



Salinity and drought induced changes in gas exchange attributes and chlorophyll fluorescence characteristics of rice (*Oryza sativa*) varieties

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ABSTRACT

The present study was conducted to assess the effect of water deficit coupled with salt stress on physiological traits and stress tolerance mechanisms of rice (*Oryza sativa* L.) varieties differing in salt tolerance. Two salt tolerant (CSR 10 and CSR 36) and two salt sensitive (IR 29 and Pusa 44) rice varieties were evaluated in different combinations of controlled water deficit coupled with salt stress conditions. The salt tolerant variety CSR 10 has shown the best morphological or phenological growth performance; while IR 29 performed the worst in terms of reduction in growth at 25 and 50% water deficit. At 50 and 100 mM NaCl along with 50% water deficit, 80% plants of IR 29 could not survive. Variety CSR 10 exhibited maintenance of higher total chlorophyll concentration (Chl_T), relative water content (RWC), gas exchange characteristics and chlorophyll fluorescence attributes with lower membrane injury (MI) and thus ultimately showed better stress tolerance than other varieties.

Key words: Chlorophyll fluorescence, Gas exchange attributes, Membrane injury, Rice varieties, Water deficit

Salinity affects soils in more than hundred countries and salinization appears to be the most rampant in arid and semi-arid regions (Rengasamy 2006). The stress on arable land to produce more food for burgeoning populations, especially in the developing world, is on margin since that land is now being cultivated which was hitherto untapped because of high salinity. Rice (*Oryza sativa* L.) is the main staple food for more than half of the world's population (Khush 2005). Since, it is one of the most sensitive cultivated species to salt and drought stresses (Lefevre *et al.* 2001), so both water/soil salinity and low water supply (drought) are rapidly becoming the major limitations to rice production worldwide (Munns 2002). The response of rice to osmotic and drought stresses vary with the crop developmental stages. The post germination stage of rice were observed as one of the most sensitive stages to osmotic stress in rice (Grattan *et al.* 2002, Suriya-arunroj *et al.* 2004).

Salinity and drought caused detrimental effects on plant growth development, physiological traits and ultimately crop productivity. The reduction in growth is the consequence of several physiological responses including modifications in ion balance, water status, stomatal behavior and photosynthetic efficiency. Plants regulate the rate of photosynthetic CO₂ assimilation, generally under severe salinity and drought conditions. This reduction is partly due to reduced stomatal conductance and consequent restriction of the availability of CO₂ for carboxylation (Parida and Das 2005). Stomatal closing is the first line of defence against dehydration (Taiz and Zeiger 2009). Plants that possess better control of stomatal function are more efficient in drought tolerance. In fact, stomata can be regulated based on the level of water deficit by only partially closing, leading to some carbon fixation during stress conditions and an increase in the efficiency of water use (Lawlor and Tezara 2009). Similarly availability, uptake, transport and partitioning of nutrients gets disturbed under both drought and salinity stresses and thereby reduce the plant growth, development and crop production. To overcome these adverse effects of salinity and drought, plants tend to develop certain physiological mechanisms that result in saving of water and overcoming the nutritional disorders during later growth periods. Therefore, studies were conducted on salt tolerant and sensitive varieties of rice to assess their adaptations in leaf relative water content, membrane injury, leaf chlorophyll content, gas exchange parameters, maximum

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photochemical efficiency (F_v/F_m) and Quantum photochemical yield [$Y(II)$]. These parameters could serve as selection criteria for the screening of varieties and improvement in their drought and salinity adaptations.

MATERIALS AND METHODS

The present study was conducted in clay/porcelain pots in net house of Crop Improvement Division, ICAR-Central Soil Salinity Research Institute (CSSRI), Karnal, (29°43'N, 76°58'E, 245 m above the mean sea level), Haryana, India. The region witnesses sub-tropical, sub-humid climate with hot summers. The mean monthly maximum temperature touched 32-34°C, while minimum temperature remained 22-24°C during the study period. The average annual rainfall of the area is around 740 mm of which nearly 80% is received during a short time span of July to mid September which is associated with high relative humidity. The pan evaporation normally exceeds rainfall throughout the year except for the monsoonal months.

A randomized complete block design experiment with 5 replications was conducted in 20 kg capacity clay/porcelain pots containing normal soil (ECe 0.43 and pH 7.1). The net house was covered with a high quality polythene sheet to avoid the rain water entry and maintain the desired salinity and water deficit stress in the pots as per treatments. Surface sterilized seeds of CSR 10, CSR 36, IR 29 and Pusa 44 were directly sown and raised in pots. Plants were supplied with Hoagland nutrient solution before imposition of stress (Hoagland and Arnon 1950). Osmotic stress was imposed 3 weeks after sowing of seeds by application of 50 and 100 mM concentration of sodium chloride (NaCl) solution in water and drought stress by withholding water. Drought stress was created by gradually decreasing the water supply to pots. Drought treatments were imposed by 100% saturation of soil in pots first and then withholding irrigation supply till the depletion of water to 25 and 50% of water in soil (25 and 50% water scarcity).

Water deficit: Twenty kg capacity clay/porcelain pots were filled with 16 kg soil (field capacity 28% v/v) at bulk density of 1.45 g/cc having approximately 40% porosity. Initially 6.5 l water (up to field capacity) was given in the pots at weekly interval and evaporation was noted through pan. During the entire study period pan evaporation was 2-3 mm/day, i.e. 21 mm/week. On this basis, 25 and 50% water deficit treatments were induced.

Salt treatment: Based on the water requirement of the pots, 50 mM and 100 mM NaCl conc. was given to pots at regular weekly interval. At final harvest, 50 mM NaCl conc. pots have mean salinity level about 4.2 dS/m and 100 mM NaCl pots have 9.1 dS/m.

Physiological parameters were studied at 10 days after the imposition of stress. Fully expanded (third leaf from top) leaves were sampled for measurement of chlorophyll content as described by Hiscox and Israelstam (1979) using DMSO. Relative Water Content (RWC) was measured following procedures of Weatherley (1950) and membrane injury was estimated with Dionisio-Sese and Tobita (1998)

method. Photosynthetic rate (Pn), transpiration (E), stomatal conductance (gs) and leaf intercellular CO₂ concentration (Ci) were measured with an infrared open gas exchange system (LI-6400, LICOR Inc., Lincoln, NE, USA). Relationships were developed to calculate instantaneous water use efficiency (WUE) ($\mu\text{mol/mol}$) as P_n/E , the instantaneous intrinsic water use efficiency (WUE intrinsic; $\mu\text{mol/mol}$) as P_n/g_s and the instantaneous carboxylation efficiency (P_n/C_i) at 15 days after imposition of osmotic and drought stress treatments.

The photochemical efficiency of plants was obtained from the fluorescent analysis of chlorophyll with measurements on the same leaves that were evaluated for gas exchange. The maximum photochemical efficiency (F_v/F_m), Quantum photochemical yield [$Y(II)$] of photosystem II were determined using a portable pulse modulated fluorescence measurer (Junior PAM Chlorophyll Fluorometer, Germany) after adapting the leaves to the dark for 5 min via special leaf clips. The readings were made after saturating 1 s light pulses to promote the closing of the photosystem II reaction centers. For Na⁺ and K⁺ content, 100 mg of oven dried and well ground plant material was digested with 10 ml of HNO₃ : HClO₄ (3:1) di-acid mixture and readings were taken with flame photometer (PFP7, Jenway, Bibby Scientific, UK) using standard NaCl and KCl.

All the data were subjected to statistical analysis using the SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA). The pair wise means were compared using Least Significant Difference (LSD) test. Correlation analysis was performed to determine the association between the traits using the Pearson coefficient procedure.

RESULTS AND DISCUSSION

Analysis of variance showed significant variability among varieties and treatments applied, as indicated by significance of mean sum of squares. The mean sum of squares of varieties \times treatment interaction exhibited positive variable response of genotype to treatments for parameters studied under water deficit conditions coupled with salt stress (Table 1). The present study was conducted to evaluate the response of salt tolerant (CSR 10 and CSR 36) and salt sensitive (IR 29 and Pusa 44) rice varieties to salt stress coupled with drought at vegetative stage. Responses of these salt tolerant and sensitive rice varieties were monitored in relation to physiological parameters, viz. total chlorophyll concentration (Chl_T), membrane injury (MI), relative water content (RWC), gas exchange characteristics and chlorophyll fluorescence attributes as well as yield attributes. Phenological/Morphological growth performance of CSR10 was the best while, IR29 showed poor growth at 25 and 50 % WD amongst all the varieties used. Rather, at 50 and 100 mM NaCl along with 50 % WD, 80% of plants could not survive in variety IR29. Such magnitude of growth reduction observed under salt stress combined with drought has been ascribed either to osmotic or ionic effects (due to salt stress) causing inhibition of cell

division and cell elongation processes associated with the seedling growth and decrease in plastic extensibility of the growing cell walls (Hu and Schmidhalter 2005).

Physiological parameters were studied 10-15 days after the imposition of stress treatments. Most of the stress treatments showed significant differences in growth and physiology within varieties and between treatments. Significant reductions for Chl_T and RWC as well as increase in cell membrane injury were observed in all the four varieties with increase in salinity and drought stress but the reductions were varietal specific. Chl_T has been known as an index for evaluation of source, therefore decrease in concentration can be considered as a stomata non-limiting factor under stress conditions. Maximum reduction in Chl_T was recorded in IR 29 (82.3) (Table 2) followed by Pusa 44 (67.09), CSR 36 (61.54), but the minimum (54.38 %) reduction in CSR 10 (19.53 $\mu\text{g/ml}$) at 100 mM NaCl +50 % WD. It is well documented in earlier reports that Chl_T of resistant and sensitive cultivars reduced in response to abiotic stress. But resistant cultivars had relatively higher chlorophyll content in response to drought and thermal stress conditions (Shamsi 2010) than sensitive ones. Chlorophyll content was negatively correlated with MI, WUE and Pn/Ci, whereas, strong positive (maximum) correlation was recorded with Pn and Ci (Table 3). RWC is useful indicator of determining the physiological water status of plants (Makbul *et al.* 2011) and the degree of drought stress. RWC decreased under salinity alone and in combination with drought. Maximum water was retained in salt tolerant variety CSR 10 at 100 mM NaCl with 50 % Water Deficit (WD), while minimum in Pusa 44 (Table 2). RWC showed positive correlation with all parameters except MI and WUE but the response was varietal specific (Table 3). Under stress, RWC of leaves was higher that may be estimated through measurements of initial stages of leaf development and declines as the dry matter accumulates and leaf matures. Mationn *et al.* (1989) represented similar report, a drop in the amount of RWC in tolerant and sensitive cultivars of barley. Significant differences in leaf water potential and RWC were recorded among the tolerant and sensitive/intolerant cultivars of wheat and these results were consistent with Subrahmanyam *et al.* (2006); Tas & Tas (2007). Salt tolerant species may possibly survive in salt stress condition using other defence mechanisms such as ion homeostasis, antioxidation and hormonal systems.

The integrity and stability of cell membrane in water deficit conditions can be considered a possible adaptive mechanism and an indicator of stress resistance. Plant membranes are subjected to changes associated with the increase in permeability and loss of integrity under abiotic environmental stresses (Blokhina *et al.* 2003). Stress conditions of salt alone or coupled with drought caused highest rate of membrane injury in Pusa 44 (73.51%) at 100 mM NaCl + 50%WD (Table 2), while minimum in CSR 10 (57.23%). Therefore, the ability of cell membranes to control the rate of ion movement in and out of cells is used as a test of damage to a great range of tissues. MI was negatively

Table 1 Analysis of variance among chlorophyll content, membrane injury, relative water content, Fv/Fm, Y [II] and gas exchange (Pn, gs, E, Ci, WUE, WUEi, Pn/Ci), of four rice varieties under interactive salinity and drought stresses

Source of variance	Degree of freedom	Means sum of squares											
		Chlorophyll content ($\mu\text{g/ml}$)	MI (%)	RWC (%)	Fv/Fm	Y [II]	Photosynthetic rate (Pn) ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)	Stomatal conductance (gs) ($\text{mmol CO}_2/\text{m}^2/\text{s}$)	Transpiration rate (E) ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)	CO ₂ assimilation ($\mu\text{mol CO}_2/\text{mol}$)	WUE	WUEi (Pn/g) (Pn/Ci)	Carboxylation efficiency (Pn/Ci)
T	6	491.98**	1555.73**	3524.13**	0.05**	0.06**	281.72**	178.18**	218.37**	18237.10**	0.34**	0.002**	860.18**
V	3	87.31**	139.91**	767.40*	0.01**	0.02**	83.21**	12.71**	15.03**	3194.20**	0.18**	0.007**	259.65**
R	2	0.10	8.89**	8.13	0.05**	0.07**	0.59	0.39	2.78	51.67	0.07**	0.00002	53.16
T × V	18	7.65**	35.99**	45.88**	0.01**	0.02**	2.59**	4.39**	3.75**	570.19**	0.06**	0.0003**	141.96**
Error	54	0.14	1.34	4.94	0.002	0.002	0.48	0.27	1.25	26.12	0.02	0.00005	25.42

Table 2 Chlorophyll content, membrane injury, relative water content, Fv/Fm, Y [II] and gas exchange (Pn, gs, E, Ci, WUE, WUEi, Pn/Ci), of four rice varieties under interactive salinity and drought stresses

Variety	Treatment	Chlorophyll content	MI (%)	RWC (%)	(Fv/Fm)	Y [II]	Pn	gs	E	CO ₂ assimilation	WUE	WUEi	Pn/Ci
CSR 10	Control	27.16 ^A	33.15 ^F	89.86 ^A	0.66 ^A	0.60 ^A	22.20 ^A	9.35 ^A	16.58 ^A	320.84 ^A	1.34 ^B	0.07 ^A	2.38 ^C
	50 mM NaCl	24.06 ^B	37.82 ^E	80.75 ^B	0.64 ^A	0.59 ^B	17.55 ^B	8.03 ^B	13.46 ^B	315.22 ^{AB}	1.31 ^{BC}	0.06 ^B	2.19 ^C
	100 mM NaCl	23.01 ^C	41.54 ^D	72.52 ^C	0.60 ^B	0.58 ^B	16.34 ^{BC}	6.21 ^C	12.22 ^C	309.01 ^B	1.34 ^B	0.05 ^B	2.63 ^C
	50 mM NaCl + 25% WD	19.44 ^D	48.85 ^C	69.16 ^C	0.59 ^B	0.56 ^C	15.14 ^C	5.27 ^D	11.54 ^{CD}	298.60 ^C	1.31 ^{BC}	0.05 ^B	2.88 ^C
	100 mM NaCl + 25% WD	16.28 ^E	52.26 ^B	63.80 ^D	0.56 ^C	0.54 ^D	12.30 ^D	4.83 ^D	11.34 ^D	284.67 ^D	1.09 ^C	0.04 ^C	2.56 ^C
	50 mM NaCl + 50% WD	14.38 ^F	56.45 ^A	57.48 ^E	0.55 ^C	0.49 ^E	11.19 ^D	1.07 ^E	8.59 ^E	276.94 ^D	1.30 ^{BC}	0.04 ^{CD}	10.48 ^B
	100 mM NaCl + 50% WD	12.39 ^G	57.23 ^A	46.19 ^F	0.55 ^C	0.45 ^F	9.45 ^E	0.76 ^E	4.92 ^F	259.21 ^E	1.98 ^A	0.04 ^D	12.62 ^A
General mean		19.53	46.76	68.54	0.59	0.54	14.88	5.07	11.24	294.93	1.38	0.05	5.11
CV(%)		0.86	2.09	4.12	1.74	0.72	5.69	6.74	4.31	1.58	9.93	5.75	19.83
SE(d)		0.138	0.796	2.305	0.008	0.003	0.691	0.279	0.395	3.815	0.112	0.002	0.827
LSD (P=0.05)		0.2999	1.7345	5.0219	0.0184	0.007	1.5063	0.6087	0.8608	8.3118	0.2439	0.0051	1.8015
CSR 36	Control	23.61 ^A	33.28 ^E	81.90 ^A	0.64 ^A	0.61 ^A	20.88 ^A	10.68 ^A	17.14 ^A	337.87 ^A	1.28	0.06 ^A	1.96 ^C
	50 mM NaCl	20.85 ^B	40.78 ^D	80.55 ^A	0.64 ^A	0.59 ^{AB}	16.06 ^B	9.65 ^A	11.94 ^B	320.75 ^B	1.35	0.05 ^B	1.68 ^C
	100 mM NaCl	17.97 ^C	42.28 ^D	74.93 ^B	0.63 ^A	0.57 ^{BC}	14.21 ^C	5.65 ^B	11.55 ^B	313.51 ^B	1.23	0.05 ^C	2.52 ^C
	50 mM NaCl + 25% WD	16.36 ^D	45.63 ^C	66.66 ^C	0.62 ^B	0.55 ^{CD}	13.09 ^D	0.51 ^C	10.98 ^B	298.02 ^C	1.20	0.04 ^C	36.02 ^A
	100 mM NaCl + 25% WD	14.71 ^E	49.23 ^B	54.89 ^D	0.57 ^C	0.53 ^D	10.32 ^E	1.36 ^C	9.51 ^{BC}	279.76 ^D	1.09	0.04 ^D	9.34 ^{BC}
	50 mM NaCl+50% WD	10.65 ^F	55.42 ^A	47.51 ^E	0.55 ^D	0.48 ^E	9.45 ^E	0.52 ^C	6.43 ^{CD}	265.70 ^E	1.47	0.04 ^D	18.20 ^{BC}
	100 mM NaCl + 50% WD	9.08 ^G	57.84 ^A	41.80 ^F	0.51 ^E	0.48 ^E	7.08 ^F	0.37 ^C	5.08 ^D	222.86 ^F	1.40	0.03 ^E	19.52 ^{AB}
General mean		16.18	46.35	64.03	0.59	0.54	13.01	4.10	10.38	291.21	1.29	0.04	12.75
CV(%)		2.55	3.09	3.56	1.21	3.39	4.68	14.43	17.83	2.38	11.67	4.86	72.98
SE(d)		0.337	1.168	1.863	0.006	0.015	0.497	0.484	1.511	5.653	0.123	0.002	7.598
LSD (P=0.05)		0.735	2.5449	4.059	0.0128	0.0328	1.0827	1.0536	3.2913	12.316	NS	0.0038	16.554
IR 29	Control	27.19 ^A	33.52 ^G	85.60 ^A	0.66 ^A	0.60 ^A	20.04 ^A	10.62 ^A	16.42 ^A	333.04 ^A	1.22 ^B	0.06 ^A	1.89 ^E
	50 mM NaCl	21.61 ^B	38.34 ^F	78.72 ^B	0.64 ^A	0.56 ^B	16.89 ^B	7.23 ^B	11.90 ^B	310.99 ^B	1.42 ^A	0.05 ^B	2.40 ^E
	100 mM NaCl	17.31 ^C	44.18 ^E	66.91 ^C	0.58 ^B	0.55 ^B	11.12 ^C	2.83 ^C	9.88 ^C	301.63 ^C	1.13 ^{BC}	0.04 ^D	4.01 ^{DE}
	50 mM NaCl + 25% WD	15.78 ^D	49.21 ^D	57.81 ^D	0.55 ^C	0.52 ^C	12.38 ^C	1.40 ^D	11.39 ^B	292.35 ^D	1.09 ^C	0.04 ^C	8.91 ^{CD}
	100 mM NaCl + 25% WD	13.64 ^E	53.05 ^C	49.81 ^E	0.52 ^D	0.47 ^D	8.94 ^D	0.70 ^{DE}	9.20 ^C	269.39 ^E	0.97 ^D	0.03 ^D	12.84 ^C
	50 mM NaCl + 50% WD	10.20 ^F	65.25 ^B	44.30 ^F	0.50 ^D	0.41 ^E	6.34 ^E	0.26 ^E	5.46 ^D	240.73 ^F	1.16 ^{BC}	0.03 ^E	25.30 ^B
	100 mM NaCl + 50% WD	4.81 ^G	71.17 ^A	36.79 ^G	0.45 ^E	0.36 ^F	5.07 ^E	0.17 ^E	3.69 ^E	204.59 ^G	1.38 ^A	0.02 ^E	31.22 ^A
General mean		15.79	50.67	59.99	0.56	0.50	11.54	3.32	9.70	278.96	1.20	0.04	12.37
CV(%)		2.35	1.97	3.41	2.76	2.46	7.12	17.56	4.60	0.82	4.79	6.28	22.88
SE(d)		0.304	0.814	1.671	0.012	0.010	0.671	0.475	0.365	1.875	0.047	0.002	2.311
LSD (P=0.05)		0.6614	1.7746	3.6411	0.0272	0.0217	1.4613	1.0358	0.7949	4.0846	0.1019	0.0044	5.0342
Pusa 44	Control	31.24 ^A	34.40 ^G	86.63 ^A	0.64 ^A	0.61 ^A	20.35 ^A	11.03 ^A	19.24 ^A	330.35 ^A	1.07 ^B	0.06 ^A	1.87 ^D
	50 mM NaCl	27.25 ^B	39.33 ^F	75.51 ^B	0.62 ^{AB}	0.58 ^{AB}	12.57 ^B	6.70 ^B	11.38 ^B	316.46 ^B	1.11 ^B	0.04 ^B	1.89 ^D
	100 mM NaCl	21.86 ^C	44.22 ^E	57.76 ^C	0.58 ^B	0.55 ^B	10.57 ^C	3.78 ^C	11.24 ^B	304.57 ^C	0.94 ^B	0.03 ^C	2.80 ^D

Contd.

Table 2 (Concluded)

Variety	Treatment	Chlorophyll content	MI (%)	RWC (%)	(Fv/Fm)	Y [II]	Pn	gs	E	CO ₂ assimilation	WUE	WUEi	Pn/Ci
	50 mM NaCl + 25% WD	18.88 ^D	47.04 ^D	49.88 ^D	0.54 ^C	0.50 ^C	9.75 ^C	2.25 ^D	9.00 ^C	263.83 ^D	1.08 ^B	0.04 ^{BC}	4.34 ^D
	100 mM NaCl + 25% WD	14.34 ^E	54.60 ^C	44.26 ^E	0.51 ^{CD}	0.46 ^D	7.48 ^D	0.80 ^E	6.88 ^D	251.18 ^E	1.09 ^B	0.03 ^D	9.34 ^C
	50 mM NaCl + 50% WD	12.47 ^F	65.63 ^B	35.83 ^F	0.48 ^D	0.42 ^E	6.20 ^E	0.27 ^E	4.18 ^E	211.26 ^F	1.48 ^A	0.03 ^D	23.20 ^B
	100 mM NaCl + 50% WD	10.28 ^G	73.95 ^A	30.33 ^G	0.42 ^E	0.35 ^F	4.83 ^F	0.18 ^E	3.20 ^E	197.08 ^G	1.53 ^A	0.02 ^E	26.63 ^A
General mean		19.48	51.31	54.32	0.54	0.49	10.25	3.57	9.30	267.82	1.19	0.04	10.01
CV(%)		2.27	2.70	3.69	4.14	4.78	5.28	15.75	11.00	1.95	7.85	4.29	16.46
SE(d)		0.360	1.130	1.637	0.018	0.019	0.442	0.460	0.836	4.262	0.076	0.001	1.345
LSD (P=0.05)		0.7849	2.4614	3.5657	0.0399	0.0421	0.9624	1.0012	1.8204	9.2856	0.1656	0.0028	2.9313

Means with at least one letter common are not statistically significant (P < 0.05) using DUNCAN's Multiple Range Test.

correlated with all the parameters except, WUE and Pn/Ci, where significant positive correlation was reported (Table 3). Valentovic *et al.* (2006) also reported that the electrolyte leakage in case of the sensitive maize cultivar increased more, i.e. 11 to 54% as compared to lesser increase in tolerant cultivars.

Studies on the maximum quantum efficiency of photosystem II, the Fv/Fm ratio (variable to maximal fluorescence) reflecting the maximum efficiency of the light absorption and its conversion into chemical energy by the antenna complex (Maxwell and Johnson 2000) and also correlated with the quantum yield of net photosynthesis, has proven to be a good physiological reference to diagnose the integrity of the photosynthetic system during water deficit (Graça *et al.* 2010, Silva *et al.* 2011). It is frequently used as an indicator of the photo-inhibition or some other injury caused to the PS II complexes (Rohacek 2002). The chlorophyll fluorescence parameter Fv/Fm ratio started declining after imposition of stress with continuous reduction as the water limitation progressed in all the varieties. However, in case of control plants, the maximal photochemical efficiency of PS II (Fv/Fm) showed no significant difference among different varieties (Table 2). Under non-stressed conditions, the Fv/Fm ratio values were about 0.65 and almost constant for different varieties. The decline in Fv/Fm value due to imposed stress in all the four varieties as compared to control (Table 2) indicated disturbances in adequate electron translocation from PS II to electron acceptor, needed for regeneration of RuBP - under stress situations (Kafi 2009).

Salt stress alone exhibited lesser change in the Fv/Fm ratio of CSR 36 as compared to the other varieties studied. When salt stress was coupled with drought (100 mM NaCl+50%WD), CSR 10 showed the highest Fv/Fm (0.55) ratio while Pusa 44 showed the lowest (0.42) value (Table 2). In the present study, water deficit conditions with salt stress were found to reduce the Fv/Fm ratio in all the rice varieties. This suggests that the total amount of light

energy transformed in PS II reaction centre was decreased. Thus, it could be implied that the changes observed in photochemical activity of PS II contributed to the limitations of photosynthesis activity under water deficit (Tezara *et al.* 2002). Salt tolerant variety CSR 10 showed a little reduction in Fv/Fm as compared to other varieties under stress condition, indicating their tolerance to combined stress. The reduction was more pronounced in variety Pusa 44 followed by IR 29, indicating that these two varieties are susceptible to water stress at vegetative stage. Fv/Fm ratio showed maximum positive correlation with Chl_T followed by RWC in IR 29, whereas minimal correlation was found with MI in IR 29 followed by Pusa 44 (Table 3).

It was observed that chlorophyll fluorescence could be used to estimate the quantum effectiveness of electron transport through PS II in leaves and this PS II is associated with CO₂ assimilation (Genty and Harbinson 1990). A decline in quantum yield of PS II observed with increasing stress conditions in our study (Table 2) might have resulted from the closure of stomata as induced by osmotic stress and the accumulation of salt (Dionisio-Sese and Tobita 2000). Chlorophyll fluorescence attributes have shown significant correlation with gas exchange parameters as well as with Chl_T and RWC (Table 3).

The degree of retardation in gas exchange parameters increased under salt stress alone but significantly higher reduction was observed under combined (osmotic and water deficit) stress treatment over the control, and the maximum retardation was recorded under 100 mM NaCl + 50% WD treatment (Table 2). Among the gas exchange characteristics, photosynthetic activities of rice varieties significantly differed but these differences could not be expressed in control conditions. The salt tolerant variety, CSR 10 maintained better gas exchange characteristics than other varieties. Maximal stress induced reduction in photosynthetic rate (Pn), was observed in the following order, i.e. Pusa 44, IR 29 followed by CSR 36 and CSR 10. Decrease in Pn under low relative water content is

Table 3 Pearson's correlation coefficients for association among chlorophyll content, membrane injury, relative water content, Fv/Fm, Y [II] and gas exchange (Pn, gs, E, Ci, WUE, WUEi, Pn/Ci), of four rice varieties under interactive salinity and drought stresses

Trait	Chlorophyll	MI	RWC	Fv/Fm	Y [II]	Pn	gs	E	Ci	WUE	WUEi	Pn/Ci
<i>CSR 10 (Salt tolerant)</i>												
Chlorophyll	1	-0.984**	0.960**	0.901**	0.923**	0.967**	0.958**	.924**	0.967**	-0.368	0.947**	-0.777**
MI (%)		1	-0.939**	-0.926**	-0.868**	-0.955**	-0.951**	-0.901**	-0.943**	0.329	-0.934**	0.721**
RWC (%)			1	0.893**	0.931**	0.955**	0.950**	.961**	0.945**	-0.470*	0.940**	-0.793**
Fv/Fm				1	0.739**	0.882**	0.866**	.822**	0.864**	-0.255	0.865**	-0.599**
Y [II]					1	0.886**	0.931**	.918**	0.930**	-0.523*	0.866**	-0.899**
Pn						1	0.933**	.927**	0.931**	-0.336	0.996**	-0.701**
gs							1	.938**	0.940**	-0.432	0.913**	-0.869**
E								1	0.934**	-0.640**	0.913**	-0.829**
CO ₂ assimilation									1	-0.511*	0.900**	-0.815**
WUE										1	-0.312	0.592**
WUEi											1	-0.672**
(Pn/Ci)												1
<i>CSR 36 (Salt tolerant)</i>												
Chlorophyll	1	-0.974**	0.971**	0.907**	0.906**	0.961**	0.883**	0.894**	0.949**	-0.249	0.937**	-0.452*
MI (%)		1	-0.938**	-0.920**	-0.885**	-0.961**	-0.867**	-0.890**	-0.939**	0.228	-0.938**	0.498*
RWC (%)			1	0.940**	0.884**	0.922**	0.854**	0.839**	0.947**	-0.214	0.892**	-0.411
Fv/Fm				1	0.798**	0.868**	0.744**	0.832**	0.934**	-0.309	0.834**	-0.356
Y [II]					1	0.881**	0.810**	0.789**	0.867**	-0.200	0.857**	-0.382
Pn						1	0.886**	0.900**	0.928**	-0.118	0.993**	-0.397
gs							1	0.741**	0.795**	0.032	0.876**	-0.654**
E								1	0.836**	-0.503*	0.902**	-0.363
CO ₂ assimilation									1	-0.215	0.884**	-0.417
WUE										1	-0.105	0.076
WUEi											1	-0.354
(Pn/Ci)												1
<i>IR 29 (Salt sensitive)</i>												
	Chlorophyll	MI	RWC	Fv/Fm	Y [II]	Pn	gs	E	Ci	WUE	WUEi	Pn/Ci
Chlorophyll	1	-0.973**	0.975**	0.966**	0.942**	0.963**	0.908**	0.963**	0.967**	0.018	0.940**	-0.896**
MI (%)		1	-0.962**	-0.953**	-0.968**	-0.937**	-0.841**	-0.947**	-0.981**	0.009	-0.916**	0.961**
RWC (%)			1	0.964**	0.934**	0.962**	0.922**	0.915**	0.945**	0.178	0.944**	-0.883**
Fv/Fm				1	0.916**	0.945**	0.914**	0.910**	0.928**	0.135	0.928**	-0.891**
Y [II]					1	0.913**	0.779**	0.929**	0.982**	-0.031	0.887**	-0.941**
Pn						1	0.923**	0.954**	0.922**	0.195	0.996**	-0.841**
gs							1	0.842**	0.791**	0.327	0.915**	-0.706**
E								1	0.952**	-0.094	0.937**	-0.879**
CO ₂ assimilation									1	-0.087	0.894**	-0.955**
WUE										1	0.242	0.117
WUEi											1	-0.812**
(Pn/Ci)												1
<i>Pusa 44 (Salt sensitive)</i>												
Chlorophyll	1	-0.939**	0.989**	0.923**	0.935**	0.948**	0.953**	0.946**	0.958**	-0.642**	0.896**	-0.831**
MI (%)		1	-0.925**	-0.940**	-0.956**	-0.868**	-0.841**	-0.896**	-0.972**	0.797**	-0.807**	0.956**
RWC (%)			1	0.922**	0.916**	0.949**	0.963**	0.933**	0.947**	-0.608**	0.898**	-0.812**
Fv/Fm				1	0.915**	0.851**	0.851**	0.845**	0.942**	-0.600**	0.790**	-0.868**
Y [II]					1	0.859**	0.848**	0.890**	0.962**	-0.756**	0.792**	-0.898**
Pn						1	0.963**	0.979**	0.874**	-0.548*	0.986**	-0.728**
gs							1	0.924**	0.872**	-0.481*	0.932**	-0.691**
E								1	0.909**	-0.675**	0.949**	-0.788**
CO ₂ assimilation									1	-0.769**	0.791**	-0.920**
WUE										1	-0.468*	0.872**
WUEi											1	-0.653**
(Pn/Ci)												1

*P< 0.05; **P<0.01; ns: Non-significant. Pearson's correlation coefficients for association using SPSS v19.

caused by impaired metabolism (shortage of ATP, limiting RuBP synthesis without or with less inhibition of photosynthetic enzyme) including Rubisco. Photosynthesis is particularly sensitive to water deficit because the stomata tend to close to conserve water under deficit conditions, reducing CO₂ diffusion to the fixation sites in the leaf mesophyll in the vicinity of the enzyme Rubisco, which causes diminished photosynthesis and consequently reduced productivity (Lawlor and Tezara 2009, Galmés *et al.* 2011). Monitoring gas exchange in plants is a common approach, with stomatal conductance (gs) reported as one of the most sensitive indicators of stress. However, minimum reduction in gs, was recorded in CSR 10 and CSR 36 followed by Pusa 44 and IR 29. The reductions in Pn were largely associated with stomatal closure, and therefore stomatal effects could be the most important to justify photosynthesis depression. Similarly, minimum reduction in transpiration rate (E) was recorded in CSR 10 and the maximal in IR 29. A significant reduction in leaf intrinsic CO₂ concentration (Ci) was also observed in all rice varieties due to salt and drought stress, but again the reduction being varietal specific. More reduction in Ci due to stresses was recorded in IR 29 and Pusa 44 followed by CSR 36 and CSR 10. Decreased stomatal conductance (gs) resulted in lesser photosynthesis (Pn) and lower CO₂ concentration inside the leaf and in the chloroplast. Possible reasons for decrease in gas exchange parameters include stomatal closure, feedback inhibition due to reduced sink activity, decreased efficiency of Rubisco, displacement of essential cations from the endo-membrane structure (leading to changes in permeability), and swelling and disorganization of the grana (Flowers and Yeo 1981), or due to the direct effects of salt on stomatal conductance via reduction in guard cell turgor and intercellular CO₂ partial pressure (Dionisio-Sese and Tobita 2000).

Among all these parameters studied, highest significant positive correlation was found between Pn and WUEi ($r = 0.99^{**}$) while highest negative correlation Chl_T and MI ($r = -0.97^{**}$) for all the varieties studied. Among gas exchange parameters, strong positive correlation was also identified between transpiration rate and photosynthetic rate ($r=0.961$) in CSR 10 followed by Pusa 44 ($r=0.933$), whereas transpiration rate displayed maximum inverse relationship with MI in IR 29. Stomatal conductance, Pusa 44 displayed maximum correlation with RWC and Pn, whereas the maximum inverse correlation was found with MI ($r=0.951$) followed by Pn/Ci ($r = -0.869$) in CSR 10. Across the varieties, maximum positive correlation of CO₂ assimilation was observed with chlorophyll followed by RWC (Table 3).

Plant water use efficiency also reduced significantly due to salt and drought stress applied. Reduction in water use efficiency (WUE) was found in all rice varieties, but CSR10 showed highest WUE at 100 mM NaCl + 50% WD treatment, whereas it is insignificant in other treatments. Application of 50 mM NaCl increased WUE as compared to control but when the salt load increased to 100 mM NaCl, the WUE decreased drastically as compared to control in IR

29 and Pusa 44. The maximum positive and negative correlation in respect to WUE was recorded with chlorophyll (Pusa 44) and MI (IR 29), respectively (Table 3).

Furthermore, intrinsic water use efficiency (WUEi) was also recorded under salt induced as well as salt coupled with drought stress in all the varieties of rice but the increasing or decreasing effect was varietal specific. In case of CSR 36, IR 29 and Pusa 44 mean WUEi was similar but it differed in CSR 10. WUEi displayed highest inverse relationship with MI in CSR 10 followed by CSR 36, however, highest positive relationship was recorded with Pn in CSR 10 and IR 29 ($r=0.996$). The plant reacts to water deficit with a rapid closure of stomata to avoid further loss of water through transpiration (Lawlor 1995) and consequently, the diffusion of CO₂ into the leaf is restricted (Flexas *et al.* 2006). The observations on mesophyll conduction/ carboxylation efficiency (Pn/Ci) showed increasing trend and the maximum value of 36.02 was observed in CSR 36 at 50 mM NaCl + 25% WD treatment; whereas the maximum Pn/Ci was observed in IR 29 under combined salt and drought stress (100 mM NaCl + 50% WD) followed by Pusa 44. The efficiency of gas exchange in the plants possessing higher photosynthetic capacity, higher water use efficiency and higher intrinsic water use efficiency has been positively correlated with the maintenance of productivity in rice (Centritto *et al.* 2009) and wheat (Monneveux *et al.* 2006).

A good supply of K⁺ to plants can minimize injurious effects of high Na⁺ under stress conditions. In the present study, the level of K⁺ gradually decreased while that of Na⁺ increased dramatically. Influx of Na⁺ into tissues is often accompanied by a decrease in K⁺ concentrations, leading to increase in Na⁺/K⁺ ratios. The decrease in K⁺ contents was due to the presence of excessive Na⁺ in growth medium because high external Na⁺ content is known to have an antagonistic effect on K⁺ uptake in plants (Sarwar and Ashraf 2003). The Na⁺/K⁺ ratio in the leaves of different rice varieties, under control and stress conditions is presented in Fig 1.

There is a large increase in Na⁺/K⁺ ratio under stress compared to control, however, varieties showed variation in

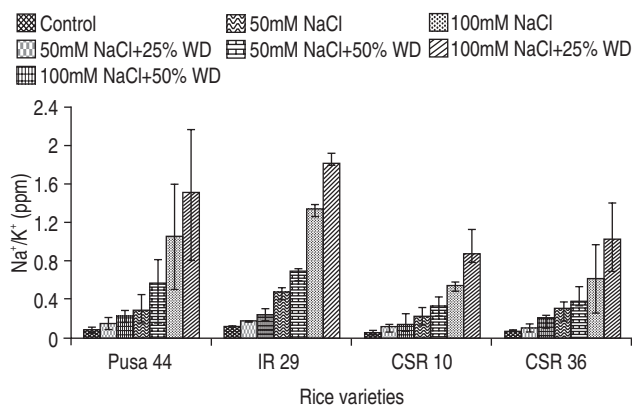


Fig 1 Effect of salt stress coupled with drought on Na⁺/K⁺ ratio in different rice varieties.

increasing pattern of Na^+/K^+ ratio in response to applied stress. Under combined stress the highest value of Na^+/K^+ ratio was recorded in IR 29 (1.83), whereas lowest in CSR 10 (0.96). It is clear from the results that all the varieties showed differing responses to salt stress coupled with drought for the accumulation of ions. Sodium (Na^+) content increased in all the varieties. The varieties CSR 10 and CSR 36 accumulated less Na^+ ion in leaves under stress. Generally, the salt tolerant varieties maintain low concentration of Na^+ in their leaves than those of salt sensitive lines, when exposed to stress (Lutts and Guerrir 1995), therefore Na^+ in the leaves of crop plants can be used as an important indicator of tolerance to stresses. Weimberg (1987) observed high levels of Na^+ inside the cells inhibit the K^+ uptake and as a result it causes an increase in the Na^+/K^+ ratio. The increase in the Na^+/K^+ ratio might be attributed to the fact that Na^+ causes a disturbance in the ion balance in plant by an increase in the Na^+ uptake (Cicek and Cakirlar 2008). Many of the deleterious effects of Na^+ seem to be related to the structural and functional integrity of membranes (Kurth *et al.* 1986). It is generally known that the maintenance of low cytosolic Na^+ concentration and Na^+/K^+ homeostasis is an important aspect of salinity tolerance and that the salt tolerant lines showed lower Na^+/K^+ levels (Chattopadhyay *et al.* 2002).

Salt stress coupled with drought accelerated all phenological growth stages, reduced the normal growth and development periods, dry matter production and final yield. Rice appears to be more sensitive in the early developmental stages after germination. But it is also sensitive at flowering (Eynard *et al.* 2005). Interaction of different levels of salinity and water deficit (Fig 2) showed that the highest grain weight (73-77 g/5 plants) exhibited at control treatment and the least grain weight (7.9-30.0 g/5 plants) at 100mM NaCl + 50 % WD in 4 rice varieties differing in their tolerance.

Abiotic stresses at the early developmental growth stages had more effect on grain yield reduction, as in comparison with control treatment, at 100mM NaCl + 50 % WD, there was 88.31% reduction in Pusa 44 followed by 86.12, 44.07 and 31.68 % reductions in grain weight of IR

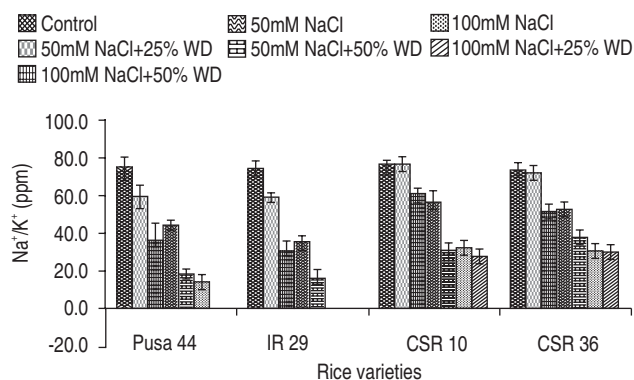


Fig 2 Effect of salt stress coupled with drought on grain yield (g/5 plants) in different rice varieties.

29, CSR 10 and CSR 36, respectively. Reduction in yield due to abiotic stresses was reported by Zeng and Shannon (2000) and Cha-um and Kirdmanee (2010). Stresses reduced yield by reducing the number of filled grains per panicle. Reduction in seed weight may be possibly due to decreased pollen viability or decreased receptivity of the stigmatic surface or both (Abdullah *et al.* 2001). It was reported that reduction in spikelet number per panicle is the major cause of yield loss due to salinity (Zeng and Shannon 2000). In summary, water deficit stress (salt and drought) affected plant growth and development by impairing physiological and biochemical mechanisms which ultimately lead to reduction in grain yield. In the present experiment, salt and drought stress imposed separately and also in combination caused more damage in salt sensitive (IR 29) rice varieties as evidenced by the lower RWC, higher membrane injury, low chlorophyll content, decrease in gas exchange characteristics, high Na^+/K^+ and yield.

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