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Zinc Biofortification of Selective Colored Rice Cultivars: Improvement of Zinc Uptake, Agronomic Traits, and Nutritional Value

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Abstract: It is difficult for ordinary rice to break the zinc-rich standard. However, employing multiple unique rice cultivar resources through biofortification of agronomic measures to achieve the target is a promising attempt. In this study, a pot experiment was conducted on seven different colored rice cultivars (GFHN 166, GFHN 168, GFHN 169, GH 1, GXHZ, GHSZ, and YXN), aiming to analyze the effect on zinc content, growth, quality, and health risk index when spraying zinc (400 g/ha) on the leaves at the heading age. The result indicated that after foliar biofortification treatment, the zinc content and the zinc accumulation of colored rice grains could reach up to 41.55 mg/kg and 2.28 mg/pot, respectively, increased by 43.92% and 65.22%. In addition, the SPAD value and grain protein content was 42.85 and 8.49%, also increased significantly by 2.15% and 2.91%, respectively. Among these, GXHZ and GHSZ could realize the zinc content of polished rice up to 69.7 mg/kg and 55.4 mg/kg, breaking through the standard of zinc-enrich rice (45 mg/kg). GXHZ plant height increased by 11.22%, and the zinc harvest index (6.44%) and zinc use efficiency (26.79%) were the highest. Meanwhile, the biofortification promoted the SPAD value of GHSZ and the protein content of GFHN 166 by 4.95% and 24.81%, respectively. Foliar-applied zinc at the heading stage is a vital practice to get better agronomic indicators, quality, and grain zinc biofortification of colored rice.

Keywords: colored rice; foliar zinc application; zinc content; zinc accumulation; agronomic indicators

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1. Introduction

Zinc is one of the essential trace elements for both plants and humans, enabling specific physiological functions such as maintaining protein synthesis [1], gene expression, enzyme structures, energy production, carbohydrate metabolism, photosynthesis, auxin metabolism, pollen formation, and resistance to certain pathogen infections [2]. It positively impacts crop yields and enhances food's nutritional quality [3]. Moreover, zinc participates in nearly all metabolic activities in the human body, enhancing immunity and promoting growth and development, earning it the nickname "the flower of life" [1]. Zinc deficiency slows the body's growth and development and weakens immunity [4,5]. Nearly one-third of the world's population is deficient in zinc [6]. Micronutrient deficiencies, including zinc, often called "hidden hunger", are a serious global health problem [7]. Zinc supplementation has become a crucial issue. Zinc biofortification of staple food crops has been recognized as an alternative, complementary, and sustainable way to overcome zinc malnutrition [8,9].

Rice (*Oryza sativa*) belongs to the genus Oryza in the grass family. It is one of the world's three major staple food crops and is a source of energy, vitamins, minerals, and rare amino acids that are essential for people who rely on rice as a staple food in their daily lives [9,10]. Over half of the world's population currently depends on rice as a staple food [11]. Ensuring that rice contains sufficient nutrients will play an important role in human health. However, the zinc content in ordinary rice grains is presently low, with the average zinc concentration in brown rice cultivars only 25.4 mg/kg [9]. Furthermore, the rice commonly consumed as polished white rice will lose most of its zinc during the milling process, only with a typical zinc content of 12.9 mg/kg, which is far below the zinc-rich standard of 45 mg/kg specified by the national standard (General Rules for Nutrition Labeling of Prepackaged Foods, GB28050) [12]. It is also unable to meet the body's zinc requirement of 11 mg (male) or 8 mg (female) per day [13]. As a treasure in rice germplasm, colored rice is a potential source of various bioactive compounds. It is rich in a large number of anthocyanins and trace mineral elements. The consumption of colored rice can reduce the risk of multiple diseases and is recognized as a functional food that promotes human health [14,15]. In recent years, colored rice has gained momentum on the international market, with growing demand worldwide [16].

Related studies manifested that the grain zinc content of five purple-milled rice cultivars and four red-milled rice cultivars in Laos ranged from 15.5~19.5 mg/kg. The grain zinc content of seventeen purple-milled rice cultivars in Thailand ranged from 19.0~41.3 mg/kg [17,18], which showed that the color rice had a solid ability to enrich zinc compared to ordinary rice [19]. However, there is still a particular gap between the previous studies and the level of zinc-rich standards. Exploring more rice germplasm or varieties to achieve zinc enrichment is a significant attempt. South China is the central region for rice cultivation [20]. Guizhou, Yunnan, and Guangxi in southern China are among the world's largest centers of rice genetic diversity and high-quality germplasm [21,22]. Landraces, especially for some colored rice varieties, are rich in genetic diversity due to their effectiveness in farmland conservation and promotion of allele variation [23]. Hence, utilizing more resources of colored rice from South China to improve zinc accumulation become a growing concern for researchers and farmers [21,22].

Meanwhile, in recent decades, there has been much research on how to reverse zinc deficiency in rice. The study found that applying zinc fertilizer could improve rice's zinc content, processing quality, nutritional value, and cooking taste [24]. However, most zinc fertilizer trials are conducted against a backdrop of managing zinc deficiency, with few studies related to zinc biofortification [25]. Biofortification employs three primary strategies: agronomic practices, traditional breeding, and genetic engineering [26]. Agronomic practice, a fertilizer-driven method [27], involves applying basal or foliar sprays to crops, enabling direct uptake of essential trace elements [28]. It is an effective way to improve the insufficient intake of nutrients by making crops contain one or several trace elements quantitatively. Foliar application, especially at the heading period, bypasses soil complexities, enhancing fertilizer efficiency with faster absorption and greater effectiveness while reducing environmental impact, which is widely accepted and applied [29,30]. According to previous reports, zinc application rates between 450~500 g/ha are most appropriate for rice [31]. Since too high a zinc concentration will have a particularly toxic effect on plants, and too low will have a negative impact on crop yield and quality [32].

Based on the above research results, we infer that the use of special colored rice cultivars combined with foliar zinc spraying can effectively make rice reach and even break the zinc-rich standard. Therefore, this study aims to select some characteristic-colored rice from the southern part of China and combine foliar zinc spraying to study the absorption and accumulation of zinc to (i) analyze the distribution and transportation of zinc in colored rice, (ii) evaluate the changes in growth and physiological quality indicators of colored rice after zinc application, (iii) identify out colored rice cultivars with strong zinc enrichment ability.

2. Materials and Methods

2.1. Experimental Location and Material

The pot experiments were applied and conducted from May to November 2023 in Pukou District, Nanjing City, Jiangsu Province, China (32°07′29.6″ N 118°38′56.8″ E). This district experiences a subtropical, humid monsoon climate characterized by four distinct seasons and abundant rainfall. The region benefits from both ample moisture and heat, with average annual temperatures ranging from 15 °C to 22 °C. The annual precipitation varies between 800 and 1600 mm, contributing to the region's rich environmental conditions. Additionally, the area sits at an average altitude of about 15 m above sea level, which complements the climatic conditions, supporting a variety of ecosystems and agricultural activities [33]. As for soil collection, we selected five sampling points randomly in the local farmland, and 50 kg of soil was taken for each point. All the five points of soil were thoroughly mixed then. The mixed soil was air-dried and crushed, passing through a 20-mesh sieve. Before the experiment, the soil properties were measured. The physicochemical properties of the soil used for the experiment are presented in Table 1.

Table 1. Properties of the experimental soil.

Parameters	Value
pH	6.365
CEC (cmol/kg)	17.9
Total nitrogen (%)	0.150
Total phosphorus (g/kg)	0.985
Total potassium (g/kg)	21.5
Available phosphorus (mg/kg)	3.3
Available potassium (mg/kg)	188
Zinc (mg/kg)	200
Sand (%)	22.35
Silt (%)	50.37
Clay (%)	27.28
Texture	Clay loam

Seven colored rice with different genetic backgrounds and characteristics were selected for the study, one of which is from East China, under the consideration of unique color and comprehensive characters. For a detailed description, see Table 2. The rice seeds were surface sterilized in 5% (*v*/*v*) H_2O_2 for 30 min, then rinsed with deionized water. Seedling cultivation began on 23 May 2023. On 13 June 2023, the seedlings were transplanted into pots.

Table 2. Information on the place of origin, color, life cycle, and breed type of the seven colored rice cultivars.

Name	Place of Origin	Color	Life Cycle (Day)	Breed Type
GFHN 166	Guangxi	Black	122	Indica-type conventional glutinous rice
GFHN 168	Guangxi	Black	124	Indica-type conventional glutinous rice
GFHN 169	Guangxi	Black	126	Indica-type conventional glutinous rice
GH 1	Guizhou	Red	150	Indica-type conventional non-glutinous rice
GXHZ	Guangxi	Red	122	Indica-type conventional non-glutinous rice
GHSZ	Guangxi	Black	127	Indica-type conventional non-glutinous rice
YXN	Jiangsu	Purple	125	Indica-type conventional non-glutinous rice

2.2. Experimental Layout and Treatments

The experiment used a completely randomized block design with three replications. Each pot (Capacity 10 dm^3) was pre-filled with 5 kg of soil.

Two treatments were set for each of the seven colored rice cultivars: without zinc fertilizer (CK) and with foliar zinc fertilizer (Zinc application). There were a total of 14 treatment groups, each replicated three times, resulting in 42 potted rice in total. Suzhou Selenium Valley Technology Co., Ltd. (Suzhou, China). Supplied the zinc-rich water-soluble fertilizer used in the experiment. According to previous literature [30], the usage rate for zinc foliar application was defined as 400 g/ha in this study, under consideration of the less negative impact on plant growth. According to the calculation, 17 g of zinc (i.e., 100 mL zinc-rich water-soluble fertilizer) was applied to each pot, diluting the water-soluble zinc fertilizer 20 times. On clear and windless days (18 August 2023), foliar zinc fertilizer was applied to the colored rice using a nano-spray bottle, making sure the droplets were evenly covered on each leaf, and zinc fertilizer was sprayed just once during the experiment. The pots were meticulously maintained under consistently saturated conditions throughout the rice cultivation period, ensuring uniform moisture levels to support optimal growth and development of the plants. Urea (0.49 g/pot), mono ammonium phosphate (MAP) (0.26 g/pot), and potassium chloride (0.31 g/pot) were applied at the recommended levels of nitrogen, phosphorus, and potassium, respectively. Urea was used as 45% base fertilizer, 25% tillering fertilizer, and 30% panicle fertilizer. MAP was applied once entirely as a base fertilizer. Potassium chloride was divided evenly, with 50% used as base fertilizer and 50% as panicle fertilizer. On 10 October 2023, rice plants were harvested.

2.3. Plant Height and SPAD Index

At harvest, three representative plants were selected from each pot. Plant height was measured by snugly placing a tape measure at each plant's base and recording the vertical distance from the soil surface to the tip of the highest leaf. The leaf's chlorophyll content was measured accurately, rapidly, and non-destructively using a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan). The three upper leaves of each rice plant were selected, and the middle part of the leaves was measured three times, and the average value was taken as the SPAD value of the leaf; three leaves of each plant were measured repeatedly, and the average value was taken as the SPAD value of the plant.

2.4. Biomass

Whole plants were collected and separated into four parts: root, stem, leaf, and grain at harvest. The samples were washed with distilled water to remove surface soil and other impurities, and the kernels were dehulled and placed in kraft paper bags. The samples were dried to constant weight at 60 °C using an electric blast drying oven (Memmert UF55, Schwabach, Germany). Each part's dry weight was measured using an electronic balance (Mettler Toledo XPR205, Greifensee, Switzerland).

2.5. Protein Content in Grain

An appropriate amount of the sample was weighed and placed in a Kjeldahl digestion tube, along with 0.4 g of copper sulfate, 6 g of potassium sulfate, and 20 mL of sulfuric acid. The sample was initially carbonized at 200 °C until foam production ceased and stabilized. The temperature was then increased to 450 °C, and the sample was heated until the liquid boiled. Once the liquid turned a transparent blue-green color, heating was continued for an additional hour. After cooling, the tube was removed, and water and alkali were added. The liquid was then distilled, with the escaping ammonia being absorbed by boric acid. The total nitrogen content in the sample was determined using a calibrated strong acid standard titration solution based on the volume and concentration of the titrant used. The nitrogen content was subsequently converted to protein content using a conversion factor of 6.25 [34].

2.6. Zinc Content

The separated dry samples were milled into powder and passed through a 100-mesh sieve. A 0.5 g sample of the dry matter was taken for chemical analysis. The zinc content in the dry matter of various plant organs was determined using an Inductively Coupled Plasma Mass Spectrometer (Agilent Technologies Agilent 7900, Santa Clara, CA, USA). HNO_3:$HClO_4$ (4:1) mixed acid-electric hot plate digestion method was used [33]. The sample preparation involved the following steps: the dried sample was ground into a powder, and 0.2 g was weighed on an analytical balance and placed in a beaker. Then, 10 mL of HNO_3:$HClO_4$ (4:1) mixed acid was added, shaken well, and left overnight. The beaker was subsequently heated on an electric heating plate at 120 °C for 1 h and then at 180 °C for 2 h. Once the solution in the beaker became clear and free of residues, the temperature of the heating plate was increased to 210 °C to initiate acid removal. Heating was continued until white fumes appeared, and the solution was reduced to 1–2 mL. The solution was then removed from the heat and cooled to room temperature. Subsequently, the solution was transferred to a 25 mL stoppered volumetric flask, mixed thoroughly, and the zinc content was measured using an Inductively Coupled Plasma Mass Spectrometer.

2.7. Translocation Factor

The rice plant Translocation Factor (*TF*) indicates the plant's ability to transfer zinc from one organ to another. A high *TF* signifies the efficient movement of zinc from one part to another, which may influence the potential for zinc bioaccumulation in the edible part of rice (grain). *TF*s were calculated via the following equations [34]:

$$TF_{a-b} = \frac{Zn_b}{Zn_a}$$

where a and b can represent distinct components of rice, such as the root, stem, leaf, and grain. $TF_{\text{root-stem}}$, $TF_{\text{stem-leaf}}$ and $TF_{\text{leaf-gain}}$ represent the ability of zinc migration from root to stem, stem to leaf, and leaf to grain.

2.8. Zinc Utilization Index

Zinc utilization-related parameters are calculated using the following formulas [30,35,36], which provide a standardized method for assessing plant zinc efficiency. These equations are widely used in studies on nutrient utilization and plant growth [37].

$$HI = \frac{GD_W}{D_W}$$

$$ZnHI = \frac{GU_{Zn}}{U_{Zn}} \times 100\%$$

$$ZnUE = \frac{ZnHI}{HI} \times 100\%$$

where GD_w and D_w represent the biomass (kg) of grain and the whole rice plant, GU_{Zn} and U_{Zn} represent the zinc content (mg/kg) in grain and rice plants, respectively.

2.9. Zinc Health Risk Index

According to previous studies, the health risk index (*HRI*) assessed noncarcinogenic health risks from individual metals and the combined health risks from all the studied metals [30]. If the $HRI \leq 1$, it suggests that zinc-treated rice poses no threat to human health; If the $1 < HRI \leq 10$, it demonstrates that zinc-treated rice poses some level of danger to human health; If the $10 < HRI$, it indicates that zinc-treated rice has chronic toxicity to human health [38,39]. The calculation formula is as follows:

$$HRI = \frac{ADI}{RfD}$$

$$ADI = \frac{C_{Zn} \times DRI \times IF \times ID}{BW \times IT}$$

$$ID = EA - AA$$

HRI is the zinc's health risk index under zinc fertilizer treatment. *RfD* (mg/kg/day) is the reference dose for daily intake, and the *RfD* value for elemental zinc is 0.30 mg/kg/day [40]. *ADI* (mg/kg/day) is the average daily intake for zinc concentrations. C_{Zn} (mg/kg) is the zinc concentration in the grain under zinc treatment. *DRI* (kg/day) is the average daily intake of rice. *IF* (day/a) is the frequency of intake per year. *ID* (a), *EA* (a), and *AA* (a) are duration of intake, life expectancy for adults and children, and average age, respectively. *BW* (kg) and *IT* (day) are meant body weight and total time intake. The values of other parameters in the health risk assessment are shown in Table 3.

Table 3. Parameters values in health risk assessment.

Category	Gender	*DRI* (kg/Day)	*TF* (Day/a)	*EA* (a)	*AA* (a)	*BW* (kg)	*IT* (Day)
Adult	Male	2.5×10^{-1}	365	81.2	46.2	72.0	12,775
	Female	1.9×10^{-1}	350	85.6	48.4	58.7	13,578
Child	Male	1.2×10^{-1}	300	6	3.6	20.7	876
	Female	8.5×10^{-2}	300	6	3.6	19.5	876

Note: *DRI*, *IF*, and *AA* are according to the data from Zhou et al. [38] and Liu et al. [41]. The *EA* is derived from household health data published by the Nanjing City Health Bureau in 2019. *BW* is according to the Communique on Constitution Monitoring about People in Jiangsu Province in 2017 issued by Jiangsu Province Sports Bureau.

2.10. Data Analysis

The data obtained were subjected to statistical analysis using Analysis of Variance (ANOVA), and the significance of differences between treatment groups was determined using the least significant difference (LSD) value at $p < 0.05$ to establish interactions. For detailed visualization, graphs were generated using Origin 2022b (Origin Lab Corporation, Northampton, MA, USA).

3. Results and Discussion

3.1. Zinc Content of Each Organ

The average zinc content (mg/kg) of the seven colored rice grains selected in this experiment was 28.87 mg/kg (dry base) under CK (Table 4). Previous reports have shown that ordinary rice grain is only 12.9 mg/kg [13]. This shows that under normal conditions, the zinc content of colored rice is higher than that of ordinary rice. After the zinc foliar treatment, the average zinc content of the grain climbed to 41.55 mg/kg dramatically, increasing by about 43.92%, indicating that zinc application significantly increased the zinc content of colored rice grain. However, the zinc content of ordinary rice grain can only be increased by 10% after zinc biofortification [9]. This indicates that colored rice has a higher zinc accumulation capacity than ordinary rice. The zinc content of GXHZ was the highest (69.7 mg/kg), followed by GHSZ with a zinc content of 55.4 mg/kg, which broke the zinc-rich rice standard (45 mg/kg) [13]. Generally, significant differences in zinc content among different rice cultivars may also result from variations in zinc uptake from the soil, which are influenced by their distinct growth behaviors [42]. The differences among cultivars and their responses to zinc fertilizer reveal the potential value of plant germplasm resources in plant nutrient management and environmental adaptation strategies. It is crucial for improving the nutrient use efficiency of rice cultivars, especially in micronutrient management.

Specifically, in the zinc content of grain (Figure 1a), most cultivars showed a significant increase after zinc application, having a 1.58, 1.65, 1.42, 1.40, 1.92, 1.62, and 1.60-fold times increase, respectively. This indicates that foliar zinc application effectively increases the zinc content in the grains of colored rice, with GXHZ showing a significant increase (92.13%), possibly due to a genetic advantage in nutrient absorption and transport efficiency. In the zinc content of the leaf (Figure 1b), all cultivars showed a substantial increase after zinc application, having a 15.29, 14.20, 12.81, 10.06, 18.67, 19.99, and 13.86-fold times increase, respectively. In the zinc content of stem (Figure 1c), all cultivars showed significant differences after zinc application, having a 4.62, 3.31, 5.78, 1.85, 3.11, 3.40, and 3.38-fold times increase, respectively. In the zinc content of root (Figure 1d), just a few cultivars (GH 1, GXHZ, and GHSZ) showed significant differences after zinc application, having a 1.02, 1.12, 1.10, 1.20, 1.41, 1.31 and 1.19-fold times increase, respectively. These results indicated that there may be significant differences in the response of foliar zinc application between cultivars and organs.

Table 4. The average values of grain zinc content, grain biomass, grain zinc accumulation, plant height, SPAD, and grain protein content of seven colored rice cultivars under CK and zinc application.

Treatment	Grain Zinc Content (mg/kg)	Grain Biomass (g/pot)	Grain Zinc Accumulation (mg/pot)	Plant Height (cm)	SPAD	Grain Protein Content (%)
CK	28.87 ± 3.33 b	45.73 ± 2.88 b	1.38 ± 0.18 a	113.48 ± 0.92 a	41.95 ± 0.54 b	8.25 ± 0.27 a
Zinc application	41.55 ± 1.94 a	51.87 ± 0.82 a	2.28 ± 0.14 a	114.86 ± 1.31 a	42.85 ± 0.13 a	8.49 ± 0.18 a

Note: Different lowercase letters in the table indicate significant differences between treatments ($p < 0.05$).

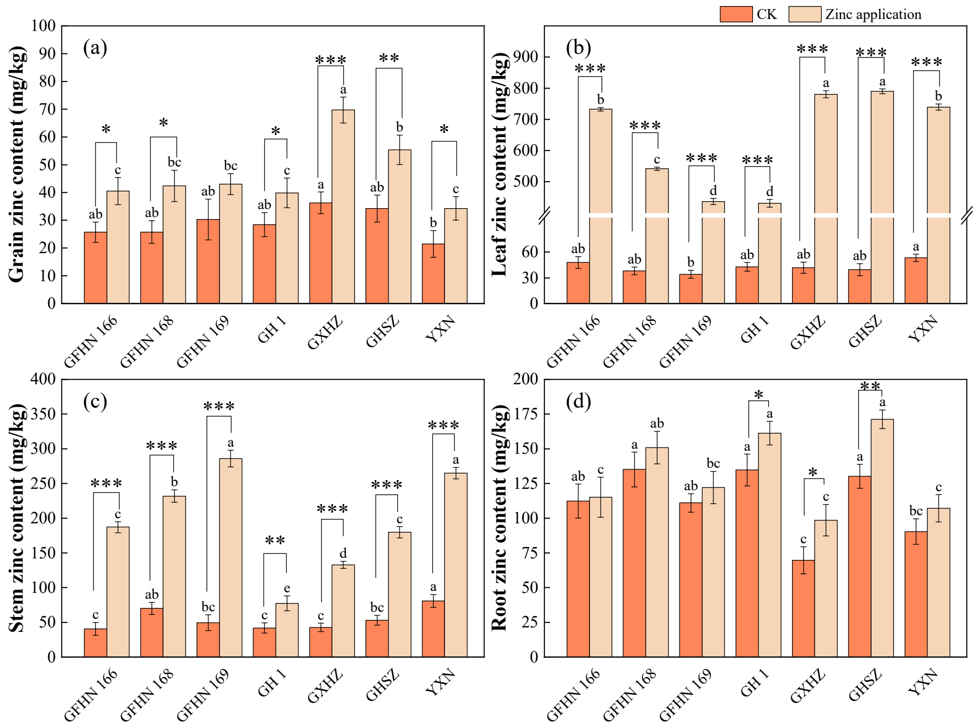

Figure 1. Effect of zinc application on zinc content in grain (**a**), leaf (**b**), stem (**c**), and root (**d**) of colored rice. Different lowercase letters (a–e) on the bar graphs indicate significant differences between cultivars ($p < 0.05$). The significance levels of *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$ indicate substantial changes in the zinc content between CK and zinc application.

The internal distribution and retention of zinc in different plant organs play a key role in the accumulation of zinc in grain [43]. The zinc content in different rice organs is affected by factors such as rice cultivars and complex mechanisms [44]. In this study, under CK, the distribution of zinc content in different organs was generally leaf > stem > root > grain, which was consistent with the study of Khanam et al. on the order of absorption, transport, and accumulation of zinc obtained from the soil in different organs of rice [45]. Under zinc application, the distribution is generally leaf > stem > root > grain. This was a discrepancy with the results of previous studies. Regardless of the treatment, the zinc content in the remaining organs was higher than in the grain, reflecting the low mobilization of zinc from different organs to the grain, resulting in the lowest zinc content [35]. For different cultivars, the effect of foliar zinc application on zinc enrichment in rice grains showed noticeable cultivar differences [35]. The internal allocation of different organs appears to be regulated in different ways, with foliar zinc spraying in which the plant first allocates additional zinc to vegetative organs (leaf and stem) with relatively low metabolic activity [46].

3.2. Biomass of Each Organ

Among the seven cultivars of colored rice, the distribution of biomass (g/pot) across different rice organs generally follows the order of root > grain > stem > leaf. In grain biomass (Figure 2a), all cultivars except GFHN 166 and GHSZ exhibited an increase under zinc spraying treatment, with GH 1 and GXHZ showing the most significant increases. In leaf biomass (Figure 2b), all cultivars except GFHN 169 and GHSZ demonstrated an increase under zinc spraying treatment, with GH 1 and GXHZ again showing the most notable increases. In stem biomass (Figure 2c), GFHN 166, GFHN 168, GFHN 169, and YXN experienced a decrease under zinc spraying treatment, while GH 1, GXHZ and GHSZ showed an increase. In root biomass (Figure 2d), all cultivars exhibited an increase under zinc spraying treatment compared to the non-zinc treatment. These findings reveal notable variations in the response of different colored rice cultivars to foliar zinc application. Some cultivars (GH 1 and GXHZ) responded positively to zinc spraying, particularly in the accumulation of biomass in grain and leaf, while other cultivars (GFHN 166 and GHSZ) showed limited responses.

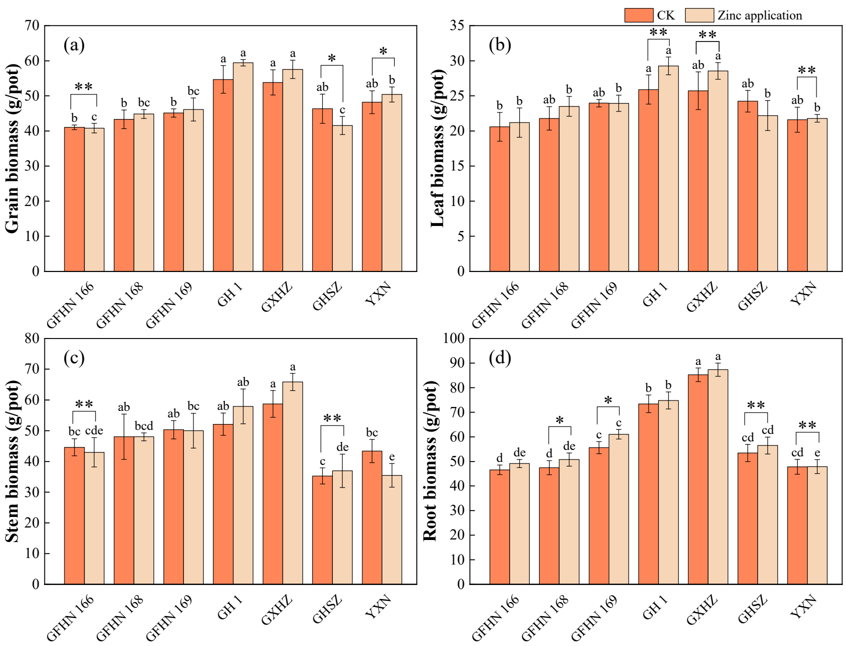

Figure 2. Effects of zinc application on biomass weight of grain (**a**), leaf (**b**), stem (**c**), and root (**d**) of colored rice. Different lowercase letters (a–e) on the bar graphs indicate significant differences between cultivars ($p < 0.05$). The significance levels of, ** $p < 0.01$, and * $p < 0.05$ indicate that there are substantial changes in the biomass between CK and zinc application.

Regarding the overall benefit of zinc spraying, foliar zinc spraying significantly increased the biomass of rice, which was consistent with the results of Zhang et al.'s [24] study that the most significant biomass accumulation period was the most significant period for rice at the heading stage. More biomasses could be collected by applying zinc fertilizer. This may be due to the role of zinc in plants and the increased activity of several enzymes that promote vegetative growth and photosynthesis [47]. In this study, the effect on stem and leaf biomass quality varies from variety to variety. The significance might be due to their varietal genetic potential, the uptake of nutrients, competition for space, light, nutrients, and differential plant height [48]. Further research is needed on the changes in biomass quality in different cultivars' organs.

3.3. Zinc Accumulation in Each Organ

Table 4 indicated that the average zinc accumulation (mg/pot) of the seven colored rice grains selected in this experiment was 1.38 mg/pot under CK, and the average zinc accumulation in the grain after zinc application was 2.28 mg/pot, with an increase of 65.22%. This is consistent with previous conclusions that foliar zinc application can increase grain and bioavailable zinc accumulation [49]. Among the seven cultivars of colored rice, overall, under CK, the distribution of zinc accumulation across different organs generally follows the order of root > stem > grain > leaf. Under zinc application, the distribution changes to leaf > stem > root > grain. In the zinc accumulation in grain (Figure 3a), all cultivars showed an increase after zinc application, having a 1.57, 1.72, 1.45, 1.52, 2.06, 1.43, and 1.65-fold times increase, respectively, with GXHZ showing a notable increase (1.06 times). In the zinc accumulation in the leaf (Figure 3b), all cultivars showed substantial increases after zinc application, having a 15.89, 15.40, 12.80, 11.30, 20.50, 18.21, and 13.93-fold times increase, respectively. In the zinc accumulation in stem (Figure 3c), all cultivars showed significant differences after zinc application, having a 4.44, 3.35, 5.80, 2.09, 3.47, 3.55, and 2.68-fold times increase, respectively. In the zinc accumulation in the root (Figure 3d), all cultivars showed significant differences after zinc application, having a 1.08, 1.20, 1.21, 1.22, 1.45, 1.39, and 1.18-fold times increase, respectively. Zinc accumulation is a complex physiological trait governed by the cumulative expression of uptake, transport, distribution, and sequestration in different rice organs. Zinc concentration varies between organs, tissues, and intracellular compartments within a rice system. This divergence arises due to the differential expression of metal transporter proteins (MTPs, ZIPs, VITs) and intracellular binding sites in a particular organ. The morphological characteristics of rice organs considerably impact plants' absorption and accumulation capacity. Several other factors, such as rice cultivars, growth stage, biomass, zinc concentration in soil, etc., also contribute to changes in zinc accumulation levels [50].

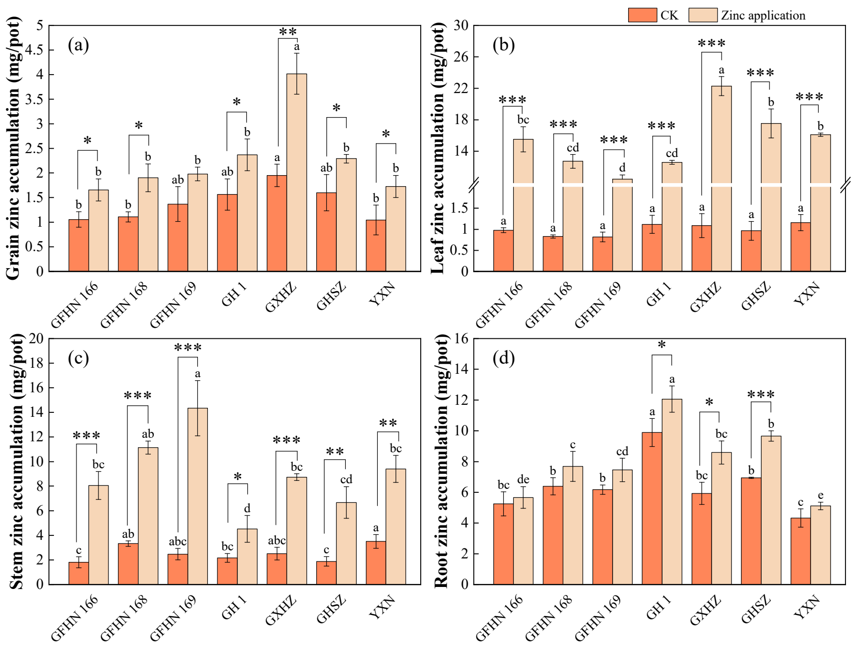

Figure 3. Effects of zinc application on zinc accumulation in grain (**a**), leaf (**b**), stem (**c**), and root (**d**) of colored rice. Different lowercase letters (a–e) on the bar graphs indicate significant differences between cultivars ($p < 0.05$). The significance levels of *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$ indicate substantial changes in the zinc accumulation between CK and zinc application.

3.4. Translocation Factor

Under the treatment of CK (Figure 4a), the plant translocation factor of different cultivars showed significant differences. It appears to be primarily confined to the plant's above-ground parts, with a notable zinc enrichment in the grain. With the treatment of zinc application (Figure 4b), The translocation factor of leaf-to-grain was significantly increased in all cultivars. Applying zinc fertilizer significantly improved the absorption and transport capacity of zinc in rice, which meant that zinc was efficiently transported from leaf to grain, which may affect the bioaccumulation potential of zinc in the edible part of rice.

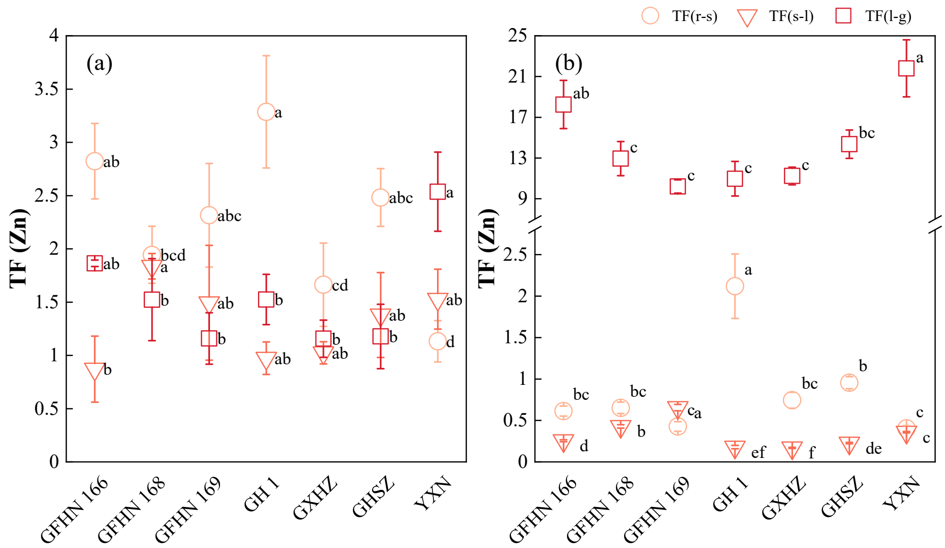

Figure 4. The translocation factor of root-to-stem, stem-to-leaf, and leaf-to-grain of seven colored rice cultivars under CK (**a**) and zinc application (**b**). Treatments were tested by LSD ($p < 0.05$). Different lowercase letters (a–f) on the bar graphs indicate significant differences between cultivars ($p < 0.05$).

In this experiment, we found that different cultivars had different responses to zinc transport from root to stem, stem to leaf, and leaf to grain under zinc application, indicating that the zinc absorption effect and zinc application source of different cultivars and organs were quite different. Differences in zinc uptake by different cultivars can also be attributed to different mechanisms within the rhizosphere and plant systems [51]. Another possible reason is the protective effect of preventing anti-superoxide radicals in soil, which improves plants' uptake, differential utilization, and zinc transport [52,53]. Kobayashi's study showed that nicotinamide efflux transport genes (ENA1 and ENA2) play a key role in the uptake and transport of zinc in plants, which determines the transport efficiency of plants. They also mentioned that the efficient transport of zinc in cereals is aided by increasing the levels of DMA (2-deoxymugineic acid) and NA (niacinamide) [54].

3.5. Zinc Utilization Index

Within the seven colored rice cultivars, *HI* (%) ranged from 24.06% to 32.39%, *ZnHI* (%) ranged from 2.99% to 6.44%, and *ZnUE* (%) rate ranged from 9.23% to 26.79% (Table 5). YXN exhibited the highest *HI* but the lowest *ZnHI* and *ZnUE*. This suggests that while YXN effectively allocates a significant proportion of biomass to the grain, the zinc content within the grain is relatively low. *ZnHI* and *ZnUE* of GXHZ and GH 1 were the highest, indicating that these two cultivars had strong zinc enrichment capacity and could be used as biofortified crops, especially in those areas where the soil lacked essential trace elements, which could improve the nutritional value of grain without increasing external inputs and help achieve the goal of sustainable agriculture.

Table 5. Harvest index, zinc harvest index, and zinc use efficiency of seven colored rice cultivars after zinc application.

Cultivar	Harvest Index (*HI*)%	Zinc Harvest Index (*ZnHI*)%	Zinc Use Efficiency (*ZnUE*)%
GFHN 166	26.48	3.77	14.24
GFHN 168	26.82	4.38	16.33
GFHN 169	25.47	4.84	19.02
GH1	26.84	5.62	20.93
GXHZ	24.06	6.44	26.79
GHSZ	26.44	4.63	17.50
YXN	32.39	2.99	9.23

HI was an important productivity index. It is an essential trait for the dramatic increase in crop yields in the 20th century. It reflects the distribution of photosynthesis between grain and vegetative plants. Zinc treatment promotes the remobilization and accumulation of biomass from source to reservoir [55]. *ZnHI* was an important production index of zinc distributed to grain. The *ZnHI/HI* ratio was used as an indicator to measure the difference in *ZnUE* of rice [35]. There is usually a negative correlation between grain weight and grain micronutrient concentration [56]. Enhancement of growth or increased production often reduces micronutrient concentrations, even when total absorption rises [57]. Cultivars with high *HI* had lower grain zinc concentrations than those with low *HI*. Therefore, while screening cultivars with strong zinc enrichment ability, grain yield must be closely monitored to select cultivars with the intrinsic ability to improve grain zinc accumulation.

3.6. Plant Height

Among the seven cultivars of colored rice, zinc application resulted in an increase in rice height (cm) across all cultivars compared to CK (Table 4), with respective increases of 3.76%, 3.12%, 3.23%, 2.28%, 11.22%, 9.80% and 4.32% respectively (Figure 5a). GXHZ demonstrated the most pronounced increase in plant height at 11.22%, reflecting its heightened sensitivity to zinc application and its exceptional efficacy in utilizing the nutrient for growth.

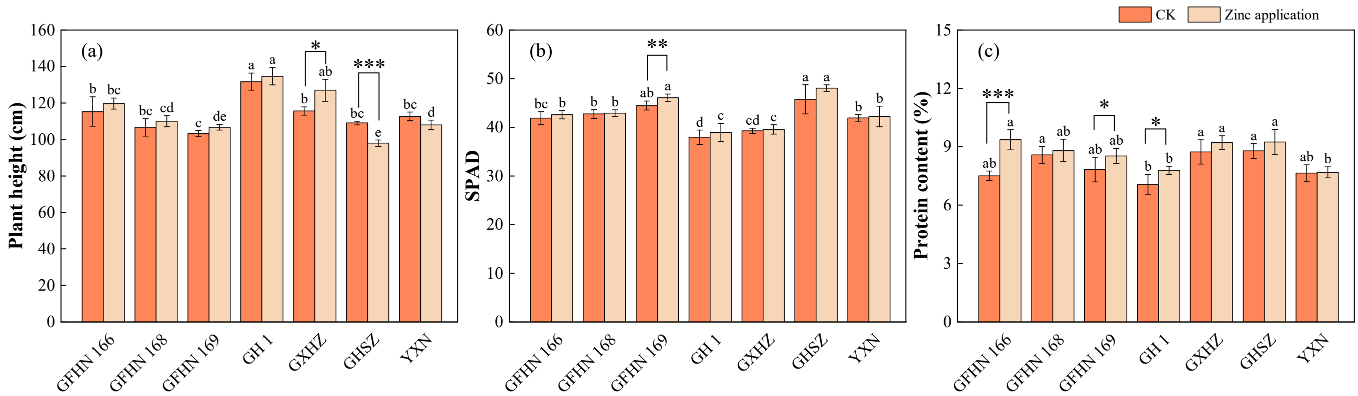

Figure 5. Effects of zinc application on plant height (**a**), SPAD (**b**), and protein content (**c**) of colored rice. Different lowercase letters (a–e) on the bar graphs indicate significant differences between cultivars ($p < 0.05$). The significance levels of *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$ indicate substantial changes in the plant height, SPAD, and protein content between CK and zinc application.

Zinc is the basis for rice's growth and quality formation [58]. As one of the spontaneous indicators of plant growth, the change in plant height reflects plants' physiological and ecological response to changes in environmental conditions. Among them, the plant height increased significantly, which is consistent with the conclusion of Chattha et al. that zinc application can significantly improve rice growth [59]. Similar findings were reported by Tuiwong et al. [47]. Khan showed that the increase in plant height after zinc application may be due to the abundant supply of zinc, which helps accelerate plants' enzyme activity and auxin metabolism [48].

3.7. SPAD Value

The SPAD value of colored rice under CK was about 41.95, and that under zinc application was about 42.85, increased by 2.15% after zinc application (Table 4). As for each of the seven cultivars, the SPAD values were 42.58, 42.91, 46.09, 38.92, 39.56, 48.03, and 42.24, with an increase of 1.67%, 0.38%, 3.60%, 2.56%, 0.75%, 4.95% and 0.72% respectively (Figure 5b). The results of this experiment showed that the SPAD value of rice leaf under zinc application was higher than that of CK, indicating that zinc fertilizer spraying could increase the chlorophyll content of rice leaf. Faizan et al. [60] also reported that the chlorophyll content of tomato plants increased after zinc treatment.

The SPAD value is an important indicator reflecting chlorophyll content and nitrogen nutritional status in plants, and it can indirectly assess photosynthesis and nutrient status [61]. The growth and development of rice are highly dependent on photosynthesis, which is one of the key physiological processes in rice [62]. As the primary organ for photosynthesis in plants, the chlorophyll content in leaves effectively indicates their nutritional status and is a reliable measure of leaf senescence. As a component of numerous plant enzymes, zinc is involved in synthesizing chlorophyll and auxin and synthesizing and converting carbohydrates [3]. Therefore, increasing zinc content can enhance photosynthesis and photosynthetic efficiency in rice, thereby increasing SPAD values [24].

3.8. Protein Content of Grain

The results showed that zinc application increased the protein content (%) of colored rice grain by 2.91% (Table 4), and each cultivar increased by 24.81%, 2.69%, 8.94%, 10.28%, 5.49%, 5.22%, and 0.62%, respectively. (Figure 5c). Foliar application of zinc at the heading stage significantly increased grain protein content. Similar results were reported by Morshedi and Farah [61], who showed that wheat grain protein content was elevated after applying a certain amount of zinc fertilizer compared to CK.

Protein content is essential to rice's cooking and eating quality [63]. Higher protein concentrations are considered beneficial for human health. Zinc activates different enzymes and protein synthesis and decomposition sugars, improving the overall quality of rice grain [64]. Studies have shown that zinc enhances the quality traits of rice by promoting an increase in the leaf area index [59]. Additionally, zinc facilitates the transfer of assimilates to the grain and activates the activity of protein synthase, thereby increasing the protein content in the grain [55,65].

3.9. Zinc Health Risk Assessment

The health risk index, described by the percentage of the safe value, was used for the risk assessment. The grain health risk index of different rice cultivars under zinc treatment is shown in Table 6. The health risk index of different zinc applications was <1. It indicated that there was no risk of zinc-treated grains on human health. During the mineral element fortification, it is worth mentioning that determining a suitable dosage for application is crucial. In this study, the foliar application usage of zinc is 400 g/ha, which is appropriate for zinc biofortification and plant growth and has no harmful health risks.

Table 6. Health risk assessment on rice grains of different rice cultivars under zinc treatment.

Cultivars	Adult		Young Child	
	Male	Female	Male	Female
GFHN 166	0.47 ± 0.06 c	0.42 ± 0.05 c	0.64 ± 0.08 c	0.48 ± 0.06 c
GFHN 168	0.49 ± 0.07 bc	0.44 ± 0.06 bc	0.67 ± 0.09 bc	0.51 ± 0.07 bc
GFHN 169	0.50 ± 0.04 bc	0.44 ± 0.04 bc	0.68 ± 0.06 bc	0.51 ± 0.05 bc
GH 1	0.46 ± 0.06 c	0.41 ± 0.06 c	0.63 ± 0.09 c	0.48 ± 0.06 c
GXHZ	0.81 ± 0.05 a	0.72 ± 0.05 c	0.91 ± 0.07 a	0.83 ± 0.06 a

Table 6. *Cont.*

Cultivars	Adult		Young Child	
	Male	Female	Male	Female
GHSZ	0.64 ± 0.06 b	0.57 ± 0.05 b	0.88 ± 0.08 b	0.66 ± 0.06 b
YXN	0.40 ± 0.05 c	0.35 ± 0.04 c	0.54 ± 0.07 c	0.41 ± 0.05 c

Note: In each column, different lowercase letters (a–c) indicate statistically significant differences at the 0.05 level between different cultivars or breeds.

4. Conclusions

Combining the foliar application of exogenous zinc with the utilization of specific cultivar resources to realize rice zinc-rich is a significant exploration. Our study showed that colored rice has more zinc enrichment potential than ordinary rice. Foliar zinc application at 400 g/ha significantly increased the zinc content of colored rice, breaking through the zinc-rich standard of 45 mg/kg while improving the agronomic traits and nutritional value without causing health risks for humans. The cultivars of GXHZ and GHSZ have better performance on the zinc absorption and accumulation that could be applied to the next zinc-enrich rice production and provide an additional resolution for tackling the hidden hunger problem.

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