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ARTICLE

Response of Contrasting Rice Genotypes to Zinc Sources under Saline Conditions

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ABSTRACT

Abiotic stresses are among the major limiting factors for plant growth and crop productivity. Among these, salinity is one of the major risk factors for plant growth and development in arid to semi-arid regions. Cultivation of salt tolerant crop genotypes is one of the imperative approaches to meet the food demand for increasing population. The current experiment was carried out to access the performance of different rice genotypes under salinity stress and Zinc (Zn) sources. Four rice genotypes were grown in a pot experiment and were exposed to salinity stress (7 dS m^{-1}), and Zn (15 mg kg^{-1} soil) was applied from two sources, $ZnSO_4$ and Zn-EDTA. A control of both salinity and Zn was kept for comparison. Results showed that based on the biomass accumulation and K^+/Na^+ ratio, KSK-133 and BAS-198 emerged as salt tolerant and salt sensitive, respectively. Similarly, based on the Zn concentration, BAS-2000 was reported as Zn-in-efficient while IR-6 was a Zn-efficient genotype. Our results also revealed that plant growth, relative water content (RWC), physiological attributes including chlorophyll contents, ionic concentrations in straw and grains of all rice genotypes were decreased under salinity stress. However, salt tolerant and Zn-in-efficient rice genotypes showed significantly higher shoot K^+ and Zn concentrations under saline conditions. Zinc application significantly alleviates the harmful effects of salinity by improving morpho-physiological attributes and enhancing antioxidant enzyme activities, and the uptake of K and Zn. The beneficial effect of Zn was more pronounced in salt-tolerant and Zn in-efficient rice genotypes as compared with salt-sensitive and Zn-efficient genotypes. In sum, our results confirmed that Zn application increased overall plant's performance under saline conditions, particularly in Zn in-efficient and tolerant genotypes as compared with salt-sensitive and Zn efficient rice genotypes.

KEYWORDS

Agronomic efficiency; antioxidant enzymes: physiology; *Oryza sativa*; salinity; Zn efficient

1 Introduction

In plant functioning, Zn plays an essential role in protein synthesis and other metabolic events [1,2]. It has been reported that about 10% of the proteins in biological systems need Zn for their structural and functional integrity [3]. This element has also been indicated to be required as a cofactor in over 300 enzymes [4]. Under salinity stress, the production of reactive oxygen species (ROS) is well known [5–7] and the role of Zn in the detoxification of ROS in plant cells has been well demonstrated in previous studies [1,2]. In addition to being essential to plants, Zn is also a vital mineral nutrient for human beings. According to an estimate, more than 30% of the world population is affected by Zn deficiency which is associated with low dietary intake. Zinc deficiency is known to have serious adverse impacts on human health, especially in children, such as impairments in physical growth, immune system, and learning ability, and causing DNA damage and cancer development [8,9]. Increasing the Zn concentration of stable food crops is, therefore, an important humanitarian challenge.

The development of salt tolerance among cereal crops over the area of land is an important goal to feed the world's increasing population [10,11]. Since, agricultural land is in decreasing trend, thus increasing yield per unit area is so important to meet the food demand and ensure global food security. Desertification and salinization sites are also increasing due to uneven rainfall distribution and the unavailability of freshwater for irrigation [12]. Salinity is among the major problems for plant cultivation with high occurrence in semiarid areas [13,14]. According to an estimate, about 800 million hectares of global land are affected by salt-alkalinization [15]. Furthermore, compared with $ZnSO_4$, various responses of rice cultivars to different Zn sources such as ZnO, zinc-coated urea, Zn phosphate, and Zn-ethylenediaminetetraacetate (Zn-EDTA) have been well established in previous studies [16–18]. For soil application, both inorganic and organic Zn sources are commonly recommended. Although soil application of organic Zn sources including Zn-EDTA is more efficient than inorganic sources (such as $ZnSO_4$) for rice crop [19], the prohibitively higher cost of Zn-EDTA deters its extensive use.

Cereal crops provide a major source of Zn for humans, especially those living in resource-poor areas. However, Zn contents of cereal-based foods are quite inadequate to meet per capita demand. The problem is especially acute for rice consumers due to the lowest Zn content in this crop when compared with other cereals [20]. Considerable variation in brown rice Zn has been found among different rice genotypes. According to a recent report by International Rice Research Institute, rice genotypes have average Zn content of 25.4 mg kg^{-1} grain, as compared with 35.0 mg Zn kg^{-1} in wheat (*Triticum aestivum* L.). Furthermore, rice-cultivated soils are distinguished with very low Zn concentrations which further, in turn, reduce Zn concentrations in grains [21,22]. Previous studies have reported that about 30% of the world's cultivated soils are Zn deficient, of which, 50% of the soils are under cereal cultivation [23,24]. Several strategies such as conventional breeding, fertilizer management, seed treatments, and fortification have been suggested to improve Zn concentration in grains [22,25]. Rice genotypes respond differentially under Zn deficit soils [26–28], but the role of this genetic variation for Zn deficiency tolerance in grain Zn accumulation is not well studied. For wheat crop, it has been well established that variable genotype response is associated with the accumulation and expression of Zn-responsive genes

[29]. As previously reported by Cakmak et al. [29], there is even an inverse relationship between high Zn-deficiency tolerance and grain Zn accumulation. Probably, grain Zn concentrations are diluted due to the higher grain yield capacity of the genotypes showing higher tolerance to soil Zn deficiency.

Keeping in view the above-discussed gaps, and beneficial effects of Zn on plant growth, a study was planned to (i) check the response of various rice cultivars to Zn sources, (ii) and evaluate the effectiveness of Zn sources in alleviating the adverse effects of salinity in different rice genotypes. Understanding morphological, physiological, and biochemical attribute changes in rice genotypes differing in salinity tolerance at different growth stages under various levels of Zn application and salinity stress is imperative for the efficient management of available resources. In this work, we hypothesized that Zn application could increase the plant performance under saline conditions by showing better growth of Zn in-efficient and tolerant genotypes relative to salt-sensitive and Zn efficient rice genotypes.

2 Material and Methods

2.1 Screening of Cultivars

Before starting the actual experiment, sufficient numbers of seeds of four selected genotypes were sown in zinc-deficient soil where standing water conditions were maintained for a month. After that, uniform and healthy seedlings were selected and transplanted into the pots in which three seedlings were transplanted in each pot. The experiment was undertaken in a wire-house located at the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad.

2.2 Experimentation

Pots were arranged according to a completely randomized design and each treatment was replicated four times. Well-sieved soil, collected from the experimental field of the department, was used for pot filling where 12 kg of soil was used in each unit. Salinity (7 dSm^{-1}) was established by considering a 50% economic threshold limit for rice crop, according to the standard protocol of Naralen et al. [30]. NaCl salt was used for salinity treatments. The calculated doses of NaCl salt and Zn from both sources ($ZnSO_4$ and Zn-EDTA; 15 mg kg^{-1} soil of each source) were applied in the respective treated pots at the time of pot filling. A control without salt and Zn was kept for comparison. The recommended dose of N, P, and K (160, 90 and 60 kg ha^{-1}, and 0.08, 0.04 and 0.03 g kg^{-1} soil, respectively) were fulfilled from urea, DAP and SOP. Full P and K and half N were applied on the 10th day after transplanting while the remaining N was divided into two equal splits and applied at 45 days after transplanting and the panicle initiation stage. All management practices were the same for all replicated pots.

2.3 Data Recorded

Data on plant growth and physiological indices including the number of tillers per plant, the number of branches per panicle, plant height, and chlorophyll contents were determined at the physiological maturity stage. The collected samples were separated into different organs, straw, and panicles, and ionic concentrations of Na^+ and K^+ and Zn were determined. After measuring fresh weights, the collected seedling samples were oven dried at 75°C for 48 h, and their dry weights (DWs) were recorded. After grounding, the dried samples were digested in a di-acid (HNO_3:$HClO_4$ ratio of 2:1) mixture, according to the prescribed protocol. The ionic concentration of Na^+ and K^+ was determined by using Sherwood-410 Flame Photometer, for that, self-prepared standard solutions prepared from reagent grade salt of NaCl and KCl, respectively, were used. The Zn concentration in the digest was estimated by an atomic absorption spectrophotometer (PerkinElmer, 100 Analyst, Waltham, USA).

2.3.1 Relative Water Content

Relative water contents were determined according to the protocol of Sairam et al. [31]. Fresh leaves samples were soaked in water for 4 h, immediately after removal, turgid leaves were quickly dried with filter paper to remove surface water and immediately weighed to obtain a fully turgid weight (TW). These samples were oven dried at 65°C for 48 h to determine the dry weight. Relative water contents were determined by the following formula:

$$\mathrm{RWC} = \frac{(FW - DW)}{(TW - DW)} \tag{1}$$

2.3.2 Agronomic and Physiological Efficiency

The physiological efficiency (PE), fertilizer recovery efficiency (RE), and agronomic efficiency (AE) were calculated according to the described method of Dobermann and Fairhurst [32], by using the below-described equations. Total Zn uptake by rice seedlings was referred to as Zn uptake by above-ground biomass (grain and straw) only.

$$\mathrm{RE} = \frac{(Zn\ uptake\ by\ treated\ plants - Zn\ uptake\ by\ untreated\ plants)}{(Applied\ Zn\ to\ each\ treatment)} \tag{2}$$

$$\mathrm{PE} = \frac{(GY\ of\ treated\ plants - GY\ under\ control)}{(Zn\ uptake\ by\ treated\ plants - Zn\ uptake\ under\ control)} \tag{3}$$

$$\mathrm{AE} = \frac{(GY\ of\ treated\ plants - GY\ under\ control)}{(Applied\ Zn\ to\ each\ treatment)} \tag{4}$$

RE, Recovery efficiency (%); PE, Physiological efficiency; GY, Grain yield; AE, Agronomic efficiency

2.4 Statistics

Recorded data on growth and yield and yield-related traits were subjected to MS excel for means calculation. The difference in mean values with control treatments was used for comparison. Graphs were prepared by using MS excel as well. A two-way ANOVA analysis technique was used for calculating the mean difference.

3 Results

3.1 Plant Height and Agronomic Traits

The effect of salinity stress on rice growth and yield under different levels of Zn was examined in terms of plant height, the number of tillers per plant, and the number of branches per panicle (Fig. 1, Table 1). Salt stress caused a significant reduction in plant height, number of tillers per plant, and number of branches per panicle in all rice genotypes. Nonetheless, Zn application significantly ($p < 0.05$) increased these traits under non-saline as well as under salinity stress. However, there was a non-significant difference observed between the Zn sources. Among all genotypes, maximum plant height (79.89 cm), number of tillers per plant (11), and number of branches per panicle (10.22) were observed in rice genotype BAS-2000 while minimum plant height (62.67 cm), the number of tillers per plant (4) and number of branches per panicle (3.44) were observed BAS-198. Salinity stress induced a reduction in all these parameters with maximum reduction in BAS-198 relative to rice genotype KSK-133. Maximum values of these parameters were observed in the Zn-inefficient genotype (BAS-2000) and minimum in the salt-sensitive genotype (BAS-A98). However, Zn application increased all attributed in all rice genotypes while effective response towards Zn application was noted in rice genotype BAS-2000 (Table 2).

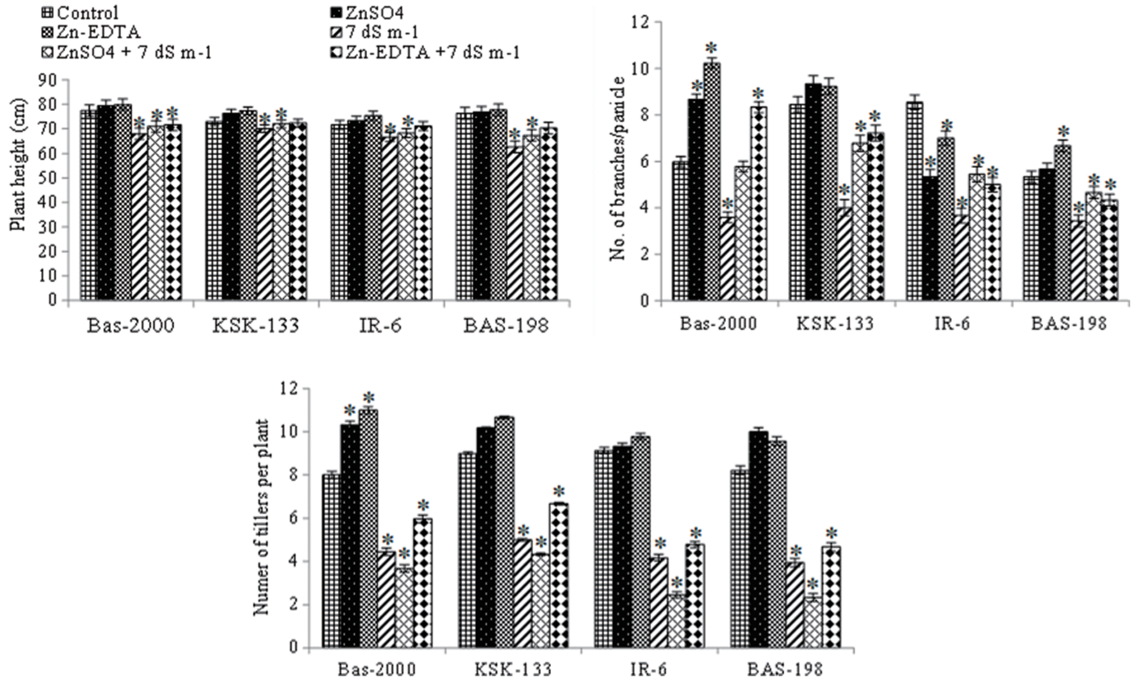

Figure 1: Influence of Zn sources ($ZnSO_4$ and Zn-EDTA) and salinity stress on plant height, number of branches per plant, and number of tillers per plant of four rice genotypes. The values are the means of four replications. $*p < 0.05$

Table 1: Three-way ANOVA analysis for different zinc sources, salinity stress, and rice cultivars

Parameters	Zinc sources (ZS)	Salinity (S)	Cultivars ©	ZS*S	ZS*C	S*C	ZS*S*C
Plant height	*	*	NS	*	NS	NS	NS
NOBPP	*	*	*	*	*	*	*
NOTPP	*	*	*	*	*	*	*
SPAD	*	*	NS	*	NS	NS	NS
RWC	*	*	NS	*	NS	NS	NS
SY	*	*	NS	*	NS	NS	NS
GY	*	*	*	*	*	*	*
S-Na	*	*	*	*	*	*	*
G-Na	*	*	*	*	*	*	*
S-K	*	*	*	*	*	*	*
G-K	*	*	*	*	*	*	*
Zn-straw	*	*	*	*	*	*	*
Zn-grain	*	*	*	*	*	*	*
S-Zn uptake	*	*	*	*	*	*	*
G-Zn uptake	*	*	*	*	*	*	*

Note: NOBPP, number of branches per plant; NOTPP, number of tillers per plant; RWC, relative water content; SY, straw yield; GY, grain yield; S-Na, Na^+ concentrations in straw; Na^+ concentrations in grains; S-K, K^+ concentrations in straw; G-K, K^+ concentrations in grains; Zn-straw, zinc concentrations in straw; Zn-grain, zinc concentrations in grains; S-Zn uptake, straw zinc uptake; G-Zn, grain zinc uptake; AE, agronomic efficiency; ARE, apparent recovery efficiency; PE, physiological efficiency. NS, non-significant effect; $*p < 0.05$.

Table 2: Influence of zinc (Zn) sources and salinity stress on the performance of rice cultivars

Treatments	Plant height	NOBPP	NOTPP	SPAD	RWC	SY	S-Na	G-Na	S-K	G-K	Zn-straw	Zn-Grain	S-Zn-Uptake	G-Zn-Uptake
Cultivars (C)														
Bas-2000	77.05 A	5.80 C	7.10 B	37.40 B	45.80 C	52.03 D	0.14 C	0.063 C	0.19 B	0.14 A	92.70 B	73.96 B	1.49 A	0.53 B
KSK-133	65.08 A	8.20 A	8.60 AB	37.82 B	60.10 A	55.50 C	0.17 B	0.071 B	0.20 A	0.11 BC	94.80 A	76.30 A	1.40 C	0.57 B
IR-6	76.10 A	7.80 B	9.70 A	39.90 A	58.09 B	63.15 A	0.19 AB	0.083 A	0.18 C	0.12 B	92.10 B	74.70 B	1.46 B	0.63 A
BAS-198	78.60 A	5.40 C	8.30 AB	36.78 B	46.90 C	60.70 B	0.20 A	0.082 A	0.18 C	0.10 C	95.70 A	73.80 B	1.44 BC	0.47 C
Zn Sources (Zn)														
Zn-EDTA	82.90 A	7.40 A	12.30 A	43.70 A	70.90 B	68.50 A	0.14 A	0.082 B	0.24 B	0.13 B	98.10 A	78.70 A	2.05 B	1.63 B
$ZnSO_4$	74.10 B	6.80 B	10.05 B	40.96 B	75.08 A	64.30 B	0.12 B	0.085 A	0.26 A	0.16 A	91.03 B	76.91 B	3.10 A	2.05 A
Salt stress (S)														
Control	77.05 A	8.70 A	9.08 A	35.49 A	55.60 A	55.30 A	0.17 B	0.07 B	0.22 A	0.12 A	21.10 A	75.14 A	1.40 A	0.56 A
Salt stress	65.10 B	4.80 B	6.96 B	29.70 B	43.90 B	29.10 B	0.25 A	0.161 A	0.13 B	0.10 B	18.05 B	76.75 B	0.96 B	0.29 B
Interactions (Cultivar*Zinc)														
Bas-2000														
Zn-EDTA	70.09 A	9.40 A	10.20 B	42.30 A	78.09 A	70.40 A	0.17 AB	0.082 C	0.22 C	0.13 C	95.11 A	72.08 B	3.33 A	2.36 A
$ZnSO_4$	72.10 A	7.05 B	9.56 C	39.70 A	69.06 A	67.40 A	0.13 D	0.078 C	0.24 A	0.18 A	93.23 A	77.09 A	2.56 B	1.86 B
KSK-133														
Zn-EDTA	74.09 A	9.15 A	10.60 B	43.90 A	62.50 A	69.80 A	0.15 C	0.11 B	0.21 D	0.17 A	80.70 B	73.40 B	2.12 B	1.50 B
$ZnSO_4$	73.07 A	9.03 A	10.03 B	40.79 A	68.23 A	65.40 A	0.12 D	0.13 A	0.20 E	0.15 B	78.25 B	69.40 C	2.40 B	1.78 B
IR-6														
Zn-EDTA	70.13 A	7.70 B	10.09 B	41.80 A	62.90 A	63.70 A	0.18 A	0.10 BC	0.19 F	0.12 C	81.10 B	69.31 C	1.76 C	1.00 C
$ZnSO_4$	72.19 A	7.00 B	10.49 B	39.30 A	64.70 A	62.83 A	0.16 BC	0.12 AB	0.22 C	0.15 B	79.50 B	73.51 B	2.20 B	1.07 C
BAS-198														
Zn-EDTA	74.05 A	7.00 B	11.90 A	38.76 A	65.90 A	66.76 A	0.18 A	0.071 C	0.21 D	0.17 A	82.70 B	70.43 BC	2.30 B	1.43 BC
$ZnSO_4$	76.20 A	5.50 C	10.70 B	37.91 A	63.69 A	68.23 A	0.19 A	0.11 B	0.23 B	0.12 C	80.25 B	72.55 B	3.10 A	1.35 BC
Interactions (Cultivar*Salinity)														
Bas-2000														
Control	74.05 A	6.50 B	9.07 A	34.50 B	45.80 C	52.43 AB	0.14 E	0.05 C	0.20 A	0.14 B	22.10 B	78.83 A	1.38 C	0.50 BC
Salt stress	72.02 A	3.00 C	4.90 B	32.90 B	37.40 D	28.54 B	0.19 C	0.13 C	0.17 BC	0.11 BC	20.40 C	77.76 A	0.96 E	0.29 D
KSK-133														
Control	70.09 A	8.40 A	9.11 A	39.80 A	60.70 A	54.37 AB	0.15 E	0.071 B	0.21 A	0.12 B	23.16 A	74.31 B	1.60 A	0.60 B
Salt stress	69.03 A	3.15 C	5.20 B	34.50 B	54.90 B	31.40 B	0.18 CD	0.093 B	0.18 B	0.10 C	19.73 C	72.50 B	1.10 D	0.33 D
IR-6														
Control	72.41 A	8.50 A	8.30 A	38.06 A	62.70 A	62.56 A	0.18 CD	0.075 B	0.19 AB	0.13 B	23.24 A	70.79 B	1.50 B	0.80 A
Salt stress	67.50 A	4.07 BC	3.70 C	32.10 B	36.80 D	29.70 B	0.23 B	0.141 A	0.15 C	0.10 C	18.65 C	69.63 B	0.76 F	0.53 BC
BAS-198														
Control	76.02 A	5.04 B	8.00 A	37.09 A	45.60 C	58.40 A	0.17 D	0.084 B	0.18 B	0.12 B	22.27 A	71.65 B	1.48 B	0.48 C
Salt stress	65.90 A	3.09 C	4.10 BC	31.97 B	30.90 E	27.90 B	0.25 A	0.149 A	0.13 D	0.17 A	19.95 C	73.24 B	0.60 G	0.22 D
Interactions (Zinc*Salinity)														
Zn-EDTA														
Control	76.05 A	8.45 A	10.27 A	44.07 A	77.80 A	71.43 A	0.17 BC	0.16 A	0.22 A	0.13 B	84.70 A	22.70 A	3.05 A	2.10 A
Salt stress	72.40 A	6.75 C	5.09 B	35.40 B	43.90 B	35.92 B	0.21 A	0.13 B	0.17 C	0.10 C	63.34 B	20.92 B	1.20 B	0.98 B
$ZnSO_4$														
Control	75.50 A	7.60 B	10.05 A	42.60 B	75.60 A	69.50 A	0.18 B	0.17 A	0.19 B	0.17 A	86.84 A	24.76 A	2.37 A	1.96 B
Salt stress	71.40 A	6.87 C	5.02 B	31.40 C	42.90 B	33.70 B	0.16 C	0.14 B	0.11 C	0.12 B	68.50 B	21.33 B	1.26 B	1.03 B
Interactions (Salinity*Zinc)														
Control														
Zn-EDTA	77.09A	8.02 A	9.04 A	43.67 A	75.04 A	70.15 A	0.25 A	0.22 A	0.19 A	0.15 A	85.40 A	25.20 A	2.30 A	1.93 A
$ZnSO_4$	74.02 B	7.84 B	7.80 B	40.29 B	70.70 B	67.40 B	0.11 B	0.19 B	0.15 B	0.10 B	66.75 B	19.40 B	1.19 B	1.00 B

(Continued)

Table 2 (continued)

Treatments	Plant height	NOBPP	NOTPP	SPAD	RWC	SY	S-Na	G-Na	S-K	G-K	Zn-straw	Zn-Grain	S-Zn-Uptake	G-Zn-Uptake
Salt stress														
Zn-EDTA	73.05 A	6.80 A	6.89 A	25.67 A	40.03 A	55.34 A	0.20 A	0.17 A	0.17 A	0.13 A	45.34 A	13.80 A	1.38 A	1.90 A
$ZnSO_4$	68.07 B	5.65 B	5.80 B	22.70 B	37.17 B	52.67 B	0.18 B	0.15 B	0.12 B	0.07 B	32.89 B	13.49 B	0.87 B	0.89 B
Interactions (Zinc*Cultivar)														
Zn-EDTA														
Bas-2000	77.06 A	9.87 A	10.40 A	45.34 A	66.45 A	63.10 A	0.27 A	0.21 A	0.22 A	0.16 B	84.90 A	25.23 A	2.34 A	1.92 A
KSK-133	72.07 A	8.34 A	9.67 A	39.67 B	62.67 B	60.56 B	0.25 A	0.19 A	0.18 B	0.19 A	82.67 B	23.78 A	2.30 A	1.84 B
IR-6	73.05 C	6.78 C	8.56 B	37.45 B	59.22 C	55.90 D	0.24 B	0.14 C	0.14 C	0.12 C	81.56 B	22.97 B	2.24 B	1,80 C
BAS-198	75.07 B	7.34 B	7.89 C	35.87 C	50.90 D	59.59 C	0.26 A	0.15 B	0.16 C	0.14 C	80.45 C	21.80 B	2.17 C	1.81 C
$ZnSO_4$														
Bas-2000	75.01 A	8.76 A	9.89 A	43.10 A	64.79 A	62.56 A	0.23 B	0.19 A	0.20 A	0.14 B	81.40 A	24.39 A	2.20 A	1.89 A
KSK-133	70.07 A	7.23 A	8.56 B	41.35 B	57.86 B	57.43 B	0.25 A	0.16 B	0.15 B	0.17 A	80.60 A	21.10 B	2.21 A	1.83 B
IR-6	69.02 C	6.89 B	7.45 B	39.90 C	57.34 C	52.07 C	0.20 C	0.17 B	0.11 C	0.10 C	78.90 B	20.67 B	2.20 A	1.77 C
BAS-198	73.00 B	7.12 A	6.89 C	40.03 C	49.80 D	53.90 C	0.21 C	0.13 C	0.12 C	0.12 C	77.90 B	19.45 C	2.13 B	1.72 C
Interactions (Salinity*Cultivars)														
Control														
Bas-2000	65.02 B	5.34 B	7.78 A	37.89 A	55.56 A	53.49 A	0.15 A	0.12 A	0.15 A	0.08 B	70.90 A	18.56 A	1.80 A	1.77 A
KSK-133	70.10 A	6.65 A	6.90 B	36.45 A	46.89 B	50.67 B	0.12 B	0.09 B	0.16 A	0.10 A	68.83 B	16.89 B	1.76 B	1.75 A
IR-6	64.23 B	5.00 B	5.34 C	32.15 B	45.67 B	47.78 C	0.11 C	0.07 B	0.09 C	0.07 B	66.34 B	15.46 C	1.71 C	1.70 B
BAS-198	61.12 C	3.67 C	4.90 D	30.67 C	41.60 C	49.80 C	0.10 C	0.11 A	0.10 B	0.10 A	65.70 C	16.67 B	1.70 C	1.72 B
Salt stress														
Bas-2000	60.19 B	4.98 B	4.46 B	19.23 B	29.70 B	28.97 C	0.09 B	0.10 B	0.09 B	0.10 A	39.42 B	13.56 B	1.05 B	0.95 B
KSK-133	63.34 A	6.23 A	5.87 A	22.56 A	32.56 A	31.80 A	0.12 A	0.12 A	0.10 A	0.11 A	42.90 A	15.49 A	1.15 A	1.02 A
IR-6	62.23 B	4.90 B	3.89 C	20.67 C	30.56 B	30.45 B	0.10 A	0.09 C	0.07 B	0.09 B	40.17 B	13.90 B	0.98 C	0.98 B
BAS-198	58.08 B	4.09 B	2.90 D	15.89 D	27.45 C	22.80 D	0.07 C	0.06 C	0.04 C	0.05 C	33.56 C	11.78 C	0.72 D	0.88 C

Note: NOBPP, number of branches per plant; NOTPP, number of tillers per plant; RWC, relative water content; SY, straw yield; GY, grain yield; S-Na, Na^+ concentrations in straw; Na^+ concentrations in grains; S-K, K^+ concentrations in straw; G-K, K^+ concentrations in grains; Zn-straw, zinc concentrations in straw; Zn-grain, zinc concentrations in grains; S-Zn uptake, straw zinc uptake; G-Zn, grain zinc uptake; AE, agronomic efficiency; ARE, apparent recovery efficiency; PE, physiological efficiency. NS, non-significant effect; $*p < 0.05$.

3.2 Chlorophyll and Relative Water Contents

Salt stress caused a significant reduction in chlorophyll and relative water contents in all rice genotypes where a prominent reduction was observed in BAS-198. However, the application of Zn increased these parameters under control as well as under saline conditions (Fig. 2). When compared the Zn sources, Zn-EDTA application increased the chlorophyll content and relative water content as compared to $ZnSO_4$. Among rice genotypes, maximum chlorophyll content (44.20) and relative water content (77%) were observed for BAS-2000 while minimum chlorophyll content (27.41) and relative water content (33%) were observed for BAS-198. For interactive effect, maximum values were observed in the Zn-inefficient genotype (BAS-2000) and minimum in the salt-sensitive genotype (BAS-A98) (Fig. 2).

3.3 Yield Attributes

Salt stress caused a significant reduction in grain and straw yield in all rice genotypes. Nonetheless, Zn application significantly increased the grain and straw yields under control as well as under saline conditions with higher values under control conditions (Fig. 3). The application of Zn increased these parameters under control and saline conditions. When compared the Zn sources, the Zn-EDTA application significantly increased the values of than $ZnSO_4$ application. Among the genotypes, maximum grain yield (35.67 g) and straw yield (70.03 g) were observed in BAS-2000 while minimum grain yield (14.63 g) and straw yield (23.86 g) were observed in BAS-198. Salinity stress caused a significant reduction in BAS-198 followed by other genotypes. For their interactive influence, higher values were observed in the Zn-inefficient genotype (BAS-2000) and minimum in salt-sensitive genotype (BAS-98) (Fig. 3).

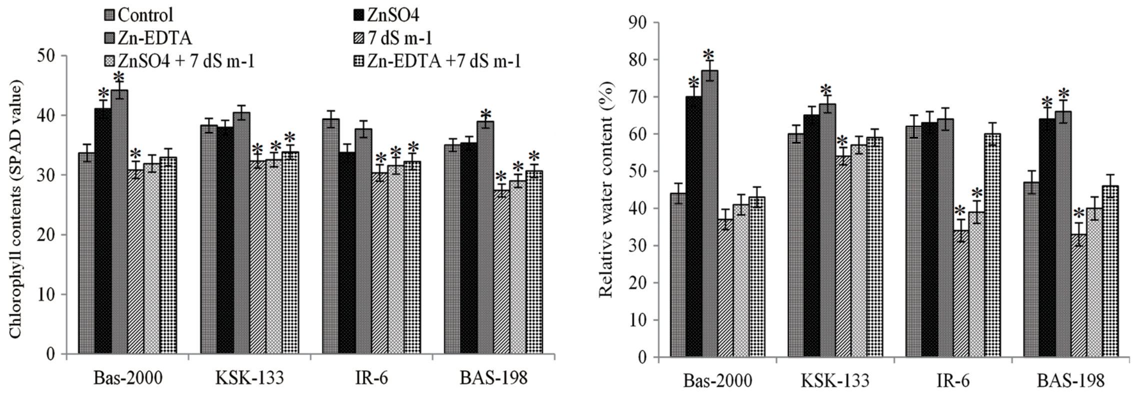

Figure 2: Influence of Zn sources ($ZnSO_4$ and Zn-EDTA) and salinity stress on chlorophyll and relative water contents of four rice genotypes. The values are the means of four replications. *$p < 0.05$

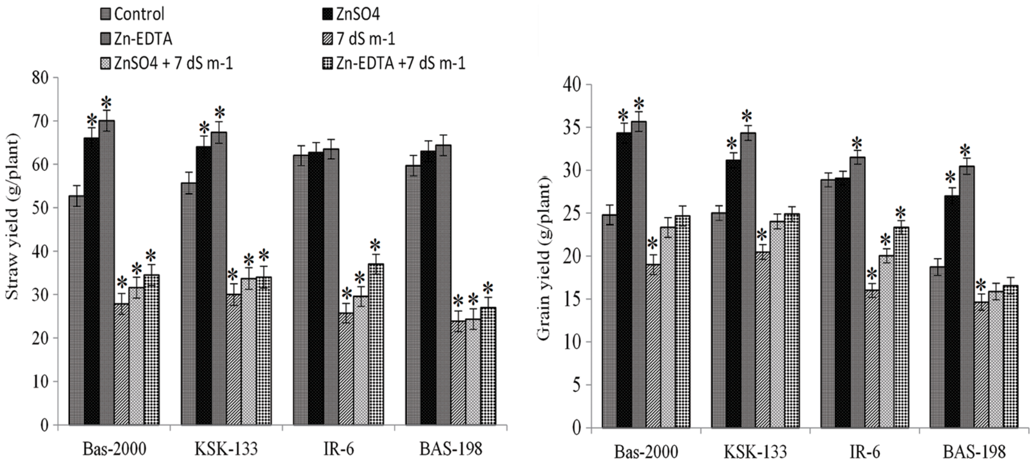

Figure 3: Influence of Zn sources and salinity stress on straw and grain yield of four rice genotypes. The values are the means of four replications. *$p < 0.05$

3.4 K^+ and Na^+ Concentrations

Salt treatment markedly increased Na^+ and decreased K^+ concentration in straw and grains of all rice genotypes (Fig. 4). Results showed that the extent of increased Na^+ and decreased K^+ concentrations were greater in rice genotype BAS-198 than in other genotypes under saline and control conditions. The maximum shoot Na^+ concentration was recorded in rice genotype BAS-198 under salinity stress while the minimum was recorded in BAS-2000 under high Zn conditions (15 mg kg^{-1}). An opposite trend was recorded for K^+ concentration (Fig. 4). Rice genotype KSK-133 markedly sustained ionic concentrations than other genotypes under saline conditions. Results also showed that Zn significantly enhanced K^+ concentration and reduced Na^+ concentration in grains under both non-saline and saline conditions. Zn application at 15 mg kg^{-1} was found most effective in improving K^+ concentrations in all rice genotypes.

And, a better response to Zn application was observed in rice genotypes BAS-2000 under non-saline and KSK-133 under saline conditions.

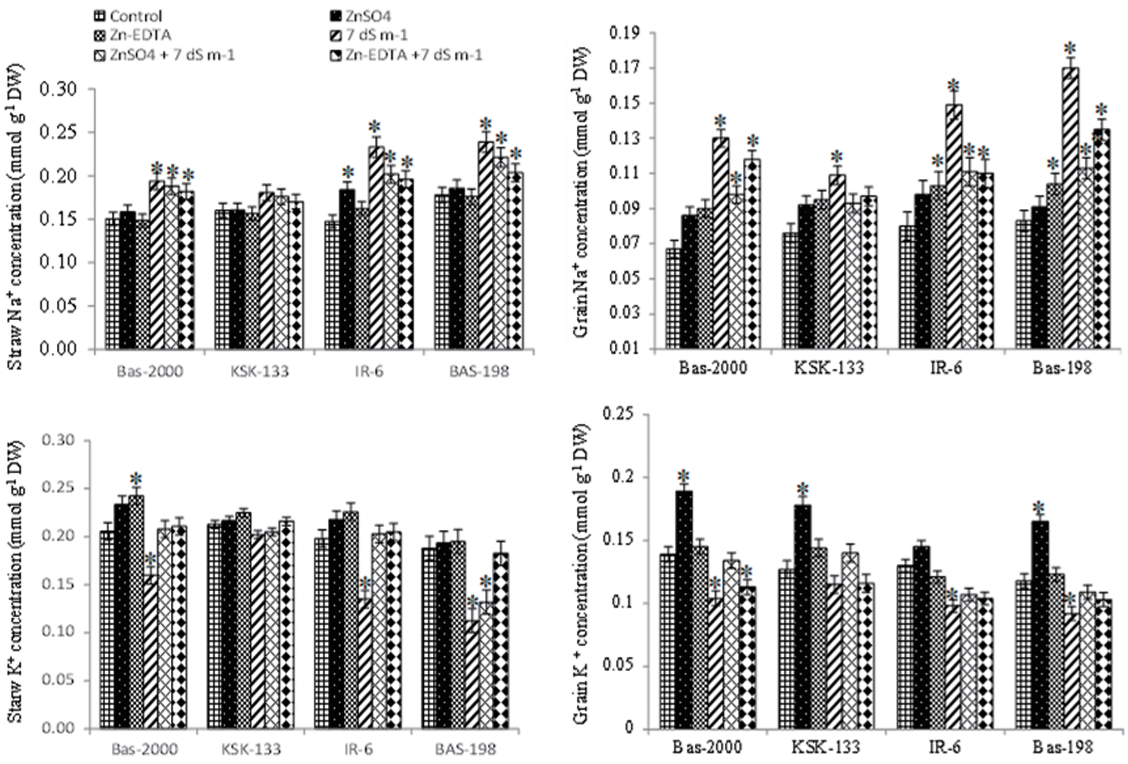

Figure 4: Influence of Zn sources ($ZnSO_4$ and Zn-EDTA) and salinity stress on straw and grain yield of four rice genotypes. The values are the means of four replications. $*p < 0.05$

3.5 Zinc Uptake

Zn application significantly increased Zn concentration in both straw and grain under non-saline conditions compared to saline conditions in all rice genotypes (Fig. 5). Results showed that the extent of increase in shoot Zn concentration was greater in rice genotype BAS-2000 than in other genotypes under both saline and non-saline conditions. The maximum Zn concentration was recorded in rice genotype BAS-2000 when treated with Zn-EDTA while the minimum Zn concentration was recorded in rice genotype IR-6. Results regarding the uptake of Zn demonstrated that higher uptake of Zn was observed in straw while minimum uptake of Zn was observed in grains of all rice genotypes. Among the genotypes, KSK-133 recorded significantly higher values followed by BAS-2000 and BAS-198 under saline conditions. Zn application at 15 mg kg^{-1} was found most effective in improving Zn concentrations in all rice genotypes. However, a better response to Zn application was observed in rice genotypes BAS-2000 under non-saline and KSK-133 under saline conditions.

3.6 Effect of Zn Application on Zn Use Efficiencies

In this work, agronomic efficiency, apparent recovery efficiency, and physiological efficiency were evaluated for Zinc sources and cultivars. Zinc application significantly increased physiological efficiency, fertilizer recovery efficiency, and agronomic efficiency in all four rice genotypes (Table 3). Among cultivars, KSK-133 recorded higher values of these parameters followed by BAS-198. The maximum physiological efficiency (12,937.14 μg μg^{-1}), fertilizer recovery efficiency (5.54 μg μg^{-1}), and agronomic efficiency (361.05 μg μg^{-1}) were recorded in rice genotype BAS-2000 treated with Zn-EDTA at 15 mg kg^{-1} while minimum physiological efficiency (6,178.32 μg μg^{-1}), fertilizer recovery efficiency (1.15 μg μg^{-1}) and agronomic efficiency (171.30 μg μg^{-1}) were recorded in IR-6.

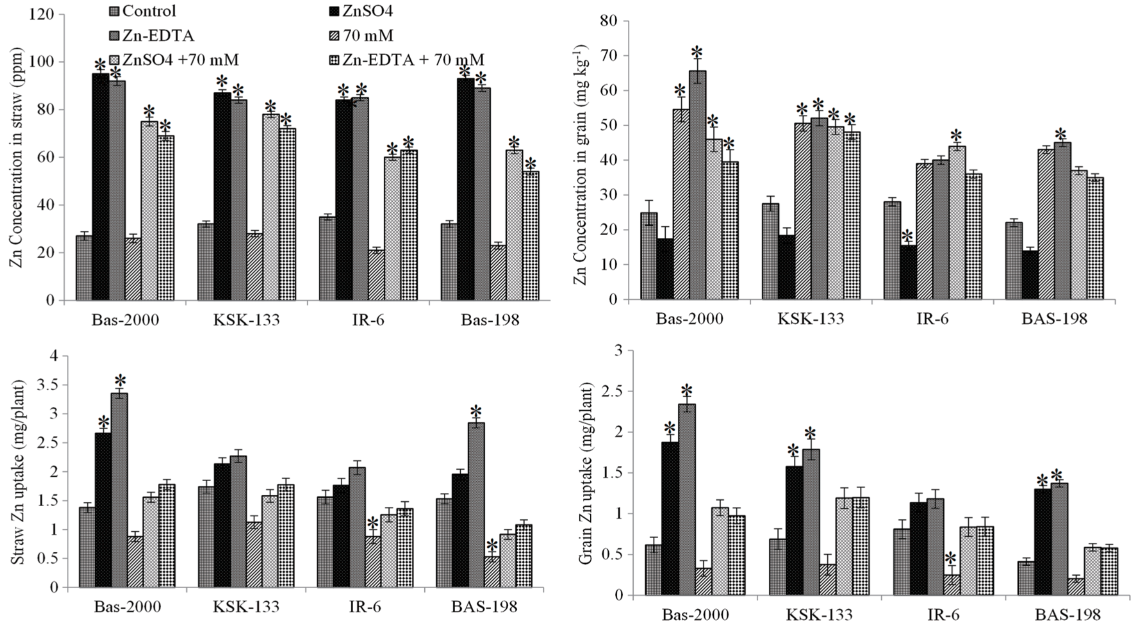

Figure 5: Influence of Zn sources ($ZnSO_4$ and Zn-EDTA) and salinity stress on straw and grain yield of four rice genotypes. The values are the means of four replications. *$p < 0.05$

Table 3: Effect of NaCl and Zn application on Zn efficiencies parameters of rice

Genotypes	Agronomic efficiency (μg μg^{-1})	Apparent recovery efficiency (%)	Physiological Efficiency (μg μg^{-1})	Means
Zn-EDTA (15 mg kg^{-1})				
Bas-2000	361.05 ± 3.56 a	5.54 ± 1.50 a	12937.14 ± 2.08 a	13303.73A
KSK-133	318.15 ± 4.51 b	5.25 ± 3.43 b	6942.30 ± 3.70 a	7265.70 C
IR-6	171.30 ± 2.90 d	1.15 ± 0.06 c	6178.32 ± 2.60 a	6350.77 D
BAS-198	264.84 ± 5.43 c	1.56 ± 0.04 d	7584.24 ± 2.68 a	7850.64 B
Mean	**278.83**	**3.37**	**8410.5**	
$ZnSO_4$				
Bas-2000	325.72 ± 4.66 a	3.60 ± 0.74 a	12237.40 ± 4.96 a	12566.73 A
KSK-133	301.92 ± 2.16 b	3.32 ± 0.55 b	8483.91 ± 3.16 b	8789.15 B
IR-6	176.20 ± 1.10 d	1.13 ± 0.88 d	5709.22 ± 2.34 d	5886.55 D
BAS-198	259.30 ± 2.09 c	1.65 ± 0.96 c	6303.51 ± 3.56 c	6564.46 C
Mean	**265.78**	**2.42**	**7183.51**	

Note: Different lower- and upper-case letters show a significant difference among treatments at $p < 0.05$.

4 Discussion

The genetic variations between the crop plants provide a precious tool in the selection of genotypes with desirable characteristics [33]. The present study, clearly demonstrated the differential response of four rice genotypes subjected to two Zn sources and salt stress. It is obvious that salinity stress reduced plant growth in terms of reduced RWC, chlorophyll content, agronomic traits, yield attributes and altered K^+/Na^+ ratios and Zn concentrations in four rice genotypes [34–37]. However, the addition of Zn significantly alleviated the harmful effects of salinity by improving growth parameters, Zn concentrations, and K^+/Na^+ ratios in four rice genotypes, as reported in previous studies [38–42]. The data also indicated that rice genotype BAS-2000 was relatively Zn-inefficient and showed a better response to Zn application as compared to IR-6 which was characterized as Zn-efficient under non-saline and saline conditions.

The results of the present work revealed that salinity caused a significant reduction in plant height, chlorophyll content, relative water content, and straw and grain yield in four rice genotypes. These results are in agreement with those of [34–37] who reported that salinity caused a reduction in plant growth and yield-related traits. This reduction in plant growth might be due to the ionic toxicity or decreased osmotic potential as well as low wall extensibility [38]. There are several reports on osmotic stress and ionic toxicity resulting from salt stress in rice genotypes [40–42]. The addition of Zn significantly reduced the lethal effects of NaCl and improved plant growth in rice genotypes. This was certified to the antagonistic effect of Zn with Na^+. Similarly, the enhancement in plant growth and dry matter production was reported in rice by the addition of Zn in saline soil [43,44].

The level of salt-induced effects on RWC has been used as one of the imperative water relations attributes for assessing the degree of salt tolerance in rice [37], *Brassica rapa* [45], and pea [46]. In this work, salinity caused a significant reduction in RWC in all rice genotypes. Salt-tolerant rice genotype KSK-133 and Zn-inefficient genotype BAS-2000 showed higher RWC under saline conditions as compared to salt-sensitive rice genotype (BAS-198) and Zn-efficient IR-6. The decreases in RWC under salinity stress in rice genotypes were confirmed by the previous study of Munns [47]. Zinc plays an important role in rice genotype's water relation by increasing the membrane stability, water relations, and photosynthetic activity, and helps the plants to absorb more water to reach turgidity [23,48,49] in C_3 and C_4 plants.

It is very clear from the results that salt stress leads to a significant reduction in leaf chlorophyll contents in all rice genotypes. A higher reduction in chlorophyll contents was observed in rice genotype BAS-198 relative to rice genotype KSK-133. These results are inconsistent with some earlier reports that showed a reduction in chlorophyll content in rice and wheat [50]. The decrease in chlorophyll content and leaf area due to the roots of some species, such as rice, being leaky and Na^+ may be taken up apoplastically and salt concentration build-up in the apoplast and caused the dehydration of cells or disorder of chloroplast structure and associated proteins [51]. In rice, lower plant available Zn in soil results in causes leaf bronzing and poor tillering at the early growth stages, leading to delayed maturity and significant yield loss [32,52]. Zinc plays a key role in various plant metabolism processes, i.e., the development of cell walls, respiration, carbohydrate metabolism and gene expression and regulation [53]. Micronutrients also enhance plant productivity, leaf area and grain yield as a result of enhancing the enzymatic system of plants [54].

Salinity stress results in high accumulation of sodium (Na^+) in rice genotype BAS-198 which could be one of the major reasons for its sensitivity to salt stress, while more K content in the case of rice genotype KSK-133 must have contributed towards its discriminating tolerance to salinity stress. The higher K^+ uptake in rice genotype KSK-133 may be related to its selectivity of K^+ over Na^+. In another study, Zhu et al. [55] also demonstrated that salt-tolerant barley plants accumulated higher K^+ due to selective absorption of K^+ and by a preferential loading of K^+ rather than Na^+ into the xylem. However, salt-tolerant rice genotype exhibited a strong affinity for K^+ over Na^+ by maintaining a higher K^+/Na^+ ratio as compared to salt-sensitive rice genotype. Working with rice and barley crops, Carden et al. [56] reported that Zn application repressed Na^+ transport in plants grown in salinized conditions, with concomitant improvement in plant growth. It was suggested that the plant's tolerance response is characterized by a distinctly higher K^+/Na^+ ratio, which may be used as an indicator of tolerance or sensitivity in crop varieties [57]. Thus, it appears that the ideal rice genotype should possess not only the capability to retain K^+ efficiently in plant roots under saline conditions but also a means of preventing Na^+ accumulation in the shoot. Thus, further breeding efforts should be rigorous in identifying, characterizing, and localizing the genes encoding these two traits in the rice genome. Comparing the sources, in the case of the pot experiment, contradictory results were observed between $ZnSO_4$ and Zn-EDTA.

5 Conclusion

In conclusion, salinity stress reduced plant growth by affecting plant agronomic traits, and yield attributes, reducing relative water contents and chlorophyll contents, decreasing Zn concentrations, and altering $K^{+/}Na^+$ ratios and efficiencies in all rice genotypes. The inhibitory effect of salt stress was more pronounced for rice genotype BAS-198 than KSK-133. However, the addition of Zn significantly alleviates the harmful effects of salinity by improving plant growth, photosynthetic and enzymatic activities, and enhancing Zn concentrations and K^+/Na^+ ratios in all rice genotypes. Zinc application (15 mg kg^{-1}) was found to be more effective in alleviating the perilous effect of salinity compared to Zn deficient and saline conditions. Salt tolerant rice genotype (KSK-133) and Zn-inefficient genotype (BAS-2000) produced more biomass, less shoot Na^+ concentrations, high shoot K^+ concentrations, high Zn concentrations, and exhibited more chlorophyll contents and relative water content under salt stress and Zn sufficient conditions compared to salt-sensitive rice genotype (BAS-198) and Zn-efficient genotype (IR-6). Using resistant rice cultivars is the topic of our oncoming research under field conditions. These results from local varieties can offer ways to better understanding the effect of Zn sources on salinity tolerance and help us to select genotypes that can maintain salinity tolerance under variable Zn sources.

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