RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
46 (1) 120-135, 2014

CHANGES IN PHOTOSYNTHESIS, CHLOROPHYLL FLUORESCENCE, GAS EXCHANGE PARAMETERS AND OSMOTIC POTENTIAL TO SALT STRESS DURING EARLY SEEDLING STAGE IN RICE (*Oryza sativa* L.)

P. SENGUTTUVEL[1,4*], C. VIJAYALAKSHMI[3], K. THIYAGARAJAN[2], J.R. KANNANBAPU[1], SUNEETHA KOTA[4], G. PADMAVATHI[4], S. GEETHA[3], N. SRITHARAN[2] and B.C. VIRAKTAMATH[3]

[1]Centre for Plant Breeding and Genetics, TNAU, Coimbatore-641 003
[2]Department of Plant Physiology, TNAU, Coimbatore-641 003
[3]Department of Plant Breeding and Genetics, ADAC, Trichy-620 009, TN, India
[4]Directorate of Rice Research, Rajendranagar, Hyderabad-500 030, Andhra Pradesh, India
*Corresponding author's email: senguttuvel@gmail.com

SU MMARY

Salinity is a serious problem all over the world with an average of 830 M ha being affected. In India, it's about 13.3 M ha with coastal and inland salinity. The present study focused on effects of salinity on chlorophyll content, chlorophyll fluorescence, gas exchange parameters and osmotic potential in different salt concentration. The chlorophyll content reduced as salinity level increases and chlorophyll a and b concentration coincides with decrease in Fv/Fm ratio. The genotype IR72593 exhibited better survival response than the susceptible genotype IR29. The sensitive cultivar IR29 responds to salinity stress quickly and did not recover after one week of salinity stress which led to complete plant death. Tolerant genotypes showed lower reduction in gas exchange parameters, while IR29 showed sudden reduction during the initial hours of salt stress and decreased at 312 hours due to complete death of plants. Osmotic potential becomes negative with increase in salinity level irrespective of genotypes; however tolerant genotype responds lower than sensitive genotype. This differential response of tolerant genotypes to salinity stress is due to the reduced transpiration rate and closure of stomatal openings during stress period, with no significant change in chlorophyll content. The transpiration rate and stomatal conductance is positively associated with intake of Na ions into the plant system.

Keywords: Rice, chlorophyll pigments, Fv/Fm ratio, stomatal conductance, transpiration rate, osmotic potential, salinity

Manuscript received: June 12, 2012; Decision on manuscript: March 19, 2014; Manuscript accepted: April 25, 2014.

Communicating Editor: Bertrand Collard

INTRODUCTION

Salinity is one of the major problem and important abiotic stress pose severe environmental and crop related problems around the world. Rice is being staple food crop for more than 50% of world population is under threat of increasing salinity. The better alternatives are reclamation of soil and breeding tolerant genotypes. Screening and breeding new genotypes for target environment is ideal and also long term goal for sustainable agriculture. Rice is being tolerant during germination, very sensitive during early seedling stage; gains

tolerance during vegetative stage becomes sensitive during pollination and fertilization and more tolerant during maturity (IRRI, 1967). Tolerance at seedling stage is the best time to access and predict the level of tolerance because it is quicker in terms of time and resources than reproductive stage screening. Physiological screening for salt tolerance is best option at early vegetative stage, especially gas exchange parameters are closely associated with photosynthetic pigments and will reflect the stress parameters directly.

Chlorophyll content and photosynthetic function were inversely proportional to salinity level (Ota and Yasue, 1962). Garg and Garg (1982) reported a marked reduction in chlorophyll content with the increasing levels of salinity. The reduction in chlorophyll content due to salt stress in rice was noticed by Krishnamurthy *et al.,* (1987). Nieves *et al.,* (1991) reported a decrease in chlorophyll content when plants were grown in saline medium. Chlorophyll fluorescence is a rapid and non-destructive method used to screen varieties for salinity tolerance (Maxwell and Johnson, 2000). Brugnoli and Lauteri (1991), Mishra *et al.,* (1991) reported that no significant change in the photosynthetic quantum yield (Fv/Fm) in response to NaCl treatments. These authors concluded that Fv/Fm was not a useful indicator of salt stress. In contrast, Smillie and Nott (1982), Bongi and Loreto (1989) and Misra *et al.,* (2001) suggested Fv/Fm was an early indicator of salt stress. Although there is some evidence in the literature that salt stress may lead to changes in the maximum quantum yield, based on the F_v / F_m ratio, the photochemical efficiency of rice cultivars grown in nutrient solution was almost unaffected, even when the carbon assimilation rate was severely restricted (Dionisio-Sese and Tobita, 2000).

The leaf gas exchange parameters i.e. transpiration rate and stomatal diffusive resistance expressed the significance of various physiological processes along with their impact on biomass production and grain yield (Dey and Rao, 1989). Dionisio-Sese and Tobita (1998) reported that subjecting plants to salinity level of 12 dS m^{-1} for 1 week during seedling stage causes retardation in growth due to direct effect of ions on photosynthesis. Dionisio-Sese and Tobita (2000) reported that the net photosynthetic rate, measured in terms of CO_2 assimilation of the youngest fully expanded leaf of 4 rice varieties, declined with increasing level of salinity stress. These authors concluded that it might be due to a direct effect of salt on stomatal resistance via reduction in guard cell turgor, leading to a reduction in intercellular CO_2 partial pressure.

High concentrations of salts disrupt homeostasis in water potential and ion distribution in plants. Altered water status most likely brings about initial growth reduction (Dash and Panda, 2001). Salinity also induces water deficit even in well-watered soils by decreasing the osmotic potential of soil solutes, thus making it difficult for roots to extract water from their surrounding media (Nemoto and Sasakuma, 2002). The deleterious effects of salinity on plant growth is attributed to the decrease in osmotic potential of the growing medium, specific ion toxicity and nutrient ion deficiency by disturbing potassium nutrition (Luo *et al.,* 2005). This study focuses on the effect of salinity on photosynthesis, gas exchange parameters and osmotic potential in rice at different levels of salinity.

MATERIALS AND METHODS

Three genotypes IR74802-3R-7-1-2, IR73104-B-1-1-3-2-1, IR72593B-19-2-3-1 (henceforth mentioned as IR74802, IR73104 and IR72593) were selected along with FL478 and IR29 served as tolerant and susceptible checks respectively. Seeds of each genotype were sown on sterilized sand media. After 5 days of growth, the seedlings were transferred to the thermocole sheets placed on plastic trays of 12 liters capacity. Each thermocole sheets had 77 holes (11 x 7) rows. Single seedling per hole was planted with a total of 11 seedlings per test entry. The thermocole sheets were floated on modified Yoshida nutrient solution and the salinity treatments were imposed after 21 days using two salinity levels of 60 and 120 mM. The treatments were replicated thrice in a randomized complete block design and 2 such sets were developed in order to reduce error. The seedlings from two sets were harvested 7 days

after initiating salinity stress for enzyme analysis. Leaves were scored apex to base *i.e.* basipetally with leaf number 3 being the oldest leaf and leaf 7 being the youngest leaf.

Chlorophyll content

The chlorophyll content (chlorophyll *a*, *b*, a/b ratio and total chlorophyll) was estimated in the youngest fully expanded by adopting the procedure of Arnon (1949) and Yoshida *et al.,* (1972) and expressed as mg g^{-1} on fresh weight basis. The absorbance of the extract was estimated at 645 and 663 nm using 80% acetone as a blank. Chlorophyll content was expressed as mg g–1 tissue fresh weight and calculated using the following equations as:

Chlorophyll a = [12.7(OD663nm)-2.69 (OD645nm)] x (V/1000w)

Chlorophyll b = [22.9(OD645nm)-4.68 (OD663nm)] x (V/1000w)

Total chlorophyll = [20.2(OD645nm)-4.68 (OD663nm)] x (V/1000w)

Chlorophyll fluorescence (Fv / Fm ratio)

Fluorescence yield determinants were measured on the same leaves used for gas exchange measurements (Dionisio-Sese and Tobita 2000). Maximum fluorescence determination was carried out for 30 min in dark adapted leaves as described by Maxwell and Johnson, (2000). Chlorophyll fluorescence in light adapted leaves was detected with a portable chlorophyll fluorometer PAM-210 WALZ. The quantum yield (Fv/Fm) was recorded after 30 min of dark adaptation. Actual quantum yield ($\Delta F/F_m$ or Φ_{PSII}) was measured on the youngest fully expanded leaves that were illuminated for 30 min in the growth chamber with actinic light after dark adaptation, with three measurements conducted per replication (Moradi and Ismail, 2007).

Chlorophyll meter (SPAD- Soil Plant Analysis Development)

Chlorophyll content was recorded using a portable chlorophyll meter (Minolta SPAD 502). The SPAD value was measured on each side of the leaf at the point of three-fourths of the way from base to leaf tip for each replication and the mean value was worked out. The Minolta SPAD-502 measures chlorophyll content as the ratio of transmittance of light at wavelengths of 650 nm and 940 nm. The 940 nm transmittance provides normalization, as it is unaffected by chlorophyll levels but is influenced by leaf thickness (Ahmad *et al.,* 1999).

Chlorophyll Stability Index (CSI)

The CSI was estimated as per the method described by Murthy and Majumder (1962) and the values expressed as a percentage.

Gas exchange measurements

Gas exchange measurements were done on the youngest fully expanded leaf at 4, 72, 168, 240 and 312 hours after treatment. Four different measurements were randomly made from the 10 seedlings of each cultivar grown in each replication.

Transpiration rate and stomatal conductance were measured using a Steady State Porometer (LI-COR) during 11.00 am to 2.00 pm. The average values were computed and expressed as mMol m^{-2} sec^{-1} for both the parameters.

Osmotic potential

The osmotic potential was recorded using osmometer. The fresh leaf samples (5 g) were taken and kept in -80 ^{o}C for overnight and next day the leaves were taken and allowed to thaw for 15 min. The cell sap was removed by inserting the leaves into a 5 ml syringe by giving pressure. The osmotic potential was expressed in Mpa.

Statistical analysis

The mean data of selected plants for each genotype per replication were subjected to analysis of variance for all the characters appropriate for randomized complete block design (Panse and Sukhatme, 1954). The analysis was done using CROPSTAT software (version 7.2).

RESULTS

The trend of values for chlorophyll 'a' content showed that the genotype IR74802 recorded highest (1.21mg g^{-1}) followed by IR72593 (1.2 mg g^{-1}) and FL478 (1.02 mg g^{-1}), IR73104 (1.18 mg g^{-1}) and IR29 (1.0 mg g^{-1}). Salinity level of 120 mM considerably reduced the chlorophyll content compared to control up to 60.8% and 30.9% in 60 mM. The average mean values between salinity irrespective of genotypes ranged from 0.62 mg g^{-1} (IR29) to 0.86 mg g^{-1} (IR72593). The variations in chlorophyll a content were analyzed and found significant and the data are appended in Table 1.

The genotype IR74802 recorded highest value of 0.59 in control followed by IR72593 (0.56), IR73104 (0.55), FL478 (0.52) and IR29 (0.53) with overall mean value of 0.55 (Table 1). In 60 and 120 mM, IR29 recorded a drastic decrease in chlorophyll b content of 0.31 and 0.175 respectively as compared to control. The average mean values in salinity irrespective of genotypes ranged from 0.55 (control), 0.34 (60 mM) to 0.21 (120 mM). The average mean values between genotypes ranged from 0.34 (IR29) to 0.39 (IR74802).

The total chlorophyll content of rice genotypes found significant and data were presented in Table 2 and Figure 1a. The mean chlorophyll content among genotypes was 1.75 (control), 1.11 (60 mM) and 0.81 (120 mM) with a decreasing trend towards increase in salinity level. Among the genotypes, IR74802 recorded highest mean total chlorophyll content of 0.91 mg g^{-1} whereas, IR73104 remains high (0.90 mg g^{-1}) and IR29 recorded lowest total chlorophyll content of 0.53 mg g^{-1} in 120 mM concentration among all the other 4 genotypes. The chlorophyll a/b ratio decreased gradually and significantly from control to stress conditions. The mean value ranged from 2.601, 2.31 to 2.018 in control, 60 and 120 mM respectively. At 120 mM, IR74802 had highest a/b ratio of 0.91, followed by IR73104 (0.90), IR72593 (0.89), FL478 (0.82) and lowest ratio recorded for IR29 (0.53) (Table 2).

The measurements of chlorophyll fluorescence were recorded from 24 to 312 h after salinization. Significant differences in response were observed in genotype and salinity interactions in all the days after salinization.

The Fv/Fm in control plants was around 0.75 to 0.87 at 24 and 312 h. However in 60 mM, the mean value decreased at 312 hr and ends in 0.75 at 72 and 168 h, same trend was also observed in 120 mM of salinization. Among the genotypes, IR72593 had maximum Fv/Fm ratio in 120 mM salt concentration (0.73 at 24 h, 0.78 at 240 h and 0.68 at 312 h after salinization). IR29 had a lowest Fv/Fm ratio of 0.50 in 312 h after salinization at 120 mM concentration (Table 3 and Figure 1e).

SPAD is the indirect measure of chlorophyll content in plant leaves, and it was significant for all the genotypes (Table 4 and Figure 1b). Irrespective of the treatment, the SPAD value increases with advancement in seedling growth. The SPAD value ranges from 22.26 (IR29 at 120 mM) to 38.28 (FL478 in control) at 10^{th} day. In 3^{rd} day after salinization in 120 mM, IR72593 had the highest value of 34.65 and lowest was recorded by IR29 (30.62), in 7^{th} and 10^{th} day after salinization, IR73104 has highest value of 34.0 and 33.0 respectively and lowest was recorded by IR29. The impact of salt stress for chlorophyll stability index was studied and the data were presented in Table 2 and Figure 1c with significant differences among genotypes and treatments. The genotype IR73104 recorded highest chlorophyll stability index (86.0, 78.0 and 64.0 %) in control as well as in stress condition whereas, IR29 showed lowest performance in all conditions (77.0, 67.0 and 49.0). The average mean value between genotypes ranges from 82.0 (control), 76.0 (60 mM) and 60.0 (120 mM) respectively, whereas between salinity ranges from 65.0 (IR29) to IR73104 (76.0).

Transpiration rate and stomatal conductance were measured at 7 days after imposition of salt treatment. The analysis of data for transpiration rate and stomatal conductance in rice genotypes showed significant variation and the data are presented in Table 5 and Table 6 respectively. Time course measurements of gas exchange were carried out to test the differential genotypic response with time that could be associated with tolerance to salt stress. Response of rice genotypes after exposure to salinity was very fast, where a reduction in all attributes measured was detected after 24 h of salinization.

Table 1. Chlorophyll a, b and a/b ratio of rice genotypes at 7th day after salinization.

No.	Genotype	chlorophyll 'a'				chlorophyll 'b'				a/b ratio			
		Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean
1	FL478	1.02	0.81	0.62	0.75	0.52	0.34	0.20	0.35	2.56	2.54	2.10	2.40
2	IR74802	1.21	0.78	0.68	0.82	0.59	0.36	0.23	0.39	2.67	2.23	2.09	2.33
3	IR73104	1.18	0.78	0.69	0.82	0.55	0.34	0.21	0.37	2.73	2.42	2.33	2.49
4	IR72593	1.20	0.885	0.68	0.86	0.56	0.35	0.21	0.37	2.54	2.36	2.29	2.40
5	IR29	1.00	0.62	0.35	0.62	0.53	0.31	0.18	0.34	2.50	2.00	1.28	1.93
	Mean	1.12	0.78	0.42		0.55	0.34	0.21		2.60	2.31	2.02	
Significance and LSD 0.05													
Genotype			0.0391***				0.0172***				0.105***		
Salinity			0.303***				0.0133***				0.0819***		
Genotype x Salinity			0.0677***				0.0298***				0.183***		
CV			4.9				5.0				4.8		

*** Significant at $P < 0.001$ %.

Table 2. Total chlorophyll content, chlorophyll stability index and osmotic potential of rice genotypes at 7th day after salinization.

No.	Genotype	Total chlorophyll				Chlorophyll Stability Index				Osmotic potential			
		Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean
1	FL478	1.76	1.15	0.82	1.24	81.2	78.8	62.0	74.0	1.09	1.29	1.39	1.26
2	IR74802	1.80	1.14	0.91	1.28	80.8	76.2	62.9	74.0	1.17	1.33	1.68	1.39
3	IR73104	1.73	1.12	0.90	1.25	86.0	78.6	64.8	76.0	1.25	1.42	1.64	1.44
4	IR72593	1.76	1.23	0.89	1.29	84.1	77.2	63.5	75.0	1.10	1.31	1.53	1.31
5	IR29	1.68	0.93	0.53	1.05	77.4	67.2	49.4	65.0	1.07	1.77	2.66	1.83
	Mean	1.75	1.11	0.81		81.9	75.6	60.5		1.13	1.42	1.78	
Significance and LSD 0.05													
Genotype			0.056***				3.25***				0.0608***		
Salinity			0.0464***				2.51***				0.0471***		
Genotype x Salinity			0.0970***				5.63***				0.1053***		
CV			4.8				4.7				4.4		

*** Significant at $P < 0.001$ %.

Table 3. Chlorophyll fluorescence (Fv/Fm ratio) of rice genotypes in different hours after salinization.

No	Genotype	24 h				72 h				168 h				240 h				312 h			
		Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean
1	FL478	0.79	0.72	0.69	0.73	0.81	0.76	0.69	0.75	0.85	0.75	0.75	0.78	0.82	0.77	0.73	0.77	0.85	0.70	0.66	0.74
2	IR74802	0.72	0.69	0.68	0.70	0.82	0.76	0.69	0.76	0.86	0.77	0.78	0.80	0.84	0.77	0.75	0.79	0.87	0.71	0.63	0.74
3	IR73104	0.76	0.75	0.71	0.74	0.86	0.78	0.68	0.77	0.82	0.72	0.79	0.78	0.83	0.79	0.77	0.80	0.88	0.73	0.62	0.74
4	IR72593	0.77	0.76	0.73	0.75	0.82	0.78	0.68	0.76	0.87	0.78	0.72	0.79	0.85	0.75	0.78	0.79	0.89	0.70	0.68	0.76
5	IR29	0.73	0.73	0.65	0.70	0.79	0.69	0.60	0.69	0.81	0.70	0.67	0.73	0.81	0.66	0.60	0.69	0.86	0.69	0.50	0.68
	Mean	0.75	0.73	0.69	0.73	0.82	0.75	0.67	0.75	0.84	0.74	0.74	0.78	0.83	0.75	0.73	0.77	0.87	0.71	0.62	0.73
Significance																					
Genotype			**				**				**				***				***		
Salinity			***				***				**				***				***		
Genotype x Salinity			ns				ns				ns				*				**		
LSD 0.05																					
Genotype			0.030				0.032				0.033				0.03				0.03		
Salinity			0.023				0.026				0.025				0.026				0.025		
Genotype x Salinity			0.053				0.057				0.056				0.057				0.055		
CV			4.5				4.5				4.2				4.4				4.4		

ns - not significant *, **, *** significant at $P < 0.05$, 0.01, 0.001% respectively.

Table 4. SPAD values of rice genotypes at different days of salinization.

No.	Genotype	3rd day				7th day				10th day			
		Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean
1	FL478	35.33	34.92	33.32	34.52	37.47	34.12	33.10	34.90	38.28	33.00	32.01	34.43
2	IR74802	33.20	33.00	32.00	32.73	35.00	32.15	31.25	32.80	35.72	31.00	30.89	32.54
3	IR73104	35.40	35.12	34.00	34.84	37.34	34.50	33.24	35.03	38.27	33.20	32.24	34.57
4	IR72593	35.74	35.40	34.65	35.26	37.14	34.89	34.00	35.34	37.83	33.10	33.00	34.64
5	IR29	32.04	31.02	30.25	31.10	36.92	30.00	27.62	31.51	37.66	27.00	22.26	28.97
Mean		34.34	33.89	32.84	33.69	36.77	33.13	31.84	33.92	37.55	31.46	30.08	33.03
Significance													
Genotype			***				***				***		
Salinity			*				**				***		
Genotype x Salinity			ns				**				***		
LSD 0.05													
Genotype			1.46				1.47				1.48		
Salinity			1.13				1.137				1.130		
Genotype x Salinity			2.530				2.674				2.662		
CV			4.5				4.5				4.6		

ns - not significant , **, *** significant at $P < 0.05, 0.01, 0.001\%$ respectively.

Table 5. Transpiration rate (mMol $m^{-2}sec^{-1}$) of rice genotypes in different hours after salinization.

No.	Genotype	24 h				72 h				168 h				240 h				312 h			
		Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean
1	FL478	10.9	8.5	3.3	7.57	11.8	7.52	3	7.4	12.3	7.5	4.4	8.07	11.6	8.6	3.9	8.03	12.1	5.25	2.5	6.62
2	IR74802	10.6	8.5	4.1	7.73	10.7	8.6	3.7	7.7	10.5	11.2	4.5	8.73	10.7	7.5	3.9	7.37	12.5	5.9	2.8	7.07
3	IR73104	10.5	8.5	4.5	7.83	11.1	9.2	4.2	8.2	12	10.2	4.3	8.83	11.8	6.6	3.5	7.30	13.2	6.5	3.2	7.63
4	IR72593	10.3	9.64	4.8	8.25	11	8.6	4	7.9	11.2	9.6	5.3	8.70	11.6	7.5	3.9	7.67	12.4	5.2	2.8	6.80
5	IR29	10.2	9.6	7.2	9.00	11	8.96	5.9	8.6	10.2	8.6	3.8	7.53	11	5.5	2.2	6.23	10.6	3.6	1.9	5.37
	Mean	10.5	8.95	4.78	8.08	11.12	8.58	4.1	7.95	11.24	9.42	4.46	8.37	11.3	7.14	3.48	7.32	12.1	5.29	2.6	6.70
Significance																					
Genotype			***				***				***				***				***		
Salinity			***				***				***				***				***		
Genotype x Salinity			***				***				***				***				***		
LSD0.05																					
Genotype			0.35				0..38				0.41				0.36				0.40		
Salinity			0.27				0.30				0.32				0.28				0.31		
Genotype x Salinity			0.61				0.65				0.72				0.62				0.70		
CV			4.6				5.0				5.2				5.2				6.3		

*** Significant at $P < 0.001\%$.

Table 6. Stomatal conductance (mMol $m^{-2}sec^{-1}$) of rice genotypes in different hours after salinization.

No.	Genotype	24 h				72 h				168 h				240 h				312 h			
		Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean	Control	60 mM	120 mM	Mean
1	FL478	1.26	1.08	0.92	1.09	1 .25	0.79	0.21	0.5	1.15	0.76	0.32	0.74	0.96	0.76	0.26	0.66	1.21	0.79	0.25	0.75
2	IR74802	1.29	1.02	0.9	1.07	1.44	0.72	0.25	0.8	1.03	0.82	0.35	0.73	1.1	0.72	0.25	0.69	1.53	0.86	0.21	0.87
3	IR73104	1.25	1.02	0.86	1.04	1.32	0.79	0.28	0.8	1.35	0.76	0.36	0.82	1.25	0.73	0.22	0.73	1.69	0.76	0.23	0.89
4	IR72593	1.23	1.07	0.92	1.07	1.29	0.82	0.24	0.78	1.34	0.72	0.39	0.82	1.15	0.78	0.29	0.74	1.3	0.86	0.24	0.80
5	IR29	1.25	1.2	1.12	1.19	1.28	0.99	0.41	0.89	0.98	0.6	0.3	0.63	0.96	0.45	0.14	0.52	1.2	0.56	0.1	0.62
	Mean	1.26	1.08	0.94	1.09	1.33	0.82	0.28	0.75	1.17	0.73	0.34	0.75	1.08	0.69	0.23	0.67	1.39	0.77	0.21	0.79
Significance																					
Genotype			***				***				***				***				***		
Salinity			***				***				***				***				***		
Genotype x Salinity			***				***				***				***				***		
LSD0.05																					
Genotype			0.044				0.041				0.040				0.039				0.056		
Salinity			0.033				0.032				0.030				0.030				0.043		
Genotype x Salinity			0.076				0.071				0.069				0.070				0.098		
CV			4.2				5.3				5.7				6.6				7.2		

*** Significant at $P < 0.001$ %.

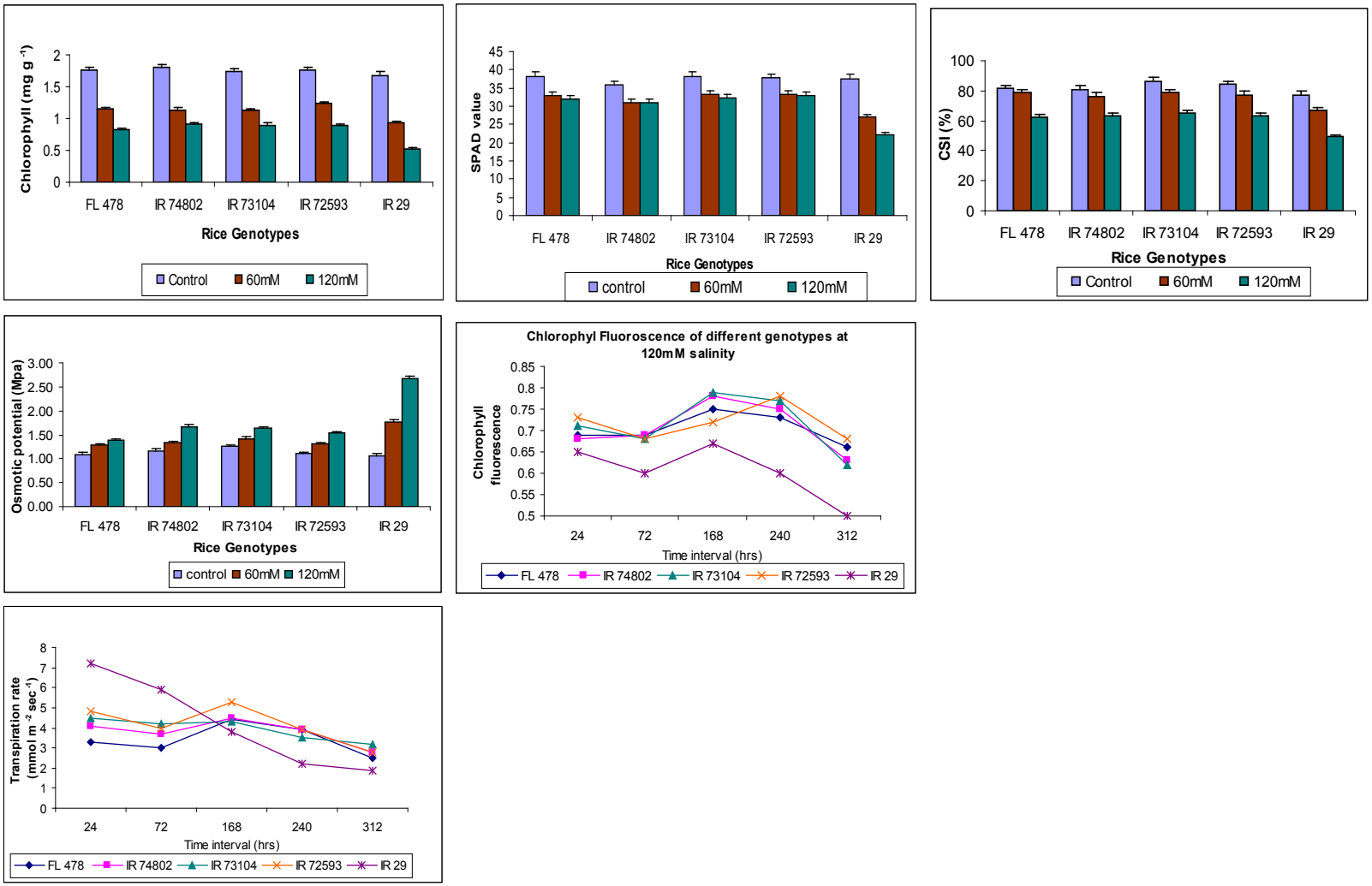

Figure 1. Effect of rice seedling at 120 mM NaCl stress on a) Total chlorophyll content b) SPAD c) chlorophyll stability index (CSI) d) Osmotic potential e) chlorophyll fluorescence f) stomatal conductance g) transpirational rate. Vertical bars indicate SE of five replicates for each treatment.

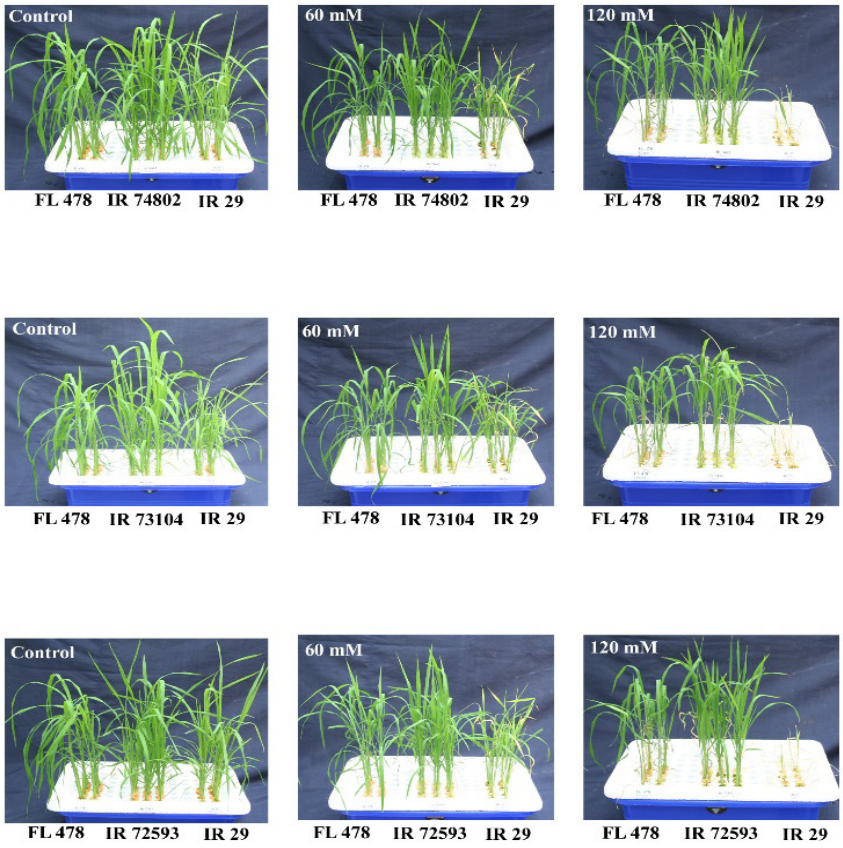

Figure 2. Comparison of IRRI lines along with checks under different salt stress.

The mean transpiration rate in control and 120 mM ranged from 10.50 to 4.78 in all the genotypes at 24 h of salinization. Likewise at 72 h the range was 11.7 to 4.1. At l68 h the mean values ranged from 11.2 to 4.4. At 240 and 312 h the mean values fall in the range of 11.3 to 3.5 and 12.2 to 2.6 respectively. The difference in mean value increased as salinity and time intervals increased. The highest transpiration rate was recorded in FL478 at 24 h (10.9), 72 h (11.8) and 168 h (12.3), while IR74802 recorded maximum value at 240 h (11.8) and 312 h (13.2). IR29 recorded highest transpiration rate in 60 mM and 120 mM concentration at 24 h (9.6 and 7.2) and 72 h (8.96 and 5.9) respectively and later it decreased and recorded lowest among all other genotypes whereas, IR74802 recorded highest value at 168 h (11.2 and 5.3), 240 h (8.6 and 4.0) and 312 h (5.9 and 3.8) 60 mM and 120 mM after salinization.

For stomatal conductance, the mean ranged from 1.26 to 0.94 at 24h, 1.33 to 0.28 at 72h, 1.17 to 0.34 at 168h, 1.68 to 0.19 at 240 h and 1.39 to 0.2 at 312 h after salinization. IR74802 recorded highest in control at 24 h (1.29) and 72 h (1.44) after salinization, whereas IR73104 recorded highest at 168 h (1.35), 240 h (1.25) and 312 h (1.69) respectively. IR29 recorded maximum in 24 h (1.2) and 72 h (0.99) in both 60 and 120 mM concentration as like that of transpiration rate noticed. IR72593 recorded highest stomatal conductance at 168 h (0.82 and 0.32) 240 h (0.78 and 0.29) and 312 h

(0.86 and 0.25) both in 60 mM and 120 mM concentration.

Percentage reduction in transpiration rate was lower in initial hours after salinization in tolerant genotypes as compared to susceptible genotype IR29 (Figure 1g). However, further increase in transpiration rate and stomatal conductance were observed in tolerant genotypes and were maintained at a steady state. The salt sensitive cultivar, IR29 had the lowest reduction in transpiration rate and stomatal conductance as time increases after 168 h after salinization, this was due to more than 60 % of the leaves were greatly damaged, while most tolerant lines FL478, IR72593, IR73104 and IR74802 still maintained active growth and production of new leaves (Figure1f & Figure1g).

The analysis of osmotic potential in rice genotypes was carried out and data were presented in Table 2 and Figure 1d. The lowest osmotic potential was recorded in FL478 with a value of 593 Mpa and 641 Mpa at 60 and 120 mM respectively. The maximum and minimum osmotic potential was recorded in IR29 is 1225 at control and 490 Mpa at 120 mM concentration. The mean values ranged from 521.8 to 819.2 Mpa. The osmotic potential increases in susceptible cultivar than resistant genotypes.

DISCUSSION

Tolerance to salinity in rice differs depending on the growth stage. In this study, early seedling stage screening was carried out; hence seedling stage is more sensitive of all other stages. Chlorophyll is an important pigment for interception of light; thereby the dry matter production might be enhanced due to increased carbon assimilation (Austin, 1989). Leaf chlorophyll content, which affects photosynthesis, has been considered to be an index of leaf injury in stressed plants (James *et al.,* 2002). Hernandez *et al.,* (2000) opined that salinity can cause leaf injury, manifested by a reduction in chlorophyll content. This study showed that the total chlorophyll content decreased with increase in salinity level up to 120 mM. Among the salt tolerant genotypes *i.e.* IR74802, IR73104, IR72593 and FL478 showed the lowest reduction in chlorophyll content under salt stress condition in comparison with their control genotypes. However, the susceptible genotype IR29 showed a drastic reduction in chlorophyll content and resulted in complete cessation of growth due to loss of chlorophyll content. Salinity affects photosynthesis either by reducing the supply of CO_2 through stomatal closure or by changing the mesophyll cell structure (Delfine *et al.,* 1998). Decrease in chlorophyll content due to salinity was reported by Panda and Khan, (2003) and Islam *et al.,* (2007). Reddy and Vora, (1985) reported that decrease in chlorophyll concentration in salinized plants could be attributed to increased activity of the chlorophyll degrading enzyme chlorophyllase or disruption of fine structure of the chloroplast and instability of pigment and protein complex ion (Djanaguiraman and Ramadass, 2004). Likewise chlorophyll a/b ratio is also decreased due to salinity treatment. Total chlorophyll and chlorophyll *a* were higher in both susceptible and tolerant cultivars of rice and chlorophyll *b* was higher only in salt tolerant rice cultivars grown in saline medium compared to control and chlorophyll a/b ratio was lower in salt tolerant and high in susceptible cultivars grown in saline medium (Peiris and Anoma Ranasinghe, 1993).

Chlorophyll stability index (CSI) is considered as an index for categorizing the crop tolerance to abiotic stress. Among the five genotypes studied, IR73104 performed well followed by IR72593 and IR74802. High CSI value indicates that the salt stress did not affect much of the chlorophyll content of tolerant plants. This is in accordance with Mohan *et al.,* (2000) who explained that a higher CSI helps the plants to withstand stress through better availability of chlorophyll.

The measurement of SPAD value, which is very simple and rapid, has been applied in rice as an index for salt tolerance screening (Hussain *et al.,* 2000; Mohan *et al.,* 2000). The SPAD value decreased upon increase in the duration of salinity. Tolerant genotypes showed lower reduction in SPAD value and susceptible genotype IR29 resulted in higher reduction after 7 days of salinization. The genotype IR72593 recorded higher SPAD values irrespective of

duration of stress and concentration of salinization.

It is noteworthy that in this study, the decrease in Fv/Fm ratios coincided with a decrease in both chlorophyll *a* and *b* concentration. The decrease in Fv/Fm indicates that loss of energy transfer from pigments to the reaction centre. The reduction of Fv/Fm ratio in NaCl stressed plants may also be due to reduction in Fm value, which indicates increased energy dissipation, dissociation of the light harvesting antennae from the PSII core, denaturation of the PSII reaction centre (Maxwell and Johnson 2000; Santos *et al.,* 2001), under accumulation of excess ions i.e. Na^+ and Cl^- or dehydration (Muranaka *et al.,* 2002). The salt treatment with 100 mM NaCl significantly decreased photosynthetic activity in IR29, as indicated by Fv/Fm and qP values (Lee *et al.,* 2013).

Both levels of salinity treatments reduced the transpiration rate and stomatal conductance (g_s). However, salt tolerant genotypes *viz.,* IR74802, IR72593, IR73104 and FL478 had higher g_s than salt sensitive genotype IR29. The reduced photosynthetic carbon assimilation may be due to reduced stomatal conductance (Bayuelo-Jimenez *et al.,* 2003). Apart from this, higher concentration of Na^+ ion are capable of reducing CO_2 assimilation because of lower Na^+ ion content in salt tolerant genotypes (IR74802, IR72593 and IR73104) than salt sensitive genotype (IR29) and this may be due to better ion compartmentation or an efficient mechanism to exclude toxic ions from the photosynthetic apparatus. Among the genotypes, IR72593 showed lowest reduction in transpiration rate and stomatal conductance, while the susceptible genotype IR29 showed higher transpiration rate during initial hours and then decreased at 312 h which resulted in the death of whole plant. Osmotic stress is effective in the beginning (h) of exposure to salt and ion toxicity becomes important in affecting plant growth after prolonged exposure (Munns, 2003). As Na accumulation is heavily dependent on transpiration rate, salinity tolerance could depend on growing medium and growth stage, selection for salt tolerance has been performed in hydroponics, where transpiration rate is low (Collins *et al.,*2008). The observed decrease in stomatal conductance and reduction in transpiration rate might be an important adaptive mechanism for salinity tolerance in rice (Flowers and Yeo, 1981).

Reduced stomatal conductance in the presence of salt stress has been suggested to be the cause of reduced metabolite assimilation in wheat genotypes (James *et al.,* 2002). Stomatal conductance has been suggested to be the most effective screening strategy for salinity tolerant genotypes (Munns and James 2003). The maximal reduction in the stomatal conductance in susceptible genotype IR29 is likely indicative of reduced metabolite assimilation in the presence of higher salt levels and this is in accordance with Singh *et al.,* (2007). The salt tolerant genotypes (IR74802, IR72593 and IR73104) showed a better control over their stomata and maintained lower transpiration rate (Moradi and Ismail, 2007). Photosynthesis is generally positively correlated with stress tolerance and therefore photosynthetic gas exchange parameters are widely used as stress indicators.

Osmotic potential of plants becomes more negative with an increase in salinity, whereas turgor pressure increases with increasing salinity (Hernandez *et al.,* 1999; Khan *et al.,* 1999; Meloni *et al.,* 2001). Leaf osmotic potential increases with an increase in salinity level (Aziz and Khan, 2001). The result indicated that all the genotypes showed an increased osmotic potential under various salinity level over control. However, the tolerant genotypes showed a lower increase in osmotic potential than sensitive genotype. On the other hand, a greater decline in osmotic potential will lead to loss in turgor under progressive or prolonged NaCl stress. Osmotic potential and stomatal conductance becomes more negative with an increase in salinity (Gulzar *et al.,* 2003). The decrease in osmotic potential may be due to the accumulation of proline as reported by Monneveux and Behhassen, (1996).

It is concluded that screening of genotypes for salt tolerance at early seedling stage is much easier to screen large set of germplasm or segregating populations, where time, labour and resources are constraint. From the findings, the reduction in growth and other biochemical factors is due to intermingled effect

of all biological attributes like chlorophyll content, stomatal conductance, transpiration rate and osmotic potential. The reduction in photosynthetic capacity with salt stress is associated with a decreased stomatal conductance (Ramanjulu *et al.,* 1998). However, at the initial response to salt stress, gas exchange parameters responds immediately thereby triggers destruction of chlorophyll pigments drastically and the mechanism differs with concentration of salt stress implied and tolerance of the genotypes. The reduction of CO_2 assimilation in salt tolerant genotypes may be due to better compartmentation and efficient excluding of Na^+ ions, restoring normal photosynthesis which avail the plants to survive the salt stress condition in tolerant genotypes. By utilizing these physiological parameters at early seedling stage quickens the selection process for breeders and tolerant lines can be forwarded to the next generation.

REFERENCES

Ahmed IV, Faiz SMA, Anwar AKM, Satter A (1989). Relation of Na^+: K^+ ratio of rice with salinity and yield. *Curr. Agric.,* 13 (1-2): 73-78.

Arnon DI (1949). Copper enzymes in isolated chlorophasts. Polyphenoloxidase in *Beta vulgaria*. *Plant Physiol.* 24 (1):1–15. doi:10.1104/pp.24.1.1

Austin RB (1989). Genetic variation in photosynthesis-Review**.** *J. Agric. Sci.* 112: 287-294.

Aziz I, Khan MA (2001). Effect of seawater on the growth, ion content and water potential of *Rhizophoro mucronata* Lam. *J. Agron. Crop Sci.,* 195: 269 – 277.

Bayuelo-Jimenez JS, Debouck DG, Lynch JP (2003). Growth, gas exchange, water relations and ion composition of Phaseolus species grown under saline conditions. *Field Crops Res.* 80: 207–222

Bongi G, Loreto F (1989). Gas exchange properties of salt-stressed olive (*Olea europea* L.) leaves. *Plant Physiol.* 90: 1408–1416.

Collins NC, Francois T, Tuberosa R (2008). Quantitative trait loci and crop performance under abiotic stress: Where do we stand? *Plant Physiology*, vol. 147, pp 469-486.

Dey SK, Rao CN (1989). Influence of nutrient deficiency on photosynthesis and productivity in early rice varieties. *Oryza*, 26: 317-319.

Dionisio-Sese ML, Tobita S (1998). Antioxidant responses of rice seedlings to salinity stress. *Plant science* 135: 1-9.

Dionisio-Sese ML, Tobita S (2000). Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance**.** *Journal of Plant Physiol.,* 157*:* 54-58.

Djanaguiraman M, Ramadass R (2004). Effect of salinity on chlorophyll content of rice genotypes. *Agric. Sci. Digest.* 24: 178-181.

Flowers TJ, Yeo AR (1981). Variability in the resistance of sodium chloride salinity within rice (Oryza sativa L.) varieties. *New Phytol.* 88: 363-373.

Garg GK, Garg OP (1982). Germination and some metabolic characteristics in green gram seedlings as affected by Na_2Co_3 and $NAHCO_3$. *Biol. Plant.* 24: 28-33

Gulzar S, Khan MA, Ungar IA (2003). Salt tolerance of coastal salt marsh grass. *Co mMun. Soil Sci. Plant Anal.* 34: 2595-2605.

Hernandez JA, Campillo A, Jimenez A, Alarcon J, Sevilla F (1999). Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. *New Phyto.,* 141: 241-251.

Hernandez JA, Jimenez A, Mullineaux P, Sevilla F (2000). Tolerance of pea to long term salt stress is associated with induction of antioxidant defenses. *Plant Cell Environ.* 23*:* 853-862.

Hussain F, Bronson KF, Singh Y, Singh B, Peng S (2000). Use of chlorophyll meter sufficiency indices for nitrogen management of irrigated rice in *Asia. Agron. J.* 92: 875–879.

Inada K (1985). Spectral ratio of reflectance for estimating chlorophyll content of leaf. *Jpn. J. Crop Sci.* 54: 261-265.

Islam MZ, Baset MA, Akter A,Rahman MH (2007). Biochemical attributes of mutant rice under different saline levels. *Int. J. Sustain. Crop Prod.* 2(3): 17-21.

James RA, Rivelli AR, Munns R, Von SC (2002). Factors affecting CO2 assimilation, leaf injury and growth in salt stressed durum wheat. *Funct. Plant Biol.* 29: 1393-1403.

Khan MA, Ungar IA, Showalter AM (1999). Effects of salinity on growth, ion content and osmotic relations in Halopyrum mocoronatum (L.) Stapf. *J. Plant Nutr.* 22: 191–204.

Krishnamurthy R, Anbazhagan M, Bhagwat KA (1987). Effect of NaCl toxicity of

chlorophyll breakdown in rice**.** *Indian Journal of Agricultural Science*, 57: 567-570.

Lee MN, Choa EJ, Wi SG, Baea H, Kim JE, Choc JY, Lee S, Kim JH, Chung BY (2013).Divergences in morphological changes and antioxidant responses in salt-tolerant and salt-sensitive rice seedlings after salt stress. *Plant Physiology and Biochemistry* (70) 325-335.

Luo Q, Yu B, Liu Y (2005). Differential sensitivity to chloride and sodium ions in seedlings of Glycine max and G. soja under NaCl stress. *J. Plant Physiol*. 162: 1003-1012.

Maxwell K, Johnson GN (2000). Chlorophyll fluorescence - a practical guide *J.Exp Bot.* 51: 659-668.

Meloni DA, Oliva MA, Ruiz HA, Martinez CA (2001). Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. *J. Plant Nutr*. 24: 599-612.

Mishra SK, Subrahmanyam D, Singhal GS (1991). Interrelationship between salt and light stress on primary processes of photosynthesis. *J. Plant Physiol*. 138: 92–96.

Misra AN, Srivastava A Strasser RJ (2001). Utilization of fast chlorophyll fluorescence technique in assessing the salt/ion sensitivity of mung bean and Brassica seedlings. *J. Plant Physiol.* 158: 1173–1181.

Mohan MM, Lakshmi SN, Ibrahim SM (2000). Chlorophyll stability index (CSI): its impact on salt tolerance in rice. *IRRI newsletter,* pp 38-39.

Monneveux P, Belhassen E (1996). The diversity of drought adaptation in the wide. In: Drought Tolerance in Higher Plants: Genetical Physiological and Molecular Biological Analysis. Ed. Belhassen, E. Kluwer Academic publications.

Moradi F, Ismail AM (2007). Responses of Photosynthesis, Chlorophyll Fluorescence and ROS-Scavenging Systems to salt stress during seedling and reproductive stages in rice. *Annals of Botany,* 99: 1161–1173.

Munns R, James RA (2003). Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant and soil,* 253: 201-218.

Muranaka S, Shimizu K, Kato M (2002). A salt-tolerant cultivar of wheat maintains photosynthetic activity by suppressing sodium uptake. *Photosynthetica,* 40: 509–515

Murthy KS, Majumder (1962). Modifications of the technique for the determination of chlorophyll stability index in relation to drought resistance in rice. *Curr. Sci.* 31: 470-471.

Nemoto Y, Sasakuma T (2002). Differential stress responses of early salt-stress responding genes in co mMon wheat, *Phytochemistry,* 61: 129-133.

Nieves M, Creda A, Botella M (1991). Salt tolerance of two lemon scions measured by leaf chloride and sodium accumulation. *J. Pl. Nutr.* 14: 623-636.

Ota K,Yasue T (1962). Studies on the salt injury in crops. XIV. The effect of NaCl solution upon photosynthesis of paddy. In Research Bulletin, Faculty of Agriculture, Gifu university. 16: 1-6.

Panda SK, Khan MH (2003). Salt stress influences lipid peroxidation and antioxidants in the leaf of an indica rice (*Oryza sativa* L.). Physiology and Molecular Biology of plants, 9(2): 273-278.

Panse VG, Sukhatme PV (1954). Statistical methods for agricultural workers. ICAR, New Delhi.

Peiris BD, Ranasinghe A (1993). Effect of sodium chloride salinity on chlorophyll content in rice (*Oryza sativa* L.) leaves. *Indian J. Plant Physiol*. 36(4): 257-258.

Ramanjulu S, Sreenivasalu N, Giridhara Kumar S, Sudhakar C (1998). Photosynthetic characteristics in mulberry during drought stress and redroughting. *Photosynthetica*, 35: 259–63

Reddy MP, Vora AB (1985). Effect of salinity on protein metabolism in Bajra leaves. *Indian J Plant Physiol.* 28: 190– 195

Santos V, Campos A, Azevedo H, Caldeira G (2001). In situ and in vitro senescence induced by KCl stress: nutritional imbalance, lipid peroxidation and antioxidant metabolism. *J. Exp. Bot.* 52: 351–360.

Singh AK, Ansari MH, Pareek A, Singla-Pareek SL (2008). Raising salinity tolerant rice: recent progress and future perspectives. *Physiol. Mol. Biol. Plants*, 14 (1&2):137-154.

Watanabe S, Hatanaka Y, Inada K (1980). Development of a digital chlorophyll meter: I Structure and performance. *Jpn. J. Crop Sci,* 49 pp 89–90.

Yoshida S, Forno DA, Cock SH, Gomez KA (1976). *Laboratory manual for physiological studies in Rice*. IRRI, Manila, Philippines.