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SALINITY INDUCED VARIABILITY IN MORPHO-PHYSIOLOGICAL TRAITS AND THEIR RELATIONSHIP WITH GRAIN YIELD IN RICE (*ORYZA SATIVA* L.)

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Abstract

Salinity is one of the most important abiotic constraint causes significant yield losses in rice crop specially when occur at flowering stage. The losses are mainly due to excessive accumulation of toxic ions mainly sodium in shoot affecting physiological processes osmotically and ionically. Twelve rice genotypes were studied under hydroponically controlled saline conditions (50 mM NaCl) along with non-saline control. Variability of sodium in shoot was assessed at vegetative, flowering and maturity stages and the impact of sodium accumulation on physiological traits of chlorophyll, proline, potassium sodium ratios and total soluble sugars were explored at flowering stage. Furthermore, significance of physiological traits in relation to adaptation of growth and yield under salt stress were also investigated. These studies revealed differential behavior of genotypes in their sodium uptake. Significant gradual increase in shoot sodium concentrations was observed under salinity at each stage of growth. Genotypic comparisons have shown that genotypes RST-177, RST -179, Shua-92-155/E, FL- 478, Kharaganja and IR-9 exhibited significantly low sodium concentrations and high potassium and sodium ratios. Highest sodium concentrations were observed in GML-528, GML-529 followed by GML-538. Results have also revealed variable increase in their total soluble sugars and proline concentrations. Highest concentrations of proline and total soluble sugars were found in GML-529, GML-538 and GML-528.Whereas, the lowest proline concentrations were observed in FL-478 followed by IR-9 and RST-177. Correlation studies among physiological traits revealed that the trait of sodium was significantly positively correlated at $p<0.01$with proline (r =0.7521) and sugars (r =0.8319) and negatively correlated with grain weight (-0.6923). The grain weight was negatively correlated with proline and total soluble sugars. Significant positive correlation of grain weight was observed only with K: Na ratio (r = 0.5355). On the basis of these studies it may be concluded that chlorophyll pigments, low sodium concentrations more specifically high potassium sodium ratios were observed as adaptive traits.

Key words: Rice (*Oryza sativa*), Salinity, Sodium, Chlorophyll and K: Na ratios.

Introduction

Rice (*Oryza sativa*) is second most important cereal crop fulfill 35-80% caloric requirement. It is grown on 160 m ha of land worldwide where, about 30% contains high level of salts, reduces normal rice productivity (Mishra, 2004). These lands are converting into nonproductive at a rate of 1.5 m ha / year due to high salinity and causing huge annual losses of US$ 12-27 billion (Wang *et al.*, 2012; Munns & Gilliham, 2015). Rice is regarded as most salt sensitive crop with threshold level of 3 dS/m exhibits varying degree of sensitivities at different growth stages, may cause more than 50% reduction in grain yield at 6 dS/m (Nam *et al.*, 2015; Ma *et al.*, 2018).

Under the present global climate change scenario, the problem of salinity is one of the major threats for food security. The expected increase in salinity would be the main obstacle to meet an additional rice requirement of 40% increased population by the year 2050 (Fageria, 2014; Ray *et al.*, 2015; Kashmir *et al.*, 2016). Thus there is a dire need to develop salt tolerant rice genotypes for sustaining rice productivity and global food security.

Salinity tolerance is a complex polygenic trait governed by interactive effects of many physiological component traits. Cambell *et al.*, (2015) suggested some of these traits may be incidental, deleterious and adaptive in nature. Yeo *et al.*, (1990) and Negrao *et al.*, (2017) proposed for trait based analysis of salinity responses (instead of studying responses at whole plant level) may contribute more beneficially to salinity tolerance. Thus there is a need to identify physiological significance of particular traits in plant adaptation/productivity under saline conditions. To date studies related to salt tolerance mechanisms have indicated that growth reduction in plants is primarily due to decreased osmotic potential of soil solution affecting water relation processes of plants and subsequently due to high concentrations of toxic ions accumulates in shoot in varying intensities at different stages of plant (Negrao *et al.*, 2017; Hussain *et al.*, 2018). Sodium accumulation is the major growth limiting factor under salinity stress. High sodium concentrations affect many metabolic processes, such as Plant water relations, nutrients uptake, stomatal conductance, photosynthesis and thereby cause negative effects on growth and yield **(**Mantri *et al.*, 2012; Gupta & Huang, 2014; Reddy *et al.*, 2017; Keisham *et al.*, 2018). Salt tolerance of plants depend mainly on their ability of osmotic adjustment, ions homeostasis through accumulating low concentrations of sodium and high potassium (Keisham *et al.*, 2018; Ouhaddach, 2018).

Large inter and intra varietal variability have been reported for sodium uptake among the rice cultivars (Munns & Tester, 2008; Platten *et al.*, 2013; Ismail & Horie, 2017). Furthermore, it has also been reported that trait of sodium transport is heritable in crosses and both low sodium transport and low sodium to potassium ratio can be selected independently (Yeo, 1992; Garcia *et al.*, 1997; Faiyue *et al.*, 2012). Selection of rice genotypes showing diversity in sodium and potassium uptake is not only important to understand plant metabolic responses but also to identify adaptive physiological traits contributing in tolerance mechanisms. The inherent diversity in specific adaptive traits may be usedfor pyramiding of traits based breeding to improve salinity tolerance in rice genotypes. Therefore we have studied variability in sodium uptake pattern at 30, 60 & 90 days exposure of salinity correspond to vegetative, flowering and maturity stage and correlated with other physiological traits at most sensitive flowering stage. Later on the relationship of these physiological responses were studied on yield at maturity.

Materials and Methods

Twelve rice genotypes (IRRI) along with known salt tolerant check (FL-478) obtained from NIBGE Faisalabad were studied for shoot sodium concentrations (at 30, 60 and 90 days exposure of salinity) and other related physiological responses at 60 days (flowering stage). Experiment was conducted under hydroponically controlled conditions in complete randomize design (CRD) with five replicates and two treatments; none saline (EC: 0.83 dS/m) and saline 50 mM NaCl (EC: 6.5 dS/m). Rice nursery at the age of four weeks (grown in sweet soil) was transplanted in sand filled cemented beds (size: 9 m x 1.2 m) at a distance of 25 cm between rows and hills. There were five rows of five plants of each genotype in a bed. The plants were irrigated with Hoagland solution (Hoagland and Arnon, 1950) and were allowed to establish for one week. Thereafter salinity treatment of 50 mM NaCl corresponding to EC: 6.5 dS/m was applied. Electrical conductivity of irrigated solutions was monitored daily and maintained on alternate days till maturity through portable EC meter. The complete renewal of nutrient solution was done weekly. Sampling of second fully expanded leaf was done at 30, 60 & 90 days exposure of salinity corresponds to vegetative, flowering and maturity stage. The leaves were analyzed for sodium and potassium (Flowers & Yeo, 1981) at each stge. Other physiological traits including proline (Bates *et al*., 1973), total soluble sugars (Riazi *et al*., 1985), potassium sodium ratios and chlorophyll contents (Lichtenthaler, 1987) were studied at floweringstage. Growth and yield traits (i.e. plant height, straw weight, productive tillers and net grain weights) were recorded on per plant basis at the time of maturity. Data were statistically analyzed for analysis of variance (ANOVA) and comparison between treatment means was performed through Tukey HSD test α 0.05. The correlation co-efficient among physiological traits and grain yield was also carried out using software Statistix 8.1[analytical software Inc., Tallahassee, FL, USA].

Results and Discussions

Growth and yield responses: The analysis of variance (ANOVA) for growth and yield related traits have shown significant differences with respect to salinity treatment, genotypes and their interactions at α 0.01 (Table 1). All rice genotypes exhibited variable degree of reduction in their growth and yield parameters under salinity stress (Table 2). The least effects of salinity stress were observed in genotypes Kharaganja and RST-177 with respect to plant height and straw weight. Comparatively more negative effects of salinity were observed in all GML lines. The genotype Kharaganja also produced maximum number of productive tillers among all genotypes. In contrast to this the genotypes RST-179, HHZ-11-Y6-Y1-Y1and all GML lines had least productive tillers. Similarly the genotype Kharaganja have also shown highest grain yield followed by RST-177and IR-6, these genotypes exhibited minimum reduction of 13, 9 and 17%, respectively under salinity as compared to their respective controls. The grain weights of genotypes GML-528, GML-538 and GML-529 reduced with greater intensities under salinity and produced least grain weights. Reduction in plant growth and yield under salinity can be related to varying intensity of osmotic and ionic effects of salinity on availability of nutrients to plant and malfunctioning of physiological processes. Excessive salts in the soil decrease water potential and cause difficulty for plants in absorbing water and creates physiological drought. Secondly the large influx of Na into plants increases the Na contents, affects the integrity and permeability of cell membrane, creates antagonistic effects in the uptake of K, Ca, Mg and Li and hampers activity of important enzymes (PEP carboxylase RUBP carboxylase) in cytosole. As a result, metabolic imbalances occur in form of reduced photosynthesis, formation of reactive oxygen species and reduction in growth and yield of plants (Shereen *et al*., 2017; Ma *et al*., 2018; Khan *et al*., 2019). Strongly negative relations were observed between grain yield and shoot sodium ions i.e. (r = -0.6923) under salinity (Table 3). Ghosh *et al*., (2016) also reported strongly negative relations between sodium contents and grain yield under salt stress.

Table 1. Mean square values (ANOVA) for agronomic traits of rice genotypes under salinity.

SoV	d.f.	Plant height	Straw weight	Productive tillers	Grain weight
Genotypes (G)	11	554.17 **	304.47 **	25.03 **	142.07 **
Treatment (T)	1	1953.13**	1449.91**	186.88 **	1132.09 **
G x T	11	97.49 **	85.02 **	12.22 *	58.12 **
Error	48	14.08	12.37	4.12	3.84
CV %		3.55	12.89	19.60	12.72

d.f.=degree of freedom ; ** = Significant @ 0.01 probability

Table 2. Growth and yield responses of rice genotypes under salinity at maturity stage.

Genotypes	**Plant height (cm)**		**Productive tiller (no)**		**Straw weight (g)**		**Grain weight (g)**	
	mM NaCl		**mM NaCl**		**mM NaCl**		**mM NaCl**	
	Control	**50**	**Control**	**50**	**Control**	**50**	**Control**	**50**
HHZ 11-Y6-Y6	132 A	103 EFG	13ABCD	5 E	41 A	17 GH	26 AB	7 HIJ
RST-177	119 B	114 BCDE	11 ABCDE	9 BCDE	41 A	32 ABCDE	18 CDE	17 DEF
Kharaganja	117 BC	116 BCD	16 A	14 ABC	39 A	34 ABCD	29 A	25 ABC
IR-9	114 BCDE	107 CDEFG	12 ABCDE	10 ABCDE	33 ABCD	32ABCDE	19CDE	14EFG
Shua-92-155/E	114 BCDE	110 BCDEF	11 ABCDE	11 ABCDE	33ABCDE	26BCDEFG	17DEF	13FGH
IR-6	113 BCDE	103 EFG	11 ABCDE	10 ABCDE	34 ABC	30 ABCDEF	21BCD	17DEF
PCT6/0/0/.	108 BCDEF	104 DEFG	15AB	11ABCDE	23DEFG	17GH	15DEF	14EFG
FL-478	107 BCDEFG	96 GH	9BCDE	8BCDE	26CDEFG	23DEFG	19CDE	8GHIJ
RST -179	105 CDEFG	103 EFG	7 DE	6 E	22 EFG	19 GH	11 FGHI	6 IJ
GML-529	103 EFG	88 HI	16A	7E	35ABC	16GH	24ABC	6IJ
GML-528	99 FGH	83 I	12 ABCDE	7E	17GH	9H	12FGHI	4J
GML 538	99 FGH	81 I	11 ABCDE	8CDE	37AB	19FGH	21BCD	7HIJ
HSD Values at α 0.05 for genotypes(G)	7.4365		4.0247		6.9702		3.8850	
Treatment(T)	1.7774		0.9619		1.6659		0.9285	
G x T	11.796		6.3842		11.057		6.1627	

Values with different letters are significantly different at $p \leq 0.05$ using Tukey- HSD all pair wise comparisons test

Table 3. Relationship (Pearson's Correlation coefficient) among grain weight and physiological traits of rice genotypes under salinity stress.

	GWT	**Chl total**	**Chl- b**	**Chl-a**	**K:Na**	**Proline**	**Sodium**
Chl total	0.1269 ns						
Chl b	-0.0188 ns	0.9067 **					
Chl~a	0.253 ns	0.8526 **	0.6603 **				
K:Na	0.5355 **	0.3046 ns	0.1902 ns	0.3266 ns			
Proline	-0.5439 **	-0.378 ns	-0.2122 ns	-0.419 *	-0.5572 **		
Sodium	-0.6923 **	-0.4261 *	-0.2668	-0.4377 *	-0.7502 **	0.7521 **	
Sugars	-0.597 **	-0.4458 *	-0.2008 ns	-0.5346 **	-0.5392 **	0.8027 **	0.8319 **

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

GWT = Grain weight, Chl total = Total chlorophyll, Chl b = Chlorophyll b, Chl-a = Chlorophyll a K: Na= Potassium sodium ratios

Fig. 1a Leaves sodium concentrations at 30 & 60 days

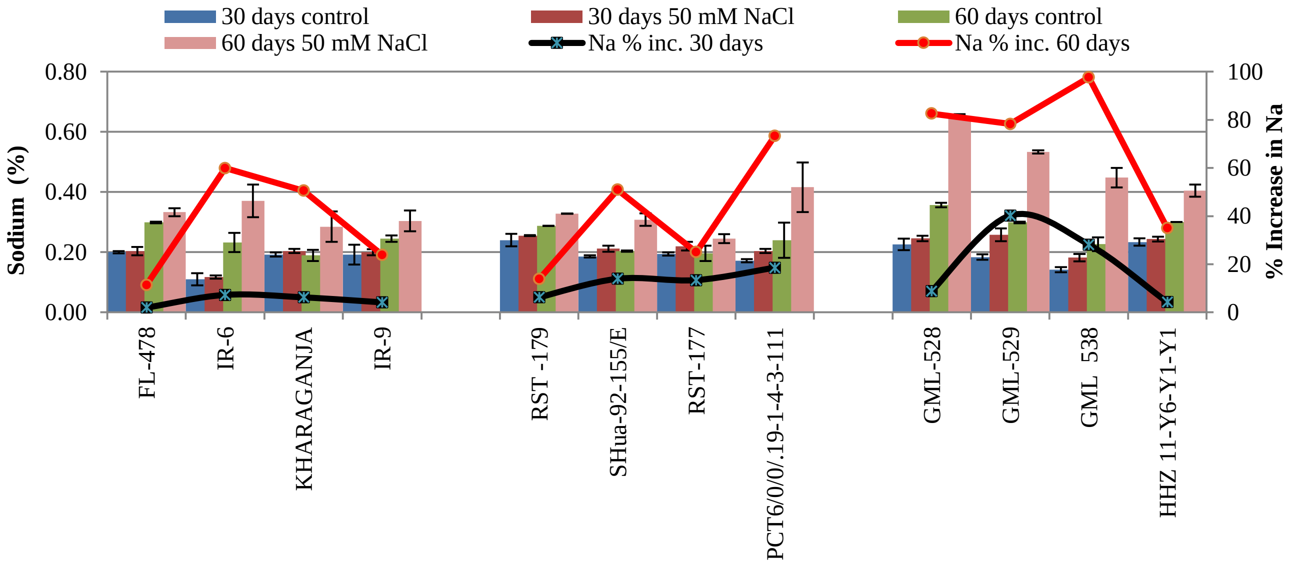

Fig. 1b Leaves sodium concentrations at 90 days

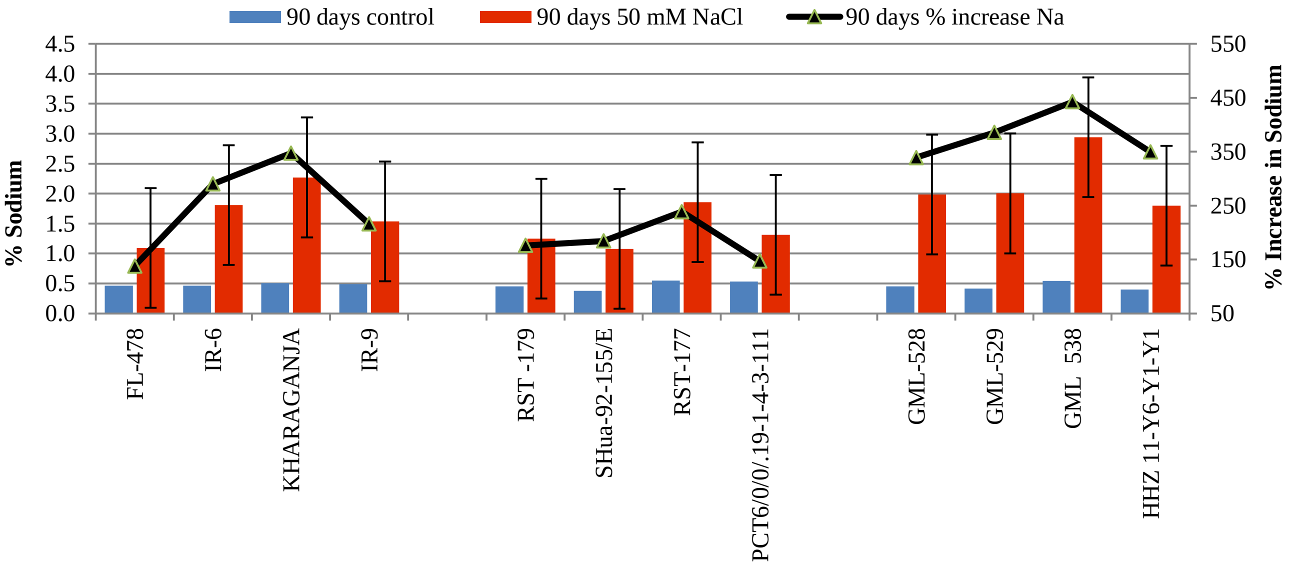

Fig. 1c Effect of salinity on potassium sodium ratios (60 days)

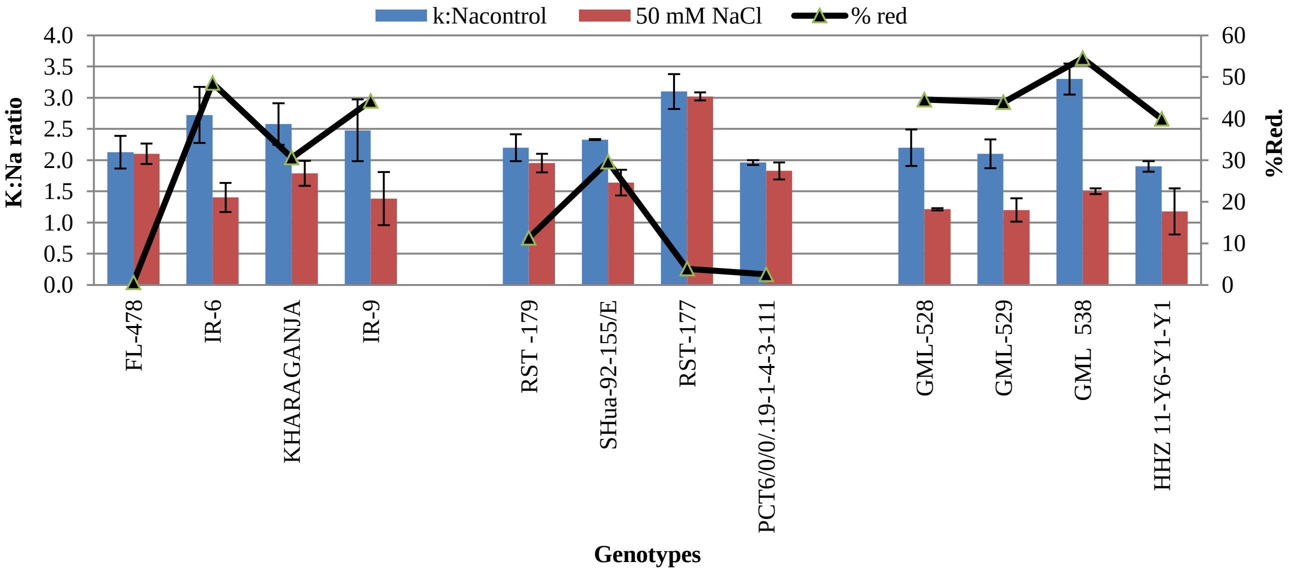

Fig. 1d

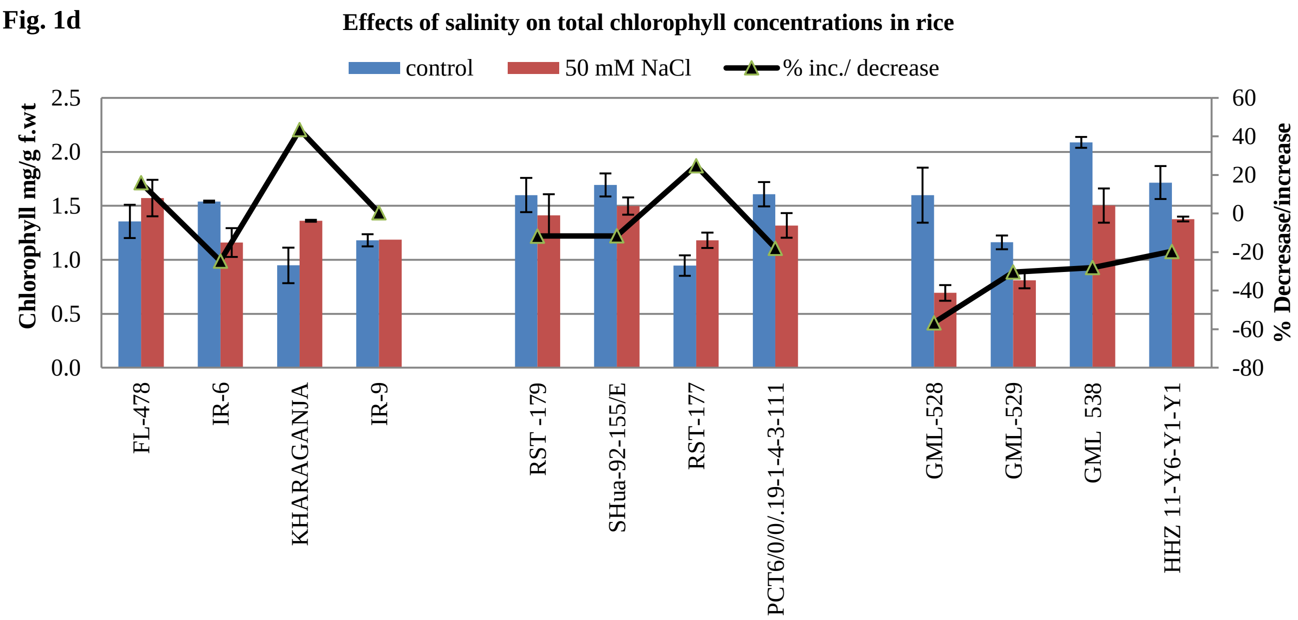

Fig. 1e

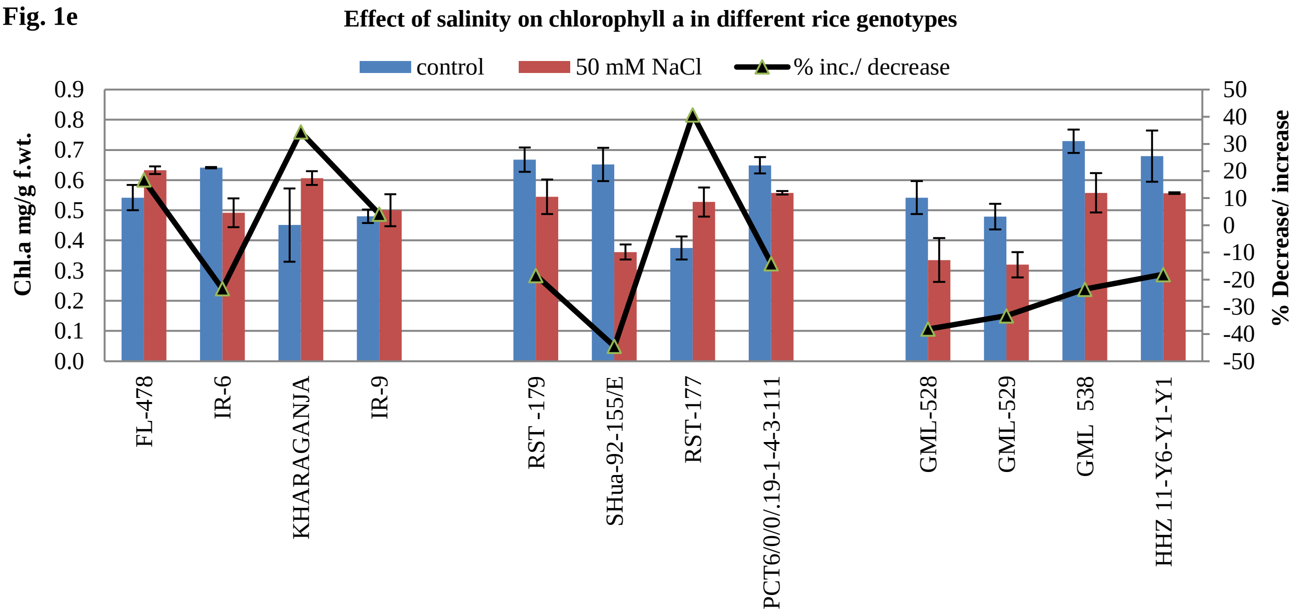

Fig. 1f

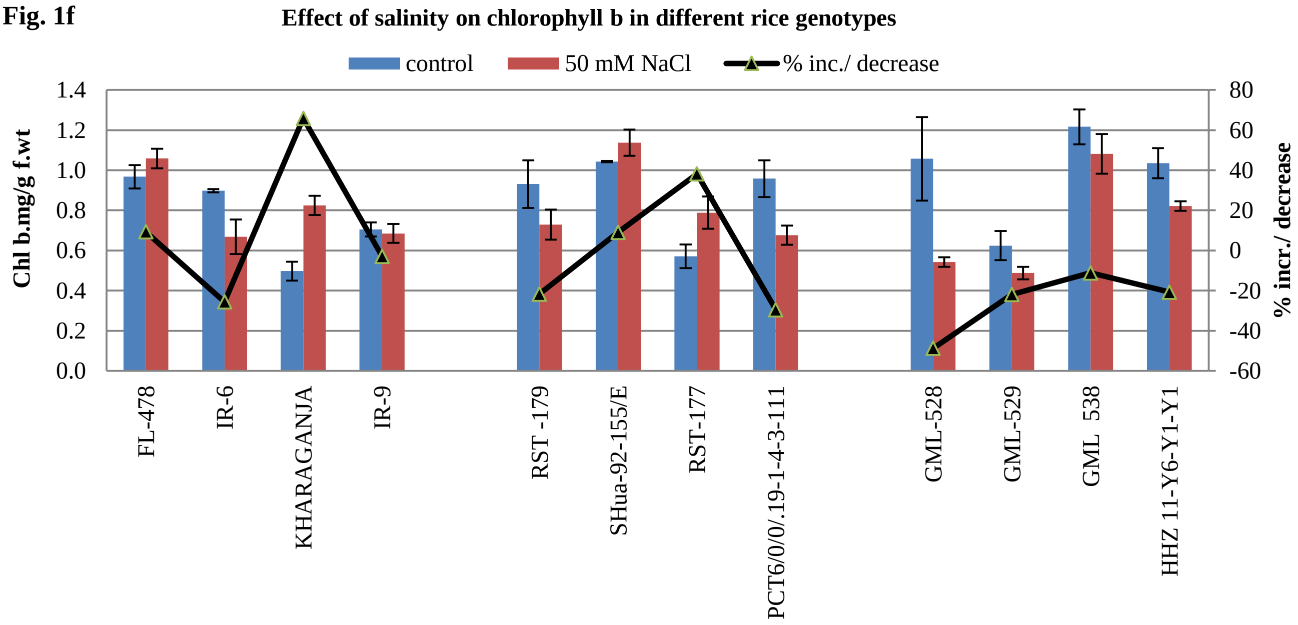

Fig. 1g

Effects of salinity on proline (uM/g.f.wt) in rice

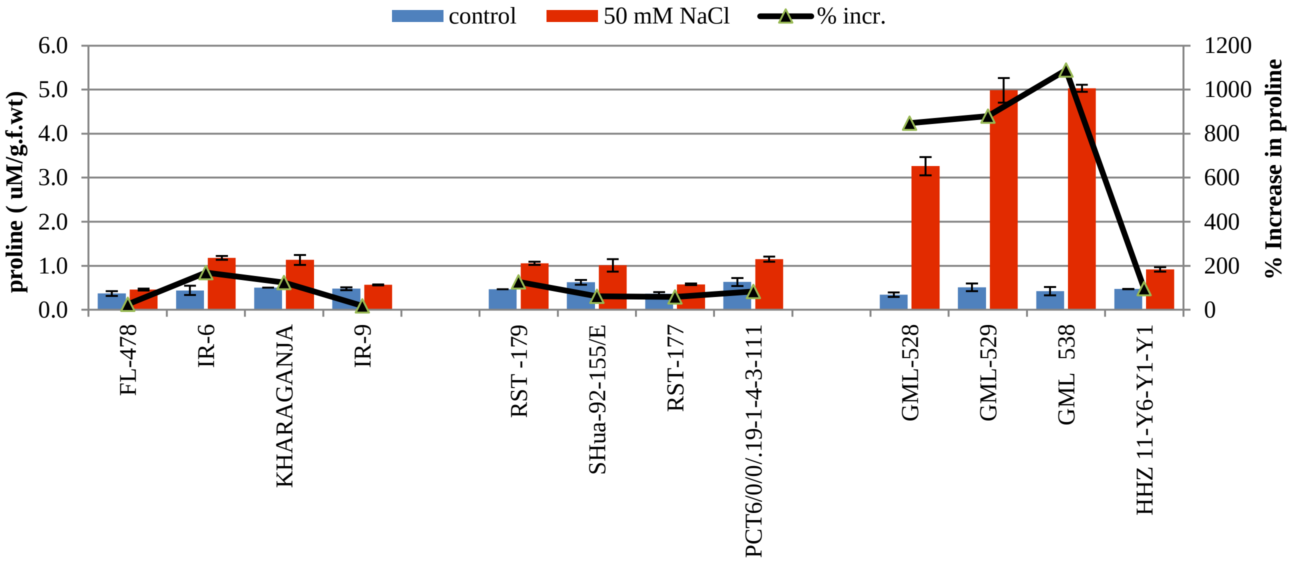

Fig. 1h

Effects of salinity on TSS (mM/Kg.f.wt) in rice

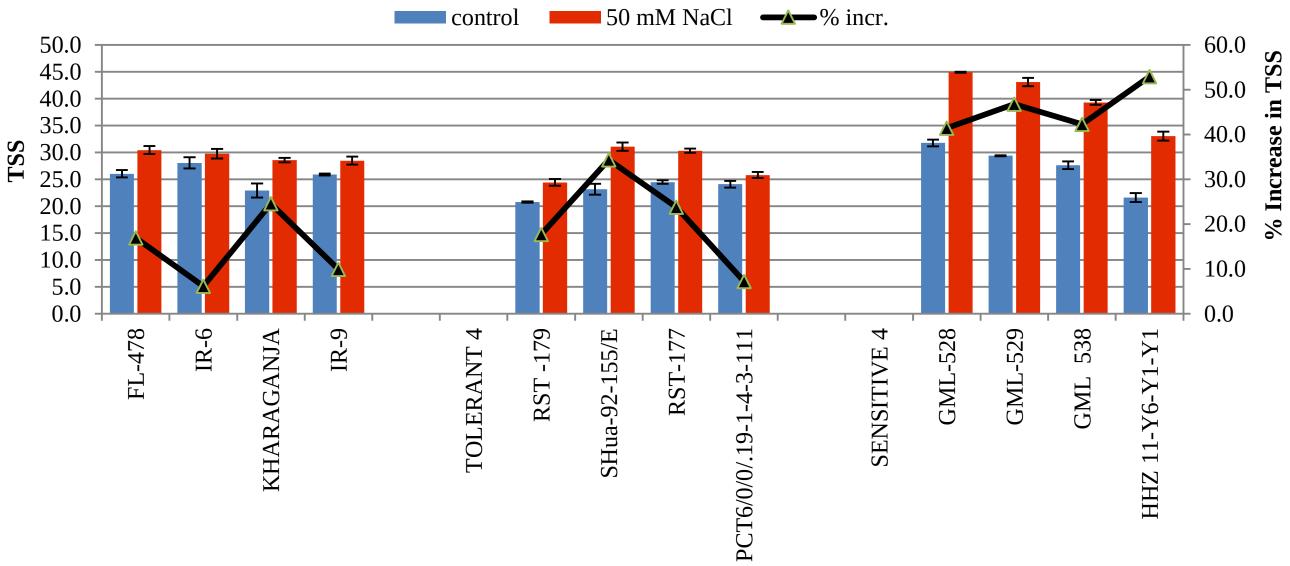

Fig. 1. Effects of salinity (50 mM NaCl) on physiological responses of rice genotypes at different stages of growth. a) Na Concentration at 30 & 60 days, b) Na Concentration at 90 days, c) Potassium sodium ratio, d) Total chlorophyll contents, e) Chlorophyll a, f) Chlorophyll b, g) Proline, h)Total soluble sugars. Bars indicate average values computed from 3 plants per treatment and per genotype. Vertical lines represent standard error (n = 3).

Physiological responses: Significant effects of salinity treatment were observed (at $p<0.05$) on physiological attributes studied i.e. inorganic and organic solutes and chlorophyll contents. Results have revealed differential behavior of genotypes in shoot sodium concentrations, which increased gradually under salinity (6.5 dS/m NaCl) at each stage of growth. Genotypic comparison at 30 days exposure of salinity treatment has shown that GML-538 and IR-6 accumulated lowest sodium concentrations initially which increases substantially at later growth stages (Fig. 1a & b). The responses of other genotypes (RST-177, RST-179, Shua-92-155/E, FL-478, Kharaganja and IR-9) were intermediary at initial stage (30 days) but at subsequent stages these genotypes have exhibited significantly differential responses in their low sodium concentrations (Fig. 1a). Among these genotypes least increase in sodium was observed in salt tolerant check genotype FL-478 (11%) followed by RST -179(14%) and RST-177 (25%). On the other hand the genotypes GML-529, GML-528 and GML-538 exhibited highest sodium concentrations at 30 and 60 days of exposure to salinity, also exhibited highest relative increase of 83, 78 and 98% respectively (60 days of exposure to salinity), when compared to their respective controls. At 90 days exposure of salinity least sodium concentration was observed in genotype FL-478 and Shua-92-155/E whereas, highest concentrations were observed in GML-538 (Fig. 1b). Inter and intra varietal variability for sodium uptake among the rice cultivars have also been reported earlier (Platten *et al.*, 2013). Sodium exclusion is one of the mechanisms for survival of plants under salinity stress. It is reported that salt tolerant rice genotypes exhibit low Na accumulation and high K/Na ratios than susceptible ones (Munns & Tester, 2008; Ismail & Horie, 2017; Chen *et al*., 2018).This was also observed in our study (Fig. 1a) all tolerant rice genotypes (Kharaganja, RST-177 and IR-6) have shown comparatively low degree of relative increase along with less absolute value of their shoot sodium concentrations (0.29, 0.25 and 0.37% respectively). In contrast to this comparatively higher relative increase in sodium concentrations were observed in sensitive genotypes (GML-528, GML-529 and GML-538).

Potassium is one of the most important essential nutrients for plant growth, plays a vital role in osmo-regulations, ionic balance, especially the K/Na ratio, which is a key factor of salt tolerance in plants. Under saline conditions high influx of sodium causes ion-specific stresses, creates K deficiency and decrease K/Na ratios. This condition in cytosole can trigger cellular Ca and activate complex signaling network regulating transport proteins involves in maintaining ionic balance of these cations (Wakeel *et al*., 2011). In the present study shoot potassium concentrations also decreased drastically under salinity with the resultant decrease in K: Na ratios (Fig. 1c). Comparison of potassium and sodium ratios among genotypes under salinity exhibited significant genotypic differences with highest ratio in genotype RST-177 and salt tolerant check (FL-478) accompanied with least relative reductions of 3.9 and 0.6% respectively under salinity. Similarly the genotypes RST -179, Shua-92-155/E and PCT6/0/0/.19-1-4-3-111 also maintained comparatively good K: Na ratios. Inhibitory effects of decreased potassium concentration on many metabolic processes including translocation of nutrient, metabolite and phytohormones and photosynthesis and protein synthesis via triggering enzymes and co- enzymes are variously reported for salinity stress (Negrao *et al*., 2017; Nounjan *et al*., 2018). Hindrances in transcription and translation steps of protein synthesis in absence of K are also reported (Wakeel *et al*., 2011). Thus low cytosolic Na and high K/Na ratios are vital to maintain ion homeostasis and biochemical equilibrium in the cytoplasm. This may be achieved through reduced influx of Na in shoot, compartmentation of Na into vacuoles and Na efflux from root (Keisham *et al*., 2018; Nounjan *et al*., 2018).

Results of present study also revealed that all tolerant genotypes maintained their K/Na ratio with least relative reductions under salinity compared to sensitive genotypes where more than 40% reduction was observed in K/Na ratios (Fig. 1c). Ghosh *et al*., (2016) suggested strong correlation between sodium content, ratio of K/Na with seedling growth and grain yield under salt stress. Other studies on salt tolerance concurred with our findings that concentration of Na in shoots negatively, while K/Na ratios positively correlated with the level of salt tolerance (Gholizadeh & Navabpour., 2011; Faiyue *et al*., 2012; Platten *et al*., 2013; Nounjan *et al*., 2018; Khan *et al*., 2019).

Plants developed adaptation mechanisms under stress to mitigate the adverse osmotic and ionic effects of salinity by osmotic adjustment (OA) with the help of inorganic and organic solutes (soluble sugars and proline). In the present studies significant increase in proline concentration was observed under salinity stress. Genotypic comparisons have shown highest proline concentrations in GML-529, GML-538 and GML-528. Whereas, the lowest proline concentrations were observed in FL-478 (salt tolerant check) followed by IR-9 and RST-177 (Fig. 1g). This alteration in proline metabolism under stress may be due to magnitude of increase or decrease activity of enzymes pyroline-5-carbooxilase reductase and proline oxidase. Extents of alteration varied among genotypes leading to maintenance of turgor and alleviation of osmotic effects caused by salinity. The other aspect of salinity effects is related to deposition of excess toxic ions, which affects metabolic processes and may results in decreased grain yield. Significantly negative relationship ($r = -0.5439$, $p<0.01$) between proline and grain weight (Table 3) was observed in the present findings. Liu *et al.,* (2018) has the opinion that proline plays more roles in survival rather than in the maintenance of plant growth functions. Results have also revealed variable increase in total soluble sugars concentrations in shoot under salinity. Highest sugar concentrations with highest relative increase under salinity were observed in GML-528, GML-529 and GML-538 (sensitive genotypes). While, the genotypes IR-9, Kharaganja and IR-6 exhibited least increase in comparison to their non-saline controls. The responses of RST-177, FL-478, and Shua 92-155/E were intermediary (Fig. 1h).

Proline and sugars are considered highly sensitive to salt stress, increased significantly in the cytoplasm and promote water retention to maintain cell turgidity as water potential dropped. There are many reports related to high accumulation of proline and total soluble sugars in the cells along with high concentrations of NaCl as was observed in our study. These organic solutes reported for their functions to stabilize and protect cellular membranes structure against dehydration, ROS scavenging system and regulate gene expressions in many metabolic processes. Therefore may be used as a tool to correlate with plant responses under abiotic stress (Abdallah *et al*., 2016; Tiwari *et al*., 2017; Perveen *et al*., 2018; Khan *et al*., 2020). Nounjan *et al*., (2018) reported accumulation of higher total soluble sugar was associated with higher osmotic adjustment in rice lines. Atabaki *et al*., (2018) have also reported increased accumulation of proline and total soluble sugars in NaCl-treated rice callus. It is well established that compatible organic solutes increase under salt stress; however, whether a greater increase in compatible solutes correlates with increased salinity tolerance in plants still remain to resolve with number of controversial reports. Chen *et al*., (2007) reported that more salt-tolerant barley varieties accumulated less compatible solutes than the sensitive varieties. Whereas, number of reports are also available for rice showed more increase in sodium and proline in sensitive rice genotypes than tolerant ones (Wang *et al*., 2016, Chunthaburee *et al*., 2016; Negrao *et al*., 2017and Shereen *et al*., 2017). This was also observed in the present study that all tolerant rice genotype including salt tolerant check (FL-478) accumulated less sodium and proline contents under salinity stress indicates sodium dependent increase of proline (Fig. 1g). Thus the findings reveal that accumulation of proline is highly dependent on shoot sodium concentrations and act as an indicator of stress rather for salt tolerance. The values of correlation co-efficient between sodium and proline further substantiate as these two traits were highly positively correlated (r =0.7521, p<0.01) and negatively correlated with grain weight (r =-0.6923 & -0.5439 respectively). In accordance with these reported results, it may be suggested that higher concentrations of compatible solutes are related to the severity of osmotic stress indicating the presence of osmotic adjustment mechanism. Even so, its physiological significance is yet to be fully understood.

Chlorophyll contents were variably increased or decreased among rice genotypes (Fig. 1d). Kharaganja and FL-478 (check genotypes) were performed comparatively better as they exhibited a relative increase of 43 and 22% in total chlorophyll contents. While the genotypes, RST-177 & IR-9 have shown an increase of 25% in their chlorophyll contents. Rests of the genotypes have shown variable reduction with least reduction in genotype HHZ 11-Y6-Y1-Y1, Shua-92-155/E and RST -179. More or less similar trends were observed for chl.a and chl.b (Fig. 1e & 1f). Findings were in consistent with many earlier reports indicating salt stress induced decrease or increase in chlorophyll contents in rice. These studies have also shown that salt-tolerant and moderately tolerant genotypes contained significantly higher chlorophyll contents (Gholizadeh & Navabpour, 2011; Hussain *et al.*, 2018; Ma *et al.*, 2018; Nounjan *et al.*, 2018; Khan *et al.*, 2019). Correlation studies (Table 3) indicated that chlorophyll pigments were negatively correlated with sugars (-0.6330, p<0.01) and positively correlated with potassium (r = 0.7710, p<0.01) and K: Na ratio (r = 0.8795, p<0.01).

Conclusions

On the basis of these studies it may be concluded that chlorophyll pigments, low sodium concentrations more specifically high potassium sodium ratios were observed as adaptive traits. The genotypes RST-177, RST -179, Shua-92-155/E & FL-478 exhibited potential for these traits may be used as a genetic resource for transferring or combining these physiological traits with some elite high yielding rice genotypes for improving their salinity tolerance.

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