeds of *Halopyrum mucronatum* was 20^0 / 30^0 C and for summer seeds was 25^0 / 35^0 C [8]. Germination promoting effects of alternate temperatures over constant temperature has also been reported for other halophytic grasses namely, *Aeluropus lagopoides* and *Sporobolus madraspatanus* [11].

The effects of various factors viz., salinity, temperature, their synergistic effects, light and of dormancy relieving compounds on seed germination behavior of *H. mucronatum* have been investigated [8, 12]. Likewise, considerable data on germination behavior of halophytes under NaCl stress is available [4]. However, little information is available about comparative effects of the salts that commonly found in saline soils on the germination of seeds [11, 13, 14]. The present investigations were therefore undertaken to monitor i) effects of various salts' treatments on germination, ii) a trend of inhibitory effects (if any) and iii) upper limit of salt tolerance and recovery behavior of *H. mucronatum* at seed germination.

MARETIALS AND METHODS

Seeds of *Halopyrum mucronatum* were harvested from mature inflorescence randomly collected from the plants growing on sand dunes at Narara Island (22^0 $27'$ N, 69^0 43' E) Marine National Park, Gulf of Kutch (India). They were selected for uniform size and color and stored at room temperature (32 ± 2 ^{0}C). Germination experiments were carried out at alternative temperature of 32 ± 2 ^{0}C during day and 10 ^{0}C during night with 12 hr photoperiod. Four replications of 50 seeds each were used for each treatment. The treatment was consisted of 0, 0.5, 1, 1.5, 2, 2.5 and 3 % concentrations of NaCl, KCl, $CaCl_2$, $MgCl_2$, Na_2SO_4, $MgSO_4$ and seawater. Seeds were presoaked in distilled water and respective test solutions for 12 hr and allowed to germinate in 20 cm diameter petridishes lined with filter papers moistened with respective concentrations of salts and distilled water. Germination (emergence of redicle) was recorded every alternative day for 16 days. Ungerminated seeds were transferred from test solutions to distilled water for recovery.

The recovery percentage (RP) was estimated by the following formula:

$$\mathrm{RP} = \frac{b}{c - a} \times 100$$

Where, a = Total number of seeds germinated under salt stress; b = Total number of seeds recovered in distilled water; and c = total number of seeds used.

The rate of germination was estimated by a modified Timson's Index.

Germination velocity (GV) = $(\sum G) / t$,

Where, G is the % seed germination at 2-day interval and 't' is the total germination period (16 days).

The data were subjected to statistical analysis. 2-way ANOVA was computed using MS-excel (Office 2000) to find out whether the effects of various salts and concentrations were significant. Least significant difference (LSD) test was further conducted if the ANOVA differences were significant.

RESULTS AND DISCUSSION

Germination under control: More than 99 % seeds germinated in non-saline control (Table. 1) and the process was adversely affected with increase in the stress of all but $CaCl_2$ and $MgSO_4$ salts. No germination was recorded beyond 2 % concentrations of NaCl, KCl, Na_2SO_4 and seawater. A 2-way ANOVA suggested that effects of different concentrations ($F=23.9$; $P<0.01$) and of 7 salts ($F=12.2$; $P<0.01$) on germination differed significantly. LSD test showed that germination was significantly decreased even in 0.5 % concentrations of NaCl, KCl, $MgCl_2$ and Na_2SO_4 (Table. 1).

Complete inhibition of or noticeable poor seed germination even in low concentrations of the salts clearly indicated that seeds of *H. mucronatum* were extremely salt sensitive. The species collected from Pakisthan coast also exhibited similar responses at seed germination [10]. The monocotyledonous halophytes were found to be sensitive to salt [5, 11, 15]. Similar behavior was noted for halophytic grasses namely, *Spergularia marina* [7], *Heleochloa setulosa* [16] and *Aeluropus lagopoides* [11] and *Chloris barbata* [17]. In contrast, seeds of *Juncus maritimus* showed 75 % germination in seawater at 26 ^{0}C [18] and 92 % germination in 24 $dS.m^{-1}$ seawater [15]. More than 60 % germination was recorded in 150 mM NaCl at 20 ^{0}/ 30 ^{0}C temperature for the seeds of *H. mucronatum* collected from Karachi coast [8]. It appears from the present investigations that not only various halophytes but different grasses also differ in the upper limit of salt endurance during germination.

Table. 1. Effects of salts on seed germination in *Halopyrum mucronatum* (mean ± SE of 4 replications)

Conc.	NaCl	KCl	$CaCl_2$	$MgCl_2$	Na_2SO_4	$MgSO_4$	Seawater
0 %	99.5 ± 0.25	99.5 ± 0.25	99.5 ± 0.25	99.5 ± 0.25	99.5 ± 0.25	99.5 ± 0.25	99.5 ± 0.25
0.5 %	62.5 ± 2.17**	90.5 ± 2.59**	98.5 ± 0.48^{ns}	62 ± 2.55**	80 ± 2.27**	98.5 ± 0.48^{ns}	94.5 ± 1.44^{ns}
1 %	9 ± 1.32**	35 ± 0.96**	97.5 ± 0.75^{ns}	58 ± 3.14**	54.5 ± 4.23**	99 ± 0.29^{ns}	39 ± 3.77**
1.5 %	0**	8 ± 0.91**	78 ± 3.19**	26 ± 1.58**	20.5 ± 3.97**	97 ± 0.5^{ns}	0.5 ± 0.25**
2 %	0**	1 ± 0.29**	39.5 ± 3.64**	13 ± 2.22**	2.5 ± 0.75**	98.5 ± 0.48^{ns}	0**
2.5 %	0**	0**	20.5 ± 3.25**	3.5 ± 0.75**	0**	93.5 ± 1.65^{ns}	0**
3 %	0**	0**	2.5 ± 0.95**	0.5 ± 0.25**	0**	82.5 ± 4.03**	0**
CD crit.							
p= 0.05	6.13	6.40	14.44	7.86	12.82	10.4	8.8
p=0.01	8.41	8.78	19.82	10.78	17.58	14.28	12.08

*^{ns}=non-significant; *=significant at p=0.05; **=significant at p=0.01.*

Table. 2. Effects salts on rate of germination (modified Timson's Index) in *Halopyrum mucronatum*

Conc.	NaCl	KCl	$CaCl_2$	$MgCl_2$	Na_2SO_4	$MgSO_4$	Seawater
0 %	21.38	21.38	21.38	21.38	21.38	21.38	21.38
0.5 %	9.56**	14.91**	21.27	9.73**	13.61**	20.73	17.69**
1 %	1.33**	4.58**	19.64	7.89**	6.16**	21.13	5.7**
1.5 %	0**	0.95**	10.78**	3.16**	2.44**	19.06**	0.08**
2 %	0**	0.08**	4.48**	1.69**	0.16**	19.59**	0**
2.5 %	0**	0**	2.78**	0.38**	0**	17.53**	0**
3 %	0**	0**	0.31**	0.06**	0**	14.11**	0**
CD crit.							
p = 0.05	1.49	1.39	2	1.85	1.86	1.99	1.39
p = 0.01	2.04	1.91	2.74	2.53	2.55	2.73	1.91

*^{ns}=non-significant; *=significant at p=0.05; **=significant at p=0.01.*

Rate of germination: Maximum rate of seed germination (GV=21.38) was recorded in distilled water and it was decreased with increasing concentrations of all salts (Fig. 1). The rate of germination was significantly affected in 7 salts ($F=14.97$, $P<0.001$) and in various concentrations ($F=30.57$, $P<0.001$). LSD test suggested that for all salts' concentrations the difference in the germination rate was statistically significant when compared with control except up to 1 % $CaCl_2$ and $MgS0_4$ (Table. 2).

While working on *H. mucronatum* growing at Karachi coast, Khan and Ungar noted that velocity of germination for all temperature regimes was reduced with increasing NaCl concentrations when compared with that in control [8]. Germination rate in inland halophytes *Hordeum jubatum, Spergularia marina, Atriplex prostrata* and *Suaeda calceoliformis* was reduced with increasing NaCl salinity and the reduction in rate was prominent for the last 2 species [7]. Guan *et al.* (2010) noted that in two species of *Suaeda* the seed germination rate was highest in non-saline controls and it decreased with the increase of salinity [19]. Present studies too showed that, for all 7 salts, rate of germination was reduced with increase in salt concentrations and the reduction was statistically significant when compared with the rate in distilled water. Furthermore, 7 salts differed significantly in their extent of inhibitory effects on the rate of germination.

Comparison between effects of different salts: Among the 7 salts tested, chlorides of Na and K along with seawater and Na_2SO_4 caused more inhibitory effects than the chlorides of Mg and Ca. The adverse effects of various salts in order from least to most inhibitory were as: $MgSO_4 < CaCl_2 < MgCl_2 < Na_2SO_4 <$ KCl ≠ seawater < NaCl. Recently, Zehra *et al.* (2012) showed that Ca alleviated the effects of NaCl salinity on seed germination of *Phragmites karka* at lower temperature regimes[20]. Vyas and Joshi (2013) noted that salts of chloride were more inhibitory than sulphide salts to *Chloris barbata* [17]. Similar results were reported for *Aeluropus lagopoides* and *Sporobolus madraspatanus* [11]. NaCl, $CaCl_2$, Na_2SO_4 and seawater concentrations were more inhibitory to *Prosopis juliflora* than KCl, $MgCl_2$ and $MgSO_4$ [13]. Chlorides of Na and K were more inhibitory to succulent halophyte *Haloxylon salicornicum* [14]. However, Macke and Ungar (1971) and Ryan *et al.* (1975) noticed no consistent effects of various salts on seed germination of grass species [21, 22].

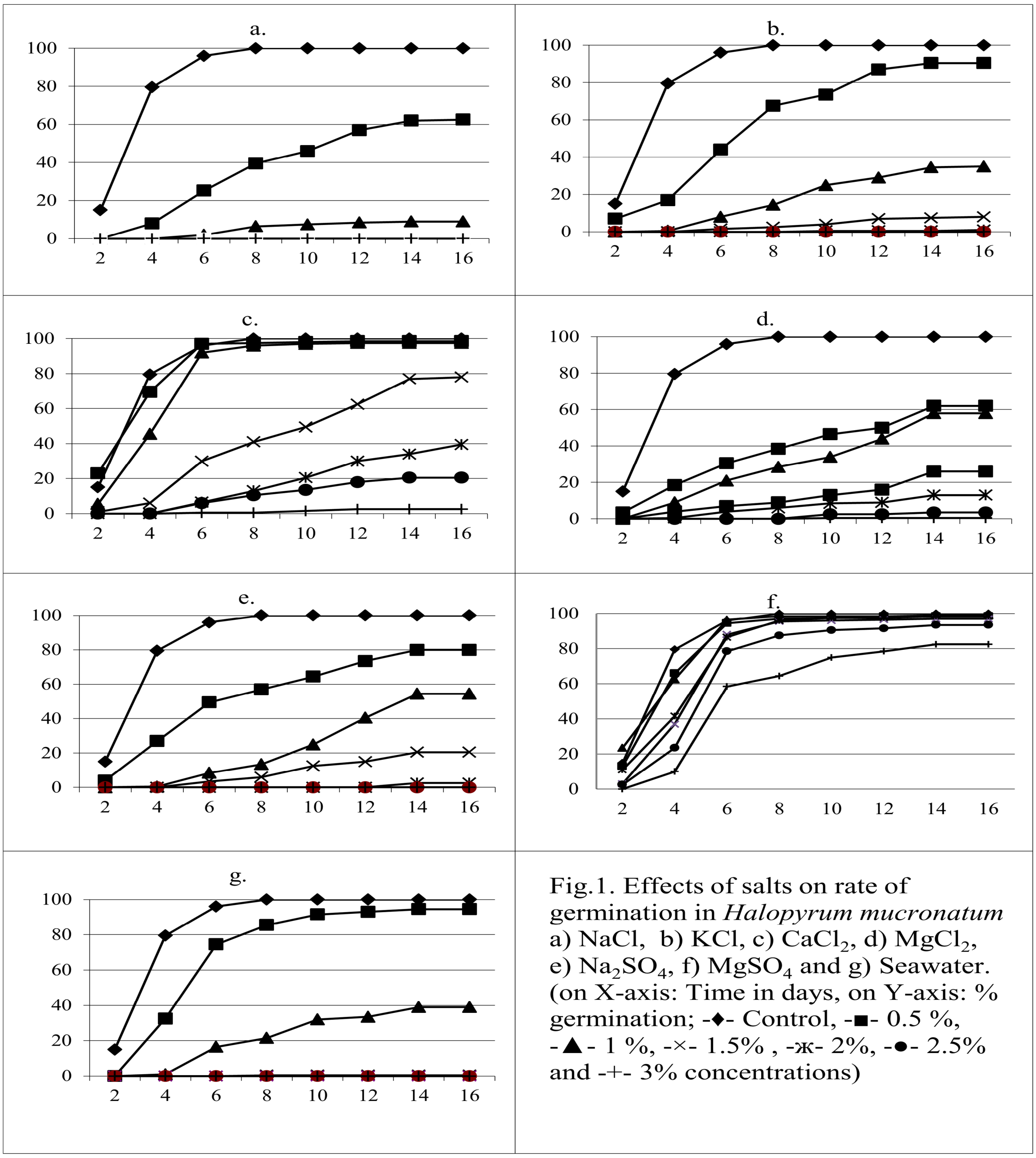

Fig.1. Effects of salts on rate of germination in *Halopyrum mucronatum* a) NaCl, b) KCl, c) $CaCl_2$, d) $MgCl_2$, e) Na_2SO_4, f) $MgSO_4$ and g) Seawater. (on X-axis: Time in days, on Y-axis: % germination; -◆- Control, -■- 0.5 %, -▲- 1 %, -×- 1.5% , -ж- 2%, -●- 2.5% and -+- 3% concentrations)

Table. 3. Recovery germination in distilled water for *Halopyrum mucronatum* pretreated with salt dilutions (mean ± SE of 4 replications).

Conc.	NaCl	KCl	$CaCl_2$	$MgCl_2$	Na_2SO_4	Seawater
0.5 %	75 ± 4.3	86.7 ± 13.3	0	41.9 ± 6.7	79.4 ± 9.8	50 ± 28.9
1 %	83.9 ± 1.5	97.7 ± 1.4	0	62.2 ± 1.1	81.7 ± 2.6	92.7 ± 2.6
1.5 %	86.6 ± 5.6	89.8 ± 7.2	87.5 ± 4.8	75.2 ± 6.2	89.7 ± 4.4	91.3 ± 1.1
2 %	73.6 ± 4.1	89.5 ± 7	92.8 ± 3.6	80.1 ± 5.1	89.3 ±1.6	74.1 ± 2.9
2.5 %	68.6 ± 1.6	87.5 ± 8.9	90.4 ± 1.4	71.7 ± 4.4	81.5 ± 3.7	60 ± 5
3 %	84 ± 4.1	84.1 ± 5.7	90.8 ± 2.6	77.4 ± 4	76 ± 3.4	59 ± 5.2

Recovery germination: The recovery germination reached up to 97.7 % when ungerminated seeds form salt treatments were transferred to distilled water (Table. 3). The less recovery germination recorded for few cases may be due to less number of seeds that were transferred from salt treatments. Effects of salt concentrations as well as that of different salts on recovery percentage were statistically non-significant (F=1.83, P=0.14 for concentrations and F=1.63, P=0.19 for salt kinds). Rate of recovery germination was higher in the seeds transferred from 1.5 % and above concentration compared to those transferred from lower concentrations (Table. 4). However, it remained less

(GV≤17.58) than that in non-saline control treatment. Statistical analysis suggested significant effects of different concentrations ($F=8.48$; $P<0.01$) used for pretreatment. However, the rate of recovery did not varied significantly due to difference in the salts used for pretreatments (Table.5).

Table. 4. Rate of recovery germination (modified Timson's Index) in distilled water for *Halopyrum mucronatum*

Conc.	NaCl	KCl	$CaCl_2$	$MgCl_2$	Na_2SO_4	Seawater
0.5 %	4.4	2.72	0	2.85	3.44	0.79
1 %	14.79	14.35	0	4.46	8.06	11.5
1.5 %	14.63	17.1	4.1	10.17	14.52	15.73
2 %	10.17	17.58	12.67	13.63	17	12.06
2.5 %	10.38	14.19	14.77	11.77	13.35	8.98
3 %	11.71	11.46	17.04	11.63	11.27	8.32

Table. 5. Two-factorial analysis of variance for various phenomenon to determine the effects of concentrations and kinds of salts

Source of Variation	Concentrations		Salts	
	F	P-value	F	P-value
Germination under stress	23.9**	3.54E-11	12.2**	1.83E-07
Rate of germination under stress	30.57**	1E-12	14.97**	1.67E-08
Recovery germination	1.83 ns	0.14	1.63 ns	0.19
Rate of recovery germination	8.48**	8.44E-05	1.42 ns	0.25

Dormancy in seeds of halophytes is a significant factor in the ecophysiology of salt marsh species. It permits seeds to remain viable in the soil during periods when the environment is not suitable for germination [23]. The seeds of *H. mucronatum* also exhibited this characteristic behavior of halophytes. Baring few exceptions, the grass showed about 60 to 97.7 % recovery germination irrespectively to the salt used to impose stress. Earlier, while working on 4 halophytes, Pujol *et al.* (2000) observed that the recovery germination of seeds did not differed significantly from the germination recovery in distilled water controls, irrespective of the iso-osmotic concentrations of 4 salts used to impose the stress [24]. Furthermore, they noted that such osmotic pretreatments promoted the rate of recovery germination in *Arthrocnemum macrostachyum* and *Sarcocornia fruticosa* to double of their rate of germination in non-saline control. Likewise, seeds of succulents namely *Salicornia europaea, Suaeda calceoliformis* and non-succulent *Spergularia marina* also exhibited more rapid germination rates following prolonged exposure to 3 and 5 % NaCl treatment [7]. In contrast, for *H. mucronatum*, the rate of recovery germination never exceeded the rate of germination in distilled water.

CONCLUSION

The present investigation showed that even though the seeds of *H. mucronatum* do not lose their viability during salt stress, they were much sensitive to osmotic stress caused by various salts including seawater. This phenomenon explains why the plants reproduce by vegetative means rather than by seeds under natural conditions. These studies further revealed that *H. mucronatum* belongs to the Type 1 of salt tolerant plants as per Woodell's modified classification of halophytes [25]. (Type 1: Dune or drift line that is rarely inundated. Plants may only be submerged in salt water during dispersal. Germination may be completely inhibited or inversely proportional to salinity. Recovery: germination rose to quite high levels, but all percentage was lower than in fresh water). NaCl and $MgSo_4$ were most and least inhibitory salts to the species. However, further study is necessary to understand synergistic effects of various salts.

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