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BIOFORTIFICATION IN RICE GRAINS VIS-A-VIS ZINC AND NITROGEN FERTILIZATION

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Abstract - The study was undertaken to investigate the effect of increasing soil N supply, and method and timing of Zn application on concentrations and bioavailability of zinc in different rice grain fractions. Grain zinc concentration was greatly enhanced by increasing soil N supply when sufficient Zn was available to the plants through soil Zn application or foliar spray at milking stage. Increasing N supply progressively decreased grain phytate concentration and increased protein concentrationin rice grains. Foliar Zn application at milking stage significantly enhanced Zn concentration while that at panicle initiation stage produced non significant effect. Soil Zn application also significantly increased grain Zn concentration. Conclusively, a proper combination of N and Zn applications could prove to be a powerful tool for zinc biofortification in rice grains.

Index Terms—bioavailability, biofortification, nitrogen, rice, zinc

I. INTRODUCTION

In the past, agricultural research especially in developing countries mostly focused on increasing food grain production. However, it is widely recognized that agriculture now must not only produce more calories to reduce hunger of food but also more nutrient-rich food to reduce hidden hunger of minerals [1]. Zinc (Zn) deficiency is ranked as the 5th leading risk factor for diseases in the developing world [2]. A key reason for this scenario is the low concentration of Zn in the most commonly consumed cereal grains [3]. Biofortification of staple food crops, therefore, has been suggested as a way to help alleviate the Zn and other micronutrient related deficiencies. Under current economic conditions, biofortification of staple food crops provides a feasible means to reach malnourished rural populations who have limited access to diverse diets, supplements, and commercially fortified foods [4].

Plant breeding (genetic) strategy of biofortification appears to be a most sustainable and cost-effective approach. This approach is, however, a long-term process requiring a substantial effort and resources [5]. On the other hand, agronomic Zn biofortification through fertilizer application is a viable alternative approach which enables us to substantially increase grain Zn concentration even in Zn-deficient soils [6]. Foliar Zn spray, especially at later growth stages was very effective in increasing Zn concentration of both whole grain and endosperm fraction, while soil Zn applications comparatively remained less effective **[7]**.

In addition to increased Zn content, a key concern in human Zn nutrition is the Zn bioavailability in rice grain. Rice grain, besides being inherently low in Zn, is also rich in substances which limit its bioavailability in the human digestive tract. The most commonly studied inhibitor of Zn and Fe bioavailability is phytic acid [8]. Because of its high polyvalent cation-chelating activity and the lack of phytase enzyme in humans, phytate significantly reduces the absorptions of dietary Zn [9]. The molar ratios of phytate to Zn in foods are often used as an indicator for the bioavailability of zinc to humans [10]. There are reports available in the literature that fertilizer Zn and N application can bring about significant drawdown in grain phytate content and as such increase zinc bioavailability [11].

We therefore studied the effect of N and Zn fertilizations simultaneously on concentration and bioavailability of Znin rice grain so as to get a holistic idea about its overall utility. To the best of our knowledge there are only few studies that have dealt with this issue till date in case of wheat [12] and no study in case of rice.

II. MATERIALS AND METHODS

Field experiments were conducted for two years during 2013 to 2014 at University Farm, SKUAST, Srinagar, India (34°08′ N, 74°83′ E and 1,587 m above the mean sea-level). The average precipitation is 780.77 mm (average of past 28 years), most of which is received from December to April in the form of snow and rains. Atmospheric temperature ranges between the lowest winter temperature of −8°C to the highest summer temperature of 33°C.

The experiments consisted of nine treatments laid out in randomized completely block design (RCBD) in three blocks. Treatments included various soil N applications at optimum soil Zn level, and various soil and foliar Zn applications at optimum soil N level (Table 1). Nitrogen was applied in three splits; first one as basal dose and second and third at active tillering and panicle initiation stages respectively.

Initial composite surface soil sample (0–15 cm depth) was collected, mixed, air dried, sieved, and analyzed for physical and chemical characteristics. The soil was clayey loam in texture, medium in N, P and K. Basal rates of 25 kg ha^{-1} each of P and K as diammonium phosphate and potassium chloride respectively were uniformly applied to all plots of 10 m^2 (4 m × 2.5 m). Thirty-five days old seedlings were transplanted manually at 15 cm × 15 cm distance.Pre-emergence herbicide Butachlor 5% G was applied 3 days after transplanting (DAT). At listed crop growth stages (Table 1), 0.5 % w/v $ZnSO_4.7H_2O$ was sprayed on plants. Spraying was done by using a foot sprayer at the time of sunset. Days from sowing to flowering, from sowing to maturity and from flowering to maturity were recorded when about 50% of the plants in a plot attained specified growth stage. At full maturity, rice crop was harvested manually. Grain yield was adjusted to 14% moisture content and straw yield was expressed on oven dry weight basis. Average of thousand grains from each plot was recorded as grain mass. Different grain fractions were separated using laboratory milling machine.

III. STATISTICAL ANALYSIS

The data obtained was subjected to analysis of variance using R software (version 3.2.0; Developer: R Core Team, University of Auckland, New Zealand). Significantly different treatment means were separated using Fisher's protected least significant difference (LSD) test.

IV. RESULTS

Grain and straw yields were significantly ($P \leq 0.05$) increased with increasing N application from N_0Zn to N_3Zn(Table 2). Response of straw yield to N application was more or less similar to those of grain yield. Soil Zn application also significantly affected grain yield as compared to Zn_0 but a non significant effect of soil Zn application was observed on straw yield. Foliar applications of Zn also non significantly influenced grain yield. Interestingly, grain weight was statistically similar in all the treatments tested. Moreover highest nitrogen level (N_3Zn) significantly extended grain filling period by about 10 days as compared to control (N_0Zn).

Grain Zn concentration was significantly ($P \leq 0.05$) affected by N and Zn fertilization (Table 3). Nitrogen supply progressively enhanced Zn concentration in all grain fractions from N_0Zn to N_3Zn. On average 19, 16 and 15% increase in grain Zn concentration of N_3Znas compared to N_0Zn was observed in unhusked grain, brown rice and white rice, respectively. Foliar Zn application at milking stage was more effective in increasing grain Zn concentration than soil Zn application at sowing, albeit both significantly increased grain Zn concentration. As compared to control (N_3Zn_0), soil Zn application alone increased grain Zn concentration in unhusked grain, brown rice and white rice respectively by 30, 25 and 21%. The increases were respectively 57, 53, and 37% with foliar application at milking stage compared with N_3Zn_0. Notably, the effect of foliar Zn application at panicle initiation was non significant for grain Zn concentration. Whereas, highest concentrations in rice grain were observed when soil Zn application was combined with foliar Zn applications at milking stage that accounted for increase of 71, 63, and 52% as compared to N_3Zn_0 respectively in unhusked grain, brown rice and white rice.

Protein concentration in rice grain was significantly increased with the increasing soil N supply and with the highest soil N level (N_3Zn), it increased by about 26% relative to the control (N_0Zn). Grain phytate content was significantly decreased with N and Zn applications. (Table 4). Greatest reduction of 23% in phytate content was observed with combined soil application at sowing and foliar Zn spray at milking stage as compared to N_3Zn_0. Phytate-Zn molar ratio were also gradually decreased with increasing soil N levels from N_0Zn to N_3Zn. Soil Zn at sowing, foliar Zn application at milking stage as well as their combination significantly decreasedphytate-Zn molar ratio.

DISCUSSION

Inherent soil N status was medium and Zn status was deficient. In such situations, increase in grain yield in response to soil applications of N and Zn is well established [13]. The increase in grain yield could mainly be attributed to the increase in yield components like number of panicles per unit area, number of seeds per panicle and panicle length (data not shown). However, mass of individual grain was only non significantly different among treatments. This is an important characteristics of agronomic biofortification as larger grain mass might related with lower grain Zn concentration owning to larger proportion of endosperm in grains [14].

Similar to our findings (Table 3), Zn concentration of rice grain was reported to significantly increase with soil N supply, soil Zn application and foliar Zn sprays at milking stage [15]. This phenomenon could be explained from the hypothesis that proteins represent a sink for Zn in the grain [16]. The positive correlation between Zn and N was however lost under 'N_3Zn_0'and 'N_3Zn_0+Zn_P' treatments, which suggests that this relationship depends on a sufficiently high Zn availability in either the growth medium or plant tissues [11]. Under Zn-deficient-N-sufficient conditions, not the sink activity but the magnitude of Zn availability to the plant is the limiting factor with regard to Zn enrichment of grains [13].

Unlike grain protein concentration which was increased with soil N application, grain phytate concentration was progressively decreased (Table 4).

This suggests that accumulation of phytate and protein in grain is probably genetically independent [17] or have antagonistic relationship. From this, it can be inferred that it is possible to simultaneously decrease phytate concentration and increase protein concentration (and therefore concentration of Zn) in grain.

Timing of foliar Zn application was critical for its efficacy in increasing grain Zn concentration (Table 3). Foliar Zn application only at milking stage significantly enhance Zn concentration in rice grain [15]. Soil Zn application also significantly affected grain Zn concentration, albeit not as powerfully as foliar application at milking stage of rice.

CONCLUSIONS

Optimum soil N application and foliar Zn application at milking stage under Zn sufficient conditions are effective tools for Zn biofortifying rice grain. Soil Zn application also contributed to increase Zn concentration in rice grain as well as its bioavailability to humans. Therefore, proper combination of N and Zn applications could prove to be a powerful tool for Zn biofortification in rice grain.

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Table 1: Details of treatments studied in the experiment

Treatments	Codes	Description
T1	N_0Zn	Soil applications of 0 kg N and 10 kg $ZnSO_4.7H_2O$ ha^{-1}
T2	N_1Zn	Soil applications of 60 kg N and 10 kg $ZnSO_4.7H_2O$ ha^{-1}
T3	N_2Zn	Soil applications of 90 kg N and 10 kg $ZnSO_4.7H_2O$ ha^{-1}
T4	N_3Zn	Soil applications of 120 kg N and 10 kg $ZnSO_4.7H_2O$ ha^{-1}
T5	N_3Zn_0	Soil applications of 120 kg N and 0 kg $ZnSO_4.7H_2O$ ha^{-1}
T6	N_3Zn_0+Zn_P	Soil applications of 120 kg N and 0 kg $ZnSO_4.7H_2O$ ha^{-1} + foliar spray of 0.5% w/v $ZnSO_4.7H_2O$ at panicle initiation
T7	N_3Zn_0+Zn_M	Soil applications of 120 kg N and 0 kg $ZnSO_4.7H_2O$ ha^{-1} + foliar spray of 0.5% w/v $ZnSO_4.7H_2O$ at milking stage
T8	N_3Zn+Zn_P	Soil applications of 120 kg N and 10 kg $ZnSO_4.7H_2O$ ha^{-1} + foliar spray of 0.5% w/v $ZnSO_4.7H_2O$ at panicle initiation
T9	N_3Zn+Zn_M	Soil applications of 120 kg N and 10 kg $ZnSO_4.7H_2O$ ha^{-1} + foliar spray of 0.5% w/v $ZnSO_4.7H_2O$ at milking stage

Table 2: Grain yield, straw yield and grain mass of rice (cv. Pusa Sugandh-3) grown with different fertilizer treatments during 2013 and 2014

Treatments	Grain yield (t ha^{-1})		Straw yield (t ha^{-1})		Grain mass (mg)	
	2013	2014	2013	2014	2013	2014
N_0Zn	3.06 c	2.86 c	5.58 b	5.31 c	23.8^{NS}	23.6^{NS}
N_1Zn	5.06 b	4.84 b	6.33 ab	6.01 b	24.0	23.7
N_2Zn	5.39 ab	5.18 ab	6.58 a	6.35 a	24.0	23.6
N_3Zn	5.52 a	5.32 a	6.81 a	6.56 a	24.1	23.8
N_3Zn_0	5.13 b	5.02 b	6.80 a	6.54 a	23.9	23.6
$N_3Zn_0+Zn_P$	5.08 b	4.87 b	6.82 a	6.59 a	24.2	24.0
$N_3Zn_0+Zn_M$	5.15 b	4.94 b	6.79 a	6.55 a	23.8	23.5
N_3Zn+Zn_P	5.51 a	5.28 a	6.81 a	6.57 a	24.0	23.7
N_3Zn+Zn_M	5.49 a	5.29 a	6.83 a	6.57 a	24.1	23.8

Within the columns, values followed by different letters are significantly different based on Fisher's LSD test at $\alpha = 0.05$. ^{NS}non significant at α= 0.05 based on one-way Analysis of Variance.

Table 3 Zinc concentration (mg kg^{-1}) in unhusked grain, brown rice and white rice of Pusa Sugandh-3 grown with different fertilizer treatments during 2013 and 2014

Treatments	Unhusked grain		Brown rice		White rice	
	2013	2014	2013	2014	2013	2014
N_0Zn	30.3 d	29.5 f	26.3 e	26.2 e	22.8 e	22.1 e
N_1Zn	31.4 d	31.2 e	28.2 d	29.2 d	23.8 d	23.6 d
N_2Zn	34.2 c	33.4 d	30.1 c	29.6 cd	24.3 d	25.6 c
N_3Zn	35.4 c	35.5 c	30.4 c	30.6 c	25.8 c	25.8 c
N_3Zn_0	27.3 e	27.2 g	24.2 f	24.4 f	21.9 e	20.6 f
$N_3Zn_0+Zn_P$	28.1 e	27.7 g	24.7 f	24.6 f	21.9 e	20.8 f
$N_3Zn_0+Zn_M$	42.5 b	42.6 b	37.4 b	37.5 b	29.1 b	29.2 b
N_3Zn+Zn_P	35.1 c	35.2 c	30.4 c	30.2 c	25.2 c	25.3 c
N_3Zn+Zn_M	46.4 a	46.5 a	39.4 a	39.6 a	32.2 a	32.3 a

Within the columns, values followed by different letters are significantly different based on Fisher's LSD test at $\alpha = 0.05$.

Table 4: Protein concentration (%), Phytate concentration (mg g^{-1}), phytate-zinc molar ratio in white rice (cv. Pusa Sugandh-3) grown with different fertilizer treatments during 2013 and 2014

Treatments	Protein concentration		Phytate concentration		Phytate to Zinc Ratio	
	2013	2014	2013	2014	2013	2014
N_0Zn	7.51 d	7.38 d	9.34 c	9.50 c	40.6 b	42.8 b
N_1Zn	8.13 c	8.31 c	9.03 d	9.01 d	37.8 c	37.9 c
N_2Zn	8.92 b	9.05 b	8.60 e	8.61 e	33.6 d	33.4 d
N_3Zn	9.48 a	9.57 a	8.63 e	8.57 e	33.2 d	33.0 d
N_3Zn_0	9.42 a	9.52 a	9.72 a	9.73 a	44.0 a	46.9 a
$N_3Zn_0+Zn_P$	9.53 a	9.46 a	9.64 b	9.65 b	43.8 a	46.1 a
$N_3Zn_0+Zn_M$	9.46 a	9.55 a	7.85 f	7.89 f	26.8 e	26.8 e
N_3Zn+Zn_P	9.51 a	9.49 a	8.52 e	8.54 e	33.5 d	33.5 d
N_3Zn+Zn_M	9.5 a	9.47 a	7.46 g	7.45 g	23.0 f	22.9 f

Within the columns, values followed by different letters are significantly different based on Fisher's LSD test at $\alpha = 0.05$

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