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GENOTYPIC VARIATION TO ZINC RESPONSE TO SALT STRESSED RICE (*Oryza sativa* L.) PLANTS IN HYDROPONIC

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Zinc (Zn) is an important micronutrient and is helpful for different metabolic processes of crop-plants, animals and human beings. In Pakistan most of the calcareous and alkaline soils are deficient in plant available Zn. Zinc concentration in the soils of rice tract in Pakistan is decreasing rapidly due to less addition of Zn in the soils and soil conditions in which rice is grown. The cultivation of tolerant varieties on saline fields may also reduce salinity by the process of biological reclamation. In this experiment 10 rice genotypes (Pak Basmati, Super Basmati, Basmati-198, Basmati-370, Basmati-385, Basmati-515, Basmati-2000, KS-282, IR-6 and KSK-133) were investigated against zinc (Zn) and salinity for total dry matter production, chlorophyll content, sodium (Na^+) , potassium (K^+) and zinc (Zn) concentrations. The treatments were: Control, Zn=15 ppm (using $ZnSO_4$), Zn=15 ppm (using Zn-EDTA), NaCl= 70 mM, Zn=15 ppm (using $ZnSO_4$) + 70mM NaCl, Zn=15 ppm (using Zn-EDTA) + 70mM NaCl. The highest biomass production, chlorophyll content, K^+ concentration was observed in rice genotype KSK-133 and reduction of these were observed in BAS-198. The application of Zn significantly (P<0.05) reduced the Na^+ concentration and improve the plant growth. By applying Zn from both sources i-e $ZnSO_4$ and Zn-EDTA, highest Zn concentration was observed in Basmati-2000 under both of the conditions saline and non-saline. On the basis of more biomass production and Zn concentration, rice genotypes Basmati-2000 is considered as Zn-inefficient and IR-6 is considered as Zn-efficient genotype.

Keywords: Chlorophyll contents, rice genotypes, Zinc EDTA, Zinc sulphate

INTRODUCTION

Soil salinity is one of the major constraints of arid and semi-arid regions where soluble salts are frequently high in the soil or in irrigation water. It adversely affects the growth of most agricultural crops through its influence on certain aspects of plant metabolism. Salinity affects the plant growth by affecting the osmotic adjustment (Bernstein, 1963), the uptake of certain essential nutrients (Greenway *et al*., 1966), photosynthesis (Downton, 1977), and enzyme activity as well as causing hormonal imbalance (Shah and Loomis, 1965).

Rice rank as a second amongst the staple food grain crops after wheat in Pakistan and it is a major source of foreign exchange earning in recent years. Pakistan grows a high quality of rice to fulfil the domestic demands and also for exports. Rice accounts for 4.9% of the value added in agriculture and 1% of GDP (Anonymous, 2011-12).

Zn addition markedly reduced the depressing effects of NaCl and increased the total biomass production and other determined plant parameters. Being an integral part of Cu-Zn SOD, directly scavenging the ROS, increase the K concentration, amino acids. phenolics content by a factor of 2.5 and a key role in the activation of antioxidant enzymes during the stress minimized the toxic effect of ROS and help the plant to survive under salt stressed conditions (Welch *et al*. 1982) . Therefore, it is strongly suggested that, in saline soils especially those low in plant available Zn, sufficient Zn should be added if rice has to be grown (Brown *et al*, 1993).

There is an impressive genotypic variation at early growth stages. Such type of variations could be useful for development of high-yielding salt tolerant genotypes for understanding the molecular and physiological mechanisms contributing to salt tolerance in rice genotypes. Some other researchers (Ashraf and Khanum, 1997; Munns *et al*., 2000) also reported such variations about the salt tolerance of wheat. Salt tolerance at early growth stages differs from the development of late stages (Ashraf and Khanum, 1997; Mano and Takeda, 1997; Almansouri *et al*., 2001). It was observed that in cereals the germination and seedling growth have a great impact on final yield (Grieve *et al*., 2001 and Willenborg *et al.*, 2005). In case of rice which is sensitive to salinity and it is observed that its sensitivity mostly at seedling and maturity stage while tolerant at germination stage. Actually rice uptake Na ions apoplastically because of leaky roots and salt accumulation in apoplast caused the dehydration of cell and results into inhibit the expansion rate of new leaves, senescence of older leaves and reduced the stomatal conductance all these inhibitory factors combined and results into smaller and thicker leaves, white strips on leaf and rolling of leaves and ultimately effect the whole plant growth. Salt tolerant genotypes cope with salinity stress by adopting different salt tolerant mechanism like such type of genotype maintain their growth by maintaining the expansion rate of new leaves and salt accumulation in the vacuole of older leaves and Na compartmentation at cellular and intracellular level to maintain the stomatal conductance which is a great threat for plant under salt stress conditions (Munns and Tester, 2008).

Considering the importance of Zn in rice crop under saline conditions, the main objectives of this experiment were:

a) To study the availability of Zn from the both sources under saline conditions.
b) To study the genotypic behaviour of rice with the application of Zn in saline conditions.

MATERIALS AND METHODS

Plant material, growth and treatment condition: A hydroponic study was performed at Saline Agriculture Research Centre (SARC), University of Agriculture, Faisalabad. Seeds were collected from Kala Shah Kaku, Research Station. Seeds of 10 rice genotypes (*Oryza sativa* L.) i.e. Pak Basmati,Super Basmati, Basmati-198, Basmati-370, Basmati-385. Basmati-515, Basmati-2000, KS-282, IR-6 and KSK-133 were sown in iron trays having washed sand. Separate iron tray was used for each rice genotype during germination. Water was spread daily over these iron trays to sustain optimum humidity. When seedling growth reached at two leaf stage (after 8 days of sowing), the seedlings of each rice genotype were transplanted in holes of thermopore sheet with the help of foam wrapped at shoot root junction, suspended on 100 L iron tub, having ½ strength Yoshida nutrient solution (Yoshida *et al.,* 1972). The culture solution was renewed once a week. Complete randomize design (CRD) with factorial arrangement was used with four replicates. After four days of transplanting, two levels of salinity (control, 70 mM) were developed by using sodium chloride salt in three increments., two Zn levels (control and 15 ppm) using $ZnSO_4$ and (control and 15 ppm) using Zn-EDTA were developed. The solution pH was adjusted to 5.5±0.5 throughout the experiment with 1 M NaOH or HCl, as required.

Plant harvest: Plant samples were placed in oven at 65 ±5°C for 48 hours to determine dry weight of root and shoot (g plant^{-1}). Plant samples were dried in an air forced oven at 60°C for 48 h (Liu *et al*., 2006). Dried samples were finely ground with a Wiley mill fitted with a stainless steel chamber and blades. Finely ground (1.0 g) samples of grains were digested in a di-acid (HNO_3:$HClO_4$ ratio of 2:1) mixture (Jones and Case, 1990). The Zn concentration in the digest was estimated by an atomic absorption spectrophotometer (PerkinElmer, 100 AAnalyst, Waltham, USA). The concentration of K^+, Na^+ was measured by using Sherwood- 410 Flame Photometer.

Determination of chlorophyll content, Na^+, K^+ and Zn^{2+} concentration: SPAD value (Soil-plant analyses development) of the leaves was determined by suing SPAD instrument (model SPAD-502; Minolta Corp., Ramsey, N.J.). Na^+ and K^+ concentration was determined in shoot by using Sherwood 410 Flame photometer while Zn^{2+} concentration was measured by using Atomic absorption spectrophotometer (PerkinElmer, 100 AAnalyst, Waltham, USA).

Zn efficiency calculation: The Zn efficiency was calculated by using the following equation:

Zn efficiency = (dry matter yield at Zn-deficient level/dry matter yield at Zn-sufficient level) x 100 (Graham *et al*, 1992).

Genotypes with Zn efficiency of 96.74% considered as Zn efficient genotypes and where efficiency of 53.24% was labelled inefficient genotypes under non-saline conditions. According to this criterion, the top Zn efficient genotype was IR-6 and the bottom inefficient genotype was Basmati-2000 (Fig. 1).

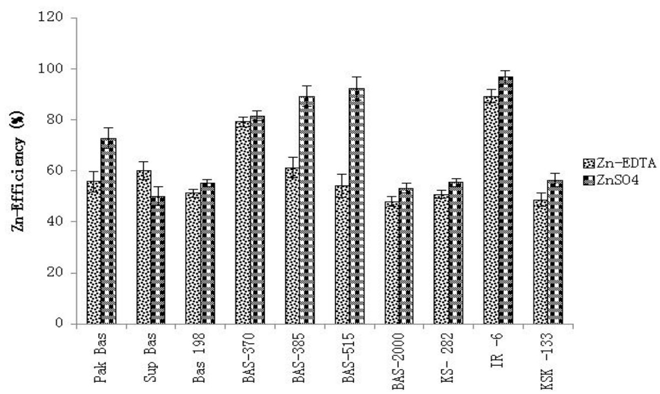

Figure 1. Performance of rice genotypes on the basis of Zn-efficiency (%) under non-saline conditions

Statistical Analysis: All data presented in this experiment are means of four replicates and standard deviation (SD). Analysis of variance (ANOVA) was performed by using a statistical package, STATIX version 16.0.

RESULTS

Effect of salinity on plant growth: Plant biomass was severely affected due to increase in salinity level consequently; shoot and root fresh weight were reduced. Four weeks of salinity treatment adversely reduced the both shoot and root fresh weight. The data indicating the means of shoot and root fresh weight of rice genotypes under control and different levels of salinity and zinc are presented in (Table 1 to 4).

Table 1. Shoot fresh weight of ten rice genotypes after 4 weeks exposure to Zn sources and its combination with NaCl

	Non- Saline			Saline		
Genotypes	**Control**	**$ZnSO_4$**	**Zn-EDTA**	**70 mM**	**$ZnSO_4$**	**Zn-EDTA**
Pak Basmati	18.30±0.52	21.50±0.30(117)	20.36±0.72(111)	10.12±0.47(55)	15.60±0.42(85)	15.18±0.75(83)
Sup Basmati	16.48±0.24	22.04±0.68(134)	20.82±0.16(126)	10.20±0.06(62)	13.46±0.23(82)	13.20±0.14(80)
Basmati-198	19.51±0.32	23.22±0.11(119)	21.38±0.40(110)	7.80±0.22(40)	12.16±0.01(62)	11.76±0.06(60)
Basmati -370	19.14±0.14	21.86±0.18(114)	21.18±0.20(110)	12.50±0.34(65)	16.90±0.04(88)	15.96±0.13(83)
Basmati -385	18.20±0.44	24.14±0.29(133)	20.72±0.04(125)	11.64±0.06(64)	15.20±0.44(83)	12.14±0.08(67)
Basmati -515	17.24±0.39	23.02±0.45(134)	20.50±0.16(119)	9.84±0.19(57)	13.94±0.21(81)	13.16±0.70(76)
Basmati- 2000	13.76±0.02	25.20±0.74(183)	22.06±0.53(160)	9.16±0.33(67)	12.94±0.46(94)	12.18±0.18(89)
KS- 282	18.78±0.37	24.50±0.63(130)	21.10±0.63(112)	14.64±0.25(78)	15.15±0.08(81)	14.68±0.10(78)
IR -6	19.92±0.27	21.44±0.17(108)	20.30±0.03(102)	14.96±0.09(75)	16.78±0.11(84)	15.74±0.26(79)
KSK -133	18.30±0.37	24.60±0.54(135)	21.90±0.47(120)	15.78±0.15(86)	17.66±0.44(97)	16.80±0.064(92)

Table 2. Shoot dry weight of ten rice genotypes after 4 weeks exposure to Zn sources and its combination with NaCl

	Non- Saline			Saline		
Genotypes	**Control**	**$ZnSO_4$**	**Zn-EDTA**	**70 mM**	**$ZnSO_4$**	**Zn-EDTA**
Pak Basmati	2.86±0.06	5.12±0.02(179)	3.93±0.29(137)	2.48±0.05(86)	2.62±0.01(92)	2.55±0.20(89)
Sup Basmati	3.06±0.02	5.09±0.01(166)	5.02±0.21(164)	2.57±0.24(84)	2.76±0.06(90)	2.68±0.04(87)
Basmati-198	3.12±0.02	5.05±0.04(163)	4.60±0.73(147)	2.40±0.05(77)	2.52±0.04(81)	2.47±0.03(79)
Basmati -370	3.24±0.01	4.97±0.01(153)	4.09±0.01(126)	2.80±0.33(86)	2.90±0.03(89)	2.86±0.32(88)
Basmati -385	3.34±0.04	5.45±0.03(163)	4.74±0.02(122)	2.88±0.12(86)	3.02±0.02(90)	2.92±0.02(87)
Basmati -515	3.40±0.02	5.27±0.18(155)	4.68±0.24(138)	2.75±0.07(81)	2.98±0.51(87)	2.89±0.25(85)
Basmati- 2000	2.74±0.02	5.71±0.05(208)	5.15±0.35(188)	2.45±0.03(89)	2.56±0.06(93)	2.48±0.06(90)
KS- 282	3.38±0.06	5.65±0.15(167)	5.10±0.40(151)	2.90±0.24(86)	3.04±0.11(90)	2.98±0.01(88)
IR -6	3.56±0.10	3.99±0.02(112)	3.68±0.40(103)	2.84±0.02(80)	2.96±0.03(83)	2.88±0.01(81)
KSK -133	3.50±0.07	5.19±0.22(148)	5.12±0.33(146)	3.14±0.02(90)	3.28±0.11(94)	3.20±0.07(91)

Table 3. Root fresh weight of ten rice genotypes after 4 weeks exposure to Zn sources and its combination with NaCl

	Non- Saline			Saline		
Genotypes	**Control**	**$ZnSO_4$**	**Zn-EDTA**	**70 mM**	**$ZnSO_4$**	**Zn-EDTA**
Pak Basmati	7.63±0.04	8.57±0.08(112)	8.22±0.08(108)	6.17±0.05(80)	6.71±0.07(88)	6.44±0.06(84)
Sup Basmati	7.18±0.05	8.45±0.03(116)	8.12±0.22(113)	5.21±0.09(72)	5.51±0.02(77)	5.42±0.05(75)
Basmati-198	6.32±0.02	8.29±0.03(131)	8.07±0.07(127)	3.90±0.06(62)	4.13±0.02(65)	3.95±0.01(62)
Basmati -370	6.74±0.06	8.62±0.04(128)	8.38±0.09(124)	5.39±0.20(80)	5.75±0.07(85)	5.60±0.03(83)
Basmati -385	6.34±0.13	8.39±0.15(132)	7.98±0.03(126)	5.36±0.03(84)	5.90±0.08(93)	5.78±0.12(91)
Basmati -515	6.89±0.07	8.77±0.05(127)	8.32±0.06(121)	5.06±0.02(73)	5.87±0.06(86)	5.63±0.12(81)
Basmati- 2000	6.17±0.04	9.71±0.05(157)	9.40±0.07(152)	4.35±0.06(70)	5.89±0.11(95)	5.59±0.08(90)
KS- 282	7.14±0.07	8.43±0.18(118)	8.17±0.13(114)	5.16±0.20(72)	6.15±0.08(86)	6.08±0.03(85)
IR -6	7.84±0.18	8.21±0.02(105)	7.96±0.08(102)	5.90±0.19(75)	6.89±0.21(88)	6.83±0.12(87)
KSK -133	7.13±0.11	8.63±0.14(121)	8.48±0.24(118)	6.76±0.08(95)	7.03±0.08(98)	6.84±0.04(96)

Table 4. Root dry weight of ten rice genotypes after 4 weeks exposure to Zn sources and its combination with NaCl

	Non- Saline			Saline		
Genotypes	**Control**	**$ZnSO_4$**	**Zn-EDTA**	**70 mM**	**$ZnSO_4$**	**Zn-EDTA**
Pak Basmati	1.34±0.02	1.61±0.02(121)	1.52±0.04(113)	1.23±0.08(91)	1.28±0.06(95)	1.20±1.24(96)
Sup Basmati	1.30±0.01	1.58±0.08(123)	1.53±0.11(119)	1.20±0.01(91)	1.25±0.02(96)	1.24±1.14(95)
Basmati-198	1.29±0.02	1.59±0.02(125)	1.50±0.04(116)	1.10±0.04(85)	1.13±0.01(87)	1.11±0.03(75)
Basmati -370	1.35±1.25	1.57±0.05(118)	1.51±0.05(113)	1.17±0.06(86)	1.20±0.02(88)	1.18±0.02(87)
Basmati -385	1.36±0.08	1.59±0.07(117)	1.56±0.08(114)	1.23±0.04(90)	1.29±0.08(94)	1.27±0.05(94)
Basmati -515	1.29±0.02	1.62±0.04(127)	1.58±0.06(122)	1.15±0.01(89)	1.19±0.02(92)	1.17±0.02(90)
Basmati- 2000	1.28±0.04	1.82±0.08(145)	1.75±0.09(140)	1.12±0.03(87)	1.15±0.04(90)	1.13±0.03(88)
KS- 282	1.33±0.08	1.65±0.07(124)	1.60±0.12(120)	1.18±0.06(88)	1.22±0.04(92)	1.20±0.02(90)
IR -6	1.46±0.04	1.56±0.05(107)	1.49±0.06(102)	1.27±0.08(86)	1.31±0.05(89)	1.30±0.06(89)
KSK -133	1.41±0.06	1.66±0.06(117)	1.53±0.16(109)	1.32±0.07(93)	1.36±0.06(96)	1.34±0.11(95)

The impact of salinity was different substantially between rice genotypes. There was a marked decrease in plant biomass exposed to NaCl salinity stress when compared with control. At 70 mM NaCl salinity rice genotype KSK-133 showed smaller reduction in shoot fresh and dry weight (86% and 90%) and (95% and 93%) root fresh and dry weight, suggesting its better tolerance against salinity stress. Rice genotype Basmati-198, on the contrary, showed much greater sensitivity to NaCl treatment on an average (40% and 77% of control shoot fresh and dry weight) and (62 and 85% of control root fresh and dry weight) at salt stress, respectively. In general, shoot fresh and dry weight of tolerant genotype was less affected as compared to salt sensitive rice genotype. All the other were in between these two genotypes at salinity level.

The application of Zn significantly increased the plant biomass. Maximum shoot fresh and dry weight (183% and 208% of control and 157% and 145% of control root fresh and dry weight observed in Basmati-2000 by Zn application while the minimum (108% and 112%) shoot fresh and dry weight and (105% and 107%) root fresh and dry weight in IR-6 . The application of $ZnSO_4$ increased the shoot fresh and dry weight as compared to Zn-EDTA application. The rice genotype Basmati-2000 was considered as Zn-inefficient genotype and IR-6 was considered as Zn-efficient genotype.

In case of combined application of salinity and zinc, zinc application increased the plant shoot/ root fresh and dry weight in all rice genotypes. NaCl addition caused significant decrease in fresh and dry weight of shoot and root as compared to control in all ten rice genotypes irrespective of salinity level. Maximum shoot fresh and dry weight (on an average, 97% and 94% of control and 98% and 96% root fresh and dry weight of control, respectively) in KSK-133 while the minimum root fresh and dry weight (on an average, 62% and 81% of control and 65% and 87% of control root fresh and dry weight, respectively) was observed in rice genotype Basmati-198. However, zinc application significantly alleviated the effects of salinity in these plant growth parameters in both salt tolerant and salt sensitive rice genotypes. By comparing the both sources of Zn, $ZnSO_4$ increased the both shoot fresh and dry weight as compared to Zn-EDTA application.

Effect of salinity and zinc application on chlorophyll contents and leaf area:

Data regarding chlorophyll content and leaf area are presented in (Table 5 and 6). The salinity (70 mM NaCl) significantly decreased chlorophyll content and leaf area in

Table 5. Leaf area (cm) of ten rice genotypes after 4 weeks exposure to Zn sources and its combination with NaCl

	Non- Saline			Saline		
Genotypes	**Control**	**$ZnSO_4$**	**Zn-EDTA**	**70 mM**	**$ZnSO_4$**	**Zn-EDTA**
Pak Basmati	9.21±0.23	11.14±1.08(121)	10.86±0.18(118)	7.92±0.45(86)	8.43±0.91(92)	8.15±0.91(88)
Sup Basmati	9.53±0.28	10.82±0.82(114)	10.48±0.33(110)	7.45±0.47(78)	8.40±0.49(88)	8.10±0.48(85)
Basmati-198	8.42±3.41	10.90±0.31(129)	10.65±0.38(126)	5.87±0.13(70)	7.01±0.63(83)	6.83±0.43(81)
Basmati -370	9.45±0.76	11.02±0.36(117)	10.97±0.62(116)	7.23±0.31(76)	8.34±0.53(88)	8.16±0.53(86)
Basmati -385	9.05±0.66	10.83±0.49(120)	10.56±0.63(117)	7.88±0.39(87)	8.38±0.55(93)	8.12±0.42(90)
Basmati -515	8.71±0.40	10.85±0.42(125)	10.47±0.64(120)	7.93±0.10(91)	8.25±0.85(95)	8.09±0.67(92)
Basmati- 2000	8.39±0.38	11.45±0.53(136)	11.19±0.45(133)	7.22±0.51(86)	8.03±0.52(96)	7.83±0.34(93)
KS- 282	9.49±0.20	10.93±0.67(115)	10.54±0.63(111)	7.18±0.82(84)	8.04±0.66(95)	7.71±0.58(91)
IR -6	10.11±0.06	10.81±0.21(107)	10.39±0.45(103)	7.33±0.43(73)	8.45±0.42(84)	7.53±0.54(92)
KSK -133	9.67±0.18	10.95±0.58(113)	10.51±0.65(109)	8.17±0.35(94)	8.48±0.55(98)	8.26±0.89(95)

Table 6. Chlorophyll content (SPAD) of ten rice genotypes after 4 weeks exposure to Zn sources and its combination with NaCl

	Non- Saline			Saline		
Genotypes	**Control**	**$ZnSO_4$**	**Zn-EDTA**	**70 mM**	**$ZnSO_4$**	**Zn-EDTA**
Pak Basmati	25.03±0.79	29.14±1.18(116)	27.14±0.60(108)	16.63±1.53(66)	23.42±0.50(93)	21.08±0.78(84)
Sup Basmati	24.93±0.91	30.18±1.17(121)	28.12±1.12(113)	17.53±1.76(70)	22.79±1.10(91)	22.05±0.83(88)
Basmati-198	16.10±0.66	22.77±1.00(141)	21.78±1.13(136)	10.25±0.33(64)	13.86±0.67(86)	13.38±1.56(83)
Basmati -370	29.42±0.27	31.60±0.57(108)	30.80±0.90(105)	22.50±1.07(76)	26.40±0.73(90)	25.26±0.39(86)
Basmati -385	23.50±1.26	28.13±1.08(120)	26.28±0.21(111)	19.04±0.42(81)	22.63±0.64(96)	21.23±1.02(90)
Basmati -515	24.62±1.01	28.30±0.50(115)	26.63±0.90(108)	20.02±0.34(81)	21.54±0.80(87)	20.85±0.65(85)
Basmati- 2000	19.91±0.70	31.69±0.61(159)	30.98±0.71(156)	17.52±0.54(88)	18.89±1.10(95)	17.61±0.73(88)
KS- 282	18.53±0.88	26.68±0.92(143)	24.70±1.43(133)	16.25±0.53(88)	17.53±0.86(94)	17.10±0.92(92)
IR -6	16.91±0.65	18.10±0.63(107)	17.73±173(104)	15.12±1.11(89)	16.23±0.10(96)	15.94±0.72(94)
KSK -133	28.07±0.75	30.10±0.52(107)	29.70±1.10(105)	26.05±0.90(93)	27.42±1.06(98)	26.63±0.74(95)

all ten rice genotypes. In salt sensitive Basmati-198, chlorophyll content and leaf area significantly decreased at salinity level (70 mM NaCl). Addition of zinc improved chlorophyll content and leaf area more effectively at adequate level (15 ppm) in salt tolerant rice genotype as compared to salt sensitive rice genotype.

Based on this data, we identified the most tolerant rice genotype KSK-133 (on an average, 93% and 94% of control chlorophyll content and leaf area at salt stress, respectively). Rice genotype Basmati-198, on the contrary, showed much greater sensitivity to NaCl treatment (on an average 64% and 70% of control chlorophyll content and leaf area at salt stress, respectively). In general, chlorophyll content and leaf area of KSK-133 was less affected as compared to Basmati-198 rice genotype. All the other genotypes values were in between these two genotypes at salt stress.

The application of Zn significantly increased the chlorophyll content and leaf area. Maximum chlorophyll content and leaf area (159% and 136%) observed in Basmati-2000 by Zn application while the minimum chlorophyll content and leaf area (107% and 107%) observed in IR-6 by Zn application. Among the both of Zn sources, $ZnSO_4$ increased the chlorophyll content and leaf area as compared to Zn-EDTA. On the basis of highest Zn concentration, Basmati-2000 was considered as Zn-inefficient genotype and IR-6 was considered as Zn-efficient genotype.

In case of combined application of salinity and zinc, zinc application increased the fresh and dry weight and root in all rice genotypes. NaCl addition caused significant decrease in chlorophyll content and leaf area as compared to control in all ten rice genotypes irrespective of salinity level. Maximum chlorophyll content and leaf area (on an average, 98% and 98% of control, respectively) in KSK-133 while the minimum chlorophyll content and leaf area (on an average, 86% and 83% of control, respectively) was observed in rice genotype Basmati-198. However, zinc application significantly alleviated the effects of salinity in these plant growth parameters in both salt tolerant and salt sensitive rice genotypes. Comparatively both of the sources application under saline conditions, $ZnSO_4$ increased the chlorophyll content and leaf area as compared to Zn-EDTA application.

Effect of salinity on chemical attributes: Considerable differences were observed for concentrations of Na^+, K^+, and K^+: Na^+ ratio in the shoot (Table 7, 8 and 9). Concentration of Na^+ differed significantly between control and 70 mM salinity. The lowest Na^+ concentrations were observed in KSK-133 and the highest in Basmati-198 at 70 mM salinity

Table 7. Sodium concentration (mM) of ten rice genotypes after 4 weeks exposure to Zn sources and its combination with NaCl

	Non- Saline			Saline		
Genotypes	**Control**	**$ZnSO_4$**	**Zn-EDTA**	**70 mM**	**$ZnSO_4$**	**Zn-EDTA**
Pak Basmati	78.10±1.22	68.23±1.64(87)	71.42±1.16(91)	125.60±0.88(161)	102.30±1.19(131)	108.05±1.18(138)
Sup Basmati	94.24±1.29	69.38±1.88(74)	72.30±2.16(77)	141.10±1.74(150)	119.80±1.49(127)	122.90±1.08(130)
Basmati-198	81.30±0.82	64.96±2.20(80)	69.10±1.38(85)	160.40±2.93(197)	126.92±1.33(156)	138.20±1.47(170)
Basmati -370	65.24±1.58	58.05±1.09(89)	62.31±1.19(95)	120.50±2.42(185)	101.50±1.80(155)	104.30±1.11(160)
Basmati -385	88.07±1.58	68.14±1.68(77)	71.42±1.47(81)	142.50±1.81(162)	114.73±2.22(130)	113.91±1.44(129)
Basmati -515	90.60±1.42	68.54±2.41(76)	70.22±1.32(78)	140.30±1.95(154)	110.62±2.44(122)	118.31±1.11(130)
Basmati- 2000	65.45±1.78	47.29±1.51(72)	49.12±1.71(75)	123.50±1.62(189)	100.32±2.78(153)	107.82±1.22(165)
KS- 282	80.58±2.29	66.39±1.22(82)	68.50±0.85(85)	133.40±2.21(166)	110.13±1.65(136)	128.43±1.71(160)
IR -6	76.56±2.76	69.64±0.62(91)	73.16±1.21(95)	143.75±1.20(188)	115.34±1.31(151)	117.74±1.65(154)
KSK -133	78.51±1.19	62.27±0.63(80)	68.06±1.38(87)	115.24±1.51(147)	99.24±1.55(126)	100.80±1.93(128)

Table 8. Potassium concentration (mM) of ten rice genotypes after 4 weeks exposure to Zn sources and its combination with NaCl

	Non- Saline			Saline		
Genotypes	**Control**	**$ZnSO_4$**	**Zn-EDTA**	**70 mM**	**$ZnSO_4$**	**Zn-EDTA**
Pak Basmati	128.85±2.02	171.92±2.17(133)	164.14±1.82(127)	99.61±0.72(77)	112.30±2.17(87)	108.12±1.11(84)
Sup Basmati	123.90±1.65	156.60±1.65(126)	148.93±1.43(120)	100.25±0.98(81)	107.50±1.08(87)	103.44±1.08(83)
Basmati-198	110.74±1.47	148.80±1.10(134)	140.87±1.55(127)	80.82±1.64(73)	92.34±1.31(83)	88.93±1.32(80)
Basmati -370	114.81±3.65	153.95±1.60(134)	146.83±0.85(128)	92.22±0.80(80)	99.74±2.17(87)	97.43±3.31(85)
Basmati -385	134.60±1.39	149.16±0.73(111)	142.13±1.85(106)	98.72±0.51(74)	116.23±2.29(86)	112.18±1.58(83)
Basmati -515	129.42±0.59	153.64±1.82(119)	144.87±1.70(112)	100.64±1.58(78)	115.18±3.36(89)	110.37±1.03(85)
Basmati- 2000	141.71±1.04	195.47±0.61(138)	182.68±2.25(129)	115.11±1.31(81)	123.24±1.85(87)	116.47±2.03(82)
KS- 282	133.32±2.16	148.45±1.27(111)	142.96±3.09(107)	106.84±0.61(80)	112.10±1.38(84)	109.83±1.65(82)
IR -6	135.23±2.10	145.48±1.06(107)	140.37±1.94(104)	99.73±1.15(74)	121.91±1.44(90)	115.47±1.75(85)
KSK -133	132.20±0.91	178.45±1.82(135)	167.12±1.19(126)	115.82±0.93(88)	123.53±1.47(93)	117.70±2.56(89)

Table 9. K^+/Na^+ Ratio of ten rice genotypes after 4 weeks exposure to Zn sources and its combination with NaCl

	Non- Saline			Saline		
Genotypes	**Control**	**$ZnSO_4$**	**Zn-EDTA**	**70 mM**	**$ZnSO_4$**	**Zn-EDTA**
Pak Basmati	1.65±0.26	2.52±0.19	2.30±0.03	0.79±0.4	1.10±2.5	1.00±3.7
Sup Basmati	1.39±0.51	2.26±0.07	2.06±0.03	0.71±0.2	0.90±2.5	0.84±2.1
Basmati-198	1.36±0.53	2.29±0.04	2.04±0.05	0.50±0.3	0.73±2.5	0.64±5.3
Basmati -370	1.77±0.65	2.65±0.10	2.36±0.02	0.77±0.1	1.03±2.6	0.93±2.4
Basmati -385	1.53±0.15	2.19±0.05	1.99±0.03	0.69±0.3	1.01±2.6	0.98±2.2
Basmati -515	1.43±0.95	2.24±0.15	2.06±0.07	0.61±0.3	0.98±2.5	0.93±4.6
Basmati- 2000	2.20±0.69	4.13±0.28	3.72±0.02	0.94±0.3	1.23±2.7	1.08±3.4
KS- 282	1.66±0.54	2.24±0.03	2.09±0.02	0.80±0.2	1.02±2.8	0.87±2.9
IR -6	1.77±0.60	2.09±0.14	1.92±0.04	0.69±0.4	1.06±2.6	0.98±2.6
KSK -133	1.68±0.35	2.87±0.03	2.46±0.01	1.01±0.4	1.25±2.8	1.17±3.6

Table 10.Zinc concentration (mM) of ten rice genotypes after 4 weeks exposure to Zn sources and its combination with NaCl

	Non- Saline			Saline		
Genotypes	**Control**	**$ZnSO_4$**	**Zn-EDTA**	**70 mM**	**$ZnSO_4$**	**Zn-EDTA**
Pak Bas	0.53±1.47	0.76±0.81(143)	0.68±1.11(129)	0.33±0.64(62)	0.49±2.50(94)	0.42±2.22(79)
Sup Bas	0.50±1.70	0.74±1.07(150)	0.71±1.11(144)	0.31±0.48(63)	0.42±1.11(84)	0.35±0.65(72)
Bas 198	0.46±1.70	0.74±0.52(161)	0.67±1.78(145)	0.26±0.71(53)	0.37±1.55(80)	0.32±2.12(70)
BAS-370	0.55±0.91	0.79±0.19(142)	0.68±1.08(122)	0.38±0.83(67)	0.50±1.87(91)	0.45±1.55(82)
BAS-385	0.54±0.69	0.75±0.83(139)	0.70±1.47(128)	0.33±0.94(60)	0.49±2.29(88)	0.40±1.55(73)
BAS-515	0.48±0.99	0.76±0.46(158)	0.72±1.71(150)	0.36±1.05(74)	0.46±2.78(96)	0.40±1.31(83)
BAS-2000	0.45±1.03	0.87±0.51(193)	0.74±1.25(166)	0.35±0.42(76)	0.43±1.29(96)	0.38±1.11(84)
KS- 282	0.48±0.85	0.77±1.41(161)	0.71±2.42(148)	0.39±0.74(80)	0.45±1.75(93)	0.41±2.06(85)
IR -6	0.57±0.58	0.73±0.60(127)	0.65±1.08(116)	0.38±2.18(65)	0.50±0.85(86)	0.39±1.75(68)
KSK -133	0.54±1.40	0.84±0.97(154)	0.72±2.80(134)	0.48±0.83(89)	0.53±1.08(97)	0.50±1.04(92)

Each value is averages of 4 replicates± S.D, figures not sharing the same letters in column differ significantly at $P \leq 0.05$ level of probability and values in parenthesis are the percent of control.

level. The trend in case of potassium concentration was almost reverse, showing decreased K^+ concentration in all ten rice genotypes. However, this decrease in potassium was more prominent in salt sensitive genotype as compared to salt tolerant rice genotype. Rice genotype KSK-133 performed better as it maintained high level of K^+ at all the salinity level in comparison to the other nine genotypes. The increasing uptake of Na^+ with increase in the salinity levels resulted in a decrease of K^+/Na^+ ratio (Table 9). The highest potassium concentration at salinity level resulted in maintaining higher K^+/Na^+ ratio in rice genotype KSK-133, showing better performance under saline conditions. Addition of zinc in solution significantly improved K^+/Na^+ ratio in salt tolerant rice genotype but no significant effect of potassium on K^+/Na^+ ratio in salt sensitive genotype.

Based on this data, we identified the most tolerant rice genotype KSK-133 (on an average, 147% and 88% of control shoot Na^+ and K^+ concentration at salt stress, respectively). Rice genotype Basmati-198, on the contrary, showed much greater sensitivity to NaCl treatment (on an average 197% and 73% of control shoot Na^+ and K^+ concentration at salt stress, respectively). In general, less Na^+ and more K^+ concentration observed in tolerant genotype compared to salt sensitive rice genotype.

The application of Zn significantly decreased the shoot Na^+ and increased K^+ concentration. Minimum shoot Na^+ and maximum K^+ concentration (72% and 138%) observed in Basmati-2000 by Zn application while maximum shoot Na^+ and minimum K^+ concentration (91% and 107%) in IR-6. The application of $ZnSO_4$ decreased the shoot Na^+ and increased shoot K^+ concentration as compared to Zn-EDTA application. The rice genotype Basmati-2000 was considered as Zn-inefficient genotype and IR-6 was considered as Zn-efficient genotype.

In case of combined application of salinity and zinc, zinc application decreased the shoot Na^+ and increased shoot K^+ concentration in all rice genotypes. NaCl addition caused significant decrease in shoot K^+ concentration as compared to control in all ten rice genotypes irrespective of salinity level. Maximum shoot Na^+ and K^+ concentration (on an average, 156% and 83% of control, respectively) in Basmati-198 while the minimum shoot Na^+ and K^+ concentration (on an average, 126% and 93% of control, respectively) was observed in rice genotype KSK-133. The addition of Zn through $ZnSO_4$ decreased the shoot Na^+ and increased the shoot K^+ concentration compared to Zn-EDTA application.

Effect of salinity and zinc application on Zn concentration: Considerable differences were observed for concentrations of Zn in the shoot (Table 10). Concentration of Zn differed significantly between control and 70mM NaCl salinity. The application of Zn significantly increased the shoot Zn concentration. Maximum shoot Zn concentration (193% of control) observed in Basmati-2000 by Zn application while minimum shoot Zn concentration (127% of control) observed in IR-6. The application of $ZnSO_4$ increased shoot Zn concentration as compared to Zn-EDTA application.

In case of combined application of salinity and zinc, zinc application increased shoot Zn^{2+} concentration in all rice genotypes. NaCl addition caused significant decrease in shoot Zn^{2+} concentration as compared to control in all ten rice genotypes irrespective of salinity level. Maximum shoot Zn concentration (on an average, 97% of control) in KSK-133 followed by Basmati-2000 (on an average, 96% of control) while the minimum shoot Zn concentration (on an average, 80% of control) was observed in rice genotype Basmati-198.

Zinc efficiency :The Zn efficiency was calculated as per formula but the ranking of genotypes were calculated by using Microsoft excel program with minimum and maximum values of given Zn efficiency of genotypes (Fig. 1). The most Zn efficient genotype was IR-6 (96.7 %) while Bastmi-2000 was considered as inefficient (53.2%) zinc efficiency.

DISCUSSIONS

A large of variation observed in plants for salt tolerance during early growth stages at 70 mM salinity. The existence of such kind of genotypic variation for NaCl tolerance could be useful for the development of high-yielding salt-tolerant genotypes (Ashraf *et al*., 1997; Munns *et al*., 2000). Results of present study revealed that salt stress significantly reduced the growth of all rice genotypes by affecting plant moropho-physiological characteristics like plant biomass and K^+/Na^+ ratio confirming the previous findings that salinity caused negative impact on plant by reducing its growth (Khan, 2001; Akram *et al*., 2010; Ahmad, 2010).

Like other workers, results of our study also depicted that salinity caused reduction in plant height, fresh weight as well as dry weight. Unlike previous report (Huang *et al*., 2006), we found that NaCl treatment firstly decreased shoot growth rather than root, resulting in a decrease in shoot to root ratio. This may have been due to the different genotype or different and fluctuating salinity treatments in our experiment. Kumar *et al*. (2009) evaluated 11 varieties of rice under saline conditions and found that tolerant varieties have greater plant height and dry matter weight.

Among all, rice genotype KSK-133 showed high root and shoot dry weight that indicates its tolerance at salinity level. It might be due to different mechanism of ion uptake and the results were in line of those obtained in previous studies (Ahmad, 2010; Akram *et al*., 2010).

Exposure of plants against salinity stress leads to decreased plant growth by reducing photosynthetic activities and high salt stress (70 mM NaCl) significantly reduced the chlorophyll contents in all genotypes which ultimately reduced plant growth as it is also in agreement with the results reported by Yadarlou and Majidihervan (2008). The decrease in SPAD value can be due to the loss of chlorophyll and yellow leaves caused by salinity.

Sodium transport from growth medium to cytoplasm of plant cells depends upon electrochemical potential gradient of Na^+ and presence of Na^+ transport channels in the plasma membranes, which permit Na^+ penetration (Jacoby, 1999). This selective uptake of Na^+ ions in plasma membrane may be a key factor in sensitivity or tolerance of rice genotypes. High salinity induces an increase in the Na^+ ions concentration which competes with the uptake of other important nutrients ions like K^+ and ultimately leads to K^+ scarcity in plant cells (Khan, 2001; Khan *et al*., 2000). These results are consistent with those obtained in other rice varieties, FL478 and IR29 (Walia *et al*., 2005), wheat (Saqib *et al*., 2005; Sharma *et al*., 2005), soybean and cucumber (Dabuxilatu and Ikeda, 2005), where there was a positive relationship between salt concentration and Na^+ concentration in root and stem. Similar results have been obtained in other rice varieties, FL478 and IR29 (Walia *et al*., 2005) and IR36 (Gong *et al*., 2006), and in wheat (Saqib *et al*., 2005). Results of current study depicted that high level of Na^+ ions in leaf sap of salt sensitive rice genotypes negatively affects their growth. Our results are in line with previous studies in which the concentration of Na^+ ions increased with increasing salinization (Munns *et al*., 1995) that leads to salt injury to plants (Serrano *et al*., 1999). In case of Zn application, these results are also in agreement with the findings of Saleh and Maftoun (2008). They approved that application of Zn reduced the toxic effect of NaCl and improve the plant growth under the saline conditions.

Zinc is very essential micronutrient for plant survival in saline environment. It contributes in the activation of many enzymes which are involved in photosynthesis of plants. The external Zn concentrations could mitigate the adverse effect of sodium chloride (NaCl) by inhibiting Na^+ and/or Cl^- uptake or translocation. Alpaslan *et al*. (1999) concluded that in the salt affected areas, Zinc application could alleviate possible Na^+ and Cl^- injury in plants. Soil salinity may reduce Zn uptake due to stronger competition by salt cations at the root surface (Khoshgoftar *et al*, 2004).Sodium transport from the external media to cytoplasm of plant cell is a passive process. It depends on electrochemical potential gradient of Na^+ and presence of Na^+ permeable channels in the plasma membranes, which allow Na^+ permeation. Regulation and selectivity of such channel seems to be responsible for Na^+ exclusion in many salt tolerant plants (Jamalomoidi *et al.* 2006). This differential selectivity of plasma membrane may be contributing factor in sensitivity/tolerance of these hybrids as high salt (NaCl) uptake competes with the uptake of other nutrient ions, leading to Zn deficiency. High NaCl induces increase in Na^+ and decrease in Zn in plants (Salama *et al*. 1996; El-Fouly *et al*. 2001).

In the present study, a higher concentration of Na^+ in plant tissues adversely affected the growth of the rice seedlings under saline conditions. Na^+ concentration increased with increasing salinity levels, similar to observations made by Munns *et al*., (1995) and this caused salt injury to plants (Serrano *et al*., 1999). Zn also greatly affects the structural and functional integrity of cell membranes (Welch *et al.*, 1982; Cakmak and Marschner, 1988). Salinity cause the Zn deficiency because of the antagonistic effect of alkaline earth metals like Na^+, K^+ and Mg which are dominant under saline conditions. Such type of cations competes with Zn for absorption at root surface and cause Zn deficiency (Chaudhry and Loneragan, 1972). It was observed that in Zn-deficient plants, loss of membrane integrity and increase in membrane permeability are very common in different crop species. Loss of membrane integrity under Zn deficiency may affect the uptake and accumulation of Na^+ at toxic levels in plants. Therefore, improving the Zn nutritional status of plants could greatly improve their salt stress tolerance because Zn involve in the orientation of macro molecules and have a strong interaction with phospholipids and sulfohydrall group and maintain the cell integrity (Cakmak and Marschner, 1988). The ability of plant cells to retain Zn is crucial for salt tolerance, rather than their ability to restrict Na^+ from uptake. As a result of a better ability to retain Zn, salt-tolerant cultivars were able to maintain higher Zn/Na^+ in the root, enabling better performance in saline conditions. This is consistent with literature reports (Norvell and Welch; 1993, Alpaslan *et al.* 1999 and Cakmak, 2000) supports the results that there is a positive correlation between seedling performance and adult plant performance. Salt tolerance rice genotypes had more plant biomass, root and shoot fresh weight than salt sensitive rice genotypes (Zayed *et al*., 2011).

Lowland rice genotypes may differ in Zn use was reported by Clark, 1990 and De Datta, 1993. It may be due to the plant growth rate and yield was well under the Zn deficient conditions are known as a Zn efficient genotype (Erenoglu *et al.,* 2000 and Graham and Rangel, 1994). Genotypic differences for Zn use efficiency for several crops have been reported by many workers (Cakmak *et al., 1*994 Graham *et al.,* 1992, Rangel 1995, Wiren *et al.,* 1994 and Xiaopeng *et al.*, 2005). In case of Zn-deficient conditions (control), among the all rice genotypes, IR-6 performed best by producing more biomass production and more shoot K and Zn concentration as compared to other genotypes. The genotype IR-6 was characterized as Zn-efficient genotype which can grow well under Zn deficient conditions. The efficiency of rice genotypes was also measured by (Singh *et al.* 2005, Chen *et al*. 2009). Our work is also supported by these researchers. They observed that Zn-efficient genotype survived well under Zn deficient conditions by adopting the following mechanisms: (i) releasing certain organic acids, (ii) more proliferation of crown root and (iii) internal Zn distribution. Singh *et al.* (2005) observed in case of cereals that Zn efficient genotype grow and yield well under Zn deficient conditions while Zn inefficient genotype can not grow and yield well.

Conclusion: Present study revealed that at seedling growth stage, shoot/root dry weight, chlorophyll contents and K^+/Na^+ ratio can be a good parameters for screening rice genotypes against salinity and to assess the Zn efficient and inefficient genotype. On the basis of these parameters, the genotype KSK-133 evaluated as a salt tolerant while BAS-198 evaluated as salt sensitive genotype. On the basis of Zn concentration, Basmati-2000 evaluated as a Zn-inefficient genotype while IR-6 as Zn efficient genotype. These results can be a good source for the plant breeders and plant physiologists engaged in the development of salt tolerant rice genotypes. These salinity tolerant genotypes could be exploited in the breeding program for the development of elite genotypes having high salinity tolerance and have the potential to grow effectively on natural salt affected soil. Therefore further work is needed to evaluate the performance of this screened material in soil culture.

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