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Gas exchange, membrane permeability, and ion uptake in two species of Indian jujube differing in salt tolerance

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Abstract

Effect of NaCl (electrical conductivity of 0, 5, 10, 15, and 20 dS m^{-1}) on growth, gas exchange, and ion uptake in two *Ziziphus* species (*Z. rotundifolia* and *Z. nummularia*) differing in salt tolerance was studied. At 30 and 45 d after first leaf initiation, the dry mass of shoot and leaves, and rates of net photosynthesis (P_N) and transpiration (E) decreased significantly with increasing NaCl concentration whereas membrane injury and accumulation of proline increased. The sodium content was highest in the roots of *Z. rotundifolia* and in the leaves of *Z. nummularia*. Potassium content did not differ much in the roots but it was significantly higher in the leaves of *Z. rotundifolia* at 30 and 45 d of observations. Thus both these species were tolerant to salinity but at high salinity *Z. rotundifolia* performed better owing to its higher P_N and E, restricted translocation of sodium from root to leaves, and larger accumulation of potassium in the leaves.

Additional key words: ion leakage; net photosynthetic rate; salinity; transpiration rate; *Ziziphus*.

Introduction

Indian jujube, commonly known as ber, is a popular fruit of arid region that is grown successfully under low and erratic rainfall, variable thermal oscillations, and salt affected soils with low fertility. Beside all available nutrients, ber fruits are a good source of vitamins A and C and vitamin B complexes (Pareek 1983).

The plant performance against adverse conditions can be judged at early growth stages by analysing certain physico-biochemical adaptations. It becomes more important in perennials. Salinity induced changes in photosynthesis (Ebert 2000), stomatal behaviour (Pradeep and Jumbhale 2000), chlorophyll content (Pandey *et al.* 1991), and accumulation of metabolites (Hooda *et al.* 1990) have already been reported at various stages in ber. Cell membranes are the main loci of various stress injuries and salt stress specially has a very high potential to disrupt cell membranes (Levitt 1980). Many recent studies also reinforced the perception that NaCl causes the growth inhibition by damaging the integrity of membranes and cell organelles and distorting ion transport (Pandey *et al.* 1993, Pathak and Pathak 2001). However, the tolerant species can avoid the situation by restricted translocation of certain anions/cations (Sibole *et al.* 2000).

Selection of a suitable rootstock for any fruit crop is important for a healthy and productive plant, particularly in problematic soils. A sizeable number of wild and domestic species of Indian jujube are available but only three species, *i.e. Ziziphus rotundifolia, Ziziphus nummularia*, and *Ziziphus mauritiana* have been used extensively in commercial production. Among these, *Z. rotundifolia* and *Z. nummularia* are commonly used as the rootstock and the *Z. mauritiana* as scion. The tolerance of above two rootstocks against adverse soil and environmental conditions is known (Pandey *et al.* 1991) but systematic information with respect to relative tolerance of one over another is lacking. Therefore, we investigated the suitability of these rootstocks with increasing soil salinity at early growth stage as well as tried to find out the physiological mechanisms involved in imparting salinity tolerance in these species.

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Abbreviations: DLFS – days after first leaf initiation; d.m. – dry mass; E – transpiration rate; EC – electrical conductivity; f.m. – fresh mass; MI – membrane injury; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density.
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Materials and methods

Experimental site: The study was conducted in the cage house of the Department of Plant Physiology, SKN College of Agriculture, Jobner, India. Seeds of *Z. rotundifolia* and *Z. nummularia* were soaked in normal water for 24 h, surface sterilised with 0.1 % mercuric chloride for 1 min, and then washed several times with distilled water. Seeds of both the species were sown in plastic bags (25×10 cm) with soil having a bulk density of 1.5 g cm^{-3}, EC 1.5 dS m^{-1}, pH 8.2, SAR 12.5, and $CaCO_3$ 0.14 %. The field capacity and permanent wilting point of the soil were 11.8 and 2.8 %, respectively. About 100 bags were utilised for each species and after germination one plant per bag was maintained. The recommended doses of manures, fertilisers, irrigation, and other inputs were provided at appropriate time. Saline water of 5.0, 10.0, 15.0, and 20.0 dS m^{-1} was prepared with NaCl and provided to these bags at regular intervals. Plants irrigated with water of 0 dS m^{-1} served as control. Observations on following parameters were recorded at 30 and 45 d after first leaf initiation in both the species.

Leaf area and dry mass: Leaf area per plant was measured directly with the help of leaf area meter (*LICOR-3100*, Lincoln, USA). The fully expanded green leaves were detached from the plants and leaf area was determined immediately to avoid wilting of leaves. Dry mass of roots and leaves was taken at 30 and 45 d after first leaf initiation stage. Three healthy plants per replication were selected and separated into leaves and roots which were then thoroughly washed with distilled water and dried in oven at 60 °C for 6 h followed by 104 °C for 1 h before weighing.

P_N **and** E were measured by infrared gas analyser (*CID-301*, Vancouver, USA). The top most fully expanded leaf was enclosed in the assimilation chamber. P_N was monitored while CO_2 concentration changed over a definite time interval. The system automatically calculated P_N on the basis of preloaded flow and leaf area. E was measured directly by *CID 301* on the same leaf. All these measurements were taken at 10:00-11:00 (Indian time) in triplicates when relative humidity, temperature, PPFD, and CO_2 concentration ranged from 50-60 %, 30-35 °C, 1 200 μmol(photon) m^{-2} s^{-1}, and 350-360 μmol mol^{-1}, respectively.

Membrane injury: For the measurement of ion leakage, 200 mg of fresh leaf sample was weighed and put in a test tube containing 10 cm^3 of double-distilled de-ionised water. Three replicates were prepared for each treatment. These tubes were incubated at 45 °C for 30 min in a water bath. Electrical conductivity of this solution was measured with the help of conductivity bridge *CM 82T* (*Elico Pvt.*, Hyderabad, India). These test tubes were then kept in boiling water at 100 °C for 10 min, cooled at room temperature, and conductivity was measured again. The method was standardised by repeated observations and uniform results (Gupta *et al.* 2000). Membrane injury was determined using the formula: MI [%] = conductivity at 45 °C/conductivity at 100 °C × 100.

Proline and ion estimation: Proline content in leaves was extracted in 3 % aqueous solution of sulphosalicylic acid and determined by the method of Bates *et al.* (1973). The contents of sodium and potassium in roots and leaves were determined using the method of Wignarajah *et al.* (1975). Dried plant material (1 g) was extracted thrice with boiling de-ionised water and the supernatant was collected after centrifuging the suspension at 6 000×*g* for 10 min. The residue was then extracted with 30 % (v/v) nitric acid for 1.0 h at 90 °C. Suspension was cooled and the supernatant was collected after centrifugation. The residue was extracted twice with 30 % nitric acid. All supernatants were pooled together and the concentrations of sodium and potassium ions were estimated using flame photometer (*Chemito 1020*, Banglore, India). Standards for sodium and potassium were prepared with NaCl and KCl, respectively.

Results and discussion

Seed germination was delayed by salinity in both the species, but after EC of 15 dS m^{-1} salinity the germinated seedlings of *Z. nummularia* did not survive. There was a significant reduction in dry mass of leaves and roots due to irrigation with saline water. *Z. rotundifolia* exhibited greater leaf mass than *Z. nummularia* at both stages of observation under both control and saline conditions. A significant reduction in leaf area was noticed only after the salinity of 10 dS m^{-1} in both the species. On day 30 after first leaf initiation, the difference between leaf area of two *Ziziphus* species was negligible, whereas on day 45 *Z. rotundifolia* had a larger leaf area than *Z. nummularia* (Table 1). The reduction in the above vegetative parameters might be due to the increased osmotic pressure in the root zone after enhanced salt concentration in the soil solution, accumulation of ions (especially Na and Cl) in the plant tissues to toxic levels, and the excessive concentration of soluble ions that might have resulted in nutrient imbalance in soil solution and plant tissues (Prisco and O'Leary 1979, Sibole *et al.* 2000). A reduction in leaf area and other vegetative growth parameters on account of salinity was reported in ber and guava (Hooda *et al.* 1990, Awasthi *et al.* 1995). The high rate of dry matter accumulation in control as well as salinity

Table 1. Effect of salinity on leaf or root dry mass [mg plant^{-1}], and leaf area [cm^2 plant^{-1}] of two *Ziziphus* species at 30 and 45 d after first leaf initiation stage. *No plant survived in *Ziziphus nummularia* at 20 dS m^{-1}; this treatment has not been included in statistical analysis.

Salinity [dS m^{-1}]	*Z. rotundifolia*						*Z. nummularia*					
	Leaf d.m.		Root d.m.		Leaf area		Leaf d.m.		Root d.m.		Leaf area	
	30 DFLS	45 DFLS	30 DFLS	45 DFLS	30 DFLS	45 DFLS	30 DFLS	45 DFLS	30 DFLS	45 DFLS	30 DFLS	45 DFLS
0	319.4	353.8	407.2	443.1	8.74	11.85	239.7	253.2	310.0	326.0	8.74	9.83
5	301.7	335.0	375.6	403.2	8.40	11.50	221.8	234.7	267.1	275.5	7.56	8.59
10	189.7	222.0	212.3	254.3	6.93	9.62	107.8	118.7	158.3	172.6	5.15	6.27
15	107.4	139.5	97.4	146.5	5.25	7.56	72.5	78.6	73.4	92.1	2.73	3.85
20	56.3	88.3	21.6	38.6	3.38	5.47	* -	-	-	-	-	-
SE	10.81	12.52	12.83	31.95	0.41	0.41	6.94	8.29	21.74	22.10	5.59	0.53
$CD_{0.05}$	32.73	37.83	38.51	86.28	1.24	1.25	21.46	25.31	66.91	68.25	1.72	1.64

treated plants of *Z. rotundifolia* might have attributed to the survival of its rootstock beyond the salinity of 15 dS m^{-1}. Variations among reactions of species and/or genotypes of the same species against salinity due to various morphological adjustments have already been reported in ber (Pradeep and Jumbhale 2000).

P_N was much higher in *Z. rotundifolia* than in *Z. nummularia* at 30 d after the first leaf stage. At 45 d, the variations between these species were lesser but *Z. rotundifolia* still maintained higher P_N in control as well as salinity-treated plants. *E* also decreased significantly with increasing salinity in both the species at 30 and 45 d after the first leaf stage. Again *Z. rotundifolia* showed higher *E* than *Z. nummularia* (Table 2). These observations suggest that the higher P_N without reducing *E* might have resulted in better growth and dry matter accumulation of *Z. rotundifolia* over *Z. nummularia*. Lower *E* in *Z. nummularia* suggests that despite similar soil and environment, the movement of water and other solutes was larger in *Z. rotundifolia*. Reductions in P_N and *E* under salinity in red and white guava fruits have also been reported (Alidinar *et al.* 1998). These authors further concluded that on the basis of chlorophyll content, photosynthesis, water relations, and stomatal parameters, red guava was more salt tolerant than white guava. However, studies on comparative efficiency of wild and domestic cultivars of India jujube with regard to physiological observations are lacking.

Table 2. Net photosynthetic rate (P_N) and transpiration rate (*E*) in salinity treated *Ziziphus* species at 30 and 45 d after first leaf initiation. *No plant survived in *Ziziphus nummularia* at 20 dS m^{-1}; this treatment has not been included in statistical analysis.

Salinity [dS m^{-1}]	P_N [μmol(CO_2) m^{-2} s^{-1}]				*E* [mmol(H_2O) m^{-2} s^{-1}]			
	Z. rotundifolia		*Z. nummularia*		*Z. rotundifolia*		*Z. nummularia*	
	30 DFLS	45 DFLS	30 DFLS	45 DFLS	30 DFLS	45 DFLS	30 DFLS	45 DFLS
0	11.46	12.57	5.52	6.57	2.11	2.43	1.92	2.15
5	10.23	11.31	4.21	5.86	2.06	2.31	1.64	1.93
10	7.25	8.17	2.86	3.37	1.64	1.69	1.23	1.45
15	5.03	6.05	1.52	1.71	1.15	1.18	0.93	1.02
20	1.31	1.91	* -	-	0.65	0.71	-	-
SE	0.55	0.63	0.38	0.41	0.13	0.13	0.10	0.09
$CD_{0.05}$	1.67	1.89	1.18	1.25	0.38	0.39	0.295	0.28

Proline accumulates in plant tissues in response to environmental stresses. In a perennial plant, accumulation of osmoregulatory substances like proline and ions contributes to maintaining leaf pressure potential and thus helps the plant to overcome the stress imposed by salinity (Gururajarao *et al.* 1999). Our results showed that the content of free proline increased with almost similar trend in both the *Ziziphus* species (Table 3). Electrolyte leakage has been widely used to differentiate the cereals in terms of drought and salinity tolerance (Blum and Ebercon 1983). In our investigation, the ion leakage increased continuously with increasing the salinity in both the species. However, the magnitude of increase was variable. A comparison of two species taken for study showed a larger ion leakage in *Z. nummularia* over *Z. rotundifolia*, again supporting the salinity tolerance of *Z. rotundifolia*.

Under salinity, due to the presence of excessive amount of toxic elements in the root zone, the availability of essential elements is hampered and, simultaneously, toxic elements are accumulated in plant parts resulting in development of deficiency/toxicity symptoms (Cherian *et al.* 1999). Our experiments show that the accumulation of sodium was higher in the roots of *Z. rotundifolia* and in the leaves of *Z. nummularia* (Table 4). Hence *Z. rotundi-*

Table 3. Membrane injury [%] and proline content in salinity treated *Ziziphus* species at 30 and 45 d after first leaf initiation. *No plant survived in *Ziziphus nummularia* at 20 dS m^{-1}; this treatment has not been included in statistical analysis.

Salinity [dS m^{-1}]	Membrane injury [%]				Proline content [g kg^{-1}(f.m.)]			
	Z. rotundifolia		*Z. nummularia*		*Z. rotundifolia*		*Z. nummularia*	
	30 DFLS	45 DFLS	30 DFLS	45 DFLS	30 DFLS	45 DFLS	30 DFLS	45 DFLS
0	39.33	20.55	45.62	23.23	0.342	0.356	0.325	0.337
5	42.65	23.60	51.39	30.62	0.363	0.385	0.342	0.356
10	53.51	31.05	64.08	39.42	0.461	0.487	0.421	0.442
15	61.31	40.13	69.85	45.65	0.574	0.597	0.523	0.542
20	64.10	51.25	*	-	0.645	0.673	*	-
SE	1.32	1.28	1.34	1.37	0.021	0.024	0.017	0.022
$CD_{0.05}$	3.89	3.84	4.13	4.21	0.063	0.074	0.052	0.066

folia accumulated this toxic element in the roots *via* restricted translocation to maintain ionic balances in leaves (Pandey *et al.* 1999, Ebert 2000). The potassium content did not much differ in roots of both the species but it was significantly higher in the leaves of *Z. rotundifolia* (Table 4). The role of potassium in ionic balances and osmotic adjustment is well known. Besides, K is essential for the formation of starch, protein synthesis, photosynthetic partitioning, stomatal regulation, and above all as an activator of a number of enzymes (Cherian *et al.* 1999). The enhanced growth and leaf area of *Z. rotundifolia* at higher salinity in present investigation might have attributed to improved K status of this species along with other physiological adaptations. A reduction in K status at higher salinity on account of higher sodium accumulation in ber was reported by Pandey *et al.* (1993).

Table 4. Sodium content [g kg^{-1}(d.m.)] in different plant parts of salinity treated *Ziziphus* species at 30 and 45 d after first leaf initiation. *No plant survived in *Ziziphus nummularia* at 20 dS m^{-1}; this treatment has not been included in statistical analysis.

Salinity [dS m^{-1}]	*Ziziphus rotundifolia*				*Ziziphus nummularia*			
	30 DFLS		45 DFLS		30 DFLS		45 DFLS	
	Root	Leaves	Root	Leaves	Root	Leaves	Root	Leaves
0	9.00	6.95	9.35	7.87	8.12	7.15	8.76	8.31
5	10.87	7.83	11.23	8.65	9.97	8.23	10.39	9.16
10	14.97	10.56	15.31	11.15	12.86	12.21	13.75	13.10
15	20.85	15.65	22.42	16.58	20.32	18.12	21.53	19.31
20	28.73	17.83	30.39	18.10	*	-	-	-
SE	0.69	0.71	0.71	0.72	0.69	0.68	0.73	0.68
$CD_{0.05}$	2.07	2.18	2.14	2.21	2.15	2.01	2.25	2.06

In conclusion, present investigation suggests that both *Z. rotundifloia* and *Z. nummularia* can grow under saline conditions but at severe salinity only *Z. rotundifolia* may survive. The high P_N, E, membrane stability, and restricted sodium translocation with higher K accumulation might have helped the *Z. rotundifolia* in imparting much tolerance against salinity over *Z. nummularia* in similar soil and environmental conditions.

Table 5. Potassium content [g kg^{-1}(d.m.)] in different plant parts of salinity treated *Ziziphus* species at 30 and 45 d after first leaf initiation. *No plant survived in *Ziziphus nummularia* at 20 dS m^{-1}; this treatment has not been included in statistical analysis.

Salinity [dS m^{-1}]	*Ziziphus rotundifolia*				*Ziziphus nummularia*			
	30 DFLS		45 DFLS		30 DFLS		45 DFLS	
	Root	Leaves	Root	Leaves	Root	Leaves	Root	Leaves
0	5.47	7.65	5.12	7.93	5.32	7.13	5.29	7.62
5	4.65	6.89	4.31	7.28	4.53	6.72	4.46	7.10
10	3.42	5.42	3.10	5.88	3.21	5.16	3.10	5.52
15	2.01	3.92	1.88	4.23	1.87	3.42	1.62	3.75
20	0.71	2.45	0.34	2.56	*	-	-	-
SE	0.35	0.42	0.37	0.45	0.37	0.39	0.40	0.41
$CD_{0.05}$	1.06	1.26	1.11	1.35	1.16	1.23	1.24	1.27

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Argyroudi-Akoyunoglou, J.H., Senger, H. (ed.): **The Chloroplast: From Molecular Biology to Biotechnology.** – Kluwer Academic Publishers, Dordrecht- Boston – London 1999. ISBN 0-7923-5577-6. 356 pp., USD 195.00.

The book covers lectures or posters presented at an international Advanced Research Workshop-Lecture Course on the Chloroplast: from Molecular Biology to Biotechnology held at the Orthodox Academy of Crete, Kolymbari-Chania, Crete from August 10 to 15, 1998.

The main purpose of the workshop was to bring together experts and students studying chloroplast biogenesis from different perpectives in an effort to propose biotechnological approaches, *via* genetic manipulation of the organelle, applicable in solving problems of economic importance.

The volume contains about 60 papers, which are divided into six main sections according to the topics of the most intense interest in the field. The papers of the first section focus on structure of photosynthetic membrane proteins and bring the three-dimensional structure of both photosynthetic units and their components. Following section is devoted to expression and regulation of the most important chloroplastic genes. The role of chloroplast envelope membrane in import and processing proteins is the main topic of the third section. Biosynthesis of chlorophylls and carotenoids is a common theme for the fourth section. Next part is devoted to regulatory mechanisms in biogenesis and turnover of photosynthetic unit. The last section was probably ment to collect contributions concerning biotechnological approaches, *i.e.* genetic manipulation of the organelle as a tool for biotechnological applications. Unfotunately, this aim has not been reached and this part of the volume seems to be a rather mixed collection of papers of various quality. If editors more or less succeeded in structuring contributions to follow above mentioned common themes, the last section was not certainly this case.

All contributions are organised as research articles including abstracts, materials and methods, illustrating figures, tables, and numerous references. Most of them are well written by active researchers and therefore present an up-to-date summary of what has been achieved. However, the book might have been improved if the editors had included a general introduction, or a final summary chapter, that brought together key elements and highlighted major trends expected in the future.

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