Journal of Tropical Agriculture 59 (2): 286-291, 2021

Short Communication

Biofortification of rice grain with zinc through inorganic fertilization

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Received 18April 2021; received in revised form 09 September 2021; accepted 16 September 2021

Abstract
Polished white rice which is widely consumed as staple food by majority of humanity has inherently low Zn content. Zn malnutrition is a major global health issue associated with rice based diets. The process of intentional enhancement of Zn in rice grain through application of Zn fertilizers causes biofortification of rice grain with Zn. The present study was thus formulated to assess the effect of method and time of Zn fertilization on yield and biofortification of rice grain with Zn. Field experiment was conducted during *kharif* 2018-19 at farmer's field in Southern Coastal Plains of Kerala. The experiment was laid out in randomized block design with eight treatments replicated thrice. Treatments comprised of different zinc sulphate concentrations *viz.,* 0.5 and 0.1% foliar application at maximum tillering, panicle initiation, booting and milking stages; zinc sulphate 20 kg ha^{-1}soil application as basal; and a control *viz.,* without Zn. Impact of lime addition to zinc sulphate spray solution was also evaluated. Foliar fertilization of zinc sulphate 0.1% at maximum tillering and milking stages, produced the highest grain (6605 kg ha^{-1}) and straw (7024 kg ha^{-1}) yield; increased Zn content in rough rice (29.8 mg kg^{-1}) and its milled fractions *viz.,*rice bran (75.8 mg kg^{-1}), brown rice (19.1 mg kg^{-1}) and white rice (9.11 mg kg^{-1}).Considering the highest apparent Zn biofortification recovery efficiency (43.8%) net returns (91213 ₹ ha^{-1}) and benefit: cost ratio (1.88), zinc sulphate 0.1% foliar fertilization at maximum tillering and milking stages can be recommended for improved yield and biofortification of rice grain with Zn.

Keywords: Apparent biofortification recovery efficiency, Biofortification, Rice, Zinc.

Rice is a major cereal crop which plays a key role in food security of India. Intensive rice cultivation with high yielding varieties, lesser application of organic manures, excessive use of high analysis fertilizers and neglecting application of Zn had led to a situation of widespread Zn deficiency in Indian soils (Mandal and Das, 2013). Zn is a cofactor of enzymes within plants concerned with photo-synthesis, carbohydrate and protein metabolism (Das and Green, 2016). In human body it is associated with providing immune function, insulin action and reproductive health (Das and Das, 2012). White rice widely consumed by humans, which is the milled fraction of harvested rough rice, has inherently low Zn content (6.1 to 8.8 mg kg^{-1}) (Sudha and Stalin, 2015). Recommended dietary allowance of Zn (12 mg day^{-1}) for an Indian adult is higher than habitual Zn intake (9 to 11 mg day^{-1}) of a moderately active Indian adult male, thus exposing Indian population to a marginal risk of inadequacy of Zn (NIN, 2009). For the huge fraction of humanity that depends on polished white rice as its staple food, low intakes of Zn is becoming severe problem (Majumder et al., 2019). Biofortiûcation is a process of intentionally enhancing the nutritional value of edible plant parts via genetic manipulation or agronomic intervention (Zulfiqar et al., 2020). Biofortification through Zn fertilization is an easy and practical approach to produce Zn enriched grains (Saha et al., 2015). Studies focusing

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specifically on biofortification of rice grain with Zn through inorganic fertilization are rare, although a large number of studies are available on the role of soil and foliar applied fertilizers in correction of Zn deficiency and increasing plant growth and yield (Cakmak, 2008).With this background, the present study was formulated with an objective to assess the effect of method and time of Zn fertilization on yield and biofortification of rice grain with Zn.

Field experiment was conducted during first crop (*kharif)* season of 2018-19 at farmer's field (8°39'N latitude, 76°48'E longitude and 9 m above mean sea level) in Southern Coastal Plains of Thiruvananthapuram, Kerala. The experiment was laid out in randomized block design with eight treatments replicated thrice. The treatments comprised of six Zn foliar fertilization *viz.,* zinc sulphate 0.5% + lime 0.25% at maximum tillering, panicle initiation, booting and milking (T_3); zinc sulphate 0.1% at maximum tillering, panicle initiation, booting and milking (T_4); zinc sulphate 0.1% at panicle initiation, booting and milking (T_5); zinc sulphate 0.1% at booting and milking (T_6); zinc sulphate 0.1% at maximum tillering and milking (T_7); and zinc sulphate 0.1% at milking (T_8), one Zn basal dressing *viz. ,*zinc sulphate 20 kg ha^{-1} (T_2), and a control *viz.,* without Zn (T_1). The experimental site was prepared by puddling, levelling and small bunds were taken all around the plots. The soil was very strongly acidic with a pH of 4.56, normal in electrical conductivity (0.09 dSm^{-1}), low in available N (168 kg ha^{-1}), high in available P (45.2 kg ha^{-1}), medium in available K (198 kg ha^{-1}), and deficient in HCl-extractable Zn (0.79 mg kg^{-1}). 'Uma', a medium duration rice variety released from Rice Research Station, Moncompu was used as the test crop. Seedlings raised in nursery were transplanted at 18 days stage to treatment plots. Zinc sulphate foliar fertilization as per treatments were done at late afternoon time until the spray solution started to run-off from the leaves as described by Cakmak et al. (2010). Recommended dose of lime @ 600 kg ha^{-1} farm yard manure @ 5 t ha^{-1}, and NPK @ 90:45:45 kg ha^{-1} were applied and all other agronomic practices were carried out in treatment plots as per package of practices recommendations of crops for Kerala (KAU, 2016). Observations on growth attributes *viz.,* leaf area index was recorded at 45 DAT from the six sample hills which were randomly selected and tagged. The yield attributing characters *viz.,* filled grains per panicle, thousand grain weight were also recorded from the sample hills. Grain and straw yield per hectare were calculated from the grain and straw yield obtained from the net plot area.

Harvested rough rice was oven dried to constant weight, milled to remove rice hull and obtain brown rice, which was further milled to remove rice bran and obtain white rice. The Zn content in rice grain was estimated by di-acid digestion and analysis using atomic absorption spectrophotometer as described by Prasad et al. (2006). The formula suggested by Shivay et al. (2016) for apparent Zn crop recovery efficiency (ZnCRE), which estimated the increase in Zn uptake by biological yield (sum of grain and straw yield) per unit quantity of Zn applied, was modified by the authors to apparent Zn biofortification recovery efficiency (ZnBRE), to estimate the increase in Zn uptake by edible part of crop plant (grain yield) per unit quantity of Zn applied, and the modified equation is given as follows.

$$\text{ZnBRE (\%)} = \frac{\begin{bmatrix}\text{Zn uptake by grain in Zn fertilized plot (kg ha}^{-1}\text{) -} \\ \text{Zn uptake by grain in Zn unfertilized plot (kg ha}^{-1}\text{)}\end{bmatrix}}{\text{Quantity of Zn applied (kg ha}^{-1}\text{)}} \times 100$$

Economics was worked out based on the prevailing market price of inputs and final produce. Data were statistically analyzed, except the net return and benefit: cost ratio.

Zn foliar fertilization had significant effect on leaf area index (LAI) at 45 days after transplanting (DAT), yield attributes *viz.,* filled grains per panicle, thousand grain weight; and yield (Table 1).Highest LAI (5.26) was observed with foliar fertilization of zinc sulphate ($ZnSO_4$) 0.1% at maximum tillering and milking and was comparable with combined

Table 1. Effect of time and method of zinc fertilization on growth, yield attributes, yield and economics of rice

Treatments	Leaf area index	Filled grains per panicle (nos.)	Thousand grain weight (g)	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Net return (₹ ha^{-1})	Benefit: cost ratio
T_1: Control (without Zn)	4.05	84	23.6	5093	5536	11412	1.11
T_2: $ZnSO_4$ 20 kg ha^{-1} basal	5.23	125	26.2	6566	7013	87125	1.85
T_3: $ZnSO_4$ 0.5% + L at MT, PI, B, M	5.20	122	25.4	6538	7017	79846	1.74
T_4: $ZnSO_4$ 0.1% at MT, PI, B, M	5.22	125	25.9	6587	7022	85721	1.80
T_5: $ZnSO_4$ 0.1% at PI, B, M	4.14	89	23.7	5155	5577	19884	1.19
T_6: $ZnSO_4$ 0.1% at B, M	4.18	90	23.6	5178	5581	23109	1.22
T_7: $ZnSO_4$ 0.1% at MT, M	5.26	127	26.0	6605	7024	91213	1.88
T_8: $ZnSO_4$ 0.1% at M	4.09	87	23.5	5128	5556	10166	1.10
SEm±	0.28	7	0.5	397	426	-	-
CD (P=0.05)	0.856	20.0	1.41	1207.2	1293.6	-	-

B: booting; L: lime 0.25%; M: milking; MT: maximum tillering; PI: panicle initiation; $ZnSO_4$: zinc sulphate

application of $ZnSO_4$ 0.5% + lime 0.25% or $ZnSO_4$ 0.1% at maximum tillering, panicle initiation, booting and milking. $ZnSO_4$ 20 kg ha^{-1} basal application also recorded comparable LAI. Ability of these foliar fertilization treatments to bring about an increase in LAI at 45 DAT comparable to $ZnSO_4$ 20 kg ha^{-1} basal application is attributed to faster crop response to foliar fertilization. Similar faster crop response was observed in rice on $ZnSO_4$ 0.5% foliar application at 20 DAT (Ram et al., 1995). Hussain (2015) reported significant leaf area expansion in rice due to increased enzymatic activity resulting from Zn fertilization. A similar increase in yield attributes *viz.,* filled grains per panicle (127 nos.) and thousand grain weight (26.0 g) was noticed in response to $ZnSO_4$ 0.1% foliar fertilization at maximum tillering and milking stages. Increased availability of Zn during vegetative phase favoured greater absorption of nutrients leading to rapid expansion of foliage, better interception of solar radiation, increased accumulation of photo-synthates, and eventually resulted in improved yield attributes. Increased number of filled grains per panicle due to $ZnSO_4$ 0.5% foliar fertilization was reported by Rani (2013).

$ZnSO_4$ 0.1% foliar fertilization at maximum tillering and milking (T_7) recorded significantly higher grain (6605 kg ha^{-1}) and straw (7024 kg ha^{-1}) yield which were 29 and 26 per cent increase, respectively over Zn unfertilized control (Table 1). Higher yields were observed only in treatments where $ZnSO_4$ was either soil applied basal or foliar fertilized during maximum tillering stage. Commencement of $ZnSO_4$ foliar fertilization beyond vegetative phase *viz.,* panicle initiation, booting and milking showed no increase in yield compared to Zn unfertilized control due to unavailability of Zn to crop during vegetative phase. Prasad et al. (2013) reported 29 per cent increase in rice grain yield with application of urea enriched with two per cent Zn as $ZnSO_4$. Increased grain and straw yield due to Zn fertilization was reported by Daivakrupa (2012), Rani (2013) and Shivay et al. (2016).

Biofortification of rice grain through agronomic intervention as $ZnSO_4$ 0.1% foliar fertilization at maximum tillering and milking stages resulted in white grain with 9.11 mg kg^{-1} Zn content, which was 33 per cent increase over Zn unfertilized control (Table 2). This application also resulted increased Zn content in harvested rough rice (29.8 mg kg^{-1}) and its milled fractions *viz.,* rice bran (75.8 mg kg^{-1}) and brown rice (19.1 mg kg^{-1}). Yadava et al. (2020) reported biofortification through genetic manipulation in Indian rice varieties DRR Dhan 45, DRR Dhan 48, DRR Dhan 49, Zinco Rice MS, CR Dhan 311 and CR Dhan 315 resulting Zn content 20.1 to 27.4 mg kg^{-1} in white rice. Sudha and Stalin (2015) studied the effect of application of $ZnSO_4$ 100 kg ha^{-1} basal plus $ZnSO_4$ 0.1% foliar fertilization at flowering, milking and dough stages in rice genotypes and reported higher Zn content in rough rice (27.3 to 43.4 mg kg^{-1}) and its milled fractions *viz.,* brown rice (16.1 to 39.5 mg kg^{-1}) and white rice (10.4 to 16.9 mg kg^{-1}). Prasad et al. (2013) reported higher Zn content in rough rice (47.4 mg kg^{-1}) with application of urea enriched with two per cent $ZnSO_4$.

High Zn content in rice grains were observed only in treatments where $ZnSO_4$ was applied either as foliar at maximum tillering and milking stages or through soil (Table 2). Direct root uptake of Zn with little dependence on retrainslocation of Zn from vegetative tissues might have contributed to increased grain Zn content in $ZnSO_4$ 20 kg ha^{-1} basal application. A three-fold increase in soil available Zn (2.83 mg ha^{-1}) at harvest recorded in this treatment evidently made adequate Zn available in the soil until grain maturation. However, instead of direct root uptake of Zn, retrainslocation of Zn from vegetative tissues and penetration of foliar applied Zn from the husk into the inner layers of rice endosperm were the factors that might have contributed to increased Zn content in rice grain in $ZnSO_4$ foliar fertilization at maximum tillering and milking stages.

It has been suggested by Johnson-Beebout et al. (2016) and Cakmak and Kutman (2018) that in situation when adequate plant available Zn in the soil is present until grain maturation, it would contribute greatly to grain Zn accumulation through continued and direct root uptake of Zn with little dependence on retrainslocation of Zn from vegetative tissues. However when soil Zn availability to plant roots is limited, grain Zn accumulation largely depends on remobilization of Zn from vegetative tissues (Stomph et al. 2009; Waters et al. 2009; Cakmak and Kutman, 2018). Wu et al. (2010) based on foliar sprayed radioactive zinc (^{65}Zn) studies, concluded that more than half of the Zn in rice grain at harvest had been taken up by crop during vegetative phase *viz.,* seedling and tillering. Lone (2015) reported that Zn foliar fertilization at milking stage increased Zn content in rice grain due to penetration of Zn from the husk into the inner layers of rice endosperm.

Application of $ZnSO_4$ 20 kg ha^{-1} basal could lead to significant soil build-up of Zn over the years, elevating Zn content in soil to levels toxic to plants. $ZnSO_4$ foliar fertilization has several advantages over soil application such as low application rates and prevention of fixation in soil (Nasri et al. 2011).During $ZnSO_4$ 0.5% foliar fertilization, addition of lime 0.25% to spray solution is recommended to avoid phytotoxicity (KAU, 2016). It was observed that $ZnSO_4$ 0.5% + lime 0.25% or $ZnSO_4$ 0.1% at maximum tillering, panicle initiation, booting and milking stages did not cause

Table 2. Effect of time and method of zinc fertilization on zinc content of rough rice and its milled fractions *viz.,* rice bran, brown rice and white rice

Treatments	Rice bran Zn (mg kg^{-1})	Brown rice Zn (mg kg^{-1})	White rice Zn (mg kg^{-1})	Rough rice Zn (mg kg^{-1})	ZnBRE (%)
T_1: Control (without Zn)	57.8	13.7	6.83	23.0	0.0
T_2: $ZnSO_4$ 20 kg ha^{-1} basal	80.5	20.4	9.48	32.1	2.0
T_3: $ZnSO_4$ 0.5% + Lat MT, PI, B, M	78.4	20.1	9.38	31.4	4.3
T_4: $ZnSO_4$ 0.1% at MT, PI, B, M	76.3	19.5	9.20	30.6	20.6
T_5: $ZnSO_4$ 0.1% at PI, B, M	60.8	14.9	7.21	24.8	3.3
T_6: $ZnSO_4$ 0.1% at B, M	59.6	14.4	7.16	23.9	3.4
T_7: $ZnSO_4$ 0.1% at MT, M	75.8	19.1	9.11	29.8	43.8
T_8: $ZnSO_4$ 0.1% at M	58.6	14.1	6.96	23.6	3.9
SEm±	3.9	0.8	0.44	1.4	5.7
CD (P=0.05)	11.80	2.54	1.33	4.13	17.28

B: booting; L: lime 0.25%; M: milking; MT: maximum tillering; PI: panicle initiation; $ZnSO_4$: zinc sulphate; ZnBRE: apparent Zn biofortification recovery efficiency any phytotoxicity in rice crop. This is likely related to $ZnSO_4$ 0.5% + lime 0.25% spray solution having almost the same Zn concentration as that of $ZnSO_4$ 0.1% spray solution. Addition of lime obviously took away some of the Zn out of $ZnSO_4$ 0.5% spray solution. Prom-u-thai et al. (2020) had reported successful biofortification of rice grain with Zn through foliar applied $ZnSO_4$ 0.5% solution alone avoiding lime without causing any phytotoxicity to rice.

Foliar fertilization with $ZnSO_4$0.5% + lime 0.25% or $ZnSO_4$0.1% at maximum tillering, panicle initiation, booting and milking stages required excessive application of Zn fertilizer for increasing rice yield and biofortification of rice grain with Zn, while the same could be achieved with substantially less Zn fertilizer in $ZnSO_4$ 0.1% foliar fertilization at maximum tillering and milking stages. Lone (2015) reported that Zn foliar application at panicle initiation did not significantly increase Zn content in rice grain. The highest apparent Zn biofortification recovery efficiency (43.8%), due to increased Zn uptake in rice grain per unit quantity of Zn applied was recorded in $ZnSO_4$ 0.1% foliar fertilization at maximum tillering and milking stages (Table 2).The highest net return (91213 ₹ ha^{-1}) and benefit: cost ratio (1.88) were also obtained with this treatment, due to lower application rate of zinc sulphate, lower frequency of zinc sulphate application, higher grain and straw yields.

Based on the present study it was concluded that zinc sulphate 0.1% foliar fertilization at maximum tillering and milking stages in rice, proved to be the best Zn fertilization strategy for improved yield and biofortification of rice grain with Zn.

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