Introduction

Zinc (Zn) deficiency is a well documented global health problem, affecting nearly half of the world population, particular in developing countries, where high proportion of cereal crops, such as rice and wheat, consumed as a staple food [1,2]. The reliance on cereal based food induce Zn deficiency related health problem, such as impairments in physical growth, immune system function [3,4]. Among the cereals, Rice (Oryza sativa L.), being one of the leading staple crop for half of the world's population, and, hence, is the main source of Zn to human [5]. Rice, however unfortunately, is a poor source of metabolizable Zn, due to inherently low in Zn content and the bioavailable Zn [6]. Enrichment of rice with high bioavailable Zn is, therefore, suggested as a way to generate major health benefits for a large number of susceptible people.

Zinc biofortification, which aims to enhance Zn concentration as well as bioavailability of rice grain, is considered as the more sustainable and economical solution to address human Zn deficiency [7]. Genetic biofortification and agronomic biofortification are two important agricultural tools to improve rice grain Zn concentration [2,8]. However, yield factor, interactions between genotype and environment, lack of sufficient genetic diversity in current cultivars for breeding program, consumer resistance and safety of genetically modified crops are the main bottlenecks of genetic biofortification [2,9,10,11]. The traditional and efficient strategy of agronomic biofortification, such as Zn fertilization is, therefore, urgent, essential and rapid solution for improving Zn concentration in rice grain to address the ongoing human Zn deficiency.

Three methods, including soil amendment, seed priming and foliar application, used in Zn fertilizations, have been extensively reviewed [2]. In recent years, a considerable progress has been made on the impact of foliar Zn fertilization on biofortification of Zn in rice grain [12,13,14], since it has the advantages of low application rates and avoiding Zn losses through soil fixation [15]. Furthermore, foliar applied Zn caused greater increases in brown rice Zn concentration than soil application [13,14]. There is evidence in literature demonstrating that foliar applied Zn can be absorbed by leaf epidermis, and remobilized and transferred into
the rice grains through the phloem [16] and several members of the Zn-regulated transporters regulate this process [17]. In most of those literatures, the reported data are mostly based on brown rice. As polished rice is the main consumed portion by human, rare information was found on Zn concentration in polished rice after foliar Zn fertilizations. Moreover, time of foliar application and the different forms of foliar Zn fertilizers may differentially influence grain Zn concentration. In recent past, several studies have been conducted to adjust time of foliar Zn application in cereal crops [2,13,18]. It is now well established that foliar Zn application after flowering stage (e.g., at early milk plus dough stages) more distinctly increase the grain Zn concentration [13]. On the other hand, different Zn fertilizers such as inorganic and organic Zn salts play a fundamental role in the way in nutrient transport from leave to the grain [19]. Unfortunately, studies evaluating the effectiveness of foliar application of different Zn forms on rice grain Zn accumulation are still rare.

The metabolizable Zn from biofortified crop grain not only depends on net Zn concentration, but also a large extent on the bioavailability of Zn. Zinc bioavailability defined as the proportion of the total amount of Zn that is potentially absorbable in a metabolically active form [20]. Phytic acid, the naturally occurring anti-nutrient presents in the seed, reduces the bioavailability of Zn, because of its ability to form complex with Zn, and inhibits Zn solubility, digestibility and absorption in human body [21]. Although, it is assume that foliar Zn fertilization improved Zn bioavailability, but till now there are rare studies on the Zn bioavailability of rice grain deserved from different forms of foliar Zn fertilization [2]. Hence, an in vivo approach to assess the potential benefits of different forms of foliar fertilization on grain Zn bioavailability is required.

Ideally, Zn bioavailability in crop grains should be evaluated through in vivo human study. However, complexity to perform large-scale screening of sample and cost limit their applicability [22]. In vivo digestion/Caco-2 cell model has been proposed as an alternative to in vivo method for estimating mineral bioavailability in diets. In recent years, in vitro digestion/Caco-2 cell culture model is being utilized for absorption studies involving Zn. This in vitro model is currently considered as the best approach, in term of cost and time, to investigate the bioavailability of different food components as a prelude to in vivo study [23]. The present study used this model to assess the bioavailability of Zn from polished rice grain fortified with different forms of foliar Zn fertilization.

Viewing the above circumstances, the current study were aimed: (i) to assess the effect of different forms of foliar Zn fertilizer on Zn concentration in brown rice and polished rice, (ii) to assess the effect of different forms of foliar Zn fertilizer on Zn bioavailability in polished rice. The findings of the current study were used to design experiment to identify some useful foliar Zn fertilizer for increasing the level of bioavailable Zn in rice grain.

### Materials and Methods

#### Field Experiment and Sampling

**Field location.** Experimental site was Longyou, Zhejiang province (29° 02’ N, 119° 11’ E), China. The climate of the experimental site is subtropical humid. The soil type of experimental field was periodical water logged paddy soil. Before the start of experiments, puddle layer (0–15 cm top soil) soil samples were taken from four random spots of the field and analyzed for various physico-chemical properties (Table 1).

**Experimental design and treatment.** Experimental design was a split plot with four replications. Foliar Zn fertilization treatments were treated as main plot and rice cultivars as sub-plot. Foliar Zn fertilization treatments comprised of four different forms of Zn fertilizer: (i) ZnNa2EDTA (Zn-EDTA), Zn-EDTA was the common Zn fertilizer which contain 9% Zn (ii) Zn-Citrate, in which Zn content was 10% (iii) ZnSO4·7H2O (ZnSO4), common Zn fertilizer, in which Zn content was 36% (iv) Zn-amino acids (Zn-AA), Zn-AA contains Zn as ZnSO4 (10%) and amino acid (25%) and, (v) Control, sprayed with distilled water. Three rice (*Oryza sativa* L.) varieties differ in their grain Zn concentration namely Hai7, rings9185 and Biyuzaonuo were selected according to our previous study [24]. Thus, there were 60 plots with each 4 m² (2×2 m).

Thirty days old seedlings of each cultivar were transplanted to the plot. Before transplanting, the standard recommended dose of NPK fertilizer was applied to all plots at rates of 187.5 kg N ha⁻¹ (70% applied as basal dose and 30% as topdressing at panicle initiation stage), 70 kg P₂O₅ ha⁻¹ and 95 kg K₂O ha⁻¹. Water management was the same as conventional rice farming practice. The foliar Zn was applied three times, one time at panicle initiation stage, two times at 7 days after flowering stage. Spray was applied after sunset. During spray, soil surface was covered to minimize the contamination of soil with foliar applied Zn. The concentration of Zn fertilizer was 0.2%. The amount of foliar Zn applied was equivalent to 2.5 kg Zn ha⁻¹. All foliar sprays contained 0.01% (v/v) Tween80 as a surfactant.

**Rice sample preparation.** Plants were harvested from the center of each plot at maturity and were manually threshed to separate grains. Rice grains were air dried; the brown rice was prepared by removing the husk using a laboratory de-husker (JLGJ4.5, Taizhou Cereal and Oil Instrument Co. Ltd., Zhejiang, China), the polished rice was prepared by polishing the bran by a laboratory de-husker (JLGJ4.5, Taizhou Cereal and Oil Instrument Co. Ltd., Zhejiang, China), the polished rice was prepared by polishing the bran by a laboratory de-husker (JLGJ4.5, Taizhou Cereal and Oil Instrument Co. Ltd., Zhejiang, China). The rice samples were powdered to make flour by using a ball mill (Retsch, MM-301, Germany), then put in the plastic bag and keep at −20°C until analysis. A part of rice was cooked for 15 min with 1:2 rice/deionized water (w/v). The cooked rice samples were then homogenized in a polytron homogenizer and then the homogenates were frozen and lyophilized before testing via the in vitro digestion/Caco-2 cell model.

### Chemical Analysis

**Mineral concentration determination.** The ground rice samples (0.3 g) of each treatment were placed in to PTFE digestion tube and, digested with nitric acid (2 mL) and hydrogen peroxide (0.5 mL). After cooling, the digestion solution was transferred to a 25 mL volumetric flask, made up the volume.

| Table 1. Selected physical and chemical properties of the soils. |
|-----------------------------|-------|
| Characteristics             | Value |
| pH (H₂O, 20 °C)             | 5.8   |
| Total N (g kg⁻¹)            | 1.36  |
| Organic matter (g kg⁻¹)     | 13.70 |
| Olsen P (mg kg⁻¹)           | 42.6  |
| CaCO₃ (%)                   | 1.98  |
| NH₄OAC-exchangeable K (mg kg⁻¹) | 90.35 |
| DTPA-extractable Zn (mg kg⁻¹) | 3.84  |
| DTPA-extractable Fe (mg kg⁻¹) | 198.45 |

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with deionized water. The concentrations of Zn, iron (Fe), calcium (Ca) in sample were determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500a, Agilent Technologies, CA, USA) following our previously described method [16].

**Phytic acid determination.** Phytic acid from the rice samples was determined by the method described by Dai et al., [25]. Briefly, 0.5 g of rice flour was extracted with 10 mL of 0.2 M HCl for 2 h by a rotary shaker and then centrifuged at 10000 g for 10 min. The clear supernatant was collected, and 2 mL of 0.2% FeCl₃ was added to 2.5 mL of supernatant. The resulting solution was mixed thoroughly, heated in a boiling water bath for 30 min, cooled in room temperature, and centrifuged at 10000 g for 15 min. Then supernatant was discarded and the residue in the tube washed three times with 5 mL of deionized water. The tube was then centrifuged again at 10000 g for 10 min after adding 3 mL of 1.5 M NaOH to it. The supernatant was discarded again, and 3 mL of 0.5 M HCl was added to the tube to dissolve the residue. Finally, deionized water was added to the solution made up to the volume of 10 mL. The Fe concentration in the solution residue. Finally, deionized water was added to the solution made up to the volume of 10 mL. The Fe concentration in the solution was determined by the method described by Dai et al., [26].

**Cell culture.** The Caco-2 cell model was calculated as the Zn content in transport plus the Zn content in transport. Solubility percentages were calculated by using following equation: solubility% = soluble fraction (µg of Zn g⁻¹ sample) × 100/C, where C = total Zn content of sample; The following equation was used for Zn retention percentage: Zn retention% = Zn retention (µg well⁻¹) × 100/C, where C = mineral soluble added (µg); The following equation was used for Zn transport percentage: Zn transport% = Zn transport (µg well⁻¹) × 100/C, where C = mineral soluble added (µg); The following equation was used for Zn uptake percentage: Zn uptake% = (retention + transport) (µg well⁻¹) × 100/C, where C = mineral soluble added (µg). Due to the differences among samples in terms of solubility of Zn after in vitro digestion, Zn uptake availability was expressed as Zn uptake efficiency, Zn uptake efficiency% = (% solubility × % uptake)/100. Bioavailable Zn (µg g⁻¹ polished rice) = Zn concentration (mg kg⁻¹) × Zn uptake efficiency%.

**Quality Control of Mineral Analysis** Standard reference material rice flour (SRM 1568a) from National Institute of Standards and Technology (Gaithersburg, MD, USA) was used to check the accuracy of Zn, Fe and Ca analysis. The measured value was 19.7±0.2 mg kg⁻¹ for Zn, 6.9±0.3 mg kg⁻¹ for Fe and 114.5±1.2 mg kg⁻¹ for Ca, which
values were in accordance with the certified ranges of 19.4 ± 0.3 mg kg⁻¹ for Zn, 7.4 ± 0.9 mg kg⁻¹ for Fe and 118 ± 6 mg kg⁻¹ for Ca.

**Statistical Analysis**

Statistical analysis of the data was performed using SPSS 12.0 (SPSS, Inc., Chicago, IL, USA). The data were subjected to a separate analysis of variance (ANOVA) for each cultivar, and Fisher’s least significant difference (LSD) at P < 0.05 was used to determine differences between treatment means. The Pearson correlation procedure and linear regression model was used to evaluate the relationship between brown rice and polished rice Zn concentration.

**Results**

**Biomass and Grain Yield**

Biomass, grain yield, harvest index and thousand seed weight of rice did not different among the four different forms of foliar Zn treatments for all three rice cultivars (Table 2).

**Zinc Concentration in Brown Rice and Polished Rice**

Foliar Zn fertilization had significant (P < 0.05) impact on Zn concentration in brown rice and polished rice (Fig. 1). Brown rice Zn concentration was significantly increased by foliar Zn fertilizations (Fig. 1A). Regardless of the three cultivars, Zn concentration was significantly increased by foliar ZnSO₄ application, to 39.84 mg kg⁻¹ in cultivar Hai7, Bing91185 and Biyuzaonuo, respectively. After application of Zn-EDTA through the foliage, Zn concentrations in brown rice were 27.90, 34.47 and 38.79 mg kg⁻¹ in cultivar Hai7, Bing91185 and Biyuzaonuo, respectively. Thus, foliar Zn fertilization could increase Zn concentration in brown rice and polished rice depending on Zn form.

The concentration of Zn in brown rice and polished rice of all cultivars were significantly increased by different forms of foliar applied Zn (Fig. 2). In control, brown rice Zn concentrations were 24.71, 30.29 and 35.82 mg kg⁻¹ in cultivar Hai7, Bing91185 and Biyuzaonuo, respectively. After application of Zn-EDTA through the foliage, Zn concentrations in brown rice were 27.90, 34.47 and 38.79 mg kg⁻¹ in cultivar Hai7, Bing91185 and Biyuzaonuo, respectively. After foliar application of Zn-Citrate, Zn concentrations in brown rice were 29.15, 36.08 and 40.96 mg kg⁻¹ in cultivar Hai7, Bing91185 and Biyuzaonuo, respectively. After foliar application of Zn-AA, Zn concentrations in brown rice were 30.46, 30.94 and 46.20 mg kg⁻¹ in cultivar Hai7, Bing91185 and Biyuzaonuo, respectively. After foliar application of Zn-AA, Zn concentrations in brown rice were 31.53, 40.62 and 47.30 mg kg⁻¹ in cultivar Hai7, Bing91185 and Biyuzaonuo, respectively. Similar trends were found in polished rice (Fig. 2B), the cultivar Biyuzaonuo had the highest Zn concentration, while Hai7 had the lowest Zn concentration in all Zn treatments.

With respect to Zn content, a significant correlation was found between polished rice and brown rice (y = 0.619x + 4.232, R² = 0.097, P < 0.01).

**Phytic Acid Content in Polished Rice**

Foliar Zn fertilization reduced the phytic acid content in the polished rice (Table 3). Regardless of cultivar, phytic acid content in polished rice ranged from 2.25 mg g⁻¹ in the control, to

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**Table 2. Effect of different forms of foliar Zn fertilization on the biomass, grain yield, harvest index and thousand seed weight of three rice cultivars.**

| Treatments | Cultivars | Biomass (t hm⁻²) | Grain yield (t hm⁻²) | Harvest index (%) | Thousand seed weight (g) |
|------------|-----------|-----------------|---------------------|-------------------|--------------------------|
|            | Hai7      | Bing91185      | Biyuzaonuo         |                   |                          |
| Control    | 19.50 a   | 18.61 a        | 18.35 a            | 7.81 a            | 7.98 a                   |
| Zn-EDTA    | 19.57 a   | 18.73 a        | 18.55 a            | 7.86 a            | 7.93 a                   |
| Zn-Citrate | 19.79 a   | 18.66 a        | 18.43 a            | 8.00 a            | 8.03 a                   |
| ZnSO₄      | 19.63 a   | 18.39 a        | 18.61 a            | 7.97 a            | 7.92 a                   |
| Zn-AA      | 19.14 a   | 18.84 a        | 18.80 a            | 8.01 a            | 8.04 a                   |
| Zn effect  | NS        | NS             | NS                 | NS                | NS                       |
| by F-test  |           |                 |                     |                   |                          |

*Different letters after number in the same column designated significant difference by LSDP < 0.05.
*Significant effects: NS = not significant at P > 0.05.

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2.09 mg g⁻¹ by Zn-EDTA, to 2.06 mg g⁻¹ Zn-Citrate, to 1.87 mg g⁻¹ by foliar ZnSO₄ and 1.92 mg g⁻¹ by Zn-AA, these showed that the decreases of 6.97%, 8.42%, 16.91% and 14.57%, respectively.

Protein, Iron and Calcium Concentration in Polished Rice

Protein content in polished rice showed an increase trend by foliar Zn-AA, but not by other foliar Zn fertilizations (Table 3). Foliar application of Zn-AA had significant impact on grain protein content in cultivar Hai7 and Bing91185, except Biyuzao-nuo. Generally, compared to the control, foliar Zn-AA could increase protein content by 1.88–4.79% depending on cultivar. Grain Fe and Ca concentration did not change by different forms of Zn treatments in all three cultivars. Foliar Zn fertilization had little impact on Fe and Ca content (Table 3).

In vitro Zinc Solubility of Polished Rice

The amount of Zn solubilized after in vitro digestion is an indicator for bioavailability. Foliar Zn fertilization had significant (P<0.05) effect on Zn solubility (Table 4). Averaged across the cultivars, in vitro Zn solubility from polished rice was ranged from 28.48% in the control, to 29.34% by application of Zn-EDTA, 29.41% by application of Zn-Citrate, 31.15% by application of ZnSO₄ and 30.67% by application of Zn-AA, these represented increase of 2.99%, 3.24%, 9.36% and 7.65%, respectively. Foliar application of Zn-AA and ZnSO₄ significant improved in vitro Zn solubility in all cultivars.

Zinc Bioavailability of Polished Rice

The soluble fraction obtained from in vitro digestion was used to carry out the retention, transport and uptake experiments in Caco-2 cell (Table 4). Foliar Zn fertilization had significant (P<0.05) impact on the percentages of Zn retention, transport and uptake efficiency in polished rice grain for all cultivars. Generally, foliar application of Zn-EDTA, Zn-Citrate, ZnSO₄ and Zn-AA could increase the percentages of Zn retention, transport and uptake efficiency in polished rice grain, but only in case of foliar application ZnSO₄ and Zn-AA could reach significant level in most of the cultivars tested (P<0.05). Regardless of cultivar, compare to the control, after foliar application of ZnSO₄, the percentages of Zn retention, transport, and uptake efficiency from the polished rice increased by 12.96%, 31.36% and 34.98%,
respectively; after foliar application of Zn-AA, the percentages of Zn retention, transport, and uptake efficiency from the polished rice increased by 19.4%, 29.47% and 35.25%, respectively. Regardless of cultivar, the amount of bioavailable Zn in the rice grain has the similar trend as the Zn uptake efficiency (Fig. 3). Regardless of cultivar, compared to the control, amount of bioavailable Zn was increased by 13.85%, 21.96%, 64.43% and 68.37% by Zn-EDTA, Zn-Citrate, ZnSO₄ and Zn-AA, respectively. Generally, the amount of bioavailable Zn could increased by the foliar Zn fertilizations (Zn-EDTA, Zn-Citrate, ZnSO₄ and Zn-AA), but only foliar ZnSO₄ and Zn-AA reach at significant level (P<0.05) in all cultivars tested.

Discussion

The DTPA extractable Zn (Table 1) in the soil of the current field study was higher than the critical level for rice (0.8 mg kg⁻¹) [28], thus the plant was in the sufficient Zn nutritional status. In the Zn sufficient soil, excess foliar application of Zn did not affect the biomass, grain yield, harvest index and thousand seed weight (Table 2). Similar results also found in previous reports [12,29].

In contrast to grain yield, foliar Zn fertilization significantly (P<0.05) increased the Zn concentration in brown rice (Fig. 1A), consisted with the previous studies [16,30]. Thus, foliar application Zn as an effective method could boost Zn level in rice grain. Here, in this study, particular attention should be given in the Zn concentration of polished rice, as this is the predominant fraction consumed by human. In current study, we found that regardless of cultivar, although polishing process decrease substantial amount Zn from mature grain, the polished rice obtained from foliar Zn applications was still contained 10.22–24.04% more Zn than those of control (Fig. 1B), and a significant positively correlation in Zn concentration between polished rice and brown rice was also found (R² = 0.897, P<0.01), indicating that Zn concentration in polished rice might be improved by increasing the Zn concentration in brown rice, consisted with previous studies [18,29], suggesting that excess foliar applied Zn could penetrate into the inner layers of rice endosperm. However, the mechanism of absorbed Zn from aleurone layer into rice endosperm still not clearly, some recent studies reported that nicotianamine and deoxymugineic acid play important role in this process [17,31].

Furthermore, the effectiveness of foliar Zn fertilization on Zn concentration of brown rice and polished rice varied with the
ZnSO₄ might be easily penetrate into the leaves than those of Zn-EDTA and Zn-Citrate and leaf penetration of different forms of foliar applied Zn fertilizer study [32]. The reasons might be due to the different capacity of brown rice and polished rice, the results agreed with the previous and Zn applications (Table 3), consisted with the previous and nutritional quality of rice, which impact on global human health. Phytic acid has long been known as a form of stored and bioavailability in rice grain [34]. In the current study, it was documented that foliar Zn applications could significantly reduced bioavailability, information about changes of anti-nutrients and deposition of foliar applied Zn with low molecular weight. Information about changes of anti-nutrients and deposition of foliar applied Zn with low molecular weight.

Although the amount of grain Zn is important for Zn bioavailability, information about changes of anti-nutrients and other nutrients in rice grain especially polished rice during foliar Zn applications is crucial, because it related with Zn bioavailability and nutritional quality of rice, which impact on global human health. Phytic acid has long been known as a form of stored phosphorus in seeds, which was considered as an inhibitor of Zn bioavailability in rice grain [14,16]. Thus, impact of foliar Zn application on grain Zn can be maximized by selecting genotypes with higher ability in leaf absorption and seed deposition of foliar applied Zn with low molecular weight.

Forms of Zn fertilizer (Fig. 1). Among the forms tested for foliar application, foliar Zn-AA and ZnSO₄ were more effective than Zn-EDTA and Zn-Citrate in improving the Zn concentration in brown rice and polished rice, the results agreed with the previous study [32]. The reasons might be due to the different capacity of leaf penetration of different forms of foliar applied Zn fertilizer [19]. Foliar fertilizer with low molecular weight like Zn-AA and ZnSO₄ might be easily penetrate into the leaves than those of Zn-EDTA and Zn-Citrate with the high molecular weight, as a result, the more plant-available Zn from foliage might be transported and accumulated in rice grain [19,33]. The response of grain Zn concentration to foliar Zn fertilization was cultivar dependent (Fig. 2). The results consisted with the previous studies reported rice genotypes differ greatly in their response to foliar applied Zn to increase grain Zn concentration [14,16]. Thus, impact of foliar Zn application on grain Zn can be maximized by selecting genotypes with higher ability in leaf absorption and seed deposition of foliar applied Zn with low molecular weight.

Table 3. Effect of different forms of foliar Zn fertilization on the grain protein, phytic acid, Fe and Ca contents of three rice cultivars.

| Treatments       | Cultivars* | Control | Zn-EDTA | Zn-Citrate | ZnSO₄ | Zn-AA |
|------------------|------------|---------|---------|------------|-------|-------|
|                  |            | Hai7    | Bing91185 | Biyuzaonuo | Hai7 | Bing91185 | Biyuzaonuo |
|                  |            | Protein (%) | Phytic acid (mg g⁻¹) | Protein (%) | Phytic acid (mg g⁻¹) | Protein (%) | Phytic acid (mg g⁻¹) |
| Control          |            | 9.06 b | 9.32 b | 9.96 a | 1.78 a | 1.64 a | 3.32 a |
| Zn-EDTA          |            | 9.15 b | 9.34 b | 9.99 a | 1.66 b | 1.48 b | 3.14 b |
| Zn-Citrate       |            | 9.16 b | 9.66 ab | 9.91 a | 1.65 b | 1.47 b | 3.05 b |
| ZnSO₄            |            | 9.15 b | 9.37 b | 9.99 a | 1.54 c | 1.39 c | 2.89 c |
| Zn-AA            |            | 9.43 a | 9.76 a | 10.17 a | 1.50 c | 1.36 c | 2.67 d |

Zn effect by f-test*

| Treatments       | Fe (mg kg⁻¹) | Ca (mg kg⁻¹) |
|------------------|--------------|--------------|
| Control          | 2.92 a       | 3.35 a       |
| Zn-EDTA          | 3.12 a       | 3.74 a       |
| Zn-Citrate       | 3.10 a       | 3.81 a       |
| ZnSO₄            | 2.98 a       | 3.88 a       |
| Zn-AA            | 2.98 a       | 3.86 a       |

Fe (mg kg⁻¹) | Ca (mg kg⁻¹)

**Different letters after number in the same column designated significant difference by LSDP at P < 0.05;*** at P < 0.01; ** at P < 0.05; NS = not significant at P > 0.05.

In vivo situation, Zn needs to be in soluble before it can be taken up by the enterocytes. In current study, we also determined the soluble Zn in polished rice obtained from the different foliar Zn treatments by in vitro digestion. The results showed that regardless of cultivar, the solubility of Zn in polished rice from foliar Zn application was higher than control, especially in the case of foliar applications of Zn-AA and ZnSO₄ (Table 4). To the best of our knowledge, no literature data on regarding foliar Zn fertilizers on the in vitro Zn solubility in rice grain are yet available. One possible reason was the in vitro Zn solubility in grain was increased by the reduction of phytic acid in grain [2,37]. The solubility method involves a simulation of gastrointestinal digestion followed by a measurement of soluble Zn in the digest and thus covers only the first phase of the overall Zn absorption process. The soluble Zn fraction obtained form in vivo digestion was used to carry out retention, transport and uptake experiment in Caco-2 cell model, which offer a more physiological tool for screening Zn bioavailability in food matrices [27,37,38,39]. In the current study, the percentage of Zn uptake efficiency of polished rice ranged from 6.01% to 12.33%, falling within the previous reported Zn uptake efficiency in Caoc-2 cell model from cereal foods (ranging from 4.1% to 48.1%) [37]. Compared to the control, foliar Zn-AA and...
Table 4. Effect of different forms of foliar Zn fertilization on the percentages of solubility, retention, transported and uptake efficiency of Zn among three rice cultivars.

| Treatments | Cultivars | Solubility (%) | Retention (%) | Transport (%) | Uptake efficiency (%) |
|------------|-----------|----------------|---------------|---------------|------------------------|
|            | *         | **            | ***           | ***           |                        |
| Control    | Hai7      | 29.17 c       | 30.58 c       | 25.70 b       | 14.53 c               |
|            | Bing91185 | 30.75 c       | 31.21 b       | 26.06 b       | 14.82 bc              |
|            | Biyuzao    | 30.90 bc      | 31.13 bc      | 26.20 b       | 14.93 bc              |
|            | nuo       |                |               |               |                        |
| Zn-EDTA    | Hai7      | 32.68 a       | 32.24 a       | 28.54 a       | 16.80 ab              |
|            | Bing91185 | 31.64 ab      | 32.62 a       | 27.73 a       | 17.53 a               |
|            | Biyuzao    |                |               |               |                        |
| Zn-Citrate | Hai7      | 31.64 ab      | 32.62 a       | 27.73 a       | 17.53 a               |
|            | Bing91185 | 32.68 a       | 32.24 a       | 28.54 a       | 16.80 ab              |
|            | Biyuzao    |                |               |               |                        |
| ZnSO4      | Hai7      | 30.90 bc      | 31.13 bc      | 26.20 b       | 14.93 bc              |
|            | Bing91185 | 30.75 c       | 31.21 b       | 26.06 b       | 14.82 bc              |
|            | Biyuzao    |                |               |               |                        |
| Zn-AA      | Hai7      | 31.64 ab      | 32.62 a       | 27.73 a       | 17.53 a               |
|            | Bing91185 | 32.68 a       | 32.24 a       | 28.54 a       | 16.80 ab              |
|            | Biyuzao    |                |               |               |                        |

*Different letters after number in the same column designated significant difference by LSDP.0.05.

**Significant effects: NS = not significant at P=0.05; *at P<0.05; **at P<0.01; ***at P<0.001.

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Figure 3. The amount of bioavailable Zn in the polished rice among three cultivars. Error bars indicate standard errors of the means (n = 4). Different letters indicate significant difference among Zn treatments according to LSD test (P<0.05).
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ZnSO₄ could significantly increase the percentages of Zn retention, transport and uptake efficiency of polished rice in most of the cultivars tested (Table 4). Regardless of cultivar, the amount of bioavailable Zn has the similar trend as Zn uptake efficiency (Fig. 3), showing the foliar Zn could improve the amount of bioavailable Zn in the polished rice, especially foliar Zn-AA and ZnSO₄ increased by 68.37% and 64.43%, respectively. The results indicated that foliar Zn application could improve the bioavailability of Zn from polished rice but depended on the forms of Zn application. Till now, few literatures on the Zn bioavailability of biofortified polished rice obtained from the foliar Zn fertilization are available. The possible explanation of our results might be foliar Zn-AA and ZnSO₄ have the higher efficiency to decrease the phytic acid, and improve the total amount of Zn than Zn-EDTA and Zn-Citrate, as a result, increase the amount of bioavailable Zn in the polished rice grain. In addition, in the current study, we also observed that the polished rice of cultivar Biyuzaomo contained the highest amount of Zn, interestingly, it was not contained the highest amount of bioavailable Zn, this is might be due to presence of significant amount of phytic acid or other anti-nutrients in this cultivar [40]. Thus, it is suggested that not only the net grain Zn concentration but also the bioavailability of grain Zn should be considered in ongoing breeding program.

In conclusion, foliar Zn fertilization was an effective agronomic practice to promote grain Zn concentration and Zn bioavailability, especially, in case of Zn-AA and ZnSO₄. On average, Zn-AA and ZnSO₄ increased Zn concentration in polished rice up to 24.04% and 22.47%, respectively. On average, Zn-AA and ZnSO₄ increased Zn bioavailability in polished rice up to 68.37% and 64.43%, respectively. The effectiveness of foliar applied Zn-AA and ZnSO₄ were higher than Zn-EDTA and Zn-Citrate to improve the Zn concentration, and reduction of phytic acid, as a results higher accumulation of bioavailable Zn in polished rice. Moreover, foliar Zn application could maintain the protein and minerals (Fe and Ca) quality of the polished rice. Therefore, it's believed that foliar application of suitable Zn form is a feasible approach to improve the bioavailable Zn status in polished rice.

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Author Contributions

Conceived and designed the experiments: YW MJIS. Performed the experiments: YW MJIS. Analyzed the data: YW MJIS XY. Contributed reagents/materials/analysis tools: YW MJIS. Wrote the paper: YW MJIS.

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