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SALINITY INDUCED VARIABILITY IN SODIUM AND PROLINE AND ITS CORRELATION WITH GRAIN YIELD IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Rice (*Oryza sativa* L.), is an important cereal crop fulfilling half of the world's food demands, is the most salt-sensitive among cereals. Approximately 30% of rice growing areas in the world are harmfully affected by soil salinity which is a major threat for global food security. High osmotic potential in rhizosphere and high sodium uptake is generally regarded as the main causative factor for reduced growth and yield. Rice genotypes exhibit variability in sodium uptake and osmo-regulation processes. The role of proline is widely documented as an osmoprotectant and controversial for enhancing salt tolerance in crop plants. In the present study eighteen rice genotypes were studied for physiological and agronomical traits under hydroponically controlled saline conditions (6 dS/m NaCl) at flowering and maturity stages. Significant differences were observed for genotypes, treatments and their interactions for evaluated traits. This study revealed negative correlation of proline and sodium with grain yield under salinity. While highly significant positive correlation ($r_= 0.800$) between sodium and proline suggest over production of proline under salinity stress is sodium dependant. Hierarchical cluster and nearest neighbor relative analysis corroborated the salinity induced physiological responses of genotypes, revealed physiological traits based genetic similarity of genotypes and classified into tolerant and sensitive groups. The genotypes Kharaganja, Shua-92-155/E and RST-177 were found relatively tolerant at 6 dS/m NaCl in term of good yield with medium sodium and low proline. High proline producing genotypes (GML-529, GML-538, HHZ5-SAL-10-DT2-DT1 and GML-534) yielded less or no grain weights. Based on these results proline over production under salinity may be regarded as an indicator of stress.

Key words: *Oryza sativa* L., Salinity, proline, sodium and yield components.

INTRODUCTION

Agricultural world has a great challenge to produce 70% more food from present resources to sustain food production and population balance of new millennium which is expected to increase over 9 billion by 2050 (UNFPA, 2014). At present one-third of food demands is being provided by irrigated system (Munns, 2002). Approximately 20% of irrigated agricultural lands and 30% of rice growing areas in the world are already harmfully affected by soil salinity (Wang *et al.,* 2012) and converting into non productive at a rate of 1.5 million ha each year due to high salinity (Munns & Tester, 2008). It is reported that global annual losses in agricultural production from salt affected lands are in excess of US $ 12 billion (Shabala, 2013); if deterioration continues as such, and it is expected that half of the cultivated lands will lose their productivity by 21st century (Mahajan and Tuteja, 2005).This will pose a major threat for present and future global food security (Mantri *et al.*, 2012).

Rice is an important staple food and cash crop provide 35 to 75% caloric intake for more than three billion people in the World and there will be an additional rice requirement of 40% by the year 2050 (Fageria, 2014). In Pakistan rice is cultivated on an area of 2.89 million hectares; earn foreign exchange of US$ 1.53 billion through share in export (Anonymous, 2014). Rice is regarded as most salt sensitive crop with threshold level of 3 dS/m exhibits varying sensitivities at different stages of growth may reduce grain yield more than 50% (USDA. 2013; Kumar *et al*., 2013; Nam *et al*., 2015) at 6 dS/m.

Growth and yield of a genotype depend on genetic makeup and environmental factors. Breeding for improved rice genotypes with enhanced adaptability for salt tolerance is an urgent need for increasing rice crop productivity to fulfill the nutritional requirements in near future. The breeding program for improvement of salt tolerance depends on identification of sources of genetic variation, screening procedures, and complete knowledge about role of different physiological/genetic traits, genetic control of traits and their relationship with yield under stress (Negrao *et al*., 2011). The traits governing salt tolerance are of dynamic and polygenic nature (Cambell *et al.*, 2015) and these traits may be categorized into adaptive, deleterious and incidental. These adaptive physiological and metabolic traits are not sufficiently applied in plant breeding (Reynolds & Langridge, 2016). Thus there is a need to understand the physiological significance of a particular trait and its contributory role in plant adaptation in terms of productivity under saline conditions.

Earlier studies have indicated that rice genotypes exhibit differential responses and poor correlation for salt tolerance between seedling and maturity stage (Shereen *et al.*, 2005; Sudharani *et al.,* 2012; Rao *et al.*, 2013). These studies have also suggested the involvement of separate sets of genes at seedling and maturity. Furthermore reproductive stage is crucial for determining grain yield. Variability among rice genotypes for salt tolerance has been reported in number of studies (Lang *et al.*, 2009; Joseph *et al.*, 2010; Kanawapee *et al.*, 2011; Ali *et al.*, 2014; Hakim *et al.,* 2014; De Leon *et al.*, 2015; Khatun *et al.,* 2015; Zafar *et al.*, 2015; Naseer *et al.*, 2015) based largely on salinity induced growth and yield reduction indicated high salt concentrations in soil cause severe toxicity/ damage to plants, while moderate to low salt concentrations affects plant growth, number of tillers, leaf scorching, spikelet sterility, number of florets per panicle, grain yield and 1000-grain weight.

Number of reports have indicated that excessive accumulation of Na^+ and Cl^- ions in soil and water is a major factor, create ion toxicity in plants, cause malfunctioning in many physiological and biochemical processes and thereby causes reduce growth and yield in salinity (Sudharani *et al.,* 2012; Boriboonkas et *et al.,* 2013; Khan & Hemalatha. 2016).There is a common consensus that both halophytes as well as glycophyte species including rice adjust salt concentrations by reducing tissue osmotic potential preferentially in sensitive metabolic sites of cells by increasing uptake of organic and inorganic solutes and their preferential compartmentation with in vacuole and cytoplasm (Munns & Tester, 2008; Sudharani *et al.*, 2012; Hakim *et al*., 2014 ; Chunthaburee *et al*., 2016). Number of solutes (sugars, sugar alcohols, amino acids, proline, allantoin and glycine betain) has been reported in crop plants under stress either with the activation of synthesis or inhibition of catabolism of osmolytes (Lee *et al.*, 2012; Wang *et al*., 2015).The genotypic differences in salt induced accumulation of proline have also been observed in many plants (Siddiqi *et al*., 2011; Bano *et al*., 2012; Hakim *et al.*, 2014; Shereen *et al*., 2015;Wang *et al*., 2015) however its actual role in salinity stress tolerance in rice is still unclear and controversial (Verbruggens and Hermans,2008; Tatar *et al.*, 2010). Some have reported positive correlation of proline and salt tolerance in several species including rice (Igarashi *et al.*, 1997; Ashraf & Foolad, 2007; Kumar *et al.*, 2013; Bilkis *et al.*, 2016) while, other reports have indicated negative correlation of proline with salt tolerance and its accumulation under stress is a symptom of injury not as an indicator of salt tolerance (Shobbar *et al.*, 2010; Kanawapee *et al.*, 2011; Kong-negern *et al*., 2012; Hakim *et al.*, 2014; Wang *et al*., 2015; Chunthaburee *et al*., 2016). Studies in this regard suggested exploring further the relationship between proline and salt tolerance in diversified rice genotypes (Shobbar *et al.,* 2010; Reynolds & Langridge, 2016). In the present study rice lines exhibiting diversified pattern for shoot sodium were selected (on the basis of our preliminary screening for relative increase in leaf sodium at 6 dS/m NaCl salinity) to investigate proline responses with particular emphasis on relationship between leaf sodium, proline and their interactive effects on yield.

MATERIALS AND METHODS

The experiment was conducted at Plant Physiology Division of Nuclear Institute of Agriculture, Tandojam during the year 2014-15. Seeds of eighteen rice genotypes (IRRI) obtained from NIBGE Faisalabad and NIA, Tandojam. Rice nursery was raised in sweet soil under normal cultural practices. The experiment was conducted in sand filled cemented beds (size: 9m x1.2m), under hydroponically controlled conditions in randomize complete block design (RCBD) with five replicates and two treatments (none saline and saline) in net house. Nitrogen, Phosphorous & Potassium was applied @ 120:60: 60 Kgha^{-1} (derived from Urea, Di-ammonium phosphate & Potassium Nitrate respectively). Six weeks old rice seedlings were transplanted in these beds at a distance of 25 cm between rows and hills. There were three rows of five plants of each genotype in each bed. The beds were flooded with Hoagland solution (Hoagland and Arnon 1950) and the plants were allowed to establish for one week. Thereafter salinity treatment of 6 dS/m was imposed and maintained throughout the growth period till maturity by monitoring and adjusting electrical conductivity of irrigated solutions in beds through portable EC meter on alternate days. The renewal of nutrient solution was done weekly. Sampling of first leaf was done for physiological parameters at the stage of flowering. The leaves were analyzed for sodium and potassium ions (Flowers & Yeo. 1981), chlorophyll (Lichtenthaler, 1987) and proline (Bates *et al.*, 1973) contents. Plants were harvested at maturity and observations on different growth; yield (i.e. plant height, number of total and productive tillers, net grains weight per plant) and sterility were recorded. The data on yield components, (panicle length, panicle weight, spikelet numbers, number of grains and grain weight) were taken on per panicle basis from three randomly selected plants. Data were statistically analyzed for ANOVA and Tukey HSD test was applied for comparison between treatment means using Statistix 8.1[analytical software Inc., Tallahassee, FL, USA] software. Physiological parameters, including chlorophyll, proline and sodium were used to classify the rice genotypes as salt-tolerant, moderately tolerant or salt-susceptible using Hierarchical cluster and nearest neighbor relative analysis in statistical software SPSS - 21.

RESULTS AND DISCUSSION

Genotypic variability for physiological traits in rice lines under salinity stress

Significant genotypic variations were observed for sodium, potassium sodium ratios, chlorophyll and proline concentrations in leaves (Fig. 1). All pair wise comparison test for genotypes and treatments were significantly different at the level of α 0.05 level. Genotypic comparison have shown highest sodium concentration in genotype HHZ5-SAL10-DT2-DT1 and lowest in salt tolerant check IR 66946-3R-178-1-1-FL-478 (FL-478) followed by IR 83142-B-20-B and GML 506. The fourteen genotypes were found in same class have shown more or less similar sodium concentrations (Table 1). Sodium concentrations in all rice lines increased with varying intensities under salinity (6 dS/m NaCl). The response of Kharaganja, Shua-92 155/E and IR88611-B-5 were more or less similar in their leaves sodium concentration as these genotypes exhibited least relative increase in their sodium concentration (10,11,17.9% respectively) under salinity (Fig. 1A).Whereas highest increase under salinity was observed in HHZ5-SAL10-DT2-DT1(1145.6%),GML-529(201.5%) & GML-538(148.3%) relative to their non saline control.

Potassium concentrations decreased variably under salinity with the resultant decrease in K: Na ratios among genotypes (Fig.1B). Highest ratio was observed in salt tolerant check genotype (FL-478) with relative reduction of 11.9%. The genotypes Shua-92 155/E, Kharaganja, and IR88611-B-5 exhibited comparatively low K: Na ratio but maintained these ratios with least relative reduction of 1.8, 4.0 and 11.8% respectively (Fig.1B) under salinity.

Salt stress induced significant increase in proline accumulation relative to non-saline controls (Fig. 1C). Significant variability among different genotypes was observed in their proline production under stress. On the basis of genotype comparison at level of α 0.05 these genotypes were observed as very high accumulator (HHZ5-SAL10-DT2-DT1 & GML-534), high accumulator (IR83141-B-18-B & GML-538), medium accumulator (UPL 79,IR 75288-144-1-3,GML 529,UPL 19, IR 83142-B-20-B & GML- 506) and low accumulator (RST -177**,** IR-72, IR 88611-B-5, Kharanganja, FL-478 & SHua-92-155/E). The data of chlorophyll pigments have shown variable responses under salinity. Significant effects of treatment were observed (at $P< 0.05$ level of significance) on chl. a, chl. b and total chlorophyll. Genotypic comparison have shown that FL-478 was comparatively better and significantly different from rest of the genotypes in chl. a, chl. b and total chlorophyll contents (Fig. 1D, E & F). While under salinity stress UPL-19, FL-478 and IR75288-144-1-3 were found comparatively better in chlorophyll contents. The genotypes HHZ5-SAL10-DT2-DT1, GML-525, GML-529, GML-534 & GML-538 exhibited least concentrations of chlorophyll pigments under salinity. Whereas the genotypes IR 88611-B-5, Kharanganja, & Shua-92-155/E were comparatively low in chlorophyll but maintained their chlorophyll concentrations under salinity. Differential responses of tolerant and sensitive rice genotypes were also observed and exhibited more chlorophyll “a” and “b” in tolerant than sensitive genotypes under salinity (Rao *et al*.,2013).

Pearson’s correlations

Correlation co-efficient among physiological traits and grain yield (Table 2) revealed highly significant positive correlation ($r =0.8000$) of proline with sodium and negative correlation with panicle weight ($r = -0.5309$) and grain weight ($r = - 0.5031$) at $p < 0.01$. The grain yield was negatively correlated with sodium ($r = -0.5227$) and positively correlated with K: Na (0.3214). The parameters chlorophyll pigments and K: Na ratio were highly positively correlated (at $P \leq 0.01$).

Effect of salinity on yield & yield contributing traits

The results of ANOVA have shown that salinity treatment, genotypes and their interactions were significantly different at α 0.05 (Table 3) in respects of all yield and yield contributory traits (panicle length, grain weight , number of gains , number of spikelets , sterility and grain yield). All yield contributing parameters were significantly reduced with varying intensities under saline conditions (Table 3). Over all genotypic comparison for panicle lengths have shown that, IR75288-144-1-13 was significantly different from rest of the genotypes. This genotype has also exhibited highest value under saline treatment followed by RST-177 and IR-72.

The effect of salinity was not so pronounced on number of spikelets / panicles. However, highest spikelet numbers under salinity were observed in RST-177 followed by IR-75288-144-1-3.Whereas least number of spikelets / panicles was observed in GML-538.

Number of grains / panicles was affected with varying intensities among the tested genotypes. Least number of grains was observed in GML-538. While highest number of grains / panicles under salinity was produced by RST-177, IR-75288-144-1-3, Shua-92-155-E and Kharaganja. Grain weight / panicles were similarly affected in these genotypes as highest grain weight was observed in RST-177 and Kharaganja followed by Shua-92-155/E and IR-75288-144-1-3. Cumulative effects of these yield components were manifested on total grain yield of a plant. Overall genotypes comparisons have shown highest grain yield under salinity was produced by kharaganga, followed by Shua-92-155/E and RST-177. These three genotypes were also found significantly different from other genotypes in their grain weight production under saline as well as under non-saline conditions (Table 2).Whereas, HHZ5-SAL10-DT_2-DT1, GML-538 and GML-529 exhibited lowest and no yield respectively were ranked as salt sensitive. The data of sterility have shown highest value in genotypes IR83142 B-20-B, GML-538 followed by GML-525 and IR83141-B-18-B.While the least sterility was observed in Kharaganja, RST-177 and Shua-92-155/E. The observed varying degree of reduction in yield and yield components may be the result of salinity genotype interaction. A large body of evidence (Boriboonkas *et al*., 2012 Zafar *et al.,* 2015; Naseer *et al*., 2015) is in support of variable degree of reduction in yield and yield components of rice genotypes under salinity. These evidences have shown delay in flowering, effects on number of spikelets, panicle length, panicle weight, pollen viability and stigma receptivity are the main cause of reduced grain yield and seed sterility.

Imbalanced nutrition, damage of membranes, and disturbed avoidance mechanisms, reduced metabolic activities, shrinkage of cell contents, reduced development and differentiation of tissues, may be the Physiological causes of lower grain yield (Hakim *et al.,* 2014).

Hierarchical cluster and K-nearest neighbor relative analysis:

Morpho-physiological parameters (proline, sodium and grain weight) were used for grouping of rice genotypes for their salt tolerance potential. Hierarchical cluster analysis of these genotypes using ward linkage method revealed that six clusters were found at 6 dS/m NaCl salinity. In cluster 01, three genotypes (IR75288-144-1-3, IR83142-B-61-B & GML 506). In cluster 02 UPL-19, GML-525 & IR 83142-B-20-B). The tolerant check IR 66946-3R-178-1-1-FL-478 (FL-478) and local check (Kharaganja) with other genotypes (IR-72, Shua-92- 155/E, IR8611-B-5; RST-177) were falling in cluster 4. This pattern indicates that these four genotypes showing more similarity with salt tolerant checks. While five genotypes IR83141-B-18-B, GML-534, HHZ5-SAL10-DT2-DT1, GML-538 & GML-529) were included in cluster 05. These genotypes have exhibited traits of high proline and high sodium along with low grain productivity. These criteria are considered most effective for classifying salt tolerance and salt sensitive genotypes and have been reported earlier in rice (Ali *et al*., 2014; Chunthaburee *et al*., 2015). K-nearest neighbor relative focal record at three selected predictors (K=3) have shown that the genotypes (shua-92-155/E , RST-177 & IR8611-B-5) were distinctly different from rest of the genotypes in their proline, sodium and chlorophyll contents as least distance was exhibited by genotype shua-92-155/E (0.1293) followed by RST-177 (0.4033) and IR8611-B-5 (0.4763) with local check Kharaganja (Fig.3).

Munns & Tester (2008) described salt tolerance of plant is dependent variably on three components i.e. sodium exclusion, sodium tissue tolerance and osmotic adjustment. Under saline stress plants suffer primarily from osmotic shock followed by ion toxicity. Sodium is the dominating ion under these conditions exerts detrimental effects on plants selective uptake of ions (K, Ca & Mg reduced) which result in disorder nutritional acquisition and many vital physiological processes (Khan and Hemalatha, 2016). Potassium is most preferred ion in plants due to its essential role in maintaining osmotic adjustment, stomatal regulation, enzymes activity and cell homeostasis. Salinity tolerance mainly depends on ion homeostasis; particularly high K: Na in cytosole (Tatar *et al*., 2010; Chunthaburee *et al*., 2016).This could be achieved by preventing K leakage through controlled activity of H^+/ATPase, outward K channels that facilitates proton pumping activity for secondary transport of toxic ion like Na from metabolic sites or deposition in other compartments like apoplast where they are less toxic. Variability in leaf sodium concentrations and maintaining K: Na ratios under salinity have been observed in present study (Fig 1). The salt tolerant check FL-478 has shown lowest sodium and higher K: Na ratio in shoot. Among the tested genotypes, tolerant genotypes (Kharaganja, Shua-92 155/E, RST-177 and IR88611-B-5) have shown least sodium increase and maintained K: Na ratios with least relative reduction under salinity (Fig 1B). The sensitive genotypes (HHZ5-SAL10-DT2-DT1, GML-538 and GML-529) exhibited highest leaf sodium concentration along with highest reduction in K: Na ratios. Increase in sodium and reduction in potassium ions in root and shoot is a common phenomenon under saline stress reported earlier in many studies (Flowers and Yeo, 1981; Joseph *et al*., 2010; Hakim *et al.,* 2014; Wang *et al*., 2015; Bilkis *et al*., 2016).These studies have shown less sodium accumulation, higher potassium concentration and less sodium translocation from root to shoot was related to salt tolerance.

In addition to this, osmotic adjustment is considered one of the important mechanisms by mean of inorganic and organic solutes. Proline accumulation is a primary response mainly appears due to any stress that causes low water potential/dehydration of plant tissue. Under saline conditions sodium is the dominating ion also exerts primarily an osmotic effect (physiological drought) on plants followed by ion toxicity. Increase accumulation of proline in plant tissues is mainly depend on enhanced biosynthesis or reduced degradation due to differential regulation of enzymes involved in proline metabolism. There are many reports which shows significant positive role of proline in osmotic adjustment (Igarashi *et al.,* 1997; Ashraf & Foolad, 2007; Rao *et al.,* 2013; Zhao *et al.,* 2014; Bilkis *et al.,* 2016).

Hakim *et al*. (2014) have reported increased accumulation of proline in the cytoplasm was associated with reduced concentrations of K and glutamate, and an increase in cytosolic water volume.

Table 1. The main effects of salinity on biochemical traits and yield of rice genotypes.

Salinity levels dSm^{-1}	Chl.a mg/g f.wt		Chl.b mg/g f.wt		Chl.total mg/g f.wt		Shoot Na %		K:Na ratio		Proline µM g^{-1} f.wt		Grain Yield g P^{-1}	
0	0.713	A	1.830	A	2.541	A	0.2313	B	3.3333	A	1.5459	B	27.252	A
6	0.634	B	1.695	B	2.329	B	0.5048	A	1.8463	B	6.7270	A	11.446	B
L.S.D Values for treatments at α 0.05	0.0348		0.0539		0.0835		0.0464		0.1487		0.4829		1.6311	
Genotypes														
IR 83142-B-20-B	0.7033	BC	1.8250	BCD	2.5283	BCD	0.1467	CD	4.5500	B	2.793	CDEF	17.083	CD
IR 83141-B-18-B	0.6717	BC	1.7933	BCD	2.4667	BCD	0.3117	BCD	2.9167	CD	9.133	B	14.567	CD
IR 66946-3R-178-1-1(FL478)	1.0150	A	2.3150	A	3.3233	A	0.1167	D	6.6000	A	0.930	EF	16.217	CD
HHZ5-SAL10-DT2-DT1	0.7117	BC	1.8467	BC	2.5567	BC	0.8150	A	3.3667	C	14.155	A	12.933	D
GML 506	0.7583	B	1.9250	B	2.6833	B	0.1867	CD	4.9833	B	2.012	CDEF	20.917	BCD
UPL 79	0.6817	BC	1.8083	BCD	2.4900	BCD	0.5083	B	2.0333	E	4.500	C	15.583	CD
UPL 19	0.7700	B	1.9217	B	2.6933	B	0.4633	B	1.6333	E	3.533	CDE	20.217	BCD
IR 75288-144-1-3	0.7983	B	1.9133	BC	2.7150	B	0.3750	BC	1.7167	E	4.358	C	21.200	BCD
GML 534	0.6367	BCD	1.7167	BCDEF	2.3517	BCDE	0.4850	B	1.8000	E	14.505	A	15.267	CD
GML 538	0.8000	B	1.7367	BCDE	2.5367	BC	0.5050	B	1.6833	E	7.518	B	12.683	D
SHua-92-155/E	0.6600	BC	1.7383	BCDE	2.3967	BCD	0.3200	BCD	1.9667	E	0.995	EF	34.117	A
KHARAGANJA	0.7133	BC	1.7983	BCD	2.5100	BCD	0.2800	BCD	1.9667	E	0.860	F	35.267	A
IR 88611-B-5	0.6500	BCD	1.7650	BCD	2.4100	BCD	0.3050	BCD	2.2833	DE	0.745	F	23.283	BC
IR 83142-B-61-B	0.5500	CDE	1.5533	DEF	2.1050	CDEF	0.3000	BCD	1.6000	E	1.557	DEF	14.783	CD
GML 525	0.4433	E	1.4367	F	1.8767	F	0.3900	BC	1.6833	E	1.468	EF	15.683	CD
GML 529	0.4617	DE	1.4667	EF	1.9233	EF	0.4483	B	1.8333	E	4.112	CD	12.683	D
RST -177	0.5650	CDE	1.6233	CDEF	2.1917	CDEF	0.2700	BCD	2.2167	DE	0.585	F	27.850	AB
IR-72	0.5333	CDE	1.5467	DEF	2.0767	DEF	0.3100	BCD	1.7833	E	0.697	F	17.950	CD
F- Test	**		**		**		**		**		**		**	
L.S.D Values for genotypes at α 0.05	0.1892		0.2931		0.4543		0.2527		0.8092		2.6271		8.874	

Means within columns with the same letters are not significantly different ($P \leq 0.05$) ** Significant at 1% level.

Table 2. Effect of salinity on yield and yield components of rice lines

Genotypes	Panicle Length (cm)		Spikelets/Panicle (no)		Grains/panicle (no)		Grain wt./panicles (g)		Grain weight (g/plant)		Sterility (%)	
	control	6 dS/m	**control**	**6 dS/m**	control	6 dS/m	control	6 dS/m	control	6 dS/m	control	6 dS/m
IR 88611-B-5	32.0	25.3	11.2	10.7	135.2	76.4	3.9	1.6	33.5	13.07	5.9	11.9
IR 83142-B-61-B	28.4	24.3	10.6	8.1	117.2	42.1	3.2	0.9	23.0	6.57	5.6	21.9
IR 83142-B-20-B	29.2	26.6	10.4	8.2	146.7	44.7	3.9	0.8	26.03	8.13	6.6	33.5
IR 83141-B-18-B	27.7	25.7	10.0	7.3	116.8	45.3	3.0	1.1	22.0	7.13	6.7	28.9
IR 66946-3R-178-1-1(FL478)	25.7	23.3	11.9	9.3	125.7	74.0	3.5	1.7	21.43	11.0	6.7	12.7
HHZ5-SAL10-DT2-DT1	26.9	24.9	9.6	8.0	123.1	61.7	3.0	1.2	20.83	5.03	6.9	17.2
GML 506	27.6	25.4	11.3	10.4	155.6	94.1	3.5	1.7	28.10	13.73	9.6	19.2
GML 525	25.1	21.6	11.1	8.9	139.0	40.8	2.8	0.7	25.57	5.80	6.4	31.0
GML 529	21.1	0.00	9.1	0.00	70.4	0.00	1.2	0.00	25.37	0.00	14.2	-
UPL 79	25.3	23.1	12.0	10.1	116.2	60.4	2.7	1.2	22.1	9.07	6.4	18.2
UPL 19	28.3	25.1	12.1	11.3	144.0	81.2	3.3	1.6	28.23	12.20	6.6	13.5
SHua-92-155/E	28.9	26.2	12.1	10.3	113.4	102.8	2.8	2.0	43.37	24.87	13.1	9.4
RST -177	29.0	27.6	12.9	13.1	142.6	126.2	3.7	2.6	33.73	21.97	7.4	8.4
KHARAGANJA	27.6	23.2	11.7	10.9	146.6	101.2	3.8	2.4	43.43	27.10	8.7	6.0
IR 75288-144-1-3	31.1	28.9	12.8	11.1	131.9	107.1	2.6	1.7	25.17	17.23	11.0	11.5
IR-72	26.4	27.3	10.7	9.6	118.7	84.8	2.6	1.6	20.57	15.33	9.5	11.9
GML 534	26.3	24.6	10.1	9.0	123.3	81.4	2.4	1.3	23.0	7.53	10.7	17.8
GML 538	25.4	18.7	9.6	6.5	81.3	22.6	1.6	0.36	25.1	0.80	14.4	34.1
HSD Value (at Alpha 0.05) for Genotypes (G)	3.5237		1.8853		42.89		0.9412		8.9774		11.414	
HSD Value (at Alpha 0.05) for Treatment (T)	0.6477		0.3463		7.8835		0.1729		1.6491		2.0967	
HSD Value (at Alpha 0.05) for G X T	5.5139		2.9503		67.117		1.4730		14.049		17.862	

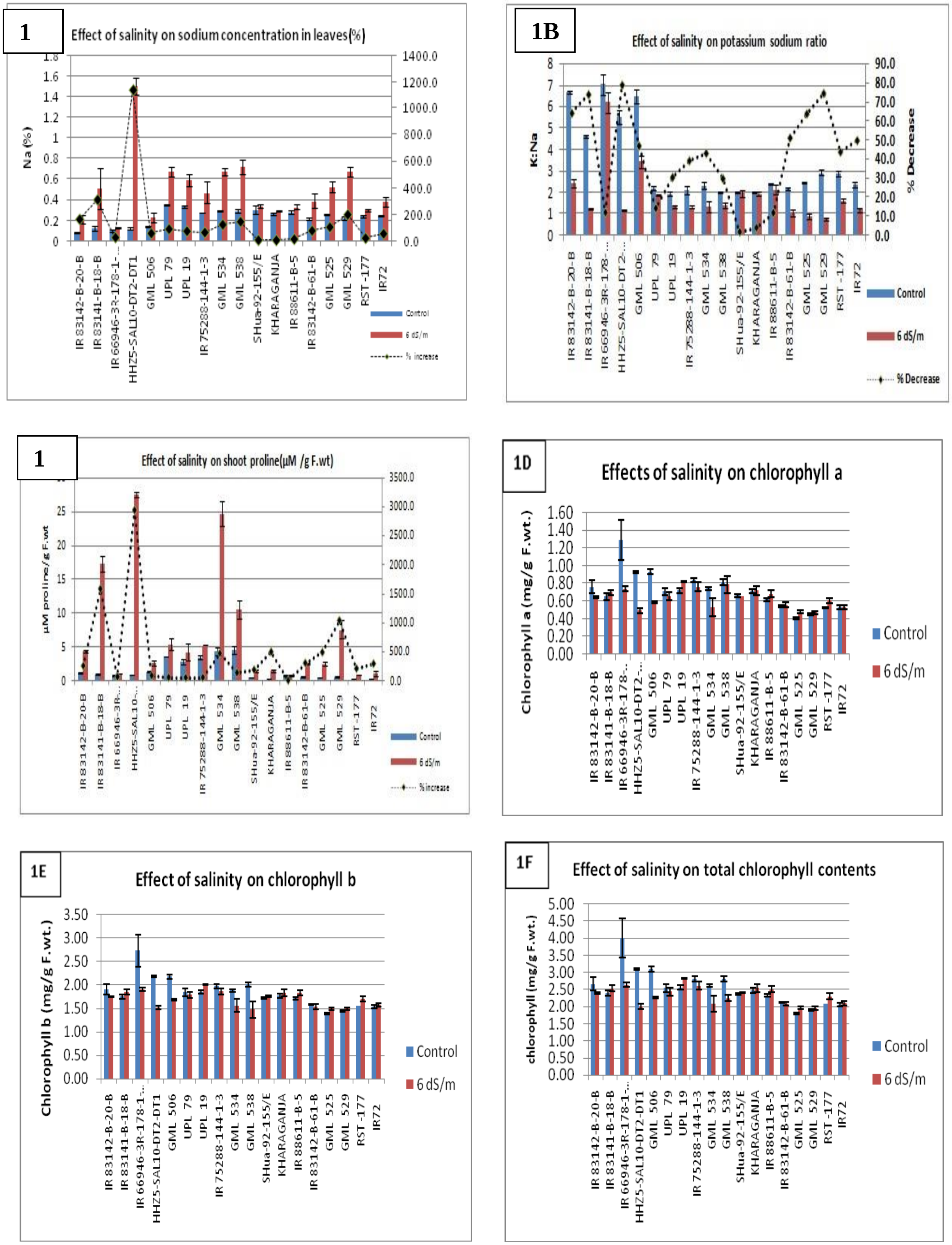

Fig.1 Physiological responses of rice genotypes under saline stress conditions.

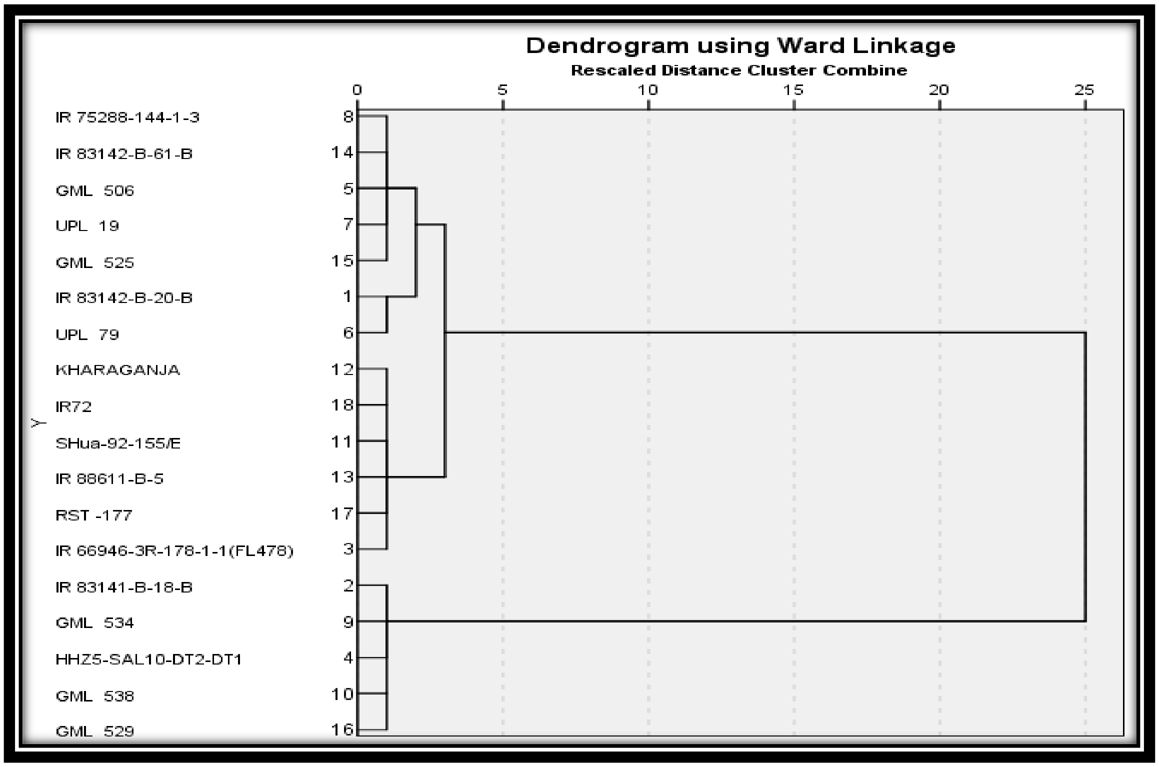

Fig. 2. Dendrogram showing relationship among rice genotypes.

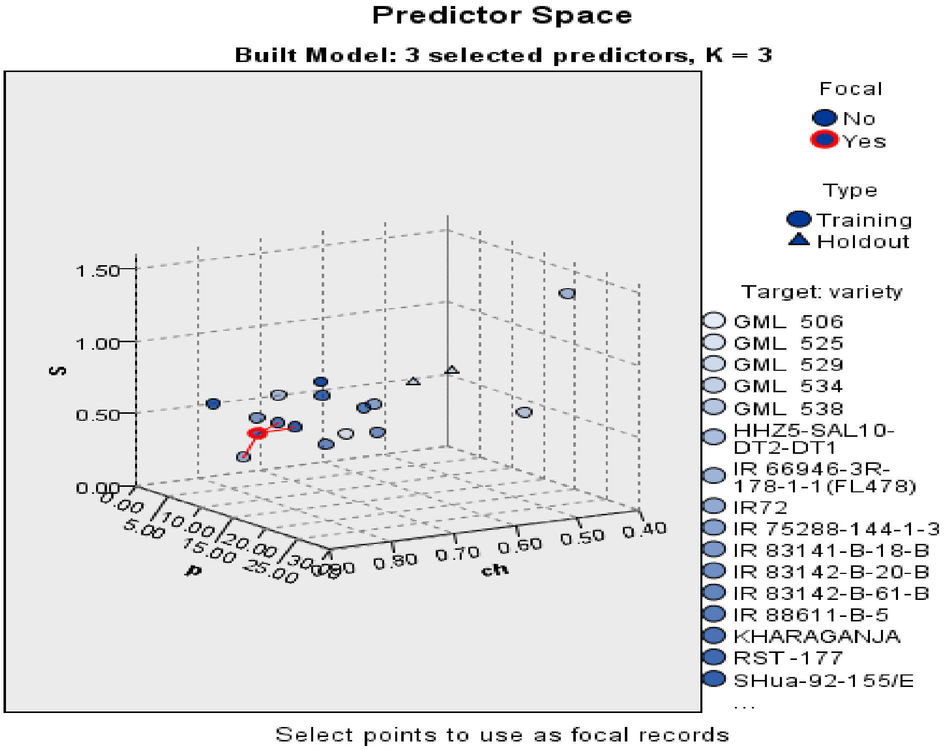

Fig. 3. K-nearest neighbor analysis of rice genotypes (K=sodium, Proline & chlorophyll).

Table 3. Pearson˝s Correlation coefficient among physiological traits and grain yield of eighteen rice genotypes under salinity.

	Proline	Na	K:Na	Chl.A	Chl.B	Total	P.wt
Na	0.8						
	**						
K:Na	-0.3419	-0.5726					
	**	**					
Chl.a	-0.1586	-0.2941	0.5628				
	ns	ns	**				
Chl.b	-0.1862	-0.3496	0.6086	0.9463			
	ns	*	**	**			
Chl.Total	-0.1759	-0.3321	0.5985	0.9797	0.9918		
	ns	*	**	**	**		
Pani.wt.	-0.5309	-0.5264	0.2869	0.1990	0.2478	0.2318	
	**	**	*	ns	ns	ns	
Grain wt.	-0.5031	-0.5227	0.3214	0.2060	0.2480	0.2348	0.9882
	**	**	*	ns	ns	ns	**

** = significant @1% prob., * = significant @ 5% probability, ns= non significant.

Our findings primarily indicated varying degree of increase in leaf sodium and proline concentrations in different rice genotypes under salinity. This observed variability for proline and sodium accumulation clearly indicated higher proline accumulating genotypes exhibiting higher tissue sodium concentrations with low yield. Furthermore the traits of shoot sodium and proline were highly positively correlated with each other and negatively correlated with grain yield (Table 2). Experimental evidences related to proline and sodium responses of known salt tolerant (Pokali and FL-478) and salt sensitive (IR-29 & KDML105) genotypes have also revealed higher accumulation of proline in salt sensitive rice genotypes than tolerant ones. These studies have indicated higher accumulation may not correspond to salt tolerance. (Kanawapee *et al*., 2011; Kong-negern *et al*., 2012; Chunthaburee *et al*., 2016). The over accumulation of proline under salinity seems to be a consequence of reduced osmotic potential due to ions. The studies conducted by Shobbar *et al.* (2010) in two contrasting rice genotypes further confirmed our findings. They observed similar response of proline in tolerant (FL-478) and sensitive (IR-29) genotypes when subjected to osmotic stress (-0.75 MPa) induced through mannitol whereas, significant difference in proline production were observed between these genotypes when the same osmotic potential induced through NaCl. Kong-negern *et al*. (2012) are of the opinion that reduction in osmotic potential due to ions and proline may play a role in osmotic adjustment in sensitive genotypes. These findings were consistent with Hakim *et al*. (2014).

There is a possibility of its accumulation only after damage /recovery period in genotypes affecting most under salinity induced reduced osmotic potential environment. Kong-negern *et al*. (2012) reported salt tolerant Pokali showed least negative OP and salt sensitive KDML105 exhibited most negative OP at 12 dSm^{-1} and this reduction in OP may be due to higher accumulation of ions and proline in sensitive genotypes. Sivakumar *et al.* (1998) reported reduced activity of photosynthetic enzyme RUBISCO at concentration of 100 mM proline in *Sesbania*, *Brassica* and *Oryza* seedlings. Garcia *et al*. (1997) comparing the effects of trehalose and proline observed opposite effects of these two osmolytes on expression of salT (salt sensitive marker gene), growth and chlorophyll in rice seedlings. Proline enhanced salT genes correlated with Na accumulation. So it may be regarded that tolerance of genotypes did not depend directly on proline contents only rather some other metabolites (sugars and other amino acids) may take part in osmotic adjustment as reported by Zhao *et al.* (2014). Wang *et al*. (2015) also reported genotypic variability for shoot proline under salinity and greater increase in salinity tolerant genotypes. Furthermore they also reported upregulation of genes control lipids and fatty acid metabolism might also enhance salt tolerance. Variability in salt tolerance of rice genotypes may be more related to some other mechanism in addition to osmotic adjustment i.e. higher membrane selective ability of genotype and compartmentation of ions which results in low shoot sodium concentrations and higher potassium sodium ratios in salinity (Fig 1B).

It can be concluded that proline over production is sodium dependent and may be regarded as indicator of stress not as an indicator of salt tolerance. The identified rice genotypes may be used in trait based breeding programme for salinity tolerance. However comparative studies on contrasting rice g enotypes imposing isoosmotic stress of ionic and osmotic nature will further be helpful in exploring its role for osmotic adjustment.

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