Chatta MB *et al.* (2023)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 51, Issue 2, Article number 13221
DOI:10.15835/nbha51213221
Research Article

Foliar applied zinc on different growth stages to improves the growth, yield, quality and kernel bio-fortification of fine rice

Muhammad B. CHATTHA[1*], Qurban ALI[1], Muhammad N. SUBHANI[1], Muhammad ASHFAQ[1], Shahbaz AHMAD[1], Zahid IQBAL[1], Muhammad IJAZ[2], Muhammad A. AYUB[2], Muhammad R. ANWAR[2], Maha ALJABRI[3], Muhammad U. HASSAN[4], Sameer H. QARI[5]

[1]*University of the Punjab, Faculty of Agricultural Sciences, Lahore, 54590, Pakistan;* bilal.agronomy@pu.edu.pk *(MBC *corresponding author);* ranaqurban8121520@gmail.com *(QA);* nasirsubhani.iags@pu.edu.pk *(MNS);* ashfaq.iags@pu.edu.pk *(MA);* shahbaz.iags@pu.edu.pk *(SA);* zahidiqbalsheikh@gmail.com *(ZI)*
[2]*Rice Research Station, Bahawalnagar, Pakistan;* cmijaz66@gmail.com *(MI);* ahsinayub@gmail.com *(MAA);* muhammad.rizwan.a@gmail.com *(MRA)*
[3]*Department of Biology, Faculty of Applied Science, Umm Al-Qura University, Makkah, Saudi Arabia;* myjabri@uqu.edu.sa *(MA)*
[4]*Jiangxi Agricultural University, Research Center on Ecological Sciences, Nanchang, 330045, China;* muhassanuaf@gmail.com *(MUH)*
[5]*Department of Biology, AlJumum University College, Umm Al-Qura University, Makkah, Saudi Arabia;* Shqari@uqu.edu.sa *(SHQ)*

Abstract

Zinc (Zn) is an essential nutrient required for plants growth and development, nonetheless, Zn deficiency is continuously increasing in our soils which is decreasing crop production. Further, the crops grown on Zn-deficient soils also contains a low amount of Zn which is also a major reason for Zn deficiency in humans. So, it is mandatory to supply the Zn to fulfil the crop needs with a corresponding increase in grain Zn. Thus, this study determined the impact of different rates of foliar applied Zn at different growth stages on the growth, yield, quality, and Zn bio-fortification of fine rice. The study comprised foliar application of distilled water (control), foliar applied Zn @ 0.5% at stem elongation stage + booting stage, foliar applied Zn @ 1.0% at stem elongation stage + booting stage, foliar applied Zn @ 0.5% at booting stage and milking stage, foliar applied Zn @ 1.0% at booting stage and milking stage, foliar applied Zn @ 0.5% at milking stage + dough stage and applied Zn @ 1.0% at milking stage + dough stage. The results indicated Zn applied different growth stages significantly improved, productivity and Zn bio-fortification of rice crop. The maximum LAI, LAD, CGR, fertile tillers (363.17 and 372.17), 1000 KW (25.50 g and 25.61 g), kernel yield (5.45 t ha^{-1} and 5.44 t ha^{-1}), biomass yield (14.22 t ha^{-1} and 14.26 t ha^{-1}) HI, chlorophyll concentration (1.60 mg/g FW and 1.52 mg/g FW), relative water content (92.33% and 90.11%), and antioxidant activities were observed with foliar applied Zn (0.5%) at booting and milking stage and lowest values of all these traits were observed in control. Likewise, the maximum kernel protein (10.55% and 10.88%), amylose (27.85% and 26.18%), kernel length (6.54 mm and 6.68 mm) and width (2.37 mm and 2.77 mm), and grain Zn concentration (32.22 mg kg^{-1} and 30.21 mg kg^{-1}) was recorded with Zn (0.5%) at the booting and milking stage, and minimum kernel protein, amylose, kernel length, and width, and grain Zn concentration was noted in control. The current study findings

Received: 21 May 2023. Received in revised form: 09 Jun 2023. Accepted: 13 Jun 2023. Published online: 20 Jun 2023.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

suggested that foliar-applied Zn (0.5%) at the booting and milking stage could be an important practice to get better productivity, quality, and grain Zn bio-fortification of rice in semi-arid conditions.

Keywords: antioxidant; bio-fortification; foliar spray; yield; zinc

Abbreviations: LAI: leaf area index; LAD: leaf area duration; CGR: crop growth rate; HI: harvest index; KW: kernel weight; OM: organic matter; Na: sodium; Ca: calcium; Mg: magnesium; DPTA: diethylentriamene pentaacetate; RCBD: randomized complete block design; DAP: ammonium phosphate; SOP: sulfate of potash; N: nitrogen; K: potassium; RWC: relative water content; CAT: catalase; H_2O_2: hydrogen peroxide; APX: ascorbate peroxidase; ANOVA: analysis of variance; LSD: least significant difference; TDM: total dry matter; POD: peroxidase; @: at the rate of

Introduction

Zinc (Zn) is an important nutrient required for plants and humans. It is involved in cell-mediated immunity and it also protects against oxidative stress and chronic diseases (Prasad, 2020). The deficiency of Zn in humans is a major problem in developing countries which disturbs the sex hormones, and testicular development, and leads to hypogonadism, inflammation, and apoptosis (Chasapis *et al.,* 2020; Beloucif *et al.,* 2021). It has been found that deficiency of Zn can cause significant yield losses (Chattha *et al.,* 2017; Hassan *et al.,* 2022). Rice is a staple food for more than 50% world's population, however, the deficiency of Zn in our soils is continuously increasing which is significantly reducing the rice growth and yield (Nawaz *et al.,* 2021; Sher *et al.,* 2022). Besides this, the concentration of Zn is also reduced in edible grain parts which also leads to Zn deficiency in humans. Therefore, increasing grain Zn concentration is considered as an important approach to increase Zn availability to humans (Ghoneim *et al.,* 2016; Pradhan *et al.,* 2020). Zn improves plant growth, chlorophyll synthesis and antioxidant activities (Zafar *et al.,* 2017) and it also protects the membranes from oxidative damage by stabilizing the membrane stability and osmolytes accumulation which resulting in substantial increase in crop yield (Al-Zahrani *et al.,* 2021).

Bio-fortification is an important way to enhance grain Zn-contents and Zn availability to plants and humans (Lividini *et al.,* 2018). The increasing grain Zn concentration through the fertilizers approach has been noticed in different crops across the globe (Kumar and Pandey *et al*., 2015). Zn fertilizers are being used across the globe to provide Zn to plants and increase Zn bio-fortification (Hassan *et al.,* 2022). The soil factors including pH, organic matter (OM), Na, Ca, and Mg, and soil phosphorus concentration strongly affect the Zn availability to plants (Chhabra and Kumar, 2018). The soil application is the most widely used method of Zn application across the globe, however, in this method, most of Zn is fixed in the soil which results in less availability of Zn to plants (Kachinski *et al.,* 2022). In this context, the foliar spray of Zn is considered an effective strategy to quickly supply the Zn to plants to remove the Zn deficiency faced by plants (Wang *et al.,* 2015; Hassan *et al.,* 2019; Sher *et al.,* 2022). Foliar applied Zn is more effective and beneficial as compared to soil application, particularly on calcareous and flooded soils where Zn availability is low (Suganya *et al.,* 2020). Further, soil applied Zn at sowing has little impact on grain Zn concentration thus, the foliar spray is very effective to improve the grain Zn (Cakmak and Kutman, 2018). However, the impacts of exogenously applied on grain Zn content is also varied according to genotypes, rate of foliar spray, and climatic conditions (Kandil *et al.,* 2022).

Foliar spray of Zn to leaves increase the Zn absorption and subsequent transformation into edible plant parts and this method is considered an effective and safe way to increase the grain Zn concentration. In foliar spray application substances directly penetrate the cuticle or stomata pathway to enter the lead and foliage feeding at the flowering stage can significantly increase the grain Zn concentration (Sher *et al.,* 2022).

Nonetheless, the sustainability of Zn ions adhering to plant leaves is very challenging and sprayed Zn can easily drop from the leaf surface or they leached away by rain which affects the absorption of Zn by leaves (Wu *et al.,* 2020). Further, foliar-applied Zn also significantly improved crop production and final quality (Yang *et al.,* 2021). In literature rare studies are conducted on the impacts of foliar feeding of Zn on yield and quality of fine rice. Therefore, in the present study, we hypothesized that foliage feeding of Zn at diverse stages of plant life can affect the yield and quality of fine rice. Thus, we performed current study to determine the optimum rate of Zn application and stage of application to improve the growth, productivity, and quality of fine rice growing in semi-arid conditions.

Materials and Methods

Experimental site

The present research was performed for two years (2015 and 2016) at the University of Punjab Lahore, to determine the impact foliar applied Zn on productivity and quality of fine rice. The studied site has hot and semi-arid conditions and further weather conditions are given in Figure 1. Before the experiment, the soil samples were taken from the diverse location of the site and soil properties were determined (Homer and Pratt 1961). The soil was sandy loam with pH 7.72, organic matter (8.0 g kg^{-1}) Ec (1.02 dS m^{-1}), total N (0.032 g kg^{-1}) and available P and K (18 and 135 mg kg^{-1}) and DPTA Zn (30 mg kg^{-1}).

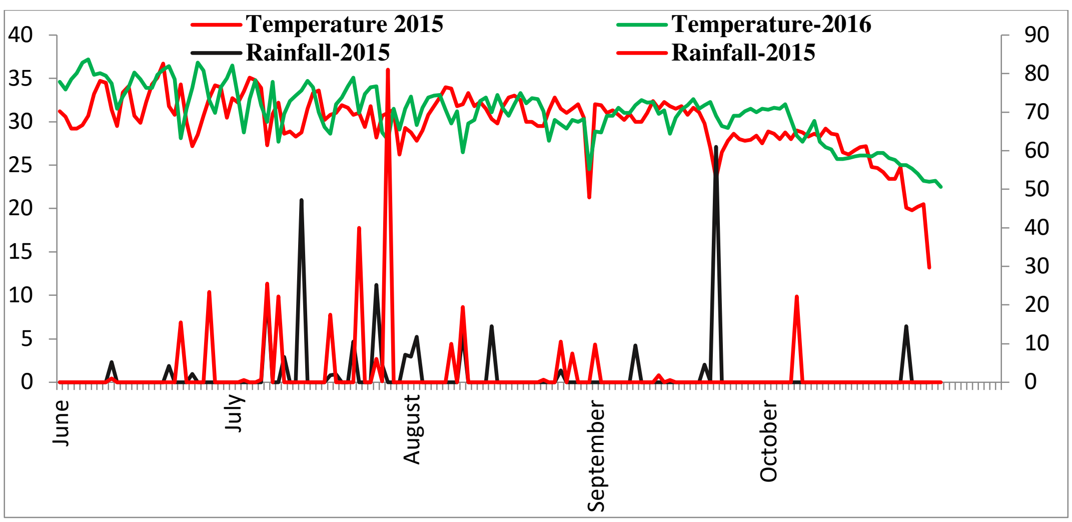


Figure 1. Weather conditions during both years of study (2015 and 2016)

Experimental treatments

The study was comprised of different treatments; foliar application of distilled water T_1: (control), T_2: foliar applied Zn @ 0.5% at stem elongation stage + booting stage, T_3: foliar applied Zn @ 1.0% at stem elongation stage + booting stage, T_4: foliar applied Zn @ 0.5% at the booting stage and milking stage, T_5: foliar applied Zn @ 1.0% at the booting stage and milking stage, T_6: foliar applied Zn @ 0.5% at milking stage + dough stage and T_7: foliar applied Zn @ 1.0% at milking stage + dough stage. The study was executed in RCBD having three replications.

Crop husbandry

The soil was flooded and cultivated three times followed by planking to prepare the seed bed. Then rice nursery was transplanted and flooded conditions were maintained for 7 days afterward, the water was drained from the studied field and again flooding conditions were created until maturity. The fertilizers NPK was applied at the rate of 120:88:68 kg ha^{-1} in the forms of urea (46%), DAP (18% N and 46% P), and SOP (50% K). The full amount of P and K and half of N were applied at sowing whilst, rest of N was applied to crop at tillering stage. Moreover, other standard management practices were kept constant in order to get a good stand establishment.

Determination of growth and yield traits

The rows of rice plants having a 1-meter length were harvested from each plot and plants were separated into leaves and roots. After that 5 g leaves were taken to determine the leaf area and LAI, while LAD and CGR were calculated with methods of Watson (1947) and Hunt (1978) respectively. Moreover, ten plants from each pot were taken to determine the plant height, panicle per plant, and kernels per panicle. After that, the complete plots were hand harvested to determine biological and kernel yield and converted into the ton per hectare basis. Additionally, a sub-sample (1000 kernels) was taken for the determination of 1000 kernel weight.

Determination of quality traits

Rice kernel's sub-sample was taken and sterile kernels were manually counted. On the other hand, 20 kernels from the harvested sample were taken and placed in light, to determine opaque kernels. Similarly, again 20% kernel was placed in light and differential into normal and abortive kernels based on size and light and not translucent to light. Kjeldhal method was for determined rice N concentration and kernel protein concentration was measured with (AOAC, 1990) method. On the other kernel, amylose concentration was determined with the procedures of Juliano (1971). For determining Zn concentration; rice kernels were over-dried and digested by the addition of a di-acid mixture ($HClO_4$: HNO_3) in and later on rice concentration was determined using an atomic absorption spectrophotometry (Prasad, 2006).

Determination of physiological and biochemical traits

To determine the relative water contents (RWC) leaf fresh samples (1 g) were taken and weight to determine the fresh weight (FW), after that samples were soaked in water for 24 hours to determine the turgid weight (TW), and then they were removed from the water and then oven dried and dry weight (DW) and RWC was following method: RWC = (FW-DW)/(TW-DW) ×100. For determination of leaf chlorophyll; 0.5 g rice leaf sample was taken and homogenized to get extract and absorbance was noted at 663 and 645 nm for determination of a and b concentration (Lichtenthaler, 1987). For CAT activity; 1 g of leaves were ground using 2.5 ml of 50 mM K-buffer and then centrifuged for 15 minutes and supernatant was taken and then 0.1 ml extract was added in 0.1 ml of H_2O_2 (5.9 mM) and 2.5 ml of 5 % TCA buffer and absorbance recorded at 240 for determination of CAT activity (Aebi, 1984). In the case of POD activity; 0.5 g rice life samples were taken and homogenized by using the K-buffer (5 ml) and centrifuged for 15 minutes and the supernatant was taken and we noted the absorbance at 470 nm (Zhang, 1992). In the case of APX activity, again 0.5% g leaf samples were taken and 5 ml of KPB was added and centrifuged for 15 minutes, and then absorbance was noted for estimation of APX activity.

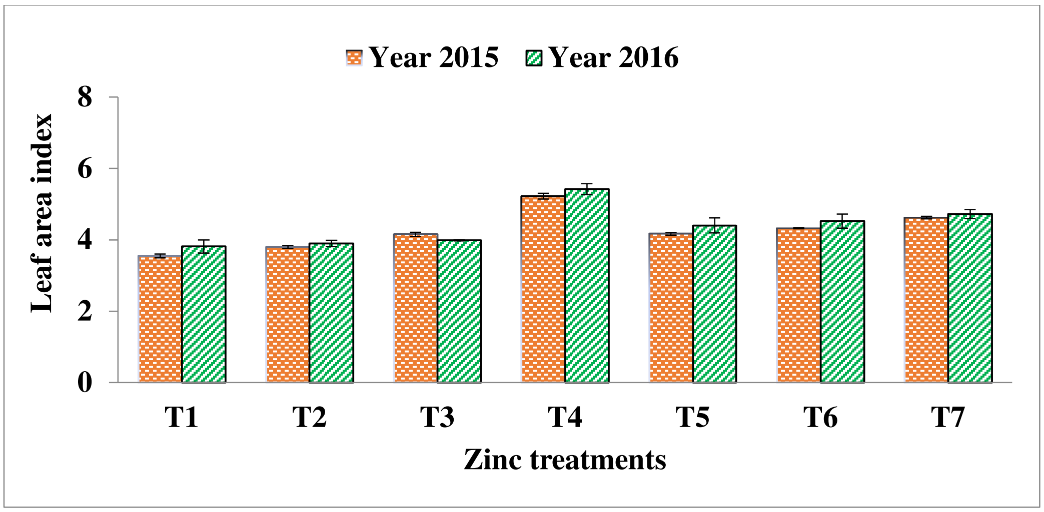
Data analysis

The collected data on different traits were analysed by one-way ANOVA and LSD test ($p \leq 0.05$) was used to detect the significant levels among ANOVA sources (Steel *et al.,* 1997). The figures were prepared by using Sigma-plot (8).

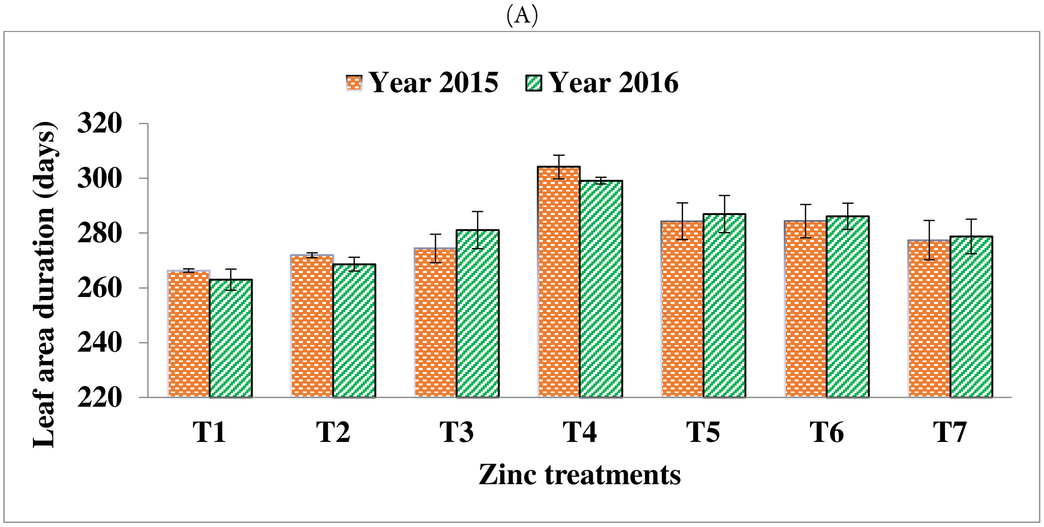
Results

Growth traits

The foliar applied Zn on different growth stages significantly affected the LAI and LAD of rice crops (Figure 1). The results indicated during both years maximum LAI and LAD were noted with foliar-applied Zn (1%) at the booting and milking stage followed by foliar-applied Zn (0.5%) at the same growth stages. Moreover, the minimum LAI and LAD during both years were recorded in the foliar application of distilled water (Figure 2). Similarly, foliar applied Zn also significantly affected the total dry matter and CGR (Figure 3). Again, maximum TDM and CGR were obtained with foliar applied Zn (1% and 5%) at the booting and milking stage followed by foliar applied Zn (1%) stem elongation and booting stages lowest TDM and CGR were recorded with foliar spray of water (control: Figure 3).



(A)



(B)

Figure 2. Effect of foliar applied Zn on different growth stages on LAI (A) and LAD (B) of rice during 2015 and 2016. The values in the above figure are means of three replicates with ±SE.

Yield traits

The study findings indicated that exogenous Zn spray at different stages of rice appreciably improved all yield traits in both study years (Table 1). The results indicates that taller plants with more tillers and kernels per panicle were recorded 0.5 foliar applied booting and milky stage followed by foliar applied Zn (1%) at the same growth stages and shorter plants with minimum tillers and kernels were obtained with foliar spray of water (Table 2). On the other hand, kernel weight, kernel yield, and HI were also significantly affected with foliar-applied Zn. Again, the maximum kernel weight (25.50 g and 25.61 g), kernel yield (5.45 t ha^{-1} and 5.44 t ha^{-1}), biological yield (14.22 t ha^{-1} and 14.26 t ha^{-1}) were noted with 0.5% Zn at booting and milking and minimum kernel weight, kernel and biomass yield and HI were recorded in control conditions with foliar spray of water (Table 2).

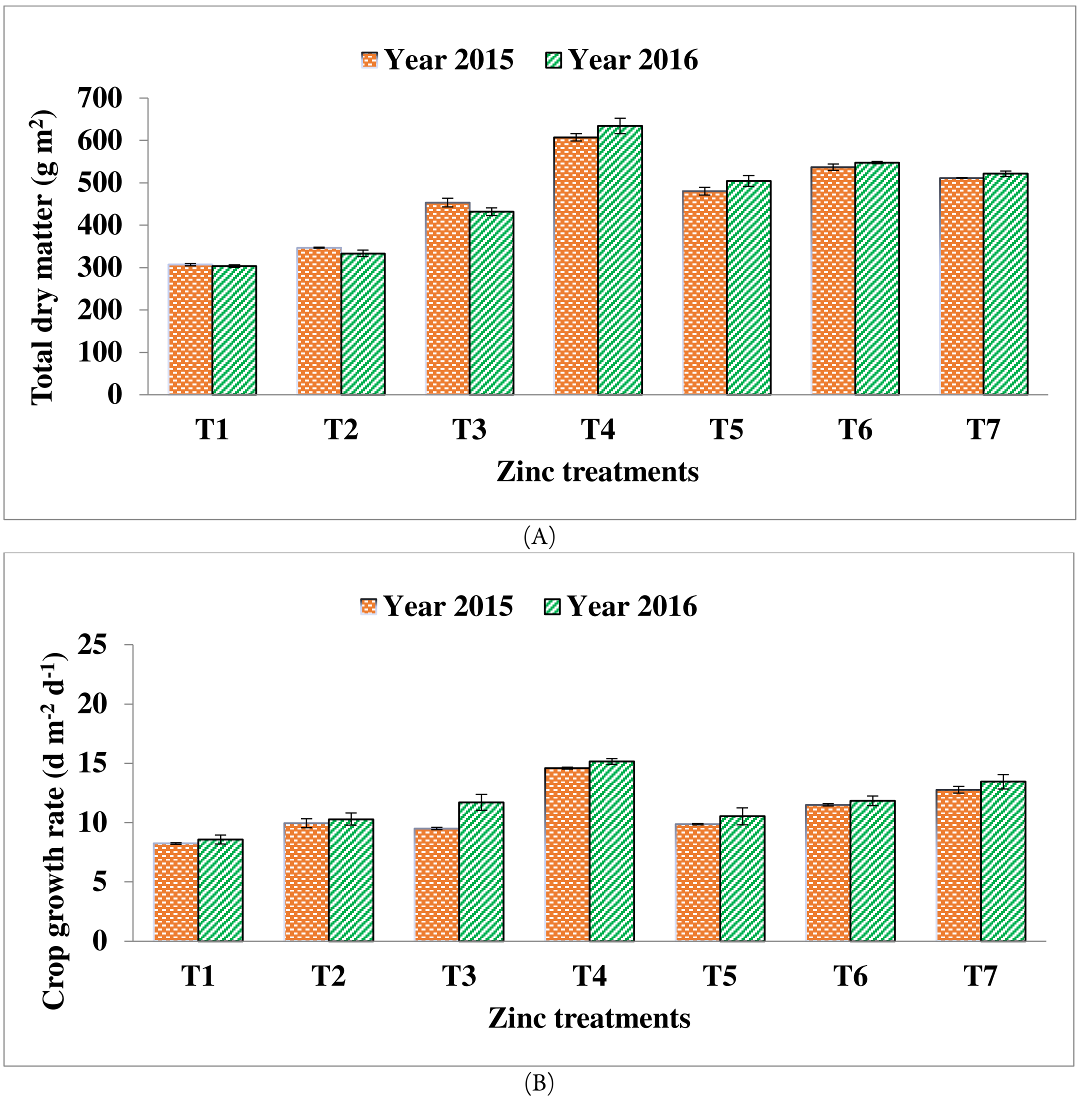


Figure 3. Effect of foliar applied Zn on different growth stages on TDM (A) and CGR (B) of rice during 2015 and 2016. The values in the above figure are means of three replicates with ±SE.

Table 1. Effect of foliar applied Zn on different growth stages on plant height, tillers and kernels/panicle of rice during 2015 and 2016

Treatments	Plant height (cm)		Fertile tillers (m^{-2})		Kernels per panicle	
	2015	2016	2015	2016	2015	2016
T1	94.68d	96.34d	309.90e	306.57e	52.67e	54.67e
T2	100.32cd	98.32cd	328.23d	324.90d	58.00cd	61.33cd
T3	106.11b	109.44b	338.20d	344.87d	61.33bc	64.67bc
T4	104.32bc	100.99bc	363.17a	372.17a	69.00a	73.67a
T5	118.15a	115.48a	346.57bc	351.23bc	68.33a	64.33a
T6	104.76bc	105.09bc	352.87d	356.20b	57.67d	60.00d
T7	105.36bc	101.69bc	342.20bd	347.87cd	61.67bc	64.67bc

The values given above are means of three replicates and diverse letters presenting the significance at $p \leq 0.05$.

Table 2. Effect of foliar applied Zn on different growth stages on 1000 KW, kernel and biological yield and harvest index of rice during 2015 and 2016

Treatm ents	1000 KW (g)		Kernel yield (t ha^{-1})		Biological yield (t ha^{-1})		Harvest index (%)	
	2015	2016	2015	2016	2015	2016	2015	2016
T1	20.16c	20.49c	2.83d	3.16d	10.01c	9.88c	28.00c	31.00d
T2	23.45ab	22.12b	4.43bc	4.46bc	11.88b	12.15b	37.00b	36.00c
T3	23.26ab	23.92ab	4.94ab	4.91ab	13.27ab	13.24ab	37.00c	37.00b
T4	25.50a	25.61a	5.45a	5.44a	14.22a	14.26a	38.00a	38.00a
T5	23.01b	23.68b	4.95ab	4.96ab	13.26ab	13.28ab	37.00b	37.00b
T6	23.07b	23.73b	4.46bc	4.47bc	12.03b	11.93b	37.00b	37.00b
T7	22.06b	23.39b	4.52bc	4.43bc	11.98b	11.72b	37.00b	37.00b

The values given above are means of three replicates and and diverse letters presenting the significance at $p \leq 0.05$.

Photosynthetic pigments and antioxidant activities

Foliar applied Zn significantly affected the RWC, chlorophyll contents, and antioxidant activities of rice crop. The maximum RWC and chlorophyll contents were obtained with foliar applied Zn (0.5%) at the booting and milky stage followed by Zn (1%) applied at the elongation stage + booting stage and minimum RWC and chlorophyll contents were recorded with foliar spray of water (Table 3). In the case of antioxidant activities maximum APX, CAT, and POD activity during both study years was noted with foliar applied Zn (0.5%) at the booting stage and milking stage and minimum APX, CAT, and POD activity was recorded in control with foliar spray of water (Table 4).

Table 3. Effect of foliar applied Zn on different growth stages on RWC and chlorophyll concentration of rice during 2015 and 2016

Treatments	Relative water contents (%)		Chlorophyll contents (mg/g FW)	
	2015	2016	2015	2016
T1	81.24e	79.21e	1.16f	1.12f
T2	85.20cd	82.13c	1.32d	1.30cd
T3	90.22a	87.32b	1.52a	1.44b
T4	92.33a	90.11a	1.60a	1.52a
T5	88.91b	85.55b	1.47ab	1.41b
T6	86.70c	83.44c	1.44c	1.35c
T7	84.50d	81.33d	1.24e	1.20e

The values given above are means of three replicates and diverse letters presenting the significance at $p \leq 0.05$.

Table 4. Effect of foliar applied Zn on different growth stages on kernel protein, amylose, and kernel length and width of rice during 2015 and 2016

Treatments	APX (U/mg protein)		CAT (U/mg protein)		POD (U/mg protein)	
	2015	2016	2015	2016	2015	2016
T1	32.44e	31.87f	5.00d	4.91e	0.38c	0.36c
T2	36.11c	34.78d	5.34c	5.14d	0.42bc	0.39b
T3	38.51b	37.13b	6.00a	5.78b	0.47b	0.44b
T4	40.32a	39.21a	6.14a	5.99a	0.52a	0.49a
T5	37.33bc	36.55bc	5.89ab	5.67b	0.46b	0.42b
T6	36.55c	35.19c	5.82b	5.55c	0.43b	0.41b
T7	35.44d	33.18e	5.12d	5.00e	0.40c	0.37c

The values given above are means of three replicates and diverse letters presenting the significance at $p \leq 0.05$.

Quality traits

The study findings indicated that foliar applied remarkably improved the quality traits of rice crop (Table 5). The minimum spikelet sterility, opaque kernels, abortive kernels, and maximum normal kernels were obtained with foliar applied Zn (0.5%) at the booting stage and milky stage followed by foliar applied Zn (1%) at the elongation stage + booting stage and maximum spikelet sterility, opaque and abortive kernels, and normal kernels were obtained with foliar spray of water (Table 5). Likewise, maximum kernel protein (10.55% and 10.88%), kernel amylose (27.85% and 26.18%), kernel length (6.54 mm and 6.68 mm), and kernel width (2.37 mm and 2.27 mm) were obtained with 0.5% Zn applied at booting and milky stages followed by 1% foliar applied Zn at booting and milking stage and 1% at same stages and minimum kernel protein (9.03% and 8.73%), kernel amylose (20.95% and 16.92%), kernel length (5.06 mm and 5.23 mm) and kernel width (1.54 mm and 1.61 mm) was recorded in with a foliar spray of water (Table 6).

Table 5. Effect of foliar applied Zn on different growth stages on kernel quality traits of rice during 2015 and 2016

Treatments	Spikelet sterility (%)		Opaque kernel (%)		Abortive kernels (%)		Normal kernels (%)	
	2015	2016	2015	2016	2015	2016	2015	2016
T1	12.64a	12.98a	15.45a	14.45a	7.54a	6.54a	75.93d	76.60d
T2	10.24b	9.91b	14.75ab	14.41ab	5.91b	5.57b	76.14cd	78.47cd
T3	10.09b	9.75b	14.06abc	13.39abc	5.72b	5.55b	77.93bc	80.27bc
T4	6.47d	6.27d	11.95c	12.15c	3.14d	3.31d	82.44a	83.94a
T5	7.62c	7.28c	13.86ab	14.12ab	3.95c	4.05c	80.01ab	81.01ab
T6	7.23c	7.23c	13.03bc	12.77bc	3.56cd	3.89cd	81.18ab	82.51ab
T7	7.19c	7.43c	12.93bc	13.13bc	3.86c	4.03c	79.61b	80.95b

The values given above are means of three replicates and diverse letters presenting the significance at $p \leq 0.05$.

Table 6. Effect of foliar applied Zn on different growth stages on kernel quality traits of rice during 2015 and 2016

Treatments	Kernel protein (%)		Kernel amylose (%)		Kernel length (mm)		Kernel width (mm)		Grain Zn (mg kg^{-1})	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
T1	9.03c	8.73c	20.95e	19.62e	5.06e	5.23e	1.54c	1.61c	17.62d	16.14d
T2	9.27b	9.80b	22.13d	21.20d	5.11e	5.31e	2.19ab	1.14ab	26.75c	25.16c
T3	9.71b	9.45b	23.28c	22.95c	5.43d	5.53d	2.21ab	1.18ab	29.11b	28.10ab
T4	10.55a	10.88a	27.85a	26.18a	6.54a	6.68a	2.37a	2.27a	32.22a	30.21a
T5	9.65b	9.98b	25.18b	24.84b	5.64b	5.90b	2.22ab	2.25ab	30.16b	29.12a
T6	9.35b	9.58b	26.12b	25.35b	5.77b	5.81b	2.18ab	2.05ab	25.66c	24.44c
T7	9.25b	9.58b	23.45c	24.39c	5.49c	5.76c	2.17ab	1.98ab	25.22c	24.40c

The values given above are means of three replicates and diverse letters presenting the significance at $p \leq 0.05$.

Grain Zn concentration

The study findings indicate that foliar-applied Zn markedly improved Zn concentration (Table 6). The results showed maximum grain Zn concentration during years was recorded from plants supplemented with foliar Zn (0.5%) used at booting and milky stages followed by 1% foliar applied Zn at stem elongation and booting stage and minimum grain Zn concentration was noted with foliar spray of water (Table 6).

Discussion

The results indicate that different rates of foliar applied Zn significantly increased the growth and yield traits of fine rice (Table 1). The foliar spray of Zn (0.5%) applied at booting and milky stages significantly enhanced rice yield. The foliar at later stages increase Zn availability in plant tissues which can substantially improve the final yield and grain Zn (Tuiwong *et al.,* 2022a). The panicle formation and development need high amount of Zn and the application of Zn to rice plants produced higher panicles with more kernels owing to improved nutrient uptake (NPK) and enhanced assimilates production (Mu et al., 2020; Mu *et al.,* 2020; Hamza *et al.,* 2022). Zn applied at booting and milky stages effectively improved the overall, LAI, CGR, TDM, and yield traits (panicles/plant and kernel/panicle) owing to better Zn accumulation improved the chlorophyll synthesis (Table 3), RWC (Table 3) and antioxidant activities (Table 4) thus resulting in a marked increase in final yield. Further, Zn plays a key role in pollination, fertilization and kernel setting which consequently improved the final kernel yield (Hassan *et al.,* 2019; Hassan *et al.,* 2020).

We noted that foliar applied Zn (0.5%) at the booting and milking stages produced high yield (Table 2). The foliar spray at early reproductive stages results in better Zn transformation to plant reproductive parts and its accumulation in grains which ensures better production and better grain Zn concentration (Shukla et al., 2009). Further, Zn applied by foliar feeding is quickly absorbed by the epidermis of leaves and after re-mobilization it is trans-located to developing seeds by phloem thus resulting in the production of bold grains with higher grain yield (Hassan *et al.,* 2019). The present increase in growth and yield is attributed to Zn mediated increase in chlorophyll synthesis (Table 3), RWC (Table 3) and antioxidant activities (Table 4: Rehman et al. 2018). These findings are the same with the outcomes of Patel *et al.* (2022) and Wang *et al.* (2017) they also found that Zn application improved the tillers, panicles and kernels/panicle owing to significant improvement in photosynthetic efficiency, chlorophyll synthesis and antioxidant activities. Likewise, other authors also found that the foliar sprays of Zn at reproductive stages improved the physiological activities which improved the overall growth and yield of plants (Kadam *et al.,* 2018; Tuiwong *et al.,* 2022a).

The foliar Zn appreciably increased LAI and CGR and decreased the LAI and CGR attenuation rates at the late stages. The increased LAI and CGR at grain filling stages allow more transformation of photosynthetic pigments from grains to leaves which results in the production of bold grains (Table 2) with more grain weight and final yield (Zhang *et al.,* 2021). The results indicate that foliar applied Zn (0.5%) at booting and milking stages significantly improved kernel protein, and kernel amylose and reduced the spikelet sterility, opaque, and abortive kernels (Table 5). Zn activates different enzymes and protein synthesis and it also activates the decomposing sugar which improves the overall quality of rice kernels (Kheyri *et al.,* 2019). Zn application (0.5%) at booting and milky stages improved kernel protein, and kernel amylose and reduced the spikelet sterility, and opaque, and abortive kernels (Table 5). The present increase in quality traits can be due to Zn mediated increase in LAI (Figure 1), photosynthetic rates and TDM (Figure 2). Previous studies also indicate that Zn application promotes the transformation of assimilates to grains and increases protein synthase activity which improved the grain protein concentration (Dimkpa *et al.,* 2020; Yang *et al.,* 2021).

The results indicate that foliar Zn enhanced grain Zn, however, foliar application (0.5%) at booting and milking stages substantially enhanced grain Zn concentration as compared to other treatments (Table 6). The time of Zn application plays a crucial role to achieve a higher grain Zn concentration (Sher *et al.,* 2022).

Likewise, Saha *et al.* (2017) noted Zn applied at flowering stages produced grains with dense Zn as compared to foliar applied Zn at tillering. While Sher *et al.* (2022) found that foliar applied at the heading stage effectively improved the grain Zn and utilization as compared to Zn applied at earlier growth stages. At earlier growth stages; plant leaves are less physiologically active, whilst matured leaves are fully active and they export assimilates from phloem to grains (Saha *et al.,* 2017). Further, in foliar application Zn ions directly enters the leaf apoplast through the pores of stomata which also increases the Zn concentration in developing grains (Gupta *et al.,* 2016). Moreover, foliar application at early grain filling stages induces re-translocation of Zn from vegetative tissues to grain and resulting in significant grain Zn contents (Wang *et al.,* 2018). Therefore, the increase in grain Zn in the current study could be due to Zn re-translocation and direct absorption of Zn by plant leaves.

Conclusions

The present study finding indicated Zn applied at different growth stages appreciably improved rice growth and yield. The maximum improvement in growth, yield, and quality was seen with 0.5% Zn applied at the booting and milking stages. The application of Zn (0.5%) at the booting and milking stages also led to an appreciable improvement in grain Zn and proved the best to address the Zn nutrition problem in humans. Therefore, foliar-applied Zn (0.5%) at the booting and milky stage could be an imperative approach to improved rice yield, quality, and grain Zn. However, more research is required to explore the role of Zn applied at the booting and milking stage on rice crop under a wide range of soil and climate conditions.

Authors' Contributions

Conceptualization: MBC, data collection; QA, writing original draft: MBC and MUH, writing, reviewing and editing: MNS, MA, SA, QA, ZI, MI, MAY, MRA, FMA, MH and MUH.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The authors would like to thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work by Grant Code (22UQU4281560DSR09).

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Al-Zahrani HS, Alharby HF, Hakeem KR, Rehman RU (2021). Exogenous application of zinc to mitigate the salt stress in *Vigna radiata* (l.) wilczek—evaluation of physiological and biochemical processes. Plants 10(5):1005. *https://doi.org/10.3390/plants10051005*

AOAC (Association of Official Analytical Chemists) (1990). Official methods of analysis. 15th ed. Association of official analytical chemists, Arlington, Virginia, USA.

Beloucif A, Kechrid Z, Bekada AMA (2021). Effect of zinc deficiency on blood glucose, lipid profile, and antioxidant status in streptozotocin diabetic rats and the potential role of sesame oil. Biological Trace Element Research 200: 3236-3247. *https://doi.org/10.1007/s12011-021-02934-5*

Bouis HE, Welch RM (2010). Biofortification—A sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Science 50:S20-S32. *https://doi.org/10.2135/cropsci2009.09.0531*

Brown KH, Rivera JA, Bhutta Z, Gibson RS, King JC, Lönnerdal B, Ruel MT, Sandtröm B, Wasantwisut E, Hotz C (2004). International zinc nutrition consultative group (IZiNCG) Technical Document# 1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutrition Bulletin 25:S99-S203.

Cakmak I, Kutman U (2018). Agronomic biofortification of cereals with zinc: a review. European Journal Soil Science 69:172-180. *https://doi.org/doi.org/10.1111/ejss.12437*

Chhabra V, Kumar R (2018). Role of zinc application on rice growth and yield. Plant Architecture 18:1382-1384.

Chattha MU, Hassan MU, Khan I, Chattha MB, Mahmood A, Chattha MU, … Khan S (2017). Biofortification of wheat cultivars to combat zinc deficiency. Frontiers in Plant Science 8:281. *https://doi.org/10.3389/fpls.2017.00281*

Chasapis CT, Ntoupa PSA, Spiliopoulou CA, Stefanidou ME (2020). Recent aspects of the effects of zinc on human health. Archives of Toxicology 94:1443–1460. *https://doi.org/10.1007/s00204-020-02702-9*

Dimkpa CO, Andrews J, Fugice J, Singh U, Bindraban PS, Elmer WH, Gardea-Torresdey JL, White JC (2020). Facile coating of urea with low-dose ZnO nanoparticles promotes wheat performance and enhances Zn uptake under drought stress. Frontiers in Plant Science 11:168. *doi.org/10.3389/fpls.2020.00168*

Ghoneim AM (2016). Effect of different methods of Zn application on rice growth, yield and nutrients dynamics in plant and soil. Journal of Agriculture and Ecology Research International 6:1-9. *https://doi.org/10.9734/JAERI/2016/22607*

Gupta N, Ram H, Kumar B (2016). Mechanism of zinc absorption in plants: uptake, transport, translocation and accumulation. Reviews in Environmental Science and Biotechnology 15:89-109. *https://doi.org/10.1007/s11157-016-9390-1*

Hamzah SM, Usman K, Rizwan M, AlJabri H, Alsafran M (2022). Functions and strategies for enhancing zinc availability in plants for sustainable agriculture. Frontiers in Plant Sciences 13:1033092. *https://doi.org/10.3389/fpls.2022.1033092*

Hassan N, Irshad S, Saddiq MS, Bashir S, Khan S, Wahid MA, Khan RR, Yousra M (2019). Potential of zinc seed treatment in improving stand establishment, phenology, yield and grain biofortification of wheat. Journal of Plant Nutrition 42:1676-1692. *https://doi.org/10.1080/01904167.2019.1630429*

Hassan MU, Chattha MU, Ullah A, Khan I, Qadeer A, Aamer M, Khan AU, Nadeem F, Khan TA (2019). Agronomic biofortification to improve productivity and grain Zn concentration of bread wheat. International Journal of Agriculture and Biology 21:615-620. *https://doi.org/10.17957/IJAB/15.0936*

Hassan M, Aamer M, Chattha MU, Haiying T, Shahzad B, Barbanti L, … Guoqin H (2020). The critical role of zinc in plants facing the drought stress. Agriculture 10:396. *https://doi.org/10.3390/agriculture10090396*

Hassan MU, Nawaz M, Mahmood A, Shah AA, Shah AN, Muhammad F, … Hasan ME (2022). The role of zinc to mitigate heavy metals toxicity in crops. Frontiers in Environmental Science 10:1712. *https://doi.org/10.3389/fenvs.2022.990223*

Homer DC, Pratt PF (1961). Methods of analysis for soils, plants and waters. Davis: University of California, Davis.

Hunt R (1978) Plant growth analysis. The institute of biology's studies in biology. Edward Arnold (Ed). Ltd, London, 96:8-38.

Juliano BO (1971). A simplified assay for milled-rice amylose. Cereal Science Today 16:334-343.

Kachinski WD, Ávila FW, Dos Reis AR, Muller MML, Mendes MC, Petranski PH (2022). Agronomic Biofortification In-creases Concentrations of Zinc and Storage Proteins in Common Bean (*Phaseolus vulgaris* L.) Grains. Food Research International 155:111105. *https://doi.org/10.1016/j.foodres.2022.111105*

Kadam S, Bhale V, Chorey A, Deshmukh M (2018). Influence of zinc and iron fortification on yield and post-harvest studies of different rice cultivars (*Oryza sativa* L.). International Journal of Current Microbiology and Applied Sciences 7:1878-1888.

Kandil EE, El-Banna AAA, Tabl DMM, Mackled MI, Ghareeb RY, Al-Huqail AA, … Abdelsalam NR (2022). Zinc nutrition responses to agronomic and yield traits, kernel quality, and pollen viability in rice (*Oryza sativa* L.). Frontier in Plant Science 13: 791066. *https://doi.org/10.3389/fpls.2022.791066*

Kheyri N, Norouzi HA, Mobasser HR, Torabi B (2019). Effect of silicon and zinc nanoparticles on growth, yield, and biochemical characteristics of rice. Agronomy Journal 111:3084-3090. *https://doi.org/10.2134/agronj2019.04.0304*

Kumar S, Pandey G (2020). Biofortification of pulses and legumes to enhance nutrition. Heliyon 6:03682. *https://doi.org/10.1016/j.heliyon.2020.e03682*

Kumssa DB, Joy EJ, Ander EL, Watts MJ, Young SD, Walker S, Broadley MR (2015). Dietary calcium and zinc deficiency risks are decreasing but remain prevalent. Scientific reports 5:10974. *https://doi.org/10.1038/srep10974*

Lichtenthaler H (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methane Enzymology 148:350-382. *https://doi.org/10.1016/0076-6879(87)48036-1*

Lividini K, Fiedler JL, De Moura FF, Moursi M, Zeller M (2018). Biofortification: A review of ex-ante models. Global Food Security 17:186-195. *https://doi.org/10.1016/j.gfs.2017.11.001*

Moura JDO, Rocha MDM, Gomes RLF, Freire FR, Damasceno e Silva KJ, Ribeiro VQ (2012). Path analysis of iron and zinc contents and others traits in cowpea. Crop Breed. Applied Biotechnology 12:245-252. *https://doi.org/10.1590/S1984-70332012000400003*

Mu S, Yamaji N, Sasaki A, Luo L, Du B, Che J, Shi H, Zhao H, Huang S, Deng F (2020). A transporter for delivering zinc to the developing tiller bud and panicle in rice. Plant Journal 105:786-799. *https://doi.org/10.1111/tpj.15073*

Nawaz A, Rehman AU, Rehman A, Ahmad S, Siddique KH, Farooq M (2021). Increasing sustainability for rice production systems. Journal of Cereal Sciences 103:103400. *https://doi.org/10.1016/j.jcs.2021.103400*

Patel PS, Singh SK, Patra A, Jatav SS (2022). Root dipping, foliar and soil application of zinc increase growth, yields, and grain zinc in rice (*Oryza sativa* L.) grown in moderate zinc soil of inceptisol order. Communication in Soil Science and Plant Analysis 53:1917-1929. *https://doi.org/10.1080/00103624.2022.2069800*

Pradhan SK, Pandit E, Pawar S, Naveenkumar R, Barik SR, Mohanty SP, … Reddy JN (2020). Linkage disequilibrium mapping for grain Fe and Zn enhancing QTLs useful for nutrient dense rice breeding. BMC Plant Biology 20:57. *https://doi.org/10.1186/s12870-020-2262-4*

Prasad R (2006). Zinc in soils and in plant: human and animal nutrition. Indian Journal of Fertilisers 2:103-119.

Prasad AS (2020). Lessons learned from experimental human model of zinc deficiency. Journal of Immunology Research 9207279. *https://doi.org/10.1155/2020/9207279*

Rehman HU, Rasool F, Awan MI, Mahmood A, Wakeel A, Hajiboland R (2018). Irrigation and Zn fertilizer management improves Zn phyto-availability in various rice production systems. Journal of Plant Nutrition and Soil Science 181:374-381. *https://doi.org/10.1002/jpln.201700412*

Rerksuppaphol L, Rerksuppaphol S (2020). Efficacy of adjunctive zinc in improving the treatment outcomes in hospitalized children with pneumonia: a randomized controlled trial. The Journal of Tropical Pediatrics 66:419-427. *https://doi.org/doi.org/10.1093/tropej/fmz082*

Saha S, Chakraborty M, Padhan D, Saha B, Murmu S, Batabyal K, … Bell RW (2017). Agronomic biofortification of zinc in rice: influence of cultivars and zinc application methods on grain yield and zinc bioavailability. Field Crops Research 210:52-60. *https://doi.org/10.1016/j.fcr.2017.05.023*

Sher A, Sarwar B, Sattar A, Ijaz M, Ul-Allah S, Hayat MT, … Askary AE (2022.) Exogenous application of zinc sulphate at heading stage of wheat improves the yield and grain zinc biofortification. Agronomy 12:734. *https://doi.org/10.3390/agronomy12030734*

Shukla AK, Dwivedi BS, Singh VK, Gill MS (2009). Macro role of micronutrients. Indian Journal Fertilizers 5:11-30.

Steel RGD, Torrie JH, Dickey D (1997). Principles and procedures of statistics: A Biometric Approach. 3rd Edition, McGraw-Hill Book Co., New York, USA, pp: 663-666.

Suganya A, Saravanan A, Manivannan N (2020). Role of zinc nutrition for increasing zinc availability, uptake, yield, and quality of maize (*Zea Mays* L.) grains: an overview. Communication in Soil Science and Plant Analysis 51:2001-2021. *https://doi.org/10.1080/00103624.2020.1820030*

Tuiwong P, Lordkaew S, Veeradittakit J, Jamjod S, Prom-U-Thai C (2022a). Seed priming and foliar application with nitrogen and zinc improve seedling growth, yield, and zinc accumulation in rice. Agriculture 12:144. *https://doi.org/10.3390/agriculture12020144*

Tyagi R, Sharma A, Srivastava PC, Shankhdhar D, Shankhdhar SC (2018). Modulation of phytic acid and phytic acid-zinc molar ratio by different modes of zinc application in rice. Indian Journal Plant Physiology 23:529-535. *https://doi.org/10.1007/s40502-018-0399-z*

Wang S, Li M, Tian X, Li J, Li H, Ni Y, Zhao J, Chen Y, Guo C, Zhao A (2015). Foliar zinc, nitrogen, and phosphorus application effects on micronutrient concentrations in winter wheat. Agronomy Journal 107:61-70. *https://doi.org/10.2134/agronj14.0414*

Wang Y, Lu J, Ren T, Hussain S, Guo C, Wang S, Cong R, Li X (2017). Effects of nitrogen and tiller type on grain yield and physiological responses in rice. AoB Plants 9:plx012. *https://doi.org/10.1093/aobpla/plx012*

Wang S, Wang ZH, Li SS, Diao CP, Liu L, Hui XL, Huang M, Luo LC, He G, Cao HB (2018). Identification of high-yield and high-Zn wheat cultivars for overcoming "yield dilution" in dryland cultivation. European Journal of Agronomy 101:57-62. https://*doi.org/10.1016/j.eja.2018.08.008*

Wang S, Wang Z, Li S, Diao C, Liu L, Huang N, Huang M, Hui X, Luo L, He G (2020). Quantify the requirements to achieve grain zn biofortification of high-yield wheat on calcareous soils. bioRxiv. *https://doi.org/10.1101/2020.04.09.033639*

Watson DJ (1947) Comparative physiological studies in the growth of field crops. I: variation in net assimilation rate and leaf area between species and varieties, and within and between years. Annals of Botany 11:41-76.

White PJ, Broadley MR (2005) Biofortifying crops with essential mineral elements. Trends Plant Science 10:586-593. *doi.org/10.1016/j.tplants.2005.10.001*

Wu F, Fang Q, Yan SW, Pan L, Tang XJ, Ye WL (2020). Effects of zinc oxide nanoparticles on arsenic stress in rice (*Oryza sativa* L.): germination, early growth, and arsenic uptake. Environmental Science and Pollution Research 27:26974-26981. *https://doi.org/10.1007/s11356-020-08965-0*

Yang GY, Yuan HY, Ji HT, Liu HJ, Zhang YF, Wang GD, Chen LG, Guo Z (2021). Effect of ZnO nanoparticles on the productivity, Zn biofortification, and nutritional quality of rice in a life cycle study. Plant Physiology and Biochemistry 163:87-94. *https://doi.org/10.1016/j.plaphy.2021.03.053*

Yin HJ, Guo XP, Stomph T, Li LJ, Zhang FS, Zou CQ (2016). Zinc concentration in rice (*Oryza sativa* L.) grains and allocation in plants as affected by different zinc fertilization strategies. Communication in Soil Science and Plant Analysis 47:761-768. *https://doi.org/10.1080/00103624.2016.1146891*

Zafar S, Ashraf MY, Saleem M (2017). Shift in physiological and biochemical processes in wheat supplied with zinc and potassium under saline condition. Journal of Plant Nutrition 41:19-28. *https://doi.org/10.1080/01904167.2017.1380825*

Zhang X (1992). The measurement and mechanism of lipid peroxidation and SOD, POD and CAT activities in biological system. Research Methods in Crop Physiology 208-211.

Zhang H, Wang R, Chen Z, Cui P, Lu H, Yang Y, Zhang H (2021). The effect of zinc oxide nanoparticles for enhancing rice (*Oryza sativa* L.) yield and quality. Agriculture 11(12):1247. *https://doi.org/10.3390/agriculture11121247*

Zulfiqar U, Hussain S, Maqsood M, Ishfaq M, Ali N (2020). Zinc nutrition to enhance rice productivity, zinc use efficiency, and grain biofortification under different production systems. Crop Science 61:739-749. *https://doi.org/10.1002/csc2.20381*