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Grain Zinc and Yield Responses of Two Rice Varieties to Zinc Biofortification and Water Management

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Abstract: Zinc (Zn) biofortification can improve grain yield and nutritional quality in rice, but its effectiveness is subject to agronomic practices and other factors. In a previous study, the application of Zn to soil enhanced grain Zn in lowland rice in well-drained and waterlogged soil, whereas grain Zn in upland rice increased only in well-drained soil. This new study explores the hypothesis that the application of foliar Zn can enhance grain Zn in upland and lowland rice grown under waterlogged and well-drained conditions. Two rice varieties, CNT1 (wetland rice) and KH CMU (upland rice) were grown in containers in waterlogged or well-drained soil with three Zn treatments (no Zn, soil Zn and foliar Zn). For the soil Zn treatment, 50 kg $ZnSO_4$ ha^{-1} was applied to the soil before transplanting. For the foliar treatment, 0.5% $ZnSO_4$ (equivalent to 900 L ha^{-1}) was applied at booting and repeated at flowering and milky growth stages. Grain yield in CNT1 was 15.9% higher in the waterlogged than in the well-drained plants, but the water regime had no effect on grain yield in KH CMU. Grain Zn concentration in CNT1 increased from 19.5% to 32.6% above the no Zn control when plants were applied with soil or foliar Zn. In KH CMU, there was an interaction between the water regime and Zn treatment. Application of foliar Zn increased grain Zn by 44.6% in well-drained and 14.7% in waterlogged soil. The results indicate strong interaction effects between variety, water regime and Zn fertilizer application on Zn biofortification in rice. Thus, the selection of rice varieties and growing conditions should be considered in order for producers to achieve desirable outcomes from high grain Zn concentrations.

Keywords: agronomy; rice crop; zinc concentration; zinc fertilizer management; zinc biofortification

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1. Introduction

Rice grains are inherently low in Zn relative to other cereals [1]. Although the average grain Zn in brown rice of 15 Thai varieties was 29 ± 13 mg kg^{-1} [2], wetland rice ecotypes can have lower Zn concentrations (19–20 mg kg^{-1}) than upland ecotypes (28–31 mg kg^{-1}) [3,4]. As rice is a staple food consumed by more than half of the world's population, there is strong interest in boosting the grain Zn concentration for human health. The three approaches used to bio-fortify staple crops are agronomic (e.g., fertilizer application and water management), plant breeding and genetic engineering. Of these, fertilizer application is a practical and effective tool for farmers in all regions as it is rapid and requires fewer resources and experience compared with other strategies [5–7]. To achieve

Zn-enriched grain with Zn fertilizer, two main factors need to be considered, rice variety and agronomy management [8]. In wheat, adding Zn fertilizer to Zn-deficient soils has long been established as an effective way to maximize grain yield and grain Zn concentration [9–11]. However, optimal practices are still being evaluated in rice to achieve desirable outcomes for productivity and premium grain quality [12–14]. Grain Zn concentration in rice is influenced by genetic and environmental factors [15]. Factors that reduce the availability of Zn in soil generally decrease grain Zn concentrations [1,7,16]. Thus, Zn fertilization and water management can be effective tools to boost grain Zn in many situations [8,17]. In rice, the response to Zn fertilization methods and growing conditions can differ among varieties [8,18,19]. Farooq et al. [20] compared four Zn application methods in Super Basmati rice grown under flooded and non-flooded fields at two locations in Pakistan. They found that the highest grain Zn concentrations were achieved with foliar Zn application (0.5% Zn) at one site, and with soil Zn application (10 kg Zn ha^{-1}) at the second site in both production systems. More generally, foliar Zn application increased grain Zn concentration in brown rice by an average of 25% [19,21]. In addition to the impact of fertilizer Zn and the rice variety, the soil water status also can influence the grain Zn concentration. For example, alternate wetting and drying cycles increased Zn concentration in rice grain compared to continuous flooding [22]. Similarly, Beebout et al. [19] showed that growing rice in well-drained conditions increased Zn concentration in both brown rice (unpolished) and white rice (polished) and attributed this to the higher extractable soil Zn under well-drained conditions than in continuous flooding conditions. In Thailand, Yamuangmorn et al. [23] grew upland and wetland rice varieties in well-drained and waterlogged conditions and found that the addition of the equivalent of 50 kg Zn ha^{-1} to the soil increased grain Zn by 45% in upland rice in well-drained soil but there was no increase in the waterlogged treatment. In contrast, grain Zn concentrations were enhanced in the wetland variety in both soil water conditions. However, it is unknown whether foliar application of Zn can improve grain Zn concentrations of upland rice in waterlogged soil. Therefore, this paper compares the effectiveness of soil and foliar Zn applications on productivity and grain Zn accumulation in the same wetland and upland rice varieties under well-drained and waterlogged conditions. The hypothesis was that the application of soil or foliar Zn would enhance grain Zn under both waterlogged and well-drained conditions. The information gained from this study will be useful for improving productivity and grain Zn concentration in rice.

2. Materials and Methods

2.1. Rice Variety and Culture

The large pot experiment was arranged in two (variety) × two (water regime) × three (Zn treatment) factorials in a completely randomized design (CRD) with three independent replications in a total of 36 large pots. The two rice varieties were Chai Nat 1 (CNT1) and Kam Hom Morchor (KH CMU). These varieties were shown in previous studies to belong to different groups with respect to their yield potential, ecotype and grain Zn concentration [2,24]: namely (1) high yielding wetland varieties with low grain Zn (CNT1); and (2) low-yielding upland varieties with high grain Zn (KH CMU). The plants were grown in a glasshouse with two water regimes (waterlogged, well-drained) with three Zn fertilizer treatments (no Zn, soil Zn, foliar Zn) during the wet season (June to September 2018) at Chiang Mai University, Thailand (18°47′ N, 98°57′ E). The soil characteristics were sandy loam soil texture (sedimentation method) [25], pH 5.82 (measured in 1:1; soil–water) [26], 1.38% organic matter [27], available phosphorus 35.06 mg kg^{-1} (Bray II) and 39.28 mg kg^{-1} exchangeable potassium (NH_4OAc, pH 7) [28]. The diethylenetriamine pentaacetic acid (DTPA)-extractable Zn concentration was 0.73 mg kg^{-1} [29]. The average temperature in the open during the cropping season was 27.5 °C with 77.0% relative humidity [30]. Ten-day-old seedlings were transplanted into cement pots of 80 cm in diameter and 50 cm in height. There were twelve plants per pot with a spacing of approximately 20 × 20 cm between plants. For the foliar treatment, 0.5% aqueous $ZnSO_4 \cdot 7H_2O$ was applied at booting, flowering and milky grain stages using a hand-sprayer, equivalent on a surface area basis to 900 L ha^{-1} or 13.5 kg ha^{-1} in total [31,32]. To prevent contamination, the pots were vertically protected with a plastic sheet during spraying. For the soil Zn treatment, 50 kg $ZnSO_4 \cdot 7H_2O$ ha^{-1} (11.9 kg Zn ha^{-1}), suggested by a previous study [23,33], was incorporated into the soil before transplanting the rice seedlings. For waterlogged soil, the plants were kept in submerged condition with 10 cm of water above the soil surface for the entire period until maturity. For well-drained soil, the pot had a hole for drainage of gravitational water and soil moisture content was maintained at field capacity by supplying water twice daily. All treatments received the equivalent of 125 kg ha^{-1} of basal 15N:15P:15K fertilizer as four equal split applications at seven days after planting, tillering, booting and flowering stages [34].

2.2. Sample Collection and Chemical Analysis

At grain maturity, the plants were harvested and evaluated for yield and yield components (number of tillers $plant^{-1}$, number of panicles $plant^{-1}$, number of spikelets $panicle^{-1}$ and percentage of filled grain). Grain yield was measured at 14% moisture content. The unpolished rice grains were oven-dried at 70 °C for 72 h before Zn analysis. Subsamples (20 g) of the unpolished rice were ground for 45 s in a hammer mill (Scientific Technical Supplies D-6072, Dreieich, Germany), dry-ashed at 550 °C for 8 h, the ash was dissolved in HCl (1:1; HCl to deionized water) heated to 120 °C for 20 min and then the final solution was analyzed in an atomic absorption spectrophotometer (Z-8230 Polarized Zeeman, Hitachi, Japan) [35]. Peach (SRM 1547) and soybean leaves were included in each batch as reference materials to validate the method.

2.3. Statistical Analysis

Analysis of variance (ANOVA) was conducted to detect any differences in grain yield, yield components and grain Zn concentration between treatments using Statistic 9 (analytical software SX). Data were tested for normality by the normal probability plot method before being analyzed in factorials in CRD. The least significant difference (LSD) test at $p < 0.05$ was applied to compare the means for significant differences between water regimes and Zn fertilizer treatments.

3. Results

3.1. Grain Yield and Yield Components

The response of grain yield and yield components to rice variety, Zn treatment and water regime is shown in Table 1. Grain yield and yield components differed between the two varieties ($p < 0.01$). Grain yield of the wetland variety CNT1 was 17% higher than the upland variety KH CMU. The number of tillers and panicles per plant was higher in CNT1, while the percentage of filled grain and the number of spikelets per plant were higher in KH CMU. Grain yield was affected by the water regime, but this depended on the rice variety ($p < 0.05$), while Zn fertilizer treatment had no effect on the grain yield. In wetland rice CNT1, the grain yield of plants grown in the waterlogged soil was 310.1 g pot^{-1}, which was 18.9% higher than that of the plants grown in the well-drained soil. By contrast, the grain yield of upland rice KH CMU was not affected by the water regime. The number of panicles per plant and the percentage of filled grain was significantly affected by the Zn treatments. Applying soil and foliar Zn increased the number of panicles per plant by 13.9% and 10.7%, and the percentage of filled grain by 6% and 3%, respectively, in both varieties compared with the no Zn plants. The CNT1 had 89.2% and 79.8% more tillers and panicles per plant, respectively, than KH CMU, regardless of Zn treatments. In addition, there was an interaction effect between rice variety and water regime on the percentage of filled grain ($p < 0.01$). In CNT1, the percentage of filled grain was 76.5% in the waterlogged soil and this was almost 6.0% higher than in well-drained soil. In comparison, the percentage of filled grain in KH CMU was 82.9% in the well-drained soil which was 5.7% higher than in the waterlogged soil.

Table 1. Yield and yield components of two rice varieties (CNT1, KH CMU) grown under two water regimes (well-drained, waterlogged) and three Zn fertilizer treatments (No Zn, Soil Zn, foliar Zn).

Variety	Water Regime	Zn Treatment	No. of Tillers Plant^{-1}	No. of Panicles Plant^{-1}	No. of Spikelets Plant^{-1}	Filled Grain (%)	Grain Yield (g pot^{-1})
CNT1	Well-drained	No Zn	8.4	8	133.8	69.4	247.2
		Soil Zn	10.4	9.8	130.4	78.6	260.3
		Foliar Zn	9.8	9.2	132.3	68.8	274.7
	Mean of well-drained		9.5	9.0	132.2	72.3	260.7
	Waterlogged	No Zn	9.7	8.8	129.5	76.4	297.9
		Soil Zn	10.3	9.3	128.7	74.3	333.8
		Foliar Zn	10.8	9.6	132.9	78.9	298.7
	Mean of waterlogged		10.3	9.2	130.4	76.5	310.1
Mean of variety			9.9	9.1	131.3	74.4	285.4
KHCMU	Well-drained	No Zn	4.5	4.1	167.2	81.3	225.6
		Soil Zn	5.6	5.3	146.7	85.1	245.3
		Foliar Zn	5.5	5.4	156.3	82.4	258.1
	Mean of well-drained		5.2	4.9	156.7	82.9	243.0
	Waterlogged	No Zn	5.5	5.4	163	74.2	247.7
		Soil Zn	5.5	5.4	150.9	81.1	212.6
		Foliar Zn	4.8	4.8	164.1	80.1	231.2
	Mean of waterlogged		5.3	5.2	159.3	78.5	230.5
Mean of variety			5.2	5.1	158.0	80.7	236.8
Analysis of variance							
Variety (V)			***	***	***	***	***
Water regime (C)			ns	ns	ns	ns	ns
Zn treatment (Zn)			ns	*	ns	*	ns
(V × C)			ns	ns	ns	**	**
(V × Zn)			ns	ns	ns	ns	ns
(C × Zn)			ns	ns	ns	ns	ns
(V × C × Zn)			ns	ns	ns	ns	ns
$LSD_{0.05}$ (V × C)			-	-	-	3.7	32.2

ns indicates no significant difference, * indicates significant difference at $p < 0.05$. ** indicates significant difference at $p < 0.01$, *** indicates significant difference at $p < 0.001$.

3.2. Zn Concentration and Content in Brown Rice

Zinc treatment significantly affected grain Zn concentration in CNT1 ($p < 0.05$) (Figure 1A). Soil and foliar Zn treatments increased grain Zn concentration in CNT1 by 19.8% and 32.9%, respectively, compared to the no Zn treatment. Unlike for CNT1, there was a water × Zn interaction in KH CMU on grain Zn concentration ($p < 0.05$) (Figure 1B). In well-drained soil, the soil and foliar Zn treatments increased grain Zn concentration in KH CMU by 44.6% and 35.2%, respectively, compared with no Zn plants. Furthermore, in this variety, the application of foliar Zn increased grain Zn concentration to 41.1 mg kg^{-1} in the waterlogged soil, an increase of 14.7% over the no Zn plants, but the soil Zn treatment had no effect.

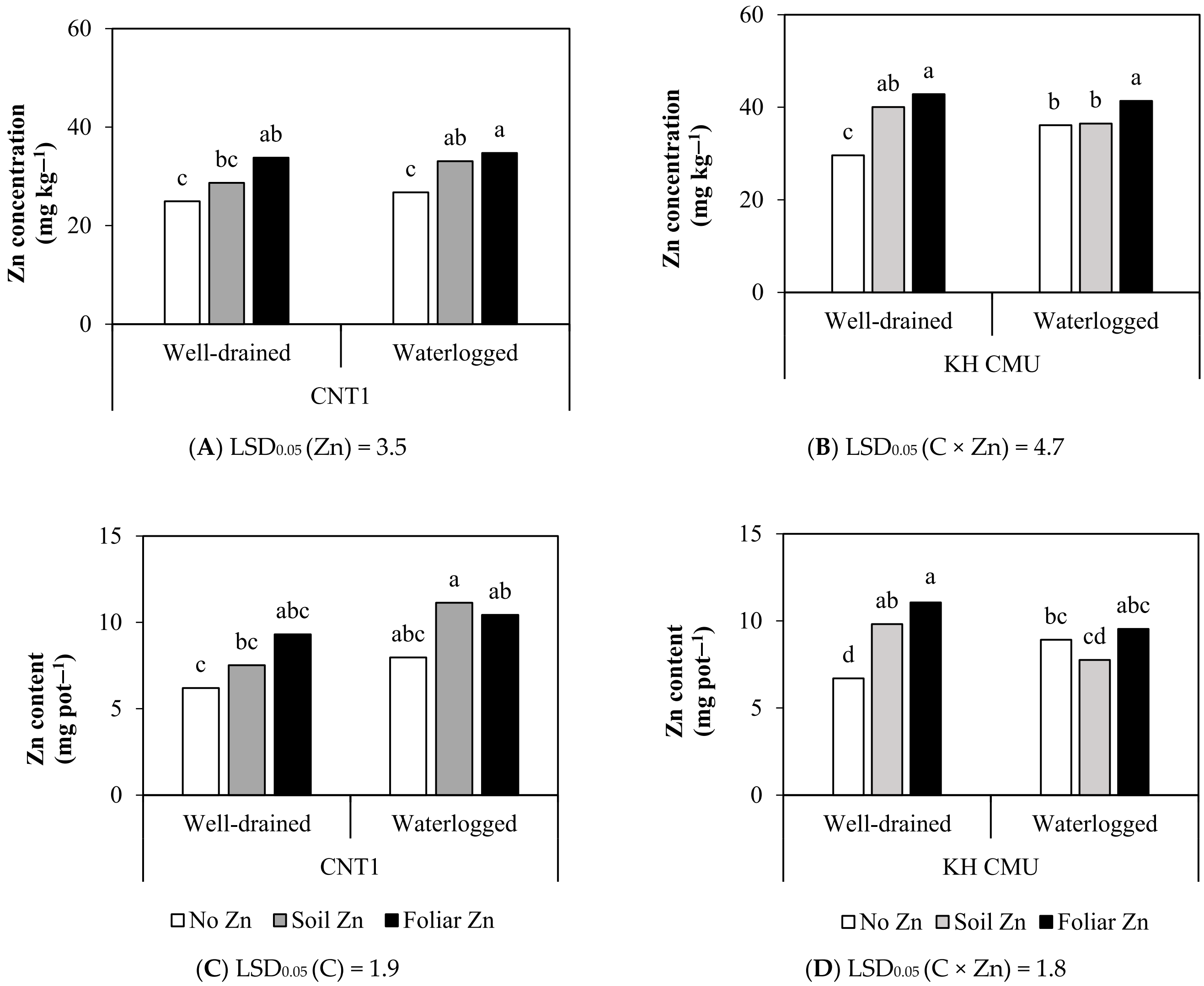

Figure 1. Grain Zn concentration (**A**,**B**) and grain Zn content (**C**,**D**) of two rice varieties (CNT1, KH CMU) grown under two water regimes (well-drained, waterlogged) and three Zn fertilizer treatments (no Zn, soil Zn, foliar Zn). The different letters indicate significant differences at $p < 0.05$.

The water regime affected the grain Zn content in CNT1 ($p < 0.05$) (Figure 1C) but Zn treatment had no effect on this property. The grain from plants grown in well-drained soil had 21.4% less Zn in comparison with the grain produced in the waterlogged plants. By contrast, there was a water × Zn interaction in KH CMU on grain Zn content ($p < 0.05$) (Figure 1D). Soil and foliar Zn treatments increased grain Zn content by 46.4% and 65.0%, respectively, in plants grown in well-drained soil compared with no Zn plants. However, grain Zn content was not increased in KH CMU in waterlogged soil.

3.3. Relationship between Grain Yield and Zn Concentration

There was a weak positive correlation between grain yield and grain Zn concentration in CNT1 ($r^2 = 0.55$ *), but no such relationship was found in KH CMU ($r^2 = 0.07$ ^{ns}) (Figure 2).

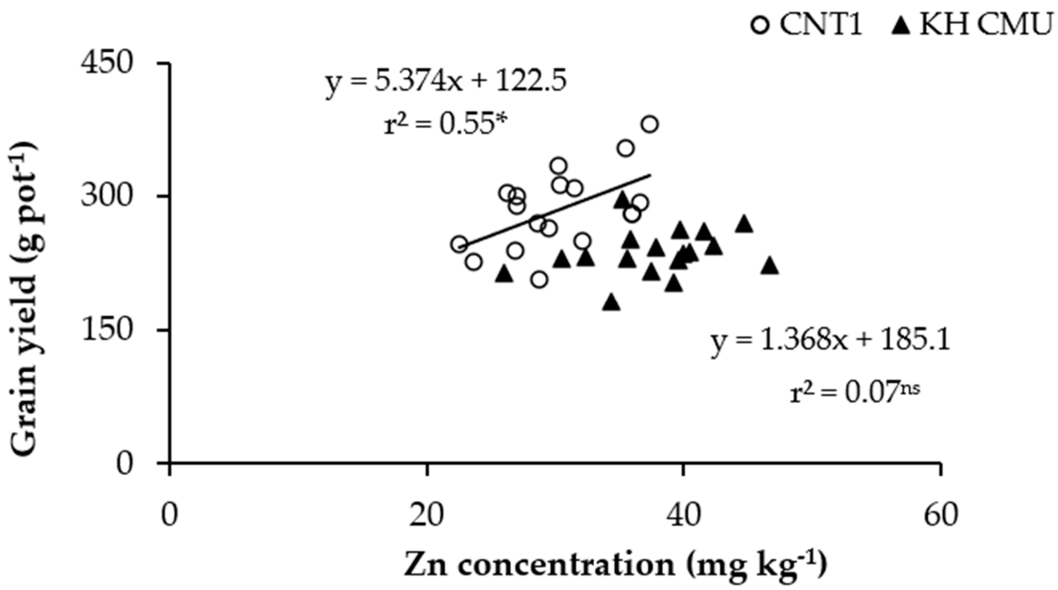

Figure 2. Relationship between grain yield and Zn concentration in brown rice of two rice varieties (CNT1, KH CMU) grown under two water regimes (well-drained, waterlogged) and three Zn fertilizer treatments (no Zn, soil Zn, foliar Zn) (n = 18); ^{ns} and * indicate no significant difference and significant difference at $p < 0.05$, respectively.

4. Discussion

The hypothesis that application of foliar Zn can enhance grain Zn in upland and lowland rice grown under waterlogged and well-drained conditions is only partly supported in this study. In KH CMU, the upland rice genotype, foliar Zn application increased grain Zn by 44.6% in well-drained soil and 14.7% in waterlogged soil. In CNT1, the lowland rice genotype, foliar Zn increased grain Zn concentration by an average of 32.8% in both well-drained and waterlogged soil. Additionally, grain yield of CNT1 was affected by water management, whereas there was no effect in KH CMU. The waterlogged CNT1 plants produced 15.9% higher grain yield than the well-drained plants. Even though the application of Zn to CNT1 had no effect on grain yield, it improved grain Zn concentration. However, the magnitude of the response differed with rice variety, especially where foliar Zn was applied. The reduction in grain yield of CNT1 in the well-drained soil was mainly due to the lower percentage of filled grain (Table 1). In contrast, KH CMU was well adapted to both growing conditions as there was no difference in grain yield between treatments. This confirms the earlier responses obtained by Yamuangmorn et al. [23]. Results of the two studies suggest that the two cultivars may differ in their response to the extraction of essential nutrients from well-drained soil. Whilst the mechanisms for nutrient acquisition have so far not been explored in these ecotypes, there are a number of factors that can be considered. These include external changes in the organic matter content and the availability of nitrogen [36], changes in soil pH [18], as well as genetic traits. Experiments have revealed the extent of soil water conditions on changes in soil chemistry [36,37] and total nutrient uptake [38]. It would be interesting to investigate root morphology, nutrient uptake, water and nutrient use efficiencies, and genetic control of grain filling capacity for the two ecotypes to assist in improving rice varieties with high productivity under different water regimes.

Advice on grain Zn biofortification is increasingly being sought by Thai producers and our research shows the importance of addressing variety as well as agronomic practice. The role of genotype, soil Zn availability and Zn fertilization were discussed by [39]. Foliar Zn application has been suggested by many researchers as a promising and effective tool to increase grain Zn concentration as foliar uptake and translocation of Zn occurs without soil chemical constraints [19,40,41]. However, our study highlights the importance of

G × E on the effectiveness of biofortification of rice using foliar Zn. The extent of grain Zn loading depends on rice variety and water conditions [32,42]. In a previous report, applying Zn fertilizer to the soil could increase grain Zn concentration in the wetland and upland varieties when grown in both well-drained and waterlogged conditions [17]. Chatzistathis [43] also reported that applying Zn fertilizer when plants were grown in well-drained soil resulted in a higher grain Zn concentration in the unpolished (brown rice) and polished rice than when plants were grown in waterlogged soil. This may be due to the oxygen supply enhancing Zn^{2+} uptake in the well-drained soil. In addition, the availability of Zn in well-drained soil can increase with a decrease in soil pH or an increase in redox potential [44]. In waterlogged soil, low redox potential and oxygen (O_2) stress may limit soil Zn availability for plants [45]. Furthermore, under continuous flooding, the formation of insoluble forms of Zn such as ZnS and Zn franklinite ($ZnFe_2O_4$) [37,46], $ZnCO_3$ [47] and $Zn(OH)_2$ [48], markedly reduces the effectiveness of Zn fertilizers [49].

The relationship between grain yield and Zn concentration suggests that increasing the grain Zn concentration could enhance the productivity of CNT1, but not KH CMU. Previous reports [32,50,51] indicate the importance of Zn for improved pollen viability and grain fill capacity in some rice varieties. This needs to be further considered when manipulating rice cultivation for maximum yield and grain Zn concentration. The information on Zn biofortification of the two rice ecotypes should be useful for extension workers to better manage rice cultivation for improving grain Zn concentration and stabilizing yield. We now intend to confirm the findings in the field with a greater number of rice varieties for each ecotype.

5. Conclusions

Improvement of grain productivity and Zn concentration in rice could benefit both rice growers and consumers. The response of rice varieties from different original ecotypes is the key factor underpinning appropriate management of the growing condition and Zn fertilizer application. In this study, the success of biofortification of grain Zn with Zn fertilizer depended on the interaction effects between the rice ecotype, soil water and the method of Zn fertilizer application. Overall, grain yield and Zn concentration can be maximized by growing rice genotypes in either well-drained or waterlogged soil and applying foliar applications of Zn at the appropriate method. Rice producers would benefit from field studies that provide recommendations on the best Zn fertilizer practices for grain Zn concentration in upland and lowland fields.

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