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VARIETAL DIFFERENCE IN ZN RESPONSIVENESS OF RICE UNDER NORMAL AND SALINE CONDITIONS

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Abstract

Being involved in several biochemical and physiological processes Zn is an essentially required micronutrient. A huge part of global population is Zn deficient due to low dietary Zn intake, leading to several health complications. The use of saline soils further decreases the concentration of Zn in crop plants. To find a sustainable solution, the current study was planned to assess the Zn responsiveness of different rice varieties in saline conditions. The high potential varieties can be used to fortify Zn in rice. In this study assessment of different rice varieties (Punjab basmati, BAS-515, KSK-133, KSK-434, PS-2, Super basmati, KS-282, Chenab basmati, Kissan Basmati, BAS-2000, Shaheen basmati, PK-386, IR-6 and Pak basmati) was done to screen out varieties with high Zn efficiency and uptake potential. Plants were grown under two salt levels (control, 75 mmol NaCl) and two levels of Zn (control, 4 µM Zn). The solution pH was kept at 5.5-6. Yoshida's nutrient solution was used and 30 days old plants were harvested. There was a significant varietal difference in response to salt stress and Zn application as evaluated through different growth and chemical attributes. Based on the salt tolerance, Zn uptake and K-mean cluster analysis, the variety IR-6 of rice was evaluated to be more Zn responsive than other tested varieties. In conclusion, varietal difference in response to salt stress, Zn application and Zn accumulation was significantly evident and varietal screening should be considerd as a pivotal step prior to consideration for bio fortification of Zn in rice either for agronomic or genetic perspective.

Key words: Zinc, Salt stress, Rice.

Introduction

Climate change due to increased biotic and abiotic stresses results in changing patterns of ecosystem processes. It has also been reported that 8.31 billion hectares of the world agricultural soils are salt stressed (Anon, 2008). Soil Salinity has become a major constraint in arid and semi-arid regions due to continuously increasing soluble salts in soil and irrigation water.

Plant growth is severely impaired by salt stress due to salinity induced imposition of osmotic effect, oxidative stress and specific ion toxicity induced nutrient deficiency (Ashraf & Haris, 2013). Soil salinity reduces water availability and absorption of essential nutrients to plants, increases ion toxicity, and reduces crop yield and quality (Grattan & Grieve, 1999; Sarwar, 2011). Ionic imbalance and high concentration of Na^+ in plants results in retarded growth and poor nutrient content of cereals (Rahnama *et al.*, 2011). Imbalanced and insufficient use of available chemical fertilizers is a common practice among farmers. Available irrigation water is also high in carbonate contents. In arid to semi-arid climate the regional temperature is usually high that results in net uphill movement of water. Ultimately there is salts accumulation on soil surface. This is the key reason for high soil pH and deficiency of most nutrients especially micronutrients like Cu, Fe, Zn and Mn etc. in most of the regions (Khalid *et al.*, 2013).

Zn is one of the widely addressed micronutrients required for healthy plant growth and development. Though Zn is not an essential component of cell structure, it acts as an enzyme regulator of more than 65 enzymes that regulate the systems associated with the water use efficiency and activation of drought tolerance in plants (Assunção *et al.*, 2010). Hence, determining the extent of its role in alleviating salt stress has piqued the interests of many researchers on a global scale. Different researches showed that the addition of Zn has markedly reduced the depressing effect of NaCl stress (Jha, 2019; Rani *et al.*, 2019).

Furthermore, Zn deficiency in humans has also become a global issue. Its deficiency results in many health issues in the human population because of its role in protein synthesis and other metabolic functions (Andreini *et al.*, 2006; Cakmak, 2008). Dependency on cereal-based diets results in lower dietary intake of Zn because cereals like wheat and rice are ranked very low in Zn contents (Cakmak, 2008). The growth of these cereal crops on saline soils further decreases their Zn contents due to poor growth and lower Zn uptake (Jan *et al.*, 2015a). About 50% of the world population is Zn deficient these days (Cakmak & Kutman, 2018). Therefore, the addition of Zn is strongly suggested to improve plant growth and their Zn contents, particularly in saline soils.

Rice is the second topmost cereal crop in the world and is one of principal food sources. It is the main nutritional source, especially in developing countries. Rice is termed as moderately sensitive to moderately tolerant crop under saline conditions (Qureshi & Barrett-Lennard, 1998), but the genotypic variation does exist in this regard. Some varieties are more tolerant while some are more sensitive, fine varieties tend to be more salt-sensitive as compared to coarse ones (Qureshi & Barrett-Lennard, 1998, Jan *et al.*, 2015a).

An impressive genotypic variation exists in salt tolerance and Zn responsiveness of rice (Jan *et al.*, 2015b). Wissuwa *et al.*, (2008) reported that in Zn fortification of grain, genotypic difference has a much larger effect than fertilization. So, screening of a Zn responsive variety is a key element while planning fortification of crop Zn contents.

Considering this importance of Zn in plant and human life and the inevitable use of salt-affected lands for crop production, Current study was planned to evaluate the Zn responsiveness of different locally used rice varieties.

The main objectives of the study are to evaluate the salt tolerance of different rice varieties and the assessment of response of these varieties to Zn application under both normal and saline conditions and to screen out most responsive rice variety to Zn application particularly under salt-stressed conditions.

Materials and Methods

Experiment and growth conditions: Seeds of fourteen rice varieties were obtained from Rice Research Institute, Kala Shah Kaku. The names of all the rice varieties used are mentioned in (Table 1). Seeds were sterilized by dipping in 1% sodium hypochlorite solution for 5 minutes and then washed with deionized water thrice. After sowing seeds in iron trays the optimal moisture contents were kept up for germination and seedling establishment. Seedlings at two leaf stage were transferred to iron tubs (lined with polythene sheets) and were transplanted to foam plugged holes, made in polystyrene sheet floating over nutrient solution. Each variety was replicated 3 times. Tubs were placed randomly according to statistical design CRD factorial.

Wire house in Institute of Soil and Environmental sciences, University of Agriculture Faisalabad, Pakistan was used to carry out the experiment. Yoshida's nutrient solution was used (Yoshida *et al.*, 1972). pH was maintained at 5.5-6 using HCl or NaOH at daily basis. Salt stress was applied using NaCl salt. Zinc sulphate was used as a source of Zn. Salt stress was applied after 1 week of transplantation in three increments. There were two salt levels, control and 75 mmol L^{-1} NaCl and two Zn levels, control and 4 µM Zn (Impa *et al.*, 2013). Treatment plan was T1= Control, T2= 4 µM Zn, T3= 100 mM NaCl and T4= 100 mM NaCl + 4 µM Zn. Plant growth measurements were done after 30 days of transplantation.

Harvesting and growth measurements: Growth parameters like root fresh weight (RFW), shoot fresh weight (SFW), root length (RL) and shoot length (SL) were measured by using weighing balance or meter rod accordingly at harvesting. To measure dry weight of shoot and root, harvested plants were first air dried and then oven dried at 65 ± 5ºC in a forced air oven until constant weight was achieved (Jones & Case, 1990).

Measurement of tolerance indices: NaCl tolerance index (TI) for general growth parameters like shoot length, shoot fresh weight, shoot dry weight etc. were calculated by using formula:

$$TI\ (\%) = (Y/X) \times 100$$

where, X = reading of a parameter of rice in control
Y = reading of a parameter of rice in a particular treatment (Zeng *et al*., 2002)

Plant chemical analysis

Sample preparation: Dried plant samples were stored and labelled accordingly in zip lock bags after finely grinding in a mechanical grinder. Each sample was digested according to modified wet digestion technique of Jones & Case, (1990) as it is good for high Zn recovery. Samples were stored after filtration in accordingly tagged plastic bottles.

Na^+ and K^+ determination: Na^+, K^+ and K^+/ Na^+ ratio was determined following the procedures in Estefan *et al*., (2013). Digested samples were run on a flame photometer (Jenway PFP7) for determination of Na^+ and K^+ by using prepared diluted digested samples and standard solutions from reagent grade NaCl and KCl. Final concentration was calculated from the standard curve equation and multiplying with the dilution factor.

Zn determination: For determination of Zn the prepared digested samples were fed to the atomic absorption spectrophotometer (model 280FS AA) according to standard procedure following Norhaizan & Ain, (2009). Firstly, working standards of 0.5, 1.0, 1.5, 2.0 and 2.5 mg L^{-1} were prepared using sub stock solution that was prepared from a stock solution of 1000 mg L^{-1} Zn, through dilution with distilled water. $ZnSO_4.7H_2O$ salt was used to prepare stock solution of Zn. After running standards and making a calibration curve, the plant samples were run on the instrument and readings were taken accordingly and the final concentration was calculated by multiplying the observed sample reading with dilution factor.

Calculation of plant Zn uptake: Zn uptake of shoot was calculated by using following formula:

$$\text{Zn uptake root or shoot (mg plant}^{-1}\text{)} = \frac{\text{[(Zn concentration in plant root or shoot x Dry weight of plant root or shoot (g)]}}{\text{Number of plants}}$$

Table 1. Names of the rice varieties used in study.

Variety name	Symbol	Variety name	Symbol	Variety name	Symbol
Punjab Basmati	V_1	Super Basmati	V_6	Shaheen Basmati	V_{11}
BAS-515	V_2	KS-282	V_7	PK-386	V_{12}
KSK-133	V_3	Chenab Basmati	V_8	IR-6	V_{13}
KSK-434	V_4	Kissan Basmati	V_9	Pak Basmati	V_{14}
PS-2	V_5	BAS-2000	V_{10}		

Cluster analysis: Data regarding different growth parameters (SFW, SDW, SL, RL, RFW and RDW) and chemical parameters (shoot Zn, root Zn, shoot Zn uptake, shoot Na^+, shoot K^+ and shoot K^+/Na^+ ratio) in normal and saline conditions was subjected to K-mean cluster analysis. No Score boundaries were set for clustering and cluster were made randomly keeping in consideration that difference in variable of standard error of different cluster means was not very high. Cluster groups were ranked from highest to lowest cluster means and the group ranking was done on basis of response of different varieties to Zn application on both salt levels (normal and saline). Group rankings on both salt levels were summed up and final ranking was done on basis of these sums in order that the varieties with lowest sum were considered most responsive to Zn application and with high sum were considered least Zn responsive varieties. The process of ranking was similar to the one used by different scientists for ranking of salinity tolerance (El-Hendawy *et al*., 2005; Naz *et al*., 2015). Only exception was that here we have used K-mean clustering for making clusters instead of Ward's minimum cluster analysis. Only dendrograms showing the separation (Euclidean distance) between clusters (groups) of rice varieties were made through Ward's minimum analysis.

Statistical analysis

Obtained data was analysed for variance (ANOVA) analysis (Steel *et al*., 1997) and significant differences were calculated among treatment means by Least Significant Difference (LSD) test using software package "Statistix 8.1".

Results

Growth responses of rice varieties: Data for growth parameters of rice varieties are presented in (Table 2) (Shoot length, Root length, SFW, SDW, RFW and RDW). Analysis of data showed that the application of NaCl salt and Zn treatments significantly affected ($p \leq 0.05$) growth of rice plants. The application of 100 mM NaCl decreased the growth parameters as compared to their respective controls.

All the growth parameters were improved by higher Zn application in both normal and saline conditions. Among all varieties for shoot growth parameters like SFW and SDW, V_{13} showed the maximum growth followed by V_{12}, V_5, V_3, V_4, and V_7 in both control and saline conditions. Percent increase in shoot weight due to Zn application in saline conditions was more as compared to normal condition in most of the varieties.

Data for all growth parameters (SFW, SDW, SL, RFW, RDW and RL) was calculated for tolerance index percentage under salt stress for both control and higher level of Zn application and results are presented in (Fig. 1). By calculating cumulative score of tolerance index percentage, varieties can be classified in decreasing order of tolerance as $V_{13} > V_3 > V_7 > V_{12} > V_{14} > V_4 > V_6 > V_9 > V_{11} > V_{10} > V_2 > V_8 > V_1 > V_5$.

Varieties like V_1, V_2, V_5, V_8, V_{10}, and V_{11} did not survive well in saline conditions and % age decrease due to salinity was more than 70% and 60% in the case of SFW and SDW respectively from respective controls. So, determinations for chemical parameters were not done for these varieties due to insufficient dry mass availability and to avoid dispensable use of resources.

Na^+ and K^+ concentration of rice: Shoot Na^+ and K^+ concentrations are presented in Table 3. Analysis of variance (ANOVA) showed significant ($p \leq 0.05$) differences in the Na^+ and K^+ concentrations among varieties at higher Zn application under NaCl salt stress. Na^+ concentration was decreased at a higher Zn level in both normal and saline conditions while the reverse was the case with K^+ concentration.

Data for was converted to tolerance index (% of control T_1) for both T_3 and T_4 that was ranked in decreasing order of $V_{13} > V_3 > V_7 > V_{12} > V_4 > V_{14} > V_9 > V_6$ and $V_{13} > V_3 > V_7 > V_4 > V_{12} > V_{14} > V_9 > V_6$ respectively. Considering Na^+, K^+ and K^+/Na^+ ratio collectively, varieties V_{13}, V_7 and V_{12} seem to be more tolerant than other varieties.

Zn concentration and plant Zn uptake of rice: The concentration of Zn in shoot and root is presented in Table 3. Rice varieties showed a more significant difference in their rice contents and Zn uptake at the higher application of Zn in both normal and saline conditions as compared to respective controls in their respective growth conditions. A significant difference among varieties was observed regarding their shoot Zn uptake. There was a significant decrease ranging between 10-45% in shoot Zn concentrations under saline conditions. At higher Zn level there was an increase in Zn concentration in both saline and normal conditions as compared to respective controls. Average shoot Zn concentration in respective control were 37 mg kg^{-1} and 28 mg kg^{-1} in normal and saline conditions respectively. Maximum shoot Zn concentration was showed by V_{13} in all four treatments. While in saline conditions it showed 37.4 mg kg^{-1} and 51.3 mg kg^{-1} at lower and higher Zn level respectively. Minimum Zn concentrations were showed by V_9 in both normal and saline conditions. Shoot Zn contents as shoot Zn uptake (Table 3) can be placed in descending order as $V_{13} > V_{12} > V_3 > V_4 > V_7 > V_6 > V_9 > V_{14}$ and $V_{13} > V_{12} > V_7 > V_3 > V_4 > V_6 > V_{14} > V_9$ respectively in normal and saline conditions.

Root Zn concentration of all rice varieties also showed a similar trend in their response to the Zn application. Varieties showed a significant difference in their root Zn contents at all treatment levels.

Cluster analysis: Ranking of rice varieties according to their Zn responsiveness is presented in (Table 4). The varieties done for all growth and chemical parameters were considered for clustering. It shows that IR-6 is in the cluster-1 under both normal and saline conditions. So, it has the lowest sum and ranked as most Zn responsive of all other varieties used in the experiment. While, Kissan Basmati was ranked as least responsive variety. Euclidean distances between varietal means are presented in (Fig. 3a and b) under normal and saline conditions.

Table 2. Effect of Zn application on growth attributes of rice varieties under normal and saline conditions.

Factors	SL (cm)	RL (cm)	SFW (g plant^{-1})	SDW (g plant^{-1})	RFW (g plant^{-1})	RDW (g plant^{-1})
	Salt (S)					
Control	57.40 A	39.11 A	9.58 A	1.72 A	3.18 A	0.51 A
75 mM NaCl	37.48 B	23.31 B	4.38 B	0.92 B	1.22 B	0.26 B
Zinc (Zn)						
Control	45.23 B	28.74 B	6.20 B	1.16 B	1.95 B	0.33 B
4 µM Zn	49.64 A	33.69 A	7.76 A	1.48 A	2.45 A	0.43 A
	Varieties (V)					
V$_1$	40.05 H	28.15 F	4.07 H	0.90 H	1.45 J	0.29 H
V$_2$	47.08 D	27.28 FG	4.89 G	0.92 GH	1.80 G	0.36 F
V$_3$	57.58 A	29.85 E	7.40 D	1.55 C	2.24 E	0.41 D
V$_4$	43.67 F	39.13 A	6.39 F	1.40 DE	2.03 F	0.33 G
V$_5$	49.89 C	32.70 D	7.91 C	1.45 D	3.16 C	0.45 C
V$_6$	46.64 D	37.53 B	6.85 E	1.18 F	2.68 D	0.39 E
V$_7$	49.67 C	29.55 E	6.92 E	1.31 E	2.28 E	0.32 G
V$_8$	45.40 E	24.15 H	3.63 I	0.72 I	1.23 K	0.24 I
V$_9$	46.66 D	40.10 A	6.57 EF	1.16 F	1.70 H	0.36 F
V$_{10}$	36.33 I	23.00 H	3.98 HI	0.75 I	0.98 L	0.20 J
V$_{11}$	41.17 G	26.67 G	3.82 HI	0.70 I	1.60 I	0.28 H
V$_{12}$	57.42 A	36.50 BC	14.73 B	2.66 B	3.96 B	0.70 B
V$_{13}$	55.33 B	35.18 C	15.61 A	2.80 A	4.27 A	0.76 A
V$_{14}$	47.21 D	27.20 FG	4.89 G	1.01 G	1.42 J	0.29 H
	F-Value					
S	18977.3^{***}	3626.56^{***}	4298.91^{***}	2046.18^{***}	11972.7^{***}	5646.2^{***}
V	539.03^{***}	131.21^{***}	640.26^{***}	399.74^{***}	886.87^{***}	681.37^{***}
Zn	930.39^{***}	355.89^{***}	386.34^{***}	346.87^{***}	773.47^{***}	922.56^{***}
S×V	221.36^{***}	47.2^{***}	88.76^{***}	50.76^{***}	234.13^{***}	114.32^{***}
S×Zn	3.68^{ns}	11.88^{**}	24.59^{***}	6.68^{*}	48.98^{***}	34.65^{***}
Zn×V	2.16^{*}	0.52^{ns}	4.29^{***}	5.61^{***}	18.4^{***}	15.12^{***}
S×Zn×V	2.16^{*}	0.57^{ns}	2.63^{**}	3.61^{***}	1.86^{*}	2.22^{*}

Means in each column with similar letters are not significantly different from each other. Values with *** are significant at $p \leq 0.001$, with ** are significant at $p \leq 0.01$and with *are significant at $p \leq 0.05$ while, ns = non-significant V1=Punjab Basmati, V2=BAS-515, V3=KSK-133, V4=KSK-434, V5=PS-2, V6=Super Basmati,V7=KS-282, V8=Chenab Basmati, V9=Kissan Basmati, V10=BAS-2000, V11=Shaheen Basmati, V12=PK-386, V13=IR-6, V14=Pak Basmati

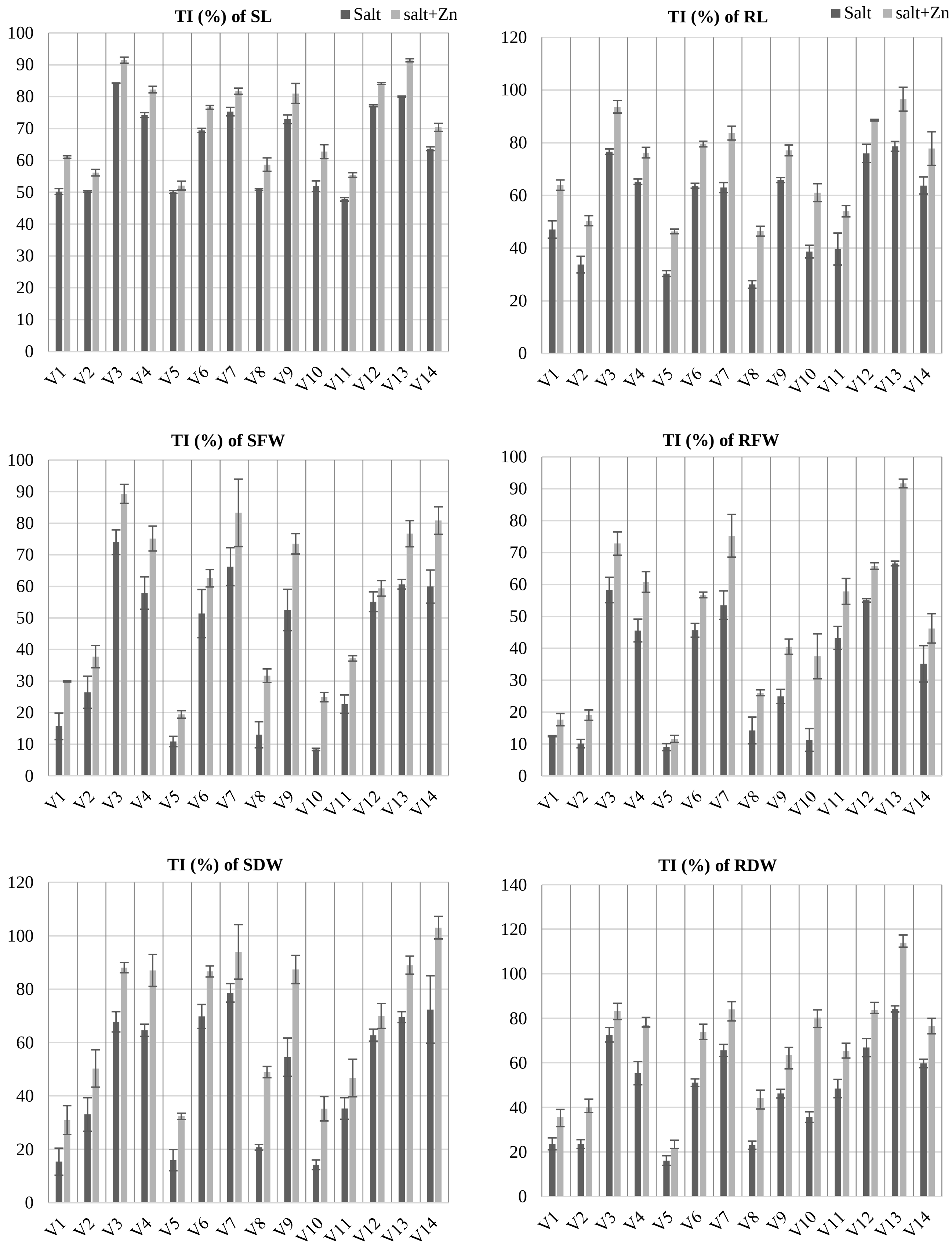
Table 3. Effect of Zn application on salt tolerance and chemical attributes of rice varieties under normal and saline conditions.

Factors	Shoot Na^+ (g kg^{-1} dry wt.)	Shoot K^+ (g kg^{-1} dry wt.)	K^+/Na^+ratio	Shoot Zn^{2+} (mg kg^{-1} dry wt.)	Root Zn^{2+} (mg kg^{-1} dry wt.)	Zn^{2+} uptake (mg $plant^{-1}$)
	Salt (S)					
Control	0.96 B	9.06 A	9.67 A	43.21 A	101.37 A	0.086 A
75 mM NaCl	2.55 A	6.11 B	2.54 B	32.88 B	81.71 B	0.047 B
Zinc (Zn)						
Control	1.95 A	7.32 B	5.29 B	32.10 B	77.25 B	0.050 B
4 µM Zn	1.56 B	7.84 A	6.92 A	43.99 A	105.84 A	0.083 A
	Varieties (V)					
V_3	1.82 B	7.77 C	5.48 D	36.90 D	90.98 BCD	0.060 C
V_4	1.84 B	7.48 CD	5.65 D	37.22 CD	88.00 D	0.053 D
V_6	1.99 A	5.30 F	4.27 E	38.20 CD	97.05 B	0.047 E
V_7	1.66 D	9.38 A	7.58 B	41.17 B	84.82 D	0.055 CD
V_9	1.88 B	5.95 E	4.58 E	31.56 E	68.92 E	0.040 F
V_{12}	1.68 D	8.66 B	7.15 C	39.41 BC	95.44 BC	0.108 B
V_{13}	1.42 E	9.18 AB	8.38 A	46.71 A	116.98 A	0.134 A
V_{14}	1.75 C	6.94 D	5.76 D	33.18 E	90.15 CD	0.035 F
	F-Value					
S	9248.36^{***}	468.67^{***}	6349.17^{***}	311.88^{***}	138.87^{***}	775.09^{***}
V	55.29^{***}	58.67^{***}	132.25^{***}	32.24^{***}	32.45^{***}	325.02^{***}
Zn	549.13^{***}	14.64^{***}	331.03^{***}	412.64^{***}	293.72^{***}	558.62^{***}
S×V	32.58^{***}	3.65^{**}	19.96^{***}	5.86^{***}	2.28^{*}	19.41^{***}
S×Zn	130.76^{***}	1.12^{ns}	74.74^{***}	5.9^{*}	5.37^{*}	25.62^{***}
Zn×V	4.23^{***}	0.17^{ns}	3.47^{**}	2.4^{*}	6.99^{***}	18.32^{***}
S×Zn×V	2.11^{*}	0.19^{ns}	0.58ns	5.72^{***}	0.64^{ns}	2.94^{**}

Means in each column with similar letters are not significantly different from each other. Values with *** are significant at $p \leq 0.001$, with ** are significant at $p \leq 0.01$and with *are significant at $p \leq 0.05$ while, ns = non-significant; V3=KSK-133, V4=KSK-434, V6=Super Basmati, V7=KS-282, V9=Kissan Basmati, V12=PK-386, V13=IR-6, V14=Pak Basmati

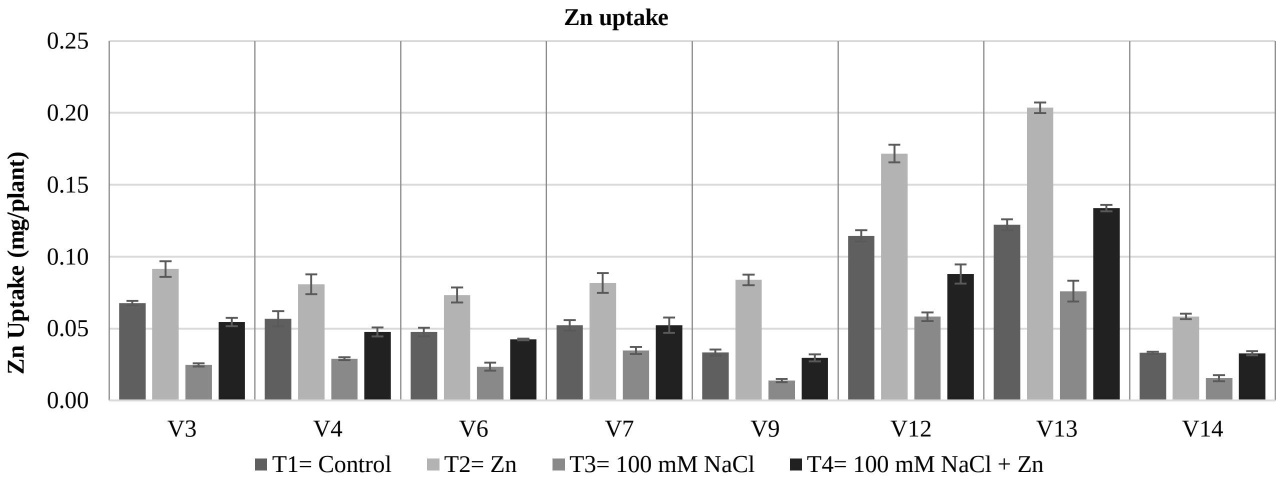
Table 4. Ranking of Rice varieties for their relative Zn responsiveness in terms of growth and ionic parameters under normal and saline conditions in a K-mean cluster analysis.

Varieties	Salt stress	Cluster group rank	Sum	Genotypic ranking	Zn responsiveness
IR-6	Normal	1	2	1	Highly responsive
	Salt	1			
PK-386	Normal	2	4	2	Fairly responsive
	Salt	2			
KSK-133, Pak Basmati	Normal	2	5	2	Fairly responsive
	Salt	3			
KSK-434, Super Basmati	Normal	3	5	2	Fairly responsive
	Salt	2			
KS-282	Normal	3	6	3	Moderately responsive
	Salt	3			
Kissan Basmati	Normal	4	8	4	Least responsive
	Salt	4			



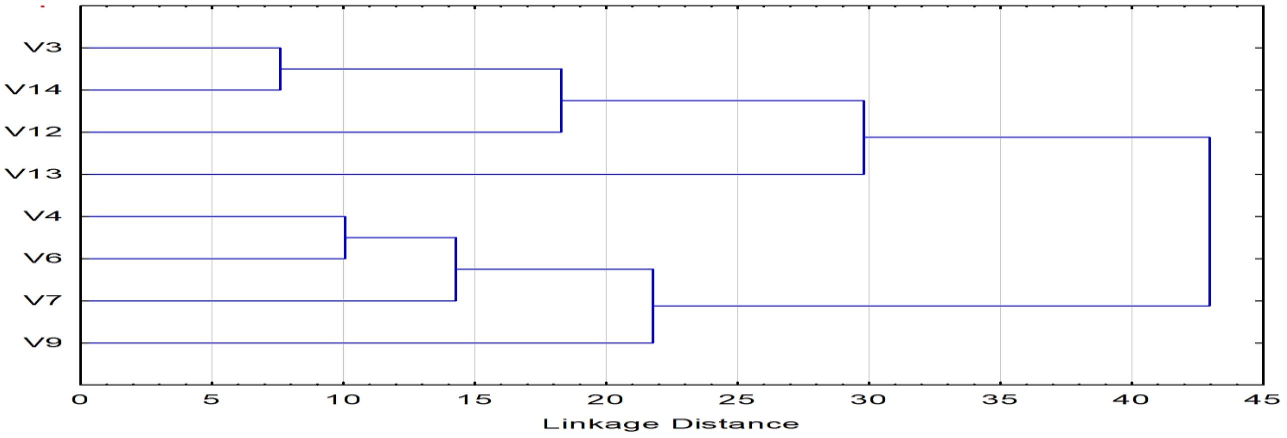
(Rice varieties are plotted along X-axis and denoted as V1 = Punjab basmati, V2 = Bas-515, V3 = KSK-133, V4 = KSK-434, V5 = PS-2, V6 = Super Basmati, V7 = KS- 282, V8 = ChenabBasmati, V9= Kissan Basmati, V10 = BAS-2000, V11 = Shaheen Basmati, V12 = PK-386, V13 = IR-6, V14 = Pak Basmati)

Fig. 1. Effect of salinity and Zn application on tolerance indices (TI, %) of shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW) and root dry weight (RDW) of rice varieties.



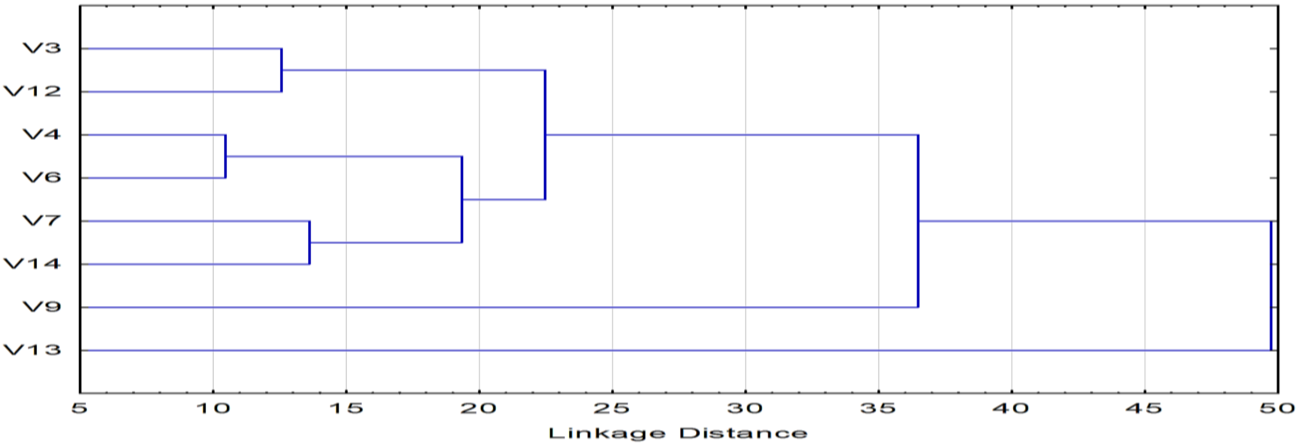
V3=KSK-133, V4=KSK-434, V6=Super Basmati, V7=KS-282, V9=Kissan Basmati, V12=PK-386, V13=IR-6, V14=Pak Basmati.

Fig 2. Effect of Zn application on Zn uptake of rice varieties under normal and saline conditions.



(Rice varieties are showed along Y-axis and denoted as V1 = Punjab basmati, V2 = Bas-515, V3 = KSK-133, V4 = KSK-434, V5 = PS-2, V6 = Super Basmati, V7 = KS- 282, V8 = ChenabBasmati, V9= Kissan Basmati, V10 = BAS-2000, V11 = Shaheen Basmati, V12 = PK-386, V13 = IR-6, V14 = Pak Basmati)

Fig. 3a. Dendrogram showing the separation (Euclidean distance) between clusters (groups) of rice varieties under normal growth conditions (Ward's method).



(Rice varieties are showed along Y-axis and denoted as V1 = Punjab basmati, V2 = Bas-515, V3 = KSK-133, V4 = KSK-434, V5 = PS-2, V6 = Super Basmati, V7 = KS- 282, V8 = ChenabBasmati, V9= Kissan Basmati, V10 = BAS-2000, V11 = Shaheen Basmati, V12 = PK-386, V13 = IR-6, V14 = Pak Basmati)

Fig. 3b. Dendrogram showing the separation (Euclidean distance) between clusters (groups) of rice varieties under saline growth conditions (Ward's method).

Discussion

Plant growth parameters like SFW, SDW, SL, RFW, RDW, and RL have fairly high correspondence with crop salt resilience and Zn supplementation responsiveness at early growth stages. These parameters can be effectively utilized as screening criteria for salt tolerance and Zn use efficiency in saline conditions (El-Hendawy *et al*., 2005, Moradi & Jahanban, 2018). In present study, the data of growth, and chemical parameters indicated that variations for salt tolerance do exist at the genetic level in tested rice varieties.

All growth parameters were reduced under saline conditions. The osmotic stress and metabolic dysfunctions can be a possible reason as excessive salt ultimately lead to higher osmotic potential and nutrient imbalance that affects plant metabolic functioning, membrane stabilization and photosynthesis resulting in growth reduction (Hasegawa *et al*., 2000; Naz *et al*., 2018). The negative impact of salt stress on shoot growth can be attributed to a decrease in photosynthetic rate and other physiological functions (Saqib *et al*., 2012; Khan *et al*., 2004, Rao *et al*., 2013). In this study, SDW and SFW were significantly reduced under salt stress. That may be due to reduced plant biochemical and physiological activity resulting in productivity loss (Carpici *et al*., 2010; Hasegawa *et al*., 2000). In a study, Ashraf *et al*., (2010) evaluated that biomass production is a function of various physiological and biochemical processes. Salinity tends to affect these activities and results in reduced crop growth (Munns & Tester, 2008; Rahnama *et al*., 2011). Results in current study are also in line with the findings of James *et al*., (2006) and Jan *et al*., (2015b) that salt stress resulted in decreased SFW, SDW, and other shoot and root growth parameters and other physiological functions of wheat and rice.

Data in this study showed that all the varieties responded differently to salt stress and there was a highly variable response towards salt stress by different rice varieties. The differential responses of varieties to salt stress might be due to their different genetic potential towards salt stress. Tolerance to salinity by varieties that survived well under saline conditions might be due to their innate ability and presence of inherent salt-tolerant genes to counter high stress.

The better growth of few tolerant varieties can be attributed to reduced accumulation of Na^+ ions as evident from results of current study and possible functioning and mobilization of plant defence mechanisms including antioxidant enzymes to suppress the transport of Na^+ ions to further plant tissues and organs (Gupta & Huang, 2014). Jan *et al*., (2015a), also reported genetic variability in salt stress responsiveness of rice varieties in which few varieties were regarded as salt-tolerant and others as salt-sensitive ones. Moradi & Jahanban (2018) evaluated that one rice cultivar showed more endurance to salt stress than others. Supplementation with essential nutrients can improve plant growth in salt-stressed conditions when applied at the proper time and in the proper amount. Hosseini & Maftoun, (2005) and Maftoun *et al*., (2003) reported a significantly high shoot growth of rice by Zn application. Khan *et al*., (2002) reported that all plant growth parameters are significantly improved by Zn application.

In this study, it was evident from the results that the Zn application improved the root and shoot growth of all rice varieties significantly in both normal and saline conditions. That may be due to the reason that salinity and Zn in combination triggers a broad range of growth and physiological responses in plants that helps in alleviating the detrimental effects of stress (Rani *et al*., 2019).

Saleh & Maftoun, (2008) reported that shoot dry weight and chlorophyll content of rice decreased considerably when NaCl salt was added in the growth medium and Zn supplementation alleviated the suppressing effect of NaCl.

In another study, it was also reported that Zn mobilization improved the physical and physiological growth and thus protects plants from salinity injuries. Also, it increases soluble carbohydrates in plant leaves helping to overcome osmotic stress in rice plants (Jha, 2019; Babaei *et al*., 2017). It was also evident from the results that different varieties showed different responses towards Zn application and their salt stress endurance at a higher Zn level. It may be due to differences in Zn responsiveness of different varieties due to some genetic factors.

Moreover, it was also shown in current study that improvement in growth responses due to Zn application was more in case of saline conditions than in normal conditions i.e., a percentage increase in saline conditions was more with respect to respective control than in normal growth conditions. Daneshbakhsh *et al*., (2013), reported similar results that 3 wheat cultivars improved their shoot and root dry matter with Zn application in saline conditions and two out of three cultivars showed more Zn efficiency at a higher salt level while one cultivar showed more Zn efficiency in normal conditions.

This is a well-known fact that Na^+ and K^+ concentrations work in an antagonistic way of determining plant NaCl stress tolerance and salt stress adaptability. Na^+ contents always showed a significant negative trend regarding plant growth and physiology under high salt treatments (Gupta & Huang, 2014; Akram *et al*., 2007). Due to considerably high concentration in a saline environment, Na^+ effectively competes with other nutrients for common transport channels and plant Na^+ contents are greatly increased (Munns, 2002; Guo *et al*., 2013; Haq *et al*., 2013). Na^+ directly competes with K^+ ion uptake sites in plasmalemma. Membrane integrity is affected due to Na^+ induced membrane depolarization and K^+ efflux (Epstein & Rains, 1987; Cramer *et al*., 1985).

Several metabolic activities of plant are disrupted by higher Na^+ concentration as it is a toxic element to plant at higher concentration levels. Genotypes with a higher ability to retain Na^+ in the root zone and its lower transport to leaves were said to be more tolerant to the salt stress (Khan *et al*., 1990; Naz *et al*., 2018). Based on the findings of this study, it is concluded that rice varieties $V_{13,}$ V_7 and V_{12} retained lesser amounts of shoot Na^+ concentrations with comparatively better growth and hence can be termed as salt-tolerant varieties. Higher Zn application decreased Na^+ contents, particularly under salt stressed conditions. Findings of this study mark with those of Saeidnejad *et al*., (2016) who reported decreased Na^+ and increased K^+ concentration with the progressive application of Zn at increasing salt stress levels particularly under 200 mmol salinity. That may be because Zn has a well-pronounced role in membrane integrity and membrane permeability. Zn deficiency results in loss of membrane integrity and poor membrane permeability in many crop species. So, under salt stress this deficiency further promotes the uptake and accumulation of Na^+ in plants and it reach to its toxic concentration levels. Hence, improved plant Zn status greatly improves crop stress tolerance. Zn is involved in the proper orientation of macromolecules with having a strong interaction with phospholipids and sulfo hydrall group (Cakmak *et al*., 1993). K^+ concentration of rice were greatly decreased under salt stress (Table 3) due to a higher concentration of Na^+ in growth medium and competition for common transport channels. Plants with a better ability to maintain normal nutrient ion contents and limit toxic ions show greater tolerance to salinity.

Zn application was reported to suppress Na^+ concentration with concomitant improvement in growth of rice and barley plants grown under salinized solutions (Aslam *et al*., 2000; Abou Hossein *et al*., 2002). Salt tolerance of plants is strongly associated with K^+ uptake and plant K^+ contents (Ashraf & Sarwar, 2002), because K^+ is a key element involved in cell osmotic regulation and always compete with Na^+ ions (Ashraf *et al*., 2005). Plants normally use the strategy of K^+ uptake regulation and efflux of Na^+ from the cell or prevention of Na^+ entry into the cell to maintain a required cytosolic K^+/Na^+ ratio (Munns & Tester, 2008). In the current study Zn application significantly increased K^+ concentrations in saline conditions. It was reported that Zn application considerably reduced Na^+ concentration of shoot and K^+ concentrations were significantly improved under saline conditions (Verma & Neue, 1984; Tavallali *et al*., 2010).

Uptake mechanism that discriminates similar ions such as Na^+, K^+ as well as K^+/Na^+ ratio could be useful selection criteria for salt tolerance (Zheng, 2004; Munns & Tester, 2008). In the present study, varieties V_{13} and V_7 of rice showed a higher K^+/Na^+ ratio than other varieties under the higher Zn application. Hence, these varieties can be termed as relatively more tolerant and responsive to the Zn application.

Data of shoot and root Zn concentrations showed that plant (root and shoot) Zn concentration was significantly increased ($p \leq 0.05$) at higher Zn application level that is because more Zn was present in available form and to compete with other nutrients for entry sites, resulting in more plant Zn uptake (Fig. 2). Results were in line with the findings of Wissuwa, *et al*., (2008) and Zaman *et al*., (2020) that application of Zn fertilizer clearly increased grain Zn concentration in paddy rice. Foliar and soil application of Zn at the proper time and dose can improve wheat Zn contents up to two folds Cakmak *et al*., 2010; Babaei *et al*., 2017; Cakmak & Kutman, 2018; Firdous *et al*., 2018).

Zn concentration in the present study was reduced due to the onset of salt stress in all rice varieties. The reduction range was 10- 45%. That may be due to high Na^+ and Cl^- concentrations that hinders plant Zn uptake due to competition, high relative concentrations of other nutrients, membrane damage, and poor nutrient uptake and suppressed plant growth. Antagonistic effects of alkaline earth metals like Mg^{2+}, Na^+, and K^+ that are dominant under saline conditions cause Zn deficiency in the salt-stressed environment. These kinds of cations compete with Zn for root absorption and cause Zn deficiency (Chaudhry & Loneragan, 1972; Jan *et al*., 2015b). However, at the application of Zn, this Zn deficiency was greatly reduced in rice varieties. Torabian *et al*., (2016) also evaluated that being part of SOD an antioxidant, Zn application helps in stress mitigation. Bettger & O'Dell, (1981) reported that Zn has a key role in the structural and functional integrity of bio membranes. So, with Zn application plant growth and nutrient uptake becomes better resulting in higher plant Zn uptake. Mitigation of salt stress by Zn application was also reported in other crops like cotton (Hussein & Abou- Baker, 2018), sage (Hendawy & Khalid, 2005), bread wheat (Selim *et al*., 2013) and pistachio (Tavallali *et al*., 2010).

The genetic difference was also evident in this study to Zn uptake in saline conditions. V_{13} and V_7 of rice showed more Zn contents with respect to other varieties. The ability of plant cells to take up Zn is important for salt resilience, as opposed to their ability to limit plant Na^+ uptake. Because of a better ability to uptake and utilize Zn, cultivars with higher tolerance to salt stress can maintain higher Zn^{2+}/Na^+ ratio in the root, empowering improved execution under salt stress (Norvell & Welch. 1993; Cakmak, 2000; Jan *et al*., 2015b). The results of the current study also showed that mostly at high Zn uptake, varieties showed better salt tolerance. Genetic difference in plant Zn uptake was also evident in many other case studies. Wissuwa *et al*., (2008) reported that the effect of genotypes is more prominent than the fertilization as for Zn concentration in grain is considered. Zaman *et al*., (2020) also reported genetic variability among paddy genotypes when Zn concentration was recorded between different genotypes. The selection for Zn accumulation was also reported by the earlier worker in paddy seed (Shivay *et al*., 2010; Hussain *et al*., 2012). Another case study also reported that varietal difference does exist in the Zn concentration of rice plants in saline conditions (Verma & Neue. 1984). The effect of foliar application also tends to be affected by genotypic variation in enhancing grain Zn concentration (Khan *et al*., 2003; Fang *et al*., 2008; Phattarakul *et al*., 2012; Yuan *et al*., 2013). Considering both Zn concentration of root and shoot and plant Zn uptake variety IR-6 of rice tends to be more Zn responsive with respect to other varieties. These results are also in-line with those of cluster analysis.

Conclusion

In the present hydroponic study, plant growth responses, Zn concentrations, Zn uptake, Plant Na^+, $K^{+,}$ and Na^+/K^+ ratio were significantly deteriorated under salt stress. But few varieties performed better than others in this constraint. Rice varieties V_{13} (IR-6) performed better and proved to be more salt-tolerant and Zn responsive than other tested varieties. Generally, recorded parameters were improved through the application of Zn in both normal and saline conditions in almost all rice varieties. Though, varietal differences were still significant in this case too. From the above result evaluations, discussions and cluster analysis ranking, it was concluded that rice variety IR-6 showed better salt tolerance and Zn responsiveness in both normal and saline conditions. In general view, among mean comparisons rice variety IR-6 can be selected for further experimentation with regard to rice bio fortification with Zn. Although, there is still a room for endosperm phytate content assessment as it is the main edible part to be considered for effective bio fortification of Zn. But assessment of the plant with high Zn uptake is still the basic step to be taken.

The varietal selection was done based on growth responses (SFW, SDW, RFW, and RDW), salt tolerance (Na^+, K^+ and Na^+/K^+ ratio), Zn contents and K-mean cluster analysis. The useful genetic differences among varieties can be further exploited for the breeding of high yielding and more Zn efficient varieties and varietal selection for general agronomic bio fortification of Zn.

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