

CELL MEMBRANE INJURY, STABILITY AND ATPase ACTIVITY AND THEIR RELEVANCE TO SEED GERMINATION, SEEDLING GROWTH TOLERANCE INDICES OF RICE UNDER ABIOTIC STRESSES

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Keywords: Cell membrane injury and stability, Rice genotypes, Electrolyte leakage, Seed germination and growth, Stress tolerance indices

Abstract

Abiotic stress tolerant germplasms of field crops are useful for cultivation under growth limiting conditions. In the present study 18 different rice germplasms for their cell membrane injury (CMI), cell membrane stability (CMS), ATPase activity and their relevance to seed germination and seedling growth tolerance index (TI) under abiotic stresses like salinity and low Ψ_w were investigated. At least 12 germplasms had relatively low membrane injury (< 10%) and consequently high membrane stability (> 90) including TM-1-5, Sadachikon, SR x P 5222, BR 23, Pokkali, Nonabokra, etc. indicating a relationship between stress tolerance and CMS. ATPase activities in some of these rice germplasms were high along with CMS related to stress tolerance. The TI of seed germinations and seedling growth of different germplasms under salinity stress were affected in different degrees and decreased more under high intensities. At 0.1 Molal NaCl all germplasms had \leq 80% TI of seed germination, at 0.2 Molal NaCl only three had \geq 80% TI and ten had \geq 50% TI and at 0.3 Molal NaCl, only nine had ~10-32% TI and the rest nine did not germinate. Osmoticum PEG-6000 at -5.5 bar Ψ_w had limited inhibitory effect on seed germination TI. NaCl as well as PEG-6000 degraded both the root and shoot growth TI of the seedlings.

Field crops face a number of abiotic stresses including salinity, drought and heat worldwide (Mantri *et al.* 2012, Lipiec1 *et al.* 2013). These stresses limit plant growth, development and yield (Qin *et al.* 2011, Krasensky and Jonak 2012). Seed germination and seedling growth stages are two critical growth phases and stresses at these stages may lead to poor seedlings establishment (Ashraf and Mehmood 1990, Albuquerque and Carvalho 2003). Screening effort for tolerant germplasms at these stages may be less time consuming and effective. Membranes have been implicated as sites of response to various abiotic stresses for a long time (Levitt 1980) and CMS under growth limiting condition has been reported to play a key role for stress tolerance ability of the plant (Sullivan 1972, Martinean *et al.* 1979, Agarie *et al.* 1995). Membranes may become disorganized, dysfunctional, etc. under stress (Mahajan and Tuteja 2005) and such membrane dysfunction increases permeability and leakage of ions out. The degree of cell plasma membrane injury induced by stress can be estimated by measuring electrolyte leakage from the cells. CMS may be applied to quantify damages to cell membranes due to abiotic stresses (Saelim and Zwiazek 2000, Sreenivasulu *et al.* 2000, Bajji *et al.* 2002). ATPase is a major enzyme protein of the plant plasma membrane and it plays important role in adaptation of plants to abiotic stresses (Ge vaudant *et al.* 2007, Janicka-Russak 2011). Cell membrane serves as the first line of plant defense under stress and that's why cell membrane features as potential breeding targets (Dhaliwal and Angeles-Shim 2022). In the present work leaf cell membrane injury, CMS and root ATPase activity of 18 rice germplasms attempts were taken to determine and compare with their salinity and moisture stress tolerance indices at seed germination and seedling growth stages.

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Seeds of 18 germplasms of rice (e.g., BR 10, BR 23, Jamainare, Kajalsail, Nonabokra and Sadachikon from BRRI, Gazipur; IRATOM-24, IRATOM-38 from INA, Mymensingh, Bangladesh; Canning-7, DMI-52, MI-48, MC-12, MC-14, Motaamon, Pokkali, SRxP, SR 26B and TM-I-5 from Bose Institute, Kolkata, India) were used in the experiment.

The electrolyte leakage technique was used for assessing leaf CMS of 18 different rice germplasms. Membrane stability was calculated according the methods initially developed by Dexter (1956) and later modified by Sullivan (1972) and Sullivan and Ross (1979). For this, rice germplasms were grown in separate 10 l pots containing mixed soil with manure (3:1) and NPK fertilizers as per Arnon and Hoagland (1940) in the open space of the Botany Department, Chittagong University, Bangladesh up to booting stage. Flag leaf samples were collected between 1300 and 1500 hrs, washed thoroughly with three changes by distilled deionized water and leaf dices (10 mm dia.) were taken at random by leaf-punch from different places of the leaf blade avoiding the midrib, transferred 10 dices/glass vials, washed with three changes by dist. deionized water, 2 ml water was added, covered with plastic wrap and incubated in a water bath at 44°C for 1 hr (treatment) while other vials were maintained at 25°C for 1 hr (control). After this, 10 ml deionized water was added to each of the control and treatment vials and kept at 10°C for 24 hrs in incubator. Next the vials were brought to 25°C, shaken well and an initial conductivity of vial contents was measured, then vials were put in boiling water bath for 30 min to completely kill leaf tissues and release all electrolytes. Subsequently vials were cooled to 25°C, shaken well and a final conductance was recorded. The membrane injury index (MII) expressed in % was calculated

$$\text{as \% MII} = 1 \frac{1 - (T_1 / T_2)}{1 - (C_1 / C_2)} \times 100, \text{ where T and C refer to mean of treatment and controls, and 1}$$

and 2 refer to initial and final conductivities, respectively. The extent of CMS was calculated from the MII as CMS (%) = 100-MII.

For this experiment, healthy surface sterilized by 0.1% Na hypochlorite solution 100 seeds/germplasm/ plastic Petridis (10 cm dia.) lined with filter paper were taken for germination in 1mM CaSO₄ (control) and in 0.1, 0.2 and 0.3 Molal NaCl solution (-4.62, -9.15, -13.68 bar) or PEG-6000 (-5.5 bar) prepared in 1mM CaSO₄ (treatment) in growth chamber at 25°C with the change of test solutions after 24 hrs interval. Seed germination count was taken up to 4 days when germination was complete in the control and 7 days old seedlings were used for the determination of root and shoot growth in lengths. Root ATPase activities of the 7 days old seedlings were determined in the extract following Hodges (1976). TI was calculated according to Fageria (1985). As statistical analysis, LSD of different results were done at 5% level of significance. There were 3- 4 replications for each set of experiment.

Results of different rice germplasms had different extent of membrane injuries (Fig. 1). The highest membrane injury was with SR-26B (42.23%), followed by IRATOM-38, 24, Canning -7, Kajalsail and others and the lowest injury were with TM-1-5 (1.73%), followed by DMI-52, BR 23, Pokkali, MC 12 Jamainare, and others. Other germplasms with less than 10% membrane injury were M C-14, Motaamon, MI-48, Nonabokra, Sadachikon and BR-10. Increased membrane leakage disrupts membrane stability to a higher extent than those with low membrane leakage. Evaluation of cellular membrane integrity as a measure of environmental stress tolerance appears to be relevant criterion (Sullivan 1972). The extent of leakage of electrolytes through the cell membrane into elute was indicated by the elute conductivity. The higher the leakage the more was conductivity in the elute (Venkateshwarlu and Ramesh 1993). Therefore, higher conductivity could be a measure of the extent of membrane injury.

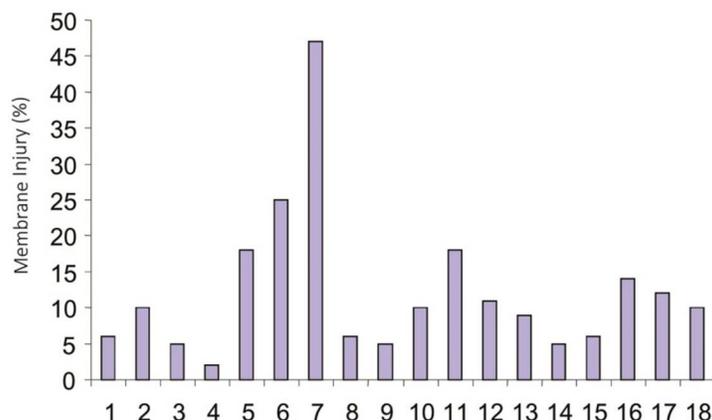


Fig. 1. Membrane injury % of different rice germplasms. 1: MC12, 2: MC-14, 3: DMI -52, 4: TM-1-5, 5: IRATOM- 24, 6: IRATOM- 38, 7: SR-26B, 8: Motaaman, 9: Pokkali, 10: MI-48, 11: Canning-7, 12: SRXP, 13: BR 10, 14: BR 23, 15: Jamainare, 16: Kajalsail, 17: Nonabokra, 18: Sadachikon

Results presented in Table 1 showed that tolerance index (TI) of different rice germplasms at seed germination stage was affected due to salinity as well as moisture stresses by NaCl and PEG-6000, respectively up to different extent. At 0.1 Molal NaCl, all germplasms had 80% TI or above, at 0.2 Molal NaCl only three (MC-12, TM-1-5 and SR 26 B) had 80% or above TI efficiency and several others had 50% or above (MC 14, DM-1- 52, IRATONI-24, Motaamon, Pokkali, MI-48, Canning-7, SR x P, BR 23, Sadachikon, etc.) whereas at 0.3 Molal NaCl, TI was very much reduced and varied between 10 and 32% in 8 germplasms and seed germination was totally inhibited in the 10 rests. At 0.3 Molal NaCl, the maximum TI was about ~30-32% (TM-I-5, Motaamon, Pokkali). BR 23 had ~22%, MI-48 had ~11%, and DM-1-52, Canning-7, SRxP and Sadachikon revealed ~10% TI. Salt stress at low intensity (0.1 NaCl) stimulated TI in 8 germplasms (BR 10, Canning-7, IRATOM-24, IRATOM-38, MC-12, MI-48, Motaamon, and Sadachikon). PEG-6000 with -5.5 bar water potential had low inhibitory effect and TI value of different germplasms varied between 67 and 94 approximately in 9 varieties (DMI-52, IRATOM-24, IRATOM-38, Jamainare, Kajalsail, MC-14, Nonabokra, SRxP and TM-1-5) while it was increased up to 115% in 9 varieties (BR 10, BR 23, Canning-7, MC-12, MI-48, Motaamon, Pokkali, Sadachikon, SR 26 B) and Kajalsail showed no effect of moisture stress on seed germination TI value. Bybordi (2010) noted significant effect of salinity due to 75-200mM NaCl on germination and seedling growth of different cultivars of canola (*Brassica napus*). Demir and Mavi (2008) showed that both NaCl and PEG-6000 had inhibitory effect on pepper seed germination and growth and opined that the inhibition at the same water potential of salt and polyethylene glycol resulted due to osmotic effect rather than ion toxicity while Kaydan and Yagmur (2008) had noted higher negative effect due to PEG- 6000 on seed germination and seedling growth of triticale than that of NaCl. While examining the response of eight indica rice varieties against six salinity levels (0- 20 dS m⁻¹) Anbumalarmathi and Mehta (2013) observed that salinity decreased germination of seeds, speed of germination, germination energy %, seedling growth, dry weight etc. Increase of stress intensities increased the inhibitory effect and at the highest salinity level (20 dS m⁻¹), six varieties did not germinate but at 12 dS m⁻¹ salinity stress ADT43, IR50 and MDU5 had greater salt tolerance during germination. Varietal difference in in seed germination response salt and moisture was also noted in the present work and in extreme case of salinity only a few germplasms showed germination activity.

Salinity and water stresses affected TI of shoot and root growth of the seedlings and in some cases, root growth was affected more and in others, the effect was higher on shoot TI, especially under the elevated NaCl stress (Table 1). At 0.1M NaCl showed stimulatory effect on root TI of MC 14, TM-I-5, IRATOM-24, MI 48 and SR x P while at 0.2 M NaCl, relatively higher root TI (> 50%) was noted in TM-I-5 and SR26B than others. At 0.3 M NaCl, only 9 showed shoot and root growth activities and among them Pokkali, MI-48, Canning 7 and SRxP had relatively higher TI for both root and shoot growth. Moisture stress due to PEG-6000 had stimulatory effect on both shoot and root TI of TM-I-5, IRATOM-24 and 38 while DMI 52, Pokkali, BR 10 and Nonabokra had relatively higher TI (> 60%) for both shoot and root. Membrane stability in 12 germplasms (e.g., MC-12, MC-14, DMI-52, TM-I-5, Motaamon, Pokkali, MI 48, BR 10, BR 23, Jamainare, Nonabokra and Sadachikon was within the range of 90-98, (e.g., IRATOM-24, Canning 7, SRxP and Kajalsail) 4 varieties had 82-89 and IRATOM-38 and SR26B had the lowest membrane stability. Bybordi (2010) noted significant effect of salinity due to 75-200 mM NaCl on germination and seedling growth of different cultivars of canola (*Brassica napus*). Demir and Mavi (2008) showed that both NaCl and PEG-6000 had inhibitory effect on pepper seed germination and growth and opined that the inhibition at the same water potential of salt and polyethylene glycol resulted due to osmotic effect rather than ion toxicity while Kaydan and Yagmur (2008) had noted higher negative effect due to PEG- 6000 on seed germination and seedling growth of triticale than that of NaCl.

High concentration of NaCl in the rooting medium results in the accumulation of Na⁺ and Cl⁻ in shoot to levels that are toxic (Greenway and Munns 1980). While working with six different sunflower germplasms under water stress due to PEG-6000. Ahmad *et al.* (2009) reported the inhibition on seed germination, seedling height and dry matter tolerance indices with increasing water stress but an increase in root length TI. Anbumalaramathi and Mehta (2013) while examining the response of eight indica rice varieties against six salinity levels (0- 20 dS m⁻¹) observed decreased germination of seeds, rate of germination, germination energy %, seedling growth, dry weight, etc. and germination was totally arrested in six varieties at the highest salinity level (20 dS m⁻¹), but at 12 dS m⁻¹ salinity stress ADT43, IR50 and MDU5 showed greater salt tolerance during germination. Varietal difference in seed germination response salt and moisture was also noted in the present work and in extreme case of salinity only a few germplasms showed germination activity.

The rate of injury of cell membrane was reported to use as a measure of tolerance of plants in stresses (Dexter 1956, Sullivan 1972, Martinean *et al.* 1979). Comparison of the tolerance indices for seed germination, seedling growth (shoot. root) with the membrane injury percentage as well as membrane stability of different germplasms showed that in most of the cases high tolerance indices were associated with the low membrane injury and high membrane stability (Table 1). The mechanism of stress tolerance in plant is yet poorly understood. Development of a suitable methodology may be helpful in this regard. Plasma membrane has been considered important for tolerance activities in plants for long time (Dexter 1956). Dell Aquila and Spada (1992) reported that in germinating seeds, salinity stress modulated the production of selected group of proteins which were not synthesized upon removal of stress. While studying the protein banding patterns (by SDS-PAGE) of 5 different genotypes of *Corchorus capsularis* (3) and *C. olitorius* (2) under different concentrations of NaCl (1000, 3000 and 5000 ppm) at germination and subsequent seedling growth Abass and Latif (2005) reported considerable increase with increasing salt stress in the seedling of different genotypes. Ibrahim (2016) observed that seed priming promoted seed vigor during germination and emergence under salinity stress as a result of increased antioxidant system activity and repair of membranes. The synthesis mechanism of salt stress protein is not better understood at present, however, the varieties difference in salt stress tolerance and protein

Table 1. Effect Of Salinity And Moisture Stresses On Tolerance Indices Of Seed Germination And Seedling Growth And Their Relation To Root AtPase And Membrane Stability.

Name of the germplasms	Tolerance index at												Root ATPase	Membrane stability, %			
	PEG-5.5 bar			Seedling growth stage						PEG-5.5 bar							
				NaCl, Molal													
	Germination stage			0.1		0.2		0.3		Shoot					Root		
	0.1	0.2	0.3	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
2. MC-14	99.85	71.12	...	78.77	71.62	27.57	38.87	59.71	50.15	112.82	93.83
3. DMI-52	94.71	56.95	10.08	87.50	101.80	28.43	38.13	37.24	68.91	105.35	90.56
4. TMI-5	89.11	89.48	30.42	71.06	93.16	18.93	28.77	6.31	8.72	8.72	6.31	67.39	64.44	51.33	94.41
5. IRAT. 24	102.15	66.58	...	83.58	132.86	30.09	62.50	4.56	5.68	5.68	4.56	133.01	110.02	120.12	98.23
6. IRAT. 38	115.42	55.91	...	74.35	120.66	24.17	42.06	146.01	113.30	80.49	82.32
7. SR 26B	91.24	95.62	...	84.25	76.12	22.04	20.00	145.26	143.41	49.78	77.91
8. Motaaman	104.78	87.21	31.98	80.34	96.89	30.92	59.65	46.27	65.97	38.12	57.77
9. Pokkali	99.54	69.57	32.62	90.59	87.73	34.47	41.55	9.97	7.79	7.79	9.97	46.63	67.42	111.68	93.38
10. MI-48	103.38	76.52	11.35	71.84	91.00	17.36	38.35	12.25	15.66	15.66	12.25	61.49	77.56	110.42	92.73
11. Canning-7	102.96	64.74	10.55	88.31	120.43	32.03	45.25	12.95	16.13	16.13	12.95	36.45	31.19	115.70	92.35
12. SRXP	95.18	62.67	10.12	68.57	86.68	25.00	48.80	12.5	17.02	17.02	12.5	37.38	32.56	45.35	82.02
13. BR 10	110.56	50.22	...	94.77	107.94	24.89	41.51	20.08	16.24	16.24	20.08	42.20	40.54	40.12	89.13
14. BR 23	89.97	61.26	22.33	58.80	86.09	17.29	29.80	81.31	70.81	55.60	91.15
15. Jamainare	87.71	21.30	...	92.22	75.88	33.44	39.29	8.44	7.33	7.33	8.44	65.46	54.43	115.16	94.05
16. Kajalsail	100.00	25.00	...	61.13	91.94	17.06	35.00	42.18	59.03	108.80	93.86
17. Nonabokra	80.00	00	...	48.48	56.67	15.53	31.83	45.87	50.00	40.00	88.64
18. Sadachikon	107.30	84.20	10.23	54.59	71.43	70.05	64.52	59.16	90.65
LSD at 5%	2.41	5.08	...	61.13	91.94	17.06	35.00	8.55	8.55	8.55	8.55	43.39	49.46	104.33	91.31
				3.15	4.57	1.29	2.89	7.06	7.63	9.81	2.75

synthesis was reported earlier (Ramagopal 1990). Such newly synthesized protein under stress may be the component of the cell plasma membrane. Appearance of a new band of membrane protein formed under stress was identified in rice and wheat by gel electrophoresis (Alamgir 1995). Cheng *et al.* (2009) also noted new changes in the plasma-membrane-associated proteome (separated by IEF/SDS-PAGE) of rice roots under 150 mmol/l NaCl stress and suggested different salt stress response mechanisms for different rice cultivars. Specific membrane proteins and/or lipids may contribute to maintenance of the membrane structure and function in salt tolerant plant species (Mansour 2013). Some of the germplasms (e.g. TM 15, BR 23, MC 12, Motaamon, etc.) revealed stimulated ATPase TI with high CMS while some others show inhibitory effects on ATPase TI and CMS (e.g. SR 26B, Kajalsail, IRAT 38, etc.) with low CMS (Table 1). Leaf membrane stability was increased in plants exposed previously to stresses and the membrane stability is considered as a measure of stress tolerance efficiency (Blum and Ebercon 1981). Salinity decreases leaf water potential and increases membrane permeability and proline content (Farkhondeh *et al.* 2012). Senguttuvel *et al.* (2013) while investigating the differential response of 6 rice germplasms at seedling stage to salinity stress found their salt tolerance was associated, among others, with their membrane stability index. Stress tolerance mechanism exists at seedling stage of maize genotypes and the higher membrane stability index and high water retention capacity might have also imparted water stress tolerance in Giza 2 (Moussa and Abdel-Aziz 2008). CMS test by determining the electrolyte leakage and conductivity of the leaf dices elutes may be a quick method for determining the stress tolerance efficiencies of crop plants.

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Manuscript received on 23 November, 2022; revised on 14 September, 2023)