EFFECTS OF SPRAYING ZINC FERTILIZER ON THE PHYSIOLOGICAL AND PHOTOSYNTHETIC CHARACTERISTICS OF MILLET PLANTS (*SETARIA ITALICA L.*) AT DIFFERENT GROWTH STAGES

CAO, M. L.[1] – LI, Y. X.[1] – DU, H. L.[2*]

[1]*College of Agricultural Science, Shanxi Agricultural University, Taigu, Shanxi 030801, People's Republic of China*

[2]*College of Arts and Sciences, Shanxi Agricultural University, Taigu, Shanxi 030801, People's Republic of China*

**Corresponding author*
e-mail: duhuiling66@163.com

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Abstract. Pot experiments were conducted from May 2017 to October of 2017 at the cultivation base of the Resource and Environment College of the Shanxi Agricultural University in China. The physiological and photosynthetic characteristics of different cultivars treated with different Zn concentrations at different growth stages were investigated to determine the optimum spraying period and spraying concentration of Zn fertilizer ($ZnSO_4 \cdot 7H_2O$) in Millet plants (*Setaria italica* L.). Results showed that spraying low Zn concentrations (20, 40, and 60 $mg \cdot L^{-1}$) solutions decreased the malondialdehyde content, Ci (intercellular CO_2 concentration), and qN (non-photochemical quenching coefficient) but increased antioxidant enzymatic activities, pigment content, photosynthetic gas exchange parameters (except Ci), and chlorophyll fluorescence parameters (except qN). By contrast spraying high Zn concentration (80 and 100 $mg \cdot L^{-1}$) showed opposite effects. Moreover, 'Jingu 21' (susceptible variety, conventional cultivar) showed more remarkable variation higher than 'Zhangzagu10' (resistant variety, hybrids). Thus, low Zn concentration promoted plant growth, while high Zn concentration inhibited plant growth, which was most pronounced during the Millet booting stage. The optimal conditions were booting period and 40 $mg \cdot L^{-1}$ of Zn. Thus, a theoretical basis for the efficient use of Zinc Fertilizer in Millet is provided.
Keywords: *foxtail millet, growth stage, zinc fertilizer, antioxidant enzyme activity, photosynthetic gas exchange parameters, chlorophyll fluorescence parameters*

Introduction

Zinc is an essential element in human health and an indispensable trace element in animal and plant growth (Gartler et al., 2013). As the only metal element to be simultaneously present in six enzymes, Zn plays an important regulatory role in plant photosynthesis, protein and nucleic acid metabolism, auxin metabolism, biofilm stability, and cell division (Prasad and Hagemeyer, 1999; Kabata-Pendias and Pendias, 2001). In recent years, plants have taken away substantial amount of nutrients from the soil because of the growing area of crops and the gradually increased yield (Feng, 2010). In addition, the amount of organic fertilizer applied in the field has generally declined (Mutegi et al., 2012), and trace elements could not be replenished in time. Thus, the available Zn in the soil gradually decreased with the passage of time. This phenomenon resulted in the prevalent deficiency of Zn in crops (Carroll and Loneragan, 1968; Alloway, 2009). Zn deficiency can cause dwarf plants, short internodes, suppression of leaf expansion and elongation, leaflets, twisted leaves, and wrinkled leaf margins. Albino corn seedlings and stiff Miller of rice seedlings are due to Zn

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deficiency (Rengel et al., 1998; Singh and Yashbir, 2017). At present, the application of Zn fertilizer to crops is the safest, most convenient, and effective approach to augment the micronutrient Zn deficiency in humans. This process can also effectively improve the symptoms of Zn deficiency in crops (Noulas et al., 2018). However, excessive application of Zn will inhibit the growth of crops, cause poisoning (Mateos-Naranjo et al., 2014), and can lead to toxicity, such as nutrient imbalances, growth inhibition, leaf chlorosis, and photosynthesis impairment (Todeschini et al., 2011; Cambrollé et al., 2012).

Photosynthesis is an important factor in plant growth and an indicator to assess plant growth status (Moss and Musgrabe, 1971; Silveira and Carvalho, 2016). Chlorophyll fluorescence is an effective tool to sense and assess the impact of Zn on the photosynthetic apparatus, and this tool has been used extensively to investigate the effects of various substances in crops (Appenroth et al., 2001; Prasad et al., 2001; Frankart et al., 2002; Drinovec et al., 2004). Zn plays a significant role in the regulation of the stomatal aperture, which accounts for the possible role of Zn in maintaining a high K content in the guard cells (Sharma et al., 1995). Cambrollé et al. (2013) showed that an appropriate Zn concentration (60 mmol/L) could increase the net photosynthetic rate (Pn), but greater external Zn concentration (90–130 mmol/L) could negatively affect plant growth probably because of the recorded decline in the Pn. This process may be linked to the adverse effect of Zn on the photosynthetic electron transport.

Various grain industries have received increasing attention because of the improved human dietary structure (Ardiea et al., 2015). The millet (*Setaria italica* L.) originated from China and is an important strategic food crop in the country (Guo et al., 2018; Yuan et al., 2017). The millet is a traditional drought-resistant crop (Bai et al., 2009; Andersen and Nepal, 2017) and the preferred crop in drought-scarce areas in northern China. The main components of millet, including starch, protein, lipid, vitamins, and minerals, satisfy the various dietary needs of the human body (Usha et al., 1996). Low Zn availability affects crop yield and food production worldwide, and this phenomenon has led to the more efficient use of Zn in agriculture. The application of Zn fertilizer is an effective measure to increase nutrient Zn (Genty et al., 1989; Bouis and Welch, 2010; Gibson, 2006), but the safety of Zn to foxtail millet is ambiguous.

Studies on Zn fertilizers are mostly concentrated on rice (Jamalomidi et al., 2006), wheat (Khoshgoftarmanesh et al., 2006; Hacisalihoglu et al., 2003), and other crops (Gunes, 1996; Hu, 1991), but few reports have focused on millet production (Zong, 2011; Guo, 2014). In this experiment, millet was used to study the effects of different Zn concentrations on the growth of millet under different stages. Moreover, the optimal combination of conditions for the growth of millet was determined to provide a theoretical basis for the reasonable application of Zn fertilizer on millet.

Materials and methods

Site and plant materials

The pot experiments were conducted from May 2017 to October of 2017 at the cultivation base of the Resource and Environment College of Shanxi Agricultural University (37°42′ N, 112°55′ E) in Shanxi, China. The area has a mean annual temperature of 9.9 °C and a frost-free period of 176 days. The precipitation is concentrated, and the mean annual rainfall is 462.9 mm. The plant materials were ‘Zhangzagu 10’ (provided by the Zhangjiakou Academy of Agricultural Sciences of

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Hebei Province, China.) and 'Jingu 21' (provided by the Institute of Millet Research, Shanxi Academy of Agricultural Sciences, China). The soil used was calcareous soil, with pH value of 8.51, organic matter content of 21.02 g/kg, alkali nitrogen content of 52.37 mg/kg, available phosphorus content is 21.98 mg/kg, available potassium content of 21.98 mg/kg, total N content of 1.180 g/kg, total P content of 1.261 g/kg, and total K content of 20.18 g/kg. The seeds were sown on May 31, 2017. After 20 days of thinning out the seedlings, five seedlings were left in each pot.

Treatment details and allocation

The experiment was conducted in a completely random design. The seeds of 'Zhangzagu 10' and 'Jingu 21' were separately sown in pots with a diameter of 32 cm and a height of 26 cm under normal water content, and each pot was filled with 10 kg of soil, the cultivation temperature was 22-35℃, relative humidity of atmosphere was 70-80%. The seeds were normally watered daily after germination to ensure the normal growth of the seedlings (by weighing method, the Moisture field was maintained at 13%-15%). Different concentrations of $ZnSO_4 \cdot 7H_2O$ solution were sprayed at the seedling stage (SL, 35 d after planting), booting stage (BT, 60 after planting), flowering stage (FE, 70 d after planting), and filling stage (FL, 90 d after planting). A control (no Zn application) was set simultaneously, and each treatment was repeated thrice. The Zn concentrations sprayed were 0, 20, 40, 60, 80, and 100 $mg \cdot L^{-1}$, and the corresponding samples were labeled as CK, Zn1, Zn2, Zn3, Zn4, and Zn5. After 7 days of Zn treatment, the physiological and photosynthetic characteristics were measured.

Determination of antioxidant enzyme activity

Fresh plant leaves (0.1 g) were placed in an ice-cooled mortar, and 1 $mg \cdot L^{-1}$ of 50 mM phosphate buffer (pH 7.8) was added. The leaves were ground on ice, transferred to a 1.5 mL centrifuge tube, and centrifuged at 12000 $\times g$ for 15 min at 4 °C. The supernatant was extracted to determine the superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities.

The SOD activity was determined using the nitroblue tetrazolium (NBT) photoreduction method (Gao, 2006). First, the NBT reaction solution was configured. The solution consisted of 50 mM phosphate buffer (pH 7.8), 13 mM L-methionine, 0.075 mM NBT, 0.002 mM riboflavin, and 0.01 mM EDTA. Then, 5 mL of the reaction solution was added to the blank and control tubes. A portion of 50 μL of the supernatant was taken from the test tube and mixed with 5 mL of the reaction solution. The control and test tubes were illuminated for 20 min under 4000 lx light conditions at 25 °C. The absorbance of the irradiated solution was measured at 560 nm, and a non-irradiated complete reaction mixture served as a control. The result was calculated by inhibiting 50% of the NBT photochemical reduction as an enzyme unit. SOD activity was presented as the number of units per gram of fresh weight (U/g FW).

The POD activity was determined by the guaiacol method (Gao, 2006). The supernatant (5 μL) was mixed with 3 mL of the reaction solution containing 3 mL of 0.1 mM phosphate buffer (pH 6.0), 28 μL of 30% H_2O_2, and 19 μL of guaiacol (0.1%). The absorbance of the complete reaction mixture was measured at 470 nm, and the 0.1 mM phosphate buffer (pH 6.0) served as a control. The change in absorbance every 3 min indicated the POD activity.

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The CAT activity was determined by the UV absorption method (Gao, 2006). The supernatant (50 μL) was mixed with 3 mL of the reaction solution containing 2.7 mL of Tris-HCl buffer (pH 7.0) and 50 μL of 200 mM H_2O_2. The absorbance of the complete reaction mixture was measured at 240 nm. The decrease of 0.1 by A240 in 1 min was the enzymatic activity unit.

Determination of lipid peroxidation

The MDA content was determined by the thiobarbituric acid (TBA) spectrophotometry method (Zhao et al., 1994). Fresh plant leaves (0.4 g) were placed in an ice-cooled mortar, and 5 mL of 0.1% trichloroacetic acid was added. The leaves were ground on ice, and 5 mL of 0.5% TBA was added. The ground leaves were transferred to a 15 mL centrifuge tube. After boiling in water for 15 min, the leaves were cooled to room temperature and centrifuged at 3000 ×*g* for 15 min. The absorbance of the supernatant was measured at 560 nm and 600 nm.

Determination of photosynthetic pigment content

The pigment contents of the leaves were determined using acetone extraction. Fresh plant leaves (0.1 g) were extracted in 10 mL of acetone (80%, v/v) and stored in the dark for 24 h (Gao, 2006). The absorbance of the supernatant was measured at 470, 646, and 663 nm (*Eqs.1-3*).

$$Ca = 12.21A663 - 2.81A646 \quad \text{(Eq.1)}$$

$$Cb = 20.13A646 - 5.03A663 \quad \text{(Eq.2)}$$

$$Cx.c = (1000A470 - 3.27Ca - 104Cb)/229 \quad \text{(Eq.3)}$$

Determination of photosynthetic gas exchange parameters

The Pn, transpiration rate (Tr), intercellular CO_2 concentration (Ci), and stomatal conductance (Gs) were measured using a CI-340 portable photosynthesis system (CID Bio-Science, Inc., USA, *Fig. 1*). The seedlings with uniform growth were randomly selected from each pot, and the photosynthetic gas exchange parameters were measured at 9:00–11:00 am on the 7th day after spraying the $ZnSO_4 \cdot 7H_2O$ solution. Photosynthetically active radiation (PAR) at the leaf surface was approximately 1000 ± 50 μmol/m^2/s. The temperature in the leaf chamber was 38 ± 2 °C, and ambient CO_2 concentration was 420 ± 30 μmol/mol.

Figure 1. *CI-340 portable photosynthesis system*

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Determination of chlorophyll fluorescence parameters

Actual photochemical efficiency (ΦPSII), photosynthetic electron transport rate (ETR), photochemical quenching coefficient (qP), non-photochemical quenching coefficient (qN), and maximum photochemical efficiency (Fv/Fm) were measured using a portable chlorophyll fluorometer PAM-2500 (Walz, Germany, *Fig. 2*). Prior to measurements, the leaves were first treated in the dark for 30 min, and a beam of measurement light (less than 0.1 μmol) was irradiated on the fully dark-adapted leaves to obtain the initial fluorescence Fo. Then, saturated pulsed light (8000 μmol $m^{-2}s^{-1}$) was turned on to obtain the maximum fluorescence Fm under dark adaptation (Marwood et al., 2001). Afterward, the endogenous actinic light (600 μmol $m^{-2}s^{-1}$) was turned on. When the actual primary light energy capture efficiency ΦPSII had stabilized, the action light was turned off, and the far-infrared light was illuminated (Schreiber et al., 1986, 2003). The ETR was automatically calculated by the instrument (Mayer et al., 2008). The different parameters were calculated as follows (*Eqs.4-7*):

$$Fv / Fm = (Fm - Fo) / Fm \quad \text{(Eq.4)}$$

$$\Phi PSII = (Fm' - Ft) / Fm' \quad \text{(Eq.5)}$$

$$qP = (Fm' - Ft) / (Fm' - Fo') \quad \text{(Eq.6)}$$

$$qN = 1 - (Fm' - Fo') / (Fm - Fo) \quad \text{(Eq.7)}$$

Statistical analysis

The data were plotted using Microsoft Excel and analyzed by ANOVA using SPSS 17.0. Duncan's test ($P < 0.05$) were used to determine the significance of differences between treatment means.

Results

Effect of Zn on the antioxidant enzymatic activity of foxtail millet

After Zn treatment, protective enzymes SOD, POD, and CAT in both cultivars increased first and then decreased with the increase in Zn concentration, and the level of change in these enzymes differed between the cultivars and among periods (*Figs. 3-5*). Among the three protective enzymes, Zn treatment of the same cultivars reached maximum effect in Zn2 and reached a significant level compared with the control ($P < 0.05$). However, in 'Zhangzagu10', no significant difference in CAT was found between the control and Zn2 treatment during the SL stage (*Fig. 3*). However, the three enzymatic activities were lower in Zn4 than in CK, and the change was more obvious after Zn5 treatment. *Figures 3-5* show that Zn treatment of the two millet varieties was most obvious at the BT stage.

Compared with CK, during the BT stage, after 7 days of Zn2 treatment, the SOD, POD, and CAT activities of 'Jingu 21' increased by 28.2%, 61.1%, and 35.1%, respectively. By contrast, the corresponding values of 'Zhangzagu 10' increased by 11.3%, 15.5%, and 10.6%. After 7 days of Zn5 treatment, compared with CK, the SOD, POD, and CAT activities of 'Jingu 21' decreased by 17.2%, 20.4%, and 16.8%' respectively, while those of 'Zhangzagu 10' were reduced by 11.7%, 12.1%, and 12.6%' respectively (*Figs. 3-5*).

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Figure 2. *Portable chlorophyll fluorometer PAM-2500*

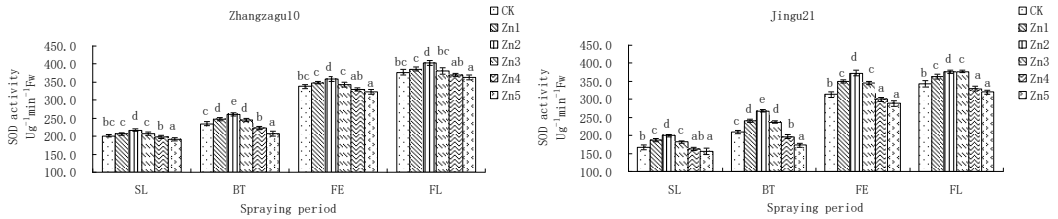

Figure 3. *Effects of Zn on the superoxide dismutase (SOD) activity in the leaves of 'Zhangzagu 10' and 'Jingu 21' at the different growth stages*

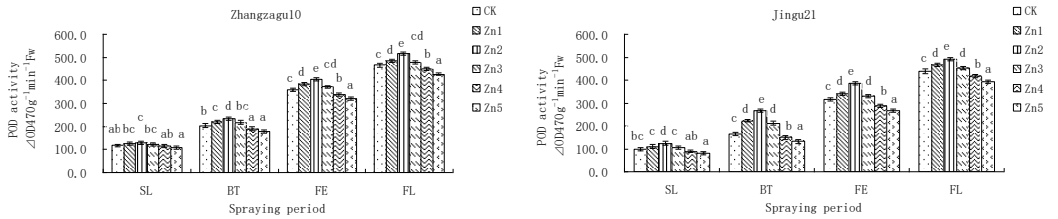

Figure 4. *Effects of Zn on peroxidase (POD) activity in the leaves of 'Zhangzagu 10' and 'Jingu 21' in the different growth stages*

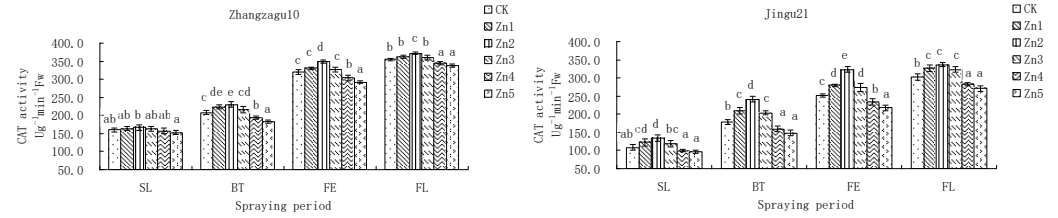

Figure 5. *Effects of Zn on the catalase (CAT) activity in the leaves of 'Zhangzagu 10' and 'Jingu 21' at the different growth stages*

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Effect of Zn on the MDA content of foxtail millet

The trend of MDA was opposite to that of the protective enzyme activity. The MDA decreased first and then increased with the increase in Zn concentration. MDA content reached minimum in Zn2 and then gradually increased until Zn4 treatment, during which the MDA content was higher than that in CK. *Figure 4* shows that the MDA content had the greatest impact during the BT stage.

Compared with CK, after 7 days of Zn2 treatment, the MDA activities of 'Jingu 21' in SL, BT, FE, and FL were decreased by 26.0%, 35.9%, 15.4% and 13.3%, respectively, while those of 'Zhangzagu10' were reduced by 14.2%, 16.7%, 11.7% and 10.7%, respectively (*Fig. 6*).

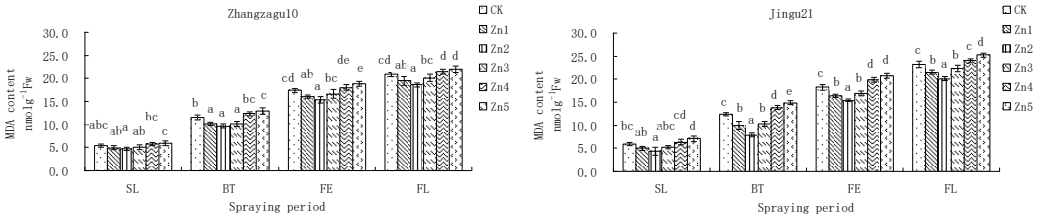

Figure 6. *Effects of Zn on the malondialdehyde (MDA) content in the leaves of 'Zhangzagu 10' and 'Jingu 21' at the different growth stages*

Effect of Zn on the pigments of foxtail millet

The average amounts of pigments in the leaves as affected by spraying period and spraying concentrations are presented in *Tables 1* and *2*. The pigment contents in the leaves of foxtail millet increased with increasing Zn concentrations until Zn2. However, the pigment contents declined at Zn4 and Zn5 treatments, and the levels were even lower than those of the CK. Although both cultivars showed similar trends in the different stages, the degrees of increase or decrease of chlorophyll contents in the cultivars were not identical. Chlorophyll a, chlorophyll b, chlorophyll, and carotenoid were most affected by Zn treatment during the BT stage.

Compared with CK, during the BT stage, after 7 days of Zn2 treatment, the chlorophyll a, chlorophyll b, chlorophyll, and carotenoid of 'Jingu 21' increased by 27.1%, 36.7%, 28.9%, and 9.7%, respectively, while those of 'Zhangzagu 10' increased by 13.3%, 27.8%, 15.9%, and 8.0%, respectively.

In addition to the carotenoid during SL of 'Jingu 21', these pigments reached significant levels. After 7 days of Zn5 treatment, compared with CK, chlorophyll a, chlorophyll b, chlorophyll, and carotenoid of 'Jingu 21' decreased by 11.6%, 31.3%, 15.3%, and 7.7%, respectively, while those of 'Zhangzagu 10' declined by 6.5%, 23.5%, 9.6%, and 6.5%, respectively. These values almost reached significant levels (*Tables 1* and *2*).

Effect of Zn on the photosynthetic gas exchange parameters of foxtail millet

After Zn treatment, the Pn, Gs, and Tr in both cultivars increased first and then decreased with the increase of Zn concentration. The change in these indices differed between cultivars and among periods (*Figs. 7-9*).

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Among these three indices, Zn treatment reached maximum effect in Zn2 and then gradually decreased. From the Zn4 treatment, these indices were lower than CK and reached a significant level between treatments. *Figures 7-9* show that the Zn treatment of the two millet varieties was most obvious during BT.

Table 1. *Effects of Zn on the pigment contents in the leaves of 'Zhangzagu 10' at the different growth stages*

Cultivar	Period	Treatment	Chlorophyll a (mg/gFw)	Chlorophyll b (mg/gFw)	Chlorophyll (mg/gFw)	Carotenoid (mg/gFw)
'Zhangza 10'	SL	CK	9.66 ± 0.18c	3.77 ± 0.17b	13.43 ± 0.11c	1.40 ± 0.04ab
		Zn1	10.13 ± 0.05d	4.07 ± 0.12cd	14.21 ± 0.17e	1.47 ± 0.04c
		Zn2	10.40 ± 0.09e	4.21 ± 0.03d	14.62 ± 0.10f	1.47 ± 0.03c
		Zn3	9.99 ± 0.07d	3.97 ± 0.11c	13.96 ± 0.04d	1.42 ± 0.01bc
		Zn4	9.35 ± 0.10b	3.47 ± 0.09a	12.82 ± 0.19b	1.36 ± 0.05ab
		Zn5	9.14 ± 0.10a	3.27 ± 0.11a	12.41 ± 0.10a	1.35 ± 0.02a
	BT	CK	16.39 ± 0.09c	3.59 ± 0.12c	19.99 ± 0.20c	4.19 ± 0.09c
		Zn1	17.48 ± 0.12d	3.99 ± 0.15d	21.47 ± 0.08d	4.42 ± 0.07de
		Zn2	18.57 ± 0.06e	4.59 ± 0.08e	23.17 ± 0.12e	4.53 ± 0.06e
		Zn3	17.28 ± 0.17d	3.96 ± 0.16d	21.24 ± 0.12d	4.33 ± 0.09d
		Zn4	15.60 ± 0.13b	3.20 ± 0.13b	18.81 ± 0.14b	4.05 ± 0.06b
		Zn5	15.33 ± 0.11a	2.75 ± 0.13a	18.08 ± 0.12a	3.92 ± 0.04a
	FE	CK	16.46 ± 0.11c	3.71 ± 0.10bc	20.16 ± 0.16c	4.79 ± 0.05c
		Zn1	17.14 ± 0.06e	3.92 ± 0.13c	21.06 ± 0.11d	4.97 ± 0.08de
		Zn2	17.48 ± 0.09f	4.43 ± 0.07d	21.92 ± 0.16e	5.04 ± 0.06e
		Zn3	16.97 ± 0.07d	3.85 ± 0.12c	20.82 ± 0.09d	4.91 ± 0.06d
		Zn4	16.03 ± 0.11b	3.51 ± 0.15ab	19.54 ± 0.16b	4.65 ± 0.10b
		Zn5	15.76 ± 0.03a	3.36 ± 0.19a	19.11 ± 0.17a	4.52 ± 0.04a
	FL	CK	16.10 ± 0.07c	3.38 ± 0.03b	19.49 ± 0.10c	4.06 ± 0.04b
		Zn1	16.42 ± 0.06de	3.54 ± 0.07bc	19.97 ± 0.10e	4.14 ± 0.06bc
		Zn2	16.56 ± 0.17e	3.75 ± 0.12c	20.31 ± 0.10f	4.23 ± 0.07c
		Zn3	16.32 ± 0.06d	3.43 ± 0.09b	19.75 ± 0.15d	4.12 ± 0.03bc
		Zn4	15.85 ± 0.03b	3.30 ± 0.12ab	19.15 ± 0.14b	4.02 ± 0.01ab
		Zn5	15.59 ± 0.12a	3.13 ± 0.23a	18.71 ± 0.11a	3.93 ± 0.11a

Values are mean ± SE (n = 3). Different letters in the same column indicate significant difference at the $P < 0.05$ level by Duncan's new multiple range test. SL, BT, FE, and FL represent the seedling, booting, flowering, and filling stages, respectively

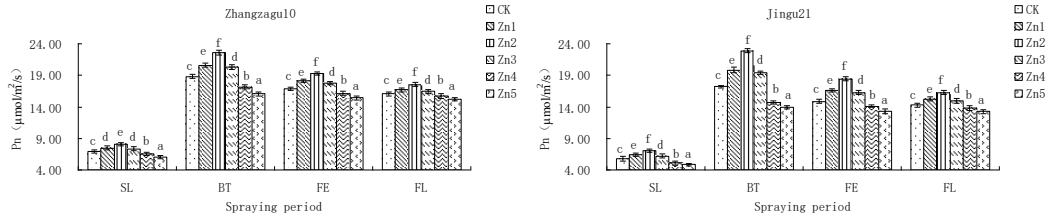

Figure 7. *Effects of Zn on the net photosynthesis rate (Pn) in the leaves of 'Zhangzagu 10' and 'Jingu 21' at the different growth stages*

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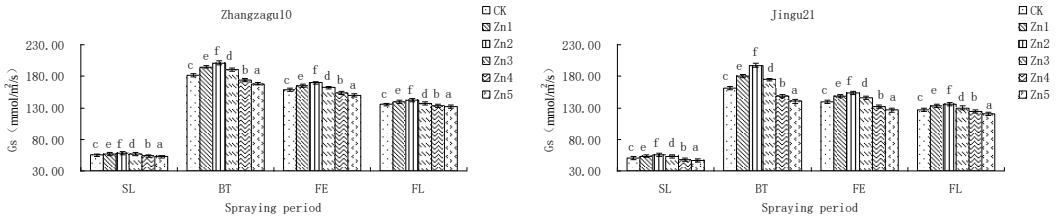

Figure 8. *Effects of Zn on stomatal conductance (Gs) in the leaves of 'Zhangzagu 10' and 'Jingu 21' at the different growth stages*

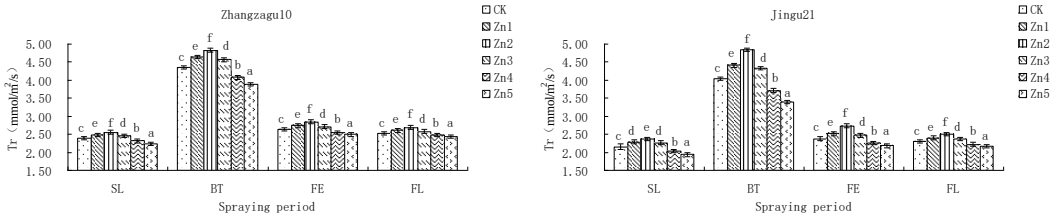

Figure 9. *Effects of Zn on transpiration rate (Tr) in the leaves of 'Zhangzagu 10' and 'Jingu 21' at the different growth stages*

During the BT stage, compared with CK, after 7 days of Zn2 treatment, the Pn, Gs, and Tr of 'Jingu 21' increased by 33.1%, 22.4%, and 20.0%, respectively, while those of 'Zhangzagu 10' increased by 19.9%, 10.5%, and 10.7%, respectively. After 7 days of Zn5 treatment, compared with CK, the Pn, Gs, and Tr of 'Jingu 21' decreased by 19.1%, 13.1%, and 16.0%, respectively, while those of 'Zhangzagu 10' declined by 14.6%, 7.7%, and 11.1%, respectively (*Figs. 7-9*).

As the Zn concentration increased, Ci decreased first and then increased, which was opposite to the trend of Pn. Among the different Zn treatments for the same period, the reduction of Zn2 treatment was the most obvious. *Figure 8* shows that, after Zn treatment, Ci had the greatest impact during the BT stage.

Compared with the control, after 7 days of Zn2 treatment, the Ci of 'Jingu 21' during SL, BT, FE, and FL decreased by 16.9%, 23.7%, 18.3%, and 13.8% respectively, while those of 'Zhangzagu 10' declined by 11.2%, 16.4%, 13.7%, and 9.5%, respectively (*Fig. 10*). During BT, the Ci of Jingu 21 was as follows: Zn5＞Zn4＞CK＞Zn3＞Zn2＞Zn1. 'Zhangzagu 10' had the same trend. Although 'Zhangzagu 10' and 'Jingu 21' showed similar trends in the different concentrations, the degrees of increase or decrease in Ci for the cultivars were not same.

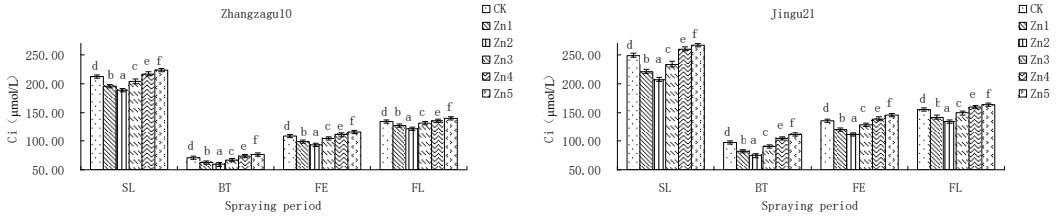

Figure 10. *Effects of Zn on intercellular CO_2 concentration (Ci) in the leaves of 'Zhangzagu 10' and 'Jingu 21' at the different growth stages*

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Table 2. *Effects of Zn on the pigment contents in the leaves of 'Jingu 21' at the different growth stages*

Cultivar	Period	Treatment	Chlorophyll a (mg/gFw)	Chlorophyll b (mg/gFw)	Chlorophyll (mg/gFw)	Carotenoid (mg/gFw)
'Jingu 21'	SL	CK	7.36 ± 0.11c	2.38 ± 0.16bc	9.74 ± 0.16c	1.31 ± 0.09ab
		Zn1	8.02 ± 0.12d	2.60 ± 0.32c	10.62 ± 0.25d	1.38 ± 0.13ab
		Zn2	8.36 ± 0.09e	2.95 ± 0.13d	11.32 ± 0.12e	1.42 ± 0.03b
		Zn3	7.86 ± 0.05d	2.56 ± 0.14c	10.41 ± 0.19d	1.34 ± 0.05ab
		Zn4	7.02 ± 0.09b	2.17 ± 0.07ab	9.19 ± 0.16b	1.27 ± 0.01a
		Zn5	6.66 ± 0.09a	1.95 ± 0.17a	8.61 ± 0.09a	1.26 ± 0.08a
	BT	CK	15.51 ± 0.07c	3.57 ± 0.14c	19.08 ± 0.12c	3.84 ± 0.03c
		Zn1	17.28 ± 0.04e	4.03 ± 0.07d	21.30 ± 0.11e	4.08 ± 0.05d
		Zn2	19.71 ± 0.12f	4.88 ± 0.03e	24.60 ± 0.14f	4.22 ± 0.03e
		Zn3	16.98 ± 0.07d	3.95 ± 0.10d	20.93 ± 0.10d	4.01 ± 0.09d
		Zn4	14.14 ± 0.07b	2.96 ± 0.10b	17.10 ± 0.08b	3.68 ± 0.08b
		Zn5	13.71 ± 0.03a	2.45 ± 0.05a	16.16 ± 0.02a	3.55 ± 0.01a
	FE	CK	16.18 ± 0.05c	2.25 ± 0.07bc	18.43 ± 0.12c	4.27 ± 0.07bc
		Zn1	17.56 ± 0.16e	2.49 ± 0.16c	20.05 ± 0.27e	4.47 ± 0.09de
		Zn2	18.18 ± 0.14f	2.83 ± 0.18d	21.00 ± 0.09f	4.58 ± 0.10e
		Zn3	17.06 ± 0.10d	2.39 ± 0.23c	19.46 ± 0.20d	4.41 ± 0.10cd
		Zn4	15.41 ± 0.05b	2.10 ± 0.13c	17.51 ± 0.17b	4.12 ± 0.08ab
		Zn5	14.82 ± 0.07a	1.95 ± 0.07a	16.77 ± 0.13a	3.99 ± 0.06a
	FL	CK	15.91 ± 0.08c	2.11 ± 0.23abc	18.01 ± 0.15c	3.55 ± 0.07bc
		Zn1	16.44 ± 0.16d	2.27 ± 0.28bc	18.71 ± 0.17d	3.66 ± 0.08cd
		Zn2	16.91 ± 0.05e	2.41 ± 0.23c	19.32 ± 0.22e	3.77 ± 0.10d
		Zn3	16.28 ± 0.05d	2.19 ± 0.17abc	18.47 ± 0.18d	3.62 ± 0.06c
		Zn4	15.39 ± 0.12b	2.04 ± 0.08ab	17.43 ± 0.19b	3.46 ± 0.05ab
		Zn5	14.87 ± 0.13a	1.86 ± 0.05a	16.73 ± 0.16a	3.37 ± 0.01a

Values are mean ± SE (n = 3). Different letters in the same column indicate significant difference at the $P < 0.05$ level by Duncan's new multiple range test. SL, BT, FE, and FL represent the seedling, booting, flowering, and filling stages, respectively

Effect of Zn on the chlorophyll fluorescence parameters of foxtail millet

Tables 3 and *4* show that the chlorophyll fluorescence parameters of foxtail millet increased with increasing Zn concentrations until Zn2. Then, at the Zn4 and Zn5 treatments, the chlorophyll fluorescence parameters declined, even lower than those at CK, except the values of qN. These indicators showed the greatest impact on millet during BT and had greater effect on 'Jingu 21' than 'Zhangzagu 10'.

During BT, compared with CK, after 7 days of Zn2 treatment, the Fo, Fm, Fv/Fm, and Fv/Fo of 'Jingu 21' increased by 5.5%, 42.5%, 11.4%, and 50.4%, respectively, while those of 'Zhangzagu 10' increased by 4.9%, 27.6%, 7.6%, and 30.9%, respectively. After 7 days of Zn5 treatment, compared with CK, the Fo, Fm, Fv/Fm, and Fv/Fo of 'Jingu 21' decreased by 6.7%, 18.5%, 6.4%, and 18.3%, respectively, while those of 'Zhangzagu 10' declined by 5.2%, 13.9%, 4.3% and 13.1%, respectively (*Tables 3* and *4*).

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Table 3. *Effects of Zn on chlorophyll fluorescence parameters of 'Zhangzagu 10' in the different growth stages*

Cultivar	Period	Treatment	Fo	Fm	Fv/Fm	Fv/Fo	Y2	ETR	qP	qN
'Zhangza 10'	SL	CK	0.226c	0.866c	0.738c	2.824c	0.214bc	69.3c	0.431c	1.144c
		Zn1	0.231d	0.935e	0.753e	3.041e	0.227de	71.8e	0.450d	1.063b
		Zn2	0.235e	0.978f	0.760f	3.159f	0.233e	74.5f	0.466e	0.982a
		Zn3	0.231d	0.913d	0.747d	2.958d	0.219cd	70.8d	0.443d	1.068b
		Zn4	0.222b	0.808b	0.726b	2.647b	0.206b	67.5b	0.409b	1.197d
		Zn5	0.217a	0.767a	0.717a	2.537a	0.196a	65.6a	0.386a	1.295e
	BT	CK	0.240c	0.803c	0.701c	2.341c	0.231c	68.2c	0.451c	1.049d
		Zn1	0.248d	0.916e	0.729d	2.697d	0.250d	72.1e	0.489e	0.881b
		Zn2	0.252e	1.024f	0.754e	3.063e	0.266e	74.9f	0.521f	0.751a
		Zn3	0.246d	0.895d	0.726d	2.646d	0.244d	71.1d	0.478d	0.911c
		Zn4	0.233b	0.738b	0.684b	2.169b	0.211b	64.7b	0.413b	1.152e
		Zn5	0.228a	0.691a	0.670a	2.035a	0.201a	62.7a	0.383a	1.237f
	FE	CK	0.236c	0.842c	0.719c	2.562c	0.219c	71.1c	0.441c	0.932d
		Zn1	0.242d	0.941e	0.743e	2.896e	0.234e	74.3e	0.464d	0.842b
		Zn2	0.246e	1.042f	0.764f	3.236f	0.244f	77.0f	0.497e	0.777a
		Zn3	0.240d	0.911d	0.736d	2.795d	0.227d	73.3d	0.460d	0.874c
		Zn4	0.231b	0.785b	0.705b	2.394b	0.205b	68.3b	0.412b	0.995e
		Zn5	0.226a	0.749a	0.698a	2.307a	0.195a	66.5a	0.398a	1.045f
	FL	CK	0.224c	0.872c	0.744c	2.899c	0.200c	71.9c	0.439c	0.866d
		Zn1	0.228d	0.933e	0.756e	3.093e	0.211d	73.7e	0.455d	0.828b
		Zn2	0.232e	1.002f	0.768f	3.314f	0.217e	75.0f	0.472e	0.779a
		Zn3	0.226d	0.914d	0.752d	3.031d	0.205cd	72.9d	0.452d	0.841c
		Zn4	0.221b	0.830b	0.734b	2.758b	0.194b	70.4b	0.430b	0.897e
		Zn5	0.218a	0.797a	0.727a	2.664a	0.185a	68.9a	0.411a	0.948f

Values are mean ± SE (n = 3). Different letters in the same column indicate significant difference at the $P < 0.05$ level by Duncan's new multiple range test. SL, BT, FE, and FL represent the seedling, booting, flowering, and filling stages, respectively

Compared with the CK, during the BT stage, after 7 days of Zn2 treatment, the Y2, ETR, and qP of 'Jingu 21' increased by 30.9%, 17.8%, and 23.1%, respectively, while those of 'Zhangzagu 10' increased by 15.0%, 9.9%, and 15.5%, respectively. After 7 days of Zn5 treatment, compared with those at CK, the Y2, ETR, and qP of 'Jingu 21' decreased by 25.8%, 10.2%, and 17.5%, respectively, while those of 'Zhangzagu 10' declined by 12.8%, 8.1%, and 15.2%, respectively (*Tables 3* and *4*).

After Zn treatment, the qN in both cultivars increased first and then decreased with increase in Zn concentration, and the level of change differed between cultivars and among periods, which was opposite to the trend of qP. In this index, the Zn treatment of the same cultivars reached the minimum effect in Zn2 and gradually increased. The levels of both cultivars from the Zn4 treatment were both lower than those in CK and reached significant levels with each treatment. *Tables 3* and *4* show that Zn treatment of the two millet varieties was most obvious during the BT stage.

Compared with CK, after 7 days of Zn2 treatment, the qN of 'Jingu 21' during SL, BT, FE, and FL decreased by 20.6%, 33.5%, 28.6%, and 13.7% respectively, while

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those of 'Zhangzagu 10' declined by 14.2%, 28.4%, 16.6%, and 10.0%, respectively (*Tables 3* and *4*). During the BT stage, the qP of 'Jingu 21' was as follows: Zn5 > Zn4 > CK > Zn3 > Zn2 > Zn1, and 'Zhangzagu 10' showed a similar trend.

Table 4. *Effects of Zn on the chlorophyll fluorescence parameters of 'Jingu 21' at the different growth stages*

Cultivar	Period	Treatment	Fo	Fm	Fv/Fm	Fv/Fo	Y2	ETR	qP	qN
'Jingu 21'	SL	CK	0.230c	0.881c	0.739c	2.828c	0.201c	72.3c	0.422c	1.162d
		Zn1	0.236d	0.966e	0.755d	3.086d	0.222d	77.2e	0.454d	1.014b
		Zn2	0.241e	1.010f	0.762e	3.197e	0.250e	80.9f	0.487e	0.922a
		Zn3	0.235d	0.949d	0.753d	3.041d	0.217d	75.7d	0.449d	1.051c
		Zn4	0.222b	0.786b	0.718b	2.546b	0.188b	68.6b	0.387b	1.278e
		Zn5	0.218a	0.726a	0.699a	2.328a	0.175a	66.4a	0.365a	1.379f
	BT	CK	0.242c	0.794c	0.696c	2.285c	0.208c	70.6c	0.451c	1.026d
		Zn1	0.250d	0.953e	0.737e	2.809e	0.257e	79.0e	0.505e	0.803b
		Zn2	0.255e	1.132f	0.775f	3.436f	0.273f	83.2f	0.556f	0.682a
		Zn3	0.248d	0.918d	0.730d	2.701d	0.244d	76.9d	0.493d	0.849c
		Zn4	0.230b	0.697b	0.669b	2.024b	0.175b	65.2b	0.399b	1.195e
		Zn5	0.226a	0.647a	0.651a	1.868a	0.155a	63.5a	0.372a	1.290f
	FE	CK	0.236c	0.823c	0.713c	2.485c	0.213c	73.1c	0.448c	0.917d
		Zn1	0.244d	0.955e	0.745e	2.922e	0.236e	77.9e	0.490e	0.790b
		Zn2	0.248e	1.097f	0.774f	3.422f	0.259f	81.5f	0.542f	0.655a
		Zn3	0.241d	0.911d	0.736d	2.781d	0.227d	75.9d	0.480d	0.824c
		Zn4	0.230b	0.746b	0.692b	2.247b	0.190b	69.1b	0.405b	1.028e
		Zn5	0.224a	0.699a	0.679a	2.114a	0.181a	67.0a	0.387a	1.113f
	FL	CK	0.229c	0.910c	0.748c	2.976c	0.205c	71.1c	0.441c	0.858d
		Zn1	0.235d	1.007e	0.767e	3.289e	0.221e	74.7e	0.464d	0.775b
		Zn2	0.239e	1.087f	0.780f	3.547f	0.236f	78.2f	0.519e	0.741a
		Zn3	0.233d	0.976d	0.761d	3.187d	0.214d	72.7d	0.459d	0.797c
		Zn4	0.224b	0.841b	0.733b	2.747b	0.195b	68.8b	0.423b	0.921e
		Zn5	0.221a	0.800a	0.724a	2.621a	0.184a	66.7a	0.403a	0.995f

Values are mean ± SE (n = 3). Different letters in the same column indicate significant difference at the $P < 0.05$ level by Duncan's new multiple range test. SL, BT, FE, and FL represent the seedling, booting, flowering, and filling stages, respectively

Discussion

Effects of Zn on the physiological characteristics

Although Zn is a micronutrient necessary for photosynthesis and plant growth at low concentration, Zn becomes toxic when its concentration reaches a threshold level (Cunha et al., 2008; Lin and Aarts, 2012). Jat et al. (2007) reported that Zn activates several antioxidant enzymes, such as SOD, POD, and CAT. Saeed et al. reported that SOD, POD, and CAT and free radical scavenging activities in cut-flowers remained maximum with the medium dose of Zn (6 mg Zn kg^{-1}). Thus, Zn applied at 6-8 mg kg^{-1} imparted greater beneficial effects on growth, production, vase life quality, and antioxidative activities in gladiolus cut flower and further higher application rates

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rendered non-significant improvement (Tariq et al., 2013). In this study, 40 $mg \cdot L^{-1}$ of $ZnSO_4 \cdot 7H_2O$ significantly increased the SOD, POD, and CAT activities of 'Jingu 21' and 'Zhangzagu 10' at different stages. However, 80 $mg \cdot L^{-1}$ of $ZnSO_4 \cdot 7H_2O$ resulted in SOD, POD, and CAT activities lower than those of CK (*Figs. 3-5*). Moreover, 40 $mg \cdot L^{-1}$ of $ZnSO_4 \cdot 7H_2O$ significantly decreased the MDA content, but 80 $mg \cdot L^{-1}$ of $ZnSO_4 \cdot 7H_2O$ increased the MDA content higher than that of CK (*Fig. 6*).

Zn plays an important role in the anti-lipid peroxidation of millet. The suitable Zn concentration can alleviate the peroxidation of membrane lipids and improve the antioxidant activity of foxtail millet (Kakade et al., 2009; Maurya and Kumar, 2014; Hajiboland and Beiramzadeh, 2008). Therefore, the application of a suitable concentration of Zn to foxtail millet will help improve its resistance to stress. The mechanism of the effect of Zn on energy metabolism needs further study.

Effects of Zn on pigments

Pigment is the most important part of photosynthesis. This parameter can reflect the ability of plant light energy conversion to a certain extent, and it will also protect the normal operation of photosynthetic apparatus to some extent (Nouet et al., 2011). The main factors in the pigments are chlorophyll and carotenoids, and Zn can prevent the oxidation of pigments (Cakmak 2000; Cherif et al., 2010). Pongrac et al. (2009) reported that an appropriate amount of Zn can increase the chlorophyll content of leaves, but excessive Zn reduces the chlorophyll content of leaves. This experiment shows that low concentration of Zn can increase the pigment content, while high concentration treatment will inhibit the pigment content. In 'Jingu 21', the pigment content was as follows Zn2 > Zn1 > Zn3 > CK > Zn4 > Zn5, and 'Zhangzagu 10' showed a similar trend (*Tables 1* and *2*). Excessive Zn affected the growth of the seedlings, causing a significant decrease in photosynthetic pigment content and yellowing of the leaves. This phenomenon may be due to the antagonistic effect of Zn and iron metabolism in plants, and the excessive Zn content inhibited the absorption of iron by plants (Wirén et al., 1996). Thus, an appropriate amount of Zn concentration can effectively increase the pigment content of the leaves (Kumar and Arora, 2000) and delay the rapid senescence of the foxtail millet.

Effects of photosynthetic gas exchange parameters

Photosynthesis plays a very important role in plants. This process is the source of plant yield, which is related to plant growth and development. This process involves photoreaction and carbon reaction to convert carbon dioxide and water into organic matter and oxygen, which are the basis for plant survival (Baron et al., 1995; Yruela, 2005). The Pn, Gs, Tr, and Ci are important indicators to affect the photosynthesis. Fernàndez-Martínez et al. (2014) reported that the photoprotective and antioxidant responses were enhanced with increasing Zn concentration, but high Zn concentration produced high toxicity levels for both clones and resulted in impaired biomass production, photochemical processes, and photosynthesis. The results showed that Zn treatment could increase Pn, Gs, and Tr and reached maximum levels in Zn2, gradually decreased. From the Zn4 treatment, these three indices were both lower than those at CK, but Ci showed an opposite trend of Pn (*Figs. 7-10*). Although the trends are similar at all times, but the degrees of increase or decrease were not the same. In summary, Zn had a regulatory effect on plant photosynthesis and exhibits different effects depending

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on the application concentration, period, and crop type (Sidhu, 2016; Camargo Gai et al., 2017).

Effects of chlorophyll fluorescence parameters

The chlorophyll fluorescence parameter is a set of variables or constant values used to describe the photosynthesis mechanism and photosynthetic physiological state of plants. This parameter reflects the "inherent" characteristics of plants and regarded as an intrinsic probe to study the relationship between plant photosynthesis and environment (Lahive et al., 2012). Information on the donor side, receptor side, and reaction center of PSII can be obtained using the chlorophyll fluorescence induction kinetics curve (Appenroth et al., 2001; Wen et al., 2002). Fv/Fm represents the primary light energy conversion efficiency of PSII. Previous studies suggested that Fv/Fm is a sensitive indicator of plant photoinhibition under stress conditions. This ratio is a measure of the maximum ability of primary light energy capture, reflecting the potential maximum photosynthetic abilities of plants (Krivosheeva et al., 1996). Fv/Fo represents the potential activity of PSII, which is more sensitive in reflecting changes in photon conversion efficiency during leaf senescence (Krivosheeva et al., 1996). In this study, the Fv/Fm and Fv/Fo values increased first and then decreased with the change in Zn concentration, indicating that low Zn concentration can enhance photosynthesis, but high Zn concentration can cause photoinhibition of foxtail millet or damage PSII complex. Y2 reflects the quantum conversion efficiency of photosynthetic electron transport and is the efficiency of PSII light energy capture in case of PSII reaction center shutdown (Longstaff et al., 2002). ETR reflects the apparent electron transfer efficiency under actual light intensity conditions (Longstaff et al., 2002). The results of this experiment indicate that as the Zn concentration increases, Y2 and ETR increased and then decreased. This phenomenon indicated that the low Zn concentration can enhance photosynthesis, but high Zn concentration inhibition of photosynthesis decreased the actual quantum yield of the PSII photosynthetic reaction center (*Tables 3* and *4*).

Photochemical quenching (qP) reflects the quantity of light energy absorbed by the PSII antenna pigment for photochemical electron transport. This parameter is a measure of the oxidation state of the original electron acceptor (QA). This parameter represents the proportion of the open part of the PSII reaction center, which can reflect the electron transfer rate of the electron photosynthetic chain and its efficiency in CO_2 fixation (Mu et al., 2016). Under the experimental conditions, 40 $mg \cdot L^{-1}$ of $ZnSO_4 \cdot 7H_2O$ significantly increased the qP of 'Jingu 21' and 'Zhangzagu 10' in different stages. However, from 80 $mg \cdot L^{-1}$ of $ZnSO_4 \cdot 7H_2O$, the qP was lower than that of the control (*Tables 3* and *4*). This result indicated that the low Zn concentration strengthened the electron transfer activity of PSII, while high Zn concentration decreased the open part of the PSII reaction center, which hindered the photosynthetic electron transfer. The parameter qN reflects the portion of the light energy dissipated in the form of heat that cannot be used for photosynthetic electron transport in the light absorbed by the PSII antenna (Mu et al., 2016; Mikulic and Beardall, 2014). The results showed that qN increased first and then decreased with the increase in Zn concentration. This parameter indicated that Zn concentration had an inhibitory effect on the heat loss of millet light energy. This process is beneficial to the accumulation of dry matter in foxtail millet.

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Conclusions

In summary, low Zn concentration could promote plant growth and enhance plant antioxidant capacity and photosynthesis. However, high Zn concentration inhibited plant growth and leaf photosynthesis. 'Jingu 21' changed more than 'Zhangzagu10'. The combination of optimal Zn spraying period and spraying concentration for the foxtail millet growth was BT and 40 $mg \cdot L^{-1}$ of Zn. Thus, a theoretical basis for the safe application of Zn fertilizer on foxtail millet is provided.

The study did not test a larger number of genotypes cultivars and more soil fertility conditions because of the limited experimental conditions. The numbers of test materials and test sites were not sufficiently comprehensive. Multiple materials, multiple points, and multiple years of tests are required to provide a clear direction for the subsequent research.

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