

Article

Improving Grain Zinc Concentration in Wetland and Upland Rice Varieties Grown under Waterlogged and Well-Drained Soils by Applying Zinc Fertilizer

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Abstract: The objective of this study was to evaluate the responses in grain yield and zinc concentration of wetland and upland rice varieties to Zn fertilizer application and different growing conditions. The wetland (Chainat 1; CNT1) and upland (Kum Hom CMU; KH CMU) rice varieties were grown under waterlogged and well-drained soil conditions with or without Zn fertilizer application. Zinc fertilizer ($ZnSO_4$) was applied at 0 and 60 kg ha⁻¹ in three stages at tillering, booting, and flowering. In the wetland variety, CNT1, grain yield decreased by 18.0% in the well-drained soil compared to the waterlogged conditions, but there was an 8.9% decrease in grain yield in the waterlogged soil compared to the well-drained soil in the upland variety, KH CMU. Applying Zn fertilizer affected yields differently between the varieties, decreasing grain yield by 11.9% in CNT1 while having no effect in KH CMU. For grain Zn concentrations in brown rice, applying Zn fertilizer increased Zn concentration by 16.5–23.1% in CNT1 and KH CMU under both growing conditions. In the well-drained soil, applying Zn fertilizer increased straw Zn concentration by 51.6% in CNT1 and by 43.4% in KH CMU compared with the waterlogged conditions. These results indicated that the wetland and upland rice varieties responded differently to Zn fertilizer application when grown in different conditions. Applying Zn fertilizer in the appropriate rice variety and growing conditions would help farmers to improve both the desirable grain yield and Zn concentration in rice.

Keywords: zinc deficiency; waterlogged; well-drained; zinc concentration; zinc fertilizer



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1. Introduction

Zinc is an essential micronutrient for growth and development, and it is involved in many of the major functions in plants including cell membrane structure, photosynthesis, hormone activity, lipid and nucleic acid metabolism, gene expression and regulation, protein synthesis, and defense against drought and disease [1,2]. In the case of Zn deficiency, plants respond by decreasing membrane integrity, increasing susceptibility to heat stress, and decreasing the synthesis of carbohydrates, cytochrome nucleotide auxin, and chlorophyll [3]. Agronomical management through application of Zn fertilizer to cereal crops has been reported as a potential tool to improve seedling germination [4] and productivity as well as grain Zn concentration [5] that could benefit human health upon consumption [6]. However, the efficiency of Zn fertilizer application has been reported to depend on several factors, including fertilizer source, application time and method, and the soil's chemical properties. The latter factor is influenced by water management [7]. The total Zn uptake into rice plants and grain Zn concentration were reported to be influenced by the ability of soil to supply Zn as well as by rice variety and water management [8]. A previous study found that alternate wetting and drying conditions increased grain yield and Zn

concentration in both brown rice (unpolished) and white rice (polished) depending on the rice variety [9]. The transition from waterlogged to well-drained soil contributed to increased Zn accumulation in the grain without compromising grain yield [10]. In comparison with the alternate wetting and drying conditions, a waterlogged condition reduced the concentration of heavy metals translocated into rice grains by reducing the root-to-shoot translocation and the availability of metals in the rhizosphere [11]. In waterlogged soil, anaerobic respiration enhances a large number of harmful substances, inhibiting the absorption of mineral ions and beneficial trace elements and resulting in disruption of root growth and development, physiological performance, and competitive ability of plants [12,13]. The waterlogged soil conditions could decrease the concentration of watersoluble Zn [14], while well-drained soil provides a favorable environment for the activity of mycorrhizal fungi, and enhanced mycorrhizal inoculation has been shown to increase plant Zn uptake [15].

Wide variation in grain Zn concentration in rice has been reported, ranging from 17–26 mg Zn kg⁻¹ dry matter in upland varieties and 39–59 mg Zn kg⁻¹ dry matter in wetland varieties [16], while in germplasm from four different states in India the concentration ranged from 14.5 to 35.3 mg Zn kg⁻¹ dry matter [17]. Generally, unpolished rice (brown rice) varieties vary widely in their response to Zn fertilizer, both in Zn uptake and accumulation [18–21]. A recent study noted that grain Zn concentration in the upland and wetland rice varieties was enhanced by Zn fertilizer application, while grain yield depended on the soil and water management [22]. The available information concerning responses of upland rice varieties to Zn fertilizer application grown under varied soil conditions is limited compared with the wetland rice varieties. Upland rice varieties are generally grown on level or sloping well-drained soils, while wetland rice varieties are grown in waterlogged soils for the entire growing season [23]. We hypothesized that the original ecotypes of wetland and upland rice varieties would respond differently to Zn fertilizer application when cultivated under waterlogged and well-drained soil conditions. Therefore, the objectives of this study were to evaluate the effects of Zn fertilizer application under different soil conditions between waterlogged and well-drained soils on yield, Zn uptake, and accumulation in rice grains among wetland and upland rice varieties. The knowledge gained from this study will support proper management of Zn fertilizer and soil conditions for specific rice varieties for improving productivity and Zn accumulation in rice grains.

2. Materials and Methods

2.1. Experimental Design and Plant Culture

This study was conducted in green house conditions during wet season (June–November) in 2018 in a demonstration field of Chiang Mai University, Thailand. The experiment was arranged in a factorial completely randomized design with four independent replications. The two rice varieties from different original ecotypes between wetland (Chainat 1; CNT1) and upland (Kum Hom CMU; KH CMU) were grown under varying two water conditions (i.e., waterlogged and well-drained) and two Zn fertilizer treatments (i.e., no Zn applied; Zn0, and applied Zn at 60 kg⁻¹ ha; Zn+) in a total of 32 pots. The plastic pots (38 cm in diameter, 30 cm deep) used in this experiment contained 12 kg sandy loam soil of the Sansai series. The characteristics of this soil were: electrical conductivity 1.5 dS m⁻¹, organic matter 2.37%, pH 6.1, total N 0.11%, available P 22.6 mg kg⁻¹, exchangeable K 113.4 mg kg⁻¹, and available Zn 0.7 mg kg⁻¹. The twenty-day-old seedlings of wetland and upland rice varieties were transplanted into the prepared pots with a single seedling per hill and three seedlings per pot. The rice varieties were grown under waterlogged and well-drained soils with or without Zn fertilizer application. Zinc sulfate at the rates of 0 (Zn0) and 60 (Zn+) kg ha⁻¹ was applied in three parts at the tillering, booting, and flowering stages. The application rate of Zn fertilizer was suggested by a previous study [5]. For waterlogged soil, the pots were kept flooded at five cm above the soil surface throughout the experiment, while in well-drained soil the pot had a hole for drainage of the excess water. Water was provided at a rate of two liters per day at two times in the morning

and evening to keep the water level at field capacity. The water was double filtered before being provided for plants in both growing conditions to avoid contamination. The fertilizer, nitrogen, phosphorus, and potassium were applied in the forms of urea, sodium dihydrogen orthophosphate, and potassium chloride at the rate of 104 N-45 P-30 K kg ha⁻¹ in total, respectively, by splitting the application amount into three portions applied equally at the tillering, booting and flowering stages.

2.2. Sample Collection and Preparation

At maturity, plants were harvested and evaluated for grain yield (14% moisture content), straw dry weight, and yield components (plant height, number of tillers and panicles per plant, panicle length, percent filled grain, and 1000 grain weight). The plant samples were washed with distilled water to avoid contamination and separated into shoot, flag leaf, and grain. The grain was subjected to removal of the husk to produce brown rice grains. The samples of shoot, flag leaf, and brown rice were oven dried at 75 °C for 72 h. The dried samples were mechanically ground in a hammer mill for Zn concentration analysis.

2.3. Chemical Analysis

The concentration of Zn in plant tissues was expressed as mg per kg dry matter and was analyzed by atomic absorption spectrophotometry (AA) (Hitachi, Z-8230 model, Japan). The samples comprised approximately 0.5 g of ground rice and were first subjected to dry ashing in a muffle furnace at 535 °C for 8 h, and the ash samples were then acid extracted by dissolving in HCl (1:1; HCl to deionized water) for 20 min [24]. The certified reference materials of ground peach leaves (SRM 1547) were obtained from the National Institute of Standard and Technology, and soybean leaves from the local lab standard were used as the standard checks for the accuracy of Zn concentration in the samples in all analyses. The content of Zn in plant parts was calculated by multiplying the concentration by dry matter and expressed in mg per plant dry weight. The shoot Zn content was the sum of the contents in all plant parts.

2.4. Statistical Analysis

Analysis of variance (ANOVA) was used to detect the differences among rice varieties and treatments of Zn fertilizer application and soil condition, and least significant difference (LSD) tests at $p < 0.05$ were used to compare the differences between means. The correlation coefficient analysis was used to detect the significant relationship between the two parameters at $p < 0.05$.

3. Results

Zinc fertilizer application and growing conditions affected grain yield differently between the wetland and upland rice varieties ($p < 0.05$) (Figure 1A,B). In the wetland rice variety, CNT1, grain yield was 205.4 g m⁻², which was a decrease of 18.0% in plants grown under the well-drained conditions compared to those grown in the waterlogged soil, but the grain yield of plants grown in the waterlogged soil only slightly decreased to 145.1 g m⁻² (8.9%) from the well-drained conditions in the upland rice variety, KH CMU (Figure 1A). In contrast, applying Zn fertilizer decreased grain yield to 213.6 g m⁻² (11.9%) in CNT1, while no effect was found in KH CMU (Figure 1B).

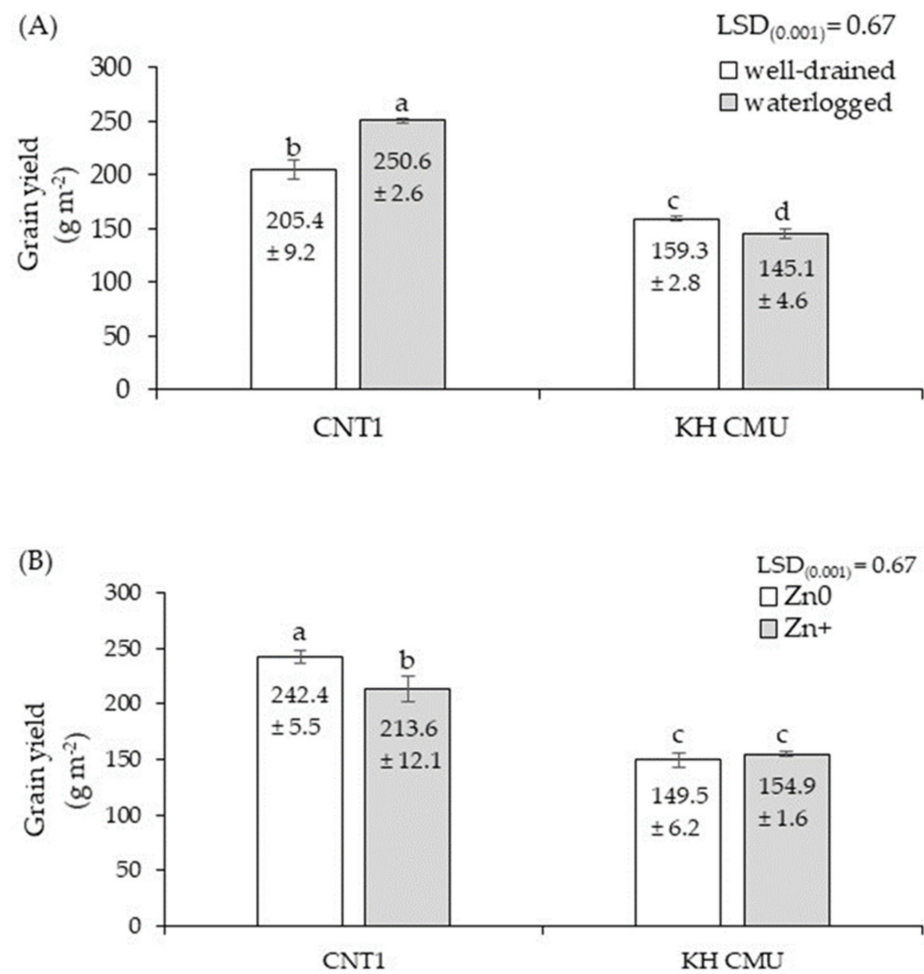


Figure 1. Grain yield of two rice varieties (Chainat 1; CNT1) and (Kum Hom; KH CMU) affected by growing conditions (A) and Zn fertilizer application (B). The data are the means of four independent replications. The lowercase letters above the bars indicate significant differences between the treatments by LSD_{0.05}, least significant difference at $p < 0.05$. The error bars are the standard error of each treatment mean from four replications.

Yield components were significantly affected by growing conditions and Zn fertilizer application, and the effects differed between the varieties in all characteristics except for plant height and 1000 grain weight ($p < 0.05$) (Table 1). In CNT1, applying Zn fertilizer to plants resulted in a higher tiller number per plant than in plants without Zn fertilizer application when grown under well-drained soil, but the opposite result was found in the waterlogged conditions. The opposite response to the growing conditions and Zn fertilizer application was found in KH CMU. Applying Zn fertilizer resulted in slightly lower panicle numbers per plant in CNT1 grown under well-drained soil, but such an effect was not found in the waterlogged conditions. In KH CMU, plants grown under well-drained soil had one more panicle per plant than when grown in the waterlogged conditions, but Zn fertilizer had no effect on panicle number. Growing plants in the waterlogged soil produced longer panicles in CNT1 compared with plants grown in the well-drained conditions, but the opposite result was found in KH CMU. In both varieties, plants with Zn fertilizer applied had lower percentages of filled grain than plants without Zn application in both growing conditions.

Table 1. Yield component of two rice varieties (i.e., CNT1 and KH CMU) grown under well-drained and waterlogged conditions with and without Zn fertilizer application. The samples were evaluated at maturity. The data are the means \pm SE of four independent replications.

Variety (V)	Growing Condition (G)	Zn Fertilizer (Zn)	Plant Height (cm)	Tillers Number Plant ⁻¹	Panicles Number Plant ⁻¹	Panicle Length (cm)	Filled Grains (%)	1000-Grains Weight (g)
CNT1	well-drained	Zn0	104.0 \pm 0.6	8.3 \pm 0.2 ^a	7.3 \pm 0.2 ^a	24.3 \pm 0.2 ^d	93.3 \pm 0.8 ^b	32.0 \pm 0.7
		Zn+	100.3 \pm 0.7	7.5 \pm 0.2 ^b	6.0 \pm 0.1 ^b	24.5 \pm 0.2 ^d	80.0 \pm 0.7 ^e	32.0 \pm 0.4
	waterlogged	Zn0	105.8 \pm 0.9	6.3 \pm 0.2 ^c	7.0 \pm 0.0 ^a	27.3 \pm 0.2 ^{bc}	95.3 \pm 0.3 ^a	31.5 \pm 0.5
		Zn+	103.5 \pm 0.7	7.0 \pm 0.1 ^b	7.0 \pm 0.1 ^a	27.3 \pm 0.3 ^{bc}	93.0 \pm 0.4 ^b	29.0 \pm 0.5
KH CMU	well-drained	Zn0	127.5 \pm 0.9	2.3 \pm 0.1 ^e	3.0 \pm 0.1 ^c	27.8 \pm 0.5 ^{ab}	89.3 \pm 0.2 ^c	35.0 \pm 1.0
		Zn+	124.3 \pm 0.2	3.0 \pm 0.1 ^d	3.0 \pm 0.1 ^c	27.5 \pm 0.5 ^b	87.3 \pm 0.4 ^d	35.3 \pm 0.4
	waterlogged	Zn0	122.0 \pm 1.0	3.0 \pm 0.1 ^d	2.0 \pm 0.1 ^d	26.3 \pm 0.5 ^c	90.0 \pm 0.4 ^c	32.5 \pm 0.3
		Zn+	119.8 \pm 0.8	2.0 \pm 0.1 ^e	2.0 \pm 0.1 ^d	28.8 \pm 0.6 ^a	74.5 \pm 0.6 ^f	31.8 \pm 0.7
F-test			ns	***	***	*	***	ns
V \times G \times Zn								
LSD _{0.05}			2.2	0.5	0.3	1.1	1.6	1.7

The lowercase letters indicate a significant difference between the treatments by LSD_{0.05}, least significant difference at $p < 0.05$. Data were statistically analyzed by F-test (ns: non-significant, * $p < 0.05$, *** $p < 0.001$).

The application of Zn fertilizer affected grain Zn concentration in brown rice, straw, and flag leaf differently between the wetland and upland rice varieties grown under waterlogged and well-drained conditions ($p < 0.05$) (Figure 2A–C). Applying Zn fertilizer increased grain Zn concentration in brown rice of CNT1 and KH CMU in both conditions (Figure 2A). In CNT1, there was 40.1 mg kg⁻¹ dry matter and 37.3 mg kg⁻¹ dry matter grain Zn concentration in brown rice; these were 23.1% and 17.4% increases compared to application of Zn fertilizer in the well-drained and waterlogged conditions, respectively, while the respective increases were 40.0 mg kg⁻¹ dry matter and 49.9 mg kg⁻¹ dry matter (16.5% and 22.7% increases) in KH CMU. With the well-drained soil, applying Zn fertilizer increased straw Zn concentration to 85.2 mg kg⁻¹ dry matter (51.6%) in CNT1 and 97.1 mg kg⁻¹ dry matter (43.4%) in KH CMU compared with the waterlogged conditions (Figure 2B). For flag leaf Zn concentration, applying Zn fertilizer in plants grown under waterlogged conditions increased the concentrations to 29.3 mg kg⁻¹ dry matter and 51.5 mg kg⁻¹ dry matter compared to the well-drained conditions in both CNT1 and KH CMU (Figure 2C).

Grain Zn content was affected by Zn fertilizer and growing conditions differently between rice varieties ($p < 0.05$) (Figure 3A). The highest Zn content in brown rice grains was 0.53 mg plant⁻¹ in CNT1 grown in waterlogged soil, while the level was slightly decreased when plants were grown under the well-drained conditions. Only a slight effect of soil conditions was found in KH CMU, where the higher content was found in plants grown in the well-drained soil compared with the waterlogged conditions (Figure 3A). In contrast, applying Zn fertilizer increased grain Zn content in both varieties, especially in KH CMU where grain Zn content was increased to 0.5 mg plant⁻¹ (29.1%) when Zn fertilizer was applied, while the corresponding increase was 0.5 mg plant⁻¹ (10.1%) in CNT1 (Figure 3B). Applying Zn fertilizer affected straw Zn content differently among soil conditions and rice varieties (Figure 4). Applying Zn fertilizer in the well-drained soil resulted in the highest straw Zn contents of 1.1 and 1.2 mg plant⁻¹ in CNT1 and KH CMU, respectively, while Zn content in straw was 0.6 mg plant⁻¹, a decrease of 18.0% compared to when plants were grown under the waterlogged conditions (Figure 4).

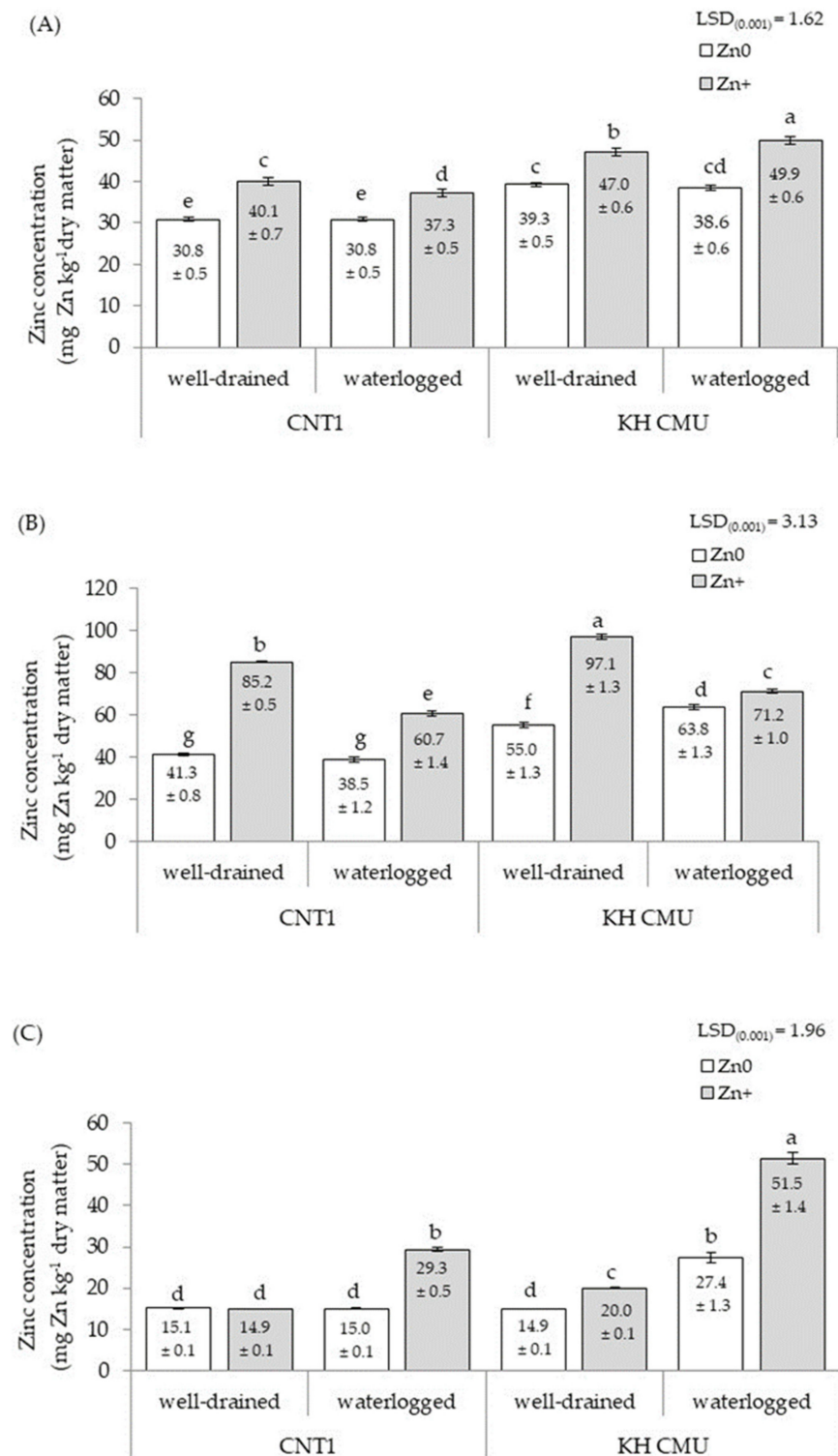


Figure 2. The concentrations of Zn in brown rice grains (A), straw (B), and flag leaf (C) of two varieties (CNT1 and KH CMU) grown under well-drained and waterlogged conditions with and without Zn fertilizer application. The samples were harvested at maturity. The data are means of four independent replications. The lowercase letters above the bars indicate significant differences between the treatments by LSD_{0.05}, least significant difference at $p < 0.05$. The error bars are the standard error of each treatment mean from four replications.

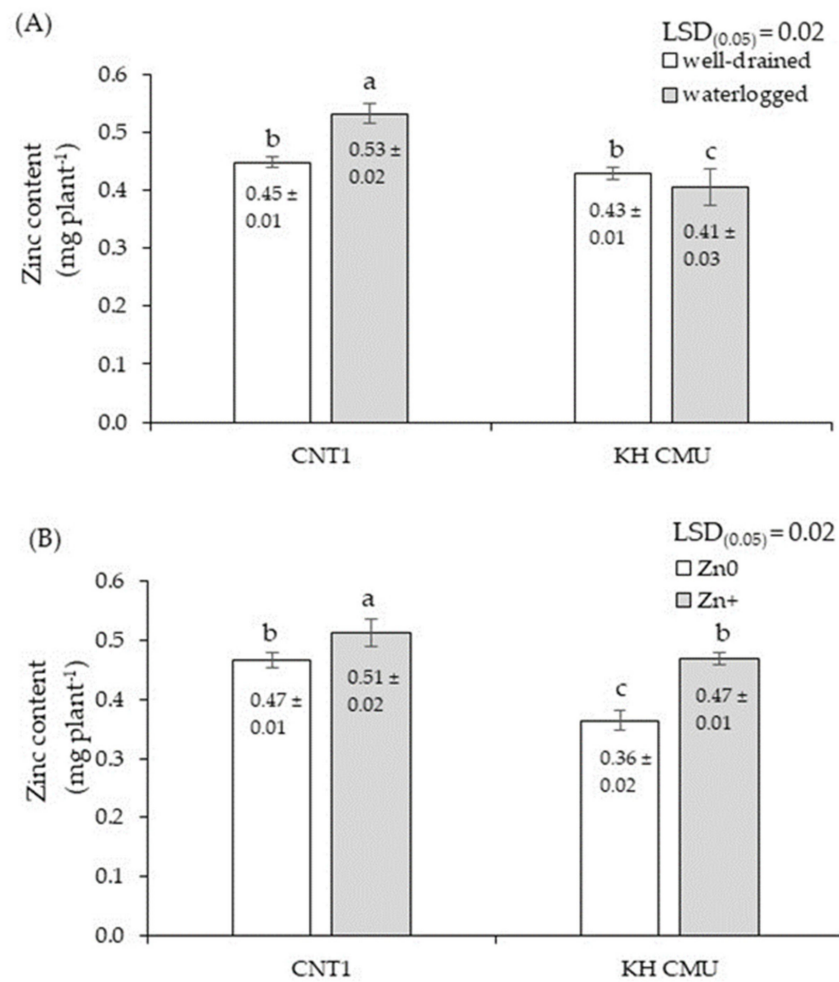


Figure 3. The content of Zn in brown rice as affected by growing condition and rice variety (A), and growing condition and fertilizer management (B). The samples were harvested at maturity. The data are means of four independent replications. The lowercase letters above the bars indicate significant differences between the treatments by LSD_{0.05}, least significant difference at $p < 0.05$. The error bars are the standard error of each treatment mean from four replications.

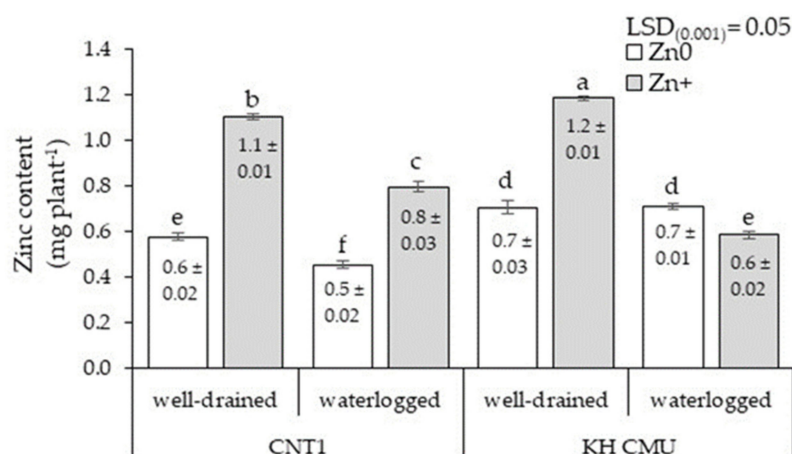


Figure 4. The content of Zn in straw of two varieties (CNT1 and KH CMU) grown under well-drained and waterlogged conditions with and without Zn fertilizer application. The samples were harvested at maturity. The data are means of four independent replications. The lowercase letters above the bars indicate significant differences between the treatments by $LSD_{0.05}$, least significant difference at $p < 0.05$. The error bars are the standard error of each treatment mean from four replications.

There was a negative correlation between grain Zn concentration and yield in the CNT1 variety ($r = -0.91$, $p < 0.01$), but there was a positive correlation for the KH CMU variety ($r = 0.92$, $p < 0.01$) (Figure 5A). The straw Zn concentration was significantly and positively correlated with grain yield in the CNT1 variety ($r = 0.95$, $p < 0.01$), but the concentration was not correlated with yield in the KH CMU variety (Figure 5B). The Zn concentration in flag leaf was not correlated with grain yield in either of the two rice varieties (Figure 5C). The Zn concentration in the grain was correlated with the Zn concentration in straw and flag leaf (Table 2). There were strongly positive correlations between grain and straw Zn concentration in CNT1 ($r = 0.94$, $p < 0.01$) and KH CMU ($r = 0.62$, $p < 0.01$). In contrast, there was no correlation between flag leaf and straw Zn concentration in either rice variety. The grain and flag leaf Zn concentrations were positively correlated in KH CMU ($r = 0.64$, $p < 0.01$), but not in the CNT1 variety. Grain Zn content was significantly positively correlated with Zn content of flag leaf in CNT1 ($r = 0.83$, $p < 0.01$) and KH CMU ($r = 0.70$, $p < 0.01$). Furthermore, there were no correlations in Zn content between straw and grain or flag leaf.

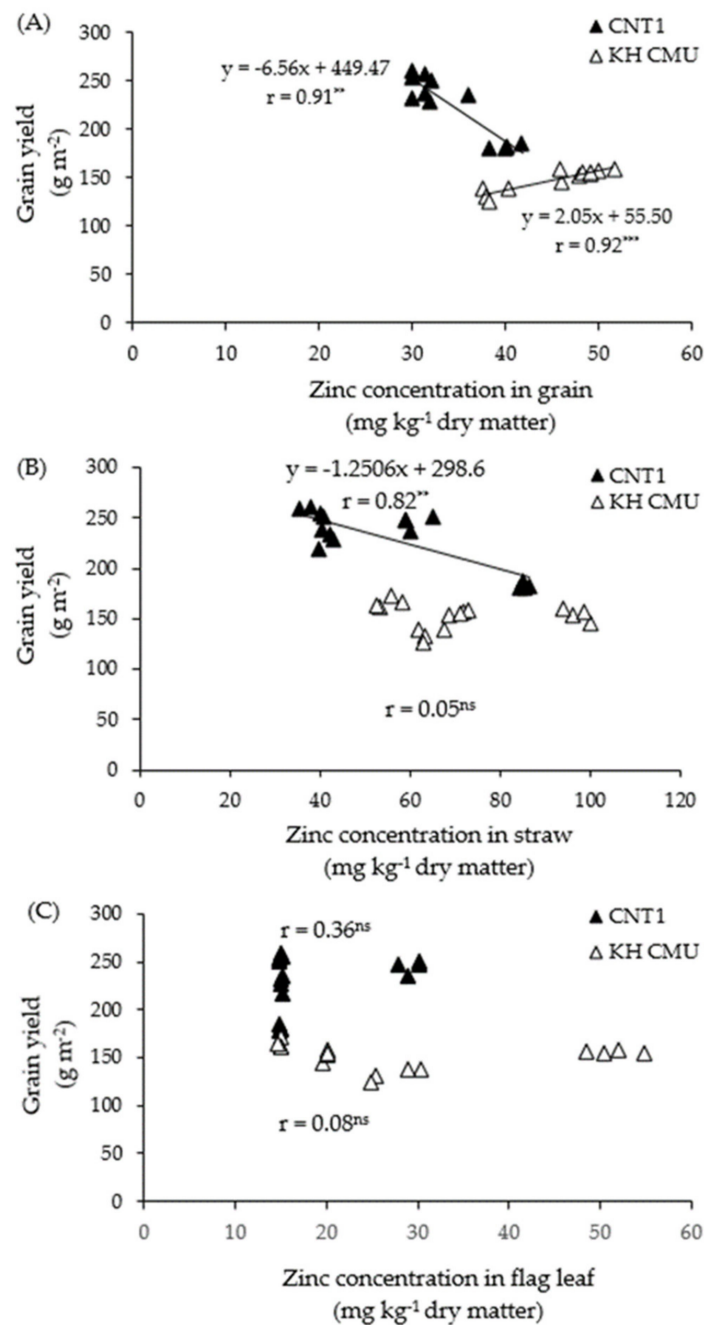


Figure 5. The relationship between grain yield and Zn concentration in grain (A), straw (B), and flag leaf (C) of two varieties (CNT1, KH CMU) grown under well-drained and waterlogged conditions with and without Zn fertilizer application ($n = 16$). The samples were harvested at maturity. Data were statistically analyzed by the F-test (ns: non-significant, ** $p < 0.01$, *** $p < 0.001$).

Table 2. Correlation analysis of Zn concentration and content between different parts of CNT1 and KH CMU rice varieties. The concentration of Zn in different plant parts was analyzed at the maturity stage.

Plant Parts	Variety	Zn Concentration		Zn Content	
		Grain	Flag Leaf	Grain	Flag Leaf
Straw	CNT1	0.94 **	0.13 ^{ns}	0.13 ^{ns}	0.10 ^{ns}
	KH CMU	0.62 **	0.01 ^{ns}	0.15 ^{ns}	0.46 ^{ns}
Flag leaf	CNT1	0.34 ^{ns}		0.83 **	
	KH CMU	0.64 **		0.70 **	

** significant at $p < 0.01$; ns: not significant.

4. Discussion

This study has shown that grain yield and Zn concentration varied depending on rice variety, growing conditions, and Zn application. The grain yield of the wetland rice variety CNT1 was decreased in the well-drained soil compared to its original waterlogged conditions. A similar response was reported by Yamuangmorn et al. [22], where grain yield of the wetland rice variety was drastically reduced in the well-drained conditions due to the reduction in the number of tillers and panicles per plant, as also found in this study. The wetland rice variety is usually very sensitive to changes in water management; the waterlogged condition is normally maintained throughout the cropping period to avoid yield reduction due to decreases in soil moisture content [25]. Thus, water management strongly influences the production of wetland rice crops compared to the upland rice varieties in which only a slight reduction of grain yield was found when changing from well-drained to waterlogged conditions. Our results indicate that changing the water conditions in rice cultivation from that of the plants' original growing ecotype could cause yield reduction depending on the response of each rice variety. Thus, the response varies according to the structure of the root system between the ecotype varieties; the structure of the root in wetland varieties is very poorly adapted to changing water conditions for the interception of nutrients in the soil, resulting in differences in grain yield compared to the upland rice variety. The rice plants growing in the well-drained or aerobic conditions need to have a deeper rooting and a greater root length density than in waterlogged soil conditions due to the limited water availability under well-drained soil compared to flooded soil [15]. A previous study noted that the waterlogged conditions improved the availability of nutrients such as nitrogen, phosphorus, and potassium [26], favoring nutrient uptake in the wetland rice variety, as these are usually major limiting factors in the productivity of wetland rice [27]. In contrast, a study of a Brazilian upland rice variety grown in well-drained conditions produced higher straw and grain yield than in the waterlogged conditions [28]. Thus, the potential of nutrient absorption and transport into rice plants to produce yield is controlled by the mechanisms of each original ecotype, but the nutrient availability in the soil for plant uptake is influenced by soil conditions. Even though the concentrations of N, P, and K in the soil were not found to be limiting factors for plant growth in this study, other nutrients may influence plant performance differently between growing conditions. In general, Zn in the soil is strongly bound by manganese (Mn) oxide and iron (Fe), which can limit Zn uptake by plants and result in failure to maintain the appropriate proportions of Zn and Fe, Cd, Cu, Ca, Mg, and Ni [29]. A study of barley found that the concentrations of Zn were reduced by applying 1.0 mM Cd [30]. However, decreasing Cd uptake by roots in rice under increasing Zn concentration in the external medium has been found [31,32]. This indicates that the relationship between available Zn in the soil and the other nutrients could explain in more detail the factors that impact rice productivity under the different growing conditions. Therefore, similar experiments with a comprehensive soil profile analysis and a greater number of rice varieties from each original ecotype will be required to confirm the present

results. This would help to enhance grain yield by managing proper growing conditions among rice varieties, as Zn fertilizer application was found to have little impact on yield.

In addition to the impact of growing conditions and Zn application on grain yield between the two original ecotype varieties, improving grain Zn accumulation is another focus of rice cultivation. The approach taken to encourage Zn intake among rice consumers in this study was to test whether applying Zn fertilizer could increase grain Zn accumulation in both varieties and under both growing conditions, especially in brown rice grains as the edible part. Based on several reports and survey studies, it has been clearly demonstrated that applying Zn fertilizer increases Zn concentration in the grain and straw of rice [33–35]. This might be due to the increasing level of available Zn in the soil solution when Zn fertilizer is applied, thereby facilitating greater absorption of Zn in rice plants [36]. The concentration of Zn in the straw was well correlated with the concentration in the grain in both rice varieties, indicating that the accumulation of Zn in the shoot tissues could influence the concentration in the grain. The concentration of Zn in the straw can be applied as an indicator of grain Zn accumulation when dealing with a large number of germplasms for the screening procedure in breeding programs for high grain Zn varieties. In general, Zn in soil is transported toward the roots by mass flow, diffusion, and root extension before being distributed and partitioned into the tissues of rice plants [16]. The distribution of Zn among plant tissues is affected by soil chemical properties such as pH, cation exchange capacity, and dissolved organic carbon. Soil organic matter has a large cation exchange capacity or surface negative charge, and Zn is usually more available in the soils with greater organic matter content. The mobility of metals or metalloids in soils is influenced by their sorption onto soil organic matter, clay minerals, and secondary Fe and Mn hydroxides [37]. The soil used in this study had low available Zn despite having quite high levels of organic matter, conflicting with the above general pattern. This may have been due to the high concentration of macronutrients causing the Zn to be unavailable or the texture of the sandy loam soil having low metal adsorption [38]. Moreover, changes in soil water content can alter the chemical and physical properties of soil; for example, the concentration of Zn in the waterlogged soil being decreased less than in well-drained soil [39]. These factors affect the concentration of Zn in plant tissues. In contrast, Zn toxicity symptoms can be observed after excessive application of Zn fertilizer, resulting in toxic Zn accumulation in plant tissues [40]. A previous study reported that the concentrations between 30 and 100 mg Zn kg⁻¹ straw dry matter were enough to support adequate plant growth and productivity, while concentrations above 300 mg Zn kg⁻¹ dry matter were toxic. However, Zn concentration in the straw at 400–600 mg Zn kg⁻¹ dry matter by foliar Zn application in rice has been reported to be without symptoms of toxicity [41,42]. The variation in response could be explained by the efficiency of Zn uptake, transport, and distribution among rice varieties. We found that Zn concentration in the grain and straw in the wetland rice variety varied after shifting from the waterlogged (original ecotype) to well-drained growing under applied Zn fertilizer. There was a negative correlation between grain yield and grain Zn as well as straw Zn concentration in the wetland rice variety CNT1 in the present study. However, a positive correlation between grain yield and Zn concentration was found in the upland rice variety, even though grain yield was not correlated with straw Zn concentration. Possibly, the wetland rice CNT1 is an improved high-yield variety that can absorb a higher input of Zn from soils and accumulate higher concentrations, but an excessive concentration could cause tissue toxicity and result in a negative impact on productivity by reducing number of panicles per plant and percent filled grains. This was observed in the present study, even though the concentration of Zn in the straw was only at 38.5–97.1 mg Zn kg⁻¹ dry matter. A previous study documented a negative correlation between the two parameters in China spring and winter wheat varieties [43]. However, it should be noted that tissue toxicity can occur at the earlier stages, since the tiller and panicle number were affected and these components caused a reduction in yield, but plants recovered at the later growing stages. Therefore, tissue analysis at different stages during plant growth may help us to deeply understand the

distribution as well as the function of Zn in rice plants at different growth periods. This study indicates that rice varieties responded differently to water and fertilizer management in the accumulation of Zn in plant tissues. The proper management of growing conditions in rice varieties from different ecotypes would be the way to increase both grain yield for farmers and grain Zn concentration for consumers.

The total Zn uptake in the whole rice plant and in the grain were expressed as the plant and grain Zn content, respectively, and were calculated by multiplying the tissue Zn concentration by plant and grain dry weight. This helps to understand how Zn is taken up and partitioned into plant parts, and how the concentration of grain Zn is diluted by its yield when grown in different conditions. Applying Zn increased the grain Zn content in both varieties, while the waterlogged conditions increased grain Zn content in the wetland variety but decreased the content in the upland variety. This result indicates that partitioning of Zn in rice grains was associated with Zn application in both the wetland and upland varieties, while growing in waterlogged soil apparently increased the partitioning only in the wetland variety. These results are related to several physiological mechanisms operating during plant growth, such as the ability of Zn uptake from root to shoot, translocation, and partitioning into different plant parts, each of which depend on the availability of Zn in the soil. Singh and Shivay [36] reported that the Zn concentration in the soil was correlated with the Zn content in dry matter. Moreover, it has been reported that Zn uptake, translocation, and remobilization to grains were affected by root growth and its matching with the availability in the soil Zn [44,45]. However, the partitioning ability from the shoot to grain should be considered, as it can be a key mechanism to increase grain Zn accumulation in rice. The current study found that applying Zn under the waterlogged conditions resulted in the highest percentages of Zn translocation from shoot to grain in the wetland and upland varieties at 41.3% and 44.7%, respectively, while applying Zn under the well-drained conditions resulted in percentages of 29.1% and 27.5%, respectively. This suggests that the total translocation of Zn from the shoot to the grain varies according to rice variety, Zn application, and growing conditions. The proper growing condition management would be one way to enhance the amount of Zn uptake from the shoot to the grain.

5. Conclusions

This study indicated that changing the water conditions in rice cultivation from that of the plants' original growing ecotype could cause yield reductions depending on the characteristic response of the rice variety. Growing in well-drained conditions strongly reduced the production of the wetland rice crop compared to the upland rice variety in which only a slight reduction of grain yield was found when changing from well-drained to waterlogged conditions. Zinc fertilizer application had little effect on yield in either variety, but this could be a promising management practice for increasing grain Zn concentration in all rice varieties and growing conditions, albeit with different degrees of improvement. However, the Zn accumulation (and possible toxicity) in rice plants should also be considered, as there were negative correlations between grain Zn and yield and between straw Zn and yield in the wetland variety, but not in the upland rice. An extensive analysis of soil properties and plant tissues at different growth stages under different growing conditions would be able to explain in more detail the factors influencing Zn accumulation and productivity. Applying Zn fertilizer in the waterlogged conditions was more efficient than in the well-drained conditions in both varieties, as Zn fertilizer application under the waterlogged conditions resulted in the highest percentages of Zn translocation from shoot to grain in the wetland and upland varieties at 41.3% and 44.7%, respectively, while applying Zn under the well-drained condition resulted in percentages of 29.1% and 27.5%, respectively. The mechanism of Zn translocation from the shoot to the grain is another issue that should be further investigated in comparisons with rice varieties from different original ecotypes and growing conditions. However, increasing the number of rice varieties of each ecotype would provide a wider range of response within

and between ecotypes in rice crops. This would enable researchers to better understand the mechanisms of Zn homeostasis in rice plants for successful biofortification to enhance grain yield and Zn concentration for the benefit of farmers and consumers.

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