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PHYSIO-BIOCHEMICAL ANALYSIS OF SALINITY TOLERANCE IN SODIUM CONTRASTING RICE (*ORYZA SATIVA* L.) GENOTYPES

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Abstract

Experiments were carried out in completely randomized design (CRD) at early seedling stage with five contrasting rice genotypes (high and low sodium rice genotypes) including salt tolerant check under different treatments of sodium chloride salinity. Multiple physiological traits were investigated in these sodium contrasting rice genotypes to identify contributory traits in salinity tolerance mechanisms. These studies have revealed variable reduction in growth among genotypes with least reduction in FL-478 (salt tolerant check) followed by IR-6 and IR-72. The genotype GML-498 exhibited highest reduction in fresh weights under salinity. The genotype IR-6 and IR-72 were observed comparatively better in most of the physiological traits studied. Comparatively less sodium (Na) concentrations along with less relative increase were observed in IR-6 and IR-72. Tolerant genotypes maintained less Na in their shoot through balancing Na in their root. On contrary to these, sensitive genotypes accumulated less Na in roots than in their shoots. Study of physiological traits revealed that low sodium accumulating rice genotypes exhibited relatively less electrolyte leakage (EL) and low malondialdehyde (MDA) concentrations, less increase of hydrogen peroxide (H_2O_2) than high sodium accumulating rice genotypes under salinity. Correlation studies have indicated that trait of sodium was significantly positively correlated with proline, total soluble sugars, MDA & EL. Whereas Na, MDA and EL were found negatively correlated with chlorophyll, while positively correlated with proline and TSS. The traits of chlorophyll, carotenoids and potassium sodium ratios were positively correlated with growth and observed as contributory traits under salinity.

Key words: Salinity, *Oryza sativa*, Sodium, Chlorophyll, Malondialdehyde and Electrolyte leakage.

Introduction

Salinity is one of the main constraints for agricultural productivity worldwide exists mostly in arid and semiarid climates. Global estimate of salinity indicates that at present 1 billion ha., of the 13.2 billion ha. world's lands are saline and sodicequivalent to 6% of the world's total cultivated area (Gerona *et al*., 2019). These lands are converting into nonproductive at a rapid rate causing huge economic losses due to decrease crop productivity (Munns&Gilliham, 2015). The reports of emerging climatic changes indicate that by the year 2050 the problem of salinity will further increase by 25%. As in future the climatic changes will be associated with problems of water shortage and food security of increasing population. The deltaic coastal regions presently contribute more than 65% of global rice production, particularly will be affected most (Radanielson *et al.*, 2018) due to expected increase of salinity. So this would be the main obstacle to meet an additional rice requirement of 40% increased population by the year 2050. New climate adapted crop varieties of rice having desired genetic variability for different stresses is a sustainable option for food security under climate change scenario.

Rice *Oryza sativa* L., a major cereal crop, provides35-80% caloric requirement to almost half of the world population, ranked as a most salt sensitive with threshold level of 3 dS/m (Radanielson *et al.*, 2018). Rice crop exhibits varying degree of sensitivities depending on crop stage, stress severity, stress duration and genotypic tolerance potential. The crop is comparatively more sensitive at early seedling and flowering stages and may cause greater than 50% reduction in grain yield in most of the rice genotypes at 6 dS/m (Radanielson *et al.*, 2018; Gerona *et al*., 2019). Enhancement of salt tolerance in current rice varieties is urgently needed to overcome food scarcity under climate changing scenario. Conventional breeding approaches mostly deals for increased yield within the gene pool under optimum environmental conditions. Most of the genotypes developed so far through conventional breeding, are for cultivation under optimal resource conditions i.e. for high yields, when these genotypes subject to stress environments suffer drastically in term of severe decline in yield. Through using this conventional approach there is a less chance of identifying genotypes which possesses all desired traits needed for salinity tolerance. This may not represent and applicable for exploring true potential of salinity tolerance among genotypes.

Salt tolerance is a polygenic character regulates by interaction of many genes control physiological traits. These physiological traits indicate genetic variability for salt tolerance. Thus there is a need to explore physiological significance of particular traits in plant adaptation and productivity under saline conditions. To date, studies related to salt tolerance mechanisms have indicated that the reduction of plant growth and yield under salinity is generally attributed to combined effects of lower osmotic potential of soil solution, disturbances in nutrients uptake and toxiceffects of harmful ions. Accumulation of toxic salts mainly sodium and chloride ions are the main causative factors for the physiological damage under salinity, disturbs cellular metabolic processes and all morpho-physiological traits. These include the suppression of many vital processes primarily starting from limited water uptake and absorption of many important nutrients, such as potassium, calcium and magnesium results in reduced turgidity and growth (Ali *et al.*, 2019; Zelm *et al*., 2020). Furthermore under stress conditions reactive oxygen species (ROS) produce in excess causes changes in normal cellular metabolism through

oxidative damage of many cellular components, peroxidation of membrane lipids, nucleic acids and proteins.ROS-mediated membrane damage under salinity has been reported to be a major cause of disruption in many physiobiochemical functions in plants cells, subsequently results in low productivity (Xie *et al.,* 2019).

Therefore, lower uptake of sodium or chloride and their subsequent transport and compartmentalization, maintenance of ion homeostasis and induction of antioxidant systems are considered the physiological basis of salt stress tolerance (Radanielson *et al.*, 2018; Gerona *et al*., 2019; Xie *et al.*, 2019). Reported variability in the salinity tolerance mechanisms among the rice cultivars (Ma *et al*., 2018; Khan *et al*., 2020) suggests the capability of different traits to cope with salt stress. There is scope to use potential physiological approaches for improving salt tolerance.

In view of this perspective, studies were conducted under salinity stress to compare the physiological and biochemical responses of earlier selected sodium contrasting rice genotypes (sodium uptake pattern) for the identification of contributory traits in salinity tolerance mechanisms.

Materials and Methods

Laboratory experiments were conducted at early seedling stage in completely randomized design (CRD). Five sodium contrasting rice genotypes: high sodium (HHZ SAL-10 DT2-DT1, GML- 498) and low sodium (IR-72, IR-6 & FL-478) including salt tolerant check (FL-478) were studied to evaluate growth and physiobiochemical responses under different treatments of sodium chloride salinity (0, 50 & 75 mM). Experiments were conducted in glass bowls (size: 8 cm Ø and 7cm height) containing inside nylon netted frame. Rice seeds were sterilized with 3% NaOCl for 20 minutes. Seeds (50 seeds) were planted on nylon netted frame in glass bowls containing treatments solution of sodium chloride (NaCl) supplemented with Yoshida culture solution adjusted at pH 5.5 (Yoshida *et al*., 1976). These glass bowls were covered with polyethylene bags to check evaporating losses and were placed under darkness in controlled incubator at temperature 28/26°C day and night respectively. Seedlings were exposed to 16 hrs photoperiod (irradiance 22 Wm^{-2}) after germination. Culture solutions were renewed twice a week to maintained required stress treatments. Growth and physiological/ biochemical parameters were measured after giving exposure of salinity treatments for the period of 10 days.

Growth parameters: Measurementsof five randomly selected plants were made for shoots & roots length; shoots & roots fresh weights. The samples were dried at 80°C in air drying oven for 72 hrs to determine their dry weights.

Physiological and biochemical parameters

Ions: Fresh weight (100 mg) of leaves was extracted in acetic acid (10 ml of 100 mM CH_3COOH) at 90°C in water bath for two hrs. (Flowers & Yeo, 1981). The solutions were filtered, volume and sodium and potassium ions were recorded at flame photometer (Jenway, Model-PFP7).

Chlorophyll: Shoot fresh weight (0.1g chopped) were taken into 10 ml acetone (80%) and kept overnight. The extracts were then centrifuge at 4000xg for 5 min. Total chlorophyll and carotenoids contents were measured (Lichtenthaler, 1987) by taking absorbance at 663.2, 646.8 and 470 nm at double beam spectrophotometer (Hitachi -150, Japan).

Electrolyte leakage (EL): Electrolyte Leakage in shoots was measured by method of Wu *et al.*, (2017) for analyzing membrane stability. Fresh leaves (0.1 g of 5 mm segments) were placed in de-ionized water (10 ml) and heated at 32°C in water bath. After two hours electrical conductivity (EC1) of water was measured on conductivity meter. These samples were autoclaved for 20 minutes at 121°C then cooled at room temperature. The electrical conductivity was again recorded (EC 2). The electrolyte leakage was computed in term of percent using formula:

$$EL = EC\ (1) / EC\ (2) \times 100.$$

Malondialdehyde (MDA): Malondialdehyde contents were determined following Dhindsa & Matowe (1981) with modification in extraction process (Moieni-Korbekandi *et al*., 2014). Fresh weight (0.5 g) of shoots were homogenized in trichloroacetic acid (10 ml; 10% TCA) then centrifuged for 20 min (4000 × *g*). Two milliliter supernatant was mixed with 2 ml reaction mixture (0.5% thiobarbituric acid in 20% TCA). The mixture was heated for 20 min at 95°C in water bath and then quickly cooled in ice. Afterwards, samples were centrifuged for 10 min (10,000 × *g*). The absorbance was read at 532 nm and at 600 nm on spectrophotometer. The MDA contents were estimated as µmol g^{-1} FW using extinction coefficient of 155 mM^{-1} cm^{-1} following Heath & Packer (1968).

Hydrogen peroxide (H_2O_2): Fresh leaves (0.5 g) were blended in 10 ml of trichloroacetic acid (0.1%) and centrifuged for 15 min at10000 rpm. Supernatant (0.5 ml) was mixed with 0.5 ml of K-phosphate buffer (100 mM; pH.7.5) and 2 ml potassium iodide (1 M w/v). Samples were kept on ice in dark for an hr. The absorbance was recorded at 390 nm at room temperature against blank of 0.1% TCA on spectrophotometer (Alexieva *et al*., 2001). The amount of hydrogen peroxide was computed against standard curve of known concentrations of H_2O_2.

Proline: Freshly chopped shoot samples (0.5g) were blended in sulphosalicylic acid (10 ml; 3%), and filtered. Two milliliter filtrate was reacted with acid ninhydrin (2 ml) and glacial acetic acid (2 ml) at 100°C for an hr. in water bath then cooled on ice. Four ml toluene was added to this filtrate and mix for on vortex mixer for 15-20 seconds. Toluene layer was aspirated and absorbance was read at 520 nm on double beam spectrophotometer (Hitachi 150, Japan). Concentrations of proline were calculated following Bates *et al.*, (1973).

$$(\mu \text{ moles proline /g FW} = [(\text{ug proline / ml x 4 ml toluene})/115.5 \text{ ug / } \mu \text{ moles}]/[0.5 \text{ g/5}].$$

Total soluble sugars: Chopped fresh leaves (1 g) were taken in 10 ml ethanol (80%v/v) and shake for overnight. 100 µL of extract was mix with 3 ml anthrone (150 mg anthrone in 100 ml of 72% sulfuric acid). The samples were heated at 97^{o}C for 10 minutes afterward cooled in an ice bath (Riaz*iet al*., 1985).The absorbance were recorded at 625 nm in double beam spectrophotometer (Model: Hitachi 150, Japan).

Statistical analysis

Two way analysis of variance was done for analyzing genotypes, treatments and genotypes treatments interactions followed by Tukey HSD test to compare treatments means (at α 0.05). Correlation studies (Pearson's) for different growth and physiological/ biochemical parameters were done by statistix 8.1.

Results

Growth responses: Allgrowth parameters have shown significant differences (at α 0.05) among genotypes, treatments and their interactions (Table 1). The effects of salinity were more pronounced on shoot growth than roots. The shoot length of all genotypes reduced with varying intensities under different salinity treatments. The genotype FL-478, IR-72 and IR-6 exhibited longest shoot lengths at both level of salinity (50 and 75 mMNaCl) along with less relative reduction when compared to their respective controls. Among these genotypes FL-478 (salt tolerant check) exhibited least reduction (9%) in shoot length at 75 mMNaCl. Highest relative reduction of 33 and 37% was observed in genotype GML-498 at 50 and 75 mMNaCl respectively. On contrary to shoot, root lengths of all genotypes increased substantially under salinity treatments. The highest increase was observed in IR-6 followed by IR-72, GML-498 and FL-478 (Table 2). Fresh and dry weights of all rice genotypes were reduced significantly at both level of salinity. Salt tolerant check genotype (FL-478) showed least reduction in their fresh weights i.e., 3 and 15% at 50 and 75 mMNaCl treatments respectively (Table 3). Whereas among the tested genotypes IR-72 and IR-6 have shown comparatively more fresh and dry weights production along with less relative reduction in their weights under salinity (Table 3).

Table 1. Mean square values (ANOVA) for growth parameters of rice genotypes under salinity.

SoV	Genotypes (G)	Treatment (T)	G x T	Error	CV %
d.f.	4	2	8	30	
Shoot length	15.72 **	69.63 **	10.67 **	2.59	8.84
Root length	1.45 *	15.95 **	1 *	0.5459	13.69
Fresh weight (shoot)	15133.5 **	31036 **	1707.6 *	845	10.02
Dry weight (shoot)	959.39 **	138.93 **	4.878 ns	6	5.2
shoot sodium	0.3364**	12.00**	0.2760**	0.0278	9.89
Root sodium	0.07146**	3.5956**	0.1635**	0.0051	5.4
K: Na (shoot)	0.5611**	1.5823**	0.1564**	0.0256	9.64
Electrolyte leakage	172.09**	8854.07**	57.62**	5.84	4.93
MDA	1.04513**	3.3110**	0.56707**	0.0142	10.79
H_2O_2	24797**	29598**	918.5*	372.9	4.65
Chlorophyll	0.0933**	0.2633**	0.0157*	0.0036	9.33
Carotenoids	0.1180**	0.4270**	0.0118ns	0.0187	12.86
Proline	5.034**	616.57**	7.714**	0.673	11.95
TSS	0.00447**	0.1678**	0.0025**	0.0011	11.38

d.f.= Degree of freedom ; ** = Significant @ 0.01 probability; ns = Non significant

Table 2. Effects of sodium chloride salinity stress on shoot & root lengths (cm) of rice genotypes.

Genotypes	Shoot length (cm)			Root length (cm)		
	Control	50 mM	75 mM	Control	50 mM	75 mM
GML-498	25.5 (0) A	17.1 (33) BCD	16.0 (37) BCD	4.6 (0) CD	5.1 (12) BCD	6.8 (47)ABC
HHZ SAL-10 DT2-DT1	19.7 (0) BC	17.1 (13) BCD	14.6 (26) D	4.3 (0) D	5.3 (23) ABCD	5.0 (16)CD
IR-72	20.7 (0) AB	19.8 (5) BC	18.2 (12) BCD	5.1 (0) BCD	5.1 (0) BCD	7.5 (46)A
IR-6	18.0 (0) BCD	16.9 (6) BCD	15.4 (15) CD	4.5 (0) D	5.0 (10) CD	7.3 (61)AB
FL-478	18.7 (0) BCD	18.5 (1) BCD	17.0 (9) BCD	4.6 (0) CD	4.5 (-3) D	6.3 (38) ABCD
HSD values at α 0.05 for genotypes (G)		2.2015			1.0103	
Treatments (T):		1.4498			0.6653	
GxT:		4.8430			2.222	

Values given in parenthesis represents (% decrease/increase) relative to control

Table 3. Growth responses of rice genotypes under different treatments of salinity (NaCl) stress.

Genotypes	Shoot fresh weight (mgs)			Shoot dry weight (mgs)		
	Control	50 mM	75 mM	Control	50 mM	75 mM
GML-498	304.2 (0) ABCD	271.5 (-11) BCD	220.1 (-28) DE	42.5 (0) DE	38.0 (-11) EF	36.7 (-14) EF
HHZ SAL-10 DT2-DT1	328.6 (0) ABC	220.3 (-33) DE	162.6 (-51) E	38.1 (0) EF	34.5 (-10) F	32.67 (-15) F
IR-72	329.2 (0) ABC	299.8 (-9)ABCD	266.1 (-19) CD	47.5 (0) CD	48.8 (3) CD	42.5 (-11) DE
IR-6	347.0 (0) ABC	304.8 (-12)ABCD	260.2 (-25)CD	59.7 (0) AB	56.7 (-5) AB	52.9 (-11) BC
FL-478	367.2 (0) A	356.4 (-3) AB	312.3 (-15) ABC	61.8 (0) A	58.9 (-5) AB	54.6 (-12) ABC
HSD values at α 0.05 for genotypes (G)		39.747			3.3493	
Treatments (T):		26.175			2.2057	
GxT:		87.439			7.3680	

Values given in parenthesis represents (% decrease/increase) relative to their respective control

Physiological and biochemical responses: Rice genotypes have shown variable relative increase in shoot and root sodium (Na) concentrations under different treatments of salinity. The least increase in shoot sodium was observed in salt tolerant check genotype (FL-478) followed by IR-6 and IR-72 at both levels of salinity. The highest sodium concentrations were exhibited by GML-498 & HHZ SAL-10 DT2-DT1 (Fig. 1a). Comparatively less Na concentrations were observed in roots (Fig. 1b). Roots of HHZ SAL-10 DT2-DT1 & GML-498 exhibited comparatively low relative increase (20 & 49% respectively) in Na at 50 mM in comparison to genotypes FL-478, IR-6, and IR-72. These genotypes showed high relative increase of 61, 99 & 93% respectively in their roots sodium concentration.Whereas, at higher level (75 mMNaCl) these differences among two category of genotypes were not observed.FL-478 exhibited least relative increase in sodium at both level of salinity (Fig. 1b). Significant differences were observed among these rice genotypes in root to shoot partitioning of sodium (roots to shoot Na ratio) at 50 mMNaCl treatment. The genotypes HHZ SAL-10 DT2-DT1 & GML-498 exhibited comparatively low ratios in their roots in comparison to FL-478, IR-6 and IR-72 (Fig. 1c).

In the present study all genotypes have shown more than 50 % decline in K/ Na ratio except salt tolerant check (FL-478) at 50 mMNaCl. However, comparatively less relative reduction was observed in IR-6, and IR-72.The reduction of more than 80% were observed in genotypes HHZ SAL-10 DT2-DT1 & GML- 498 at 50 and 75 mMNaCl (Fig. 1d).

Exposure of these rice genotypes to salinity exhibited varying level of increase in electrolyte leakage (EL). The values were comparatively low under non stress conditions. The genotype IR-72 exhibited least relative increase (129%) followed by genotypes FL-478 and HHZ SAL-10 DT2-DT1 at 50m M NaCl treatment. At 75 mMNaCl salinity FL-478 and IR-6 exhibited comparatively less relative increase (177 &194 %) in EL compared to their respective controls. GML- 498& HHZ SAL-10 DT2-DT1 showed highest relative increase in electrolyte leakage (Fig. 2a).

The data of lipid peroxidations measured by malondialdehyde (MDA) contents indicated that MDA in shoots was increased variably among all rice genotypes under salinity. The rice genotype FL-478 exhibited least increase (25 &32%) followed by IR-72 and IR-6 at both levels of salinity. The highest increase was observed in HHZ SAL-10 DT2-DT1& GML-498 (Fig. 2b). These two genotypes also exhibited higher relative increase in H_2O_2.Whereas least increase was observed in IR-72 (Fig. 2c).

The chlorophyll and carotenoids contents of these genotypes decreased with different intensities under salinity with least reduction in salt tolerant check (FL-478) and highest in HHZ SAL-10 DT2-DT1 & GML- 498 (Fig. 3a & b).In the present study excessive increase of organic solutes (proline and soluble sugars) in shoots was observed under salinity (Fig. 4a & 4b). IR-72, FL-478 and IR-6 exhibited comparatively higher proline contents along with high relative increase at both level of salinity (Fig. 4a). In contrast to proline, total soluble sugars were increased with higher intensities in genotypes HHZ SAL-10 DT2-DT1 & GML- 498 (Fig. 4b).

Discussion

Rice genotypes exhibited a significant variation in all tested parameters under salinity stress. Present study revealed that all growth parameters related to shoot were reduced variably under salinity. Whereas; all genotypes exhibited increased root lengths under salinity with highest increase in tolerant genotypes. Pradheeban *et al.*, (2017) also reported that a salt tolerant rice genotype, Pokkali produced more roots growth under salinity. They regarded this trait as plants adaptation, may be beneficial for absorbing more water under salinity. Based on relative reduction in shoot growth under salinity, the order of sensitivity in five rice genotypes was HHZ SAL-10 DT2-DT1 > GML- 498 > IR-6> IR-72> FL-478 (Table 3). The reduction in growth under salinity is a general response attributable to combined osmotic and ionic effects of salts as reported in earlier studies (Radanielson *et al.*, 2018; Fogliatto *et al.*, 2019; Khan *et al.*, 2019). These osmotic and ionic factors primarily cause imbalance in water and nutrients uptake of plants, transport of carbohydrates for newly developed tissues and ultimately reduces plant biomass production. Induced Ca_2^+ signaling, H^+ transport, K^+ transport, phospholipid modifications, increased production of reactive oxygen species and compatible solutes are also some of the earliest salt stress induced responses (Zelm *et al*., 2020).

Secondary causes of reduction in growth may include disorganization in functions of cellular structure like membrane, mitochondria and chloroplasts (Che-Othman *et al*., 2020).

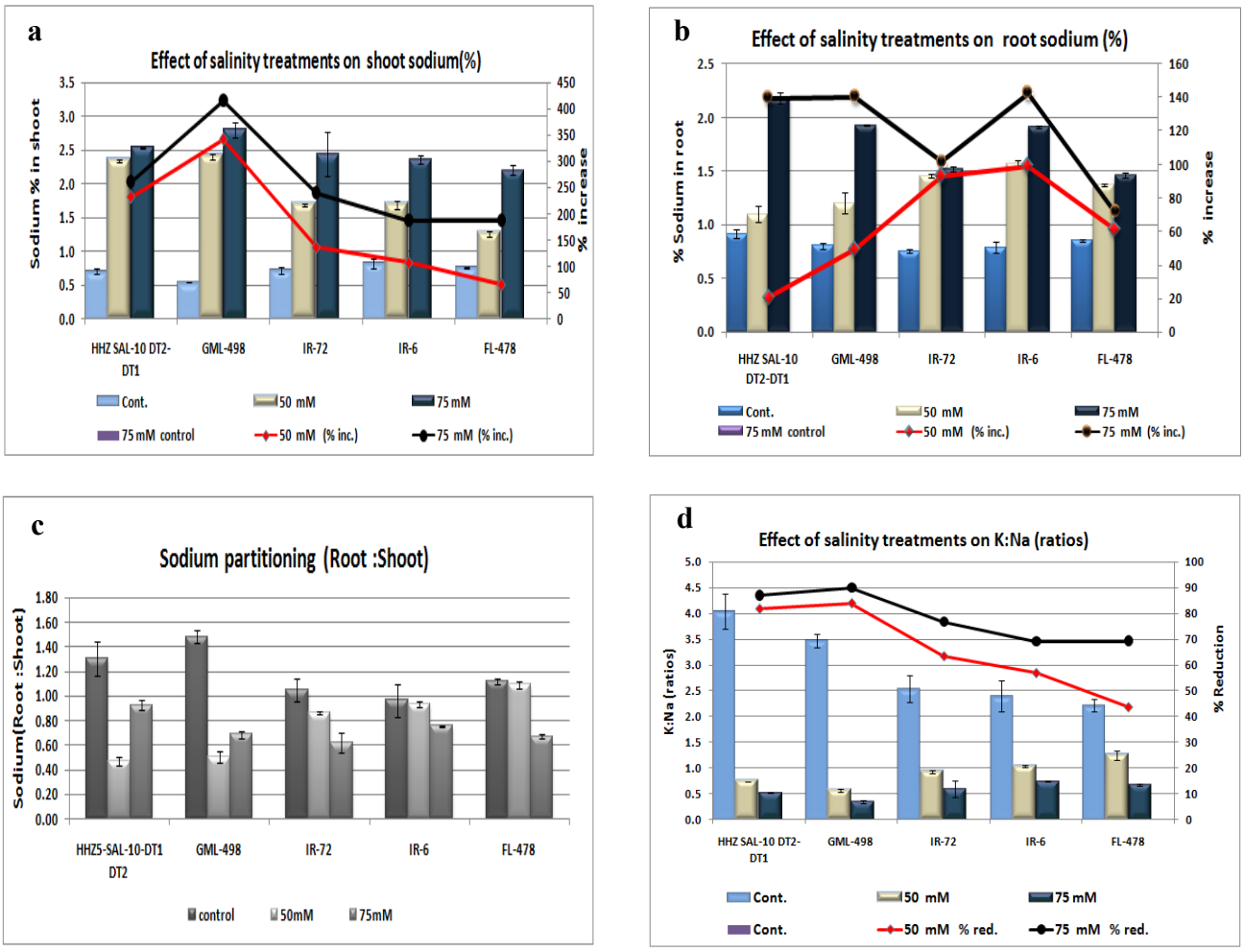

Fig. 1. Ionic responses of rice genotypes under different treatments of sodium chloride salinity stress.
Bars indicate average values computed from three plants / treatment / genotypes.
Vertical line represent standard error (n=3).

Table 4. Relationship (Pearson´s Correlation coefficient) among physiological traits of rice genotypes under salinity stress.

	EL	FWT	H_2O_2	K:Na	MDA	Na (shoot)	Na (root)	Proline	TSS	Carotenoids
FWT	-0.7678 **									
H_2O_2	0.6021 *	-0.4787 Ns								
K: Na	-0.9001 **	0.5847 *	-0.6082 *							
MDA	0.7401 **	-0.6488 **	0.2266 Ns	-0.5702 *						
Na (shoot)	0.9316 **	-0.8186 **	0.5462 *	-0.9053 **	0.635 *					
Na(Root)	0.9099 **	-0.6882 **	0.4263 Ns	-0.7531 **	0.7682 **	0.8067 **				
Proline	0.8238 **	-0.4301 *	0.4697 Ns	-0.78 **	0.4902 ns	0.7877 **	0.8269 **			
TSS	0.9415 **	-0.6966 **	0.6692 **	-0.914 **	0.5932 *	0.9085 **	0.8855 **	0.8424 **		
Carotenoids	-0.806 **	0.7915 **	-0.3652 Ns	0.5953 *	-0.8501 **	-0.7533 **	-0.7985 **	-0.6308 *	-0.6996 **	
Chlorophyll	-0.856 **	0.7877 **	-0.3759 Ns	0.6626 **	-0.875 **	-0.7829 **	-0.7985 **	-0.6103 **	-0.7264 **	0.9403 **

** = Significant @ 1% prob., * = Significant @ 5% probability, ns = Non-significant

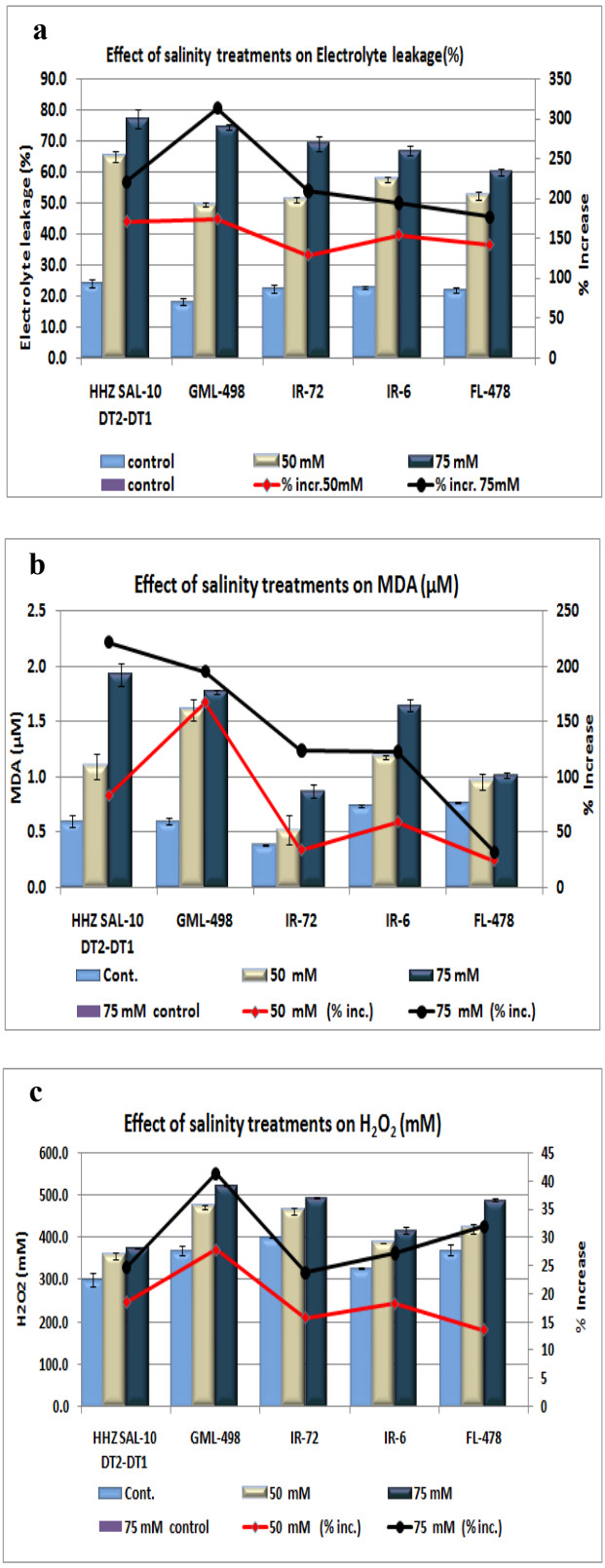

Fig. 2. Biochemical responses of rice genotypes under different treatment of salinity (NaCl). Bars indicate average values computed from three plants / treatment / genotypes. Vertical line represent standard error (n=3).

The present study affirmed that the sensitive genotypes of rice accumulated a larger amount of sodium and absorbed a smaller amount of potassium in shoots than the tolerant ones (Fig. 1a, 1b & 1d). The differential sensitivities among these genotypes may be due to differences in sodium accumulation in shoots and their adaptive capability to toxic sodium ions as evidenced from strong negative correlation ($r_=$ - 0.8186) between shoot growth and sodium concentrations (Table 4). Excess of sodium in the cytosol impairs optimal potassium sodium ratio, which results as potassium deficiency in plants under stress. Therefore optimum K: Na ratios under stress often regarded as a key parameter for measuring balanced physiological condition of plants. We have also observed significant positive correlation of K: Na ratios with chlorophyll, carotenoids contents and plant growth (Table 4). This indicated that ability to retain potassium in photosynthesizing shoots can impart substantially in keeping low cytosolic sodium, homeostasis between Na and K and thus contribute in salt tolerance. Khan *et al.*, (2019) were also of the view that varying K: Na ratio in shoot might have impacts on physiological responses and growth.

Sodium ion absorption, transportacross cell membrane and its distribution among organs is considered as a main factor for determining salt tolerance (Zelm *et al.*, 2020).Plasma membrane is one of the first targets under stress conditions. Sodium may be sensed intercellular, extracellular, or by ion channels/transporters at plasma membrane.Salinity induces structural changes in bi-layer lipid membrane and caused depolarization which opens outward rectifying channels allows Na influx and K efflux affects permeability and ions selective ability of plasma membrane (Demidchik *et al.*, 2014; Zelm *et al.*, 2020; Solis *et al.*, 2020). Liu *et al.*, (2019) reported two such channels for potassium (OsGORK. OsSKOR) over expressed in roots of tolerant rice varieties which helped in K accumulation in roots in the presence of salinity stress.

Therefore maintenance of selective ability of root to absorb/ retain salts in roots or minimum transport to shoot may play an initial protective role for minimizing salt load on physiologically active tissues. As was observed in present study at moderate salinity (50 mM), the genotypes IR-6, IR-72 and FL-478 (check genotype) exhibited comparatively higher partitioning of sodium (roots to shoot Na ratio) in roots than the SAL-10 DT2-DT1 & GML- 498 (Fig. 1c). This depicts differential selective ability of roots and this may relate to genotypic variability in shoot sodium. Studies have indicated that accumulation of sodium in shoots depends on specific transporters proteins (high-affinity K^+ transporter: HKT) and multiple variants of HKT1-type regulate Na transport from the root to xylem, and also restricts sodium transfer from leaf sheath to leaf blade thereby minimizing Na in shoots. These transporters have been recently reported in many plant species including Arabidopsis, wheat, rice, sorghum, and tomato; mediate the equilibrium between Na and K ions (Ali *et al.*, 2019; Mahi *et al.*, 2019). Zhang *et al.*, (2018) reported variability in salt tolerance of weedy and cultivated types of rice linked with variants of *HK*T1; regulating transport mechanisms and K/Na balance. Alnayef *et al.*, (2020) were of the opinion that *HK*T1:5 induce feedback regulation of other transporters activities and also involved in ions homeostasis and signaling processes.

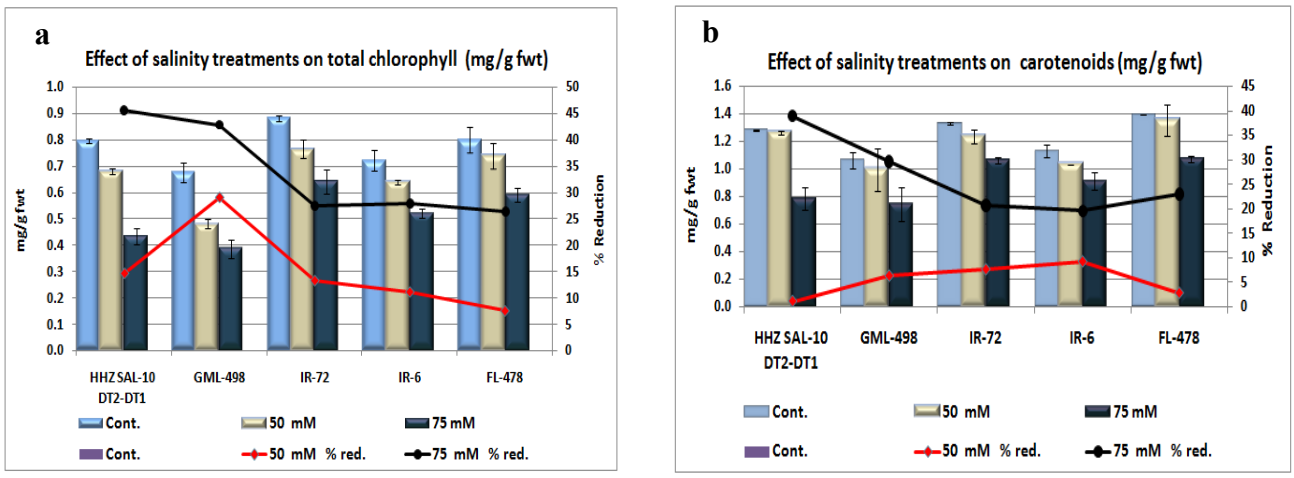

Fig. 3. Effects of salinity stress (NaCl) on pigment composition in rice genotypes. Bars indicate average values computed from three plants / treatment / genotypes. Vertical line represent standard error (n=3).

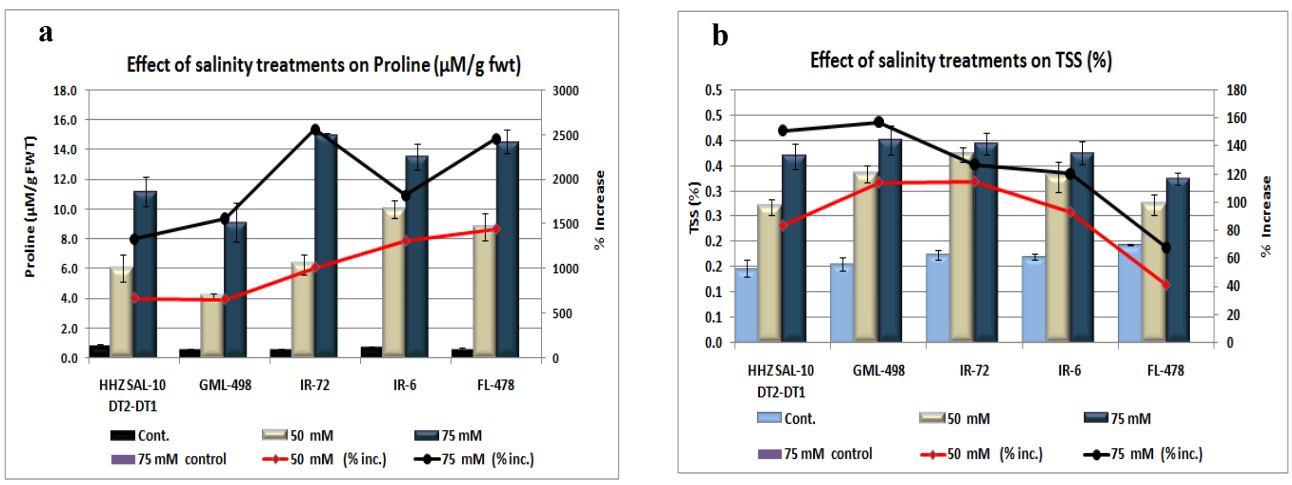

Fig. 4. Effects of salinity treatments (NaCl) on osmolyte production in rice genotypes. Bars indicate average values computed from three plants / treatment / genotypes. Vertical line represent standard error (n=3).

In the present study salt stress elicited symptoms of oxidative stress including high electrolyte leakage, increased concentrations of H_2 O_2 andMDA in shoots of sensitive rice genotypes (high sodium accumulating) with greater extents (Fig. 2a, b & c). We have also observed that these traits were positively correlated with sodium and negatively correlated with growth (Table 4). Under normal conditions, H_2O_2 is produced in low quantities in cells, acts as signal transduction molecules. Whereas during stress, their rate of synthesis is considerably high, functions as a toxic molecules in addition to signal transduction causes oxidative damage (Caverzan *et al*., 2019). Das & Roychoudhury (2014) reported that excess accumulation of H_2O_2 depends on the balance between its production and detoxification from cell. Failure of this balance may cause lipid peroxidation in biological membranes, inactivation of CO_2-fixingenzymes, damage to organelles like mitochondria; chloroplasts, chlorophyll contents, photo-chemical processes, ATP production and reduction of photosynthetic rate, degradation of proteins and denaturing DNA molecules, (Che-Othman *et al*., 2020; Munns *et al*., 2020). Some other studies reported that higher lipid peroxidation and higher electrolyte leakagewere observed under salinity in salt sensitive plant species including rice, wheat and maize whereas, low MDA contents was associated with salt tolerance (Mekawy *et al*., 2018; Xie *et al*.,2019; Yong *et al*., 2020). These reports corroborate the present findings.

Osmoregulation under salinity stress is another adaptation mechanism; in which higher accumulation of organic solutes help in osmotic adjustment by lowering osmotic potential of cells (Munns *et al*., 2020). We have also observed that proline and sugars were increased variably under salinity. Present study demonstrated differential osmotic adjustments among rice genotypes. The genotype IR-6, IR-72 and FL-478 produced comparatively more proline. Whereas, the genotypes SAL-10 DT2-DT1 & GML- 498 synthesize more soluble sugars under salinity stress (Fig. 4a & b). These two osmolytes have shown negative correlation with plant growth (Table 4). This may be due to fact that under salinity stress, key metabolic enzymes involves in production of ATP and reductants during TCA cycle are inhibited physio-chemically thereby affect adaptive processes i.e., ion exclusion, osmoregulation and detoxification of reactive oxygen species (Che-Othman *et al*., 2020). Under stress conditions

the production of organic solutes for osmotic adjustment depends on reallocation of available resources is energy deviating process from normal growth results in decreased plant growth (Zelm *et al*., 2020).

Conclusions

Physio-biochemical analysis of these rice lines indicated that among all tested genotypes IR-6 and IR-72 showed moderate level of salinity tolerance. Our results suggested that salt tolerance ability of the genotypes may be resulted from higher K/Na ratio, high chlorophyll contents, better osmotic adjustment, and less MDA & H_2O_2 production under salt stress conditions. These physiological traits may be utilized for the improvement of evaluation / selection processes for developing salt-tolerant genotypes.

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