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Efficacy of Nitrogen and Zinc Application at Different Growth Stages on Yield, Grain Zinc, and Nitrogen Concentration in Rice

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Abstract: Zinc (Zn) is an essential element involved in human metabolism, which can be supplied by an appropriate diet. Enhancing Zn enrichment in rice grains through agronomic biofortification is advocated as an immediate and effective approach to combat micronutrient malnutrition in hu-man. It has been well-documented that high grain Zn accumulation in rice can be achieved by Zn fertilizers management. This study evaluated the effects of foliar nitrogen (N) and Zn applied at the flowering and milky stages of brown rice plants with and without soil Zn application. A glasshouse pot experiment was conducted using a completely randomized design with four replicates. Soil Zn in the form of $ZnSO_4$ was applied at 0 and 50 kg ha^{-1}. Foliar fertilizer of 1% urea along with 0.5% $ZnSO_4$ was applied and assigned as (1) nil foliar N and Zn (N0Zn0), (2) foliar N with nil Zn (N+Zn0), (3) nil foliar N with foliar Zn (N0Zn+), and (4) foliar N and Zn (N+Zn+) at flowering and milky stages. Foliar application of N and Zn increased grain yield and yield components in both soil Zn conditions. Grain Zn concentration in brown rice was the highest when foliar N and Zn were applied under nil soil Zn conditions; however, grain N concentration decreased by 13.1–28.5% with foliar application at flowering and 18.8–28.5% with application at the milky stage. The grain Zn content was increased by foliar application of N0Zn+ and N+Zn+ at flowering and milky stages. Applying foliar N and Zn at flowering or milky stages tended to increase the grain N content when Zn was applied to the soil, while nil soil Zn decreased the N content by 26.8% at flowering and milky stages under N0Zn+. The results suggest that the milky stage is the most suitable for foliar application of Zn for increasing (i) grain yield and (ii) N and Zn concentrations in brown rice without having a dilution effect.

Keywords: rice; nitrogen; foliar zinc; zinc fertilizer; growth stage

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1. Introduction

Zinc is present in all living organisms and is essential for the proper operation of the body. Zinc is involved in cell-mediated immunity, the response to oxidative stress, and the generation of chronic inflammatory cytokines [1]. Deficiencies in Zn remain major public health problems that are common among people in developing countries, contributing to hypogonadism, delayed testicular development, impaired spermatogenesis, sex hormone disturbances, oxidative stress and inflammation, and apoptosis [2]. Recently, Zn has been found to enhance the treatment outcomes of pneumonia by reducing the resolution period and normalizing oxygen levels in the body [3]. There is a heightened risk of prolonged hospital stay in COVID-19 patients that are Zn-deficient [4]. A recent study has reported that although the national prevalence of Zn deficiency in Thailand is currently unknown, it has been estimated that around 40% of Thai people are at risk of Zn deficiency due to insufficient intake [5]. The deficiency of Zn feed has been confirmed to provoke a decrease in Zn levels in tissues (femur, liver, and pancreas); Zn also affects glutathione concentration and the activities of lactic dehydrogenase, amylase, catalase, superoxide dismutase, and glutathione-S-transferase [6]. Rice is widely consumed in developing countries, including Thailand [7]. Increasing the grain Zn content in rice has been suggested as an ideal strategy to improve the intake of Zn among the members of the population who consume rice as their staple food [8]. Zinc biofortification through fertilizer management is one of the potential pathways to improving grain Zn concentration in rice grains [9,10].

Zinc fertilizers are used in the prevention of Zn deficiency and in the biofortification of cereal grains [11]. The main soil factors affecting the availability of Zn to plants are low Zn content; high pH; high calcite and organic matter contents; and high concentrations of Na, Ca, Mg, bicarbonate, and phosphate in the soil solution or in labile forms [11,12]. It has been well-documented that foliar Zn spray is an effective strategy to biofortify cereals with Zn [13–15]. Foliar Zn application has tremendous benefits, but the most notable ones include rapid regulation of nutrient deficiencies; fast growth response of plants to foliar treatments; and improved Fe, Ca, and N concentrations [16]. It can be beneficial and more effective than soil application of nutrients, especially when plants are grown on calcareous soils and flooded soils, or where the available Zn status of soils is very low [17]. Soil Zn applications at the time of sowing had little effect on the concentration of Zn in the grain under field conditions, whereas foliar Zn sprays were very effective in improving the grain Zn [18]. However, the effect of Zn application on Zn accumulation in rice grain is likely due to response differences among genotypes. Kandil et al. [19] showed significant differences among the five rice cultivars regarding plant height, grain yield, straw yield, biological yield, harvest index, 1000-grain weight, panicle length, protein percentage, and grain Zn content when the plant is grown under applied Zn fertilizer.

Applying N fertilizer is a common practice in rice crop cultivation for farmers in all regions, and the proper use of N together with foliar Zn application has been shown to be an efficient agronomic measure to improve Zn accumulation in rice grains [20]. The application of N has been reported to increase grain weight, grain yield, and grain Zn accumulation in rice [21,22] and wheat crops [23]. Recent studies have shown that applying a combination of N and Zn fertilizer during the panicle stage can significantly increase Zn concentration in unpolished (brown) and polished (white) rice [24]. Additionally, seed priming and foliar application using a combination of N and Zn have been used to improve the productivity and grain Zn concentration of rice [25]. These results demonstrate that the application of N and Zn can influence plant growth, productivity, and grain Zn accumulation in rice crops. However, limited information is available concerning how applying foliar N and Zn fertilizers at different growth stages and in various soil conditions will affect plant growth, productivity, and grain N and Zn accumulation. This study aimed to investigate the effects of different application regimes of N and Zn fertilizers applied through the soil and foliar routes at two different growth stages on rice growth, yield, and Zn accumulation in grains and straw of the rice variety SPT1. The information gained from this study will be very useful for improving the productivity and grain Zn concentration of rice crops.

2. Materials and Methods

2.1. Plant Culture

The rice seeds of the variety SPT1 (San Pa Tong 1) were obtained from the rice seed center in Chiang Mai, Thailand. The plastic pots (30 cm-diameter, 25 cm-deep) used in this experiment contained 15 kg of loam soil of the Sansai series. The soil was composed of 47.90% sand, 38.50% silt, and 13.60% clay. The soil contained 0.11% total nitrogen (N), 24.97 mg kg^{-1} available phosphorus (P), 77.52 mg kg^{-1} exchangeable potassium (K), 6.60 cmol (+) kg^{-1} cation exchange capacity (CEC), and 3.11% organic matter. The pH of the soil was 6.95, and the concentration of DTPA-extractable Zn in the soil was 2.02 mg kg^{-1} soil. About 100 g of SPT1 seeds were washed three times with water. The seeds were soaked in the water for 24 h, similar to the common practice among farmers, before being rinsed thoroughly with water and incubated in moistened fabric for 48 h. The germinated seeds were transferred into prepared seedling trays. Rice seeds were germinated until seven days old, and the seedlings were transplanted into the pots with a single seedling per hill, three seedlings per pot, under waterlogged conditions. The water was kept at around 5 cm above the soil surface in the pots throughout the experiment. Basal fertilizer containing nitrogen (N), phosphorus (P), and potassium (K) was applied at the rate of 90–60–60 kg ha^{-1} in total by equally splitting into three applications at two, three, and four weeks after transplanting.

2.2. N and Zn Fertilizer Applications

This experiment was conducted during the wet season from September 2019 to January 2020 at the research station field, Chiang Mai University, Thailand. The experiment was arranged as a completely randomized design with four independent replications. Plants were grown under two fertilizer conditions of Zn in the soil: no addition of soil Zn and soil Zn applied at 50 kg ha^{-1} before the transplanting of seedlings. The eight fertilizer treatments of (1) nil soil Zn + nil foliar N and Zn (N0Zn0), (2) nil soil Zn + foliar N with nil Zn (N+Zn0), (3) nil soil Zn + nil foliar N with foliar Zn (N0Zn+), (4) nil soil Zn + foliar N and Zn (N+Zn+), (5) 50 kg soil Zn ha^{-1} + nil foliar N and Zn (N0Zn0), (6) 50 kg soil Zn ha^{-1} + foliar N with nil Zn (N+Zn0), (7) 50 kg soil Zn ha^{-1} + nil foliar N with foliar Zn (N0Zn+), and (8) 50 kg soil Zn ha^{-1} + foliar N and Zn (N+Zn+) were applied at two different growth stages. Plant samples were separated into two sets for foliar application. The foliar N was applied as 1% urea, while Zn was applied as 0.5% $ZnSO_4$ at the rate of 1000 L ha^{-1} at the flowering stage (50 days after transplanting) and with the same fertilizer treatments at the milky stage (63 days after transplanting).

2.3. Data Collection

2.3.1. Yield and Yield Components

At maturity, rice was manually harvested from three plants per replication, and the seeds were threshed by hand. The number of tiller and panicle numbers per plant were evaluated. Five random panicles of each sample were evaluated for the number of spikelets per panicle. The paddy rice samples were cleaned and air-dried to reach 14% moisture content prior to determining grain yield. The samples of straw were oven-dried at 75 °C for 72 h to determine straw yield. The grain was subjected to removal of the husk to produce brown rice grains for N and Zn concentration analysis after being dried with no moisture content.

2.3.2. Zn Concentration and Content

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) on approximately 0.5 g of the ground sample that was weighed before being subjected to dry-ash burning in a muffle furnace at 535 °C for 8 h; the ash samples were then acid-extracted by dissolving in HCl (1:1; HCl to deionized water) for 20 min. Certified reference materials of ground peach leaves (SRM 1547) received from the National Institute of Standards and Technology and soybean leaves were used as the standard checks for the accuracy of Zn concentration in the samples in all analyzed batches. The grain Zn contents were calculated by multiplying the grain Zn concentration by the mass of grain dry matter and were expressed in mg m^{-2}.

2.3.3. N Concentration and Content

The brown rice was analyzed by titration after Kjedahl digestion [26]. Digestion was carried out at 120 °C for 20 min, 200 °C for 30 min, 220 °C for 20 min, 240 °C for 20 min, 260 °C for 20 min, and 360 °C for 4 h with concentrated sulfuric acid (digestion); $CuSO_4$ was added to the digest. The sample solutions were distilled through sodium hydroxide (NaOH). Ammonium gas was trapped by a boric acid solution. Ammonium ion concentration in the boric acid, and thus, the amount of N in the sample, was measured via titration. The grain N contents were calculated by multiplying the grain N concentration by the mass of grain dry matter and were expressed in mg m^{-2}.

2.4. Statistical Analysis

Analysis of variance (ANOVA) was performed using Statistix 8 (Analytical software, SXW). The least significant difference (LSD) at $p < 0.05$ was used to compare the treatment means. The responses of grain yield and straw yield to the Zn and N concentrations in grain were described by linear regression.

3. Results

Foliar application of N and Zn at two different growth stages affected grain yield differently in rice plants grown under the two soil Zn conditions (Figure 1). No foliar application of N or Zn (N0Zn0) produced grain yields of 93.3 to 140.1 g m^{-2} when grown under nil soil Zn conditions, but the grain yield increased from 34.0 to 74.4 g m^{-2} when grown under soil Zn application. With foliar N and Zn applied at the flowering stage, under nil soil Zn conditions, foliar N+Zn+ increased grain yield by 27.6% compared with N0Zn0, N+Zn0, and N0Zn+; the latter three treatments were not significantly different, while with added soil Zn, foliar application of N+Zn0 and N+Zn+ increased grain yield 10.5% compared with N0Zn0 and N0Zn+. With the foliar application of N and Zn at the milky stage, under nil soil Zn conditions, N+Zn0 and N+Zn+ increased grain yield by 31.0% compared with N0Zn0 and N0Zn+, while with Zn added to the soil, N+Zn0, N0Zn+, and N+Zn+ increased grain yield by 9.2%, 21.3%, and 33.0%, respectively. The highest grain yield of 186.3 g m^{-2} was found with foliar application of N and Zn (N+Zn+) at the milky stage. Additionally, the response of straw yield was similar to that of grain yield (Figure 2).

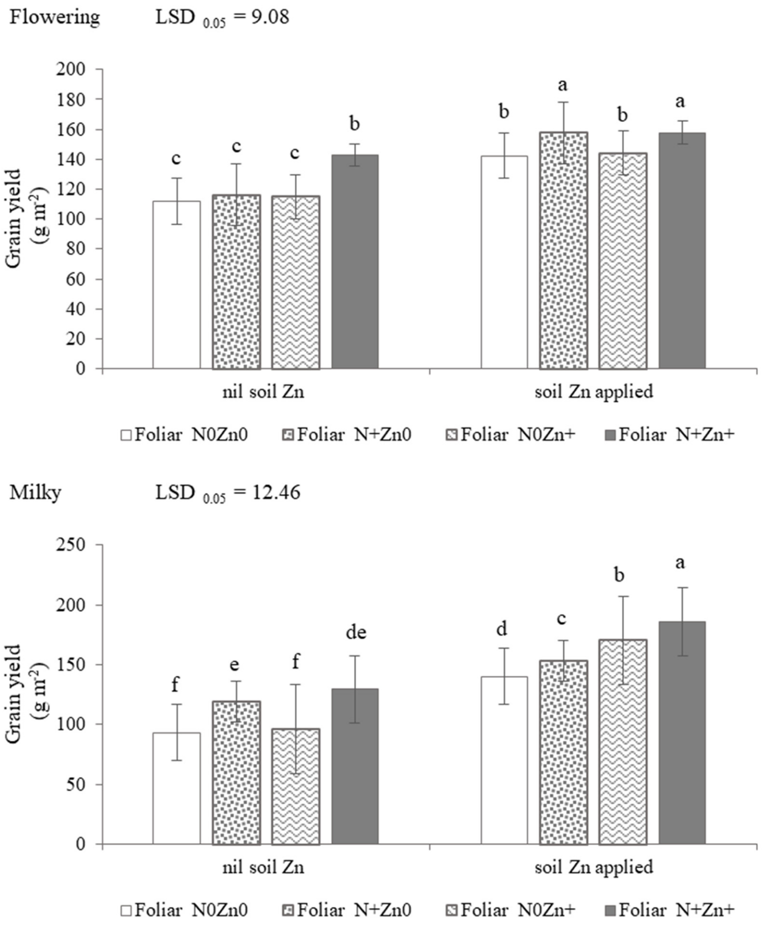

Figure 1. Grain yield in the SPT1 rice variety grown at different foliar N and Zn application rates under two soil Zn conditions at the flowering and milky stages. Each value is the mean of four independent replications. Different letters above the bars indicate a significant difference by LSD (0.05). The error bars indicate the standard deviation of each treatment mean (SD) (n = 4).

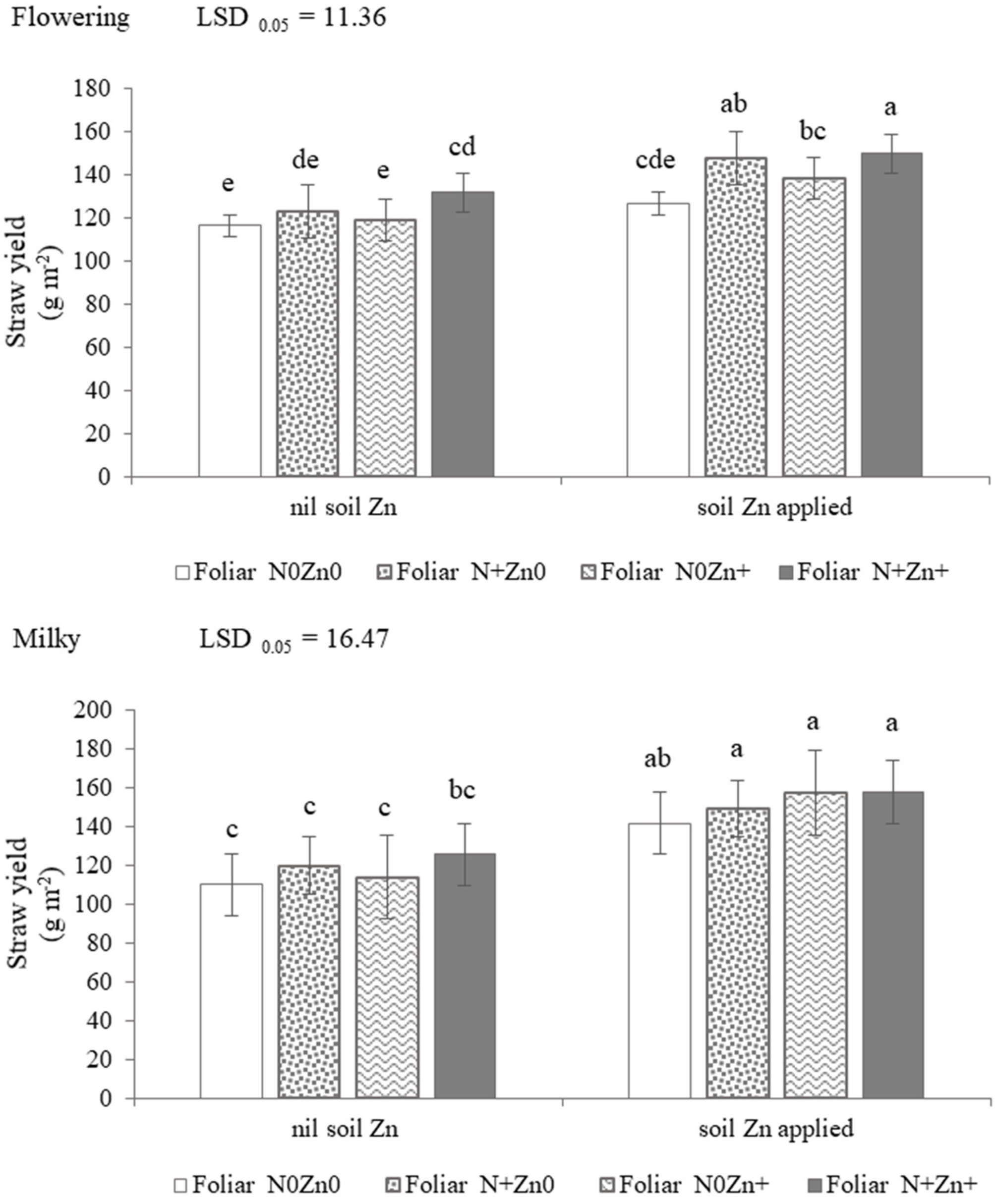

Figure 2. Straw yield of the SPT1 rice variety grown at different foliar N and Zn fertilizer rates under two soil Zn treatments at the flowering and milky stages. Each value is the mean of four independent replications. Different letters above the bars indicate significant differences by LSD (0.05). The error bars indicate the standard deviation of each treatment mean (SD) (*n* = 4).

The yield components contributing to the variation in grain yield responded to fertilizer treatments similarly in the number of tillers and the number of panicles per plant (Figures 3 and 4). With Zn added to the soil, the number of tillers and panicles per plant increased by one tiller and two panicles within the N+Zn+ treatment at the flowering stage compared with the other treatments, with approximately four tillers and three panicles per plant. The number of spikelets per panicle was significantly affected by fertilizer treatments differently when N and Zn were applied at different growth stages and soil Zn conditions (Figure 5). The number of spikelets ranged from 117.5 to 152.2 per panicle with nil soil Zn and from 120.4 to 155.4 spikelets per panicle under soil Zn treatment with foliar N and Zn applied at the flowering and milky stages. Applying N+Zn+ at the flowering stage increased the number of spikelets by 14.2 and 13.5% under nil soil Zn and added soil Zn conditions, respectively, compared with N0Zn0. In contrast, applying N+Zn+ during the milky stage of plants grown under nil soil Zn conditions increased the number of spikelets by 13.4% compared with N0Zn0, while with added soil Zn, N+Zn0, N0Zn+, and N+Zn+ increased the number of spikelets by 8.4, 10.2, and 11.8%, respectively.

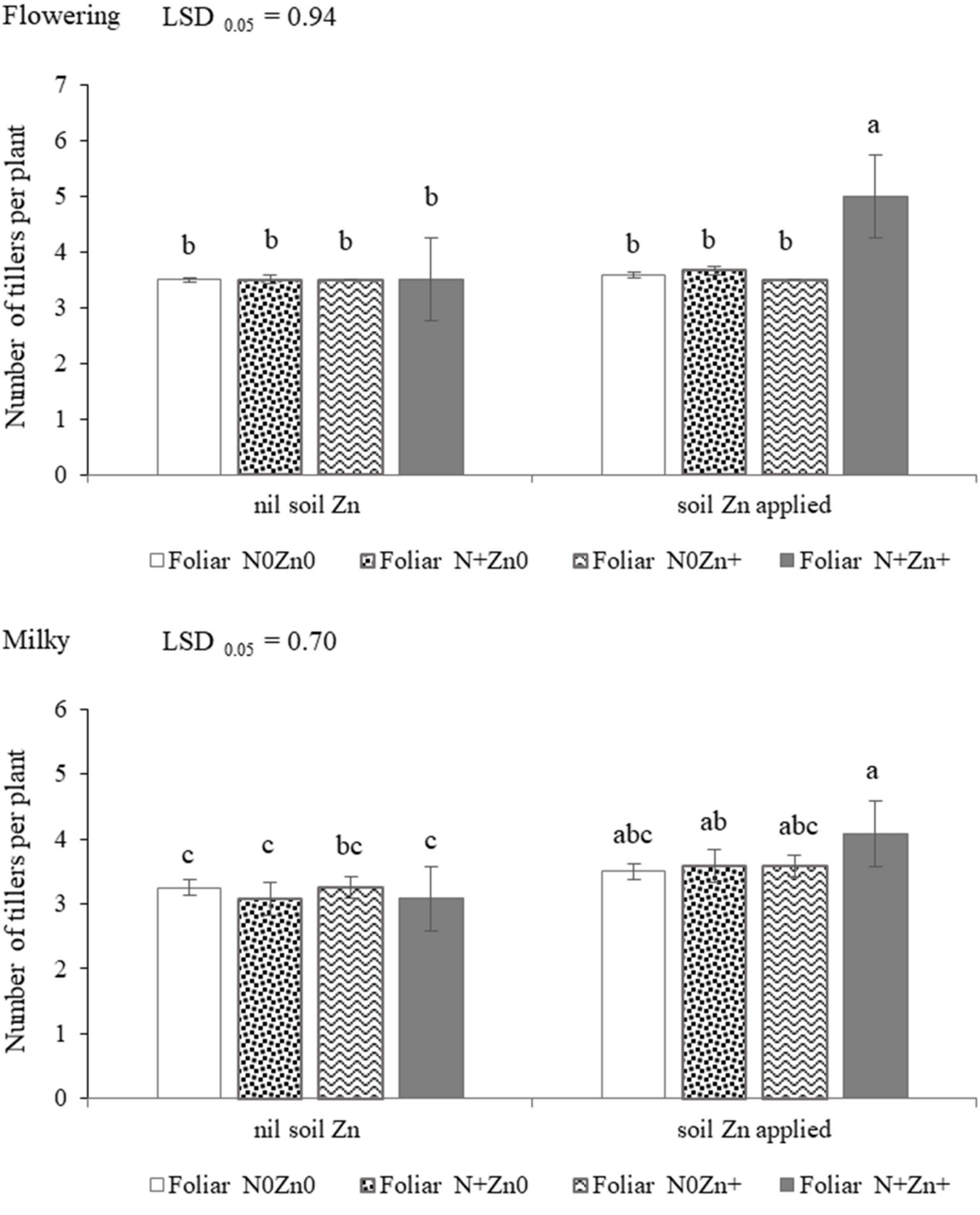

Figure 3. Number of tillers per plant of the SPT1 rice variety grown at different foliar N and Zn fertilizer rates under two soil Zn treatments at the flowering and milky stages. Each value is the mean of four independent replications. Different letters above the bars indicate a significant difference by LSD (0.05). The error bars indicate the standard deviation of each treatment mean (SD) (n = 4).

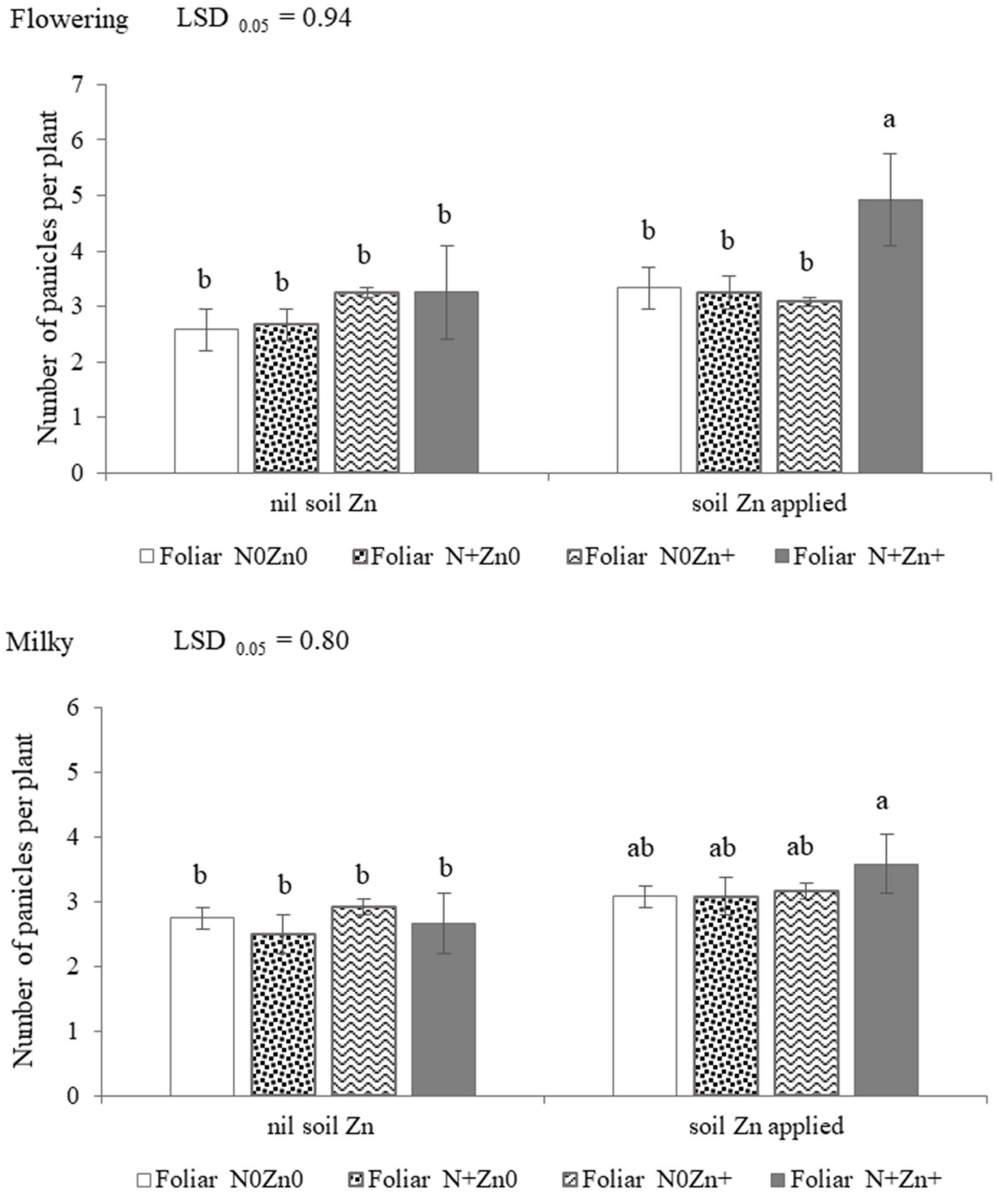

Figure 4. Number of panicles per plant of the SPT1 rice variety grown at different foliar N and Zn fertilizer rates under two soil Zn treatments at the flowering and milky stages. Each value is the mean of four independent replications. Different letters above the bars indicate significant differences by LSD (0.05). The error bars indicate the standard deviation of each treatment mean (SD) ($n = 4$).

Foliar application of N and Zn fertilizers at flowering and milky stages affected Zn and N concentrations in brown rice differently when the plants were grown under nil and added soil Zn ($p < 0.05$) (Table 1). With nil foliar application (N0Zn0) at the flowering stage, grain Zn concentrations in brown rice were 7.2 and 10.8 mg Zn kg^{-1} when plants were grown under nil soil Zn and added soil Zn, respectively, while at the milky stage, application of grain Zn yielded concentrations of 14.0 and 26.1 mg Zn kg^{-1}, respectively.

Foliar application of Zn and N increased grain Zn concentration in all treatments compared with nil foliar application (N0Zn0), especially with the foliar application under application of soil Zn. For nil soil Zn, foliar application of N and Zn fertilizers at the flowering stage increased grain Zn concentration from 58.6% to 196.6% compared with N0Zn0, and the respective increase ranged from 32.6 to 127.9% with added soil Zn. In addition, foliar application of N and Zn fertilizers at the milky stage under nil soil Zn increased grain Zn concentration from 71.4% to 103.6% compared with N0Zn0, but the same increase was only 26.0% in the N+Zn+ treatment with added soil Zn.

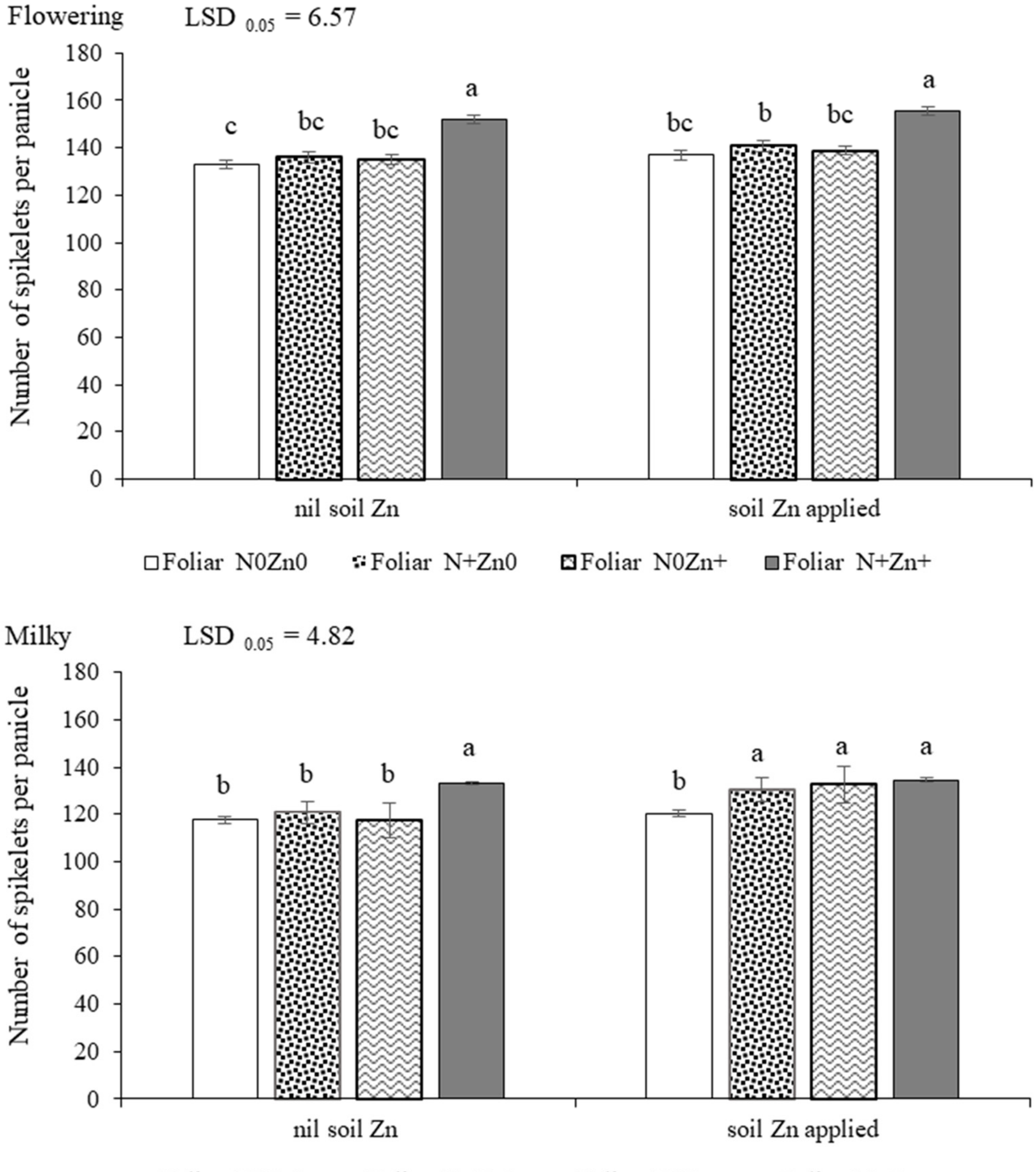

Figure 5. Number of spikelets per panicle of the SPT1 rice variety grown at different foliar N and Zn fertilizer rates under two soil Zn treatments at the flowering and milky stages. Each value is the mean of four independent replications. Different letters above the bars indicate a significant difference by LSD (0.05). The error bars indicate the standard deviation of each treatment mean (SD) (n = 4).

Table 1. Zn and N concentrations in brown rice SPT1 variety grown at different foliar N and Zn fertilizer rates under variation of soil Zn application at the flowering and milky stages. Each value is the mean of four independent replications ± standard deviation of the mean ($n = 4$).

Fertilizer Condition	Zn Concentration (mg kg^{-1})		N Concentration (g kg^{-1})	
	Flowering Stage	**Milky Stage**	**Flowering Stage**	**Milky Stage**
Nil soil Zn				
Foliar application				
N0Zn0	7.25 ± 0.5 e	14.00 ± 1.8 d	13.71 ± 0.1 a	14.44 ± 0.3 b
N+Zn0	11.50 ± 1.0 cd	16.00 ± 0.9 d	11.89 ± 0.1 c	11.70 ± 0.04 e
N0Zn+	21.50 ± 0.9 ab	24.00 ± 1.1 c	9.87 ± 0.2 e	10.27 ± 0.3 f
N+Zn+	21.50 ± 1.9 ab	28.50 ± 0.5 b	10.42 ± 0.3 d	10.35 ± 0.4 f
Soil Zn applied				
Foliar application				
N0Zn0	10.75 ± 1.4 d	26.00 ± 2.0 bc	11.31 ± 0.1 c	12.90 ± 0.03 d
N+Zn0	14.25 ± 0.9 c	28.00 ± 1.2 b	12.74 ± 0.2 b	12.72 ± 0.4 d
N0Zn+	21.00 ± 1.3 b	29.25 ± 0.5 ab	12.62 ± 0.3 b	13.66 ± 0.1 c
N+Zn+	24.50 ± 0.4 a	32.75 ± 1.3 a	12.58 ± 0.1 b	15.30 ± 0.2 a
F-test	<0.001	<0.001	<0.001	<0.001
LSD$_{0.05}$	3.37	3.68	0.60	0.75

The lowercase letters indicate a significant difference between the treatments by LSD 0.05, the least significant difference at $p < 0.05$.

Moreover, under nil foliar application (N0Zn0) at the flowering stage, N concentration in brown rice was 13.7 and 11.3 g kg^{-1} when plants were grown under nil soil Zn and added soil Zn, respectively, while at the milky stage, the respective N concentrations were 14.4 and 12.9 g kg^{-1}. All foliar treatments of Zn and N under nil soil Zn conditions decreased grain N concentration by 13.1% to 28.5% at the flowering stage and by 18.8% to 28.5% at the milky stage compared with nil foliar application (N0Zn0). With applied soil Zn, foliar application of Zn and N at the flowering stage increased grain N concentration in all treatments; the average increase was 11.5% compared with nil foliar application (N0Zn0). In addition, at the milky stage, a higher increase was found for foliar N+Zn+ with applied soil Zn, as N concentration increased by 18.6%.

There was a significant regression between grain Zn concentration and grain yield among the foliar applications at flowering ($R^2 = 0.36$, $p < 0.001$) and milky ($R^2 = 0.52$, $p < 0.001$) stages (Figure 6). A relationship was also found between grain Zn concentration and straw yield among the foliar applications at flowering ($R^2 = 0.26$, $p < 0.001$) and milky ($R^2 = 0.45$, $p < 0.001$) stages. There was no relationship between grain N concentration and grain yield ($R^2 = 0.02$, $p > 0.05$) under the foliar application of N and Zn fertilizers at the flowering stage of rice. In contrast, with the foliar application at the milky stage, N concentration was positively related to grain yield ($R^2 = 0.24$, $p < 0.001$) (Figure 7).

Foliar application of N and Zn fertilizers in both at flowering and milky stages affected grain Zn and N contents in brown rice differently when plants were grown under nil soil Zn and added soil Zn (Table 2). With nil foliar application (N0Zn0) at the flowering stage, grain Zn content was 0.8 and 1.5 mg m^{-2} when plants were grown at nil soil Zn and added soil Zn, respectively, while at milky application, grain Zn content was 1.3 and 3.7 mg m^{-2}, respectively. At nil soil Zn, the Zn content was increased by foliar N+Zn0, N0Zn+, and N+Zn+ at the flowering and milky stages; grain Zn content increased by 65.0%, 207.4%, and 278.1%, respectively, compared with N0Zn0 at the flowering stage. Under soil Zn conditions, N+Zn0, N0Zn+, and N+Zn+ treatments increased the grain Zn content by 45.9%, 97.6%, and 155.5% from N0Zn0, respectively, with foliar treatment at flowering stage, while applying N+Zn0, N0Zn+, and N+Zn+ increased the grain Zn content by 17.7%, 36.3%, and 66.6%, respectively, with foliar application at the milky stage.

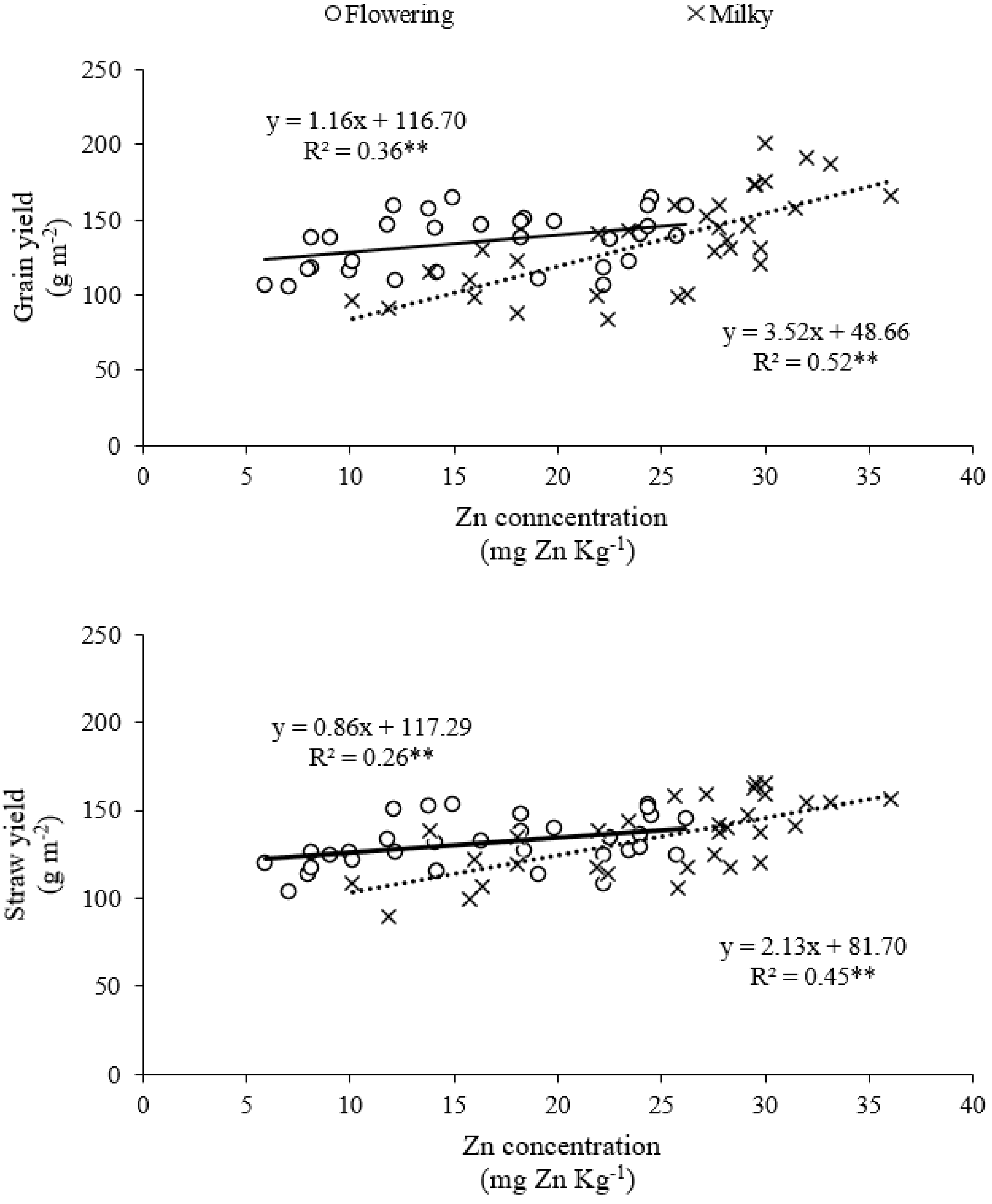

Figure 6. The relationship between Zn concentration and grain yield, Zn concentration, and straw yield in the brown rice SPT1 variety grown at different foliar N and Zn fertilizer rates under variations of soil Zn fertilizer at the flowering and milky stages. ** indicates signification linear regression at $p < 0.001$ ($n = 32$).

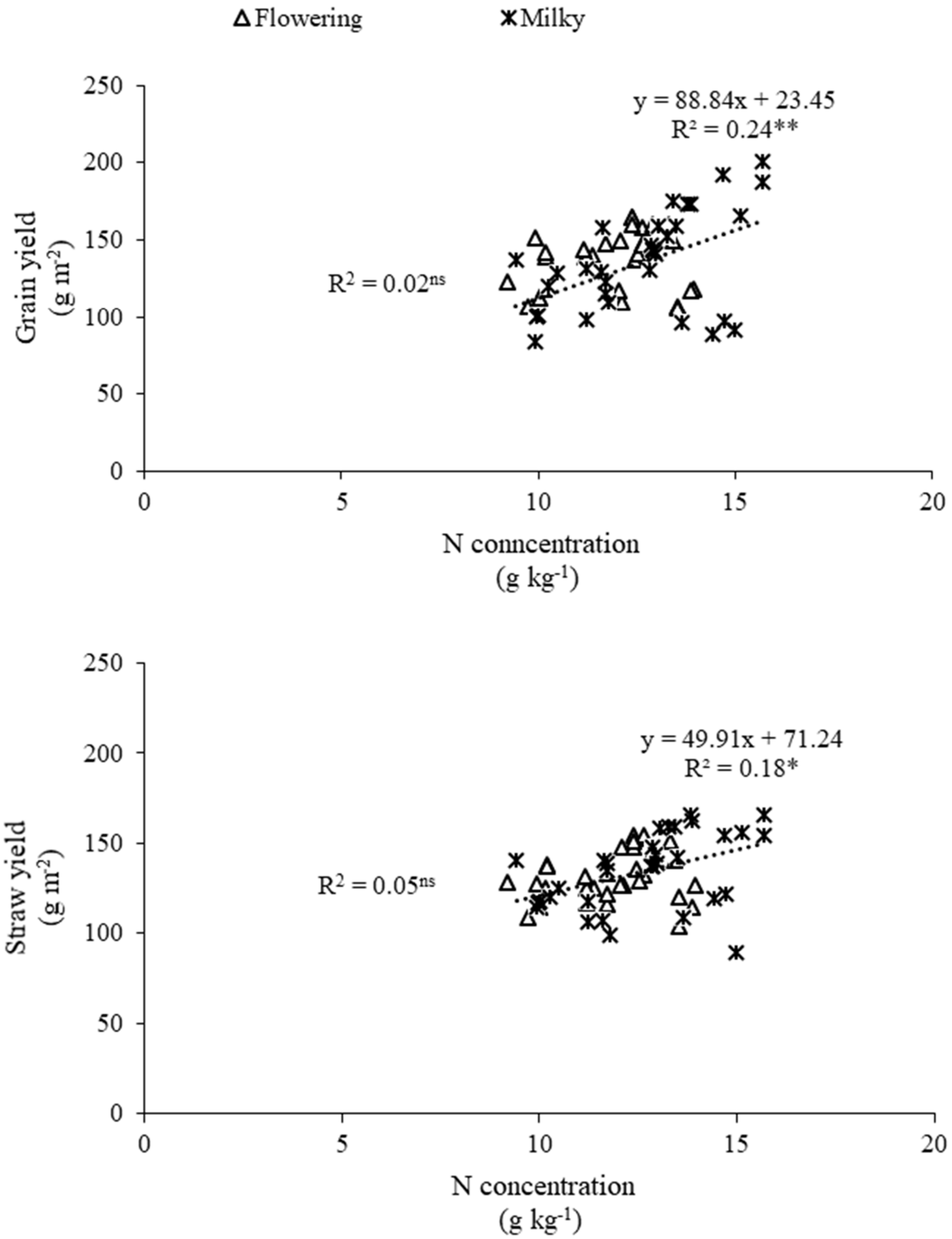

Figure 7. The relationship between N concentration and grain yield. N concentration and straw yield in brown rice of SPT1 variety grown at different foliar N and Zn fertilizer rates under variations of soil Zn fertilizer at the flowering and milky stages. ^{ns}, **, * indicate nonsignificant and significant linear regression at $p < 0.01$ and $p < 0.05$ ($n = 32$), respectively.

Table 2. Grain Zn and N contents in brown rice SPT1 variety grown at different foliar N and Zn fertilizer rates under variations in soil Zn application at the flowering and milky stages. Each value is the mean of four independent replications ± standard deviation of the mean (n = 4).

Fertilizer Condition	**Grain Zn Content (mg m^{-2})**		**Grain N Content (g m^{-2})**	
	Flowering Stage	**Milky Stage**	**Flowering Stage**	**Milky Stage**
Nil soil Zn				
Foliar application				
N0Zn0	0.81 ± 0.1 e	1.30 ± 0.2 f	1.50 ± 0.1 cd	1.35 ± 0.04 d
N+Zn0	1.34 ± 0.1 d	1.91 ± 0.1 e	1.38 ± 0.1 d	1.38 ± 0.1 d
N0Zn+	2.50 ± 0.2 c	2.32 ± 0.2 e	1.10 ± 0.03 e	0.98 ± 0.1 e
N+Zn+	3.07 ± 0.2 b	3.67 ± 0.1 d	1.48 ± 0.04 d	1.33 ± 0.05 d
Soil Zn applied				
Foliar application				
N0Zn0	1.53 ± 0.2 d	3.65 ± 0.3 d	1.63 ± 0.04 c	1.80 ± 0.05 c
N+Zn0	2.24 ± 0.1 c	4.30 ± 0.2 c	2.00 ± 0.1 a	1.95 ± 0.1 c
N0Zn+	3.03 ± 0.1 b	4.98 ± 0.2 b	1.83 ± 0.1 b	2.35 ± 0.1 b
N+Zn+	3.92 ± 0.1 a	6.08 ± 0.1 a	2.00 ± 0.05 a	2.83 ± 0.01 a
F-test	< 0.001	< 0.001	< 0.001	< 0.001
LSD $_{0.05}$	0.46	0.51	0.13	0.19

The lowercase letters indicate a significant difference between the treatments by LSD 0.05, the least significant difference at $p < 0.05$.

At the flowering stage, in the N0Zn0 treatment, the grain N content in brown rice was 1.5 and 1.6 g m^{-2} when plants were grown under nil and added soil Zn, respectively, while at the milky stage, grain N content was 1.4 and 1.8 g m^{-2}, respectively. Foliar treatments of N0Zn+ at nil soil Zn conditions decreased grain N content by 26.8% at the flowering and milky stages compared with nil foliar application (N0Zn0). Under soil Zn application, foliar grain Zn and N applied at the flowering stage increased N content in all treatments; the average increase was 20.5% compared with nil foliar application (N0Zn0). The grain N content at the milky stage with foliar N0Zn+ and N+Zn+ applications under soil Zn conditions increased by 28.7% and 57.7%, respectively.

4. Discussion

One of the most popular glutinous rice varieties in Thailand's north and northeast regions, recommended by the Thailand Ministry of Agriculture and Cooperatives, is the San pa tong 1 (SPT1). One reason for this is that SPT1 has elite characteristics, such as high yield potential, shorter growth duration, good eating quality, and enhanced resistance to several diseases and insect pests. This rice type has very low light sensitivity; stable harvesting time; can be grown throughout the year; and is classified as an odorless, white glutinous rice variety [25,27]. This study has established that management using appropriate N and Zn fertilizers in rice crops has the potential to improve productivity and grain Zn accumulation. Foliar application of N and Zn fertilizer either at flowering or milky stages substantially enhanced N and Zn availability in plant tissues and consequently improved yield and grain Zn accumulation. The increases occurred with and without soil Zn application, with the higher magnitude of increase observed with foliar spray under soil Zn application at the milky stage.

Previous studies have consistently reported that foliar application of N and Zn fertilizer significantly increased yield in various field crops such as rice [25], common bean [28], wheat [29,30], and onion [31]. The current study found that rice plants grown under added soil Zn had higher yields than under nil soil Zn, even when foliar N and Zn fertilizers were not applied to the plants (N0Zn0). Thus, Zn availability in the soil had a significant effect on yield by improving the number of panicles per plant and the number of spikelets per panicle. The formation and development of panicles require high amounts of Zn for active cell growth [32]. Applying Zn to rice crops was reported to produce a higher number of panicles per plant and a greater number of spikelets per panicle [33] due to the increase in the total NPK uptake [34] and the enhancement of photosynthetic production [34]. However, this study showed little variation in grain yield between the nil soil Zn and added soil Zn treatments for the two growing stages; this could be due to the samples being collected in different plants even though they experienced similar treatments. Foliar application of either N and/or Zn fertilizer in the N+Zn0 and N+Zn+ groups when the plants were grown in the added soil Zn condition had the highest grain yield, while in nil soil Zn conditions only foliar application with N+Zn+ produced a grain yield higher than N0Zn0. The phenomenon was similar to our previous reports, where foliar application with N+Zn+ increased grain yield by 33% [25]. This indicates that applying N and Zn fertilizer is essential for yield improvement in rice crops, the fertilizer source can be from the soil and/or foliar application, and the highest yield occurs when both sources are continuously supplied to the plants. When Zn is limited in the soil, which was not the case in this study that used soil containing 2.02 mg kg^{-1}, the critical concentration for rice crop growing is approximately 0.5 mg kg^{-1} [35]. Foliar application of N and Zn at the flowering stage can recover up to 90% of grain yield compared with added soil Zn conditions, and Zn plays key roles in pollination, fertilization, and grain setting [36]. The sufficiency of Zn in plant tissues by soil and/or foliar application can increase seed formation and thereby increase yield. In addition, Zn plays key roles in DNA polymerase activity, sugar and protein metabolism, as well as in cell division, processes that can increase grain size and grain weight [37,38]. We found that it was not just foliar application of N and Zn fertilizer at the flowering stage that improved plant growth performance; foliar application at the milky stage also produced a higher yield with a greater rate of increase. Foliar application of Zn at the early reproductive stage results in the translocation of the Zn to the reproductive structures of the plant and it is then accumulated in grains [39]. Zinc applied by foliar spray is readily absorbed by the leaf epidermis and, after remobilization, it is transferred via the phloem to developing seeds [40]. A previous study by Pandey et al. [41] stated that the increased grain weight of chickpeas was attributed to fully developed grains as a result of increased starch content due to the increased activity of starch synthase after foliar application of Zn at the flowering stage. Drought is one of the most important factors limiting grain yield [42]. Drought negatively affects plant processes such as the formation of proteins, nucleic acids, lipids, and carbohydrates [43]. Toor et al. [44] reported that foliar application of Zn reduced the negative effects of drought on plants by reducing photo-oxidative damage. The foliar application of Zn enhances drought tolerance by maintaining leaf water status or by higher expression of aquaporins [45], thereby increasing the grain yield in winter wheat under drought conditions [46].

The number of tillers per plant of the SPT1 rice variety was increased by foliar N+Zn+ together with soil Zn fertilizer at both the flowering and milky stages. This may have been due to the fact that the SPT1 rice variety obtained more nutrients through soil Zn application that, in return, produced more tillers per plant. Secondly, the SPT1 rice variety may have a greater response to foliar N+Zn+ application in terms of increased vegetative growth, as in regenerated tiller growth. Third, the effect may be due to increases in photosynthesis, chlorophyll synthesis, protein synthesis, or N fixation in response to the application of Zn. These results are in accordance with those of Rehman et al. [47], who reported that the number of tillers increased with soil fertilizer application of Zn. Similarly, Patel et al. [48] also reported that the foliar application of Zn increased the number of tillers. Wang et al. [49] also reported an increase in the number of tillers in response to the application of N. Moreover, the tiller number per plant has a close relationship with the number of panicles per plant. The tillering of rice, which determines the number of panicles per plant, is an important agronomic trait for grain production [50]. The number of panicles depends on the number of tillers per plant and the proportion of effective tillers for a particular variety [51]. However, an increasing number of panicles per plant obtained by combining the application of foliar N+Zn+ and soil Zn fertilizer increases the use efficiency of added nutrients supplied to the plants throughout the crop growth period. This will promote various physiological activities in the plant that are indispensable for the proper growth and development of rice panicles. There were parallel responses of yield and straw dry weight to N and Zn fertilizer application. Thus, the result of yield performance is attributed to the concentrations of N and Zn in plant tissues.

Our previous study reported that grain Zn concentration was the highest under foliar application of N+Zn+, with a 38% increase without applied soil Zn [25]. In the present study, grain Zn concentration of plants grown under added soil Zn were 30 to 50% higher than in the nil soil Zn, in accordance with the results for productivity, although the variation of grain Zn within nil Zn and added soil Zn conditions without foliar application was found between the two growth stages as for grain yield. Foliar application of the N0Zn+ and N+Zn+ treatments drastically increased grain Zn concentration by 95 to 197% when applied at the flowering stage and by 13 to 104% at the milky stage. Grain Zn concentration of plants grown under N and Zn application ranged from 11 to 33 mg kg^{-1}, which was higher than the Zn recommended dietary thresholds if about 500 g of rice is consumed per day. The recommended dietary allowance of Zn for adults is 11 mg per day for men and 8 mg per day for women [52]. The magnitude of grain Zn concentration increase indicates that it could be due to the differences in soil type, genotype, and timing of foliar Zn fertilizer [53]. The timing of application is one of the key factors for achieving the highest increases in grain Zn concentration [54]. Saha et al. [55] reported that foliar application of Zn at the flowering stage yielded more Zn-dense grains than application at the maximum tillering stage. Li et al. [56] showed that foliar Zn application at the primary grain filling stage considerably increased the utilization efficiency and grain Zn concentration compared with the application at early growth stages. This was probably due to the fact that at the early stage, immature leaves are less physiologically capable of exporting nutrients, while mature leaves at the reproductive stage export nutrients directly via the phloem to develop grains and other organs [55]. In foliar application, Zn^{2+} ions enter the plant (leaf apoplast) directly through stomatal pores and increase Zn concentration in the phloem tissue of leaves, from where it can be directly translocated to grains [57]. The foliar application of Zn at early grain filling causes Zn to re-translocate into grains from vegetative tissues, resulting in high Zn accumulation in grains [56]. In addition, the increase in grain Zn concentration relies on high Zn distribution to grain [58]. The adequate Zn distribution to grain was defined, from the view of Zn biofortification, as the situation where the Zn distribution to grain (Zn harvest index) increased to the observed maximum of 91.0% and the Zn concentration of vegetative parts (straw Zn concentration) decreased to the observed minimum of 1.5 mg kg^{-1}; all the extra Zn was remobilized from straw to grain and the grain Zn concentration increased to its highest attainable level [59]. The previous study measured Zn concentrations and isotope ratios to elucidate Zn pathways and processes in the shoot during grain filling. They reported that Zn mass decreased while heavy Zn isotopes accumulated in straw during grain filling. Three-quarters of the Zn mass in the shoot moved to the grains, which were enriched in light Zn isotopes relative to the straw [60]. This study has confirmed that applying N and Zn fertilizer can act synergistically in improving grain Zn and N concentrations [61]. Nitrogen promotes the production of dry matter and ensures the nutritional needs for the development of young panicles, resulting in an increase in the number of differentiated branches and spikelets [62]. Zinc application strongly affects N accumulation and metabolism by influencing glutathione metabolism, amino acid metabolism, and the metabolic processes of the chloroplast [63]. Supplying Zn upregulated the expression levels of OsAMTs in both the roots and shoots of rice plants, thereby contributing to higher uptake, translocation, and preferential distribution of N into growth centers including new leaves and spikelets, ultimately increasing shoot biomass and yield production [64].

The N status of plants contributes to grain Zn accumulation. Enhancement of grain Zn concentrations by N application has suggested that foliar spray of N at reproductive stages improves productivity via slowing the synthesis of abscisic acid, accelerating the production of cytokinin, increasing the rate of photosynthesis, and facilitating the translocation of photosynthates to reproductive organs [65]. Moreover, N promoted the accumulation of protein, decreased the accumulation of amylose in grains, and increased the Zn content in grains [66]. The increases in grain Zn concentrations were achieved by foliar application of N+Zn0 and N+Zn+. A recent study showed that no or low N supply led to low grain Zn concentration, while a high N supply caused higher Zn concentration [67]; however, under both nil soil Zn and soil Zn conditions, foliar N application as well as foliar Zn application did not further increase grain N concentration, and rather caused a decrease in N concentration. There are several possible reasons for this decrease. The decrease in grain N concentration may be due to a shift in dry-mass partitioning from plant organs high in N to plant organs lower in N [67]. There was one exception in which foliar spraying increased N concentration; with foliar N+Zn+ application at the milky stage under soil Zn conditions, the N concentration was increased by 18.6%. It seems that the N+Zn+ application at the milky stage significantly enhanced the N transport and, as a result, the re-translocation of N from leaves into grains under soil Zn conditions. The milky stage is the first stage of seed ripening. The milky stage (7–15 days after pollination) is the period when the rice endosperm is in liquid, semisolid, and solid forms [68]. Storage protein in rice starts to accumulate four days after pollination (DAP) and peaks at 8 DAP [69]. The highest accumulation of storage protein in grains occurs at the milky stage; therefore, an application of foliar N+Zn+ at the milky stage as demonstrated in this study would be ideal for enhancing grain N concentration in rice. Many researchers have focused on the effect of foliar application of N and Zn at this growth stage [70–73].

Due to the different physiological characteristics of rice at different growth stages, the absorption and utilization of Zn also differ among stages. Foliar Zn (N0Zn+ and N+Zn+) was applied at the flowering stage when the peak number of panicles occurs and at the early milky stage, and this was well-matched with the best application times to increase the grain Zn content. The highest Zn concentration in brown rice with foliar N+Zn+ spraying at the milky stage under both soil conditions (with or without Zn fertilizers) indicated efficient absorption of Zn by rice plants compared with foliar N+Zn+ spraying at the flowering stage. These results were in line with the findings of Mabesa et al. [74], who reported that foliar application at the milky stage emerged as the most effective stage for increasing Zn accumulation in brown rice. The accumulation of Zn in rice grains primarily occurs in the first 20 days after anthesis [75]; thus, an application of foliar Zn at the milky stage, as demonstrated in the present study, would be ideal for enhancing grain Zn content in rice. However, these studies have indicated that the foliar application of N and Zn was weakly effective in enhancing grain N content compared with the application of soil Zn fertilizer, as foliar N0Zn+ spraying without soil Zn fertilizer decreased the grain N content.

5. Conclusions

Foliar application of N and Zn enhanced the agronomic traits of the SPT1 rice variety, including grain and straw yield. Additionally, the treatments effectively increased grain Zn concentration through foliar N+Zn+ fertilizer. The highest grain yield was obtained by foliar N+Zn+ application at the milky stage, either with or without soil Zn fertilizer, indicating that foliar N+Zn+ application is the key to achieving the highest Zn concentration in rice grains for addressing the problem of Zn nutrition in humans. In addition, the combined application of foliar N and Zn (N+Zn+) at the milky stage under soil Zn application led to a further significant increase in grain N concentration. Foliar application of N or Zn alone significantly reduced grain N concentration, while foliar Zn fertilizer combined with or without soil Zn fertilizer significantly increased grain Zn content. Therefore, the foliar application of N and Zn, especially foliar N+Zn+ application at the milky stage, is a promising way to improve yield, yield components, and grain Zn accumulation in rice, although further studies are needed to explore the effect of the foliar application of N and Zn under field rice-growing conditions.

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