An Authenticated Approach towards the Confirmation of Tolerance and Susceptibility against Zinc Stress Toxicity in Reciprocal Introgression Lines of Rice (Oryza sativa L.)

Aijaz Ahmed Soomro, Zhang Jian

Abstract— The acid soils and lands of zinc mining areas are mostly contaminated with zinc toxicity. Therefore, keeping in view the importance of cultivation of rice in such Zinc contaminated areas, this study had been conducted to observe the ill-effects of Zinc (Zn) toxicity on the growth of rice and Zinc content in root and shoot of rice plants and to develop the zinc biofortified rice. Two parents MH63 indica and 02428 japonica along with six reciprocal introgression lines of each highly tolerant and highly susceptible from both of the respective backgrounds to zinc stress brought under study in green house conditions. Zinc in the form of $ZnSO_4.7H_2O$ at the rate of 200 ppm and control was consequently applied to the rice seedlings for 21 days in hydroponic culture. A decrease in all parameters of the treated seedlings was observed. The highly susceptible RI lines had more content than highly RI tolerant lines. However, the phenotypic performance of tolerant RI lines was better than highly susceptible RI lines. Therefore, rice crop has been brought under further study to know its complete mechanism for zinc toxicity tolerance.

Index Terms /Key words: Green house, Extreme lines, Zinc, Root, Shoot, Seedlings, Rice

1 Introduction

International Zinc association (IZA) [1] has informed that zinc (Zn) is essential for humans, animals and plants. On the one side it is important for many biological functions and on the other side it plays a central role in more than 300 enzymes in human body. Zinc has been found in all parts of the body, such as, fluids and cells, tissues, bones, and organs. Of the 100% zinc in body, only the muscles and bones contain 90%. Hence, it is essential for cell division, fertility, immune system, skin, hair and nails, taste, smell, appetite and vision (eyesight). The daily intake rate of zinc is 5 mg for infants, 10 mg for children, 12 mg for women with no pregnancy, 15 mg for pregnant women, 16 mg for lactating women and 15 mg for men. However, pregnant as well as lactating women need more zinc for optimum development of fetus and for new born baby. The working people in coal mines, working people with refining and smelting zinc metals and peoples living near to wastes sites and smelting operation areas may be exposed to high levels of zinc.

In 1998, Meng Ling et al., [2] have studied the response of wheat to lead, cadmium and zinc. They conducted their study in contaminated area named Huize Lead-Zinc Mine, Yunnan Province of China. They found that cadmium, lead and zinc might inhibit DNA transcription and had much more effect on gene expression than structure in wheat, which might acclimate to metal pollution after having grown in polluted area for long time. They also found that the interference of these metals in gene expression might be one of the main mechanisms of plant adaptation to metal toxicity. Eric et al., (2004) [3] have studied the properties of soil affecting the toxicity of zinc to microbial properties in 15 top-soils of Europe, which were freshly spiked with $ZnCl_2$, with concentration of total range of zinc from 7 to 191 mg/kg. And they also conducted their research in three sites under galvanized pylons where concentration of zinc was ranging from 2,100 to 3,700 mg Zn/kg. They suggested that the concentrations of background Zn in soil affect the sensitivity of soil micro organisms to added zinc was confirmed there by the significant correlations between toxicity threshold and background Zn for the potential nitrification rate (PNR) and substrate induced respiration (SIR) tests. Annette L., et al., (2005) [4] have conducted a research on 11 contaminated soils in Australia and two contaminated soils in USA, in total 13 metal contaminated soils with Zn, Cd, Pb, and Cu. They concluded that the kinetically labile solid phase pool of metal which was included in Diffusive Gradients in Thin Films (DGT) measurement, played an important role in zinc (Zn) and cadmium (Cd) uptake by wheat along with the labile metal in soil solution.

Therefore, by keeping in view the importance of Zn this study was conducted to study the ill-effects of zinc on crop plants, to find out part of the plant body which absorbs more Zn, to evaluate the concentration of Zn in both root and shoot of rice and to initiate the development of zinc biofortified rice variety to control the hidden hunger among human population of the zinc deficient areas of the world.

2 Materials and method

2.1 Plant material

Two sets of 24 (six highly tolerant and six highly susceptible

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Reciprocal Introgression Lines RILs) from each MH63 indica and 02428 japonica background were selected from already used 200 RI line populations from three regular experiments conducted to find out zinc stress toxicity tolerant and susceptible Reciprocal Introgression Lines (RILs) were brought under careful study to re-confirm the extreme lines (highly tolerant and highly susceptible) for zinc related traits (as per following formula) and then zinc concentration from root and shoot of each extreme line was also detected through atomic absorption spectrophotometry.

Extreme line= control-stress/control

2.2 Methodology for phenotypic evaluation Zn^{2+}-related traits from Extreme (highly tolerant and highly susceptible) RI lines

The seed of extreme lines was surface sterilized with 1% hypochlorite solution for 10 minutes and rinsed well with distilled water. Then seed was soaked in distilled water in the dark at 30^oC for 48 hours. The most uniform 10 emerged seeds for each extreme RI line per replication were directly sown into perforated Styrofoam sheets covered with nylon net at the bottom. For each experimental condition (i.e. control and treated) most uniform 10 emerged seeds from parents MH63 indica and 02428 japonica were also sown in each container randomly.

The Styrofoam sheets were allowed to float on water up to 7 days and then transferred to Yoshida culture solution [5] without applying suitable concentrations of Zn^{2+} stress for first 15 days. On the 16th day of seeding at 3rd leaf stage, the zinc in the form of $ZnSO_4.7H_2O$ at the rate of 0 (control) and 200 ppm (stress) was applied for 21 days. The pH of the solution on alternative day was adjusted to 5.0 with 1 *N* NaOH/HCl. The solution was renewed every fifth day. The experimental materials were laid out in two replications for all experiments (control and treated) under green house conditions at around 32/25^oC in day/night, 70-75% of relative humidity and average 12 hrs photoperiod. The largest root and shoot length and fresh root and shoot weight were recorded after 21 days of the treatment. Then the samples were kept in oven for 72 hours (3 days) at gradually increasing temperature maximum 65^oC [6]. Finally dry weight of root and shoot was recorded to get the standard value for concentration of zinc per line.
The relative variation among all traits root length (RRL) and shoot height (RSH) as well as root dry weight (RDW) and shoot dry weight (SDW) were calculated by comparison between plants under control and treated conditions to the formula [7]:
1-Relative variation of length=
[(Length of treated plant-Length of control plant/Length of control plant)]X100

2-Relative variation of dry weight=
[(DW of treated plant-DW of control plant/DW of control plant)]X100

3 Results

3.1 Phenotypic performance of zinc toxicity tolerance-related traits in Extreme Reciprocal Introgression Lines in MH63 indica and 02428 japonica backgrounds (21 days after stress)

The two parents MH63 indica and 02428 japonica were similar in all traits of control condition except shoot height (24.25 cm) and shoot weight (1.96 mg) under control condition, where MH63 indica had significantly higher values than those of 02428 japonica parents which has shoot height (20.45 cm) and shoot weight (1.16 mg). (Table1).

Under Zn^{2+} stress condition, 02428 japonica parents had higher trends for root length (4.16 cm significantly higher) and shoot weight (1.24 mg) than that of MH63 indica parents which has root length (3.51 cm) and shoot weight (1.12 mg).

For zinc^{2+}-relative value % in control environmenet 02428 japonica parents had higher values in all traits of evaluation with significantly higher root length at (35.23) and shoot dry weight (6.90).

When losses were compared between MH63 indica parents under control condition to their respective tolerant RI lines in MH63 indica background under control condition; the MH63 indica parents had achieved significantly higher trends at root length (RT) lost 24.69% at tolerant root length (TRL), 20% at tolerant root weight (TRW), 0.74% at tolerant shoot height (TSH), and 14.29% at tolerant shoot weight (TSW).

However, when losses were compared between 02428 japonica parents under control condition to their respective 02428 japonica RI lines under control condition, the 02428 RI lines had showed significantly higher trends at all traits except TRW, where 02428 japonica parents had indicated significantly higher root weight (4.0.4 mg) than that of 02428 japonica RI lines' root weight (0.03 mg). In this respect, 02428 japonica parents had lost 27.39% at root length (RL), 23.30% at shoot height (SH), 35.91% at shoot weight (SW) except root weight (RW), where 02428 japonica parents had gained 25% more root weight than that of their respective RILs.

In case of comparison between MH63 indica parents under control with those of their respective MH63 indica susceptible RI lines under control;, the susceptible RI lines had lost 13.45% for susceptible root length (SRL), 40% (significantly different) for susceptible root weight (SRW), 1.07% for susceptible shoot height (SSH), and 17.35% for susceptible shoot weight (SSW).

When losses were compared between 02428 japonica parents under control condition with those of their respective susceptible 02428 japonica RI lines under control condition, the susceptible RI lines lost 0.97% for susceptible root length (SRL), 50% (significantly different) for susceptible root weight (SRW), 1.66% for susceptible shoot height (SSH) and 10.34% for susceptible shoot weight (SSW) respectively.

When losses and gains were compared between the MH63 indica parents under zinc stress condition with those of

- *Corresponding Author: Aijaz Ahmed Soomro, Assistant Professor, Department of Agronomy, FCPD, Sindh Agriculture University, Tandojam, Pakistan.Post Code#:70060. PH- 00923023473482. E-mail: professoraijazahmed@gmail.com*
- *Co-Author: Zhang Jian, Institute of Crop Sciences, Chinese Academy of Agricltural Sciences, Beijing, China, PH-008615801498374. E-mail: 0603zhj@163.com*

their respective MH63 indica tolerant RI lines under zinc stress, almost all the RI lines had greater values than those of related parents in both backgrounds except TRW for which MH63 parents and MH63 tolerant RI lines had same values. In this respect, the parents in MH63 indica 11.59% at RL, 0.09% at SH, and 10.4% at SW

When losses and gains were compared between 02428 japonica parents under zinc stress condition with those of their respective 02428 japonica tolerant RI lines under zinc stress condition, almost all the RI lines had greater values than those of related parents in 02428 japonica backgrounds except TSW for which 02428 japonica parents had same values with that of 02428 tolerant RI lines.; 02428 japonica had lost 7.35% for RL, 20% for RW, and 14.22% for SH.

In respect of comparison of losses between MH63 indica parents under zinc stress and MH63 indica susceptible RI lines under zinc stress; the RI lines lost 9.40% at SRL, 20% at SRW, 7.8% at SSH, 13.39% at SSW. While losses were compared between 02428 japonica parents under zinc stress with those of their respective 02428 japonica susceptible RI lines under zinc stress, almost all of the susceptible RI lines had showed smaller trends than those of their related parents except SRW, where 02428 japonica parents and 02428 japonica susceptible RI lines had similar means, susceptible reciprocal introgression lines (RILs) lost 22.84% at SRL (significant difference), 1.69% at SSH, and 18.55% at SSW respectively.

The ratio of Reciprocal Introgression lines showed transgressive segregations for all Zn^{2+} related traits and showed continuous variations in MH63 indica and 02428 japonica backgrounds (Table1).

3.2 Concentration of Zn^{2+} at cellular level in related traits of extreme RILs and parents under MH63 indica and 02428 japonica backgrounds (after 21 days of stress)

Under normal control conditions, MH63 parents had significantly higher concentration of zinc in root than that of 02428 japonica parents with the mean of 0.34 mg/L and 02428 japonica parents had lost 47.06%. But 02428 parents had significantly higher concentration of zinc at shoot parameter than that of MH63 indica parents with the mean of 0.9 mg/L and MH63 indica parents lost 37.78%.

While 02428 japonica parents under zinc stress condition had higher concentration of zinc in root (5.73 mg/L) than that of MH63 parents (5.08 mg/L) under zinc stress condition, therefore, MH63 indica parents lost 11.34%. The concentration of zinc in shoot (13.98 mg/L) of MH63 indica parents under zinc stress had significantly higher values than those of 02428 japonica parents (10.79 mg/L) under zinc stress; the 02428 japonica parents lost 22.82%.

In Zn^{2+} relative value %, MH63 indica parents had significantly higher relative values at root (1394.12) than that of 02428 japonica parents (3004.01). In case of shoot, the 02428 japonica parents had significantly higher relative values% at shoot parameters (1098.89).

While the losses and gains were compared for zinc concentration between MH63 indica under control condition with those of their respective MH63 tolerant RI Lines under control condition, the tolerant RILs in MH63 indica background lost 23.53% at zinc concentration in root (ZCRT) which is (significant difference), and MH63 indica parents had lost 26.32%, at zinc concentration in shoot (ZCS), it is also (significant difference).

In case of comparison between 02428 japonica parents with those of their respective 028428 japonica reciprocal introgression lines (RILs), the 02428 japonica parents had lost 25% at zinc concentration in root (ZCR) (significant difference) and 02428 tolerant RILs lost 37.78% at ZCShT (significant difference) than their respective parents.

When losses for concentration of zinc were compared in MH63 indica parents under control environment with those of their respective susceptible RILs under control environment, the susceptible RILs in MH63 indica background had lost 20.59% at zinc concentration in root of susceptible line (CZRS). In case of zinc concentration in shoot, MH63 indica parents had significantly lost 26.32% at zinc concentration in shoot (ZCSh).

In terms of comparison between 02428 japonica parents under control environment whit those of their respective susceptible RILs under control environment, 02428 japonica parents had lost 21.74% at ZCR, and susceptible RILs had lost 20% at ZCShS.

While losses for zinc concentration in MH63 indica parents under zinc stress environment were compared with those of their respective tolerant RILs under zinc stress environment, MH63 indica parents had higher values at all parameters. In this regard the tolerant RILs in MH63 indica background had comparatively lost 13.19% at ZCRT, 5.72% at ZCShT. When losses for zinc concentration in root and shoot of 02428 japonica parents under zinc stress environment were compared with those of their respective tolerant RILs under zinc stress, 02428 japonica parents had higher concentration of zinc in their root (5.73 mg/L) than that of their respective tolerant RILs, which had (4.70 mg/L) tolerant RILs in 02428 japonica background had comparatively lost 17.98% at ZCRT, except zinc concentration in shoot of tolerant lines (ZCShT) in 02428 japonica background (11.08mg/L), where tolerant RILs had higher values than that of parents and 02428 japonica parents had lost 2.62% at ZCShT.

In terms of comparison of losses and gains for concentration of zinc in MH63 indica parents under zinc stress environment with those of susceptible RILs under zinc stress environment, all the parameters of MH63 indica parents had higher values than those of susceptible RILs. In this respect, susceptible RILs in MH63 indica background had lost 2.17% at ZCRS, 12.30% at ZCShS. In case of comparison of losses and gains between 02428 japonica parents with those of their respective susceptible RILs, the susceptible RILs in 02428 japonica background had lost 16.93% at ZCRS, and 02428 japonica parents had lost 18.81% at ZCShS.

In case of comparison of all traits in MH63 indica parents as well as 02428 japonica parents under control condition with those of MH63 indica parents under zinc stress condition as well as 02428 japonica parents, all traits of evaluation in both of the backgrounds under zinc stress condition had predictable significantly higher concentration of zinc than those of the traits of evaluation under control condition respectively.

When means for concentration of zinc in roots of tolerant RI lines of MH63 indica background under zinc stress condition were compared with those of susceptible RI lines in MH63 indica background under zinc stress condition, mostly the averages for roots of the susceptible RI lines under zinc stress condition (4.97mg/L) with the range of (3.4 to 6.61 mg/L) were higher than those of roots of tolerant RI lines under zinc stress with the mean (4.41 mg/L) and range from (0.01 to 6.5 mg/L.

In terms of concentration of zinc in shoot of tolerant RI lines of Mh63 indica background with those of susceptible RI lines in MH63 indica background, the susceptible RI lines in MH63 indica background has higher concentration of zinc with the mean of (13.26 mg/L ZCShS) and range from (9.51 to 18.08 mg/L ZCshS). When same parameters were compared between tolerant RI lines in 02428 japonica background under zinc stress condition as well as susceptible RILs in 02428 japonica background under zinc stress condition, the susceptible 02428 japonica RILs had higher values than those of tolerant RILs in 02428 japonica background with the means of (13.29 mg/L in ZCRS) and range from (8.06 to 18.66 mg/L ZCRS) (Table2).

The ratio of Reciprocal Introgression lines showed transgressive segregations for all Fe^{2+} related traits and showed continuous variations in MH63 indica and 02428japonica backgrounds (Table2).

4 Discussion

The results of phenotypic data of this study for zinc toxicity stress tolerance in rice crop revealed that the excess of zinc is highly toxic to the rice crop. Therefore, rice plants showed decreased root length, stunted/shortened shoot elongation, lesser root and shoot weight than rice plants under control condition. It has been keenly observed that the plants under control environment had not only got higher quantitative-related trait values but also the plants under control condition were always looking fresh and normal green than plants under zinc toxicity stress condition. It gave us the strong support to write that excess of zinc has both qualitative and quantitative harmful effects on rice crop. As susceptible Reciprocal Introgression lines (RILs) from both MH63 indica as well as 02428 japonica backgrounds had higher concentration of zinc in root and shoot than respective tolerant RILs. It indicated that the susceptible RI lines possess higher ability of zinc absorption during toxicity period under zinc stress condition than respective tolerant lines. Hence phenotypic growth parameters of tolerant RI lines had higher trends than those of susceptible RI lines (Table1).

Another important point to note that concentration of zinc in roots of both tolerant as well as susceptible RI lines is significantly lower than those of tolerant and susceptible shoots in both of the MH63 indica and 02428 japonica backgrounds indicated that zinc is mobile to the upper parts of the plants, so for this, zinc was highly accumulated to the upper parts (shoots) of the plant body (Table2). Hence, it was accumulated more in shoots than roots, where it could produce its physiological and morphological harmful effects to the plants, which are mentioned in "Introduction and Review" as given above. Our findings are strongly supported by Zhang (2012-2013) [8]. In his latest findings for mobility of zinc from root to shoot and higher concentration of zinc in shoot than root were recorded (Zhang, 2012-2013). He had also detected the higher concentration of zinc in shoot than root under control condition and zinc toxicity stress environments. These all evidences for higher concentration of zinc in shoot than root gave strong support to the idea of harmfulness of zinc excess toxicity to the upper parts of plants is induced through root absorption, which is the first entrance to zinc absorption, then greatly moves to the upper parts of the plants.

However, the results of phenotypic performance of extreme RI lines (highly susceptible and tolerant RILs) under control and Zn^{2+} excess toxicity conditions indicated that MH63 indica and 02428 japonica backgrounds had some highly tolerant RI lines which could survive well under zinc excess toxicity condition. It is very much interesting to note that study on zinc content of Bean Plants by applying very low to high levels of zinc was also carried out by F.G. et al., in 1953 [9]. This paper is an evidence for the interest of Scientists in zinc content in plants and also a historical record for study of zinc content in plants. They detected different contents of the zinc, such as in stem 15.2 ppm at Z_0 and 32.8 ppm at Z_1; in mature leaves 22.6 ppm at Z_0 and 32.5 ppm at Z_1. Our results are in similarity with those of [10] and [11] who had also found same results for higher zinc concentration in shoots than in roots. The findings of [12] were also similar to our findings which indicated that the concentration of zinc in zinc toxicity stressed plants was expectedly higher than controlled plants.

5 Conclusion and Recommendations

The results of phenotypic data showed that all the traits of evaluation under this study, such as, root length, shoot height, root fresh as well as dry weight and shoot fresh as well as dry weight were highly susceptible to Zn^{2+} excess toxicity. It was because of that the plants under normal control conditions achieved higher trends of evaluation than those of plants under zinc (Zn) treatment/ toxicity stress. However, it can hopefully be estimated that as MH63 indica and 02428 japonica RI lines had some important highly zinc toxicity tolerant RI lines which could be highly effective to develop/release the new highly zinc excess toxicity tolerant lines/varieties. In the light of this study, it is suggested that these two backgrounds MH63 indica and 02428 japonica should be avoided to cultivate in zinc contaminated soils until and unless, new highly tolerant varieties/lines to these toxicities could be evolved and released for further replication up to the farmers who are real-end-users of agricultural inventions as well as innovations

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REFERENCES

[1] International Zinc Association (IZA), "Zinc: Essential for human health",

http://www.zinc.org/health/

http://www.zinc.org/health-learning-annex/

[2] Meng Ling Tan, De-yong, Wang, Huan-xiao, Duan, Chang-qun, Duan, Pei-shang, Gao, Shang-yi, "Study on the response of wheat to lead, cadmium and zinc", Journal of environmental sciences, vol. 10 (2): pp. 238-244, 1998.

[3] Erik Smolders, Jurgen, Buekers, Lan, Oliver, and J. Mike, McLaughlins, "Soil properties affecting toxicity of zinc to soil microbial properties in laboratory-spiked and filed contaminated soils," Environmental toxicology and Chemistry. 23 (11), 2633-2640. SETAC. Printed in USA, pp. 0730-7268/04, 2004.

[4] Annettte, L. Nolan, Hao, Zhang, and Mike, J., McLaughlin, "Prediction of Zinc, Cadmium, lead, and Copper Availability to Wheat in contaminated soils using chemical speciation, diffusive gradients in thin films, extraction and isotopic dilution techniques," J. of Environmental quality. 34: pp. 496-507, 2005.

[5] S. Yoshida, D. A. Forno, J. A. Cock, and K. A. Gomez, "Laboratory manual for plant physiological studies of rice," 3rd edn. International Rice Research Institute, Manila, The Philippines, 1976.

[6] Jian lin Wan, Hu qu Zhai, Jian Min Wan, and H. Iqihashi, "Detection and analysis of QTLs for ferrous iron toxicity tolerance in rice, Oryza sativa L.," Euphytica 131: pp. 201–206, 2003.

[7] Dufey Ine`s, Hakizimana Patrice, Draye Xavier, Lutts Stanley , Bertin Pierre, "QTL mapping for biomass and physiological parameters linked to resistance mechanisms to ferrous iron toxicity in rice," *Euphytica*, 167: pp. 143–160, 2009.

[8] Zhang Jian, "Mapping of QTLs for ferrous and zinc toxicity tolerance at seedling stage using two sets of Reciprocal Introgression lines in rice," (unpublished, submitted Thesis of M.Sc.) at Institute of Crop Sciences, Graduate School of Chinese Academy of Agricultural Sciences, Beijing, China, 2013.

[9] F. G. Viets, J. R., L. C. Boawn, and C. L. Crawford, "Zinc content of Bean Plants in relation to deficiency symptoms and yield," Plant Physiology. Scientific Paper No. 1186. Washington Agricultural Experiments Stations. Pullman, Washington, pp. 76-79, 1953.

[10] Santa Maria, G. E., and D. H. Cogliatti, "Bidirectional Zn fluxes and compartmentation in wheat seedling roots," J. of Plant Physiology. 132: pp. 312-315, 1988.

[11] I. Cakmak and W. J. Horst, "Effect of aluminum on lipid per oxidation, super oxide dismutase, catalase and peroxidase activities in root tips of soybean (Glycine max)" Physiology of Plants, 83: pp. 463-468, 1990.

[12] D. J. Reuter, "Distribution of copper and zinc in subterranean clover in relation to deficiency diagnosis," (PhD Thesis), 1980.

Table1. Phenotypic performance of Zinc-related traits in Extreme RILs and parents under MH63 indica and 02428 japonica back grounds (21 days after stress)

		Parents			MH63 RILs			02428 RILs		
Treatment	Trait	MH63	02428	P1-P2	Mean ± SD	CV%	Range	Mean±SD	CV%	Range
Control	TRL	4.09	3.08	1.01	3.87±0.73	18.92	3.71-4.38	4.24±0.86	19.87	3.61-6.27
	SRL				3.54±0.21	6.07	3.27-3.86	3.05±0.44	14.43	3.03-4.23
	TRW	0.05	0.04	0.01	0.04±0.007	16.89	0.04-0.06	0.03±0.01	33.33	0.03-0.04
	SRW				0.03±0.007	25.51	0.03-0.05	0.02±0.01	50	0.03-0.04
	TSH	24.25	20.45	3.8*	24.07±1.01	4.18	21.75-25.61	27±4.45	16.48	19.62-24.26
	SSH				23.99±1.29	5.41	22.3-25.12	20.11±2.35	11.69	19.24-28.96
	TSW	1.96	1.16	0.8*	1.68±0.28	16.44	1.25-2.01	1.81±0.31	17.04	1.40-2.40
	SSW				1.62±0.34	21.08	1.04-2.22	1.58±0.26	16.51	1.10-1.87
Zn^{2+} Stress	TRL	3.51	4.16	-0.65	3.97±0.85	21.5	3.38-6.59	4.49±0.71	15.87	4.27-5.97
	SRL				3.18±0.48	15.11	2.84-4.56	3.21±0.22	6.9	2.74-3.64
	TRW	0.04	0.03	0.01	0.04±0.01	15.98	0.04-0.06	0.04±0.01	25	0.03-0.05
	SRW				0.03±0.01	33.33	0.03-0.04	0.03±0.01	33.33	0.02-0.04
	TSH	21.54	20.14	1.4	21.56±2.25	10.41	20.28-22.68	23.48±4.14	17.67	20.27-31.1
	SSH				19.86±0.35	1.75	19.15-20.41	19.8±0.39	1.95	19.11-20.1
	TSW	1.12	1.24	-0.12	1.25±0.22	18.05	1.00-1.58	1.24±0.19	15.07	1.01-1.66
	SSW				0.97±0.20	20.86	0.70-1.36	1.01±0.15	15.07	0.70-1.27
Zn^{2+} Relative Value (%)	TRL	-14.2	35.23	21.05**	2.58±16.44	13.64	-13.05-8.88	5.90±-17.4	-20.1	-28.9-29.97
	SRL				-10.2±128.57	148.93	-36.23-74.32	-15.97±-50	-39.4	-39.56-5.97
	TRW	-20	-25	5	0.00±42.86	-5.39	-25-294.44	33.33±0.00	-25	-22.9-86.96
	SRW				0.00±42.86	30.65	-47.72-30	50.00±0.00	-33.3	-40-75.86
	TSH	-11.2	-1.52	-9.66	-10.43±122.8	149.04	-21.70--4.98	-13.04±-7	7.22	-30.54-2.80
	SSH				-17.22±-72.9	-67.65	-29.53--13.2	-21.2±-83.4	-79.1	-48.02--12.0
	TSW	-42.9	6.90	-49.75	-25.60±-21.4	9.79	-45.22-26.4	-31.5±-38.7	-11.6	-51.44--10.8
	SSW				-40.12±-41.2	-1.04	-59.09--4.42	-36.1±-42.3	-8.72	-56.79--10.9

TRL= Root length of tolerant line, **SRL**= Root length of susceptible line, **TRW**= Root weight of tolerant line, SRW= Root weight of susceptible line, **TSH**= Shoot height of tolerant line, **SSH**= Shoot height of susceptible line, **TSW**= Shoot weight of tolerant line, SSW= Shoot weight of susceptible line

Mean= Average of values belonging to Reciprocal Introgression Lines (RILs) in MH63 *indica* background. **SD**= Standard Deviation. **CV**= Co-efficient co-variation=SD/Mean*100

Range= Minimum values and maximum values in RILs under MH63 *indica* background.

*= Level of significance at p< 0.05, ** = Level of significance at p< 0.01, *** = Level of significance at the p < 0.001.

Table2. Concentration of Zinc-related traits in Extreme RILs and parents under MH63 indica and 02428japonica backgrounds (21 days after stress)

		Parent1	Parent2		MH63 RILs			02428RILs		
Treatment	Traits	MH63	2428	P1-P2	Mean ± SD	CV%	Range	Mean ± SD	CV%	Range
Control	ZCRT	0.34	0.18	0.16*	0.26±0.05	19.4	0.21-0.35	0.24±0.06	26.4	0.22-0.39
	ZCRS				0.27±0.07	26.5	0.15-0.41	0.25±0.06	24	0.17-0.4
	ZCShT	0.56	0.9	-0.34*	0.76±0.20	26.12	0.5-1.18	0.56±0.12	22.22	0.36-0.77
	ZCShS				0.99±0.27	27.07	0.53-1.39	0.72±0.25	35.03	0.51-1.44
Zn^{2+} Stress	ZCRT	5.08	5.73	-0.65	4.41±0.74	16.75	0.01-6.5	4.70±0.94	19.99	4-7.7
	ZCRS				4.97±0.77	15.41	3.4-6.61	4.76±1.49	31.27	3.31-8.50
	ZCShT	13.98	10.79	3.19*	13.18±2.18	16.56	9.51-18.08	11.08±3.07	27.74	7.9-17.78
	ZCShS				13.26±2.20	16.59	7.42-15.4	13.29±2.89	21.72	8.06-18.66
Zn^{2+} Relative value%	ZCRT	1394.12	3004.01	-1609.89	1376.92±3960	172.27	1312.1-	1858.3±1466.7	-24.28	982.8-3841.6
	ZCRS				1740.74±1000	-41.85	88.2-96.8	1969.6±2383.3	11.52	1164.8-3613.5
	ZCShT	2396.43	1098.89	1297.53*	1634.21±990	-36.60	1203.4-281.8	1878.6±2458.3	24.84	1119.1-3364.4
	ZCShS				1138.38±714.81	-33.84	831.8-1631.4	1745.8±1056	-38.00	1192.1-2950.3

All the related information for this Table is as same as for Table1.
*= significant difference
ZCRT= Zinc concentration in tolerant root, **ZCRS**= Zinc concentration in susceptible root, **ZCShT**= Zinc concentration in tolerant shoot, **ZCShS**= Zinc concentration in susceptible shoot