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Zinc nutrition application augments morpho-physiological attributes, productivity and grain zinc bioavailability of Paddy Rice

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Summary

Zinc (Zn) deficiency is the most important micronutrient disorders affecting plants and human health. Present study evaluated the potential of various Zn application methods in improving the performance of selected rice genotypes and Zn bioavailability in grains. Pre-selected Zn application methods through pot experiments were validated in the field. Harvested grains were fed to albino rats for Zn bioavailability. Results revealed that soil + foliar application of Zn was effective in improving the seedling growth of rice genotypes by modulating the agronomic, water related and biochemical attributes. The rats gained more body weight fed with rice genotype Accession-164 (high Zn accumulator) compared with the minimum for Super Basmati (low Zn accumulator) feed. In crux, soil application of Zn at 15 kg ha^{-1} followed by foliar application of 0.25% $ZnSO_4.7H_2O$ solution at tillering and heading stages produced the highest grain yield (26.25%, 29.11%) with maximum bioavailable Zn (21.02%, 22.50%) during both years, respectively, in the grains for combating malnutrition in the tested rats.

Keywords: application methods, genotypes, growth, micronutrient, rice, zinc.

Introduction

Human beings need at least 22 mineral elements for fitness and proper growth (Welch and Graham, 2004). Nutrient demand of human body is met through balanced food. Recent studies indicate that of the total global population, about 60, 30 and 20% of people are deficient in iron, iodine and zinc, respectively (Graham et al., 2007). Apart from unbalanced food intakes, such minerals deficiencies also owe substantially to growing food crops in soils, which are extremely low in phyto-availability of minerals (Graham et al., 2007).

Zn deficiency is common in soils of various countries including Pakistan (Kumssa et al., 2015). Cereals are more prone to the Zn deficiency as compared with legumes, and often result in reduction of yield and nutritional quality of the former (Kumssa et al., 2015). Malnutrition in human beings regarding Zn can be rectified through nutritional diversification, food fortification by Zn, or by escalating Zn concentrations in staple crops (biofortification). Biofortification of cereal crops using the inorganic fertilizers and cultivation of such verities having greater capability of absorbing nutrients is the best approach for both improving the yield and nutritional value of staple crops. Out of different practical approached, agronomic biofortification through the foliar application is economically sustainable and practically adoptable solution to overcome the Zn deficiency issue in rice (Zaman et al., 2017).

Zinc is the major micronutrient limiting rice growth and yield in many of the rice growing areas in Pakistan and elsewhere (Quijano-Guerta et al., 2002). Reduced tillering, increased spikelet sterility and extension in the maturity period of the rice crop are major symptoms of Zn deficiency and playing major role in yield and quality reduction (Lopez-Millan et al., 2005). Zn is often applied for improving Zn availability in the soil and its bioavailability for plants. Zinc can be applied via the soil, seeds, leaves and roots (Esfandiari et al., 2016; Rehman et al., 2017). The efficiency of Zn can be evaluated in terms of the ratio of shoot dry matter or grain yield produced between the treatments with or without Zn application. In the sub soils, the Zn deficiency problem can be solved by the foliar spray (Hussain et al., 2013). Generally, the Zn applied through the foliar and soil application improved the yield and quality status of rice using $ZnSO_4$ (Zaman et al., 2018) or by using the seed treatment and foliar application of Zn (Farooq et al., 2018). Proper availability at the peak periods of crop demand and the costs involved in Zn fertilization are crucial for harvesting good rice yields and making final recommendations regarding the method of its application under field conditions. Keeping in view the importance of Zn for plant growth, the severity of its deficiency in soils and plants, and the significance of its bioavailability, an attempt has been made to optimize the most suitable method of Zn application in rice under field conditions. A field appraisal of selected Zn application methods was carried out for enhancing productivity, quality, and Zn biofortification of rice grains. Such Zn-biofortified rice grains were also assessed for Zn bioavailability in albino rats.

Materials and methods

To evaluate the performance of different Zn application methods for rice crop, the experiments were conducted at glass-house and field conditions and later on tested for Zn bioavailability in albino rats.

A – Pot experiment

Study site and experimental design

A glass-house experiment was conducted at Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan in 2014. Experiment was designed in a completely randomized design with two factors (5 rice genotypes and 15 Zn application methods) and each treatment was replicated thrice.

Experimental treatments: Five Zn efficient Basmati genotypes of rice viz., Super Basmati, Accession-126, Accession-154, Acces-

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sion-164 and Accession-175 selected from a screening experiment containing 183 accessions/genotypes on the basis of grain Zn contents by following the protocols JONES and CASE (1990) and on yield potential (data not shown) was used in this study. Seeds were obtained from the Plant Genetic Resource Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad, Pakistan. The experiment comprised of 15 treatments for Zn application using $ZnSO_4.7H_2O$ as a Zn source: control (nil Zn), seed priming (0.5% solution for 10 h), soil application (15 kg ha^{-1} at the time of sowing), foliar application (0.25% solution at 30 DAS), root dipping (0.5% solution for five minutes at the time of transplanting), seed priming + soil application, seed priming + foliar application, seed priming + root dipping, soil application + foliar application, soil application + root dipping, foliar application + root dipping, seed priming + soil application + foliar application, seed priming + soil application + root dipping, soil application + foliar application + root dipping, and seed priming+ soil application + foliar application + root dipping.

Crop husbandry: Ten seeds of each genotype (with around 12% moisture content) were sown in each earthen pot (45 cm depth × 30 cm diameter) filled with 10 kg soil. For priming, 50 g seeds were soaked in well aerated $ZnSO_4.7H_2O$ solution for 10 h at 25 ± 2 °C, and dried back to their initial weight. Aquarium pump was used for aeration purpose. For seedling root dipping, seedlings raised in a nursery were uprooted 15 days after sowing (DAS) and dipped in the $ZnSO_4.7H_2O$ solution for 5 minutes, and were planted in the pots. Foliar application was done 30 DAS. For soil application, all the Zn was applied in pots at the time of sowing. Basal doses of urea, di-ammonium phosphate and potassium sulphate were applied at the rates of 24 (N), 40 (P), and 62 (K) gram per pot, respectively. The plants were harvested at 45 DAS for subsequent determinations.

Data collection: Three randomly selected plants were tagged for measuring plant height. At harvesting, the tagged plants from each pot were counted for number of tillers. For chlorophyll contents, fresh leaves were cut into 0.5 cm segments and extracted overnight with 80% acetone at -10 °C after 30 DAS. The extract was centrifuged at 14,000 rpm for 5 min at 25 °C and absorbance of supernatant was read at 645 and 663 nm using a spectrophotometer (T60 U Spectrophotometer PG Instruments, Limited, USA). The readings were taken according to NAGATA and YAMASHITA (1992). For relative leaf water content (RWC), fresh leaves (0.5 g) were weighed and were floated on water for 4 h, and saturated weight (WS) was measured thereafter. These leaves were oven-dried for 24 h at 85 °C to determine dry weight (Wd) and readings were taken by following the method of BARRS and WEATHERLY (1962). To determine membrane permeability, leaf electrolyte leakage was measured following the protocol of BLUM and EBERCON (1981). Six leaf segments of similar size were lightly washed with distilled water and immersed in a test tube having 6 ml distilled water for 12 h at room temperature. Then electrical conductivity (EC_1) of solution was measured with a conductivity meter (Model DDS-11A, Shanghai Leici Instrument Inc., Shanghai, China). Samples were then heated in boiling water for 20 min and cooled to room temperature. The conductivity of killed tissues (EC_2) was again measured. Electrolyte leakage was measured as the ratio of EC_1 to EC_2 and expressed in percentage. For shoot Zn concentration, a di- acid mixture (HNO_3:$HClO_4$ ratio of 2:1) method was used by following the protocols of JONES and CASE (1990).

B – Field experiments

Study site and experimental design

The field experiments were conducted at Research Farm of Nuclear Institute for Agriculture & Biology (NIAB), Faisalabad, Pakistan during kharif, 2014 and 2015. The experiments were laid out in a randomized complete block design with factorial arrangement of treatments and were replicated thrice having net plot size 2.0 m × 6.0 m.

Experimental treatments: The same set of rice genotypes was used as in the pot experiments. There were four Zn ($ZnSO_4.7H_2O$) treatments including control (non-Zn application), soil application (SA) at 15 kg ha^{-1}, foliar application (FA) at 0.25% (at tillering and heading) and soil application followed by two foliar applications as in previous treatments.

Crop husbandry: Rice seeds of selected genotypes were sown on 20th of June 2014 and 25th June of 2015 using hand drill in a nursery. Seedlings (30 day-old) were manually transplanted in puddled fields by maintaining R × R and P × P distance of 22.5 cm. At transplantation, uniform application of fertilizer in the form of 100 N, 67 P and 60 K kg ha^{-1} was done by applying urea, di-ammonium phosphate and potassium sulphate, respectively. At 25 days after transplantation, second dose of N at 60 kg ha^{-1} was applied. All the Zn by soil application method was applied at the time of sowing while foliar application of Zn was performed at tillering and heading stages in both years. To maintain the submerged soil conditions during the crop growth canal water was used. Irrigation water was maintained at a depth of 3-4 cm at transplantation, and one week afterwards at a depth of 5-6 cm throughout the growing season till one week before harvesting. For weed control, mixture of Ethoxy sulphuran and Phenoxyprop-p-ethyle at 200 g and 370 ml ha^{-1}, respectively was applied at specific intervals. Carbofuran was broadcasted at 25 kg ha^{-1} to shield the crop from the insects. Crop was harvested manually on 15th and 21st November, during 2014 and 2015, respectively. Each plot was harvested and threshed separately.

Data Collection: To determine plant water relations, three penultimate leaves from five selected rice plants of each treatment were harvested at tillering (75 days after sowing). Pressure chamber was used to determine the leaf water potential. After the determination of water potential, leaf tissues were frozen for 48 h and thawed. The sap was extracted by centrifuging at 5000 × *g* and osmotic potential was determined (Digital Osmometer, Wescor, Logan, UT, USA). Chlorophyll contents, relative water contents, and electrolyte leakage were determined as described in the pot experiment by getting the samples from the selected plants. Net assimilation rate (NAR) was estimated following HUNT (1978). Stomatal conductance (*gs*), photosynthetic rate (*A*), and transpiration rate (*E*) of 3rd leaf from top of each selected plant were recorded by using a photosynthetic system (LCA-4; Analytical Development Company, Hoddesdon, England). All the gas exchange attributes were recorded at the tillering stage between 10:00 AM to 01:00 PM. During data recording, leaf chamber molar gas flow rate was 248 µmol s^{-1}, ambient CO_2 conc. (Cref) was 352 µmol mol^{-1}, temperature of leaf chamber (Tch) varied from 36.1 to 40.4 °C, ambient pressure (P) 98.01 kPa, molar flow of air/leaf area 221.06 mol m^{-2} s^{-1}. PAR was maximum up to 1050 µmol m^{-2} s^{-1} and leaf chamber volume gas flow rate (v) was 380 mL min^{-1}. A meter rod was used to measure plant height at maturity starting from the base of plant up to the tip of flag leaf. Unit area was selected randomly from each plot for counting the number of tillers. Panicle length was measured by measuring tape from 20 randomly selected panicles from each plot. After harvesting and threshing of crop, five random samples were taken from each treatment for 1000-kernel weight on electronic balance. Each plot was harvested and threshed separately and clean paddy rice was air-dried. Yield of each plot was weighed and values were adjusted to 12% moisture and expressed in t ha^{-1}. Micro-Jheldahl digestion and ammonia distillation process was used to determine the protein contents of the kernels which were then converted to protein contents by multiplying with the factor 5.95. Milled rice kernels were grinded in a grinding mill for determination of kernel amylase contents (Juliano, 1971). Kernel dimension (length) was taken on 100 normal kernels from each treatment with the help of a digital vernier caliper.

C – Animal Feeding Experiment

Study site and experimental design

This experiment was carried out at Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan during winter, 2014 and 2015 and was laid out in completely randomized design (CRD).

Experimental treatments: Zinc biofortified rice kernels of five selected genotypes (Super Basmati, Accession-154, Accession-175, Accession-164 and Accession-126) were produced as described above in both years in field experiments. After harvesting, kernels of all five genotypes of best treatment (best one from the four Zn treatments "Soil + Foliar Application of $ZnSO_4$" for rat experiment) was analyzed and grouped into five different categories of grains on the basis of having different levels of Zn (i.e. 27, 45, 50, 55 and 59 mg kg^{-1} dry weight, respectively. Kernels of rice genotype G_1 (Super Basmati) were considered as control.

Experimental conditions for animals: Forty male Wister strain albino rats having initial body weight of 100 ± 10 g were purchased from the same department. The rats were acclimated for five days prior to experiment with an evaluation of their health status in both years. Animals were kept in metallic cages and maintained in a controlled room 22 ± 2 °C with relative humidity of air 55 ± 5%, and having the optimum lightening in a daily cycle, (i.e. 12 h light: dark periods). The study was reviewed and approved by an Animal Care and Use Committee prior to the commencement of the research and the experiment was performed according to the rules and regulation protocols accepted by Local Ethical Commission for Investigation on Animals. Rats were maintained in collective cages (4 rats per cage) as treatment plan. Rat feed was made by following AIN standards and during the whole period (one month) of experiment, all rats were supplied with deionized water to eliminate additional Zn sources. After one month feeding of Zn biofortified kernels to male albino rats, their body weight was recorded in both years.

Statistical analysis

The collected data from each experiment were analyzed using Statistics 8.1 software. Highest significant difference (HSD) test at 5% probability level was used to compare the treatment means. Correlation coefficients among different traits were calculated using a computer assisted program MINITAB 14.

Results

Pot experiment

All Zn application methods significantly improved plant water-relations, and agronomic as well as biochemical attributes of rice genotypes, compared with control at 45 DAS (Tab. 1). Soil + foliar application of $ZnSO_4.7H_2O$ at 15 kg ha^{-1} and 0.25% solution, respectively

Tab. 1: Agronomic, water and biochemical traits of rice genotypes influenced by different Zn application methods in a pot experiment till 45 DAS

Factor	Agronomic Trait		Water Related Trait	Biochemical Trait				
	PH (cm)	T (per plant)	RWC (%)	*CHL* a (mg L^{-1})	*CHL* b (mg L^{-1})	T *CHL* (mg L^{-1})	S Zn (mg kg^{-1})	EL (%)
Genotype (G)								
G_1 (Super Basmati)	51.79 A	4.42 A	55.42 A	3.37 B	1.74 C	5.12 D	52.29 D	15.27 C
G_2 (Accession-126)	46.65 CD	3.35 D	46.99 C	3.06 D	4.08 B	7.15 C	68.19 A	20.41 A
G_3 (Accession-154)	44.52 D	3.13 D	44.54 D	3.26 C	0.61 E	3.87 E	59.36 C	21.07 A
G_4 (Accession-164)	48.69 BC	3.71 C	53.10 B	0.52 E	7.22 A	7.75 B	67.80 A	17.59 B
G_5 (Accession-175)	50.12 AB	4.08 B	53.45 AB	7.10 A	0.95 D	8.06 A	63.60 B	17.81 B
Application Method (M)								
Control	37.16 HI	1.73 H	44.94 GH	3.28 HI	2.78 IJ	6.06 KL	40.81 J	22.95 AB
Seed priming (SP)	40.62 GH	2.33 G	46.62 FH	3.33 G-1	2.81 H-J	6.14 JK	43.86 IJ	21.96 BC
Soil application (SA)	47.23 EF	2.86 FG	48.44 D-G	3.41 E-G	2.86 F-I	6.27 H-J	47.86 G-J	19.99 CD
Foliar application (FA)	49.96 DE	3.33 EF	49.27 D-G	3.43 D-G	2.89 E-H	6.33 G-I	50.03 G-I	19.37 C-E
Root dipping (RD)	43.44 FG	2.66 G	47.73 E-G	3.37 F-H	2.83 G-J	6.20 I-J	46.01 H-J	20.89 B-E
SP + SA	54.51 CD	4.66 D	53.26 A-D	3.55 A-D	2.98 B-D	6.53 C-E	52.78 F-H	16.15 F-H
SP + FA	58.06 BC	5.00 CD	54.37 A-C	3.57 A-C	3.01 A-D	6.58 B-D	55.97 D-G	15.46 G-I
SP + RD	52.77 D	4.53 D	52.04 B-E	3.52 B-E	2.96 C-E	6.48 D-F	53.05 E-H	17.29 E-G
SA + FA	64.99 A	6.20 A	57.53 A	3.64 A	3.09 A	6.74 A	61.72 D	13.36 I
SA + RD	61.16 AB	5.33 BC	55.40 A-C	3.59 A-C	3.03 A-C	6.62 A-C	58.48 D-F	14.55 HI
FA + RD	62.28 AB	5.66 AB	56.53 AB	3.62 AB	3.07 AB	6.69 AB	60.95 DE	13.96 HI
SP + SA + FA	39.46 G-1	3.53 E	50.83 C-F	3.49 C-E	2.92 D-G	6.42 E-G	88.16 B	18.40 D-F
SP + SA + RD	38.99 G-1	3.33 EF	50.85 C-F	3.48 C-F	2.93 D-F	6.42 E-G	76.56 C	18.43 D-F
SA + FA + RD	39.16 G-1	3.53 E	50.83 C-F	3.47 D-F	2.92 D-G	6.40 F-H	94.75 AB	18.49 D-F
SP + SA + FA + RD	35.56 I	1.40 H	41.86 H	3.24 I	2.75 J	5.99 L	102.81 A	25.19 A
HSD (G) ($p \leq 0.05$)	2.18	0.25	2.29	0.05	0.04	0.06	3.76	1.21
HSD (M) ($p \leq 0.05$)	4.71	0.55	4.94	0.11	0.09	0.13	8.11	2.61
G × M ($p \leq 0.05$)	NS	NS	NS	NS	NS	NS	NS	NS

PH = Plant height; T = Tillers; EL = Electrolyte leakage; RWC = Relative water contents; S Zn = Shoot Zn contents; *CHL* a = Chlorophyll *a*; *CHL* b = Chlorophyll *b*; T *CHL* = Total chlorophyll contents; HSD = Highest significant Difference; SP = Seed priming; SA = Soil application; FA = Foliar application; RD = Root dipping. Any two means within a column followed by same letter are not significantly different at $p \leq 0.05$, NS = non-significant, n = 3.

recorded the maximum plant height (64.9 cm), number of tillers (6.2 per plant), RWC (57.5%), chlorophyll *a* (3.6 mg L^{-1}), chlorophyll *b* (3.1 mg L^{-1}), total chlorophyll contents (6.7 mg L^{-1}) and minimum electrolyte leakage (13.4%). Root dipping (0.5% $ZnSO_4.7H_2O$) followed by the foliar application (0.25% $ZnSO_4.7H_2O$) was the second best treatment for these attributes. The maximum shoot Zn contents (102.8 mg kg^{-1}) were observed in the treatment 'seed priming + soil application + foliar application + seedling root dipping', which showed toxic and detrimental effects on seedling growth. Among various rice genotypes, Super Basmati performed best for agronomic and water related traits. However, chlorophyll contents and shoot Zn contents were higher in Accession-175 and Accession-126, respectively, compared with other genotypes. The minimum electrolyte leakage was observed in Accession-154 (Tab. 1). Interaction between rice genotypes and Zn application methods was not significant for all agronomic, water related and biochemical attributes. Correlation analysis revealed that electrolyte leakage had negative relationship with plant height, RWC, shoot Zn contents, tillers per plant and total chlorophyll contents (Tab. 2). However, total chlorophyll contents and tillers per plant were positively correlated with shoot Zn contents, RWC and plant height. Likewise, RWC was positively linked with plant height and chlorophyll *b*, however, chlorophyll *a* was negatively related to chlorophyll *b* (Tab. 2).

Field experiment

Zinc application methods and rice genotypes significantly influenced the plant water-relations in both years (Tab. 3). However, there was non significant interaction between genotypes and application methods. Zn application treatment 'foliar + soil application' significantly enhanced the water potential, osmotic potential, and RWC of rice genotypes in both years, compared with the non-Zn application control. Foliar Zn application was found to be more effective than soil Zn application for all of these attributes. Zn application significantly improved chlorophyll contents of rice genotypes in both years, compared with control (Tab. 3). Total chlorophyll contents were the highest in 'soil + foliar application' treatment followed by foliar Zn application alone, and soil Zn application alone (Tab. 3). In different genotypes, Zn-induced increase in water related traits and chlorophyll contents in rice genotypes followed the order of Super Basmati > Accession-175 > Accession-164 > Accession-126 > Accession-154 (Tab. 3). Electrolyte leakage in rice genotypes was significantly decreased by different Zn application methods, compared with the control. However, soil + foliar application of Zn recorded the maximum reduction in the electrolyte leakage in both years. The Zn-induced reductions in electrolyte leakage of different rice genotypes followed the order of Super Basmati < Accession-175 < Accession-164 < Accession-126 < Accession-154 (Tab. 3).

Tab. 2: Correlation matrix of agronomic, water related and biochemical attributes of rice genotypes influenced by different Zn application methods in a pot experiment

Variable	*CHL* a	*CHL* b	EL	PH	RWC	S Zn	T *CHL*
CHL b	-0.754**						
EL	-0.066 NS	-0.074 NS					
PH	0.099 NS	0.039 NS	-0.691**				
RWC	0.110 NS	0.136*	-0.837**	0.572**			
S Zn	-0.059 NS	0.184**	0.153*	0.330**	-0.078 NS		
T *CHL*	0.148**	0.537**	-0.196**	0.187**	0.347**	0.202**	
T	0.148**	-0.001NS	-0.851**	0.845**	0.743**	0.145*	0.189**

PH = Plant height; EL = Electrolyte leakage; RWC = Relative water contents; S Zn = Shoot Zn contents; T = Tillers; *CHL* a = Chlorophyll a; *CHL* b = Chlorophyll b; T *CHL* = Total chlorophyll contents; * = Significant at $p \leq 0.05$; ** = Significant at $p \leq 0.01$; NS = Non-significant

Tab. 3: Influence of Zn applications on water-related and biochemical traits of five rice genotypes for two growth seasons in a field experiment

Factor	Water related trait						Biochemical trait			
	Water potential (MPa)		Osmotic potential (MPa)		Relative water contents (cm)		Total chlorophyll contents (mg L^{-1})		Electrolyte leakage (%)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Genotype (G)										
G_1 (Super Basmati)	2.07 A	2.18 A	2.51 A	2.59 A	57.03 A	58.22 A	5.23 D	5.57 D	14.92 C	14.38 D
G_2 (Accession-126)	1.61 D	1.66 D	1.87 C	1.96 D	47.77 B	48.67 B	7.29 C	7.42 C	19.92 A	19.83 B
G_3 (Accession-154)	1.23 E	1.33 E	1.72 D	1.84 E	44.40 C	46.56 B	4.06 E	4.10 E	21.20 A	21.02 A
G_4 (Accession-164)	1.76 C	1.86 C	1.96 BC	2.04 C	53.88 A	54.89 A	7.95 B	8.03 B	17.82 B	17.72 C
G_5 (Accession-175)	1.95 A	2.06 B	2.04 B	2.14 B	55.57 A	57.20 A	8.28 A	8.38 A	17.87 B	17.79 C
Application method (M)										
Control	1.51 C	1.70 D	1.78 C	1.99 D	45.25 D	46.50 D	6.38 D	6.51 D	23.10 A	22.97 A
Soil application (SA)	1.59 B	1.79 C	1.87 B	2.08 C	50.43 C	51.32 C	6.51 C	6.64 C	20.23 B	20.16 B
Foliar application (FA)	1.61 B	1.85 B	1.92 B	2.15 B	53.72 B	54.90 B	6.62 B	6.75 B	17.02 C	16.92 C
SA × FA	1.70 A	1.92 A	2.02 A	2.24 A	57.53 A	59.71 A	6.74 A	6.90 A	13.04 D	12.90 D
HSD (G) ($p \leq 0.05$)	0.13	0.06	0.13	0.07	3.24	3.38	0.11	0.09	1.38	1.17
HSD (M) ($p \leq 0.05$)	0.07	0.05	0.07	0.06	2.72	2.83	0.09	0.07	1.15	0.98
G × M ($p \leq 0.05$)	NS	NS	NS	NS	NS	NS	NS	NS	3.66	**

Any two means within a column followed by same letters are not significantly different at $p \leq 0.05$, NS = non-significant, n = 3.

Zinc application methods and rice genotypes significantly ($p \leq 0.05$) affected the physiological growth attributes (Fig. 1). Maximum transpiration rate, stomatal conductance, photosynthetic rate and net assimilation rate were observed in soil + foliar application followed by foliar Zn application alone and soil Zn application alone in both years. Among different rice genotypes, the highest values of these attributes were recorded in Super Basmati, followed by Accession-175, Accession-164, Accession-126, and Accession-154 (Fig. 1).

All the Zn application methods and rice genotypes significantly ($p \leq 0.05$) affected the plant height, number of tillers, panicle length, 1000-kernels weight and kernel yield in both years at field levels (Tab. 4). The interactive effect between the both factors was also sig-

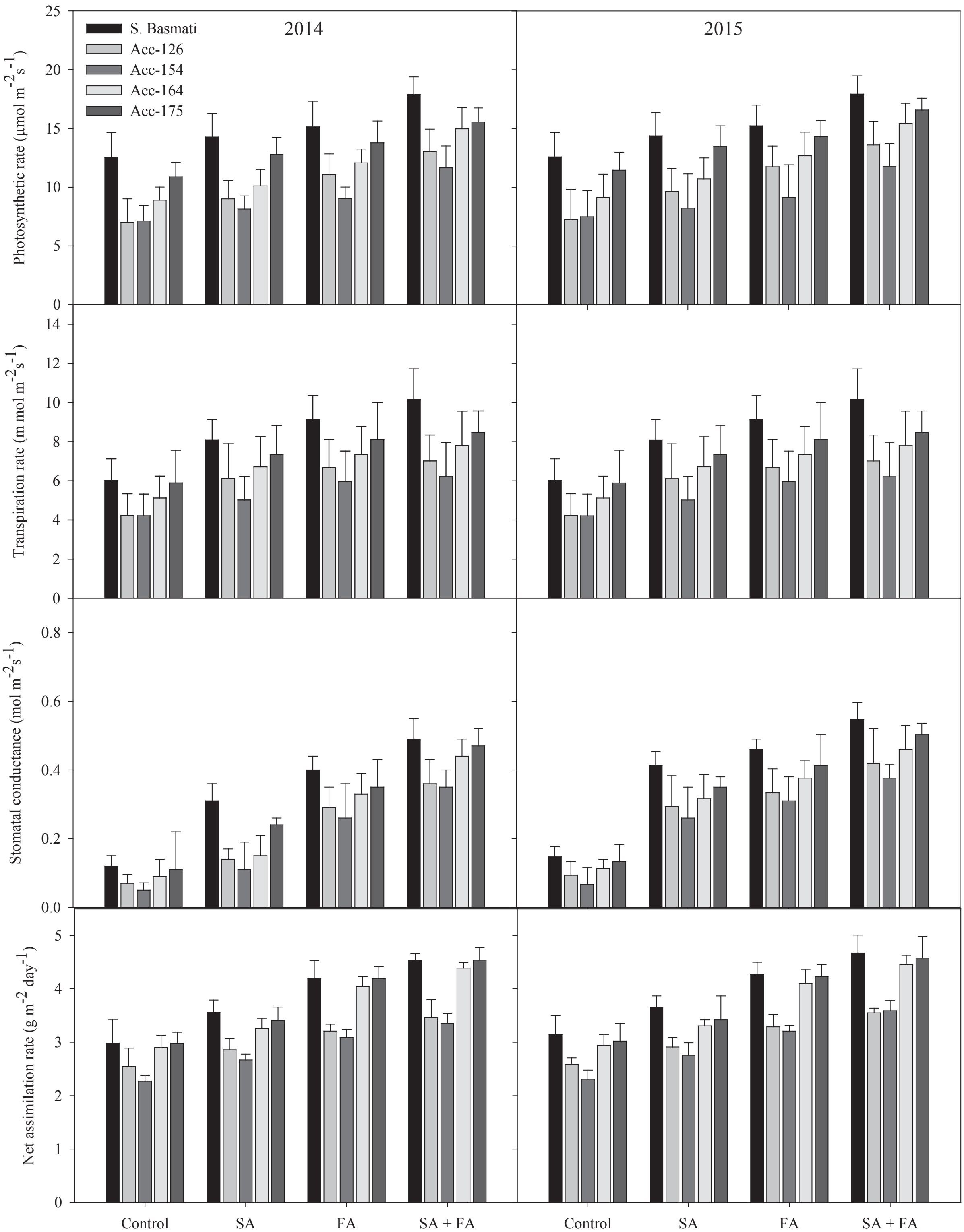

Fig. 1: Effect of Zn fertilization treatments on physiological and growth attributes of rice genotypes. Vertical bars above mean denote the standard error of three replicates.

nificant for total tillers for first year, 1000-kernels weight and kernel yield during both years. Soil + foliar application of Zn had the maximum plant height, number of tillers, panicle length, 1000-kernels weight and kernel yield in both years (Tab. 4), followed by foliar application and soil application treatments. Among genotypes, the maximum plant height was recorded for Accession-164, while for the values of all yield related traits were in order of Super Basmati > Accession-175 > Accession-164 > Accession-126 > Accession-154.

Zinc application methods significantly ($p \leq 0.05$) affected the shoot Zn and kernel Zn contents of rice in both years, while kernel protein contents varied significantly ($p \leq 0.05$) in the second year of the study only. There was no significant effect on kernel amylose contents and kernel length by Zn application methods in both years (Tab. 5). Maximum shoot Zn and kernel Zn contents were recorded in the treatment 'soil + foliar application of Zn' followed by foliar Zn application alone, and soil Zn application alone in both years. All the grain quality attributes varied significantly among different rice genotypes. Shoot Zn content, kernel Zn content, kernel protein content, and kernel amylose content in different rice genotypes followed the order of Accession-126 > Accession-164 > Accession-175 > Accession-154 > Super Basmati, while kernel length was in the order of Super Basmati > Accession-175 > Accession-154 > Accession-164 > Accession-126 (Tab. 5).

Correlation analysis indicated negative relationship between electrolyte leakage and plant height, panicle length, photosynthetic rate, total tillers, grain yield and 1000-kernels weight, respectively (Tab. 6). Grain Zn contents were also negatively correlated with 1000-kernels weight and grain yield. However, total chlorophyll contents and 1000-kernels weight were positively linked with total tillers, photosynthetic rate, plant height and panicle length.

Tab. 4: Influence of Zn application on agronomic and yield-related traits of five rice genotypes for two growth seasons in a field experiment

Factor	Plant height (cm)		Total tillers (m^{-2})		Panicle length (cm)		1000-kernels weight (g)		Kernel yield ($t\ ha^{-1}$)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Genotype (G)										
G_1 (Super Basmati)	141.58 B	140.77 B	448.50 A	454.33 A	26.60 A	26.90 A	24.03 A	24.09 A	4.50 A	4.60 A
G_2 (Accession-126)	139.28 B	137.58 B	411.08 C	419.50 C	22.97 C	23.19 CD	20.55 D	20.61 D	3.23 D	3.31 D
G_3 (Accession-154)	110.48 C	110.50 C	398.00 D	404.33 D	21.53 D	21.65 D	19.24 E	19.28 E	3.02 E	3.11 E
G_4 (Accession-164)	150.08 A	148.40 A	432.17 B	438.92 B	23.89 BC	23.75 BC	21.59 C	21.59 C	3.52 C	3.60 C
G_5 (Accession-175)	137.78 B	136.18 B	444.67 A	449.33 A	25.09 B	25.21 B	22.77 B	22.80 B	4.09 B	4.20 B
Application method (M)										
Control	130.66 C	128.49 B	414.13 C	423.93 C	21.74 D	21.83 C	20.48 C	20.49 C	3.39 C	3.58 C
Soil application (SA)	133.60 C	132.47 B	419.33 B	430.40 B	23.29 C	23.34 B	21.11 BC	21.17 B	3.46 BC	3.71 B
Foliar application (FA)	137.71 B	137.10 A	436.33 A	435.87 B	24.64 B	24.68 B	21.75 B	21.79 B	3.55 B	3.80 B
SA × FA	141.39 A	140.69 A	437.73 A	442.93 A	26.38 A	26.72 A	23.21 A	23.25 A	4.28 A	4.37 A
HSD (G) ($p \leq 0.05$)	3.82	4.98	4.30	7.41	1.22	1.67	0.81	0.79	0.15	0.10
HSD (M) ($p \leq 0.05$)	3.20	4.18	3.61	6.22	1.02	1.40	0.68	0.66	0.13	0.09
G × M ($p \leq 0.05$)	NS	NS	11.41	NS	NS	NS	2.16	2.11	0.41	0.28

Any two means within a column followed by same letters are not significantly different at $p \leq 0.05$, NS = non-significant, n = 3.

Tab. 5: Influence of Zn application on kernel quality and grain Zn biofortification of five rice genotypes for two growing seasons in a field experiment

Factor	Shoot Zn contents ($mg\ kg^{-1}$)		Kernel Zn contents ($mg\ kg^{-1}$)		Kernel protein contents (%)		Kernel amylose contents (%)		Kernel length (mm)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Genotype (G)										
G_1 (Super Basmati)	42.34 D	42.24 D	27.75 E	27.70 E	7.02 C	7.05 C	24.23 E	24.30 E	10.92 A	10.91 A
G_2 (Accession-126)	62.28 A	62.33 A	59.08 A	59.16 A	7.31 A	7.37 A	27.27 A	27.30 A	10.58 C	10.58 D
G_3 (Accession-154)	50.42 C	50.89 C	45.16 D	45.23 D	7.08 C	7.10 C	25.23 C	25.28 C	10.67 C	10.66 C
G_4 (Accession-164)	60.60 A	60.78 A	55.20 B	55.32 B	7.21 B	7.23 B	27.23 A	27.33 A	10.61 C	10.61 C
G_5 (Accession-175)	56.19 B	56.37 B	50.51 C	50.64 C	7.16 B	7.17 B	26.12 B	26.20 B	10.75 B	10.74 B
Application method (M)										
Control	45.20 D	45.23 D	43.16 D	43.10 D	7.11	7.09 C	25.46	25.90	10.71	10.72
Soil application (SA)	52.69 C	52.78 C	46.24 C	46.55 C	7.16	7.14 B	26.64	26.70	10.68	10.67
Foliar application (FA)	57.70 B	57.82 B	48.10 B	40.94 B	7.16	7.16 B	26.90	26.93	10.66	10.66
SA × FA	61.72 A	61.78 A	52.67 A	52.80 A	7.17	7.19 A	27.12	27.22	10.70	10.71
HSD (G) ($p \leq 0.05$)	3.76	3.23	1.35	1.67	0.09	0.08	0.69	0.54	0.09	0.07
HSD (M) ($p \leq 0.05$)	3.15	3.49	1.13	1.27	NS	0.04	NS	NS	NS	NS
G × M ($p \leq 0.05$)	NS	NS	3.59	NS	NS	NS	NS	NS	NS	NS

Any two means within a column followed by same letters are not significantly different at $p \leq 0.05$, NS = non-significant, n = 3.

Tab. 6: Correlation matrix of agronomic, physiological and biochemical attributes of rice genotypes influenced by different Zn application methods (Means of both years used in the analysis)

Variable	EL	G Zn	KW	KY	T *CHL*	TT	PR	PH
G Zn	0.03^{NS}							
KW	- 0.74**	- 0.26*						
GY	- 0.66**	- 0.30*	0.87**					
T *CHL*	$- 0.14^{NS}$	0.61**	0.26*	0.14^{NS}				
TT	- 0.73**	$- 0.22^{NS}$	0.88**	0.77**	0.41**			
PR	- 0.85**	$- 0.17^{NS}$	0.89**	0.75**	0.27*	0.86**		
PH	- 0.52**	0.20^{NS}	0.59**	0.45**	0.71**	0.69**	0.57**	
PL	- 0.87**	$- 0.16^{NS}$	0.85**	0.74**	0.22**	0.82**	0.88**	0.58**

PH = Plant height; EL = Electrolyte leakage; G Zn = Grain Zn contents; TT = Total tillers; T *CHL* = Total chlorophyll contents; KW = 1000 kernel weight; KY = Kernels yield; PR = Photosynthetic rate; PL = Panicle length; * = Significant at $p \leq 0.05$; ** = Significant at $p \leq 0.01$; NS = Non-significant

Animal feeding experiment

Feeding of Zn biofortified rice to rats significantly ($p \leq 0.05$) increased their body weight in all groups except the last group assessed weekly (Fig. 2) in both years. Compared to initial body weight, the maximum increase in body weight of rats was observed for rats fed with kernels of Accession-164 (treated with 55 mg kg^{-1} of Zn) at 1^{st}, 2^{nd}, 3^{rd} and 4^{th} week in 2014 (35.22%, 52.28%, 68.22% and 80.59%) and 2015 (36.57%, 54.56%, 71.00% and 83.73%), respectively (Fig. 2). However, body weight of rats was not improved after feeding of kernels of Accession-126 regardless high kernel-Zn concentration in this group. The increase in the body weight of male Wister rats was in the order of $G_4 > G_3 > G_5 > G_2 > G_1$ in both years.

Discussion

Different Zn application methods considerably improved the crop stand establishment, water relations, photosynthetic pigments, tillering, kernel quality and biofortification of the tested rice genotypes. Application of Zn in the Zn-deficient calcareous soil followed by foliar spray significantly ($p \leq 0.05$) improved the agronomic traits. CAKMAK (2008) reviewed a number of studies and documented the positive effects of Zn application on plant growth and productivity in Zn-deficient calcareous soils.

Increasing doses of Zn improved crop water relations that might be ascribed to better root growth and increased the water uptake by plants (ZHAO et al., 2016). Better root development enabled the plants to extract water efficiently, and hence showed healthy water relations (Tabs. 1 and 3). RWC increased gradually with the increasing Zn concentration and achieved the highest (27.13% and 27.33%) during both years under field study where 15 kg ha^{-1} Zn was applied in soil followed by foliar application of 0.25% Zn solution. Nonetheless, higher concentration of Zn gradually decreased RWC of rice leaves, which might be due to disturbed osmotic relations at higher levels of Zn. Decline of water uptake rate or decrease of hydraulic conductivity of root at higher Zn levels might also have resulted in lower RWC of the leaves (CHEN et al., 2008). There are also reports showing disturbed water relations of the leaves exposed to Zn-deficient environments (ZHAO et al., 2016). The minimum electrolyte leakage in rice leaves was observed for soil + foliar application of Zn which might also be related with higher leaf RWC, which also corresponded to higher *gs* (Fig. 1). Similar responses have been reported for various filed crops (TAS and TAS, 2007). As Zn has major role in the membrane system. Zinc has a unique property of existing in a divalent state, without any redox cycling, and is thereby stable in biological medium in which oxido-reductive potential is subjected to continuous flux. As a result of these properties, membrane lipid packing is protected from ROS per-oxidation, which in turn prevents ion leakage from ion channels (SAMREEN et al., 2013).

Gas exchange characteristics like stomatal conductance (*gs*), photosynthetic rate (*A*) and transpiration rate (*E*) showed significant increase with increase in the Zn application. Wang and Jin (2005) reported that deficiency of Zn in the soil depressed photosynthetic activity because of decrease in *gs* and increase in sub- stomatal CO_2 concentration (*Ci*). The reduction in photosynthetic activity under control plots (0.31 mg kg^{-1} Zn) of rice genotypes might be due to low chlorophyll contents. Zinc also provides building material for leaf chlorophyll contents. Soil + foliar application of Zn improved the chlorophyll *a*, *b* and total chlorophyll contents in all rice geno-

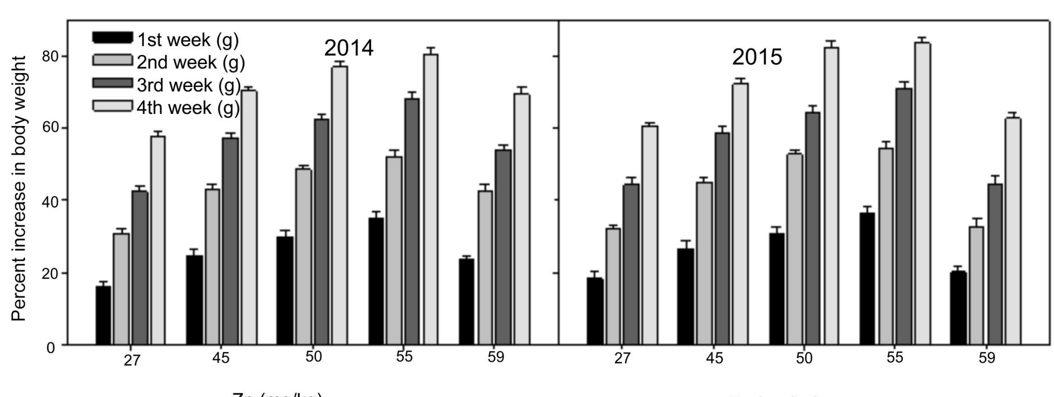

Fig. 2: Percent increase in body weight of groups of male rats after feeding with Zn biofortified rice of both years. Vertical bars above mean denote the standard error of three replicates.

types showing involvement of Zn in chlorophyll synthesis (Abadi and Sepehri, 2016). Application of Zn in soil followed by foliar spray had positive effect on chlorophyll formation which ultimately promoted the photosynthetic rate. There was a linear increase in transpiration rate (*E*) of rice genotypes that might be linked to optimum Zn nutrition. Similar finding has been given by Tariq et al. (2014) who reported that application of Zn increased the photosynthesis and chlorophyll production. Marschner (2012) reported an instant decline in transpiration efficiency of leaves exposed to deficiency of Zn. Our findings suggested that balanced application of Zn in Zn-deficient soils improved gas exchange characteristics of the rice genotypes. Similar trend, as in *E,* with varying Zn application methods was also observed for *gs*. Zinc deficiency was associated with lower *g*s, which is consistent with the findings of Khan et al. (2013). Zinc application in soil followed by foliar application substantially increased the *gs*. Zn presumably involved in regulation of stomata due to its role in maintaining integrity of the membranes (Khan et al., 2013). Our data showed that Zn deficiency not only resulted in poor water uptake, but also depicted poor transpiration efficiency. Any potential benefit of lower *g*s on water relations appears to be negated by a reduction in net carbon fixation.

In the present study, application of Zn increased the height of rice plants (8 and 9%), total tillers (5 and 8%), panicle length (21 and 21.55%) and kernel yield (26.25%, 29.11%) during both years respectively, over control. Zn has been reported to be involved in cell elongation and/or improved cell division rates (Cakmak, 2000b; Ramesh et al., 2014), and meristematic growth (Abaid-Ullah et al., 2015). Chang et al. (2005) also opined that Zn is necessary for cell division and elongation as the fundamental events of growth. Such basic event of growth as enhanced cell division and/or cell elongation, better enzymatic activity and auxin metabolism might have improved morphological traits of rice with Zn supply in the present study. Zn application increased its availability and resulted in reduced spikelet sterility and increased number of panicle bearing tillers (Abaid-Ullah et al., 2015). Our findings also supported this phenomenon. Khan et al. (2013) reported maximum panicle length, kernel per panicle and grain yield with the application of Zn at 9 kg ha^{-1}. Increase in kernels per panicle and 1000-grain weight was due to the involvement of Zn in grain partitioning (Gomez-Coronado et al., 2016).

Shoot Zn concentration ranged from 40.8 to 102.8 mg kg^{-1} in various Zn application treatments (Tab. 1). Usually a concentration greater than 50 mg kg^{-1} Zn in rice plant is considered a threshold level for achieving a positive impact in the grain Zn concentration that ultimately brings a positive impact on human health for combating malnutrition (Cakmak, 2008). Application of Zn by all methods simultaneously proved to be toxic and suppressed rice seed germination and its seedling growth. Higher Zn concentration may restrict the seedling growth by suppressing the cell division (Gomez-Coronado et al., 2016). Moreover, higher concentration of Zn may diminish the root and leaf development owing to substantial decrease in NADPH (nicotinamide adenine dinucleotide phosphate) production in chloroplasts (Mousavi, 2011). Nonetheless, Zn concentration in shoots of rice plants grown in field experiments ranged from 42 to 62 mg kg^{-1} under different Zn applications. Phattarakul et al. (2012) reported that Zn concentration in brown rice was increased by 25% and 32% by foliar and foliar + soil Zn applications as compared with control, respectively. Soil application of Zn followed by foliar sprays significantly increased Zn concentration (22.03 and 22.50%) during both years respectively in rice kernels. Foliar applied Zn is easily absorbed and transported through phloem (Haslett et al., 2005). Increase in Zn concentration of all rice genotypes would provide significant nutritional benefits to rice consumers, particularly for those who have limited access to Zn from other food sources. High grain Zn has also important agronomic benefits for plants grown under low Zn supply. It has been reported that nearly 2800 proteins require Zn for their structural and functional integrity (Hippler et al., 2015), and hence suggesting a high need for Zn during root and coleoptiles development for active protein synthesis and/or other related functions. Application of Zn to rice not only improved the crop stand establishment, water relations, photosynthetic pigments, tillering ability, kernel yield but also significantly improved the kernel length, Zn content, amylose and protein contents. Rashid et al. (2004) also reported that deterioration of the kernel quality of rice was associated with Zn deficiency.

Feeding of Zn excessive and deficient rice kernel diets resulted in poor body and organ weight gain of albino rats. These results are in accordance with Della Lucia et al. (2014) who stated that enhanced Zn dietary intake would result in an increase in the weight gain of test animals but the dose above optimal concentration cause severe complications including growth retardation, poor appetite, impaired immunity and diabetes that might be the reasons for the reduction in the body weight (Prasad, 2014; Maares and Haase, 2016). Another report concluded that the food conversion efficiency of Zn-sufficient animals was higher than Zn-deficient animals (Lazarte et al., 2015). Gain in body weight of rats might be associated with increased feed intake per day.

Conclusion

Present study concluded that soil application of 15 kg ha^{-1} $ZnSO_4.7H_2O$ followed by foliar application at 0.25% $ZnSO_4.7H_2O$ solution at tillering and heading stage of rice improved the crop stand establishment, water relations, membrane permeability, photosynthetic pigments, tillering ability, kernel length, kernel yield, kernel Zn, amylose and protein contents, and was effective in kernel biofortification. These promising genotypes (Accession-175 > Accession-164 > Accession-126 > Accession-154) may be further utilized in breeding programs for genetic potential to transfer the desirable gene(s) to improve Zn as well as yield potential of evolving genotypes and may help in developing Zn biofortified rice which is the need of the hour. Nonetheless, various minerals have been reported to interact and influence the uptake of Zn from roots to developing grains (Fageria 2001; Lopez et al., 2002). Future studies aimed at biofortifying rice kernels need to look for such interactive effects of other essential mineral nutrients. Moreover, specific role of Zn in stomatal regulation requires further investigation.

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Conflict of interest

No potential conflict of interest was reported by the authors.

References

Abadi, V.A.J.M., Sepehri, M., 2016: Effect of *Piriformospora indica* and *Azotobacter chroococcum* on mitigation of zinc deficiency stress in wheat (*Triticum aestivum* L.). Symbiosis. 69, 9-19.

Abaid-Ullah, M., Hassan, M.N., Jamil, M., Brader, G., Shah, M.K.N., Sessitsch, A., Hafeez, F.Y., 2015: Plant growth promoting rhizobacteria: an alternate way to improve yield and quality of wheat (*Triticum aestivum*). Int. J. Agri. Biol. 17, 51-60.

Barras, H.D., Weatherley, P.E., 1962: A re-examination of relative turgidity technique for estimation of water deficit in leaves. Australian J. Biol. Sci. 15, 418-428.

Blum, A., Ebercon, A., 1981: Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. 21, 43-47.

Cakmak, I., 2000b: Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. New Phytol. 146, 185-205.

Cakmak, I., 2008: Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant Soil. 302, 1-17.

Chang, H.B., Win, L.C., Huang, H.J., 2005: Zinc induced cell death in rice (*Oryza sativa* L.) roots. Plant Growth Regul. 46, 261-266.

Della Lucia, C.M., Menezes Santos, L.L., da Cruz Rodrigues, K.C., da Cruz Rodrigues, V.C., Duarte Martino, H.S., Pinheiro Sant'Ana, H.M., 2014: Bioavailability of zinc in Wistar Rats fed with rice fortified with zinc oxide. Nutrients 6(6), 2279-2289.

Fageria, V.D., 2001: Nutrient interactions in crop plants. J. Plant Nut. 24, 1269-1290.

Farooq, M., Ullaha, A., Rehman, A., Nawaz, A., Nadeem, A., Wakeel, A., Nadeem, F., Siddique, K.H.M., 2016: Application of zinc improves the productivity and biofortification of fine grain aromatic rice grown in dry seeded and puddled transplanted production systems. Field Crop Res. 216, 53-62.

Gomez-Coronado, F., Poblaciones, M.J., Almeida, A.S., Cakmak, I., 2016: Zinc (Zn) concentration of bread wheat grown under Mediterranean conditions as affected by genotype and soil/foliar Zn application. Plant Soil 401, 331.

Graham, R.D., Welch, R.M., Saunders, D.A., Ortiz-Monasterio, I., Bouis, H.E., Bonierbale, M., de Haan, S., Burgos, G., Thiele, G., Liria, R., 2007: Nutritious subsistence food systems. Adv. Agron. 92, 1-74.

Esfandiari, E., Abdoli, M., Mousavi, S.B., Sadeghzadeh, B., 2016: Impact of foliar zinc application on agronomic traits and grain quality parameters of wheat grown in zinc deficient soil. Indian J. Plant Physiol. 21, 263-270.

Haslett, B.S., Reid, R.J., Rengel, Z., 2001: Zinc mobility in wheat: uptake and distribution of zinc applied to leaves and roots. Ann. Bot. 87, 379-386.

Hippler, F.W.R., Boaretto, R.M., Quaggio, J.A., Boaretto, A.E., Abreu-Junior, C.H., Mattos, J.D., 2015: Uptake and distribution of soil applied zinc by citrus trees addressing fertilizer use efficiency with 68Zn labeling. PLoS ONE. 10, e0116903.

Hunt, R., 1978: Plant growth analysis. Studies in Biology No. 96. Edward Arnold, London, 26-38

Hussain, S., Maqsood, M.A., Rengel, Z., Aziz, T., Abid, M., 2013: Estimated zinc bioavailability in milling fractions of biofortified wheat grains and in flours of different extraction rates. Int. J. Agri. Biol. 15, 921-926.

Jones, J.R.J., Case, V.W., 1990: Sampling, handling, and analyzing plant tissue samples. In: Westerman, R.L. (ed.), Soil testing and plant analysis, 3rd Ed., 389-428. Soil Science Society America, Madison, USA.

Juliano, B.O., 1971: A simplified assay for milled rice amylase. Cereals Sci. Today 16, 334-340.

Khan, P., Yousaf, M.Y., Imtiaz, M., Depar, N., Aslam, M., Memon, M.S., Shah, J.A., 2012: Determination the zinc requirements of rice genotype sarshar evolved at Nia, Tandojam. Sarhad J. Agri. 28, 236-240.

Kumssa, D.B., Joy, E.J.M., Ander, E.L., Watts, M.J., Young, S.D., Walker, S., Broadley, M.R., 2015: Dietary calcium and zinc deficiency risks are decreasing but remain prevalent. Sci. Rep. 5, 10974.

Lazarte, C.E., Vargas, M., Granfeldt, Y., 2015: Zinc bioavailability in rats fed a plant-based diet: a study of fermentation and zinc supplementation. Food Nut. Res. 59, 27796.

Lopez, H.W., Leenhardt, F., Coudray, C., Remesy, C., 2002: Minerals and phytic acid interactions: Is it a real problem for human nutrition? Int. J. Food Sci. Technol. 37, 727-739.

Lopez-Millan, A.F., Ellis, D.R., Grusak, M.A., 2005: Effect of zinc and manganese supply on the activities of superoxide dismutase and carbonic anhydrase in *Medicago truncatula* wild type and raz mutant plants. Plant Sci. 168, 1015-1022.

Maares, M., Haase, H., 2016: Zinc and immunity: an essential interrelation. Arch. Biochem. Biophys. 611, 58-65.

Marschner, P., 2012: Marschner's Mineral Nutrition of Higher Plants, 3rd Edn. Academic Press, London.

Mousavi, S.R., 2011: Zinc in crop production and interaction with phosphorus. Australian J. Basic App. Sci. 5, 1503-1509.

Nagata, M., Yamashita, I., 1992: Simple method for the simultaneous determination of chlorophyll and carotenoids in tomato fruits. J. Japan Soc. Food Sci. Feclinol. 39, 925-928.

Phattarakul, N., Rerkasem, B., Li, L.J., Wu, L.H., Zou, C.Q., Ram, H., Sohu, V.S., Kang, B.S., Surek, H., Kalayci, M., Yazici, A., Zhang, F.S., Cakmak, I., 2012: Biofortification of rice grain with zinc through zinc fertilization in different countries. Plant Soil. 361, 131-141.

Prasad, A.S., 2014: Impact of the discovery of human zinc deficiency on health. J. Trace Elem. Med. Biol. 28, 357-363.

Quijano-Guerta, C., Kirk, G.J.D., Portugal, A.M., Bartolome, V.I., McLaren, G.C., 2002: Tolerance of rice germplasm to zinc deficiency. Field Crop Res. 76, 123-130.

Rashid, M., Khalil, S., Ayub, N., Alam, S., Latif, F., 2004: Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. Pak. J. Biol. Sci. 7, 187-196.

Ramesh, A., Sharma, S.K., Sharma, M.P., Yadav, N., Joshi, O.P., 2014: Inoculation of zinc solubilizing *Bacillus aryabhattai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in Vertisols of central India. App. Soil Ecol. 73, 87-96.

Rehman, A., Farooq, M., Ozturk, L., Asif, M., Siddique, K.H.M., 2017: Zinc nutrition in wheat-based cropping systems. Plant Soil 422(1-2), 283-315.

Samreen, T., Shah, H.U., Ullah, S., Javid, M., 2013: Zinc effect on growth rate, chlorophyll, protein and mineral contents of hydroponically grown mung beans plant (*Vigna radiata*). Arabian J. Chem. 7, 145-152.

Tariq, M., Hameed, S., Malik, K.A., Hafeez, F.Y., 2007: Plant root associated bacteria for Zn mobilization in rice. Pak. J. Bot. 39, 245-253.

Tas, S., Tas, B., 2007: Some physiological responses of drought stress in wheat genotypes with different ploidity in Turkiye. World J. Agri. Sci. 3, 178-183.

Wang, H., Jin, J.Y., 2005: Photosynthetic rate, chlorophyll fluorescence parameters and lipid peroxidation of maize leaves as affected by zinc deficiency. Photosynthetica. 43, 591-596.

Welch, R.M., Graham, R.D., 2004: Breeding for micronutrients in staple food crops from a human nutrition perspective. J. Exp. Bot. 55, 353-364.

Zhao, A.Q., Tian, X.H., Chen, Y.L., Li, S., 2016: Application of $ZnSO_4$ or Zn-EDTA fertilizer to a calcareous soil: Zn diffusion in soil and its uptake by wheat plants. J. Sci. Food Agri. 96, 1484-1491.

Zaman, Q.U., Aslam, Z., Yaseen, M., Ihsan, M.Z., Khaliq, A., Fahad, S., Bashir, S., Ramzani, P.M.A., Naeem, M., 2017: Zinc biofortification in rice: leveraging agriculture to moderate hidden hunger in developing countries. Arch. Agron. Soil Sci. 64, 140-161.

Zaman, Q.U., Aslam, Z., Rashid, M., Khaliq, A., Yaseen, M., 2018: Influence of zinc fertilization on morpho-physiological attributes, growth, productivity and hematic appraisal of paddy rice. J. Anim. Plant Sci. 28(3), 778-790.

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