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SEED GERMINATION, AMINO ACIDS AND SUGARS IN SEEDLINGS OF *JUNCUS MARITIMUS* AND *J. ACUTUS* UNDER SALT STRESS

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69% seeds of *J. maritimus* germinated in 24 dS.m^{-1} seawater but higher concentrations inhibited the process. Recovery of the seeds subjected to 32 and 48 dS.m^{-1} seawater was quite high. The Australian species *J. acutus* exhibited only 17% germination in 8 dS.m^{-1} and no germination beyond this level. Alanine, asparagine, aspartic acid, glutamic acid, phenylalanine, proline, glutamine, glycine, serine and threonine constituted major fraction of amino acids in 40 day old seedlings. Concentrations of asparagine, aspartic acid, glutamic acid and proline increased while those of glycine, phenylalanine and serine decreased in response to salinity stress. However, the total amount of the amino acids increased under saline condition. Seawater caused a slight reduction in total and reducing sugars and in amounts of glucose, galactose and arabinose in both the species.

Key Words : Germination, amino acids, *J. maritimus*, *J. acutus* Salt stress.

Although large number of halophytes contribute to the biology of maritime areas on 5700 km. long coastline of India, their ecophysiology is not fully understood. Similarly, investigations on effects of salinity on germinability followed by early establishment of salt tolerant plants are not many (Joshi, 1986). Such studies are inevitable if halophytes having economic potential are to be recommended for reclamation of saline wastelands which spread in about 8 million hectares in our country. The present investigation was undertaken to examine effects of seawater salinity on germination and seedling growth of *J. maritimus* and *J. acutus*, which can be used in paper industries like other species of *Juncus* (Zahran and Wahid, 1982).

MATERIALS AND METHODS

Seeds of *J. maritimus* were collected from Ghogha (21°45' N 27° 14' E) while those of *J. acutus* from the Grazing Valley in Western Australia by the senior author in 1984. Seeds of uniform size, shape and colour were selected with the help of magnifying glass because of their minute size. Seawater dilutions (8 to 48 dS.m^{-1}) were prepared by mixing distilled water and natural seawater. Five replications of fifty seeds each were maintained and the experiments were conducted at control temperature (25±1°C) in continuous illumination. The data were statistically analysed for the standard error of mean.

The seedlings were raised from the seeds in the plastic pots containing acid washed sand and having holes at the bottom for uniform soaking of sand bed, at 25±1°C in continous illumination. After hot water (60°C) treatment the seeds were first subjected to 4dS.m^{-1} seawater, the concentration of which was gradually increased to 20 dS.m^{-1} with an increment of 4dS.m^{-1} per week. The seedlings were collected after 40 days. However, seeds of *J. acutus* did not require hot water treatment before sowing in pots and seedlings could withstand salinity up to 8dS.m^{-1}.

Table 1: The effects of seawater dilutions on germination in *J. maritimus*. Each value respresents mean±SEM of four replications.

	% untreated			% Hot water treatment		
Treatments. dS.m^{-1}	Germination 22 days	Recovery 18 days	Total 40 days	Germination 22 days	Recovery 18 days	Total 40 days
0	98±1.0	-	98	100	-	100
8	86±4.8	15±4.2	91	98±0.5	1.±0.2	99
16	79±5.0	18±4.9	97	95±0.6	5.±0.6	100
24	69±1.3	30±1.8	99	92±0.9	6.5±1.2	98
32	8±2.4	88±2.2	96	7±0.7	88±0.8	95
48	-	95±0.9	95	-	86±4.1	86

The method of extraction and estimation of free amino acids described in detail elsewhere (Joshi, 1986) was followed. Total and reducing sugars were estimated respectively by anthrone reagent and by the method of Umbreit *et al.* (1959), whereas for estimation of ethanol soluble sugars paper chromatographic method (Khairatkar, 1986) was followed. Na^+ and K^+ were estimated by flame photometry; Ca^{2+} and Mg^{2+} by EDTA titration and chloride on chloride meter Elico EE 34.

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Table 2: The effects of seawater dilutions on germination in *J. acutus*. Each value represents mean±SEM of four replications.

Treatments dS.m⁻¹	% Germination 40 days	Recovery 12 days	Total 32 days
0	80±4.3	-	80
8	17±4.7	59±20.8	76
16	-	62±11.4	62
24	-	63±15.3	63
32	-	79±4.7	79
48	-	-	-

Table 3: Accumulation of free amino acids (μl.g⁻¹d.m) in 40 day old seedlings of *J. maritimus* and *J. acutus* and in leaves of *J. maritimus*.

Amino acids	*J. maritimus*		*J. acutus*		*J. maritimus* leaves	
	Con-trol	20 dS.m⁻¹	Con-trol	20 dS.m⁻¹	Young	Mature
Alanine	450	269	683	1600	206	86
Asparagine	2128	5400	202	1023	256	271
Aspartic acid	2524	4083	4316	11183	294	364
Glutamic acid	536	565	628	1850	94	119
Glutamine	467	1619	567	Tr	90	113
Glycine	1033	450	465	Tr	203	128
Isoleucine	56	Tr	Tr	350	25	18
Leucine	139	Tr	Tr	333	50	39
Methionine	-	-	782	717	84	61
Phenylalanine	1599	708	2550	272	195	125
Proline	816	1024	1316	2224	366	549
Serine	650	533	619	Tr	106	54
Threonine	133	233	283	-	85	24
Valine	-	-	412	494	54	40

RESULTS

Results of seed germination (Table1) showed that 98% seeds of *J. maritimus* germinated in distilled water and the process was remarkably inhibited in higher concentrations of seawater. However, recovery percentage were progressively greater for those seeds which were exposed to 8 to 48 dS.m⁻¹ seawater. It was further evident that hot water (60°C) treatment had favourable effects on germination and 92% seeds germinated even in 24 dS.m⁻¹ salinity.

Only 17% seeds of *J. acutus* germinated in 8dS.m⁻¹ seawater and no germination beyond this salinity level (Table 2) reflected salt sensitive nature of the species. High percentage of recovery of ungerminated seeds suggested that process was inhibited due to osmotic effects of sea water dilutions. The seeds lost their capacity to recover after exposure to 48dS.m⁻¹.

Table 4: Accumulation of sugars (mg.g⁻¹d.m) in 40 days old seedlings of *J. maritimus* and *J. acutus* and in leaves of *J. maritimus*.

Species	Treat-ment	Total	Reduc-ing	Glucose	Galac-tose	Arabi-nose
J. maritimus	Control	43	18	3.0	4.5	3.3
	20dS.m⁻¹	38	16	1.67	3.16	1.33
J. acutus	Control	40	16	5.5	2.5	6.67
	8 dS.m⁻¹	36	15	2.67	7.0	Tr
J. maritimus	Leaves (Natural habitat)					
	Young	15.0	7.55	4.65	1.44	1.21
	Mature	13.2	6.62	3.14	1.26	0.87

Table 3 represents observations on amino acids in seedlings grown in non-saline and saline conditions. A remarkable increase in accumulation of asparagine, aspartic acid, glutamic acid and proline in both the species was noticed under seawater stress. In contrast, concentrations of glycine, phenylalanine and serine decreased in saline condition.

Accumulation of total and reducing sugars and that of glucose, galactose and arabinose in both the species was adversely affected by seawater salinity (Table 4). Attempts were also made to compare the amounts of free amino acids and sugars in the seedlings and leaves collected from plants growing in nature. These results (Tables 3,4) evidently showed greater concentrations of asparagine, aspartic acid, phenylalanine and of some other amino acids including proline in seedlings than in leaves of *J. maritimus* growing in saline habitat. Accumulation of sugars was also greater in seedlings. Thus, seedlings of two species of *Jancus* contained greater amounts of organic metabolites than mature leaves collected from nature.

DISCUSSION

Chapman (1974) reported that 50 and 18% seeds of *J. maritimus* germinated respectively in distilled water and in 1% NaCl but later on Rozema (1976) observed 75% germination in seawater *per se* at 26°C under flooded condition. Although 100% increase in germination in distilled water over the earlier report (Chapman, 1974) was observed during present study, germination in 32 dS.m⁻¹ seawater was quite less than reported by Rozema (1976). This evidently shows that seeds of *J. maritimus* collected from different continents have different degrees of salt tolerance.

Zahran (1975) had recorded 15 and 5% germination of *J. acutus* in 0.5 and 1% NaCl. Quite similar behaviour by the seeds of the same species collected from the Western Australia was observed (Table 2). Recovery of germination in distilled water (Tables 1,2) further suggests that temporary inhibition of the process was due to osmotic effects of seawater. These findings also explain the complete failure of seed germination of *J. maritimus* in nature as essential submerged condition hardly exists in habitats.

The present investigation (Table 3) evidently showed that seedlings of two *Juncus* species contained all major amino acids occurring in mangroves, succulent halophytes and halophytic grasses (De La Cruz and Poe, 1975; Popp *et al.*, 1984; Joshi and Sagar Kumar, 1989) and their concentrations were remarkably greater than plants growing in nature (Khairatkar, 1986). Moreover, some amino acids including alanine, aspartic acid, glutamic acid, proline, glycine and serine have protective effects on cell membrances in seedlings in halophytes (Tyankova, 1970; Popp *et al.*, 1984). Thus, an increase in amounts of these amino acids in seedlings of *J. maritimus* and *J. acutus* would be of survival value under salt stressed condition.

Experimental evidences indicate either increase or decrease (Rozema, 1976; Gorham *et al*, 1981) or no effects (Jefferies *et al.*, 1979) on accumulation of sugars in halophytes when they are exposed to salt stressed condition. Our findings suggests that *J. maritimus* and *J. acutus* belong to the second group of halophytes and that sugar metabolism in halophytes has species specific response to salinity.

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