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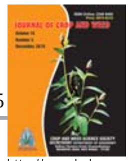

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Physiological and biochemical parameters as a basis of screening salinity tolerance at seedling stage in Rice (*Oryza sativa* L.)

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ABSTRACT

Twelve rice genotypes were evaluated at seedling stage with response to salinity stress at 60 mM NaCl for fourteen days in hydophonics in Yashida solution at B.C.K.V., Mohanpur, Nadia, West Bengal, following 12 × 2 factorial experimental design in RCB with 2 replications. Three physiological and biochemical parameters considered for study viz., chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyall), relative water content (RWC) and Na^+/K^+ ratio. There was reduction in chlorophyll a, chlorophyll b and total chlorophyll content, increase in Na^+/K^+ ratio and decrease in relative water content (RWC) in the saline condition as compared to control. Chlorophyll a ranged from 0.26 (Annada) to 1.57 (IR10206-29-2-1-1) mg/g of fresh leaf tissue, Chlorophyll b value varied from 0.08 mg g^{-1} fresh leaf tissue (CSR 22) to 0.58 mg/g fresh leaf tissue (IR06M143). Under saline condition, the highest and lowest RWC was obtained for the genotype IR06M143 (90.84) and Lalat (75.35) respectively whereas the genotype IR10206-29-2-1-1 recorded highest value (0.77) and genotype IR06M143 lowest value (0.24) with respect to shoot Na^+/K^+ ratio. Visual scoring for salt stress injury showed that six rice genotypes viz., IR10206-29-2-1-1, PUSA NR 580-6, BRRI Dhan 53, CSR 22, Annada and Lalat fall under susceptible category and rest six genotypes viz., IR11T138, Lal Minikit (WGL20471), IR66946-3R-149-1-1, IR06M143, IRRI 147 and BRRI Dhan 47 fall under moderately tolerant category. The genotypes IR11T138, Lal Minikit (WGL20471) and IR66946-3R-149-1-1 showed lower increase in Na^+/K^+ ratio as well as lesser decrease in RWC and chlorophyll content hence may be considered as tolerant to salinity whereas genotypes BRRI Dhan 53 and Lalat showed greater increase in Na^+/K^+ ratio and greater decrease in RWC and chlorophyll content hence may be considered as susceptible to salinity. Hence Na^+/K^+ ratio, RWC and chlorophyll content were found to be critical physiological parameters for evaluating rice genotypes for salinity tolerance.

Keywords: Chlorophyll content, relative water content, rice, salinity tolerance, seedling stage

Salinity is the second most important abiotic stress that severely limits crop growth, crop stand and productivity, and causes the continuous loss of arable land, which results in desertification in semi-arid and arid regions of the world (Pons *et al*., 2011). It is estimated that more than 800 million hectares of land across the world are adversely affected by high salinity (Munns and Tester, 2008). Saline soils are characterized by excess of sodium ions with dominant anions of chloride and sulfate resulting in higher electrical conductivity (>4 dSm^{-1}) (Ali *et al.*, 2013). In general, salinity stress induces an initial osmotic stress and subsequent toxicity as a consequence of accumulation of ions. However, damage can also ensue as a result of excessive reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) produced at a high rate and commonly accumulated in plant tissues due to ion imbalance and hyperosmotic stresses. ROS accumulation leads to lipid oxidation and has a negative effect on cellular metabolism and physiology, thus adversely ruining the membrane integrity (Munns *et al.*, 2006).

Rice is the most important staple food crop that feeds over half of the world population and more than 400 million people in rice producing areas of Asia, Africa and South America are still getting a major proportion of their energy requirement from rice and its derived products with the demand for food expected to increase by another 38 per cent within 30 years (Surridge, 2004; Joseph *et al*., 2010). Under saline condition, plants response to salt stress in two sequential ways, in first way plants exhibit a typical water stress, due to the high osmotic potential of soil solution caused by accumulation of soluble salts, and in second way, show ion toxicity and nutritional imbalances caused by ionic stress. Maintenance of high relative water content (RWC) of rice plant under salinity stress could be associated with salt tolerance. RWC measures the water content of a leaf relative to the maximum amount that leaf can take under full turgidity and hence is considered as an appropriate measure of plant water status under stress. RWC is, therefore, an appropriate estimate of plant water status, because it accounts for both leaf water potential and osmotic adjustment (OA). The salt tolerant genotypes could, therefore, be selected based on their ability to maintain high relative water content during the initial phase of salt stress. The study on these physiological parameters was conducted to determine the association

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between RWC and salinity tolerance in rice, and to investigate the possibility of using RWC as one of the screening criteria by the breeders to incorporate tolerance to salt stress.

Salinity tolerance in glycophytic crops including rice is predominantly associated with the maintenance of ion homeostasis, particularly low Na^+/K^+ or high K^+/Na^+ ratios, through exclusion, compartmentation, and partitioning of Na^+ (Blumwald, 2000). The two main mechanisms showing salt tolerance in plants are reducing the entry of salt into the plant and reducing the concentration of salt in the cytoplasm. Moreover, salt tolerant plants have lower rate of Na^+ and Cl^- transport to leaves and higher ability to prevent the build-up of ions in cytoplasm or cell wall. The compartmentation of ions into vacuoles is also more efficient in salt-tolerant than salt-sensitive plants (Munns 2002). The protection of chloroplast functions involve plant salt tolerance mechanisms. Generally, chlorophyll content of leaves is decreased under salt stress and salt-sensitive cultivar had often-lower chlorophyll content than salt-resistant ones (Lutts *et al*., 1996; Molazem *et al.*, 2010).

Evaluation of physiological and biochemical traits of rice genotypes subjected to salinity stress are considered as one of the useful way for breeders to better understand physiological responses during development of rice varieties grown on salt-affected soils, which are essential and may help in formulating efficient breeding programs to improve and development of rice with higher salt tolerant trait. In addition to physiological and biochemical parameters, selection for salt tolerance in rice could be achieved by visual scoring of seedlings grown under salt stress for sufficient time.

The present study was, therefore, conducted to explore different physiological changes in seedlings subjected to salinity stress in rice varieties differing in their level of salt tolerance along with classification of rice varieties with diverse growth and physiological parameters including visual score. The information regarding these studied physiological and biochemical parameters may be used to assist the evaluation of relative field performance of different rice genotypes and may be employed as reliable parameters for breeding and selection for salt tolerance.

MATERIALS AND METHODS

Study area and experimental details

The experiments were conducted at departmental laboratory of Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal during 2013.The materials comprising twelve rice (*Oryza sativa* L.) genotypes, were collected from ICAR-Central Soil Salinity Research Institute, RRS, Canning Town, South 24 Parganas, West Bengal. These genotypes were evaluated for salt tolerance levels at the seedling stage. For establishment of seedlings, rice seeds were surface sterilized with 0.1% $HgCl_2$ solution for 2 minutes then rinsed with distilled water and germinated on wet filter papers embedded in Petri dishes. Subsequently, six-days-old seedlings of each rice genotype raised in petri dishes were transplanted into plastic tray containing nutrient solution prepared as per Yoshida *et al*. (1976), covered with thermo-cal having suitable holes to place one seedling in each hole in such a way that the root part of each seedling remained immersed in solution. After 4 days of transplanting, seedlings of each rice genotype were subjected to salinity stress at 60 mM NaCl for 14 days along with the respective control. The experimental set up was done in indoor laboratory condition under sufficient light, 70-80 per cent relative humidity and at a temperature range of 25-30^{0}C. For maintaining the optimum pH of the nutrient solution, it was changed after 8 days because any deviation to the extent beyond (±1) from 5.0 would make some nutrient toxic and consequently deficient of some minerals.

Plant sampling and physiological parameters

Sampling was performed at the end of the experiments and the observations were recorded on Chlorophyll content as per Arnon (1949), shoot Na^+/K^+ ratio by Flame Photometer (Systronics-128).Relative water content (RWC) was estimated as per Perez *et al.* (2002). Randomly collected fresh leaves were weighed (F_W) immediately after collection and then submerged in distilled water for 4 hours at room temperature. The turgid weight (T_W) was recorded after blotting the surface moisture. Dry weights (D_W) of the samples were finally taken after drying them at 80°C till they attained constant weight. RWC was expressed as

$$RWC\ (\%) = \frac{F_W - D_W}{T_W - D_W} \times 100$$

The scoring of visual salt stress injury and growth reduction of rice seedlings treated with 60 mM NaCl were performed using the modified standard evaluation system of rice (Gregorio *et al.*, 1997).

Statistical analysis

Randomized Complete Block (RCB) factorial design was followed in statistical analysis of data by the GENRES software. Treatment combination of 12 genotypes × 2 salinity levels was tested with two replications. Analysis of variance (ANOVA) as applicable to RCB (Gomez and Gomez 1984) was followed to test the significance of treatments. Treatment means were compared by critical difference (CD) at probability level of d”0.05.

RESULTS AND DISCUSSION

Chlorophyll content

The data on contents of chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll in leaves of twelve genotypes at 0 mM (control) and 60 mM salinity level have been presented in table 1.

Chlorophyll a

The observation showed that inter-genotypic differences in the respective salinity level were significant when compared with genotypic C.D. value (critical differences). The range of chlorophyll ‘a’ content under control was from 0.44 mg/g fresh leaf tissue {Lal Minikit (WGL20471)**}** to 4.63 mg/g fresh leaf tissue (PUSA NR 580-6), but when the genotypes were subjected to salinity stress, the values varied from 0.26 mg/g fresh leaf tissue (Annada) to 1.57 mg/g fresh leaf tissue (IR10206-29-2-1-1). Due to salinity treatment all the genotypes had conspicuous reduction in chlorophyll ‘a’ content.

Chlorophyll b

In case of chlorophyll ‘b’ the value under control varied from 0.24 mg/g fresh leaf tissue (CSR 22) to 0.83 mg g^{-1} fresh leaf tissue (Lalat) but due to imposition of salinity the values varied from 0.08 mg g^{-1} fresh leaf tissue (CSR 22) to 0.58 mg g^{-1} fresh leaf tissue (IR06M143). Similarly, due to salinity treatment all the genotypes had conspicuous reduction in chlorophyll ‘b’ content like ‘a’ content.

Total chlorophyll

The total chlorophyll content reflects mainly the additive result of the two types of pigments studied; therefore, the mean values for this character were the reflection of the results of the former observations. The mean values for this character reduced significantly in all the genotypes due to treatment.

Relative water content (RWC) and Na^+/K^+ ratio

The results obtained with respect to relative water content (RWC) and Na^+/K^+ ratio in the leaf tissues of control and treated genotypes have been presented in table 2.

Relative water content (RWC)

The respective CD values revealed that there was significant inter genotypic, inter treatment and interaction difference for relative water content. The highest and lowest relative water content was obtained in the genotype IR06M143 (94.23) and IR11T138 (87.45) in control whereas IR06M143 (90.84) and Lalat (75.35) in saline. The comparative studies in control and saline showed that under saline condition the value of relative water content were reduced as compared to control for all the genotypes. The highest decrease incurred in Lalat (17.9) followed by Annada (14.73), BRRI Dhan 53 (13.33) and CSR 22 (12.18) and lowest in IRRI 147 (2.05), IR66946-3R-149-1-1 (2.10), Lal Minikit (WGL20471) (3.10) and IR11T138 (3.12). Similar findings were earlier corroborated by Arunroj *et al.* (2004) in rice genotypes under similar condition.

The basis of relative water content technique is the use of water soluble carbohydrate as an indicator for selecting salt tolerant genotypes (Kerepesi and Galiba, 2000)), which accumulate more soluble carbohydrate than the sensitive ones. The accumulation of solutes (such as fructans) has been documented as an adaptive mechanism to maintain osmotic pressure under salinity stress. The principal role of osmotic adjustment is to maintain cellular water potential and to provide metabolites which would act as surfactant to protect sensitive molecules under stress. With the increase in solute concentration, water potential decreases and water moves spontaneously from regions of high water potential to regions of low water potential, (Bray *et al.*, 2000). Therefore, plants possessing high solute concentration absorb more water and will therefore, have high water content in their cells. This mechanism is called osmotic adjustment. Usually, when rice plants are subjected to salinity stress, the tolerant plants will markedly accumulate a number of solute particles in cells. Based on these findings, RWC can effectively be used for screening for salt tolerance in rice. Sinclair and Ludlow (1985) studied the water status in two different conditions (leaf relative water content and water potential) and concluded that leaf relative water content was the better indicator of water status than was water potential. The use of RWC in evaluation of tolerant and susceptible genotypes could have many advantages over the current evaluation techniques because RWC is cheap, and eliminates the need for the high cost and time consuming mineral analysis commonly used for screening.

Shoot Na^+/K^+ ratio

The highest value for shoot Na^+/K^+ ratio in control was obtained from the genotype CSR 22 (0.55) and lowest for IR06M143 (0.17); whereas in saline condition, highest value of shoot Na^+/K^+ ratio was recorded for IR10206-29-2-1-1 (0.77) and lowest for IR06M143(0.24). The comparative studies in control and saline showed that highest increase incurred in IR10206-29-2-1-1 (0.35) followed by BRRI Dhan 53 (0.34),

Table 1: Chlorophyll-a, chlorophyll-b and total chlorophyll in seedlings of twelve rice genotypes under control (0 mM) and 60 mM salinity stress treatment

Genotypes	Ch-a* Salinity level			Ch-b* Salinity level			Total-Ch* Salinity level		
	0 mM	60 mM	Mean	0 mM	60 mM	Mean	0 mM	60 mM	Mean
IR10206-29-2-1-1	1.96	1.57	1.77	0.46	0.12	0.29	2.42	1.69	2.06
PUSA NR 580-6	4.63	1.44	3.04	0.27	0.10	0.19	4.90	1.54	3.22
BRRI Dhan 53	1.43	1.05	1.24	0.29	0.11	0.20	1.72	1.16	1.44
CSR 22	1.78	1.19	1.49	0.24	0.08	0.16	2.02	1.27	1.65
Annada	0.54	0.26	0.40	0.80	0.47	0.64	1.34	0.73	1.04
Lalat	0.51	0.29	0.40	0.83	0.46	0.65	1.34	0.75	1.05
IR11T138	0.73	0.62	0.68	0.54	0.49	0.52	1.27	1.11	1.19
Lal Minikit (WGL20471)	0.44	0.34	0.39	0.62	0.51	0.57	1.06	0.85	0.96
IR66946-3R-149-1-1	0.46	0.32	0.39	0.67	0.57	0.62	1.13	0.89	1.01
IR06M143	0.45	0.39	0.42	0.63	0.58	0.61	1.08	0.97	1.03
IRRI 147	0.57	0.48	0.53	0.44	0.37	0.41	1.01	0.85	0.93
BRRI Dhan 47	0.72	0.65	0.69	0.53	0.47	0.50	1.25	1.12	1.19
Mean	**1.19**	**0.72**	**0.95**	**0.53**	**0.36**	**0.45**	**1.71**	**1.08**	**1.39**
GENOTYPE	SEm(±)	0.006			0.003			0.008	
	LSD (0.05)	0.013			0.006			0.017	
TREATMENT	SEm(±)	0.003			0.001			0.003	
	LSD (0.05)	0.005			0.002			0.007	
INTRACTION (G×S)	SEm(±)	0.009			0.004			0.012	
	LSD (0.05)	0.018			0.008			0.025	

**Data expressed as mg g^{-1} fresh weight*

Table 2: Mean values of physiological and biochemical parameters in twelve rice genotypes exposed to 60 mM NaCl

Genotypes	Relative water content (%) Salinity level			Shoot Na^+/K^+ ratio Salinity level		
	0 mM	60mM	Mean	0 mM	60mM	Mean
IR10206-29-2-1-1	89.64	82.250	85.94	0.42	0.77	0.60
PUSA NR 580-6	89.33	81.125	85.23	0.51	0.69	0.60
BRRI Dhan 53	91.55	78.220	84.89	0.40	0.74	0.57
CSR 22	92.61	80.430	86.52	0.55	0.66	0.61
Annada	90.84	76.110	83.48	0.50	0.63	0.56
Lalat	93.25	75.35	84.30	0.48	0.62	0.55
IR11T138	87.45	84.33	85.89	0.35	0.40	0.38
Lal Minikit (WGL20471)	89.84	86.74	88.29	0.27	0.35	0.31
IR66946-3R-149-1-1	88.23	86.13	87.18	0.24	0.31	0.28
IR06M143	94.23	90.84	92.53	0.17	0.24	0.21
IRRI 147	90.86	88.81	89.83	0.37	0.48	0.43
BRRI Dhan 47	91.76	87.54	89.65	0.23	0.30	0.26
Mean	**90.80**	**83.16**	**86.98**	**0.42**	**0.48**	**0.45**
GENOTYPE	SEm(±)	0.021			0.001	
	LSD (0.05)	0.043			0.003	
TREATMENT	SEm(±)	0.009			0.001	
	LSD (0.05)	0.018			0.001	
INTRACTION(G×S)	SEm(±)	0.029			0.002	
	LSD (0.05)	0.061			0.004	

Table 3: Standard evaluation scores (SES) for twelve rice genotypes subjected to 60 mM NaCl salinity stress

Genotypes	Score	Observation	Tolerance level
IR10206-29-2-1-1	7	Complete cessation of growth, most leaves dry, some plants dying	Susceptible
PUSA NR 580-6	7	-do-	Susceptible
BRRI Dhan 53	7	-do-	Susceptible
CSR 22	7	-do-	Susceptible
Annada	7	-do-	Susceptible
Lalat	7	-do-	Susceptible
IR11T138	5	Growth severely retarded, most leaves rolled, only a few are elongating	Moderately Tolerant
Lal Minikit (WGL20471)	5	-do-	Moderately Tolerant
IR66946-3R-149-1-1	5	-do-	Moderately Tolerant
IR06M143	5	-do-	Moderately Tolerant
IRRI 147	5	-do-	Moderately Tolerant
BRRI Dhan 47	5	-do-	Moderately Tolerant

PUSA NR 580-6 (0.18) and Lalat (0.14), and lowest increase incurred for IR11T138 (0.05) preceded by IR66946-3R-149-1-1, IR06M143 (0.07) and Lal Minikit (WGL20471) (0.08). Salt tolerant plants have high affinity for K^+ uptake and low for Na^+ uptake (Munns *et al.*, 2002; Munns and James, 2003).

This indicated that under stress conditions, plants with low Na^+/K^+ ratio and high RWC are tolerant to salinity stress. Based on these findings, RCW and Na^+/K^+ ratio may effectively be used to screen the rice genotypes for salt tolerance.

Visual scoring

The visual scoring for salt stress injury was calculated using modified standard evaluation system of rice (SES) as per Gregorio *et al.* (1997) for twelve rice cultivars ranging from 5 to 7 (Table 3).

Six rice genotypes *viz*., IR10206-29-2-1-1, PUSA NR 580-6, BRRI Dhan 53, CSR 22, Annada and Lalat secured a score point of 7 and hence fall under susceptible category, and the rest six genotypes viz., IR11T138, Lal Minikit (WGL20471), IR66946-3R-149-1-1, IR06M143, IRRI 147 and BRRI Dhan 47 secured score point of 5 leading to its inclusion under moderately tolerant category.

The genotype IR11T138, Lal Minikit (WGL20471) and IR66946-3R-149-1-1 showed lower increase in Na^+/K^+ratio and lower decrease in relative water content and chlorophyll content hence considered as tolerant to salinity; whereas genotypes BRRI Dhan 53 and Lalat showed higher increase in Na^+/K^+ ratio, and higher decrease in relative water content and chlorophyll content hence considered as susceptible to salinity. These results were also confirmed by visual scoring. Hence, Na^+/K^+ ratio, relative water content and chlorophyll content were found to be the effective characters for evaluating rice cultivars for salinity tolerance.

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