

Effects of short-term NaCl stress on water relations and gas exchange of two jute species

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Abstract

Thirty-day-old seedlings of two jute species (*Corchorus capsularis* L. cv. JRC 212 and *C. olitorius* L. cv. JRO 632) were subjected to short-term salinity stress (160 and 200 mM NaCl for 1 and 2 d). Relative water content, leaf water potential, water uptake, transpiration rate, water retention, stomatal conductance, net photosynthetic rate and water use efficiency of both jute species decreased due to salinity stress. The decrease was greater in *C. olitorius* than in *C. capsularis* and with higher magnitude of stress. Greater accumulation of Na^+ and Cl^- and a lower ratio of K^+/Na^+ in the root and shoot of *C. olitorius* compared with *C. capsularis* were also recorded. Pretreatment of seedlings with kinetin (0.09 mM), glutamic acid (4 mM) and calcium nitrate (5 mM) for 24 h significantly improved net photosynthesis, transpiration and water use efficiency of salinity stressed plants, the effect being more marked in *C. olitorius*. Among the pre-treatment chemicals, calcium nitrate was most effective.

Additional key words: ion accumulation, leaf water potential, net photosynthetic rate, relative water content, stomatal conductance, transpiration rate, water use efficiency.

Introduction

For clarifying the mechanism of salt tolerance and for improving salt tolerance of crop plants, it is necessary to identify the physiological and biochemical responses of crop species to short-term sublethal salinity. It should be borne in mind that several responses may be species or even cultivar specific.

Salinity-induced cellular disturbances may be caused by both osmotic and ionic effects (Greenway and Munns 1980, Prat and Fathe-Ettai 1990, Alarcon *et al.* 1993),

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Abbreviations. E - transpiration rate; g_s - stomatal conductance; LWP - leaf water potential; P_N - net photosynthetic rate; RWC - relative water content; WUE - water use efficiency.

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although it is not always possible to demarcate which of the two is more damaging. Most of the studies on salinity stress with crop plants were carried out with continuously treated plants which might not reflect the immediate responses of plants subjected to salinity stress for a short duration.

The present study was therefore undertaken to analyse the responses of two jute species (the important fibre crop of India and Bangladesh) to short-term NaCl stress to assess their NaCl tolerance and to find out the possible reason for differences in tolerance between the two. Besides, effects of kinetin, glutamic acid and calcium nitrate in ameliorating the deleterious effects of salinity were also examined.

Materials and methods

Healthy uniform seeds of *Corchorus capsularis* L. cv. JRC 212 and *C. olitorius* L. cv. JRO 632 were sown in earthen pots, each containing 4 plants. After 30 d, plants were carefully uprooted, adhering soil particles were removed by washing thoroughly with distilled water and then separately dipped in distilled water (control) and in 160 and 200 mM NaCl solution (osmotic potentials -0.8 and -1.0 MPa, respectively) for 1 and 2 d. For pre-treatment study, 30-d-old seedlings were firstly immersed in solution of kinetin (0.09 mM), glutamic acid (4 mM) or calcium nitrate (5 mM), separately for 24 h. Thus there were four different sets of seedlings: 1) untreated control, 2) pretreated control, 3) NaCl stressed seedlings, and 4) pretreated and NaCl stressed seedlings. Each set contained ten seedlings and after treatments, the plants were thoroughly washed and analysed for the following parameters.

RWC was measured gravimetrically. 25 leaf discs of 1 cm diameter were floated onto distilled water for 5 h at 25 °C and RWC was determined following the formula of Barrs and Weatherley (1962). LWP was estimated using Scholander pressure bomb (Scholander *et al.* 1965).

Water uptake, transpiration and water retention were simultaneously measured gravimetrically according to the method described by Chowdhury and Choudhuri (1987). To prevent evaporation, the mouth of each vial (30 cm³) was covered with a polythene sheet through which one seedling was introduced into the vial. The initial weights of the set-up without plant (W1) and with plant (W2) were recorded and the whole set-up was kept in the laboratory under natural light for transpiration. After 24 h, the weight of the whole set-up was again recorded (W3). Finally, the weight of the vial without plant was recorded (W4) and the following calculations were made:

amount of water uptake = W1-W4

amount of water transpired = W2-W3

amount of water retained = (W1-W4) - (W2-W3).

Stomatal aperture (pore width) was measured according to Weyers and Travis (1984) on epidermal peels made from the leaf abaxial surface. K⁺ content of the guard cell was determined according to Willmer and Mansfield (1970). The epidermal peels from the abaxial surface of leaves were immersed in ice-cold freshly prepared sodium-cobalt nitrite reagent for 45 min at 0 °C. The tissue was then washed in ice-cold distilled water fixed in 10 % ammonium sulphide solution at 0 °C

for 10 min and rinsed in ice-cold water thoroughly. The peelings were then mounted on a slide for observation under microscope. The amount of K^+ was expressed as the percentage black stained area occupied in the guard cell.

Net photosynthetic rate, transpiration rate and leaf diffusive conductance were simultaneously measured with a *Licor-6200 Portable Photosynthetic System* (Licor, Lincoln, USA) equipped with a chamber (1.0 dm^3). The leaf lamina was totally enclosed within a cuvette being stirred continuously with a fan inside. Ten consecutive measurements of each parameter were taken at intervals of 5 s. The CO_2 analyser was calibrated daily with a series of CO_2 : air mixtures. The WUE was calculated as the ratio of CO_2 assimilation and transpiration rates.

For estimation of Na^+ and K^+ , shoot and root parts of the seedlings were separately dried and ground in 1 M HCl. The mixture was then filtered and Na^+ and K^+ contents were estimated by flame photometric method (Prat and Fathe-Ettai 1990). Chloride was estimated titrimetrically following the method of Ross (1984).

Results

Short-term salinity stress reduced both RWC and LWP in the two jute species, the effect being greater with higher NaCl concentration and duration of stress. The extent of reduction in either parameter was marginally greater in *C. olitorius* than in *C. capsularis* (Table 1).

Table 1. Effects of short-term NaCl salinity stress on relative water content (RWC) and leaf water potential (LWP) of 30-d-old seedlings of two jute species (mean \pm S.E.; $n = 10$).

NaCl concentration [mM]	Duration of stress [d]	<i>Corchorus capsularis</i>		<i>Corchorus olitorius</i>	
		RWC [%]	LWP [MPa]	RWC [%]	LWP [MPa]
0	1	95.21 \pm 0.27	-0.60 \pm 0.05	94.23 \pm 0.15	-0.66 \pm 0.09
	2	93.5 \pm 0.35	-0.64 \pm 0.03	91.00 \pm 0.23	-0.69 \pm 0.07
160	1	93.25 \pm 0.14	-0.84 \pm 0.05	88.54 \pm 0.17	-0.99 \pm 0.05
	2	90.88 \pm 0.18	-1.2 \pm 0.06	83.25 \pm 0.11	-1.32 \pm 0.14
200	1	89.21 \pm 0.25	-0.90 \pm 0.08	79.55 \pm 0.21	-1.2 \pm 0.12
	2	89.32 \pm 0.13	-1.38 \pm 0.07	72.25 \pm 0.25	-1.56 \pm 0.13

The uptake of water was more significantly inhibited in *C. olitorius* than in *C. capsularis* over controls due to salinity stress. The rate of transpiration was also reduced by salinity stress but more severely in *C. capsularis*. The amount of water retained was negative in both the species exposed to salinity stress but it was marginal in *C. capsularis* and more marked in *C. olitorius* which showed wilting (Table 2).

Table 2. Effects of short-term [24 h] NaCl salinity stress on water uptake, water transpired and water retained [g d^{-1}] in 30-d-old seedlings of two jute species after imposition of stress (160 and 200 mM NaCl) (mean \pm S.E., $n = 10$).

NaCl concentration [mM]	<i>Corchorus capsularis</i>			<i>Corchorus olitorius</i>		
	Water uptake	Water transpired	Water retained	Water uptake	Water transpired	Water retained
0	2.70 \pm 0.12	2.50 \pm 0.15	0.20 \pm 0.03	2.65 \pm 0.15	2.55 \pm 0.13	0.10 \pm 0.12
160	0.75 \pm 0.02	0.79 \pm 0.02	-0.04 \pm 0.02	0.55 \pm 0.08	1.25 \pm 0.03	-0.70 \pm 0.03
200	0.69 \pm 0.03	0.75 \pm 0.06	-0.06 \pm 0.03	0.44 \pm 0.06	1.18 \pm 0.12	-0.74 \pm 0.02

Stomatal aperture, guard cell K^+ content and stomatal conductance (g_s) of the two jute species declined under salinity stress and the decline was more pronounced in *C. capsularis* than in *C. olitorius* (Table 3)

Table 3. Effect of short-term NaCl salinity stress on the relative potassium content of guard cells [% stained area], stomatal aperture [μm^2] and stomatal conductance [$\text{mol m}^{-2} \text{s}^{-1}$] of 30-d-old seedlings of two jute species (mean \pm S.E., $n = 10$).

NaCl [mM]	Duration of stress [d]	<i>Corchorus capsularis</i>			<i>Corchorus olitorius</i>		
		K^+ content	Stomatal aperture	Stomatal conductance	K^+ content	Stomatal aperture	Stomatal conductance
0	1	58 \pm 0.21	6.5 \pm 0.15	8.015 \pm 0.09	61 \pm 0.13	6.9 \pm 0.12	8.012 \pm 0.11
	2	55 \pm 0.15	6.3 \pm 0.11	7.762 \pm 0.05	60 \pm 0.11	6.8 \pm 0.09	7.514 \pm 0.08
160	1	19 \pm 0.22	5.8 \pm 0.13	3.113 \pm 0.11	29 \pm 0.09	6.5 \pm 0.11	3.678 \pm 0.07
	2	17 \pm 0.16	5.7 \pm 0.17	3.014 \pm 0.07	27 \pm 0.15	6.4 \pm 0.16	3.222 \pm 0.12
200	1	12 \pm 0.13	4.5 \pm 0.12	3.013 \pm 0.06	25 \pm 0.17	5.8 \pm 0.13	3.159 \pm 0.05
	2	10 \pm 0.11	4.2 \pm 0.05	2.052 \pm 0.12	23 \pm 0.12	5.7 \pm 0.10	2.558 \pm 0.07

Table 4. Effects of short-term NaCl salinity stress on net photosynthetic rate, P_N [$\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$], transpiration rate, E [$\text{mol}(\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$] and water use efficiency (WUE) [$\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}(\text{H}_2\text{O})$] in 30-d-old seedlings of two jute species (mean \pm S.E., $n = 10$).

NaCl [mM]	Stress [d]	<i>Corchorus capsularis</i>			<i>Corchorus olitorius</i>		
		P_N	E	WUE	P_N	E	WUE
0	1	29.151 \pm 0.85	0.0795 \pm 0.02	0.341 \pm 0.06	28.951 \pm 0.45	0.0872 \pm 0.01	0.332 \pm 0.09
	2	26.923 \pm 0.31	0.0713 \pm 0.01	0.377 \pm 0.11	26.053 \pm 0.83	0.0810 \pm 0.02	0.322 \pm 0.07
160	1	7.510 \pm 0.27	0.0571 \pm 0.01	0.131 \pm 0.09	5.618 \pm 0.83	0.0592 \pm 0.03	0.095 \pm 0.12
	2	5.751 \pm 0.19	0.0568 \pm 0.03	0.101 \pm 0.07	4.051 \pm 0.65	0.0575 \pm 0.02	0.070 \pm 0.08
200	1	4.928 \pm 0.23	0.0546 \pm 0.02	0.090 \pm 0.03	2.199 \pm 0.25	0.0576 \pm 0.01	0.038 \pm 0.11
	2	3.242 \pm 0.46	0.0537 \pm 0.02	0.060 \pm 0.02	1.659 \pm 0.18	0.0557 \pm 0.04	0.029 \pm 0.07

Net photosynthetic rate (P_N), transpiration rate (E) and water use efficiency (WUE) greatly declined, higher magnitude and duration of stress having greater

effects. The decline in the above parameters was more pronounced in *C. olitorius* than in *C. capsularis* (Table 4.).

Under NaCl stress there was a marked increase in concentration of Na^+ with concomitant decrease in concentration of K^+ in the seedlings of two jute species, the effect being more prominent with increased duration and magnitude of salinity stress. There was also a marked increase in Cl⁻ along with Na^+ in both the jute species. Such effects of salinity stress were more pronounced in *C. olitorius* than in *C. capsularis*. Although the roots of *C. capsularis* and *C. olitorius* showed almost similar accumulation of Cl⁻ ion, its accumulation in the shoot of *C. olitorius* was markedly greater than in *C. capsularis* (Table 5).

Table 5. Effects of short-term NaCl salinity stress on the content [$\text{mg g}^{-1}(\text{DM})$] of sodium (Na^+), potassium (K^+) and chloride (Cl^-) in 30-d-old seedlings of two jute species (mean \pm S.E., $n = 10$).

NaCl [mM]	Stress [d]	Organ	<i>Corchorus capsularis</i>			<i>Corchorus olitorius</i>		
			Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
0	1	root	15.05 \pm 0.23	12.10 \pm 0.13	40.96 \pm 0.25	14.95 \pm 0.21	12.00 \pm 0.26	39.87 \pm 0.45
		shoot	13.95 \pm 0.17	11.75 \pm 0.09	39.24 \pm 0.52	13.65 \pm 0.17	11.75 \pm 0.19	38.77 \pm 0.23
	2	root	15.02 \pm 0.19	12.05 \pm 0.21	40.24 \pm 0.94	14.43 \pm 0.15	11.96 \pm 0.35	39.03 \pm 0.71
		shoot	13.62 \pm 0.26	11.79 \pm 0.13	39.15 \pm 0.67	13.13 \pm 0.25	11.51 \pm 0.27	38.19 \pm 0.16
160	1	root	49.24 \pm 0.35	5.59 \pm 0.09	161.25 \pm 0.51	61.15 \pm 0.31	3.29 \pm 0.12	197.15 \pm 0.63
		shoot	33.05 \pm 0.71	7.25 \pm 0.12	131.05 \pm 0.95	54.50 \pm 0.35	4.85 \pm 0.32	175.25 \pm 0.15
	2	root	57.86 \pm 0.43	5.47 \pm 0.11	177.13 \pm 0.70	73.08 \pm 0.28	3.02 \pm 0.15	209.25 \pm 0.85
		shoot	40.54 \pm 0.93	7.03 \pm 0.25	141.25 \pm 0.82	65.58 \pm 0.72	4.69 \pm 0.45	187.15 \pm 0.61
200	1	root	53.15 \pm 0.81	3.27 \pm 0.05	165.19 \pm 0.23	72.35 \pm 0.41	2.80 \pm 0.17	205.25 \pm 0.97
		shoot	35.85 \pm 0.19	5.19 \pm 0.27	137.23 \pm 0.57	61.29 \pm 0.28	3.95 \pm 0.16	186.25 \pm 0.75
	2	root	62.05 \pm 0.75	3.15 \pm 0.08	181.05 \pm 0.29	87.19 \pm 0.19	2.15 \pm 0.13	217.38 \pm 0.10
		shoot	44.13 \pm 0.35	5.04 \pm 0.19	144.89 \pm 0.95	69.15 \pm 0.65	3.45 \pm 0.12	198.21 \pm 0.89

The decline in P_N and E due to imposition of short-duration salinity stress was markedly alleviated in the 24-h pretreated seedlings. Such pretreatments also improved the WUE of the jute seedlings. Among three pre-treatment chemicals, calcium nitrate proved most effective and kinetin the least in mitigating stress effects (Table 6).

Discussion

The greater decline in RWC and LWP in *C. olitorius* than in *C. capsularis* at the two magnitudes and durations of salinity stress indicates greater susceptibility of the former to short-term salinity stress.

While salinity stress reduced water uptake more markedly in *C. olitorius*, the reduction in E was, however, less pronounced in this species. Thus, reduced water uptake concomitant with relatively higher E led to a more negative water retention in

C. olitorius than in *C. capsularis* under short-term salinity stress. This appears to be one of the major reasons for relatively greater susceptibility of the former to salinity. Similar observations were also reported for these jute species subjected to water stress induced by polyethylene glycol (Chowdhury and Choudhuri 1985).

Table 6. Effects of short-term NaCl salinity stress on photosynthetic rate [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$], transpiration rate [$\text{mol}(\text{H}_2\text{O}) \text{ m}^{-2} \text{ s}^{-1}$] and water use efficiency (WUE) [$\text{nmol}(\text{CO}_2) \text{ mol}^{-1}(\text{H}_2\text{O})$] in 30-d-old seedlings of two jute species pretreated [24 h] with 0.09 mM kinetin, 4 mM glutamic acid and 5 mM calcium nitrate.

Pretreatment	NaCl [mM]	Stress [d]	<i>Corchorus capsularis</i>			<i>Corchorus olitorius</i>		
			P_N	E	WUE	P_N	E	WUE
Control	0	1	27.15±0.85	0.0795±0.02	0.341±0.06	28.95±0.45	0.0872±0.01	0.332±0.09
	0	2	26.92±0.31	0.0713±0.01	0.377±0.11	26.05±0.83	0.0810±0.02	0.322±0.07
H_2O	160	1	7.51±0.27	0.0571±0.01	0.131±0.09	5.62±0.83	0.0592±0.03	0.095±0.12
		2	5.75±0.19	0.0568±0.03	0.101±0.07	4.01±0.65	0.0575±0.02	0.070±0.08
	200	1	4.92±0.23	0.0546±0.02	0.090±0.03	2.19±0.25	0.0576±0.01	0.038±0.11
		2	3.24±0.46	0.0537±0.02	0.060±0.02	1.65±0.18	0.0557±0.04	0.029±0.07
Kinetin	160	1	8.08±0.28	0.0585±0.01	0.138±0.07	11.49±0.15	0.0651±0.01	0.176±0.15
		2	6.25±0.19	0.0575±0.02	0.108±0.04	10.11±0.21	0.0693±0.03	0.146±0.08
	200	1	7.75±0.21	0.0561±0.03	0.138±0.08	8.22±0.13	0.0591±0.03	0.139±0.07
		2	5.24±0.23	0.0553±0.02	0.094±0.07	7.12±0.17	0.0567±0.01	0.125±0.19
Glutamic acid	160	1	10.92±0.25	0.0612±0.01	0.178±0.02	19.01±0.26	0.0675±0.05	0.282±0.16
		2	8.12±0.19	0.0605±0.04	0.134±0.04	18.21±0.31	0.0662±0.04	0.275±0.09
	200	1	8.22±0.15	0.0569±0.01	0.144±0.02	9.01±0.12	0.0595±0.01	0.151±0.15
		2	6.05±0.31	0.0555±0.03	0.109±0.07	8.22±0.11	0.0572±0.03	0.143±0.21
Calcium nitrate	160	1	12.17±0.32	0.0635±0.05	0.192±0.08	22.21±0.45	0.0695±0.06	0.319±0.08
		2	10.31±0.18	0.0628±0.02	0.164±0.06	20.24±0.27	0.0668±0.03	0.303±0.13
	200	1	10.11±0.12	0.0591±0.06	0.171±0.04	11.05±0.19	0.0605±0.01	0.183±0.14
		2	8.20±0.09	0.0575±0.05	0.142±0.05	10.12±0.15	0.0593±0.02	0.170±0.08

The reduction in P_N and E in the salt affected plants is primarily due to stomatal closure (e.g. Behboudian *et al.* 1986). In the present study, a close correlation between reduced E and g_s could be discernible. Although g_s of *C. capsularis* was slightly greater compared with that of *C. olitorius* in the control set, the same was reduced to a greater extent in the former under identical salinity stress, thereby preventing water loss more effectively than in the latter species.

Despite a lower g_s , P_N remained greater in *C. capsularis* than in *C. olitorius*. This suggests that P_N depends not only on g_s .

That the deleterious effects of salinity stress on P_N are associated with Na^+ and Cl^- toxicity besides dehydration, are revealed by the fact that there was a marked increase in the levels of both ions with the concomitant decrease in K^+ level resulting in lower K^+/Na^+ ratio. The greater accumulation of Na^+ and Cl^- in root and shoot of

C. olitorius than in *C. capsularis* under saline stress might again be the reason for the formers' greater susceptibility to salinity.

It was observed that pre-treatment of seedlings separately with kinetin, glutamic acid and calcium nitrate markedly improved P_N , E and g_s of the jute species subjected to short-duration salinity stress and such improvements were better displayed in the more susceptible species (*C. olitorius*). Shoot growth of salinity affected plants is adversely affected since production of cytokinin-like substances in roots and their translocation to shoot is reported to be also hampered (Munns and Termaat 1986, Nilsen and Orcutt 1996). This is further corroborated by the present study where the application of kinetin profoundly enhanced P_N in salt-stressed seedlings, possibly due to antagonistic effects of cytokinin on ABA-induced stomatal closure. Some amino acids such as proline, glutamic acid, asparagine and glycine-betaine are known to be associated with stress tolerance (Lehle *et al.* 1992, Lenc *et al.* 1987). In the present study, glutamic acid ameliorated the adverse effects of salinity on P_N , E and WUE in the jute seedlings. Since glutamic acid is a precursor of putrescine which is associated with stress (Flores 1990), it may be speculated that the ameliorating effects of glutamic acid on salinity-induced inhibition of gas exchange parameters is possibly mediated through putrescine production. Some direct and indirect evidence support the involvement of calcium in maintaining membrane integrity and its deficiency alters membrane permeability (Läuchli and Epstein 1970, Cramer *et al.* 1985). Also, there are reports that the presence of Ca^{2+} inhibits Na^+ uptake and maintains K^+/Na^+ selectivity (Cramer *et al.* 1985, Shah *et al.* 1990). The present study shows that pre-treatment of jute seedlings with calcium nitrate markedly improved gas exchange parameters under short-term saline stress. Among the three pre-treatment chemicals, calcium nitrate proved most effective ameliorant of salinity stress effects on P_N , E and WUE and kinetin the least.

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