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A safe, high fertilizer-efficiency and economical approach based on a low-volume spraying UAV loaded with chelated-zinc fertilizer to produce zinc-biofortified rice grains

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ABSTRACT

Agricultural spraying unmanned aerial vehicles (UAVs) can be used to avoid human exposure to agrochemical mists and to obtain many extra benefits in crop protection, and concentrated Zn fertilizer is needed for low-volume UAV sprays applied for Zn biofortification. Applying the widely used $ZnSO_4$ fertilizer at high spraying rates improved Zn biofortification, but it also caused leaf burn, yield losses, and fertilizer losses. To determine whether there is an alternative UAV-supported Zn source that further improves Zn concentration in rice seeds without compromising agronomic performance, a 3-year field experiment was conducted. Initial tests at different spraying rates showed that Zn-EDTA, Zn-Gly, and Zn-Glc had approximately two-fold higher safe threshold rates ($\leq$0.8%) than $ZnSO_4$ and $ZnNO_3$. Furthermore, Zn biofortification was optimally improved by applying $ZnNO_3$ at a normal rate, and Zn-Gly was the best at a high spraying rate. The third-year results demonstrated that two sprays of 0.81% Zn-Gly by UAV achieved equivalent Zn biofortification levels as those after three sprays of 0.27% $ZnNO_3$ by manual spraying. In addition, UAV-based spraying greatly lowered the risk of fertilizer residual in rice paddies because of its low Zn input and its high Zn recovery rate. This safer and cleaner technology will expand the existing fertilizing options to produce highly Zn-biofortified grain with low input costs.

1. Introduction

Zn homeostasis is important for human health (Hambidge et al., 2010). Adequate Zn intake achieved by taking Zn supplements boosts immunity and can even fight the global burden of the COVID-19 pandemic, according to the latest reports (Brasiel, 2020; McPherson et al., 2020; Skalny et al., 2020). Nevertheless, the Zn intake among billions of people is insufficient due to the low Zn level in common grains, which is associated with risks of Zn deficiency diseases or hidden hunger (Geyik et al., 2020; Wessells and Brown, 2012). The commercialization of genetically modified (GM) or non-GM Zn-rich grain crop varieties has just begun, and they are not widely used owing to concerns about GM food, long breeding cycles, low yields, or poor variety adaptability (Cakmak, 2007; Bouis and Saltzman, 2017; Agarwal et al., 2018). In contrast to breeding processes, foliar application of Zn fertilizer is a quick method to grow Zn-biofortified grains globally, independent of crops, cultivars, and soil types (Cakmak, 2007; Zou et al., 2012).

As a classical agrospraying device, knapsack sprayers play an important role in crop spraying work. However, to cover 1 ha of the farming area, the typical scenario is as follows: an elderly man (as it is presently different to find younger employees to perform burdensome farm work in developed rural regions in China) walks across the field carrying a 10–25 L sprayer on his back and is directly exposed to harmful agrochemicals for 2 days. Moreover, paddy fields have to be drained to allow walking across the flooded areas. This scenario does not match the expectations of modern agriculture.

The remote operation of unmanned aerial vehicles (UAVs) can

Abbreviations: UAV, unmanned aerial vehicle; Zn-EDTA, zinc ethylene diamine tetraacetic acid; Zn-Gly, glycine chelated zinc; Zn-Glc, zinc gluconate; *Pn*, net photosynthetic rate; ARE, apparent recovery efficiency; GM, genetically modified; GR, guaranteed reagent; EC, electrical conductivity; CK, blank control; SPAD, Soil-Plant Analysis Development.

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remove the risk of direct exposure to agrochemical solutions and the constraints associated with various field conditions, and it greatly reduces both labor intensity and labor time (Kim et al., 2019; Yang et al., 2017). And electric multi-rotor UAV with a volume of 10 L has increasingly become the mainstream model used in China (Yang et al., 2017; Kharim et al., 2019; Zhou et al., 2017; Wang et al., 2020). The low payload capacity of this UAV model requires high solute (agrochemical) concentrations (Kharim et al., 2019). Usually, the total solvent (water) input per unit of UAV is not more than a tenth of that of the knapsack sprayer, and it is necessary to apply several times the concentration of agrochemical that is usually used to obtain effective spraying results. According to the reports published so far, UAVs have not been used for crop biofortification. The use of UAVs for spraying Zn fertilizer has the potential to broadly apply Zn for biofortification and achieve considerable cost reductions for Zn fertilizer.

Zn sulfate is the most widely used Zn fertilizer (Li et al., 2009). Zn sulfate is severely irritating to the eyes and toxic after long-term inhalation and dermal exposures during the manual spraying process (Bodar et al., 2005). Although Zn toxicity exposure to humans can be avoided by remote operation of a UAV, Zn phytotoxicity or even leaf burn caused by high Zn spraying concentrations cannot be avoided. The most frequently used $ZnSO_4 \cdot 7H_2O$ rate is 0.1%–0.5% (w/v), which is empirically used for all other Zn formulations as well. Interestingly, with further increases in the foliar spraying rate of $ZnSO_4 \cdot 7H_2O$, the Zn concentration steadily increased in the edible parts of wheat (Zhang et al., 2012; Dapkekar et al., 2018) and green beans (Almeida et al., 2020). However, when the spraying rate exceeded the critical range, $ZnSO_4$ induced visible foliage phytotoxicity in corn (Drissi et al., 2015; Golden et al., 2016) and even caused yield losses in tomatoes (Kaya and Higgs, 2002), accompanied by decreased photosynthesis. Golden et al. (2016) ranked Zn fertilizers according to the severity of foliar injury that was induced and reported that ZnEDTA caused the highest extent of foliar injury, followed by $ZnSO_4$. The reason was possibly because the pH and electrical conductivity (EC) of ZnEDTA are more suitable for leaf spray applications than those of $ZnSO_4$. However, the basic physicochemical properties of the Zn fertilizers used were not specified. It is possible that ZnEDTA has a higher spray concentration safety threshold than $ZnSO_4$. To our knowledge, no other studies have explored $ZnSO_4$-induced foliar burn mitigation. In addition, different spraying rates of other Zn sources have been rarely studied, and differences in the rate effect among Zn sources were also not clear because almost all previous studies tested only a frequently used rate range to compare the Zn-fortified effect of different Zn formulations (Wei et al., 2012; Ghasemi et al., 2013; Tabesh et al., 2020).

In this study, rice (*Oryza sativa* L.) was chosen as the targeted crop because it is the foremost crop in southern China and one of the three major grain crops worldwide. The field experiments were designed with the following objectives: (1) are there existing Zn sources that can be applied at high concentrations more safely than the formulations presently used? (2) which is the optimal Zn source with highest grain allocation efficiency? and (3), whether low-volume, high Zn-concentration foliar applications of this source by UAV is an alternative and preferable method for fortifying grain Zn concentration?

2. Materials and methods

2.1. Experimental conditions and management measures

From 2018 to 2020, consecutive annual experiments were conducted at the field trial bases in Langya Town (30°27′ N, 119°46′ E, 89 m asl), Jinhua City, Zhejiang Province, China and/or Fenshui Town (29°54′ N, 119°24′ E, 62 m asl), Hangzhou City, Zhejiang Province, China. Both locations have a typical subtropical monsoon climate with four distinct seasons, and the date for monthly average temperatures, rainfall and hours of sunlight are provided in Table S1 in the Supplementary Material. Before transplanting rice seedlings every year, three soil core samples from a depth of 0–20 cm were randomly collected for soil chemical analysis, and the basic properties are shown in Table S2 in the Supplementary Material. The following fertilizer ingredients were incorporated as base fertilizers prior to manual transplanting: N, 180 kg nitrogen (N)/ha as coated urea; P, 90 kg phosphorus pentoxide (P_2O_5)/ha as triple superphosphate; K, 150 kg potassium oxide (K_2O)/ha as potassium chloride. The cultivars Yongyou 538 (YY538) and Yongyou 1540 (YY1540) are both hybrid japonica rice varieties, which were recommended as single cropping rice varieties by the local agricultural sector and were widely cultivated in the local province. The 21-day-old seedlings of YY538 were transplanted in the first half of June and harvested in late October every year. YY1540 was cultivated later; the 19-day-old seedlings were transplanted in early July and harvested in early November 2020. The transplant density was 137,174 holes/ha, and spacing was 27 × 27 cm (plant space × row space). Other management measures, except the foliar fertilizer application, were consistent.

Table 1
pH and EC (electrical conductivity, mS/cm) values of different Zn source solutions at different Zn application rates (25 °C).

Zn rate	Index	$ZnSO_4$	$ZnNO_3$	Zn-EDTA	Zn-Gly	Zn-Glc
0.1%	pH	6.18	5.47	6.01	4.80	5.68
	EC	0.53	0.66	0.55	0.54	0.21
0.2%	pH	5.94	5.2	5.8	4.67	5.99
	EC	0.90	1.12	1.04	0.91	0.38
0.4%	pH	5.73	5.05	5.89	4.47	5.94
	EC	1.64	2.21	1.95	1.65	0.64
0.8%	pH	5.61	4.94	5.71	4.32	5.92
	EC	2.43	4.35	3.43	2.58	0.83
1.6%	pH	5.36	4.73	5.84	4.21	5.81
	EC	4.16	8.1	6.39	4.37	1.24
3.2%	pH	5.01	4.55	5.98	4.05	5.85
	EC	7.24	16.15	12.09	8.32	2.31
6.4%	pH	4.53	4.37	5.79	3.97	insoluble
	EC	12.4	33.02	21.61	17.81	

Note: The pH and EC values of CK (water) were 7.08 and 0.00 mS cm^{-1}, respectively; for 6.4% NaCl (w/w), the values were 7.04 and 89.24 mS cm^{-1}, respectively. A 10-fold serial dilution method was used when the conductivity meter (DDS-307, Leici, China) range was exceeded. The pH was measured using a pH meter (FE28-Meter, Mettler Toledo, Switzerland).

2.2. Experimental design of foliar fertilizer application

Test 1: Efficacy comparison of different Zn sources at different Zn spraying rates in 2018

Two inorganic Zn fertilizers, zinc sulfate heptahydrate ($ZnSO_4$) and zinc nitrate hexahydrate ($ZnNO_3$), and three chelated Zn fertilizers, zinc EDTA(Zn-EDTA), glycine chelated zinc (Zn-Gly), and zinc gluconate (Zn-Glc), were tested in the field. $ZnSO_4$ was prepared at seven different concentrations using zinc sulfate heptahydrate and the following weight per volume percentages (w/v): 0.1%, 0.2%, 0.4%, 0.8%, 1.6%, 3.2%, and 6.4%. The other four Zn sources were prepared using the same concentrations adjusted for the same percentage of Zn^{2+} used at each $ZnSO_4$ concentration step. Zn-Glc was not tested at 6.4% due to its limited solubility, and 3.2% Zn-Glc was used as the highest concentration instead. Two controls were prepared: water was used as the blank control (CK), and 6.4% sodium chloride (NaCl) was used to check the enrichment effect. The surfactant Tween 80 was used in every treatment, including the controls, at 0.01% (v/v). The pH and electrical conductivity (EC) values of the Zn formulation solutions at different Zn concentrations are shown in Table 1.

The test was conducted in Langya Town in 2018 using the cultivar Yongyou 538. All 108 treatment plots were arranged in a randomized complete block design with three replicates. Each plot had 50 plants, covered an area of 2.70 m × 1.35 m, and was separated from other plots by a guard row of 1.35 m. Spraying was performed after 17:00 on

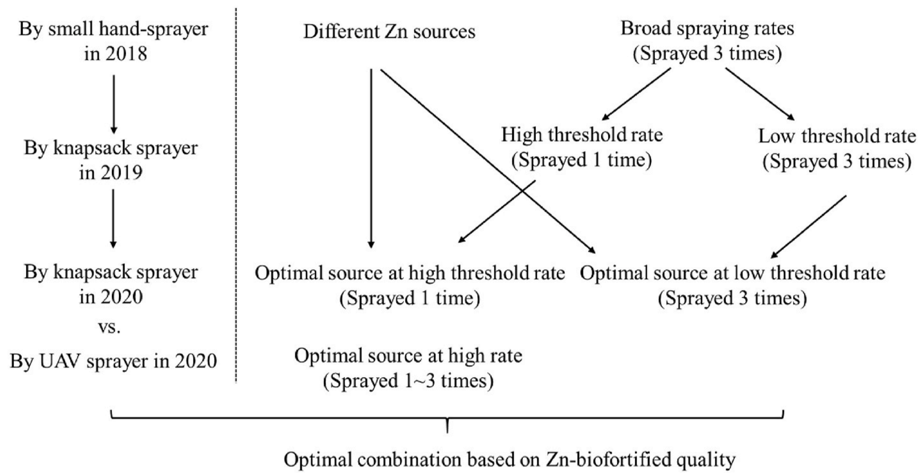

Fig. 1. The flowchart of the experimental design.

rainless and windless days with 2 L artificial compression sprayers (702C, LvTai, China) after the flowering stage. A total of three sprays were performed in 7-day intervals. The amount of each spray was 600 L/ha. The orifice diameter of the tangential entry of the hollow cone nozzle was 1.0 mm, and the range of droplet sizes was 40–110 μm. The flow rate was 0.5 L/min.

Test 2: Efficacy comparison of split application and one-off application in 2019

Test 2 was a partial repeatability test of Test 1, but the spraying rate and frequency were modified (Fig. 1). The safety threshold rate range based on Test 1 was separated into a low and a high threshold rate for each formulation at an approximate ratio of 1:3. To compare the difference between splitting the sprays and one-off spraying of the same Zn input amount, one-off spraying of 0.81% rate and three sprays at a rate of 0.27% and one-off spraying at a rate of 0.81% were performed with the five Zn sources. Water was used for CK sprays, and Tween 80 was added at 0.01% (v/v) to every treatment, including the control.

The test was conducted in Langya Town in 2019 using the cultivar YY538. All plots were arranged in a randomized complete block design with three replicates. Each plot had 2500 plants and covered an area of 13.5 m × 13.5 m. The sprays were performed using a typical manual sprayer (Electric knapsack sprayer, Gelv 3WBD-16, China). The orifice diameter of the tangential entry of the hollow cone nozzle was 1.0 mm, and the range of droplet sizes was 70–120 μm. The flow rate was 0.5 L/min.

Test 3 Efficacy comparison between knapsack sprayer and UAV application in 2020

The formulation with an optimally high safety threshold rate based on the preceding two-year test was used for sprays performed with a remotely controlled flight sprayer (Electric quad-rotor plant protection UAV, Zhitian 3WD4-10, China). The frequencies were 1, 2, and 3 times with 7-day intervals. The volume of each spray was 60 L/ha. The type of fan nozzle was XR11001VS, and the range of droplet sizes was 136–177 μm. The flight altitude was 2.5 m, and the flow rate was 1.0 L/min. The UAV sprays were performed by a skilled UAV operator.

Based on the test in 2019, the optimal Zn formulation for the high-Zn rate with one-off sprays and the optimal Zn formulation for the low-Zn rate with three sprays were tested again in 2020 to compare the Zn-biofortification effect of the knapsack sprayer application treatment with that of the UAV sprayer application. Water was used for the CK sprays, and Tween 80 was added at 0.01% (v/v) to every treatment, including the control.

In 2020, the test was synchronously conducted at both locations, Langya Town and Fenshui Town. Cultivars YY538 and YY1540 were planted at both locations. All plots were arranged with three replicates for each cultivar in each location. For knapsack sprayer treatments, including CK, each plot covered an area of 13.5 m × 13.5 m. For UAV treatments, each plot covered an area of 667 m^2.

2.3. Assessment of leaf health

The leaf health status was assessed by the same researcher in the field, 7 days after the last foliar application. Representative examples of injured plants and leaves were photographed to compare the degree of fertilizer-induced damage. Healthy foliar areas unaffected by fertilizer-induced damage were visually estimated as a percentage of the total leaf area. SPAD (Soil-Plant Analysis Development, Minolta SPAD-502, Japan) readings of 15 flag leaves randomly sampled from each replicate were performed at 5 cm from the flag leaf tip. The photosynthetic performance was measured at the flag leaf base using a portable photosynthesis measurement system (LI-COR LI-6400 XT, USA) to monitor the net photosynthetic rate (*P*n, $\mu mol \cdot m^{-2} \cdot s^{-1}$). Individual measurements started at 9:30 a.m. and were finished within 2.5 h.

2.4. Yield, sampling, and analytical methods

All rice plants from 2018 to 50 randomly sampled plants from 2019 to 2020 were collected to measure the grain yield, and three representative plants of every treatment group from each year were selected to analyze yield components and element quality individually. Every sample was heat-treated to a constant weight at 65 °C (after an initial period of 30 min at 105 °C). Then, the seeds (brown rice) were crushed into powder and packed in bags.

In preparation for the Zn analysis, the seed samples were ground in a sample grinder with an agate mortar (model MM301, Retsch, Germany) and digested in 20 mL of nitric acid (HNO_3) (guaranteed reagent, GR), perchloric acid ($HClO_4$) (GR), and hydrogen peroxide (H_2O_2) (volume ratio 5:2:1). The digestion solutions were cooled to room temperature and diluted to 50 mL using double-distilled deionized water. The Zn concentration was determined using an inductively coupled plasma mass spectrometer (ICP-MS, model 7500a, Agilent, USA). The reference material (polished rice powder, GBW (E) 080684) and the blank controls were also analyzed for Zn concentration.

To quantitatively evaluate the utilization efficiency of foliar Zn fertilizer, the apparent recovery efficiency (ARE) of Zn fertilizer was defined as the percentage of Zn ions from the Zn fertilizer that was theoretically transferred to rice seeds and calculated using the following equation (Almeida, 2020):

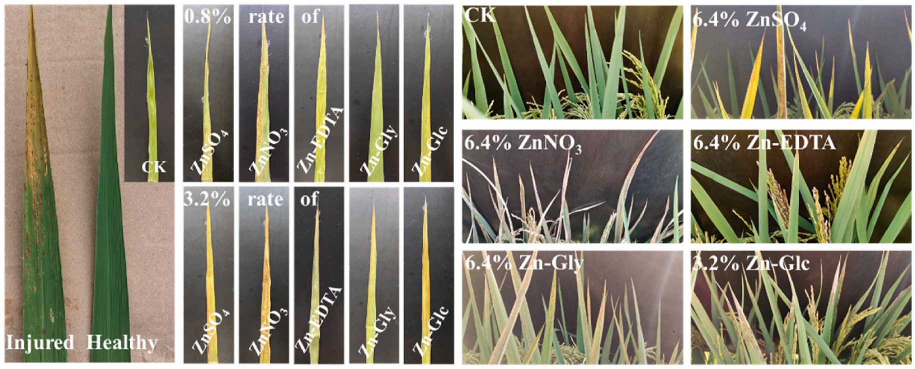

Fig. 2. Zn fertilizer-induced foliar burn photographed 7 days after foliar application in 2018.

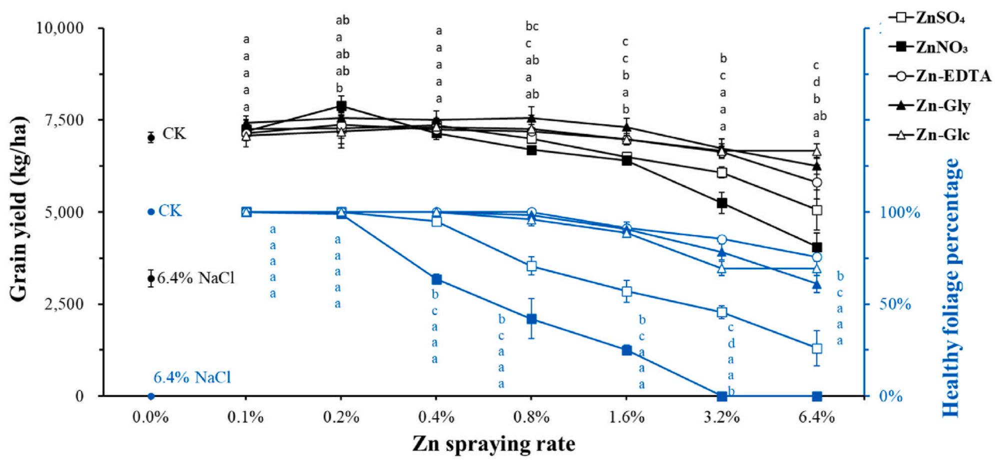

Fig. 3. Effect of manual spraying using different Zn sources and rates on rice yield and healthy foliage percentage in 2018. Small letters from top to bottom at each Zn rate correspond to the order of the Zn sources in the legend, and different small letters indicate significant differences at the 5% level.

$$ARE\ (\%) = \frac{C_t \times W_t - C_0 \times W_0}{F} \times 100$$

where C_t and C_0 is the Zn concentration (mg/kg) in seeds treated with or without foliar Zn fertilizer, respectively; W_t *and* W_0 is the dry seed weight (kg/ha) treated with or without foliar Zn fertilizer, respectively; F (mg/ha) is total Zn ion input of the foliar Zn fertilizer.

2.5. Statistical analysis

The results were analyzed using STATISTICA software 5.5. Tables and figures were created using Microsoft Excel software 2016. The data analyses were performed using average values derived from three replicates (n = 3) for each treatment, and results were expressed as means ± standard deviation. Significant differences among mean values were determined using Turkey's multiple range test at $p < 0.05$.

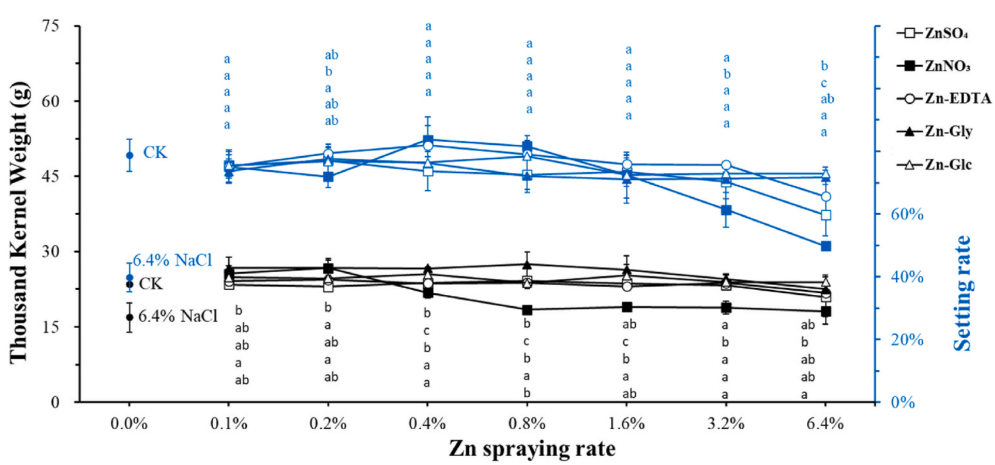

Fig. 4. Effect of manual spraying using different Zn sources and rates on thousand kernel weight and setting rate in 2018. Small letters from top to bottom at each Zn rate correspond to the order of the Zn sources in the legend, and different small letters indicate significant differences at the 5% level.

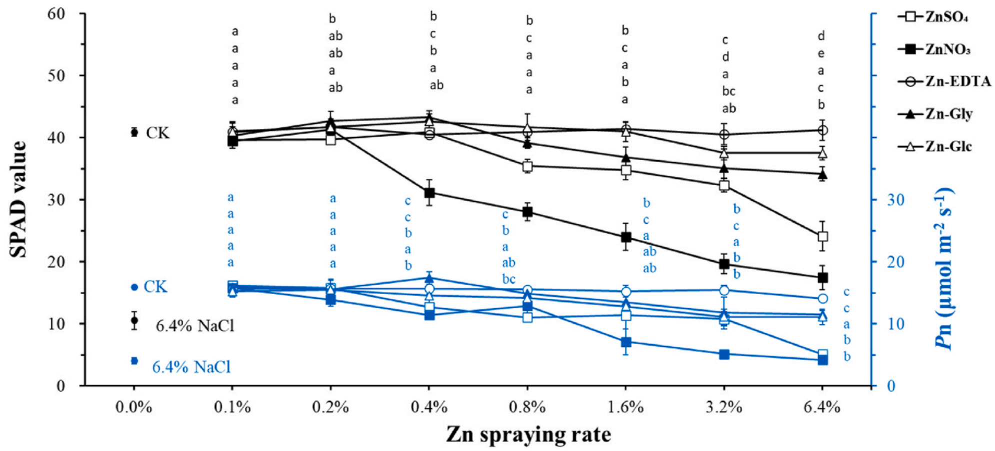

Fig. 5. Effect of manual spraying using different Zn sources and rates on SPAD value and net photosynthetic rate (*Pn*) in 2018. Small letters from top to bottom at each Zn rate correspond to the order of the Zn sources in the legend, and different small letters indicate significant differences at the 5% level.

3. Results

3.1. *Effect of different Zn sources at different spraying rates on YY538 in 2018*

3.1.1. *Foliar burn and yield components*

Fertilizer-induced foliar injuries were associated with visible brown mottles, yellow streaks, or even senesced margins and white rolled leaves, among which the upper leaves often had greater damage on the blade tip (Fig. 2). White and brown hulls were found using the 6.4% Zn-EDTA treatment even when the leaves looked green. At spraying rates of 0.1% and 0.2%, all Zn sources were safe for leaves, and some of them even increased rice yield (Fig. 3). At a rate of 0.4%, $ZnSO_4$ induced negligible damage in leaves, whereas $ZnNO_3$ caused visible phytotoxicity that could not be ignored. At a rate of 0.8%, $ZnSO_4$ and $ZnNO_3$ severely damaged the leaves, while all chelated fertilizers caused minor injuries (Figs. 1 and 2). At exposure rates >0.8%, all fertilizers caused an increase in foliage damage accompanied by growing yield losses. Moreover, the visible phytotoxicity symptoms associated with inorganic fertilizers were more severe than those associated with the chelated fertilizers. The average of the safe Zn application rate thresholds calculated for chelated-Zn fertilizers (0.8%) was approximately three times higher than that calculated for the inorganic fertilizers based on the values for $ZnSO_4$ (0.4%) and $ZnNO_3$ (0.2%).

The foliar Zn fertilizer effect on two yield components factors is shown in Fig. 4. The total descent range was larger for the setting rate than for the thousand kernel weight. This means that the yield losses were mainly caused by the effect of Zn fertilizers on the setting rate. None of the fertilizers had a substantial effect on the setting rate when the spraying rate was ≤1.6%, while the inorganic fertilizers were always associated with a lower setting rate than the chelated fertilizers when the spraying rate was >1.6%. Moreover, the lowest thousand kernel weight among all fertilizers was associated with $ZnNO_3$ when the spraying rate was >0.2%.

3.1.2. *Photosynthetic performance*

For some fertilizers, such as Zn-EDTA, foliar damage was not always associated with decreased SPAD or *Pn* values (Fig. 5). For other fertilizers, the SPAD and Pn values declined as the Zn rate increased. The severity of the effect, according to the decline in SPAD and *Pn* values, depended on the fertilizer (sorted from the highest decline to the minimal effect at spraying rates >0.2%): $ZnNO_3$ > $ZnSO_4$ > chelated fertilizers.

3.1.3. *Zn concentration in rice seeds and Zn utilization efficiency*

The Zn concentration in rice seeds increased with the Zn rate for all Zn sources (Fig. 6). At a rate of 0.1%–0.4%, the highest Zn concentration in seeds was associated with $ZnNO_3$, followed by Zn-Gly. At a rate of 0.8%, Zn-Gly had a significantly stronger effect than $ZnSO_4$. There were no significant differences among the other Zn sources. At a spraying rate

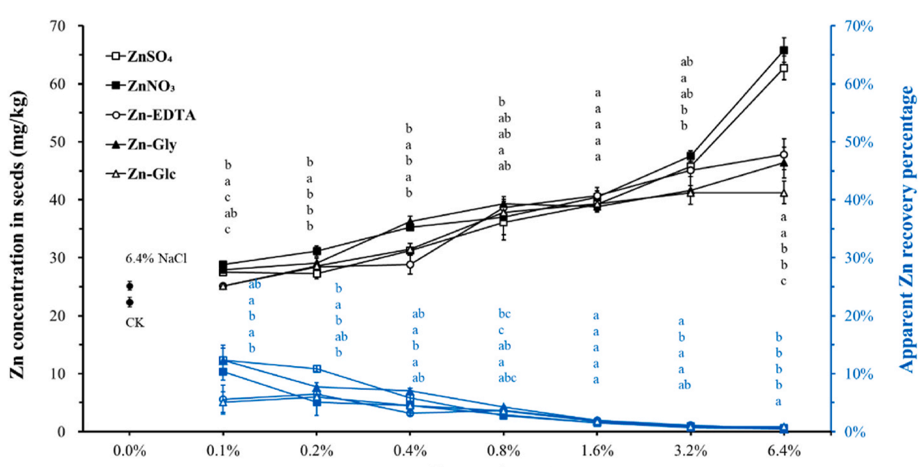

Fig. 6. Effect of manual spraying using different Zn sources and rates on Zn concentration in rice seeds and apparent Zn recovery percentage in 2018. Small letters from top to bottom at each Zn rate correspond to the order of the Zn sources in the legend, and different small letters indicate significant differences at the 5% level.

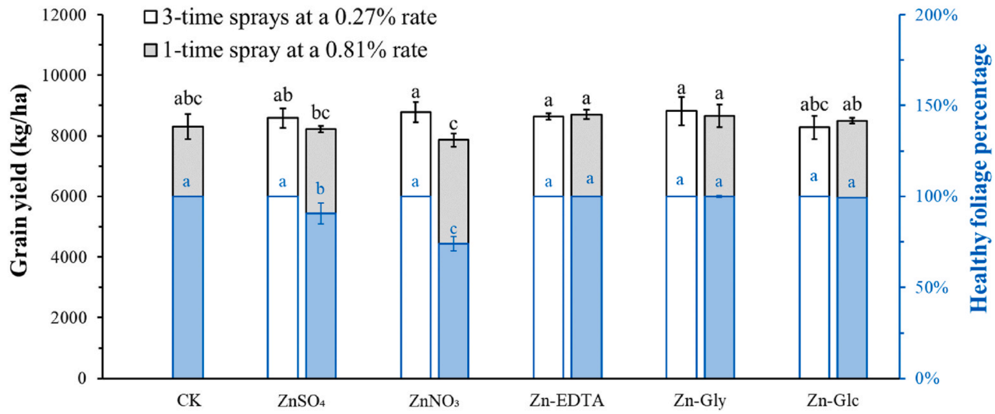

Fig. 7. Effect of split sprays and one–off sprays on rice yield and the healthy foliage percentage from manual spraying in 2019. Values of treatments marked with different small letters varied significantly at the 5% level between treatments.

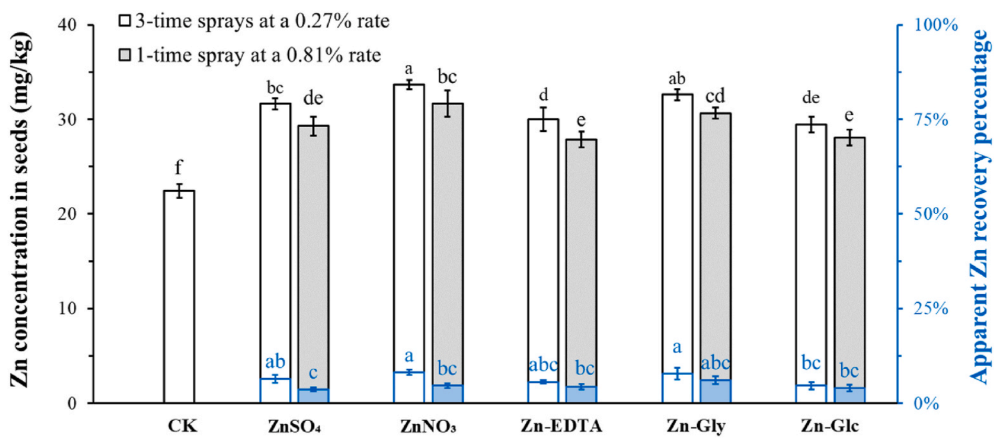

Fig. 8. Effect of split sprays and one–off sprays on the Zn concentration in seeds and apparent Zn recovery percentage from manual spraying in 2019. Values of treatments marked with different small letters varied significantly at the 5% level between treatments.

of 1.6%, the Zn concentration in rice seeds did not vary substantially relative to the Zn sources. At 0.32% and 0.64%, inorganic Zn fertilizers achieved a higher Zn concentration in seeds than chelated Zn fertilizers. In addition, spraying 6.4% NaCl significantly increased the Zn concentration to an average of 2.80 mg kg^{-1}, which was significantly higher than that associated with spraying water (CK).

However, the apparent Zn recovery percentage decreased with the increasing Zn rate for all Zn sources (Fig. 6). At a rate of 0.1%, the utilization percentage ranged from 5% to 12%, and Zn-Glc had the lowest. At the Zn rates of 0.2% and 0.4%, Zn-EDTA had the lowest utilization percentage, and Zn-Gly had the highest. At a Zn rate of 0.8%, the utilization rates of the inorganic Zn fertilizers were lower than those of the chelated-Zn fertilizers. The utilization percentage was lower than 2% when the Zn fertilizer rate reached 1.6%, and there were no

Table 2
The cost inputs of different spraying treatments for application to operation one 1 ha for one worker.

Sprayer type (spraying flow)	Spraying frequency, Zn rate & Zn source	Zn input (kg/ha)	Water input (L/ha)	Time cost (h/ha)	Labor cost (RMB/ha)	Total cost (RMB/ha)	Allocated cost (RMB/kg seeds)
Knapsack sprayer (0.5 L/min)	three times, 0.27% $ZnNO_3$	1.10	1800	60	600	646	0.08
	one time, 0.81% Zn-Gly	1.10	600	20	200	302	0.04
UAV sprayer (1 L/min)	one time, 0.81% Zn-Gly	0.11	60	1	150	160	0.02
	two times, 0.81% Zn-Gly	0.22	120	2	300	320	0.04
	three times, 0.81% Zn-Gly	0.33	180	3	450	481	0.06

Note: The estimated cost is based on the 2020 local market, and the USD/RMB exchange rate was 6.8974. Total cost is the sum of fertilizer cost and labor cost, and allocated cost was calculated by dividing total cost by total grain yield. The labor time was calculated as 10 h of work per day for one person; labor cost was calculated as 200 RMB/ha or 100 RMB/day for knapsack spraying, and as 150 RMB/ha or 1500 RMB/day for UAV spraying. The UAV spraying flow was set conservatively in the range of 0.75–3 L/min. The price of $ZnNO_3$ and Zn-EDTA was 9500 RMB/ton and 21,000 RMB/ton, respectively; the price of Zn-Gly was calculated relative to that of Zn-EDTA. The spraying flow rate that of UAV used was conservative and conducted in 0.75–3 L/min sets usually.

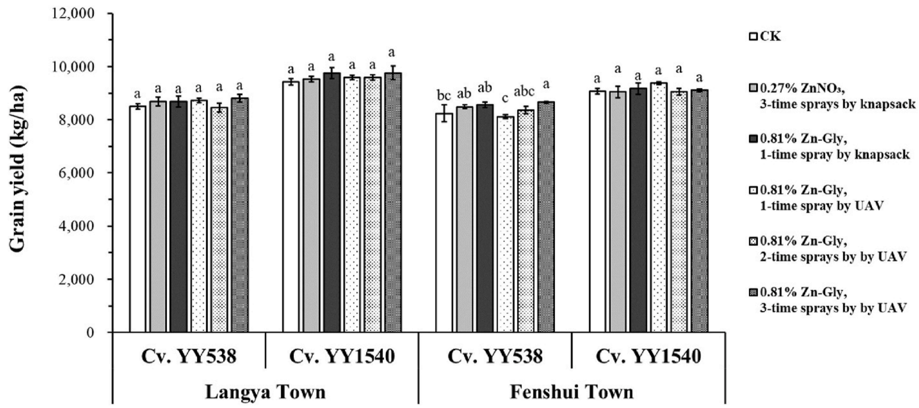

Fig. 9. Effect of different spray treatments on rice yield in 2020. Values of the same cultivar at the same location marked with different small letters varied significantly at the 5% level between treatments.

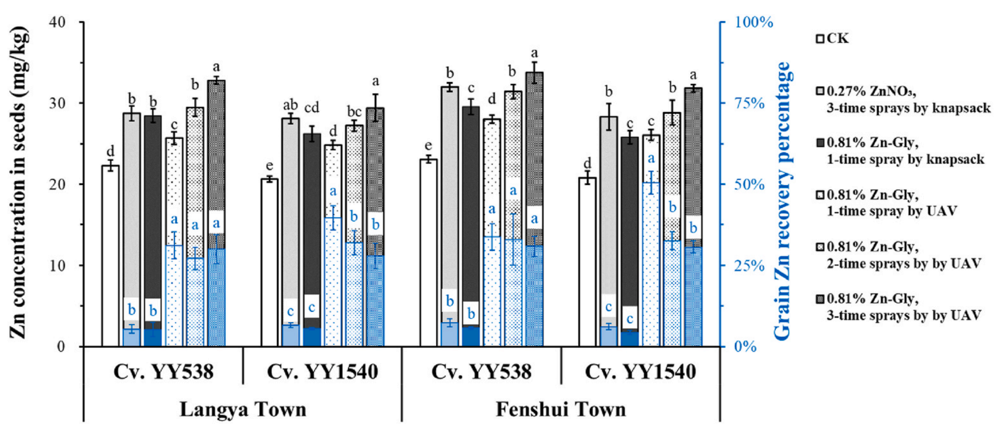

Fig. 10. Effect of different spray treatments on Zn concentration in rice seeds and apparent Zn recovery percentage in 2020. Values of the same cultivar at the same location marked with different small letters varied significantly at the 5% level between treatments.

significant differences among the different Zn sources.

The safe threshold application rates of different Zn sources sorted according to the Zn concentration in the seeds were as follows: 0.8% chelated Zn fertilizers >0.2% $ZnNO_3$ ≥ 0.4% $ZnSO_4$. The recovery percentages had the following order: 0.8% chelated Zn fertilizers <0.4% $ZnSO_4$ < 0.2% $ZnNO_3$. The 2019-year experiment was derived from the repetitive testing in 2018, and it compared the differences between split applications and one-off applications using the same Zn input to confirm whether one-off applications can reach the same grain Zn concentration as that resulting from split applications. Thus, a one-off spray at a Zn rate of 0.81% and three sprays at a rate of 0.27% were conducted in 2019.

3.2. Effect of split application and one-off application on YY538 in 2019

For $ZnNO_3$ and $ZnSO_4$, the spraying rate of 0.81% caused visible foliar burn again in 2019 (Figs. 2 and 7). The damage led to significant yield losses when compared with the highest yield. Other treatments had no apparent adverse effects on rice foliage and yield production at harvest (Fig. 7). The $ZnNO_3$ formulation achieved the highest Zn concentration in rice seeds independent of the split or one-off application, followed by Zn-Gly and $ZnSO_4$ (Fig. 8). The results were consistent with those of the 2018 test.

Regardless of the Zn concentration in rice seeds and the apparent Zn recovery percentage, the split application was superior to the one-off application for all Zn sources (Fig. 8). However, the cost of the split application was three times that of the one-off application (Table 2).

Based on the two-year results, $ZnNO_3$ was the optimal formulation for a normal rate of application, and Zn-Gly was the optimal formulation for a high rate of application, which was the ideal fertilizer formulation to be applied by agricultural spraying UAVs.

3.3. Effect of knapsack sprayer and UAV application in 2020

None of the rice yield responses to foliar Zn fertilizers was lower than that of the CK treatment in 2020 (Fig. 9). Using equal Zn input for application by knapsack sprayer, three sprays of 0.27% $ZnNO_3$ were superior to the one-off spray of 0.81% Zn-Gly (Fig. 10), which resembled the conclusions from the test in 2019 (Fig. 8).

For applications by UAV, the Zn concentration in rice seeds increased with increasing spraying frequency (Fig. 10). Using the one-off application of 0.81% Zn-Gly, the Zn concentration in rice seeds was lower with the UAV than with the knapsack, except for cultivar YY1540 in Fenshui Town. Two applications of 0.81% Zn-Gly by UAV achieved a higher Zn concentration in rice seeds than one application of 0.81% Zn-Gly by knapsack, and it was equal to that achieved by three applications of 0.81% Zn-Gly. Furthermore, three applications of 0.81% Zn-Gly by UAV achieved the highest Zn concentration in rice seeds.

The apparent Zn recovery percentages ranged from 27.16% to 50.47% for application by UAV, which were much higher than those of the knapsack sprayer applications, which ranged from 4.35% to 7.37% (Fig. 10). An increase in spraying frequency did not substantially change the apparent Zn recovery percentage in cultivar YY538, while it showed a downward trend in YY1540. Overall, when compared with the most effective treatment option by knapsack sprayer (three sprays of 0.27% $ZnNO_3$), two to three sprays of 0.81% Zn-Gly by UAV had an equivalent effect on Zn biofortification associated with a much higher apparent Zn recovery percentage.

3.4. Cost input

The cost inputs of the different spraying treatments are presented in Table 2. Under the same spraying frequency, the labor efficiency (reciprocal of labor time) of the UAV was 20 times that of the knapsack sprayer, and using the UAV consumed only one-tenth of the water quantity and 3/4 of the labor cost compared with those of the knapsack sprayer. Under conditions that had an almost equivalent effect on Zn biofortification using a knapsack sprayer for three sprays of 0.27% $ZnNO_3$ and a UAV for two sprays of 0.81% Zn-Gly (Fig. 10), the total cost and allocated cost of the UAV were both only half of the knapsack, and the spraying efficiency of the UAV was much higher than that of the knapsack sprayer due to a considerable reduction in the farming operation time, the total Zn input, and the total water input. All cost burdens for farmers, including time, materials and money, become more acceptable. In addition, UAV application greatly lowered residual Zn pollution in rice paddies because of its low Zn input and its resulting high Zn-utilization rate (Fig. 10).

4. Discussion

4.1. Leaf burn and the alleviation effect

It is commonly assumed that the lower the pH value, the higher the EC value, the more serious the foliar damage in crops. The pH value of foliar Zn fertilizers is usually adjusted to 5–8 to avoid acid damage, and the Zn rate for spraying (typically $ZnSO_4{\cdot}7H_2O$) is usually in the range of 0.1%–0.5% (w/w, as to prevent salt damage) (Li et al., 2009). However, this study demonstrated that pH < 5 and/or Zn rate $>$0.5% did not always cause foliar damage, and the type of Zn source affects the response in rice (Table 1 and Fig. 3). Although the pH of Zn-Gly was lower than that of $ZnSO_4$ and the EC value of Zn-EDTA was higher than that of $ZnSO_4$, all three chelated fertilizers caused fewer foliar injuries than the inorganic Zn fertilizers, which has rarely been reported before. Even for the two inorganic fertilizers, $ZnNO_3$ was associated with more serious toxicity than $ZnSO_4$ at the same excess Zn^{2+} rate. This observation can be attributed to the anion difference rather than the difference in pH because simulated acid rain studies showed that at the same pH, NO_3^--rich rain had stronger inhibitory effects than SO_4^{2-}-rich rain (Li et al., 2014; Sun et al., 2012).

A recent study showed that Zn-EDTA can be immediately absorbed in chelated form and moves slower than $ZnSO_4$ in wheat leaves (Doolette et al., 2018). In addition, the concentrations of remaining free Zn^{2+} ions in fertilizers were in the order of CK $<$ chelated Zn fertilizers $<$ inorganic fertilizers. Based on these facts, the probable mechanism for the alleviating effect of chelated Zn fertilizers on foliar damage was not only the reduced concentration of harmful Zn^{2+} ions in the solution formula but also the relatively low toxicity of the chelated-Zn fertilizer complexes compared to that of Zn^{2+} ions. Moreover, in corn seedlings, Zn-EDTA induced fewer foliar injuries than ZnSO4 (Golden et al., 2016). Chelated fertilizers can be used in cases requiring high safety thresholds or to alleviate foliar burn in plants sensitive to exogeneous Zn^{2+} applications. However, some chelating agents, such as citric acid, cannot alleviate the harmful effects of Zn^{2+} ions (Golden et al., 2016).

4.2. Variability of the Zn-biofortification level

The characteristic differences among the Zn fertilizers not only induced different levels of foliar damage but were also associated with variations in the Zn-biofortification level. At the rates $>$1.6%, inorganic Zn fertilizers were better biofortifying agents than chelated-Zn fertilizers. Considering the observed effect of the treatment with 6.4% NaCl, inorganic Zn fertilizers may have had an enrichment effect on grain Zn concentration because they induced sharp yield losses (Figs. 3 and 6). When the Zn rate was $<$0.8%, $ZnNO_3$ had better Zn-biofortification level than $ZnSO_4$. Oxidation by NO_3^- is stronger than by SO_4^{2-} and causes stronger disruptions to the membrane permeability of leaves (Li et al., 2014; Sun et al., 2012). This disruption could enhance the amount of exogenous Zn^{2+} ions entering rice leaves, stimulating the transfer of Zn^{2+} ions from $ZnNO_3$ to rice seeds.

Existing research on the physiological mechanisms through which chelated fertilizers act is insufficient given their many potential applications. Despite a few different examples, most studies have shown that the Zn-biofortification levels of foliar Zn-EDTA and Zn-Glc are lower than those of $ZnSO_4$ (Golden et al., 2016; Wei et al., 2012; Zhang et al., 2015). The molecular weight and stability constant of Zn-EDTA chelate and Zn-Gly chelate are higher than that of other Zn sources. The weak absorption capacity by leaves and the rate of movement between leaves and other plant tissues are related to the limited ability of rice to transfer Zn from the foliar fertilizers to seeds; hence, Zn-EDTA chelate and Zn-Gly chelate were always associated with the lowest Zn concentrations in the rice seeds. Several studies have proved that the Zn-biofortification effect of amino acid-chelated Zn fertilizers are better than those of $ZnSO_4$ and/or Zn-EDTA (Wei et al., 2012; Ghasemi et al., 2013; Mohammadi and Khoshgoftarmanesh, 2014; Tabesh et al., 2020). Information is scarce regarding the mechanisms whereby leaves absorb amino acids and amino acid-chelated Zn. Isotope tracer techniques and synchrotron radiation analysis should be used to elucidate these mechanisms in the future (Souri, 2016; Doolette et al., 2018).

In addition to the differential effects among different Zn sources, the level of Zn biofortification is linked to the Zn input amount. In our study, an increase of the Zn input achieved by using a higher Zn rate was associated with improved Zn biofortification in the rice seeds (Fig. 5); three sprays were better than one or two sprays (Fig. 10), and the knapsack sprayer was better than the UAV using the same spraying rate but a low volume spray condition (Fig. 10). Similar results have been made in previous studies (Zhang et al., 2012; Dapkekar et al., 2018; Almeida et al., 2020). The split application was better than the one-off application because of the higher outflow associated with the latter (Fig. 8). However, low Zn input by the UAV sprayings also had a positive effect on Zn biofortification (Fig. 10), due to the high efficiency of the spraying process.

4.3. Vital differences among chelated-Zn sources

Zn-EDTA is the most widely used chelated-Zn fertilizer (Du et al., 2014). However, EDTA application entails environmental accumulation risks due to degradation resistance, which has limited its use in some industries by the European Union (Claudia, 2003; Muthu et al., 2014). In contrast to EDTA, amino acids, usually glycine, are used in modern formulations of chelate fertilizers. As a naturally organic chelator, glycine has no environmental risks and is highly compatible with current sustainable agriculture (Dolev et al., 2020; Souri, M.K., 2016). Glycine can also function as an effective nutrient source providing organic nitrogen for plants (Souri and Hatamian, 2018). Despite insufficient scientific data and information on fertilizer responses, amino acid-chelated fertilizers have increasingly dominated the competitive fertilizer markets in many countries (Souri, 2016).

In addition to the poor Zn-biofortification effect, the low solubility of Zn-Glc in water also limits its application (Table 1). To perform Zn-Glc foliar spraying, a pre-dissolved bulky solution was prepared and carefully transported to the field. Another option is to dissolve the solid Zn-Glc material in the field, which is difficult to implement and associated with the risk of clogging the spray nozzle if the material does not dissolve well. Other Zn sources are much more easily soluble in water (Montalvo et al., 2016).

4.4. Differences between manual spraying and the UAV spraying process

The manual spraying process depends on subjective experience and

Fig. 11. The spraying operation of a knapsack sprayer and an agricultural spraying UAV in a rice paddy.

intuition and requires footpaths across the field (Wang et al., 2020). It is inevitable that manual spraying variability occurs due to uncontrollable walking speed and that spraying direction causes spatial variability in the extent of Zn biofortification. Although these approaches allow spraying close to the target crop with low fertilizer drift and high spray coverage (Fig. 11), walking or driving through the rice field also leads to the destruction of healthy rice plants and paddy soil compaction.

The UAV spraying process did not damage crops and soils, and high-quality spraying can be performed by skilled UAV operators. Because of the high UAV price and laborious operation knowledge for small farmers, agricultural UAV services for smallholdings in China have been dominated by specialized UAV companies that purchase mass UAV equipment and train vocational operators (Yang et al., 2017; Zhou et al., 2017). The introduction of the first national standard in agricultural UAV use in China, the Technical Specification of Quality Evaluation for Crop Protection UAS (NY/T 3213–2018), has also promoted the standardization of foliar spraying processes and the regulation of market quality. The rotor of the UAV blows downward in favor of the downward penetration of the droplets to the rice canopy and the base and the front and back of the leaves (Fig. 11). This is the primary reason why the spraying efficacy of the low-volume sprays of the UAV is not inferior to that of high-volume sprays of artificial sprayers (Fig. 10; Table 2). In addition, the optimal operation parameters for UAV spraying have been widely investigated indoors under controlled conditions and under real field conditions in recent years (Wang et al., 2018, 2020; Kim et al., 2019; Kharim et al., 2019; Ahmad et al., 2020; Guo et al., 2020). Meanwhile, the autonomous control mode of UAVs can minimize the spatial variability in the Zn-biofortification quality because of its fully automatic flight control, which includes takeoff, landing, and fixed spraying settings, flying conditions, and even flying routes. Therefore, fine spraying homogeneity results can be achieved by continuous improvement of UAV-based spraying technologies.

5. Conclusion

This study addressed the knowledge gap that existed regarding the application of chelated-Zn fertilizers. At high spraying rates, the inorganic fertilizer-induced phytotoxicity not only reduced photosynthesis, but also impaired the normal development of the spikelet, causing a decline in grain production at harvest. Owing to their lower toxicity than that of Zn^{2+} ions, all chelated-Zn fertilizers produced much less foliar damage than the inorganic Zn fertilizers, a finding that has not previously reported. The chelated fertilizers with high safety thresholds can be used in cases that requiring high fertilizer use rates or to alleviate foliar burns in plants sensitive to exogenous Zn^{2+} applications. However, there are limitations because Zn-EDTA is bio-refractory, and Zn-Glc has low solubility. Zn-Gly achieved the highest level of Zn biofortification among all the chelated fertilizers. Based on this, Zn-Gly was considered suitable for the UAV requirement of "low volume sprays with a high rate of agrochemical application." The quality evaluation of the UAV spraying test showed that the Zn-utilization efficiency of UAV-based spraying reached a level that will probably never be attained by knapsack spraying, and two or three sprays of 0.81% Zn-Gly achieved at least the equivalent Zn-biofortification level of the best treatment regimen by manual spraying (three sprays of 0.27% $ZnNO_3$ at high spraying quantity).

To our knowledge, this was the first scientific investigation about the use of UAVs for agricultural spraying to achieve a high level of Zn biofortification. In summary, compared to knapsack sprayer applications, the UAV-based application: (1) greatly reduced Zn input and water quantity input; (2) increased the high Zn utilization rate, which lowered potentially residual Zn risks in the rice paddies; (3) diminished contact exposure to Zn fertilizer mist; (4) eliminated of artificial disturbance brought to the soil and plants; (5) an equal or greater biofortification level; (6) increased labor efficiency and reduced work time and working strength; and (7) had no restrictive conditions due to the flooded paddy environment. Considering the major purpose of spraying UAVs is to apply fungicides and insecticides for crop protection, the possibility of combined application of pesticide along with chelated-Zn fertilizers should be explored to further reduce Zn-biofortification costs in the future.

CRediT authorship contribution statement

Meng Xu: Investigation, Resources, Formal analysis, Writing – original draft. **Mengjiao Liu:** Methodology. **Fan Liu:** Visualization. **Nan Zheng:** Software. **Sheng Tang:** Formal analysis. **Jingjie Zhou:** Auxiliary experiment. **Qingxu Ma:** Writing – review & editing. **Lianghuan Wu:** Project administration, Funding acquisition, Resources, Supervision, Data curation, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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