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Employ the Colored Rice Cultivars under the Foliar Biofortification to Facilitate the Zinc Enrichment

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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 this target is a novel attempt that has rarely been reported. In this study, the effects of foliar application of water-soluble zinc-rich fertilizer (400 g/ha Zn) on seven different cultivars (GFHN 166, GFHN168, GFHN 169, GH 1, GXHZ, GHSZ and YXN) of colored rice at heading stage were investigated through pot experiment. The result indicated that foliar biofortification significantly increased the zinc content of colored rice grains by 43.89%, and the zinc accumulation in grains increased by 64.50%. In addition, the SPAD value and grain protein content also increased significantly by 2.15% and 2.86%, respectively. Among these, GXHZ and GHSZ could realize the zinc content of polished rice up to 69.7 mg/kg and 55.4mg/kg, breaking through the standard of zinc-enrich rice (45mg/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. And zinc-treated colored rice at the concentration used in this study has no risk to human health. Combining the foliar application of exogenous zinc with the utilization of specific cultivars resources could realize zinc-enrich rice production, and provide an additional resolution for tackling the hidden hunger problems.

Keywords: colored rice; foliar zinc application; zinc content; plant height; biomass; SPAD values; protein content

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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 [7,8]. 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 [9,10].

Rice 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 [10,11]. More than half of the world's population currently depends on rice as a staple food [12]. 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 [10]. 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) [13]. It is also unable to meet the body's zinc requirement of 11 mg (male) or 8 mg (female) per day [14]. 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 it is recognized as a functional food that promotes human health [15,16]. In recent years, colored rice has gained momentum on the international market, with growing demand worldwide [17].

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, and the grain zinc content of seventeen purple-milled rice cultivars in Thailand ranged from 19.0 ~ 41.3 mg/kg [18,19], which showed that the color rice had a solid ability to enrich zinc when compared with the ordinary rice [20]. However, there is still a particular gap between the previous studies and the level of zinc-rich standard. Exploring more rice germplasm or varieties to achieve zinc enrichment is a significant attempt. South China is the main region for rice cultivation [21]. Guizhou, Yunnan and Guangxi in southern China are among the world's largest centers of rice genetic diversity and high-quality germplasm [22–24]. Landraces, especially for some colored rice varieties, are rich in genetic diversity due to their effectiveness in farmland conservation and promotion of allele variation [25]. Hence, utilizing more resources of colored rice from South China to improve zinc accumulation become a growing concern for researchers and farmers [23,24].

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, along with processing quality, nutritional value and cooking taste [26]. However, most zinc fertilizer trials are conducted against a backdrop of managing zinc deficiency, with few studies related to zinc biofortification [27]. Biofortification employs three primary strategies: agronomic practices, traditional breeding, and genetic engineering [28]. Agronomic practice, a fertilizer-driven method [29], involves applying basal or foliar sprays to crops, enabling direct uptake of essential trace elements [30]. 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 [31,32]. According to previous reports, zinc application rates between 450 - 500 g/ha are most appropriate for rice [33]. Since too high a zinc concentration will have a certain toxic effect on plants, and too low will have a negative impact on crop yield and quality [34].

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 achieve (i) analyze the distribution and transportation of zinc in colored rice, (ii) identify colored rice with strong zinc enrichment ability, (iii) explore a viable solution to the problem of "hidden hunger".

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). The collected soil was air-dried and then crushed, all through a 20-mesh sieve. Before the experiment, the soil properties were measured. The soil textural class was clay loam contained 22.35% sand, 50.37% silt, 27.28% clay, 200 mg/kg Zn, 6.365 pH, 17.9 cmol/kg CEC, 0.150% total nitrogen, 0.985 g/kg total phosphorus, 21.5 g/kg total potassium, 3.3 mg/kg available phosphorus and 188 mg/kg available potassium.

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 1. The rice seeds were surface sterilized in 5% (v/v) H_2O_2 for 30 minutes, then rinsed with deionized water. Seedling cultivation began on May 23, 2023. On June 13, 2023, the seedlings were transplanted into pots.

Table 1. 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 (27 cm in diameter and 23 cm in height) was pre-filled with 5 kg of soil.

Two treatments were set: 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. On clear and windless days, foliar zinc fertilizer was applied to the colored rice using a nano-spray bottle. Suzhou Selenium Valley Technology Co., Ltd supplied the zinc-rich water-soluble fertilizer used in the experiment. According to previous pieces of literature [32], the usage rate for zinc foliar application was defined as 400 g/ha in this study under consideration less negative impact on plant growth. 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), monoammonium 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.

2.3. Plant Height and Chlorophyll 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, Japan).

2.4. Biomass

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

2.6. Zinc Content

The separated dry samples were then 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, America). HNO_3:$HClO_4$ (4:1) mixed acid-electric hot plate digestion method was used. 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 hour and then at 180 °C for 2 hours. Once the solution in the beaker became clear and free of precipitates, 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:

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

a and b can represent distinct components of rice, such as the root, stem, leaf and grain. $TF_{root\text{-}stem}$, $TF_{stem\text{-}leaf}$ and $TF_{leaf\text{-}gain}$ 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 [32–34], which provide a standardized method for assessing plant zinc efficiency. These equations are widely used in studies on nutrient utilization and plant growth [35].

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

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

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

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*) was used to assess noncarcinogenic health risks from individual metals and the combined health risks from all the studied metals [32]. If the $HRI \leq 1$, it indicates that zinc-treated rice poses no threat to human health; If the $1 < HRI \leq 10$, it indicates 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 [36,37]. The calculation formula is as follows:

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

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

$$ID = EA - AA$$

where, *HRI* is the health risk index of zinc under zinc fertilizer treatment. *RfD* (mg/kg/d) is the reference dose for daily intake and the *RfD* value for elemental zinc is 0.30 mg/kg/d [38]. *ADI* (mg/kg/d) is the average daily intake for zinc concentrations. C_{Zn} (mg/kg) is the zinc concentration in the grain under zinc treatment. *DRI* (kg/d) is the average daily intake of rice. *IF* (d/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* (d) are meant body weight and total time intake. The values of other parameters in the health risk assessment are shown in Table 2.

Table 2. Parameters values in health risk assessment.

Category	Gender	*DRI* (kg/d)	*TF* (d/a)	*EA* (a)	*AA* (a)	*BW* (kg)	*IT* (d)
Adult	Male	2.5×10^{-1}	365	81.2	46.2	72.0	12775
	Female	1.9×10^{-1}	350	85.6	48.4	58.7	13578
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. [36] and Zhu et al. [39]. 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. Statistical Data Treatment

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), a widely recognized data analysis and graphing tool.

3. Results and Discussion

3.1. Zinc Content of Each Organ

The average zinc content of the seven colored rice grains selected in this experiment was 28.87 mg/kg under CK (Table 3), after the zinc foliar treatment, the average zinc content of the grain climbed to 41.55 mg/kg dramatically ($p<0.05$), increased by about 40%, indicating that zinc application significantly increased the zinc content of rice grain, and have a better performance when compared with the zinc content of ordinary rice grains at around 12.9 mg/kg [14]. 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 [46,47]. 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 important for improving the nutrient use efficiency of rice cultivars, especially in micronutrient management.

Table 3. 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)	Grain zinc accumulation (mg)	Plant height (cm)	SPAD	Grain protein content (%)
CK	28.87±3.33b	45.73±2.88b	1.38±0.18a	113.48±0.92a	41.95±0.54b	8.25±0.27a
Zinc application	41.55±1.94a	51.87±0.82a	2.28±0.14a	114.86±1.31a	42.85±0.13a	8.49±0.18a

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

Specifically, in the zinc content of grain (Figure 1a), most cultivars showed a significant increase after zinc application, with increases of 57.76%, 64.64%, 42.12%, 40.23%, 92.13%, 61.67% and 59.53%, respectively. This indicates that foliar zinc application is effective in increasing 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, with increases of 1428.72%, 1319.57%, 1181.01%, 905.61%, 1766.72%, 1898.96%, and 1286.33%, respectively. In the zinc content of stem (Figure 1c), all cultivars showed significant differences after zinc application, with increases of 361.87%, 230.84%, 477.76%, 85.44%, 211.23%, 239.65% and 228.16%, respectively. In the zinc content of root (Figure 1d), just a few cultivars (GH 1, GXHZ, and GHSZ) showed significant differences after zinc application, with increases of 2.49%, 11.71%, 9.95%, 19.68%, 41.48%, 31.47% and 18.57%, respectively. These results indicated that there may be significant differences in the response of foliar zinc application between cultivars and organs.

The internal distribution and retention of zinc in different organs of the plant play a key role in the accumulation of zinc in grain [40]. The zinc content in different organs of rice is affected by factors such as rice cultivars and complex mechanisms [41]. 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 [42]. 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 that in the grain, reflecting the low mobilization of zinc from different organs to the grain, resulting in the lowest zinc content in the grain [33]. For different cultivars, the effect of foliar zinc application on zinc enrichment in rice grains showed obvious cultivar differences [33]. 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 [43].

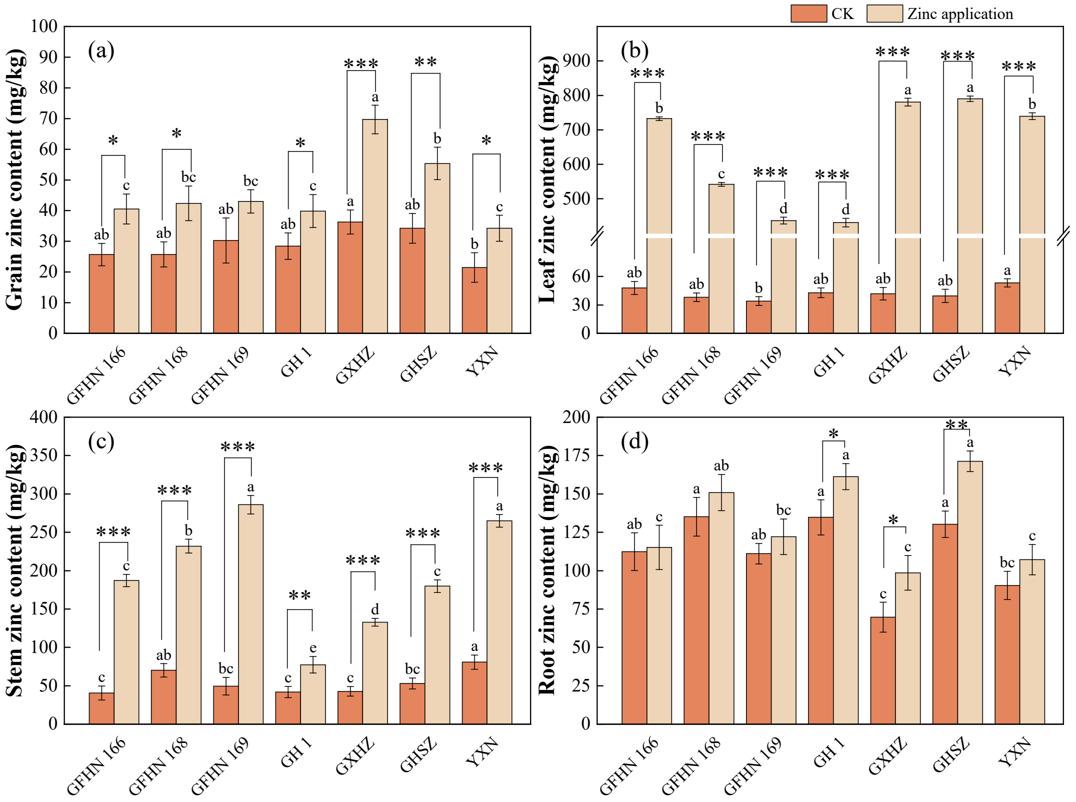

Figure 1. Effect of zinc application on zinc content in grain (a), leaf (b), stem (c) and root (d) of colored rice. Different lowercase letters on the bar graphs indicate significant differences between cultivars ($p<0.05$). The significance levels of ***$p \leqslant 0.001$, **$p \leqslant 0.01$ and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

3.2. Biomass of Each Organ

Among the seven cultivars of colored rice, the distribution of biomass across different rice organs generally follows the order: 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 3c), 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 3d), 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.

In terms of 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 [26] study that the most significant biomass accumulation period was the most significant period for rice at the heading stage, and more biomass 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 [48]. 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 [49]. Further research is needed on the changes in the quality of biomass in different organs of different cultivars.

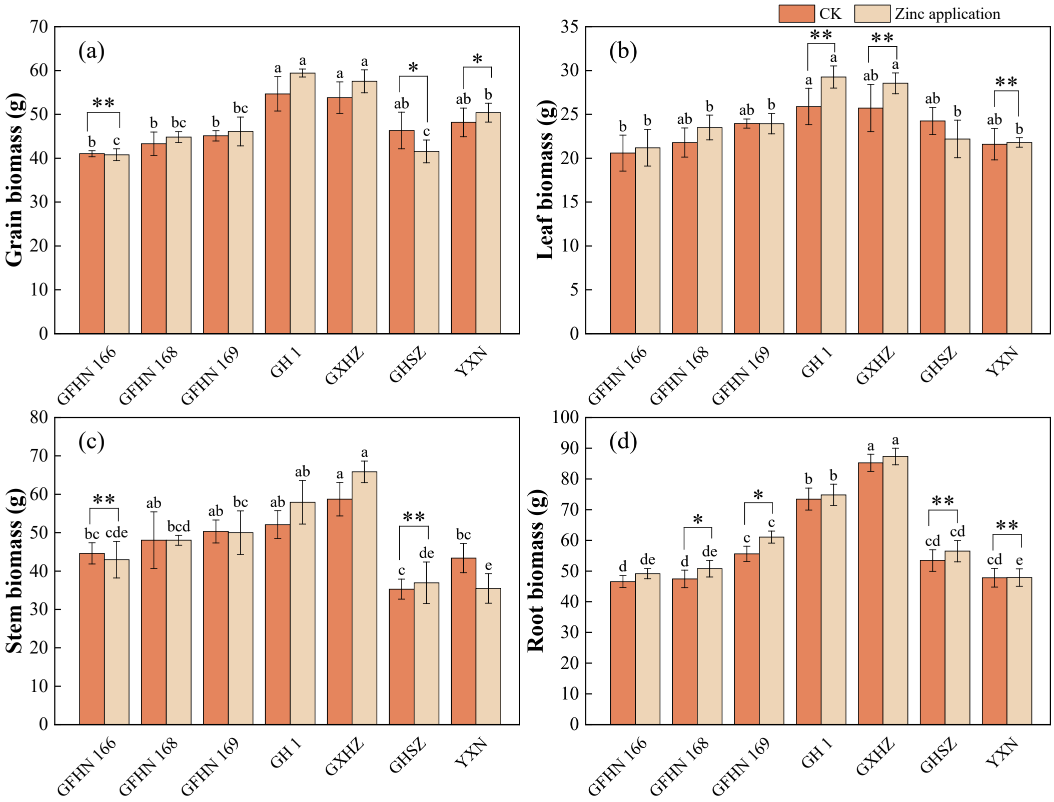

Figure 2. Effects of zinc application on biomass weight of grain (a), leaf (b), stem (c) and root (d) of colored rice. Different lowercase letters on the bar graphs indicate significant differences between cultivars ($p<0.05$). The significance levels of ***$p \leqslant 0.001$, **$p \leqslant 0.01$ and *$p \leqslant 0.05$ indicate that there are substantial changes in the biomass between CK and zinc application.

3.3. Zinc Accumulation in Each Organ

Table 3 indicated that the average zinc accumulation of the seven colored rice grain selected in this experiment was 1.38 mg under CK, and the average zinc accumulation in the grain after zinc application was 2.28 mg with an increase of 70%. This is consistent with previous conclusions that foliar zinc application can increase grain zinc accumulation and bioavailable zinc [50]. Among the seven cultivars of colored rice, overall, under CK, the distribution of zinc accumulation across different organs generally follows the order: 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, with respective increases of 56.92%, 71.75%, 44.50%, 51.59%, 105.87%, 43.27% and 65.23%, with GXHZ showing a notable increase (105.87%). In the zinc accumulation in leaf (Figure 3b), all cultivars showed substantial increases after zinc application, with increases of 1488.64%, 1440.38%, 1179.79%, 1029.90%, 1949.76%, 1720.71% and 1292.82%. In the zinc accumulation in stem (Figure 3c), all cultivars showed significant differences after zinc application, with increases of 344.23%, 234.58%, 479.58%, 108.75%, 247.29%, 254.76% and 167.92%. In the zinc accumulation in the root (Figure 3d), all cultivars showed significant differences after zinc application, with increases of 7.80%, 20.06%, 20.83%, 21.96%, 44.90%, 39.16%, and 18.24%. Accumulation of zinc is a complex physiological trait which is governed by 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 have a huge impact on the absorption and accumulation capacity of plants. Several other factors, such as rice cultivars, growth stage, biomass, zinc concentration in soil, etc., also contribute to changes in zinc accumulation levels [44].

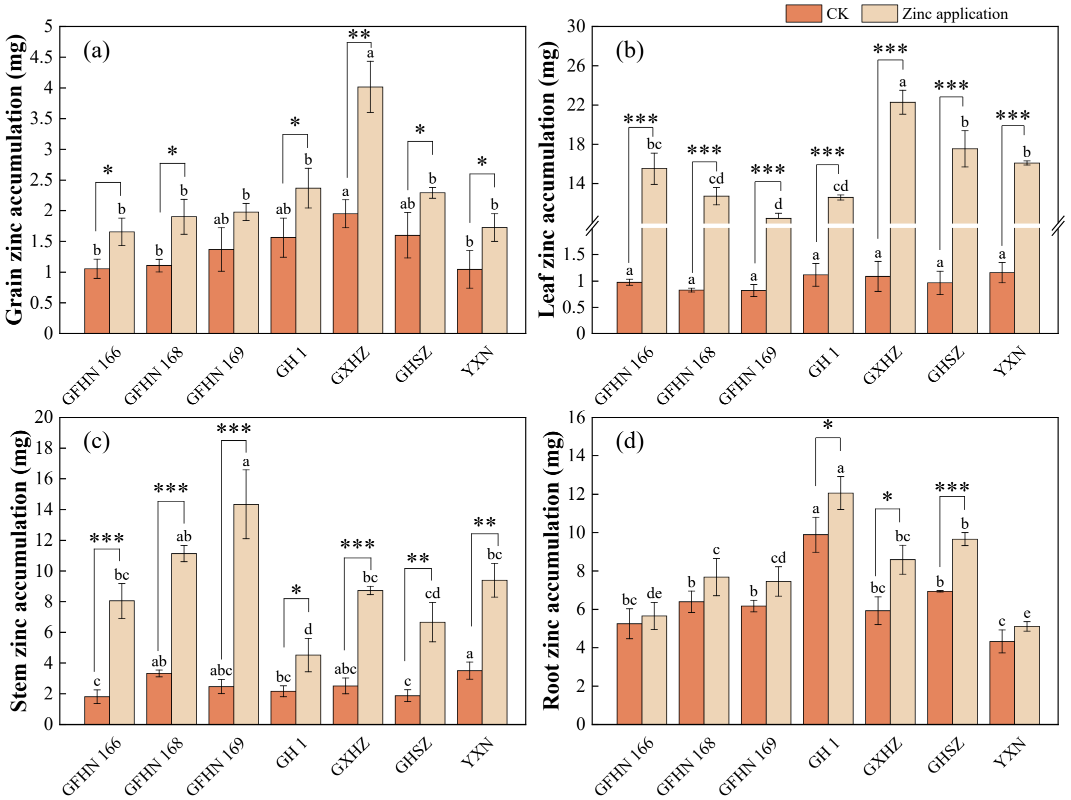

Figure 3. Effects of zinc application on zinc accumulation in grain (a), leaf (b), stem (c) and root (d) of colored rice. Different lowercase letters on the bar graphs indicate significant differences between cultivars ($p<0.05$). The significance levels of ***$p \leqslant 0.001$, **$p \leqslant 0.01$ and *$p \leqslant 0.05$ indicate that there are 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. Appears to be primarily confined to the above-ground parts of the plant, with a notable enrichment of zinc in the grain. With the treatment of zinc application (Figure 4b), The translocation factor of leaf-to grain was significantly increased in all cultivars. The application of 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.

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 different 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 the uptake, differential utilization, and transport of zinc by plants [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].

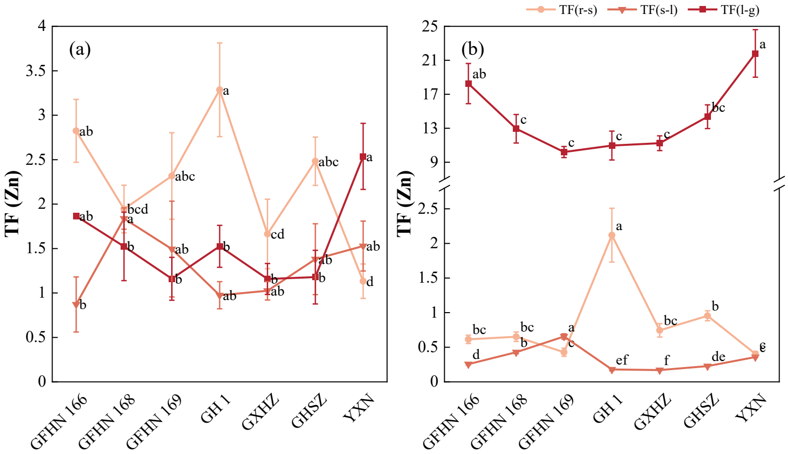

Figure 4. The translocation factor of root-to-stem, stem-to-leaf and leaf-to-grain of seven colored rice cultivars under CK (a) and zinc application (b). Treatments were tested by LSD ($p<0.05$).

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 4). 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.

HI was an important productivity index. It is an important trait for the dramatic increase in crop yields in the 20th century [55]. It reflects the distribution of photosynthesis between grain and vegetative plants [47]. Zinc treatment promotes the remobilization and accumulation of biomass from source to reservoir [56]. *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 [33]. There is usually a negative correlation between grain weight and grain micronutrient concentration [57]. Enhancement of growth or increased production often reduces micronutrient concentrations, even when total absorption increases [58]. That is, cultivars with a high *HI* had lower grain zinc concentrations than cultivars with a 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.

Table 4. 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

3.6. Plant Height

Among the seven cultivars of colored rice, zinc application resulted in an increase in rice height across all cultivars compared to CK (Table 3), 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.

Zinc is the material basis for the growth and quality formation of rice [59]. As one of the spontaneous indicators of plant growth, the change in plant height reflects the physiological and ecological response of plants 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 [60]. Similar conclusions were reported by Tuiwong et al [48]. Khan showed that the increase in plant height after zinc application may be due to the abundant supply of zinc, which helps to accelerate the enzyme activity and auxin metabolism of plants [49].

3.7. SPAD Value

The SPAD value of colored rice increased by 2.15% after zinc application (Table 3). Among the seven cultivars of colored rice, compared to CK, all cultivars showed an increase in SPAD values under the zinc spraying treatment, increased by 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 was higher than that CK, indicating that zinc fertilizer spraying could increase the chlorophyll content of rice leaf. Faizan et al. [61] 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 [62]. The growth and development of rice are highly dependent on photosynthesis, which is one of the key physiological processes in rice [63]. As the primary organ for photosynthesis in plants, the chlorophyll content in leaves is not only an effective indicator of their nutritional status but also a reliable measure of leaf senescence. Zinc, as a component of numerous plant enzymes, is involved in the synthesis of chlorophyll, auxin, and the synthesis and conversion of carbohydrates [64]. Therefore, an increase in zinc content can enhance photosynthesis and photosynthetic efficiency in rice, thereby increasing SPAD values [26].

3.8. Protein Content of Grain

The results showed that zinc application increased the protein content of colored rice grain by 2.86% (Table 3), 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 an important factor that affects the cooking and eating quality of rice [65]. Higher protein concentrations are considered beneficial for human health. Zinc activated different enzymes and protein synthesis, and also activated decomposition sugars, improving the overall quality of rice grain [66]. Studies have shown that zinc improves the quality traits of rice by promoting an increase in the leaf area index [60]. 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 [56,67].

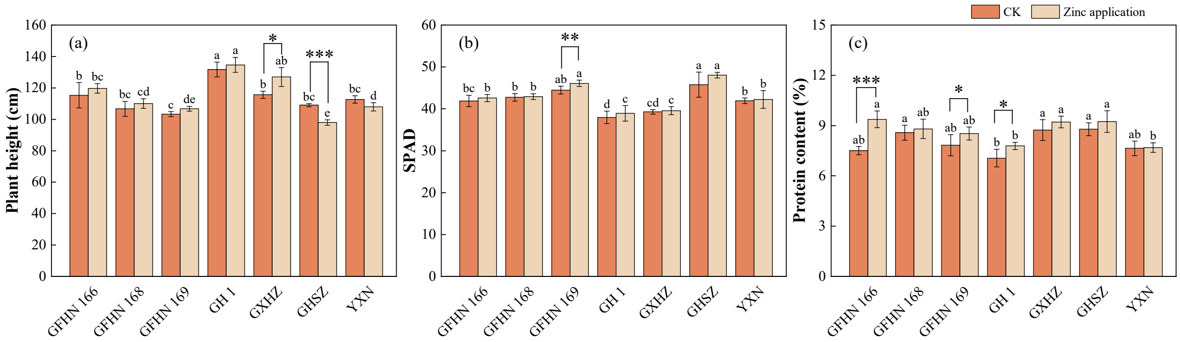

Figure 5. Effects of zinc application on plant height (a), SPAD (b) and protein content (c) of colored rice. Different lowercase letters on the bar graphs indicate significant differences between cultivars ($p<0.05$). The significance levels of $^{***}p \leqslant 0.001$, $^{**}p \leqslant 0.01$ and $^{*}p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

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 5. 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 how determining suitable application dosage 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 also has no harmful health risks.

Table 5. 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±0.06c	0.42±0.05c	0.64±0.08c	0.48±0.06c
GFHN 168	0.49±0.07bc	0.44±0.06bc	0.67±0.09bc	0.51±0.07bc
GFHN 169	0.50±0.04bc	0.44±0.04bc	0.68±0.06bc	0.51±0.05bc
GH 1	0.46±0.06c	0.41±0.06c	0.63±0.09c	0.48±0.06c
GXHZ	0.81±0.05a	0.72±0.05c	0.91±0.07a	0.83±0.06a
GHSZ	0.64±0.06b	0.57±0.05b	0.88±0.08b	0.66±0.06b
YXN	0.40±0.05c	0.35±0.04c	0.54±0.07c	0.41±0.05c

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

4. Conclusions

The study indicated that foliar zinc fertilizer application significantly increased the zinc content of colored rice. Rice with high zinc provided both agronomic and nutritional benefits. Among them, GH 1 and GXHZ showed the most prominent zinc enrichment ability and could achieve higher zinc content levels with less zinc fertilizer supplementation. This not only reduced the dependence on chemical fertilizers and reduced the pressure of agriculture on the environment, but also increased the plant height and leaf SPAD value of rice, promoted the accumulation of biomass, and increased the protein content in the grain. In addition, zinc-treated rice posed no risk to human health. Since the widespread occurrence of zinc deficiency in human populations is associated with low dietary zinc intake, special attention should be paid to foliar treatments of staple food crops with zinc. The combination of foliar zinc spraying with specific colored rice cultivars can effectively improve the nutritional value of rice and has a wide range of application potential.

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