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ZINC APPLICATION IMPROVES PRODUCTIVITY AND BIOFORTIFICATION OF MINI CORE RICE HYBRIDS

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ABSTRACT

Zinc (Zn) is essentially required by plants and humans for proper growth and development but its widespread deficiency has become a global concern. In this study we evaluated the role of Zn in improving rice productivity, Zn concentration and bioavailability. Zn was applied as basal dose of $ZnSO_4.H_2O$ (10 kg Zn ha^{-1}) to ten rice genotypes. Application of Zn increased average grain yield, grain Zn concentration and uptake by the factor of 1.08, 2.48 and 2.47, respectively. Average Phytate to Zn ratio of tested genotypes was significantly decreased by 57%. Estimated Zn bioavailability to human from rice was also increased. Highest recorded Zn bioavailability was 4.27 mg/300g/day. Average Zn bioavailability of genotypes improved by the factor of 2.06. In conclusion, agronomic Zn biofortification of rice can be an easy, adaptable and short term approach to increase crop yield, Zn concentration and bioavailability.

Keywords: biofortification, food security, hidden hunger, micronutrient, nutrition

INTRODUCTION

Micronutrients deficiencies are one of the major causes of malnutrition in humans. Soils, deficient in micronutrients affect plant nutrition directly and human health indirectly (Sanchez and Swaminathan, 2005). Over two billion people are suffering from Zn deficiency, worldwide (WHO, 2016). Vegetarian diets, low in micronutrients are commonly consumed in resource poor populations of developing countries (Joy *et al.,* 2014). Therefore, increasing health issues due to malnutrition have become matter of concern worldwide (FAO, 2015). The magnitude of the problem is even bigger in low to middle income developing countries of Africa and Asia where multi-nutrient deficiencies have been reported (Joy *et al.,* 2014). Soils in these countries cannot supplement desired concentration of nutrients to the crops and cause low yield and inferior quality produce (Kumssa *et al.,* 2015). Thus, the deficiency has shifted from soil to crops and then to human. Micronutrient deficiency symptoms in human are seldom visible. Therefore, it is often called as “hidden hunger” (Black *et al.,* 2013). However, chronic deficiency has serious health implications in masses, especially in women and children (Finkelstein *et al.,* 2015). Ruel *et al.* (2013) proposed various direct and indirect approaches to resolve the problem of hidden hunger. Direct approaches are nutrition specific and are focused to changing dietary habits, expansion of dietary choices, medication and industrial fortification of food products. Indirect approaches are nutrient sensitive. These approaches address the primary causes of hidden hunger through biofortification (Bouis *et al.,* 2011). Agronomic and genetic mediation during growth and development of crops to increase their mineral contents is called biofortification. Conventional breeding, genetic engineering and agronomy are the tools of biofortification (Saltzman *et al.,* 2013). Among these, agronomic biofortification is less time consuming, effective, economic and highly adaptable approach (Cakmak, 2014; de Valenca *et al.,* 2017). Soil and foliar applications of micronutrients to the crops are required to achieve agronomic biofortification (Cakmak *et al.,* 2010; Phattarakul *et al.,* 2012).

Rice crop has great economic and nutrition value, consumed by large proportion of world population. FAO (2018) estimated consumption of 509.1 million tons milled rice worldwide against the production of 510.6 million tons during the year 2018-19. Pakistan is ranked second after India among fine rice exporting countries. Rice contributes 0.6% in gross domestic product and 3.0% in value added

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agriculture of the country (GoP, 2019). Pakistan achieved all time high rice production of 11.1 million tons (7.4 million tons milled basis) during 2017-18 due to favorable growing conditions, attractive price and input assistance provided by the state (FAO, 2018). Contrary to estimate of 1% increase, rice production in the country remained 3.3% short of targeted yield during 2018-19 due to reduction in cultivation area, water scarcity and climate shift scenario (FAO, 2018; GoP, 2019). Rice is not only important for its exports earning but also termed as second major staple food of the country. Thus, interventions to sustain rice production are needed to ensure food security and good farm earning.

Multiple factors control crop production. Soil is one of these factors which play pivotal role in crop production. In the country, soils are mostly alkaline and calcareous (FAO, 2017). In such soils, Zn is often found deficient but its deficiency in rice fields is more pronounced. According to Alloway (2009), Zn gets precipitated in soils with high pH ($ZnOH_2$), low redox potential (ZnS) and high calcareousness ($ZnCO_3$). Nutrient-nutrient interactions also influence Zn bioavailability to plants. For instance, P addition to soil can stimulate Zn uptake while higher application rate of same P fertilizer can precipitate smaller Zn concentration present in soil solution (Zingore *et al.,* 2008). However, soil managements like Zn fertilization and increasing organic matter contents can stimulate Zn availability to the crop (Smith and Read, 1997; Vanlauwe *et al.,* 2015). Second most crucial factor is lack of awareness among farming community. Currently, they are more inclined to macronutrient fertilization despite of the fact that micronutrients are equally important for crop growth and production (FAO, 2017). Resultantly our soils are becoming non-responsive to even higher doses of NPK due to micronutrient deficiencies (Voortman and Bindraban, 2015).

The Zn bioavailability to human is also challenging aspect. Crops with high Zn concentration do not ensure high Zn supply to human. Genotypes with greater Zn re-translocation potential are desired for biofortification. Plant acquired Zn must be re-translocated to edible portion of crop so that bioavailability to human can be ensured (de Valença *et al.,* 2017). Secondly, food processing is an important factor, controlling Zn bioavailability from food. In rice Zn is generally stored in the proteins present in the external surface of grains. This surface is often removed during de-husking, milling and polishing (Haas *et al.,* 2005; Zimmermann and Hurrell, 2007). Whereas, parboiling of rice increases Zn and Fe contents in rice by drawing nutrients from germ layer and bran to the endosperm of the rice (Hotz *et al.,* 2015; Prakash *et al.,* 2016). Thirdly, Zn availability to human is controlled by the food-host relation. Bioavailability depends on kind of food, chemical form, age, sex, physiology, ethnicity and interaction among dietary components, which can increase or decrease mineral absorption in gastrointestinal tract of human (Gibson, 2007). For example, ascorbic acid present in fruits and vegetables positively affects Zn and Fe absorption in human, while phytate acts contrary to it (Perera *et al.,* 2018). Thus, Zn bioavailability from soil to plant, plant to food and food to human is quite a challenge (de Valença *et al.,* 2017).

The present study aims to improve both quantity and quality factors of rice genotypes and to elaborate variable responses of rice genotypes to Zn application in terms of yield, Zn concentration, accumulation and bioavailability.

MATERIALS AND METHODS

Field experiment was carried out at the research farm of Nuclear Institute of Agriculture (NIA), Tandojam, Sindh-Pakistan, during Kharif, 2017 (April-October, 2017). The experimental site had elevation of 12 m above sea level situated at the latitude of 25°42’24.19” North and longitude of 68°52’68.76” East (NAMC, 2017). The field was ploughed twice, followed by laser leveling, cultivation and planking. Soil samples were taken from the field at the depths of 0-15 cm and 15-30 cm and were analyzed for various physio-chemical properties of soil (Table 1). Briefly, at experimental site soil was non-saline, alkaline in reaction, low in organic matter, nitrogen and zinc, marginal in AB-DTPA extractable P and high in K.

Before transplanting, the field was heavily irrigated to establish submerged conditions which were maintained till the maturity of the crop. Minicore hybrid rice genotypes, MC-24, MC-50, MC-53, MC-57, MC-84, MC-90, MC-95, MC-98, MC-101 and MC-109, obtained from National Institute of Biotechnology and Genetic Engineering (NIBGE) Faisalabad, were grown on raised soil beds for the development of nurseries. Thirty- five-days old nurseries of the genotypes, were manually transplanted into the field maintaining plant to plant and row to row distances of 22.5 cm, respectively. Each genotype was treated with two Zn application rates (0 and 10 kg Zn ha^{-1} from $ZnSO_4.H_2O$). Genotypes and Zn treatments were independently assorted in sixty experimental units, each of dimension 2.5 m × 2 m, following split plot arrangement where genotypes were placed in main plot and treatment were assorted in sub plots. Each treatment was repeated thrice.

Phosphorus and potassium were applied as DAP (90 kg P_2O_5 ha^{-1}) and SOP (30 kg K_2O ha^{-1}) during land preparation. The nitrogen at the application rate of 150 kg ha^{-1} was supplemented in the form of Urea in three equal splits at critical growth stages *viz.,* at the time of sowing, tillering, and panicle initiation. Zn was applied after 15 days of transplanting along with second split of Urea. Chemical and manual eradication of weeds was performed when needed. At maturity biological, grain and straw yields of genotypes were recorded.

Straw and brown rice samples from each treatment were collected and dried at 70°C in forced air oven. Dried plant samples were then grinded and wet-digested in di-acid mixture of HNO_3 and $HClO_4$ (5:1) by following Jones and Case (1990) and were subsequently analyzed for Zn concentration in plant tissues using Atomic Absorption Spectrophotometer (Analytik Jena Nova 400, Germany).

Table 1. Physico-chemical properties of soil at experimental site

Soil properties	Unit	Value	Reference/ method
Textural class	--	Silt loam	Bouyoucos, (962)
EC $_{(1:2.5)}$	dS m^{-1}	0.31	Anderson and Ingram, (1993)
pH $_{(1:2.5)}$	--	8.2	Anderson and Ingram (1993)
Organic matter	%	0.61	Nelson and Sommers 1982
Kjeldhal N	%	0.029	Jackson (1962)
AB-DTPA extractable P	mg kg^{-1}	6.21	Soltanpour and Workman (1979)
AB-DTPA extractable Zn	mg kg^{-1}	0.67	Soltanpour and Workman (1979)
AB-DTPA extractable K	mg kg^{-1}	140	Soltanpour and Workman (1979)

AB-DTPA=Ammonium biocarbonate-Diethylene triamine pentoacetric acid

Zn accumulation in rice was calculated following Farooq *et al.* (2018) by using formula:

$$Zn\ uptake\ (g\,ha^{-1}) = Zn\ concentration\ grain/straw\ (\mu g\ g^{-1})$$
$$x\ Yield\ grain/\ straw\ (kg\ ha^{-1})$$

To evaluate Zn complexation with phytate (PA), the total inositol phosphorus determined according to the method of Haug and Lantzsch (1983). Briefly, rice grains were grinded and extracted with dilute hydrochloric acid. The 0.5 mL extract was homogenized with 0.4 mM Iron (III) sulfate solution (prepared in 0.2 N HCl) solution in a caped glass tube. Then, tubes were heated for 30 minutes in a boiling water bath and then cooled for 15 minutes in ice water. Tubes were kept resting to attain room temperature. Upon addition of 2 mL of 2, 2'-bi-pyridine solution (prepared by dissolving 5g 2, 2'-bi-pyridine in 500mL water having 1% v/v thioglycollic acid) into each sample tube, pink color appeared due to unreacted ferrous ions. Absorbance was measured at 519 nm using UV-Visible Spectrophotometer (U-2900, Hitachi, Japan). Standard phytate solutions prepared from sodium phytate (*Sigma-Aldrich P-8810,* St. Louis, USA) were processed similarly for the preparation of standard calibration curve and phytate concentration in the samples was calculated accordingly. Molar concentrations of phytate and Zn were used to calculate [phytate]-[Zn] ratio in rice grains of tested genotypes. Trivariate Zn absorption model proposed by Miller *et al.* (2007) was used to estimate the bioavailability of Zn to human. Following is the model equation based on Zn homeostasis in human digestive tract. Total available Zn was calculated on reference adults consuming 300 g rice per day as the only daily Zn source (Rosado *et al.,* 2009).

$$TAZ=0.5\left(A_{max}+TDZ+K_R\times\left(1+\frac{TDP}{K_P}\right)\right)-$$
$$-\sqrt{\left(A_{max}+TDZ+K_R\times\left(1+\frac{TDP}{K_P}\right)\right)^2-4\times A_{max}\times TDZ}$$

Where:

$TAZ = \text{Total daily absorbed Zn (mg Zn/day)}$

$A_{max} = maximum\ Zn\ absorption$

$TDZ = \text{Total daily dietary Zn (mmol Zn/day)}$

$K_R = Equilibrium\ dissociation\ constant\ of\ the\ Zn$
$-\ receptor\ binding\ reaction$

$TDP = \text{Total daily dietary PA (mmol PA/day)}$

$K_P = Equilibrium\ dissociation\ constant\ of\ the\ Zn$
$-\ PA\ binding\ reaction$

For Zn homeostasis in human intestine 0.091, 0.680 and 0.033 are the constant values for A_{max}, K_P and K_R (Hambidge *et al.,* 2010).

The data associated to different yield and Zn attributes were statistically analyzed by analysis of variance technique. Significantly different means for different parameters were individually separated at each Zn level using Tukey's HSD test at 95% confidence level. (Steel *et al.,* 1997). The data were analyzed using Microsoft Excel

2010® (Microsoft Cooperation, USA) and Statistix 8.1® (Analytical Software, Tallahassee, USA).

RESULTS

Crop yield

A significant variation was observed in dry matter accumulation by tested genotypes upon application of Zn in comparison with their respective control (Table 2). The MC-57 produced highest dry matter (30.29 t ha^{-1}) among the genotypes with the application of 10 kg Zn ha^{-1}. The lower yield was observed in the plots where Zn was not added. The application of Zn showed significant effects on grain yield of genotypes used in this study. The grain yield of the genotypes was significantly increased by the application of Zn. The MC-90 showed maximum yield of 6.98 tons ha^{-1} with Zn application. The genotypes MC-109 showed second highest yield of 5.42 tons ha^{-1} in response to Zn. Lowest grain yield was produced by MC-53.

The Zn application also influenced straw yield significantly. Higher straw yields of genotypes were recorded when supplied with Zn. Maximum straw yield was in the genotype MC-57 (25.40 t ha^{-1}) upon Zn application. Straw yield of MC-95 was ranked second which increased from 20.31 to 22.52 t ha^{-1} with Zn application. On an average, Zn application improved straw yield of genotypes by 8.82%.

Table 2. Biological, grain and straw yield of rice genotypes affected by Zn application

Genotypes	**Biological yield (t ha^{-1})**			**Grain yield (t ha^{-1})**			**Straw yield (t ha^{-1})**		
	Zn application rate (kg ha^{1})			**Zn application rate (kg ha^{1})**			**Zn application rate (kg ha^{1})**		
	0	**10**	**Mean**	**0**	**10**	**Mean**	**0**	**10**	**Mean**
MC-24	14.06 h	15.85 f-h	14.96 E	3.75 de	4.06 c-e	3.91 CD	10.31 j	11.79 h-j	11.05 F
MC-50	15.10 gh	16.67 e-h	15.89 DE	4.74 b-d	4.90 b-d	4.82 BC	10.36 j	11.77 h-j	11.07 F
MC-53	16.67 e-h	18.23 d-f	17.45 CD	2.71 e	2.81 e	2.76 D	13.96 fg	15.42 f	14.69 D
MC-57	26.93 bc	30.29 a	28.61 A	4.27 c-e	4.90 b-d	4.58 BC	22.66 b	25.40 a	24.03 A
MC-84	17.71 d-g	18.23 d-f	17.97 C	4.90 b-d	5.10 b-d	5.00 BC	12.81 g-i	13.13 g-i	12.97 E
MC-90	17.71 d-g	19.79 d	18.75 C	6.46 ab	6.98 a	6.72 A	11.25 ij	12.81 g-i	12.03 EF
MC-95	25.00 bc	27.10 b	26.05 B	4.69 b-d	5.42 a-d	5.05 BC	20.31 c-e	22.52 b	21.42 B
MC-98	17.53 d-g	19.27 de	18.40 C	5.23 a-d	5.79 a-c	5.51 AB	12.29 g-j	13.48 f-h	12.89 E
MC-101	25.63 bc	26.69 bc	26.16 B	4.38 c-e	4.79 b-d	4.58 BC	21.25 b-d	21.89 bc	21.57 B
MC-109	24.15 c	25.00 bc	24.57 B	5.21 a-d	5.42 a-d	5.31 B	18.94 e	19.58 de	19.26 C
Mean	20.05 B	21.71 A		4.633 B	5.017 A		15.42 B	16.78 A	
Tuckey’s HSD (*p<0.05*)									
Genotype	1.827			1.396			1.281		
Zinc	0.443			0.187			0.357		
Genotype × Zinc	2.71			1.144			2.181		

Means sharing similar letter(s) are statistically at par with each other *(P≤ 0.05)*

Table 3. Zn concentrations in grains and straws of rice genotypes affected by Zn application

Genotypes	**Grain [Zn] (μg g^{-1})**			**Straw [Zn] (μg g^{-1})**		
	Zn application rate (kg ha^{-1})			**Zn application rate (kg ha^{-1})**		
	0	**10**	**Mean**	**0**	**10**	**Mean**
MC-24	16.51 h	30.36 e	23.44 E	6.28 k	14.33 f	10.30 G
MC-50	8.99 k	34.01 d	21.50 G	10.24 h	18.40 e	14.32 E
MC-53	18.52 g	39.73 a	29.12 A	11.51 g	24.55 a	18.03 A
MC-57	11.35 j	33.70 d	22.52 F	8.27 j	19.44 d	13.85 F
MC-84	12.17 j	25.04 f	18.61 H	6.63 k	13.81 f	10.22 G
MC-90	14.41 i	35.26 c	24.84 D	9.47 i	20.49 c	14.98 D
MC-95	16.51 h	39.95 a	28.23 B	11.47 g	21.98 b	16.73 B
MC-98	10.01 k	36.06 bc	23.04 EF	10.48 h	20.49 c	15.49 C
MC-101	15.80 h	37.02 b	26.41 C	8.60 j	20.48 c	14.54 E
MC-109	15.37 hi	35.72 c	25.55 D	10.18 h	19.99 cd	15.08 D
Mean	13.964 B	34.686 A		9.313 B	19.394 A	
Tuckey’s HSD (*p<0.05*)						
Genotypes	0.825			0.3386		
Zinc	0.1704			0.1204		
Genotype × Zinc	1.043			0.737		

Means sharing similar letter(s) are statistically at par with each other *(P≤ 0.05)*

Table 4. Zn uptake in rice genotypes affected by Zn application

Genotypes	Grain Zn uptake (g ha^{-1})			Straw Zinc uptake (g ha^{-1})			Total Zn uptake (g ha^{-1})		
	Zn application rate (kg/ha^{-1})			Zn application rate (kg/ha^{-1})			Zn application rate (kg/ha^{-1})		
	0	**10**	**Mean**	**0**	**10**	**Mean**	**0**	**10**	**Mean**
MC-24	61.86 m	123.55 h	92.71 G	64.65 q	168.95 l	116.80 J	126.51 q	292.50 i	209.51 I
MC-50	42.61 p	166.78 f	104.69 F	106.14 o	214.04 h	160.09 H	148.75 p	380.82 g	264.78 G
MC-53	50.06 no	111.64 i	80.85 H	160.79 m	378.25 d	269.52 E	210.86 m	489.88 f	350.37 E
MC-57	48.60 o	164.53 f	106.56 F	187.19 j	493.53 a	340.36 B	235.79 l	658.05 b	446.92 B
MC-84	59.46 m	127.83 g	93.65 G	84.95 p	181.12 k	133.03 I	144.41 p	308.95 h	226.68 H
MC-90	92.96 j	246.02 a	169.49 A	106.49 o	262.45 f	184.47 G	199.45 n	508.46 e	353.96 E
MC-95	77.37 k	216.42 b	146.89 B	232.97 g	494.95 a	363.96 A	310.34 h	711.37 a	510.85 A
MC-98	52.42 n	211.34 c	131.88 D	129.39 n	276.04 e	202.72 F	181.82 o	487.38 f	334.60 F
MC-101	69.20 l	177.56 e	123.38 E	182.80 k	448.93 b	315.86 C	252.00 k	626.49 c	439.25 C
MC-109	80.01 k	193.44 d	136.72 C	192.81 i	391.54 c	292.17 D	272.81 j	584.98 d	428.90 D
Mean	63.46 B	173.91 A		144.82 B	330.98 A		208.27 B	504.89 A	
Tuckey's HSD (*p<0.05*)									
Genotypes	2.761			2.018			3.599		
Zinc	0.466			0.787			0.638		
Genotype × Zinc	2.852			4.817			3.904		

Means sharing similar letter(s) are statistically at par with each other *(P≤0.05)*

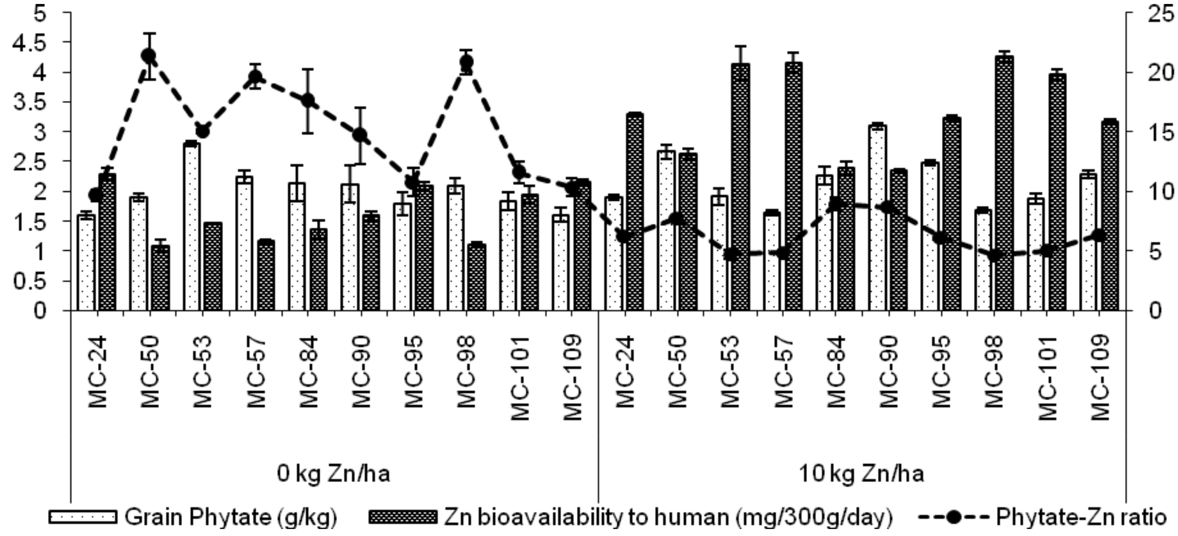

Figure 1. Grain phytate, phytate/Zn ratio and Zn bioavailability of rice genotypes affected by Zn application

Zinc concentration

Zinc concentrations in grains of rice plants varied with the soil application of Zn. Efficient hybrids managed to absorb more Zn from deficient soil. Grain Zn concentration in tested genotypes at Zn deficient level ranged between 8.99 to 18.52 µg g^{-1} (Table 3). Upon Zn application, highest grain [Zn] was recorded in MC-95 (39.95 µg g^{-1}) which was statistically comparable to the grain [Zn] in MC-53 (39.73 µg g^{-1}). Grain [Zn] of MC-101 (37.02 µg g^{-1}) and MC-98 (36.06 µg g^{-1}) were statistically ranked second. Least grain [Zn] was recorded in MC-84 (25.04 µg g^{-1}). Overall 2.48 times increase in mean grain [Zn] of genotypes was recorded. Straw [Zn] in genotypes was also enhanced by using Zn fertilizer. Highest straw [Zn] in MC-53 (24.55 µg g^{-1}) was recorded due to Zn application, followed by MC-95 (21.98 µg g^{-1}). The MC-90 (20.49 µg g^{-1}), MC-98 (20.49 µg g^{-1}) and MC-101 (20.48 µg g^{-1}) had third highest straw [Zn]. Lowest straw [Zn] was recorded in MC-84 (13.81 µg g^{-1}).

Zn accumulation

Zinc application significantly increased Zn accumulation in rice hybrids (Table 4). Highest increase in grain Zn uptake from 92.96 to 246.09 g ha^{-1} was in MC-90, followed by MC-95 which amassed 216.42 g Zn ha^{-1} in response to Zn application. Grain Zn uptake of 111.64 g ha^{-1} was lowermost in MC-53 in response to Zn application. Straw Zn accumulation was also positively affected by Zn application. Zn accumulation in straw was highest in MC-94 (494.95 g ha^{-1} in response to 10 kg Zn ha^{-1}), followed by MC-101, MC-109 and MC-53 which accumulated 448.93, 391.54 and 378.25 g Zn ha^{-1}, respectively. Lowest Zn accumulation in straw in response to Zn application was recorded in MC-24 (168.65 g ha^{-1}). Tested genotypes responded to Zn application with increase in total Zn uptake over their respective control. In MC-95, MC-57 and MC-101 total Zn accumulation increased from 310.34 to 711.37 g ha^{-1}, from 235.79 to 658.05 g ha^{-1} and from 252 to 626.49 g ha^{-1}, respectively. Lowest total Zn accumulation in response to Zn application was recorded in MC-24 (292.5 g ha^{-1}).

Zn bioavailability to human

The results of tested rice genotypes suggested that Zn application did not affect grain phytate concentration (Figure 1). Minimum phytate concentration (1.76 g kg^{-1}) was observed in MC-24 which was comparable to grain phytate concentration in MC-57 (1.95 g kg^{-1}), MC-84 (2.21 g kg^{-1}), MC-95 (2.14 g kg^{-1}) MC-98 (1.90 g kg^{-1}), MC-101 (1.87 g kg^{-1}) and MC-109 (1.95 g kg^{-1}). Maximum phytate (2.61 g kg^{-1}) was observed in MC-90. Phytate to Zn ratio is another important parameter to estimate the extent of Zn bioavailability in cereals. The present study showed that Zn application appreciably decreased phytate-Zn ratios of rice genotypes against their respective control. Maximum decrease was observed in MC-98 where the ratio changed from 20.84 to 4.69 with the application of Zn. In MC-57, the ratio decreased from 19.67 to 4.88. Overall, average decrease in Phytate-Zn ratio was 57.9% among tested genotypes. Bioavailabilities from MC-98 (4.27 mg/300g/day), MC-57 (4.16 mg/300g/day) and MC-53 (4.15 mg/300g/day) in response to Zn fertilization were statistically identical and highest among the tested rice genotypes. The Zn bioavailability from MC-98 increased appreciably from 1.11 mg/300g/day to 4.27 mg/300g/day as an effect of Zn application. The minimum bioavailable Zn was observed in MC-50 when grown without Zn fertilizer.

DISCUSSION

The outcomes of the present study supported the hypothesis that Zn application increases crop yield, grain Zn concentration and ultimately Zn bioavailability to humans. Zn influences grain formation by affecting pollen tube development and fertilization (Kaya and Higgs, 2002). Zn also stimulates plant growth and development by enhances enzymatic activities by acting as co-factor of all classes of enzymes (Cakmak *et al.,* 2010; Imran *et al.,* 2015). Similar responses for Zn fertilization were observed for yield, grain [Zn] and Zn accumulation. Average grain and straw yields of the genotypes improved by 8.2 and 9% with Zn application, respectively (Table 2). Likewise, there were increments in average grain [Zn], straw [Zn], grain Zn accumulation, straw Zn accumulation and total Zn accumulation (Table 3 and 4) 2.48, 2.08, 2.74, 2.29 and 2.42 times increase over control, respectively. Zn helps in translocation of carbohydrates to the grains which increase grain weight and yield (Ajiboye *et al.,* 2015). Moreover, Zn availability to plants is improved in flooded condition which is responsible for lowering redox potential and subsequently improving Zn concentration and uptake (Chen *et al.,* 2017). In Pakistan, Zn application in rice enhances grain yield, grain [Zn] and improves rural economics (Farooq *et al.,* 2018).

Phytate or phytic acid is the anti-nutrient element in the cereals. It binds metal ions and make them unavailable to human. Phytate/polyphenols restricts mineral absorption in gastrointestinal tract of human (Clemens, 2014). The effects of Zn application on grain [phytic acid] varied among genotypes. Most genotypes responded with insignificant increase in phytic acid concentration to Zn application (increase of 8.32%) (Figure 1). Phytic acid concentration could not be regulated by Zn application. Hence, it is independent of grain [Zn] and uptake (Zhang *et al.,* 2012; Wang *et al.,* 2015). Molar ratio of grain Phytic acid and grain Zn concentrations of genotypes decreased with application of Zn. Average decrease was 2.4 times in comparison to control. These results support the hypothesis that agronomic fortification improves yield and availability of Zn. Hence, to achieve Zn biofortified rice grains, lower PA/Zn ratio is desired (Hussain *et al.,* 2013). Zn application provided appreciable bioavailable proportion of grain [Zn] supports the hypothesis of agronomic biofortification. Average estimated human available Zn was 3.36 mg/300g/day which surpassed the desired bioavailable Zn concentration from 300 g cereals for an adult human by 12%. It is desired that consumption of 300 g cereals by adult must provide him with 3 mg Zn in a day (Rosado *et al.,* 2009).

CONCLUSION

Zn application enhanced grain [Zn] which was desired for higher yield and better Zn bioavailability to human. Grain phytic acid concentration was not affected by Zn application. However, Zn application improved bioavailability to the desired level of biofortification.

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AUTHOR'S CONTRIBUTION

M. A. Akram: Joint research.
N. Depar: Joint research.
M. Irfan: Data analysis and interpretation.

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