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Evaluation of Different Methods of Zinc Application on Growth, Yield and Biofortification of Zinc in Rice (*Oryza sativa* L.)

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Zinc (Zn) fertilization, not only gives better yield of cereals but also provides better nutrition to the population. This study evaluated the effect of different Zn application methods on growth, yield and biofortification of Zn in rice. Zinc treatments included were: soil application (5 kg Zn ha^{-1}) (SA), root dipping (2% ZnO slurry) (RD), foliar spray (0.5% $ZnSO_4.7H_2O$ + 0.25% lime) at tillering and milking stage (FS) and combinations of these treatments. Significantly higher grain yield was obtained in SA + RD + FS and RD + FS (27.2 and 22.6% increase over RDF, respectively). Zinc content in grains had a significant positive correlation with grain yield (r = 0.395, $p< 0.05$). Efficiencies of applied Zn decreased with increasing Zn application rates, except crop recovery efficiency. Maximum Zn content in grains and bioavailability of the fortified Zn was recorded in SA + FS treatment. However, less than 1% of the fortified grain Zn was bioavailable. Zinc enrichment had a negative ($p< 0.01$) impact on Fe content in both grain (r = -0.538) and straw (r = -0.603), but showed a positive ($p< 0.01$) correlation in respect of Mn content in grain (r = 0.556).

Key words: Zinc, application methods, rice, yield, biofortification, use efficiencies, phytate, molar ratios, bioavailability

Micronutrients are essential for increasing crop production and enhancing animal and human health. About one third of agricultural soils in the world are estimated to be low in available zinc (Zn), resulting in poor crop yields and nutritional quality of the harvested grains (Alloway 2008; Cakmak 2008). As per All India Coordinated Research Project (AICRP) on Micro and Secondary Nutrients and Pollutant Elements in Soils and Plants (Shukla *et al*. 2016), 39.9% of 169,290 soil samples collected from all over the country and 32.4% of 8,072 samples from Uttar Pradesh are deficient in available Zn. However, latest estimate (Shukla *et al*. 2018) showed a decline in Zn deficiency (36.5%) in Indian soils. Proper Zn nutrition of the crop is needed for maintaining crop and productivity (Cakmak 2004). Cereals contain high Zn in grain when grown on soils having high content of plant available Zn. In order to achive desirable quantities of grain Zn concentration in rice for human nutrition, rice requires more Zn in soil than it needs for maximal grain yield.

Zinc deficiency ranked fifth among the ten leading causes of illness and disease worldwide (WHO 2002). For millions of children, Zn malnutrition affects their ability to learn and work by impairing growth and development (The World Bank 2006). Low Zn level in serum (cutoff level 65 μg dL^{-1}) were reported in Indian children (< 5 years) with the highest occurrence in Orissa, followed by Uttar Pradesh, Gujarat, Madhya Pradesh and Karnataka (Kapil and Jain 2011). During green revolution, traditional agriculture has been modified to high yielding and nutrient responsive agriculture to achieve food security. Thus, essential multinutrient deficiency is a common phenomenon in India (Katyal 2003). There is consensus among human nutritionists that the best way to tackle micronutrient deficiency in human is through diversification in diet (FAO and WHO 2002). However, this is not always possible, especially for people with a low income. Other strategies including fortification and supplementation require national infrastructure, purchasing power, or access to markets or health-care systems, which are often inadequate in developing countries (Mayer *et al.* 2008).

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Biofortification is an approach aiming to increase micronutrient concentrations in the edible parts of plants through crop breeding or the use of biotechnology or through agronomic practices in a cost effective manner to deliver the benefits of micronutrient enhancement to the rural populations in developing countries (Cakmak 2008). Breeding for Zn fortification is a long-term process involving identification of the required traits and the most promising parent, crossing and back crossing, environmental stability of the trait and adaptation of the plant to the new environment (Cakmak 2008). For the success of a breeding program, it is very important that the Zn level in the soil is not limiting and if found deficient, Zn should be provided to the plant through fertilizer application. Application of Zn fertilizers or Zn-enriched NPK fertilizers offers a rapid solution to the problem and represents useful complementary approach to ongoing breeding programs. Recent work on agronomic biofortification of rice through application of Zn in soil has not been proved to be useful (Wissuwa *et al*. 2008) but foliar Zn application significantly enhanced grain Zn concentration over control treatments in rice (Mabesa *et al*. 2013). Seedling dipping in ZnO suspension has been proved effective in correcting Zn deficiency (Yoshida *et al*. 1973) in rice. But no attempt has been tried to find the effect of root dipping treatment in enhancing Zn content in the grains.

A new era for agriculture has started linking farming to human health because of the fact that agriculture is a primary source of the nutrients required by humans and animals. Successes have been achieved to produce nutritionally rich legume and pulse crops in some countries (Welch and Graham 2004). The bioavailability of the fortified Zn is of concern than the Zn content in the biofortified crop as it is governed by various anti-nutritional factors like phytic acid. As the phytic acid content of the diet increases, the intestinal absorption of Zn decreases because phytic acid forms very stable complexes with mineral ions rendering them unavailable for intestinal uptake (Flanagan 1984). Rice (*Oryza sativa* L.) is cultivated widely across the world to feed millions of people. On the basis of recommended daily allowances (RDA) and bioavailability values, 24 μg g^{-1} grain Zn contents in polished rice grains have been decided (HarvestPlus 2012), whereas consumable rice grains supply only 20% of the daily Zn requirements of the human (Welch and Graham 2004). In view of the above, this research work was initiated with the sole aim to understand the response of rice to different methods of Zn application with respect to growth, yield and agronomic biofortification.

Materials and Methods

Experimental details

A pot experiment was conducted during July to October 2014, in the net house of the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Bulk soil sample was collected from a dry-land field under continuous maize cropping situated in the Agricultural Research Farm of the University. The soil (Typic Ustrochept) was ground to pass through 2-mm sieve and filled in polythene lined pots of 10 kg capacity. The physicochemical properties of the soil used for experiment are presented in table 1.

Rice variety HUR-105 (Malviya sugandh 105, NBPGR I.C. No. 560495) developed by Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi was chosen for this study. It is a popular variety widely grown in the area and is suitable for cultivation in irrigated areas of entire Uttar Pradesh. It can be sown in any time of the year depending on the water availability and takes 140 days to mature. A recommended dose of fertilizers (RDF) at the rate of 150:60:60 kg ha^{-1} was applied as nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) in the form of urea, mono-potassium dihydrogen phosphate and muriate of potash, respectively. Treatments consists of absolute control (without fertilizers), RDF, soil application of 5.0 kg Zn ha^{-1} through $ZnSO_4.7H_2O$ (RDF+SA), dipping the roots of rice seedling before

Table 1. Properties of the experimental soil

Parameters	Value
pH (1:2.5)	8.33
EC (dS m^{-1})	0.23
Organic carbon (g kg^{-1})	3.7
$CaCO_3$ (%)	0.47
Available N (kg ha^{-1})	75.3
Available P (kg ha^{-1})	40.9
Available K (kg ha^{-1})	212
Available S (mg kg^{-1})	19.1
Available Fe (mg kg^{-1})	34.6
Available Mn (mg kg^{-1})	7.13
Available Zn (mg kg^{-1})	0.72
Available Cu (mg kg^{-1})	2.14
Sand (%)	59.3
Silt (%)	25.2
Clay (%)	15.5
Texture	Sandy clay loam

transplanting in a 2% ZnO slurry (RDF+RD), foliar spray of 0.5% $ZnSO_4.7H_2O$ + 0.25% lime at both tillering and milking stage (RDF+FS) and combinations of different methods of Zn application (RDF+SA+RD; RDF+SA+FS; RDF+RD+FS and RDF+SA+RD+FS) were applied. Each treatment was replicated thrice. The treatments were arranged in completely randomized design (CRD). Fertilizers were applied in solution form. Forty per cent of the N and full dose of P and K was applied as basal, while the rest of the N was applied in two splits *i.e.* 40% at 30 days after transplanting (DAT) and 20% at 60 DAT. To reduce grasshopper (*Omocestus viridulus* L.) infestation, Cypermethrin EC (trade name: Super Kill 25) was sprayed at 0.2% at 47 DAT. Continuous submergence was maintained in the pots up to physiological maturity.

Growth and yield attributes of rice

Different growth attributing characters like plant height, number of tillers, greenness index (SPAD value) of plants were recorded at 30, 60 and 90 DAT. At maturity, average number of panicles per pot, number of grains per panicle and panicle length were also recorded from each pot. After harvesting of the grains, plant samples were cut leaving 5 cm from base to avoid contamination from soil. The plants were then washed sequentially with 0.2% detergent solution, 0.1*N* HCl and finally with doubled distilled water, dried in hot air oven at 70 °C till constant weight and the straw yields were recorded. Grain yield and 1000 grain weight per pot were recorded after the seeds were dried up to 14% moisture content. Harvest index was calculated as follows:

Harvest Index (%) = [Economic yield (g pot^{-1})/Total biological yield (g pot^{-1}) × 100]

Soil and plant analyses

Soil samples were processed and analyzed for pH and electrical conductivity (EC) (1:2.5) using glass electrode pH meter and digital EC meter, respectively, organic carbon (Walkley and Black 1934), available N by alkaline potassium permanganate method (Subbiah and Asija 1956); available P by Olsen's method (Olsen *et al*. 1954), available K by extraction with 1*N* ammonium acetate at pH 7.0 (Hanway and Heidal 1952) and available sulphur (S) by turbidimetric method (Chesnin and Yien 1951) following the procedure outlined in Sparks (1996). For analysis of iron (Fe), copper (Cu), manganese (Mn) and Zn, soil samples were extracted by 0.005*M* DTPA containing 0.01 *M* $CaCl_2$ and 0.1 *M* TEA (pH-7.3) (Lindsay and Norvell 1978) and analyzed by atomic absorption spectrophotometer (UNICAM 696). Finely ground straw and grain samples were digested with di-acid mixture (HNO_3:$HClO_4$: 3:1, v/v) and analyzed for Zn using atomic absorption spectrophotometer (Tandon 2001).

Estimation of Zn use efficiencies

Efficiencies of applied Zn, such as, partial factor productivity (PFP), agronomic efficiency (AE), crop recovery efficiency (CRE), agro physiological efficiency (APE) and Zn harvest index (Zn HI) were computed using the following expressions (Fageria and Baligar 2003) and (Dobermann 2005):

$$PFP\ (kg\ kg^{-1}) = [Y_{Zn}/Zn_a]$$
$$AE\ (kg\ kg^{-1}) = [(Y_{Zn} - Y_{AC})/Zn_a]$$
$$CRE\ (\%) = [(U_{Zn} - U_{AC})/Zn_a \times 100]$$
$$APE\ (kg\ kg^{-1}) = [(Y_{Zn} - Y_{AC})/\ (U_{Zn} - U_{AC})]$$
$$Zn\ HI\ (\%) = [GU_{Zn}/U_{Zn} \times 100]$$

where, Y_{Zn} and U_{Zn} refer to the grain yield (kg ha^{-1}) and total Zn uptake (kg ha^{-1}), respectively of rice in Zn applied plots; Y_{AC} and U_{AC} refer to the grain yield (kg ha^{-1}) and total Zn uptake (kg ha^{-1}), respectively of rice in absolute control plots. Zinc sulphate was applied at 5 kg Zn ha^{-1} *i.e.* 3.9 mg Zn pot^{-1}, 2% ZnO root dipping treatments were done at 2.1 mg Zn $hill^{-1}$ *i.e.* 4.2 mg Zn pot^{-1}, 0.5% $ZnSO_4.7H_2O$ foliar spray was done at 6 0.1 L m^{-2} *i.e.* 5.2 mg Zn pot^{-1}.

Estimation of phytates and human bioavailability of the fortified Zn

Phytates of the seeds were extracted by Na_2SO_4 in HCl under magnetic agitation and their content was determined by back-titration using EDTA as developed by García-Estepa *et al.* (1999). The mole ratio of phytate and Zn were obtained by dividing their amount by their molecular weight and the respective phytate: Zn molar ratios were obtained. The human bio-availability of the fortified Zn was estimated using a trivariate model developed by (Miller *et al.* 2007).

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

Table 2. Effect of methods of zinc application on growth attributing characters in rice at 30, 60 and 90 days after transplanting (DAT) in rice

Treatments	Plant height (cm)			Greenness index (SPAD value)			Number of tillers pot^{-1}		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT (effective tillers)
Absolute Control	71.5d	82.9c	84.8c	24.9c	28.0b	26.8ab	3.00e	3.00c	2.86b
RDF	77.5c	98.5b	91.2bc	27.5bc	34.4a	26.2ab	3.93d	6.33b	5.00a
RDF + SA	78.0bc	99.5ab	90.2bc	29.9ab	33.4a	28.1ab	4.26cd	6.26b	5.13a
RDF + RD	85.9a	100.0ab	96.8b	32.7a	34.3a	27.6ab	6.20a	6.93ab	5.06a
RDF + FS	79.3bc	100.0ab	96.2b	31.5ab	34.6a	25.4ab	4.46cd	7.60a	5.40a
RDF + SA+RD	79.9bc	98.7b	92.5b	30.9ab	33.4a	23.1b	4.60c	6.53ab	5.06a
RDF + SA+FS	79.5bc	104.0a	103.0a	30.5ab	35.8a	30.5a	4.80c	7.06ab	5.00a
RDF + RD+FS	82.7ab	101.0ab	93.6b	32.1ab	34.2a	27.9ab	4.73c	7.13ab	5.33a
RDF + SA+RD+FS	81.0bc	100.0ab	95.7b	30.4ab	36.2a	27.3ab	5.53b	7.26ab	5.20a

Please see Materials and Methods section for treatment details.
Means with similar lower-case letters within a column are not significantly different at $p < 0.05$ according to DMRT test.

where, A_{max} = maximum absorption, K_R = equilibrium dissociation constant of Zn-receptor binding reaction, K_P = equilibrium dissociation constant of Zn-phytate binding reaction and has a value of 0.091, 0.68 and 0.033 (Hambidge *et al*. 2010), TAZ = total daily absorbed Zn, TDP = total daily dietary phytate and TDZ = total daily dietary Zn.

Statistical analysis

The data obtained were subjected to analysis of variance (completely randomized design) using statistical software R (R version 3.3.1 (2016-06-21). Significant differences in mean values were identified using the Duncan's multiple range test (DMRT) method at the 0.05 level of significance. Other calculations including regression and correlation analysis were also performed with the same software. All figures were drawn using Microsoft Office Excel 2013.

Results and Discussion

Growth and yield attributes

At 30 DAT, treatments receiving Zn as root dipping (RDF+RD) significantly increased plant height (Table 2) over all other treatments and the increase was 10.5 per cent over control (RDF). The maximum tiller number was recorded in RDF+RD followed by RDF+SA+RD+FS. The maximum greenness index (SPAD value) was also recorded in RDF+RD with an increase of 18.8 per cent over RDF. However, at 60 DAT, only RDF+SA+FS significantly increased plant height over RDF. At maturity, only RDF+SA+FS significantly increased plant height over RDF. But the number of effective tillers produced and greenness index did not differ significantly between different treatments. It was noted that none of the treatments involving Zn application improved growth parameters of plant except RDF+RD. Although RDF+RD, significantly increased plant height which may be attributed to the direct contact of the roots with Zn in this method of application. At 60 DAT and later growth stages, the effectiveness of RDF+RD treatment decreased, which might be due to decreased ZnO level with increasing root growth. However, RDF+FS appears to provide Zn in later growth stages thereby increased plant growth. Saha *et al.* (2013) also reported that soil + foliar application of Zn was more effective in increasing plant height (7.3% increase) than soil application alone (3.6% increase) over control.

Levels of Zn application and grain yield had a positive quadratic relationship and the R^2 values suggest that the variation can be adequately explained by the quadratic function of the variable Zn levels (Fig. 1A). The maximum grain yield was obtained when Zn was applied by all the three methods (RDF+SA+RD+FS) followed by RDF+RD+FS (Table 3). Only these two treatments produced a significantly higher grain yield over RDF with a respective increase of 27.2 and 22.6 per cent. Zinc content in grains had a significant positive correlation with grain yield (r = 0.395, $p < 0.05$). However, the treatments involving root dipping (RDF+RD) recorded a significantly higher straw yield over RDF (33.7%) and RDF+SA. It has been found (Table 3) that harvest index was not significantly affected by any of the treatments. Straw yield and Zn levels had a positive quadratic relationship and the variation in straw yield could be explained adequately by variation of Zn levels from the R^2 value (Fig. 1B) as in the case of grain yield. Out of the various methods of Zn application, none was effective to produce a significant increase in 1000 grain weight over RDF. In the present study, application of Zn by any methods did not affect the yield attributing characters (like number of panicles, panicle length and number of grains per panicle) significantly over RDF. Root dipping treatment (RDF+RD) showed a significantly lower harvest index than soil application (RDF+SA) because of more straw yield in the former. It has been observed (Table 3) that application of Zn by more than one method had higher grain yield than with any one mode of application. It appears that regular supply of Zn is maintained throughout the life cycle of rice through conjoint application (SA, RD and FS) which may be considered as a probable explanation for more number of productive tillers per hill and higher Zn uptake resulting increased grain yield. Imran *et al.* (2015) also found similar responses of Zn supply on grain yield of lowland rice.

Zinc efficiencies

Zinc use efficiencies were assessed in terms of partial factor productivity (PFP), agronomic efficiency (AE), crop recovery efficiency (CRE), agro-physiological efficiency (APE) of Zn and Zn harvest index (Zn HI) in different treatments (Table 3). Zinc use efficiency decreased as more Zn was supplied. The PFP was highest (13032 kg grain kg^{-1} Zn) in RDF+SA and significantly decreased (3234 kg grain kg^{-1} Zn) when all the three modes of Zn application were combined. Similar trend was also found for AE and APE which varied from 7290 to 2023 kg grain kg^{-1} Zn and 43074 to 2745 kg grain kg^{-1} Zn, respectively. However, CRE of the treatments involving FS of Zn was higher (except RD+FS) than SA or RD or SA+RD treatments, thus suggesting that the efficiency of foliar applied Zn was better than SA or RD. Zinc HI was significantly higher in absolute control (44.7%) and RDF (40.6%) and decreased to a minimum of 15.5% with all the three modes of Zn application. The regression analysis results indicated that the relationship between different Zn use efficiencies and Zn levels can be adequately described by the linear equation (Fig. 1C, D, E, F and G), being significant at 5% level of significance. The computed R^2 values were 0.818, 0.559, 0.503, 0.683 and 0.826 in case of PFP, AE, CRE, APE and Zn HI, respectively, which indicate a respective variation of

Table 3. Effect of Zn treatments on yield attributing characters, partial factor productivity (PFP), agronomic efficiency (AE), crop recovery efficiency (CRE), agro-physiological efficiency (APE) and Zn harvest index (Zn HI) of applied Zn in rice

Treatments	Number of panicles	Panicle length (cm)	Number of grains $panicle^{-1}$	Grain yield (g pot^{-1})	Straw yield (g pot^{-1})	Harvest index (%)	1000 grain weight (g)	PFP for Zn (kg kg^{-1})	AE (kg kg^{-1})	CRE (%)	APE (kg kg^{-1})	Zn HI (%)
Absolute Control	2.86b	22.1b	69b	22.4d	24.6c	47.1ab	20.6c	-	-	-	-	44.8a
RDF	5.00a	24.3a	120a	47.0c	45.1b	51.1ab	22.4a	-	-	-	-	40.6ab
RDF + SA	5.13a	24.3a	111a	50.8abc	45.4b	55.0a	22.3a	13032a	7291a	18.0bc	43075a	37.8ab
RDF + RD	5.06a	24.6a	116a	49.1bc	60.3a	44.7b	22.3a	11694a	6363ab	18.4bc	3355a	33.4b
RDF + FS	5.40a	24.6a	108a	49.5bc	49.0ab	50.6ab	22.2a	4759c	2606c	73.7a	5280b	21.3c
RDF + SA+RD	5.06a	24.0a	129a	54.4abc	47.2ab	52.6ab	22.4a	6722b	3957bc	12.4c	31990a	33.8b
RDF + SA+FS	5.00a	24.8a	123a	55.7abc	56.8ab	49.6ab	22.4a	3898c	2332c	64.6a	3648b	21.1c
RDF + RD+FS	5.33a	24.5a	124a	57.7ab	57.8ab	50.5ab	21.7b	3950c	2417c	55.4ab	4531b	22.2c
RDF + SA+RD+FS	5.20a	24.3a	119a	59.8a	56.9ab	51.1ab	22.5a	3234c	2024c	79.9a	2746b	15.6c

Please see Materials and Methods section for treatment details.
Means with similar lower-case letters within a column are not significantly different at $p < 0.05$ according to DMRT test.

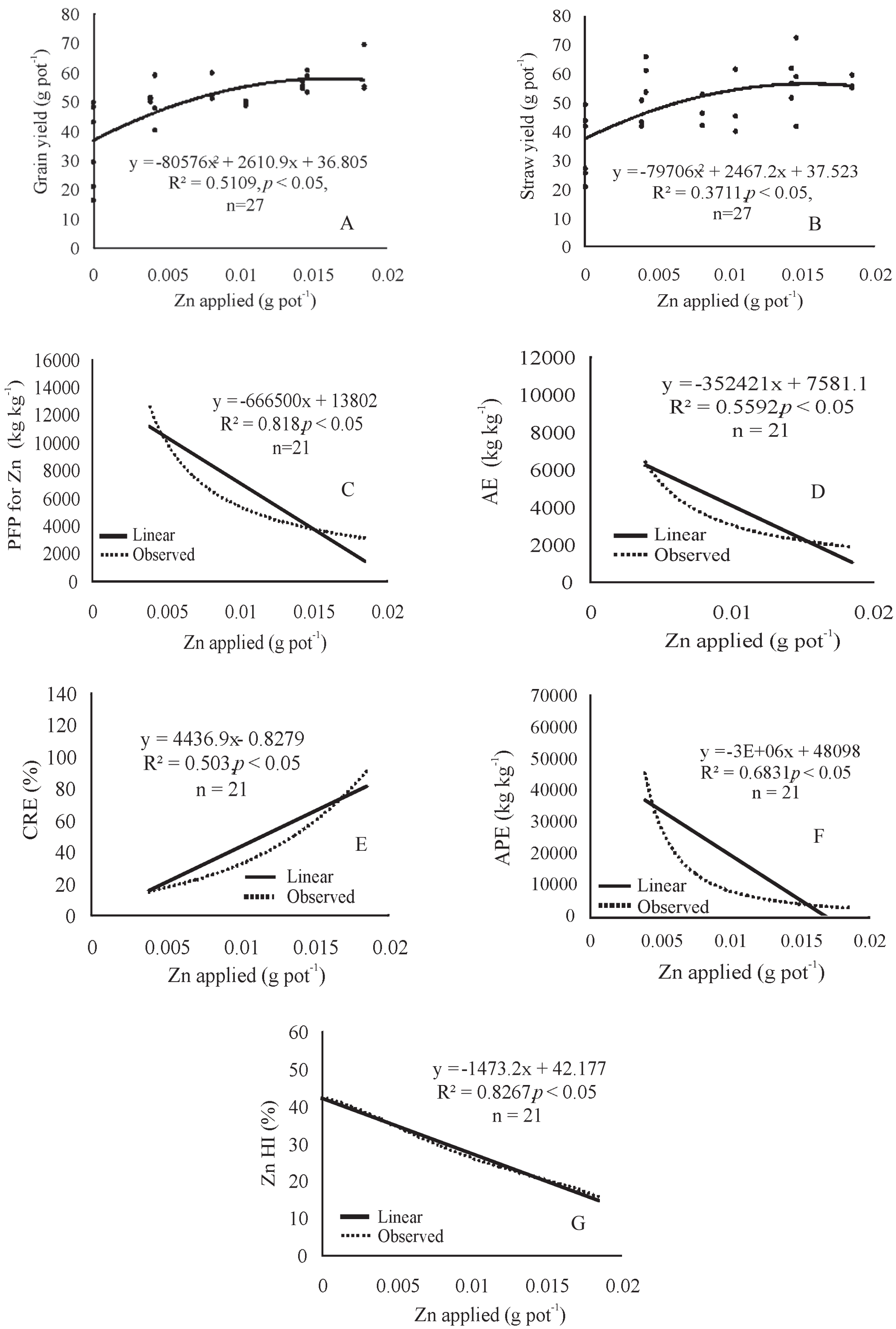

Fig. 1. Relationship between levels Zn applied (g pot^{-1}) and (A) grain yield (g pot^{-1}), (B) straw yield (g pot^{-1}), (C) partial factor productivity (PFP), (D) agronomic efficiency (AE), (E) crop recovery efficiency (CRE), (F) agro-physiological efficiency (APE) and (G) Zn harvest index (Zn HI) in rice

81.8, 55.9, 50.3, 68.3 and 82.6 per cent in mean Zn use efficiencies. The PFP, AE and APE of Zn decreased as the level of Zn supplied increased. The highest PFP, AE and APE of applied Zn were recorded with the lowest level of Zn application involving soil Zn application. Increasing the level of Zn decreased the difference in PFP, AE and APE of applied Zn. A higher CRE or a lower Zn HI was found in the Zn treatments applied as foliar spray. The Zn content in the straw was significantly higher in these treatments, therefore, total Zn uptake was also higher.

Zinc accumulation in rice grains and straw

Zinc concentration in the grains of unpolished rice varied from 22.3 to 45.0 mg kg^{-1} with different mode of Zn application (Fig. 2A). Treatments in which foliar spray of Zn was applied (FS, SA+FS, RD+FS and SA+RD+FS) showed significantly higher Zn concentration in grain than RDF. However, it did not differ significantly among them. The maximum Zn concentration was recorded when Zn was applied in soil as well as through foliar sprays (SA+FS) which registered an increase of 102 per cent over RDF. This was followed by SA+RD+FS, RD+FS and FS with respective increase of 95.7, 82.2 and 75.9 per cent over RDF. Zinc application by any single method (except FS) failed to increase Zn content to a significant level over RDF. It was also observed that SA+RD showed significantly lower Zn concentration in the grains than other treatments comprised of more than one mode of Zn application.

Zinc concentration in straw varied from 29.2 to 260.9 mg kg^{-1} and was significantly higher over control (RDF) in the treatments which received FS (Fig. 2B). Treatment in which Zn was applied through all the three means (SA+RD+FS), significantly increased Zn concentration in straw over other treatments and was 6.61 times higher than control followed by FS 4.09 times, SA+FS 3.81 times and RD+FS 3.32 times. However, there was no significant difference between latter three treatments in terms of Zn concentration in straw.

It was observed that FS of Zn is necessary for increasing biofortification of Zn in the grains. A higher remobilization capacity of foliar-applied Zn from leaves to grain, along with the capacity to continue taking up Zn from the roots throughout the growth stage might be the cause of higher Zn concentration of unpolished rice grains. Yilmaz *et al.* (1997), Saha *et al.* (2013) and Imran *et al.* (2015) also found similar kind of results. However, different genotypes may have different grain Zn sources depending on the availability of Zn from leaves or soil (Mabesa *et al.* 2013).

Phytate content in rice grains

Zinc concentration in the unpolished rice grains had significant negative correlation with phytate (r = -0.848, $p = 0.01$). Phytate content in the grains reduced with increasing levels of Zn. Zinc application by any one method (except FS) failed to significantly decrease the phytate content in the grains. The maximum decrease in phytate content over RDF was to the tune of 33.9 per cent when Zn was applied SA+RD+FS (Fig. 2C). Although FS alone or in combination with RD significantly decreased phytate content over RDF (22.6 and 26.4% decrease, respectively), but did not differ significantly between themselves.

Bioavailability of the fortified Zn

Foliar spray of Zn either alone or in combination with other mode of Zn application significantly reduced the phytate: Zn molar ratios in unpolished rice grains over RDF. The lowest phytate: Zn molar ratio and hence a higher bioavailability of the fortified Zn was found in case of SA+FS followed by SA+RD+FS, RD+FS and FS with a corresponding decrease of 66.7, 66.3, 59.3 and 55.4 per cent over RDF (Fig. 2D). Although RD+SA significantly reduced phytate: Zn molar ratio than RDF, but showed a significantly higher ratio than the treatments receiving foliar spray of Zn. Zinc application by SA or RD individually failed to decrease the ratio than RDF and therefore, indicated a lower bioavailability of the fortified Zn. Zinc content of the grains was significantly and negatively correlated with phytate: Zn molar ratios (r = -0.951, $p < 0.01$). The maximum daily total absorbed Zn (TAZ) was in SA+RD+FS followed by SA+FS with a respective increase of 29.6 and 26.5 per cent over RDF (Fig. 2E). Zinc treatments involving FS, SA+RD and RD+FS significantly increased TAZ over RDF but did not differ significantly between themselves. Total absorbed Zn had a significant negative relationship with both the phytate content and phytate: Zn molar ratios (r = -0.614, $p < 0.01$ and r = -0.551, $p < 0.01$, respectively).

Zinc application increased its concentration in grain but little amount of Zn fortified is bioavailable. The bioavailability of the fortified Zn is governed by the phytate content of the seed. The maximum decrease in phytate content was found in the treatments where Zn was applied through foliar spray. Treatments involving foliar sprays of Zn also showed

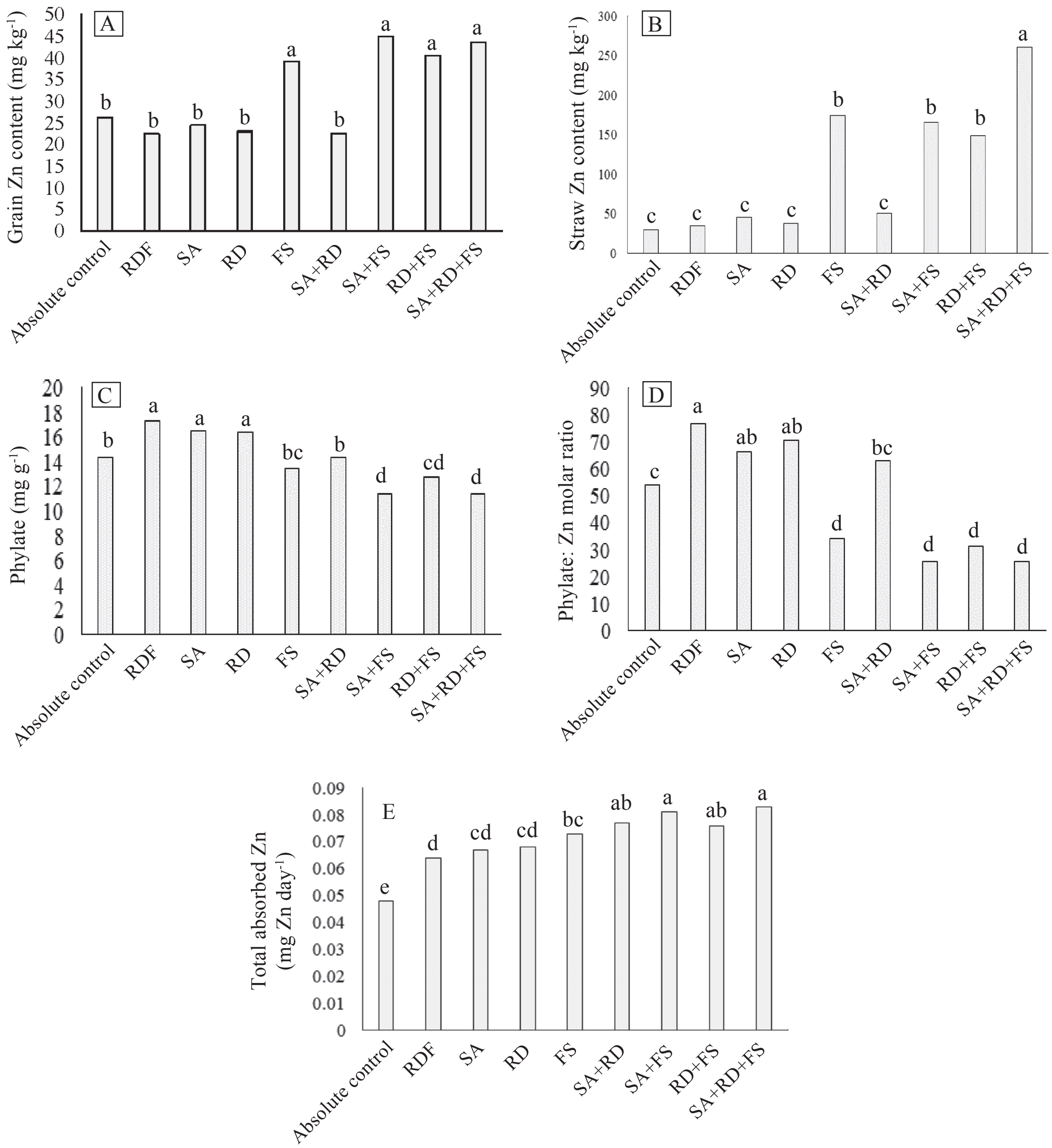

Fig. 2. Effect of different treatments on (A) grain Zn content and (B) straw Zn content, (C) grains phytate content, (D) phytate: Zn molar ratios and (E) total absorbed Zn per day in rice

Bars with similar lower-case letters are not significantly different at $p < 0.05$ according to DMRT.

significant reduction in phytate to Zn molar ratios, therefore, the maximum Zn bioavailability could be expected in these treatments. The decrease in concentration of phytate might be related to an effect of Zn application on uptake, translocation and metabolism of P (Marschner and Cakmak 1986). Imran *et al.* (2015) reported a minimum phytate: Zn molar ratio of 44 (35% less than control) in rice grains with soil + foliar Zn application. But actually, the estimated TAZ per day was found to be quite low (<1% of the grain Zn content) in all the treatments.

Zinc enrichment influencing Fe, Cu and Mn contents in rice grains and straw

Zinc enrichment in rice grain and straw affected the concentration of Fe, Cu and Mn. The variation in grain Fe and Mn content (Fig. 3A and 3B) and straw Fe content (Fig. 3C) could be explained by the estimated linear equations R^2 significant values of 0.289, 0.309 and 0.448, respectively. It has been observed that increase in Zn concentrations of the grains significantly decreased Fe concentration which was corroborated by a significant negative correlation

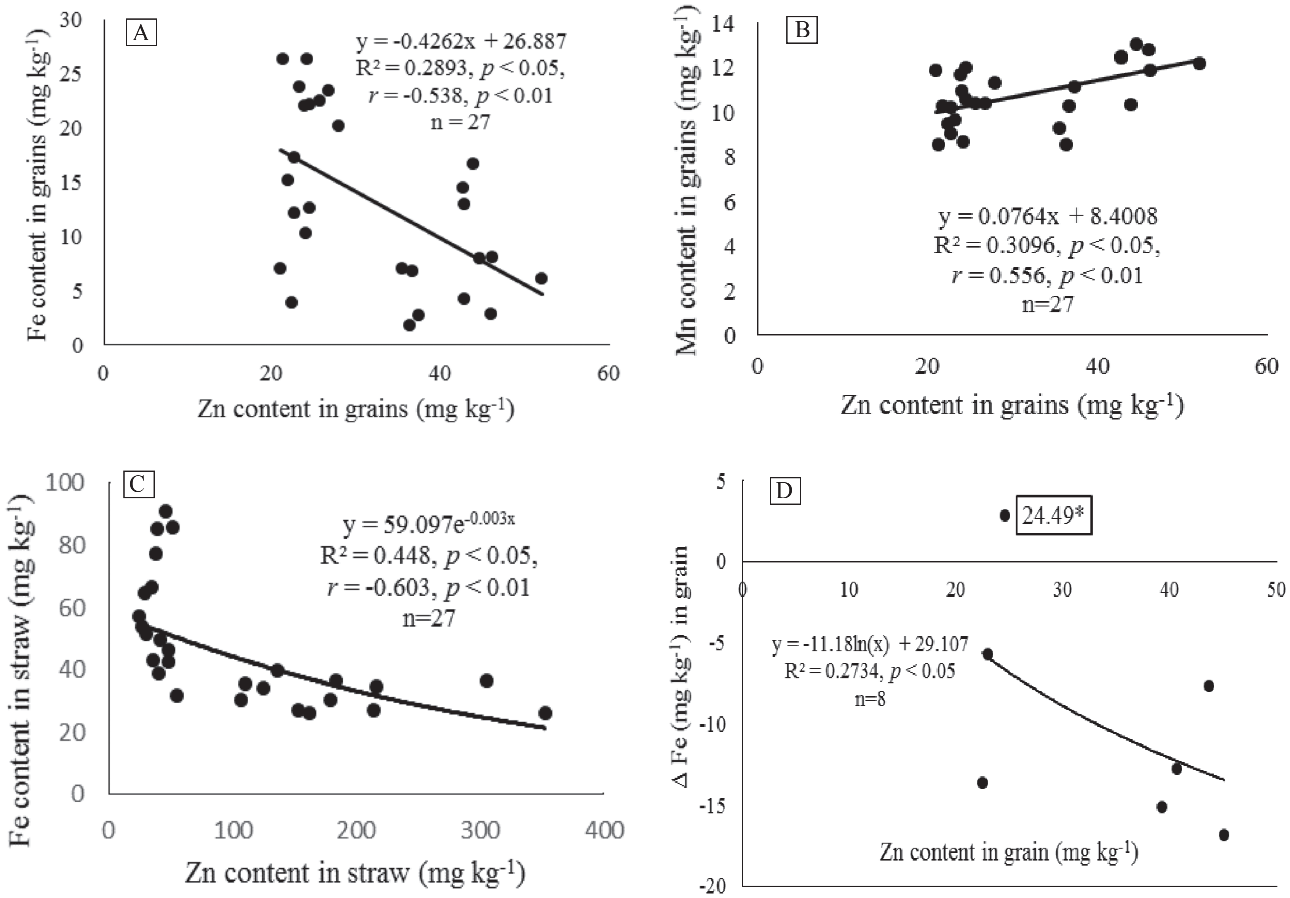

Fig. 3. Relationship between (A) Zn and Fe content in grains, (B) Zn and Mn content in grains, (C) Zn and Fe content in straw, changes in Fe concentration (ΔFe) over control (nil Zn application) in (D) grain and (E) straw of rice
*Data callouts show threshold Zn concentration in grains and straw for a positive ΔFe value.

between them (r = -0.538, $p< 0.01$). However, Zn and Mn concentrations in the grains were found to be positively correlated (r = 0.556, $p < 0.01$). Zinc enrichment in the grains did not significantly affect Cu concentration. Increase in Zn concentration in the straw had a significant negative effect on Fe concentration (r = -0.603, $p < 0.01$). The maximum threshold concentrations up to which Zn could be enriched in the grains and straw were found to be 24.49 and 45.49 mg kg^{-1}, respectively, so that the Fe concentration in them is not lower than that of RDF (Fig. 3D and 3E). The regression analysis showed that the variation of ΔFe value (the changes in Fe concentration over RDF) due to Zn enrichment in the grains and straw can be explained by the estimated logarithmic equations. The reason for antagonism between Fe and Zn concentration in the grains might be attributed to the similarity between the ionic radii of Fe^{2+} and Zn^{2+} (Woolhouse 1983) or the same translocation pathway for Zn and Fe movement within the plant (Goirdano and Mortvedt 1972). The level of antagonism between Fe and Zn was observed more in straw than in grains, might be due to dilution of Fe concentration in the former due to more biomass production. Enriching rice grain or straw with Zn over the threshold concentration may invite Fe deficiency.

Conclusions

It can be concluded that soil application or root dipping of rice in ZnO suspension help the plant in improving initial growth. But to ensure optimum Zn supply at later growth stages, foliar spray of Zn is effective. For increasing the grain yield, combination of Zn application methods hold promise. Application of Zn through foliar spray has been found effective for biofortification of Zn in rice. Further research is needed to reduce the phytate concentration while increasing the Zn in the grains to make it more bioavailable. Increasing the density of Zn in grain decreased Fe concentration essential for human nutrition. Therefore, Fe fertilization may also be kept in mind to keep balance of these micronutrients.

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