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Research Article

Improved yield and grain zinc enrichment of rice (*Oryza sativa* L.) varieties through ferti-fortification in southern coastal plains of Kerala

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ABSTRACT

Field experiment was conducted in farmer's field during rainy (kharif) season 2020-21 at Southern Coastal Plains of Kerala, to study the enhancement in grain zinc (Zn) content and yield improvement through foliar Zn fertilization. Four medium duration rice varieties viz., Uma, Pournami, Gouri and DRR Dhan 45, planted in factorial randomised complete block design constituted the first factor treatments. The second factor comprised of four fertilization treatments applied foliar at maximum tillering and milk stages viz., an unfertilized control, and $ZnSO_4$ @ 0.1, 0.5, and 1%. DRR Dhan 45 recorded significantly higher Zn content in rough rice, brown rice, white rice, rice bran, and cooked rice of 28.6, 33.7, 25.3, 97.7, and 16.6 mg kg^{-1}, respectively. Foliar $ZnSO_4$ @ 0.5% fertilization produced higher grain yield (4.62 t ha^{-1}) that was comparable to $ZnSO_4$ @ 1% and resulted in 12% yield increase over the unfertilized control. $ZnSO_4$ @ 0.5% also resulted in greater Zn content in rough rice, brown rice, white rice, rice bran, and cooked rice of 22, 26, 19.5, 75.3, and 12.8 mg kg^{-1}, respectively. Foliar $ZnSO_4$ @ 0.5% ferti-fortification in rice was economical since it generated greater net income and benefit cost ratio.

Keywords: Biofortification, cooked rice, foliar fertilization, grain Zn, rice, and zinc

Zinc (Zn) is an essential micronutrient for human growth and development through its role in physiological processes, including immune function, DNA synthesis, and wound healing. It is estimated that 4.2% of the Indian population faces exposure of risk to inadequate Zn intake (Myers *et al.*, 2015). Globally, India ranks as the second largest producer of rice (*Oryza sativa* L.) with a production of 129 MT during the year 2021-22. Rice consuming south and northeast Indian states have a much higher pervasiveness of inadequate Zn intake (38%) than the predominantly wheat consuming states in western, central, east, and north India (17%) (Smith *et al.*, 2019). The most common form of rice consumed is polished white rice, obtained by removing the bran and hull from harvested rough rice. Zn content of white rice is inherently low. Thus, there is a need to improve Zn content in rice grain (Bouis and Welch, 2010).

Traditional strategies of delivering mineral micronutrients to humans relied on food fortification, dietary diversification and mineral supplementation (White and Broadly, 2005). These efforts have had limited success. Biofortification or biological fortification presents an alternate approach to combat the issue of insufficient Zn intake in humans. This entails deliberate improvement of nutritional content of edible plant parts by means of either genetic modification or agronomic methods (Zulfiqar *et al.*, 2020).

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Genetic biofortification involves conventional plant breeding and transgenic methods, which can be a lengthy process with substantial development costs. On the other hand, agronomic biofortification can be accomplished through appropriate use of fertilizers (Cakmak and Kutman, 2018). Ferti-fortification describes the process of agronomic biofortification where nutrients are physically applied to crops through fertilizers to improve nutritional status of edible plant parts, thus improving human nutrition.

Ferti-fortification through foliar fertilization reduces application rates compared to soil fertilization since it minimises losses caused by soil fixation (Nasri *et al.*, 2011). Most rice varieties remobilize foliar fertilised Zn from their vegetative parts such as leaves and stem to the grain, however this ability varies depending on the plant's genetic composition and the Zn availability (Mabesa *et al.*, 2013). According to Cakmak (2008), problems with physical and chemical soil properties in most cereal growing countries make it difficult for biofortified rice varieties to uptake adequate Zn from soil and translocate them to grain. The present study was conducted with the objective of determining the optimal concentration of Zn in foliar spray solution that would maximise the Zn content in rice grain while simultaneously improving the grain yield.

MATERIALS AND METHODS

A field trial in rice was carried out in farmer's field, which lies at 8° 43' N latitude and 76° 45' E longitude at 52 m above mean sea level, in the Southern Coastal Plains of Kerala during rainy *(kharif)* season 2020-21. The seasonal rainfall received was 935.8 mm and rainy days were 50 during 2020 which is beneficial for crop growth and grain development. The mean seasonal maximum and minimum temperature ranged from 30.3° to 32.7°C and 24.5° to 25.9°C respectively. The field experiment was laid out in a two-factor factorial randomised complete block design, replicated thrice, with 16 treatment combinations. The treatments comprised of four medium duration rice varieties as factor (V) *viz*., V_1: Uma, V_2: Pournami, V_3: Gouri, and V_4: DRR Dhan 45, and four nutrient levels as factor (F) *viz.*, F_1: control treatment without application of Zn source, F_2: $ZnSO_4$ @ 0.1%, F_3: $ZnSO_4$ @ 0.5%, and F_4: $ZnSO_4$ @ 1%. Foliar Zn fertilisation of the rice crop was done in two splits with a single concentration of spray solution. The first Zn spray was given during the maximum tillering stage, while the second spray was applied during the milk stage. The study utilized both biofortified and non biofortified rice varieties. DRR Dhan 45 is a biofortified rice variety released by the Indian Institute of Rice Research, Hyderabad. Gouri is a rice variety that accumulates high Zn content in grains, released from Rice Research Station, Moncompu. Despite not being a biofortified variety, Uma is popular among rice farmers of Kerala, suited for cultivation during all three growing seasons. Pournami is a recently released non biofortified rice variety by the Rice Research Station, Moncompu.

The experimental site was puddled, levelled, and small bunds were formed surrounding the plots. The clay loam soil of the experimental site was very strongly acidic (pH 5.4), non saline in electrical conductivity (0.19 dS m^{-1}), high in organic carbon (1.32%), medium in available N (282 Kg ha^{-1}), P (12.7 Kg ha^{-1}), and K (187 Kg ha^{-1}), and sufficient in HCl-extractable Zn (1.05 mg Kg^{-1}). Acidic soils in Kerala having Zn higher than 1.00 mg Kg^{-1} are considered to be 'sufficient' in Zn (KAU, 2016). The nursery raised seedlings were transplanted to treatment plots. According to the treatments, spray solution for foliar Zn fertilization was sprayed on the crop late in the afternoon till the spray solution just began to trickle off the leaves, as suggested by Cakmak *et al.* (2010). All other agronomic practices were followed as per standard recommendation for Kerala, including lime (600 Kg ha^{-1}), farm yard manure (5 t ha^{-1}), and fertilizer (90:45:45 Kg $N:P_2O_5:K_2O$ ha^{-1}) application at recommended dose (KAU, 2016). Observation on growth parameter such as leaf area index at panicle initiation stage was recorded from six randomly chosen and tagged sample hills in each plot. Yield attributing characters namely panicle numbers and filled grains were also recorded from these sample hills. After a thorough sun drying of harvested produce from net plot area, weight for grain and straw were taken and then converted to grain and straw yield per hectare.

The rough rice that was harvested from field was dried in an oven to a constant weight prior to being milled to remove the rice hull and get brown rice, which was then polished to produce white rice by removing the rice bran. White rice was cooked in accordance with procedure described by Suman (2011), to get cooked rice. The analysis of Zn content in rough rice and its milled fractions, and cooked rice, was performed by di-acid digestion and atomic absorption spectrophotometry. The economic parameters of various treatments were calculated considering the market price of inputs and final produce. The data were subjected to statistical analysis, and critical difference at 5% significance level was calculated for each parameter.

RESULTS AND DISCUSSION

Fert-fortification depends on nutrient availability. Optimal availability of essential plant nutrients from soil as well as fertilizer application is mandatory for enhanced nutrient content in rice grain. Foliar $ZnSO_4$ @ 0.5% fertilization at maximum tillering and milk stages of rice, resulted in significantly higher growth parameter and yield attribute such as leaf area index, panicle number and filled grains, than $ZnSO_4$ @ 0.1% and unfertilized control treatments (Table 1). Comparable increase in leaf area index (3.61 and 3.87) was observed with foliar $ZnSO_4$ fertilisation at 0.5 and 1%, respectively. According to Fageria *et al*. (2009), crops respond more quickly to fertilizers administered through the foliage than through the soil. Foliar application made it easier for phloem to absorb and transport Zn. Foliar application prevents Zn from being lost through soil fixation (Nasri *et al.*, 2011). Hussain (2015) noted considerable expansion of leaf area in rice as a result of increased enzymatic activity brought on by Zn fertilization. This had contributed to better interception of solar radiation and increased photosynthesis. Foliar fertilization with $ZnSO_4$ @ 0.5%, caused considerable leaf area expansion owing to rapid cell division and enlargement, fast growth, and consequently improved vegetative development, as evidenced by the increase in leaf area index.

Table 1: Effect of Zn ferti-fortification on leaf area index, panicles per square metre, filled grains per panicle, grain and straw yield of rice

Treatment	Leaf area index	Panicles per sq. m (nos.)	Filled grains per panicle (nos.)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Factor V: Variety					
V_1: Uma	3.39	434	78.0	4.62	4.84
V_2: Pournami	3.09	411	83.0	4.29	4.49
V_3: Gouri	3.03	428	78.9	4.15	4.35
V_4: DRR Dhan 45	3.08	430	70.3	4.48	4.69
SEm (±)	**0.13**	**8**	**1.76**	**0.15**	**0.16**
LSD (0.05)	**NS**	**NS**	**5.21**	**NS**	**NS**
Factor F: Fertilizer					
F_1: No fertilizer	2.42	395	71.4	4.12	4.33
F_2: $ZnSO_4$ @ 0.1 %	2.69	403	74.7	4.16	4.38
F_3: $ZnSO_4$ @ 0.5 %	3.61	446	80.8	4.62	4.81
F_4: $ZnSO_4$ @ 1 %	3.87	459	83.3	4.65	4.85
SEm (±)	**0.13**	**8**	**1.76**	**0.15**	**0.16**
LSD (0.05)	**0.38**	**23.40**	**5.21**	**0.43**	**0.42**

Note: Interaction (V × F) non-significant

Similar increases in yield attributes, such as the panicle numbers (446 and 459 nos. m^{-2}) and the filled grains (80.8 and 83.3 nos. $panicle^{-1}$) were noticed in response to $ZnSO_4$ @ 0.5 and 1% fertilization, respectively (Table 1). The panicle numbers in rice plant have a substantial positive relationship with the productive tiller number of the plant (Chen *et al.*, 2015). According to Rani (2013), $ZnSO_4$ 0.5% foliar fertilization enhanced Zn uptake in plants, thereby increasing the filled grains in each panicle. $ZnSO_4$ enhances the chlorophyll content in leaves and maintains turgidity of leaves, facilitating more photosynthates to accumulate and increase rice yield (Mukherjee *et al.*, 2020). Crop fertilisation with $ZnSO_4$ @ 0.5% lead to improved nutrient uptake, photosynthesis, increased number of panicles and carbohydrate transfer to the grain, which in turn increased the filled grain numbers in each panicle. However, there was no significant difference among $ZnSO_4$ @ 0.5% and 1% fertilization with regard to nutrient intake, panicle numbers, and number of filled grains in each panicle. These results confirm the findings of Daivakrupa (2012), Hussain (2015) and Shivay *et al.* (2016). Higher filled grains (83 nos. $panicle^{-1}$) observed in Pournami variety was comparable to varieties such as Gouri (78.9 nos.), and Uma (78 nos.) (Table 1). Variety DRR Dhan 45 recorded less filled grains (70.3 nos. $panicle^{-1}$). According to Praneeth (2013), the number of filled grains is influenced by significant differences in the spikelet sterility among various rice varieties. Similar varietal differences affecting the filled grain numbers was reported by Fukushima (2019).

Various nutrient levels significantly influenced the grain and straw yield of rice. Better vegetative crop growth and higher yield attributes resulted in significantly higher grain yields (4.62 and 4.65 t ha^{-1}) in foliar fertilization with $ZnSO_4$ @ 0.5 and 1%, respectively. These yields were comparable and increased grain yield by 12 to 13% over the unfertilized control (Table 1). Similarly, $ZnSO_4$ @ 0.5 and 1% recorded higher straw yields (4.81 and 4.85 t ha^{-1}, respectively) which were comparable. The interaction effect between varieties and nutrient levels on yield was not found to be significant. According to Shivay *et* *al.* (2016), foliar fertilization with $ZnSO_4$ @ 0.5% increased the grain and straw yield. This outcome is consistent with findings of Suresh and Salakinkop (2016), who noted that increased grain yield due to foliar Zn fertilization was brought on by significantly greater yield components, namely productive tillers and filled grains.

The results revealed that effect of varieties and nutrient levels on Zn content in grain were significant while their interaction were not significant. Biofortified variety DRR Dhan 45 recorded significantly higher rough rice Zn content (28.6 mg kg^{-1}), and in its milled fractions such as brown rice, white rice, and rice bran of 33.7, 25.3 and 97.7 mg kg^{-1}, respectively, and also in cooked rice (16.6 mg kg^{-1}) (Table 2). Rice variety Gouri came in second with much lower Zn content. Uma, the popular non biofortified variety among farmers of Kerala recorded significantly lower Zn content in rough rice, brown rice, white rice, rice bran, and cooked rice of 13.8, 16.3, 12.2, 47.1, and 8 mg kg^{-1}, respectively. High Zn content in white rice (20.1 to 27.4 mg kg^{-1}) of Indian biofortified rice varieties such as CR Dhan 311, CR Dhan 315, Zinco Rice MS, DRR Dhan 45, DRR Dhan 48 and DRR Dhan 49 were reported by Yadava *et al.*, (2020). Rice varieties differ in their ability to remobilize the Zn from their vegetative parts causing variation in rice grain Zn content in those varieties (Fernandez and Brown, 2013). According to Impa *et al.* (2013), in some rice varieties net remobilization of Zn from vegetative parts such as shoot and root predominated, whilst in others continued root uptake remained the main source for loading Zn in grains.

Table 2: Effect of Zn ferti-fortification on Zn content of rough rice, brown rice, white rice, rice bran, and cooked rice

Treatment	Rough rice Zn (mg kg^{-1})	Brown rice Zn (mg kg^{-1})	White rice Zn (mg kg^{-1})	Rice bran Zn (mg kg^{-1})	Cooked rice Zn (mg kg^{-1})
Factor V: Variety					
V_1: Uma	13.8	16.3	12.2	47.1	8.0
V_2: Pournami	15.4	18.2	13.6	52.6	8.9
V_3: Gouri	21.5	25.4	19.1	73.5	12.5
V_4: DRR Dhan 45	28.6	33.7	25.3	97.7	16.6
SEm(±)	0.73	0.86	0.69	2.49	0.42
LSD (0.05)	2.11	2.49	1.99	7.21	1.24
Factor F: Fertilizer					
F_1: No fertilizer	15.9	18.8	14.1	54.4	9.3
F_2: $ZnSO_4$ @ 0.1 %	17.3	20.4	15.3	59.2	10.1
F_3: $ZnSO_4$ @ 0.5 %	22.0	26.0	19.5	75.3	12.8
F_4: $ZnSO_4$ @ 1 %	24.0	28.3	21.2	82.0	13.9
SEm(±)	**0.73**	**0.86**	**0.69**	**2.49**	**0.42**
LSD(0.05)	**2.11**	**2.49**	**1.99**	**7.21**	**1.24**

Note: Interaction (V × F) non-significant

Accumulation of Zn increased substantially in white rice, being significantly higher in $ZnSO_4$ @ 0.5% (19.5 mg kg^{-1}), which remained statistically at par with $ZnSO_4$ @ 1% (21.2 mg kg^{-1}) (Table 2). Moreover, $ZnSO_4$ @ 0.5% also increased the Zn content in harvested rough rice, brown rice, rice bran, and cooked rice of 22, 26, 75.3, and 12.8 mg kg^{-1}, respectively, to levels comparable to $ZnSO_4$ @ 1%. Under field conditions, foliar applied Zn fertilizers are highly effective and practical technique to maximize rice grain Zn content (Wissuwa *et al.*, 2008). Increased rice grain Zn content resulting from $ZnSO_4$ @ 0.5% foliar application was reported by Prom-u-thai *et al.*, (2020). According to Beebout *et al.* (2016), direct root uptake accounted for the majority of grain Zn content when Zn availability in soil is sufficient until grain maturation. Remobilization of Zn from vegetative plant parts, however, accounts for a significant portion of the grain Zn content when soils have low Zn levels (Stomph *et al.*, 2009; Cakmak and Kutman, 2018).

Jiang *et al.* (2007) reported that in studies with radioactive Zn (^{65}Zn), most of the Zn in grain was remobilized from vegetative plant parts that include stem and leaves, and was translocated to grains at the reproductive stage of crop. Radioactive Zn (^{65}Zn) foliar applied was remobilized and translocated through phloem to the grains (Wu *et al.*, 2010). Over half of the Zn in harvested rough rice was taken up by the crop when it was in vegetative phase (Wu *et al.*, 2011). The vascular system that connects hull to caryopsis provides passage for photosynthates and nutrients (Cakmak *et al.*, 2010). The increased transfer of Zn into rice grain after flowering is attributed to substantial increase in production of protein during the early stages of grain development (Martre *et al.*, 2003; Ozturk *et al.*, 2006). Zn accumulation in seed is particularly high during early milk stage which is the first half of the seed development stage (Ozturk *et al.*, 2006). According to Stomph *et al.* (2009), reduced Zn loading in rice endosperm is not brought about by transport barriers, but primarily caused by limited sink capacity in the high starch filled endosperm cells and therefore continuity in the Zn loading of endosperm during grain filling requires genetically improved uptake capacity along with properly managed Zn availability. $ZnSO_4$ @ 0.5% or 1% foliar fertilized at maximum tillering and milk stages in the current study may have got remobilized to the grains from vegetative plant parts such as stem and leaves, and hull, thus substantially raising the rice grain Zn content.

Regardless of the treatments, the higher Zn content among the milled fractions observed in rice bran (47 to 98 mg kg^{-1}), indicated that bran removal during milling caused the major Zn loss (Table 2). According to Lu *et al.* (2013), the *in vivo* mineral Zn distribution pattern in various rice grain fractions is in this order: rice bran > brown rice > white rice. They reported a 75 mg Kg^{-1} Zn content in rice bran, which was three times more than what they observed in their corresponding white rice. Nevertheless, the Zn content in white rice of the biofortified DRR Dhan 45 variety (25.3 mg kg^{-1}) was twice that of Uma (12.2 mg kg^{-1}), the widely cultivated non-biofortified variety among farmers of Kerala. White rice Zn content (19.5 mg kg^{-1}) was 38% higher after ferti-fortification with $ZnSO_4$ @ 0.5% compared to the unfertilized control. Increased Zn content of white rice also lead to increased Zn content in cooked rice as well. These results indicate that, use of the biofortified rice variety DRR Dhan 45 and ferti-fortification with $ZnSO_4$ @ 0.5% could greatly improve nutritional status of rice grain. Decreased content of Zn in white rice following milling was reported by Jiang *et al.* (2007) and Wang *et al.* (2014).

$ZnSO_4$ @ 0.5% fertilisation in rice varieties Uma, Pournami, Gouri, and DRR Dhan 45, resulted in higher net income of ₹ 46055, 36804, 32469, and 41340 ha^{-1}, respectively. However, net income drastically decreased by 29 to 39 % and 22 to 33 % respectively, when Zn in the spray solution was reduced to $ZnSO_4$ @ 0.1% and in the unfertilized control (Table 2). Ferti-fortification with $ZnSO_4$ @ 1% had 10 per cent higher additional input cost than ferti-fortification with $ZnSO_4$ @ 0.5%, which increased the cultivation cost, but did not significantly increase the yield (Tables 1, 2). $ZnSO_4$ @ 0.5% ferti-fortification in rice varieties Uma, Pournami, Gouri, and DRR Dhan 45, could achieve higher benefit cost ratios of 1.49, 1.39, 1.34, and 1.44, respectively (Table 3). Biofortified rice was not priced differentially from conventional rice grain in Kerala's consumer market due to a lack of awareness about the health benefits of this type of rice. This prevented biofortified rice grain from being sold at a greater price than conventional rice grain, which would have increased net income.

Table 3: Economics of Zn ferti-fortification as influenced by rice varieties and fertilizer levels

Treat-ment	Zinc sulphate (kg ha^{-1})	Additional cost due to treatment (₹ ha^{-1})	Cost of cultivation (₹ ha^{-1})	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Gross income (₹ ha^{-1})	Net income (₹ ha^{-1})	Benefit cost ratio
$V_1 F_1$	0	0	90221	4367	4596	126342	36121	1.40
$V_1 F_2$	1	3880	94101	4383	4614	126808	32707	1.35
$V_1 F_3$	5	4200	94421	4860	5063	140476	46055	1.49
$V_1 F_4$	10	4600	94821	4887	5090	141253	46432	1.49
$V_2 F_1$	0	0	90221	3977	4186	115060	24839	1.28
$V_2 F_2$	1	3880	94101	4067	4281	117665	23564	1.25
$V_2 F_3$	5	4200	94421	4540	4729	131225	36804	1.39
$V_2 F_4$	10	4600	94821	4570	4760	132091	37270	1.39
$V_3 F_1$	0	0	90221	3887	4091	112456	22235	1.25
$V_3 F_2$	1	3880	94101	3940	4147	113990	19889	1.21
$V_3 F_3$	5	4200	94421	4390	4573	126890	32469	1.34
$V_3 F_4$	10	4600	94821	4400	4583	127178	32357	1.34
$V_4 F_1$	0	0	90221	4233	4456	122468	32247	1.36
$V_4 F_2$	1	3880	94101	4240	4463	122670	28569	1.30
$V_4 F_3$	5	4200	94421	4697	4892	135761	41340	1.44
$V_4 F_4$	10	4600	94821	4757	4955	137497	42676	1.45

Note: Cost of cultivation excluding treatment ₹ 90221 ha^{-1}; cost of zinc sulphate heptahydrate ₹ 80 kg^{-1}; cost of labour for foliar Zn spraying with a knapsack sprayer ₹ 3800 ha^{-1} @ ₹ 950 manday^{-1}; price of grain ₹ 26.30 kg^{-1}; price of straw ₹ 2.50 kg^{-1}

CONCLUSION

The results concluded that higher Zn content in rough rice, its milled fractions, and cooked rice was obtained in biofortified rice variety DRR Dhan 45. Foliar fertilization in rice with $ZnSO_4$ @ 0.5% at maximum tillering and milk stages is an economic ferti-fortification strategy to improve yield and enrich Zn in rice grain.

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