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Effects of Zinc on Growth and Physiological Characters of Flag Leaf and Grains of Winter Wheat after Anthesis

Lina Jiang, Daijing Zhang, Fei Song, Xinmin Zhang, Yun Shao and Chunxi Li
College of Life Sciences, Henan Normal University, Xinxiang 453007, P.R. China

Abstract: The study explored the effects of supplementing 0, 200, 400 and 800 mg/kg Zn in cultivating soils on the growth and yield characters of winter wheat Zhoumai 18 and Aikang 58, the two popular wheat varieties currently in Henan, China, planted in pots in a screen house after anthesis during 2009-2010. The results indicated that the differences in activities of Peroxidase (POD), Superoxide Dismutase (SOD) and Catalase (CAT) in flag leaf and grains at 5 and 25 days after anthesis mainly came from differences in Zn concentration, not varieties. In addition, the Photosynthetic rates (Pn) of flag leaf in the two varieties were the same. Zn treatment significantly influenced CAT activity and Pn of flag leaf at 5 days after anthesis, SOD activity in flag leaf at 25 days after anthesis, SOD activity in grains at 5 days after anthesis, as well as POD and CAT activities in grains at 25 days after anthesis ($p<0.01$). Adequate Zn enhanced SOD, POD and CAT activities and Pn of flag leaf after anthesis, SOD and POD activities in grains at 5 days after anthesis and POD activity in grains at 25 days after anthesis, but reduced CAT activity in grains after anthesis. Pn of flag leaf was higher at 200 mg/kg Zn content. Moreover, the yield characters such as grains per spike, grain weight per spike and 1000-grain weight tended to be the highest at 200 mg/kg Zn content and lowest at 800 mg/kg Zn content. The correlation analysis showed that 1) Pn of flag leaf at 25 days after anthesis was positively correlated with grain number per spike, grain weight per spike and 1000-grain weight ($p<0.05$); 2) the activities of SOD, POD and CAT in grains at 25 days after anthesis were positively correlated with grain yield characters. In conclusion, appropriate Zn amount could promote the growth of flag leaf and grains after anthesis and improve yield characters.

Keywords: Flag leaf, grain, physiological characters, winter wheat, zinc

INTRODUCTION

Zinc (Zn) is one of the most functional micronutrients in plants, animals and humans and plays an important physiological role in their growth and development (Cakmak, 2008). Zn deficiency is common in both crop plants and human beings, causing various serious health complications such as stunting, increased susceptibility to infectious diseases, impaired brain function and mental development, poor birth outcomes and anemia (Hotz and Brown, 2004; Fraga, 2005). It is estimated that more than one third of the population is affected by Zn deficiency, particularly children and pregnant women and low dietary intake of Zn has been discussed as a major reason (Bouis, 2003; Hotz and Brown, 2004; White and Broadly, 2009). Enrichment of cereal crops with Zn is an important global challenge and a high-priority research area. Zn is required for the growth and grain nutritional quality of wheat, a staple food in a number of developing countries in the world and essential to ensure food safety and healthy diet (Welch and Graham, 1999; Pfeiffer and McClafferty, 2007; Cakmak, 2008). It has been reported that Zn concentration in wheat grains is different among wheat cultivars and subjects to the regulation of the environment and cultivation measures (Gao *et al*., 2001; Zhang *et al*., 2004; Hao *et al*., 2008; Jiang *et al*., 2010; Cakmak *et al*., 2010). Genetic biofortification and agronomic biofortification are two important agricultural tools to improve Zn concentration in grains (White and Broadly, 2005; Brinch-Pedersen *et al*., 2007; Pfeiffer and McClafferty, 2007; Cakmak, 2008; Cakmak *et al*., 2010). It has been reported that grain Zn concentration can be enhanced to a certain extent by increasing the application of Zn fertilizer (Rengel *et al*., 1999). Application of Zn fertilizers not only improves nutritional quality but also contributes significantly to grain production in Zn-deficient soils (Graham *et al*., 1992; Yilmaz *et al*., 1997; Cakmak, 2008; Peck *et al*., 2008). Hao *et al*. (2003) found that after applying Zn fertilizer, Zn absorption in wheat increased significantly and its concentration in wheat grains increased by 18.8%. Studies have shown that exogenous Zn^{2+} can significantly improve the dry weight of seedling roots and shoots (Jiang *et al*., 2008), ease winter wheat

Corresponding Author: Lina Jiang, College of Life Sciences, Henan Normal University, Xinxiang 453007, P.R. China, Tel.: 13937361762

seedlings peroxidation, enhance SOD and CAT activities and promote chlorophyll synthesis, thereby affecting photosynthesis (Han *et al*., 2003). Adequate Zn can accelerate the wheat growth, tillers and anthesis, while excessive Zn content in the environment will inhibit the growth of wheat seedlings, exacerbate flag leaf membrane peroxidation at the grain filling stage and lead to yield loss (Chen *et al*., 2003; Han *et al*., 2004; Shao *et al*., 2006; Feng *et al*., 2007). Current studies on the influence of Zn on crops are mainly concentrated on the influence of Zn on seedling growth, photosynthetic physiology and grain yield. Only few researches studied the effects of zinc on the relationship between wheat physiological characteristics after anthesis and the growth of flag leaf and grains. In this study, we treated two currently popular wheat varieties in Henan, China with different zinc contents by supplementing zinc fertilizer to the culture pots in screen house, measured the effects of zinc on the growth and physiological characteristics of their flag leaves and grains and analyzed its relationship with grain yield and traits.

MATERIALS AND METHODS

This pot experiment was conducted in the screen house of Henan Normal University in the northern Henan Province, China, during 2009-2010 using winter wheat, Zhoumai 18 (Zhoukou Academy of Agricultural Sciences, Henan) and Aikang 58 (Henan Science and Technology University). These two varieties and soil Zn treatment were arranged by randomized blocks design. Pot with diameter of 28 cm and height of 30 cm was filled with 10 kg of soil supplemented with $ZnSO_4 \cdot 7H_2O$ (AR) to zinc concentration of 0, 200, 400 and 800 mg/kg soil, as expressed as Zn_0, Zn_2, Zn_4 and Zn_8, respectively. In addition to Zn fertilizer, pot soil is supplemented and thoroughly mixed with urea (1.82 g), diammonium phosphate (1.57 g) and potassium chloride (0.94 g) before planting. 20 grains were planted in each pot in Oct. 13, 2009 and 10 final singled seedlings were selected at three-leaf stage. The experiment was performed in quadruplicate. Flag leaf and grain were collected 5 days (May 1, 2010) and 25 days (May 21, 2010) after anthesis and used to measure their physiological activities. Mature wheat were harvested and examined.

Methods: Peroxidase (POD), Catalase (CAT) and Superoxide Dismutase (SOD) activities were measured as previously reported (Zhang and Qu, 2003; Shanghai Institute of Plant Physiology and Shanghai Plant Physiology Society, 1999; Feng *et al*., 1997).

Net photosynthetic rate (Pn, μmol CO_2/ ($m^2 \cdot s$)) of flag leaf was measured by LI-6400 portable photosynthetic locator with an open gas line with CO_2 concentration of 365 μmol CO_2/mol. Ten leaves in each treatment were measured and each leaf was measured for three times.

The grain number, grain weight per spike and 1000-grain weight were measured using mature wheat.

Analysis methods: Data were analyzed using Microsoft Excel and SPSS statistical software and expressed as mean±S.D. Differences between samples were analyzed using analysis of variance and LSD method. A p value less than 0.05 was considered as statistic significance.

RESULTS AND DISCUSSION

Effects of Zn on SOD activity in flag leaf and grains: The SOD activities in flag leaf and grains at 25 days after anthesis were higher than those at 5 days after anthesis. In general, SOD activity in flag leaf was higher than that in grains (Fig. 1).

At 5 days after anthesis, SOD activity in flag leaf of Zhoumai 18 in Zn_0 group was lower than that of Aikang 58. SOD activity in flag leaf was higher in Zn treatment groups than that in Zn_0 group. With Zn concentration increasing, SOD activity in flag leaf of Zhoumai 18 slightly increased in the order of $Zn_2 > Zn_4 > Zn_8$, while SOD activity in flag leaf of Aikang 58 first increased, then slightly decreased, which was in the order of $Zn_2 < Zn_4 < Zn_8$. Aikang 58 had higher SOD activity in flag leaf in Zn_4 and Zn_8 group. In addition, SOD activity in flag leaf of Zhoumai 18 in Zn_4 and Zn_8 groups was higher than that of Aikang 58 in Zn_4 and Zn_8 groups. Moreover, Zn treatment improved SOD activity in grains of the two varieties significantly ($p<0.01$). And SOD activity in grains was the highest in Zn_8 group and the lowest in Zn_0 group. Zhoumai 18 had lower SOD activity in grains than Aikang 58 in all Zn groups except Zn_8 group.

At 25 days after anthesis, SOD activities in flag leaf of the two varieties differed significantly among treatments, which in Zn treatment groups is higher that in Zn_0 group, indicating that Zn treatment can improve SOD activity in flag leaf at 25 days after anthesis. Moreover, SOD activity in flag leaf of Zhoumai 18 was lower than that of Aikang 58 under Zn_0 and Zn_2 treatments, but higher than Aikang 58 under Zn_4 and Zn_8 treatments. SOD activities in grains of Zhoumai 18, which was in the order of $Zn_8 > Zn_4 > Zn_2$, were lower than that in Zn_0 treatment. SOD activity in grains of Aikang 58 increased first with Zn concentration increasing but then decreased in Zn_8 group, showing a trend of $Zn_2 > Zn_4 > Zn_0 > Zn_8$. These results indicated that Aikang 58 is more sensitive to Zn fertilizer. Excessive Zn decreased SOD activity in grains and is harmful for anti-aging.

Influence of Zn on the POD activity in flag leaf and grains: The results of POD activity (Fig. 2) show that:

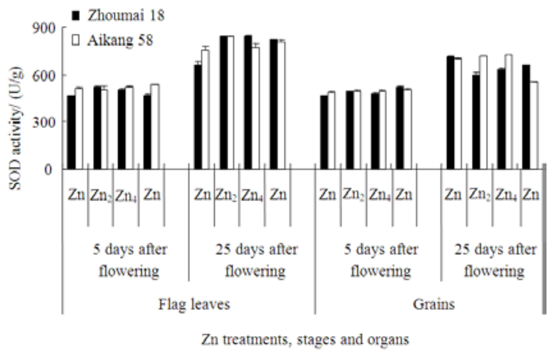

Fig. 1: SOD activity in flag leaves and grains at 5 and 25 days after anthesis

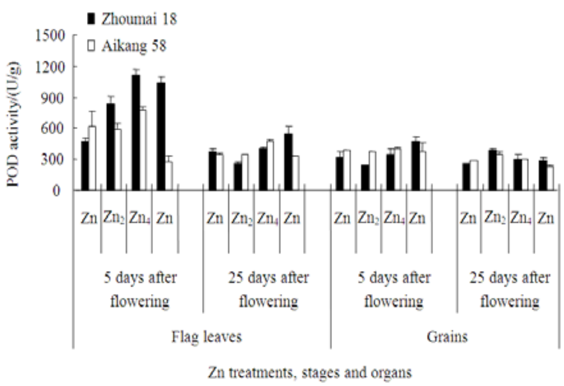

Fig. 2: POD activity in flag leaves and grains at 5 and 25 days after anthesis

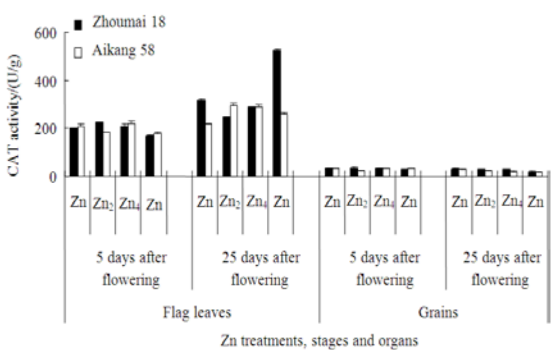

Fig. 3: CAT activity in flag leaves and grains at 5 and 25 days after anthesis

- POD activity in flag leaf of both varieties at 5 days after anthesis was less than that at 25 days after anthesis
- The change of POD activity in grains at 5 and 25 days after anthesis was not obvious
- POD activity in flag leaf was higher than that in grains
- POD activity in flag leaf at 5 days after anthesis was different among varieties (p<0.05)

POD activity in flag leaf at 5 days after anthesis was lower in Zhoumai 18 than Aikang 58 in Zn_0 group, but higher in other groups. In addition, the order of POD activity in flag leaf of Zhoumai 18 was $Zn_4>Zn_8>Zn_2>Zn_0$, while that of Aikang 58 was $Zn_4>Zn_0>Zn_2>Zn_8$. Flag leaf POD activities were increased by 1.36 times and 0.25 times in Zhoumai 18 and Aikang 58 under Zn_4 treatment, respectively, compared to those under Zn_0 treatment. Grain POD activity did not show significant difference among varieties and treatments. Grain POD activity was lower in Zhoumai 18 than in Aikang 58 except Zn_8 treatment. With Zn concentration increasing, POD activity in grains of Zhoumai 18 increased with Zn treatment, but decreased in grains of Aikang 58. POD activity in grains of Zhoumai 18 under Zn_8 treatment increased by 49.6% than that under Zn_0 treatment.

With Zn concentration increasing, flag leaf POD activities at 25 days after anthesis were different between species. The flag leaf POD activity of Zhoumai 18 presented a down and then up trend, in the order of $Zn_8>Zn_4>Zn_0>Zn_2$, while flag leaf POD activity of Aikang 58 showed a up and then down trend, in the order of $Zn_4>Zn_2>Zn_0>Zn_8$. Grain POD activity was significantly different between species (p<0.01). The correlation analysis showed that at 25 days after anthesis, flag leaf POD activity was significantly, positively correlated with grain POD activity at 5 days after anthesis (r = 0.84, p<0.05).

Effect of Zn on the CAT activity in flag leaf and grains: It can be seen from Fig. 3 that CAT activity in flag leaf was significantly higher than that in grains after anthesis. In flag leaf, CAT activity at 5 days after anthesis was less than that at 25 days after anthesis, while in grains, CAT activity at 5 days was slightly higher than that at 25 days. At 5 days after anthesis, Zn treatment has significant influence on the CAT activity in flag leaf (p<0.05). CAT activity in flag leaf of Zhoumai 18 increased by 11.6% in Zn_2 group compared to that in Zn_0, reaching its highest. CAT activity in flag leaf of Aikang 58 increased by 7.9% in Zn_4 group compared to that in Zn_0, reaching its highest. In addition, CAT activity in flag leaves of the two varieties was the lowest in Zn_8 group. CAT activity in grains in Zn_2, Zn_4 and Zn_8 only slightly increased compared to that in Zn_0.

At 25 days after anthesis, with Zn concentration increasing, CAT activity in flag leaf of Aikang 58 first increased and then decreased, showing a trend of $Zn_2>Zn_4>Zn_8>Zn_0$, while that of Zhoumai 18 first decreased and then increased, showing a trend of $Zn_8>Zn_0>Zn_2>Zn_4$. CAT activity in flag leaf of Zhoumai 18 in Zn_8 group significantly increased by 64% compared with that in Zn_0 (p<0.01). CAT activity in grains varied significantly between species and between Zn treatments (p<0.01). CAT activity in grains of Zhouami 18 was greater than that of Aikang 58. In addition, CAT activity in grains of both Zhouami 18 and Aikang 58 declined with Zn concentration the increasing, showing a trend of $Zn_0>Zn_2>Zn_4>Zn_8$.

It can be seen from Fig. 2 and 3 that variation trends of CAT activity and POD activity in flag leaf were similar. Correlation analysis showed that CAT activity and POD activity in flag leaf at 5 and 25 days after anthesis had significant positive correlation (r = 0.78, $p<0.05$ and r = 0.80, $p<0.05$, respectively). Therefore, appropriate Zn amount can simultaneously contribute to the improvement of flag leaf CAT and POD activities after anthesis.

Effect of Zn on photosynthetic rate of wheat flag leaf: It can be seen from Fig. 4, the flag leaf photosynthetic rate at 5 days after anthesis was higher than that at 25 days and Zn treatment significantly affected flag leaf Pn at 5 and 25 days after anthesis ($p<0.01$). At 5 days after flowering, flag leaf Pn of Zhoumai 18 had a trend of $Zn_2>Zn_8>Zn_4>Zn_0$, while that of Ailkang 58 had a trend of $Zn_8>Zn_2>Zn_4>Zn_0$. With the exception of Zn_8, the flag leaf Pn of Zhoumai 18 was always higher than that of Aikang 58. At 25 days after anthesis, flag leaf Pn of the two varieties reached their highest in Zn_2 group. In addition, flag leaf Pn of Zhoumai18 was lower than that of Aikang 58 under Zn_0 condition, while that of Zhoumai 18 was higher than that of Aikang 58 when supplemented with Zn. Therefore, Zn treatment resulted in a higher Pn in zhoumai 18 at 25 days after anthesis to maintain adequate photosynthesis.

Correlation analysis showed that flag leaf Pn and grain SOD activity had a significant, positive correlation at 5 days after anthesis (r = 0.84, $p<0.01$) and at 25 day after anthesis (r = 0.95, $p<0.01$).

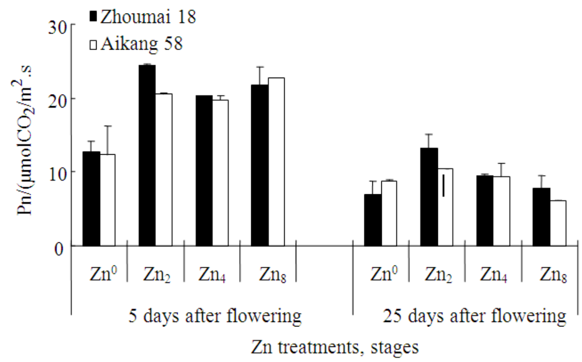

Fig. 4: Pn in flag leaves at 5 and 25 days after anthesis

Effects of Zn on the traits of grain yield: Zn has great impact on the traits of grain yield (Table 1). Grains per spike, grain weight per spike and 1000-grain weight were higher in Zn_2 group compared to that of in Zn_0, by 14.74, 24.02 and 8.09% in Zhoumai18, respectively and by 1.61, 10.53 and 8.85% in Aikang 58, respectively. By contrast, grains per spike, grain weight per spike and 1000-grain weight reduced in Zn_8 group compared to that in Zn_0, by 22.00, 31.28 and 0.47% in Zhoumai 18, respectively and by 18.95, 30.00 and 5.74% in Aikang 58, respectively.

Grains per spike, grain weight per spike and 1000-grain weight of Zhoumai 18 showed a trend of $Zn_2>Zn_4>Zn_0>Zn_8$. Variance analysis showed that they were not significantly different between Zn_2 and Zn_4 groups, but significant different between Zn_2 and Zn_0 groups. In addition, they were significantly lower in Zn_8 group compared to those in all other groups. Grains per

Table 1: Effects of Zn on grain yield characters

Variety	Treatments	Grain number per ear	Grain weight per ear (g)	1000-grain weight (g)
Zhoumai 18	Zn_0	44.1b	1.79b	40.68b
	Zn_2	50.6a	2.22a	43.97a
	Zn_4	46.2ab	2.01ab	43.59ab
	Zn_8	34.4c	1.23c	40.49c
Aikang 58	Zn_0	49.6a	1.90ab	38.32ab
	Zn_2	50.4a	2.10a	41.71a
	Zn_4	45.9a	1.75b	40.69b
	Zn_8	40.2b	1.33c	36.12c

Different letters in the same column indicate significant difference at 0.05 level

Table 2: Correlation analysis of between physiological indexes and yield characters

Stages	Physiological index	Grain number per ear	Grain weight per ear	1000-grain weight
5 days after anthesis	Pn of flag leaf	-0.106	-0.031	0.245
	CAT activity in flag leaf	-0.014	0.156	0.581
	CAT activity in grains	0.126	0.157	0.105
	POD activity in flag leaf	-0.092	0.154	0.699
	POD activity in grains	-0.653	-0.722*	-0.450
	SOD activity in flag leaf	0.105	-0.117	-0.379
	SOD activity in grains	-0.723*	-0.744*	-0.353
25 days after anthesis	Pn of flag leaf	0.734*	0.841**	0.781*
	CAT activity in flag leaf	-0.793	-0.611	0.074
	CAT activity in grains	0.457	0.526	0.283
	POD activity in flag leaf	-0.686	-0.611	-0.057
	POD activity in grains	0.588	0.734*	0.811*
	SOD activity in flag leaf	0.039	0.121	0.347
	SOD activity in grains	0.246	0.213	0.120

* & **: Significant correlation at 0.05 and 0.01, respectively

spike and grain weight per spike of Aikang 58 showed a trend of $Zn_2>Zn_0>Zn_4>Zn_8$, while 1000-grain weight showed a trend of $Zn_2>Zn_4>Zn_0>Zn_8$. In addition, grains per spike, grain weight per spike and 1000-grain weight in Zn_8 group were significantly lower than those in all other groups.

The phenotypic correlation coefficient showed that with Zn treatment, flag leaf Pn at 25 days after anthesis stage was positively correlated with grain yield traits including grains per spike (r = 0.73, $p<0.05$), grain weight per spike (r = 0.84, $p<0.01$) and 1000-grain weight (r = 0.78, $p<0.05$). In addition, grains per spike, grain weight per spike and 1000-grain weight were positively correlated with grain SOD activity, POD activity and CAT activity at 25 days after anthesis and their correlation with grain POD activity was closer than others. By contrast, grains per spike, grain weight per spike and 1000-grain weight were negatively correlated with grain SOD and POD activity at 5 days after anthesis. In detail, grains per spike was significantly negatively correlated with grain SOD activity at 5 days after anthesis (r = -0.72, $p<0.05$), grain weight per spike was significantly negatively correlated with grain SOD activity (r = -0.74, $p<0.05$) and POD activity (r = -0.72, $p<0.05$) at 5 days after anthesis (Table 2).

Discussion: Zn is an essential trace mineral element to plant growth and development (Han *et al*., 2004), but if used excessively, it is toxic to plants. Compared with Cu and Cd, Zn stress is lower for plant growth (Feng *et al*., 2007; Chen *et al*., 2003; Shao *et al*., 2006). Studies have shown that only 2.39% Zn was used by above-ground part of wheat, while the majority of Zn resides in the soil in various forms (Zhang *et al*., 1999, 2008). Thus long-term excessive application of Zn fertilizer may cause potential danger to plant and Zn pollution. Wheat seeds grow mainly after anthesis. Flag leaf, as the main component of the canopy, plays an important role in the production and accumulation of materials during grain filling. Meanwhile, grain maturation happens concurrently with flag leaf senescence after anthesis. Decrease in effective photosynthesis is unfavorable for grain yield (Zhang *et al*., 2008). Therefore, delaying flag leaf senescence and enhancing the photosynthetic capacity of flag leaf after anthesis are two key factors to improve grain weight. Wheat leaf aging process is accompanied by the cumulative process of reactive oxygen species. Cells have a variety of ways to clear these reactive oxygen species. SOD, CAT and POD are important protective enzymes of intracellular reactive oxygen scavenging system and can inhibit membrane lipid peroxidation and delay senescence (He *et al*., 2009).

Zn^{2+} can affect physiological activity of wheat seedlings. Hydroponic experiment showed that with Zn^{2+} concentration (0-360 mg/L) increasing:

- The content of soluble sugar, MDA content and POD activity increase in wheat seedling leaves
- SOD activity shows a trend of first decreasing and then increasing
- CAT activity shows a trend of first increasing and then decreasing
- Soluble protein and chlorophyll content decrease
- Expression of isozymes of POD, SOD and CAT increase (Feng *et al*., 2007)

Excessive Zn in soil could inhibit plant growth in the grain filling process, reduce chlorophyll content in flag leaf and increase respiration rate, soluble sugars, free proline and malondialdehyde levels (Shao *et al*., 2006). This study demonstrated that Zn could affect the growth and physiology of wheat flag leaf and grains after anthesis in all tested varieties at slightly different extent. Zn has significant effects on CAT activity and Pn of flag leaf, as well as SOD activity in grains at 5 days after anthesis and SOD activity in flag leaf and POD and CAT activity in grains at 25 days after anthesis ($p<0.01$). Appropriate amount of Zn could promote SOD, POD and CAT activity and Pn in wheat flag leaf after anthesis, SOD activity and POD activity in wheat grains at 5 days after anthesis and POD activity in grains at 25 days after anthesis, while reduce CAT activity in grains after anthesis. SOD, POD and CAT activities in wheat flag leaf after anthesis were higher than those in grains. Compared with those at 5 days after anthesis, SOD activities in flag leaf and grains at 25 days increase while POD activities decrease. Therefore, in the early grain filling stage (for 5 days after anthesis), POD is the major antioxidant enzyme. By contrast, in the late of grain filling stage (25 days after anthesis stage), SOD is the major scavenging enzyme of reactive oxygen species.

Zn affects the photosynthesis by influencing the carbon anhydride enzyme activity and the chlorophyll content. In a certain Zn concentration range, photosynthesis intensity is enhanced with Zn concentration increasing (Han *et al*., 2004). This study shows that application of defined Zn amount to the soil could improve wheat flag leaf Pn after anthesis. With Zn level increasing, flag leaf Pn of the two wheat varieties decreases after increasing. With Zn_2 treatment, flag leaf photosynthetic rates of Zhoumai 18 and Aikang 58 are significantly higher than that without Zn treatment ($p<0.05$). Therefore, the appropriate amount of exogenous Zn could extend the functional period of flag leaf and be beneficial to accumulating and transfering photosynthetic products. By 17 years of long-term Zn fertilizer positioning test, Hao *et al*. (2003) found that application of Zn fertilizer improved wheat Zn nutritional status and increased plant tiller number, earbeared tiller percentage, spike number, grain number per spike and 1000-grain weight per unit area. Wang *et al*. (2003) tested cinnamon soil of Zn deficiency using micro-positioning and found that Zn fertilizer improved wheat tiller number, grain number per spike, spike length and corncob length, grain number per corncob and grain weight, by which significantly increased the production of wheat and corn. This study showed that Zn treatment to soil had a greater influence on wheat yield. In this experiment, grain number per spike, grain weight per spike and 1000-grain weight in Zn_2 group were the highest and significantly higher than those in Zn_8 group, which were significantly lower than those in Zn_0 group. Moreover, considering the correlation of grain yield traits with flag leaf growth and physiological characters after anthesis, flag leaf Pn at 25 days after anthesis has significant, positive correlation with grain number per spike, grain weight per spike and 1000-grain weight ($p<0.05$). The grain yield traits in Zn_2 group are the highest.

Overall, the appropriate application of Zn could promote the growth of wheat flag leaf and grains after anthesis and improve yield traits. In addition, the amount of Zn fertilizer should be strictly controlled in agricultural production. In this experiment, application of 200 mg/kg of Zn to soil is the best for wheat grain growth.

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