A golden gateway towards the development of zinc bio-fortified Rice (Oryza sativa L.)

Aijaz Ahmed Soomro, Zhang Jian

ABSTRACT— In tropical and sub-tropical developing countries of the world peoples are suffering from zinc deficiency. Therefore, keeping in view the scope of zinc bio-fortified rice in future, this study had been conducted to develop the zinc bio-fortified rice for controlling the hidden hunger from the peoples of zinc deficient countries. Two parents MH63 indica and 02428 japonica along with their respective 200 reciprocal introgression lines (RILs) 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 the extended period of 26 days in hydroponic culture solution. Highly supplemented zinc efficient RILs were discovered. However, the phenotypic performance of zinc supplemented RI lines was better than zinc supplemented parents.

Index Terms /Key words: Green house, Extreme lines, Supplemented zinc, Reciprocal Introgression lines, Rice

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1 INTRODUCTION

About one third population of the world is suffering from zinc (Zn) deficiency. Therefore deficiency of zinc in food for humans is one of the major worldwide health issues [1], [2]. In world-human population, especially in developing countries zinc is less available in human diet which results in widespread of Zn deficiency in peoples of the related regions. It is estimated that in South and Southeast Asia about more than half a billion people are suffering from Zn deficiency [2], [3]. In these regions due to inadequacy of Zn in human food there are many infectious diseases in children which are directly associated with zinc deficiency [4]. Therefore, to overcome this major health issue the concentration of zinc in rice grain should be increased to improve the health of rice consumers especially belonging to poor population. Besides that, in zinc deficient soils the seeds which contain higher zinc concentration possess the potency to improve germination, seedling vigor, crop growth and yield [5]. This practice has been already applied in USA where Zn coated rice seeds with (1.0 – 4.7 g Zn kg^1) resulted in better germination, longer root as well as shoot growth than untreated seeds [6].

It is very much informative for the 'Medical Doctors" as well as for Diabetic patients that zinc plays its vital role during metabolic phases of insulin, such as insulin production> secretion> utilization> storage. Zinc controls the destruction of pancreatic beta cells whereas its deficiency affects their ability to produce as well as secrete insulin. When pancreas cannot produce or secret required amount of insulin then glucose level increases in blood. Therefore the lack of insulin sensitivity is concerned with the decreased zinc concentration in the body [7], [8].

High micronutrient deficiencies including zinc in human beings are characterized by severe poverty, insufficient nutritional pattern, reduced cleanliness as well as hygienic conditions [9]. Therefore, international organizations such as WHO, MI and USAID are seriously involved in set up the strategy for micronutrients' fortification of staple food crops, such as wheat flour and rice [10].

It is unanimously agreed that rice is one of the important nutritional cereal crops of the world which is consumed by 2.7 billion peoples of the world daily [11] (Amar et al., 2007). However, in Asian countries it provides it provides about 80% daily energy intake [12] (saharay and Islam, 2008). The increasing population of the world will also increase the demand of rice to over 700 million tons by 2025. Therefore, in next 10-20 years Asian countries will face food deficiency [13] (M.K. Papademetriou 'FAO', 2000).

Therefore, keeping in view the future demand of zinc bio-fortified rice this study has been conducted to achieve very much important goals, these are to evaluate the phenotypic performance of two different rice populations (MH63 indica and 02428 japonica backgrounds) at extended period of supplemented zinc for 26 days, to analyze the concentration of zinc in root and shoot of both of the backgrounds as well as

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their respective parents at extended period of supplemented zinc for 26 days and to open a gateway towards the development of zinc bio-fortified rice to control the hidden hunger from zinc deficient peoples and poor population of rice consuming countries of world.

2 Materials and method

2.1 Plant material

This study was carried out thrice to develop the zinc bio-fortified rice (Oryza sativa L.). In first two consecutive experiments, 200 Reciprocal Introgression Lines (RILs) of rice from each MH63 indica and 02428 japonica were brought under study. These both of the backgrounds were also used first time for mapping QTLs against different heavy metal toxicities. On the basis of phenotypic performance and detection of QTLs for supplemental zinc application during earlier two experiments, this study has been carried out third time for supplementation of zinc in each background MH63 indica and 024282 japonica and their respective parents. Then zinc concentration from root and shoot of each extreme line was also analyzed through atomic absorption spectrophotometer.

Zinc supplemented tolerant RI lines (extreme lines) were detected with the following formula:
Extreme lines= control-supplemental zinc/control

2.2 Phenotypic evaluation for supplemental Zn^{2+} tolerant-related traits from Extreme lines

The seed of Reciprocal Introgression lines (RIls) from each background MH-63 indica and 02428 japonica was surface sterilized with 1% hypochlorite solution for 10 minutes and then rinsed well with distilled water. The seed was soaked in distilled water in dark at 30°C for 72 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 (control and zinc treated) most uniform 10 emerged seeds from parents MH63 indica and 02428 japonica were also sown in each container at random [14](Ines et al., 2009). The Styrofoam sheets were allowed to float on water up to 7 days and then transferred to Yoshida culture solution [15](Yoshida et al. 1976) without application of supplemental Zn^{2+} for first 15 days. When seedlings reached at 3rd leaf stage 15 days after sowing (DAS) the zinc in the form of ($ZnSO_4 . 7H_2O$) at the rate of 0 (control) and 200 ppm (supplemented) was applied. This application was continued for 26 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 temperature around 32/25°C in day/night, 70-75% of relative humidity and average 12 hours photoperiod were maintained. The experimental materials were laid out in two replications for all experiments conditions (control and zinc treated) in green house of Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China. The largest root and shoot length and fresh root and shoot weight were recorded after 26 days of the zinc treatment. Then the samples were kept in oven for 72 hours (03 days) at maximum 65°C [16](Jian lin, et al., 2003). Finally dry weight of root and shoot was recorded to get the standard value for zinc concentration per each RI line from each background MH63 indica and 02482 japonica as well as their respective parent lines. Then, the data was recorded for root length, shoot height, root-shoot fresh weight and root-shoot dry weight (Table1) as well as concentration of zinc in root and shoot of all RILs and their respective parental lines (Table2).

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:
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 (Ines et al., 2009)

3 Results

3.1 Phenotypic performance of zinc bio-fortified-related traits in RILs and their respective parents in MH63 indica and 02428 japonica backgrounds (after supplemented zinc for 26 days)

The results for phenotypic performance of zinc bio-fortified traits in RILs and their respective parents in MH63 indica and 02428 japonica backgrounds are shown in table1. These results indicated that two parents MH63 indica and 02428 japonica under control conditions had no any significant difference in all traits of evaluation, supplemented zinc and relative value %. However, under control condition MH63 indica parents had higher trends of evaluation except fresh root weight (FRW), for which 02428 japonica had higher values (0.45 mg). The averages for MH63 indica parents were root length (03.82 cm), root dry weight (0.019 mg), shoot height (23.78 cm) than that of 02428 japonica parents average root length (03.19 cm), dry root weight (0.015 cm) and shoot height (22.7 cm). In terms of fresh root weight (FRW) 02428 japonica parents had higher fresh root weight (0.45 mg) under control condition than that of MH63 indica parents at (0.39 mg) (Table1). In case of shoot height (23.78 cm) and shoot weight (2.2 mg) under control, MH63 indica showed higher trends than that of 02428 japonica parents.

When losses were compared between MH63 indica parents under control condition to tolerant RI lines in MH63 indica background under control; RI lines had got higher trends than those respective parents at all traits RL, SH, SW and RW respectively, and RI lines gained RL 10.54%, RW 17.02%, SH 0.91%, SW 3.08%. Furthermore, when losses between 02428 japonica parents under control condition were compared with those of respective 02428 RILs under control condition, RL 26.50% (significantly higher), RW 4.26%, SH 15.01%, and SW 11.21% respectively.

In supplemented zinc environmental condition MH63 indica parents achieved higher trends for all traits of evaluation than those of 02428 japonica parents except root length, for which 02428 japonica had higher values (4.45 cm) than that of MH63 indica parents (4.1 cm).

For Zn^{2+} relative value %, MH63 indica parents had higher values in all traits than those of 02428 japonica parents except root length relative value %, for which 02428 japonica had significantly higher values (39.5).

When losses were compared between MH63 indica parents under supplemented zinc condition to Reciprocal Introgression lines (RILs) in MH63 indica background under supplemented zinc condition, RIls gained higher trends of evaluation than those of MH63 indica parents except dry weight of both root and shoot for which both populations have almost same trends. Therefore, MH-63 indica RILs gained root length (2.61%), fresh root weight (62.15%) significantly higher, shoot height (2.61%), fresh shoot weight (1.20%).

In case of comparison between 02428 japonica parents under supplemented zinc condition with those of supplemented zinc 02428 japonica RI lines, 02428 RI lines had got higher trends than those of their respective parents 02428 at all traits, RL(28.45%) significantly higher, shoot height (SH), fresh shoot weight (FSW) and RW respectively, and RI lines gained root fresh weight (34.62%), RW 34.62% (significantly higher), SH 0.91%, SW 3.08%; RL 26.50% (significantly higher), RW 4.26%, SH 15.01%, and SW 11.21% respectively.

The ratio of Reciprocal Introgression lines showed transgressive segregations for all supplemented Zinc-related traits and showed continuous variations in MH63 indica and 02428 japonica backgrounds (Table1).

3.2 Concentration of zinc at cellular level in RILs and their respective parents in MH63 indica and 02428 japonica backgrounds (after supplemented zinc for 26 days)

The results for concentration of zinc at cellular in RILs and their respective parents in MH63 indica and 02428 japonica backgrounds are shown in table2. These results revealed that there was no any significant difference between two parents MH63 indica and 02428 japonica backgrounds under control and supplemented zinc environmental conditions. Even though they were similar in all traits of control and supplemented zinc conditions while compared the level of significance; but, 02428 japonica parents had higher concentration of zinc at all parameters of measurement, such as, root and shoot respectively. It indicated that 02428 japonica parents had absorbed higher amount of zinc from the solution.

While the concentration of zinc in root and shoot of MH63 indica and 02428 japonica parents under control condition had been compared with those of their respective RI lines under control condition, all the tolerant RI lines in MH63 indica and 02428 japonica parents had higher concentration of zinc in root and shoot except root of RI lines in 02428 japonica background under control condition, for which 02428 japonica parents had higher values than that of respective RI lines with the mean of 0.17 mg/L and with the higher percentage of 17.65% mg/L. In this respect, when the losses for concentration of zinc in roots and shoots of MH63 indica parents were compared with those of their respective RILs under control condition, the MH63 indica parents lost 7.14% mg/L for zinc concentration in root (ZCR), 14.26% mg/L for zinc concentration in shoot (ZCSh). In case of comparison of losses between 02428 japonica parents under control condition with those of respective RILs in 02428 japonica backgrounds, the 02828 japonica parents absorbed significantly lesser zinc at 57.5% mg/L for zinc concentration in root (ZCR) and 8.57% mg/L for ZCSh, respectively.

The results for MH63 indica and 02428 japonica parents under supplemented zinc condition for concentration of Zn were not similar, because MH63 indica parents' root contained 1.8 mg of Zn and 02428 japonica parents' root contained 2.08 mg of Zn. When losses of zinc absorption in MH63 indica parents were compared with those of their respective RI Lines in MH63 indica background, the RILs gained 19.64% mg/L higher concentration of zinc in root and 9.32% mg/L higher in shoot.

In case of comparison between 02428 japonica parents under supplemented zinc condition with those of their respective 02428 RI lines under supplemented zinc condition, mostly the average concentration of Zn in roots (2.49 mg) and shoots (11.87 mg) of the RI lines were higher than those of their respective 02428 japonica parents' roots (2.08 mg) and shoots (11.67 mg) in supplemented zinc environment. The parents 02428 japonica under supplemented zinc environment lost 16.47% mg/L for ZCR and 1.60% mg/L for ZCSh.

While in case of comparison between both of the Reciprocal Introgression lines (RILs) in each MH63 indica and 02428 japonica backgrounds, the RILs in 02428 japonica backgrounds under supplemented zinc condition had higher concentration of zinc in roots (2.49 mg/L) as well as in shoot (12.87 mg/L) than that of MH63 indica RILs which contained (2.24 mg/L) in root and (11.8 mg/L) in shoot. In this way, 02428 japonica RILs absorbed higher zinc from the solution at about (8.94% mg/L) in root and (8.31% mg/L) in shoot (Table2).

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

4 Discussion

The results of phenotypic data of MH63 indica and 02428 japonica background populations have leaded us for the development of zinc bio-fortified rice. These findings revealed that when zinc was applied at the rate of (200 ppm Zn) showed that rice crop-plants remained under some stress than untreated (controlled) plants. Therefore, zinc supplemented rice plants showed decreased root length, stunted/shortened shoot height, lesser root and shoot weight than the respective rice plants under control condition. It has been keenly observed that the plants under control condition had not only got higher quantitative-related trait values but also these plants were always looking fresh and normal green than the rice plants under supplemented zinc environment. It gave us the novel theory to write that supplemental/additional application of zinc at 200 ppm can exert both qualitative and quantitative effects on rice crop-plants.

By keeping in view the importance of comparative study of ferrous in rice [17](Aijaz et al., 2014) and zinc in rice, we had laid down two separate experiments simultaneously, one for the supplemented Zn^{2+} in rice and the second for supplemented Fe^{2+} in rice under same environmental conditions at the rates of zinc (200 ppm Zn) and ferrous (300 ppm Fe). In this regard, it had been clearly observed from the phenotypic performance of the populations under study that excess of zinc was more harmful than the excess of ferrous in rice. Because mostly all the traits of measurement under supplemented Zn^{2+} environment showed smaller values (Table1 and Table2) than the trait values of measurement under supplemented Fe^{2+} [17](Aijaz et al., 2014) which indicated that a little excess of zinc application to rice plants is more harmful than the excess of ferrous application. Therefore, it enhanced our enthusiasm to develop zinc bio-fortified rice and to screen out zinc toxicity tolerant RILs of MH63 indica and 02428 japonica backgrounds.

However, the results of phenotypic performance of RI lines under control and supplemented Zn^{2+} indicated that MH63 indica and 02428 japonica backgrounds had some highly tolerant lines which could survive well under supplemented zinc condition (200 ppm Zn). The concentration of zinc in roots and shoots of highly tolerant lines showed that shoots of both RILs had higher concentration than those of roots. It also proved that zinc is mobile from root to shoot through xylem. Therefore, its concentration was higher in shoot than root of the same plant populations.

In order to know the similar results of previous studies with our this study, luckily we could download two ancient and important publications, these are, Sommer and Lipman (1926) [18] studied the indispensible nature of zinc in barley and sunflower; Sommer, (1928) [19] studied the essentiality of zinc in buckwheat, Windsor beans and red kidney beans. These two papers are great evidences for the interest of scientists in impacts of zinc on higher plants and also a historical record for study of zinc in higher plants. It is noteworthy that the findings of F.G. et al., (1951) [20] also in agreement with the findings of this study. They also studied the concentration and accumulation of zinc in bean plants at different levels of applied zinc. 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 findings are also similar with those of Reuter (1980) [21]; Santa and Cogliatti (1988) [22]; Cakamak and Marschner (1990) [23], because they had also recorded similar results for higher zinc concentration in shoots than in roots. The latest findings for mobility of zinc from root to shoot and higher concentration of zinc in shoot than root were recorded by Zhang Jian et al., (2013) [24] in their fresh study during 2012-2013. They had also detected the higher concentration of zinc in shoot than root under control as well as zinc stress environments. These all evidences for higher concentration of zinc in shoot than root gave strong support to the idea of impact of zinc excess to the upper parts of plants is induced through root absorption, which is the first entrance to zinc absorption, where from it greatly moves to the upper parts of the plants.

The most important findings of our study are, even though we extended the exposure of rice plants to supplemented zinc at similar rate (200 ppm) from 21 (Aijaz and Zhang, 2015) [25] to 26 days, the rice plants of tolerant Reciprocal Introgression Lines could withstand well with that extended period of 26 days. In the light of such successful growth of rice seedlings at zinc supplemented rate (Toxicity) for 26 days, we become able to move forth to develop the zinc bio-fortified rice in future to overcome the hidden hunger among the zinc deficient human populations.

5 Conclusion and recommendations

The results of phenotypic data in MH63 indica and 02428 japonica backgrounds showed that all the traits under this study, such as, root length, shoot height, root-shoot fresh weight as well as root-shoot dry weight at some extent were stressed to supplemented zinc. It was because of that the rice-plants under normal control conditions achieved higher trends of evaluation than those of plants under supplemented zinc treatment. In-spite of that, the moderate concentration of zinc at cellular level of each MH63 indica and 02428 japonica background populations has opened a gateway for some highly zinc efficient RILs which could absorb supplemented zinc at the level of their tolerance. It is very much important point to note that level of absorbed supplemented zinc in RILs would also be beneficial for the consumption of human beings. Because human beings intake all essential nutrients as well as vitamins from crop-plants. These novel findings of our study have enabled us to develop zinc bio-fortified rice from these two backgrounds MH63 indica and 02428 japonica to control the hidden hunger from zinc deficient peoples and poor population of the world as well.

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Table1. Phenotypic performance of zinc biofortified-related traits in extreme RILs and parents under MH63 indica and 02428 japonica

		Parents			MH63 RILs			02428 RILs		
Treatment	Trait	MH63	2428	P1-P2	Mean ± SD	CV%	Range	Mean±SD	CV%	Range
Control 0 supplemental zinc	RL	3.82	3.19	0.63	4.27±0.57	13.35	2.6-4.4	4.34±0.98	22.66	2.66-6.01
	FRW	0.39	0.45	-0.06	0.47±0.09	19.52	0.34-0.67	0.47±0.06	13.05	0.4-0.6
	DRW	0.019	0.015	0.004	0.008±0.002	25.66	0.002-0.017	0.005±0.002	41.45	0.003-0.06
	SH	23.78	22.7	1.08	24±1.61	6.72	21.58-26.6	26.71±5.80	21.72	19.49-35.7
	FSW	2.2	1.98	0.22	2.27±0.51	22.47	1.49-2.93	2.23±3.34	15.12	1.62-2.77
	DSW	0.058	0.035	0.023	0.048±0.013	27.971	0.011-0.111	0.058±0.103	131.61	0.02-1.02
Zn^{2+} (Supplemental)	RL	4.1	4.45	-0.35	4.21±0.36	8.55	3.51-4.53	6.22±1.23	19.9	4.64-7.97
	FRW	0.36	0.34	0.02	0.95±0.54	56.32	0.34-1.84	0.52±0.10	19.6	0.38-0.66
	DRW	0.006	0.007	-0.001	0.006±0.001	20.74	0.002-0.01	0.006±0.001	29.235	0.001-0.013
	SH	20.84	17.66	3.18	21.08±0.82	3.87	19.9-22.9	**24.82**±4.76	19.18	20.68-31.78
	FSW	1.64	1.13	0.52	1.66±0.23	13.86	1.14-1.91	**1.68**±0.37	21.95	1.26-2.27
	DSW	0.05	0.04	0.01	0.05±0.007	19.794	0.02-0.08	0.069±0.015	24.23	0.024-0.09
Zn^{2+} Relative Value (%)	RL	7.33	39.5	-32.17**	24.16±36.84	-49.62	-0.57-70.64	43.32±25.51	-12.18	-22.75-122.84
	RW	-7.69	-24.44	-16.75	102.13±500	188.53	-31-33.33	10.64±66.67	50.19	-27.6-50
	SH	-12.36	-22.2	-9.84	-12.17±-49.01	-42.41	-20.84-4.34	-7.08±-17.93	-11.69	-37.52-31.45
	SW	-25.45	-42.93	-17.47	440.74±-54.90	-36.65	-58.7-5.37	-24.66±-88.92	45.17	-43.5-2.47

***RL**= Root length, ***FRW**=Fresh root weight, ***DRW**=dry root weight, ***SH**= Shoot height, ***FSW**= Fresh shoot weight, ***DSW**= Dry shoo weight, **Mean**= Average of values belonging to Reciprocal Introgression Lines (RILs) in MH63 indica and 02428 japonica backgrounds. **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 $p < 0.001$.

Table1. Concentration of zinc at cellular level in MH63 indica and 02428 japonica parents and their perspective RILs of MH63 indica and 02428 japonica backgrounds

					RL1		RL2		RL3		RRE	
Trait	QTL	Chr	Marker intervals	Position	LOD	Ad	LOD	Ad	LOD	Ad	LOD	Ad
RL	QRl1	1	M31-M32	152	2.29	0.4					1.32	1.9
	QRl2	2	M39-M40	48	2.27	-0.2			1.48	-0.17	4.58	1.63
	QRl9	9	M199-M200	36	1.63	-0.2	1.49	-0.26	1.31	-0.19		
	QRl10	10	M209-M210	30	1.44							

Abbreviations and other related information for this Table is as same as for Table1.