Co-application of molybdenum and zinc increases grain yield and photosynthetic efficiency of wheat leaves

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Abstract: The effects of the co-application of molybdenum (Mo) and zinc (Zn) on winter wheat grain yield, leaf photosynthetic efficiency, and antioxidant activity were investigated using pot culture experiments with sandy soil. Four treatments were investigated, including a control (CK), 0.15 mg Mo/kg soil (Mo), 1 mg Zn/kg soil (Zn), and 0.15 mg Mo/kg + 1 mg Zn/kg soil (Mo + Zn). The results showed that the soil application of Mo and Mo + Zn increased the winter wheat grain yield, spike number, and thousand kernel weight, coupled with significant enhancement in nitrate reductase activity, chlorophyll a and chlorophyll a + b contents, as well as the photosynthetic rate of the leaves, which were also positively correlated with grain yield. Furthermore, the co-utilization of Zn and Mo + Zn significantly increased the activities of superoxide dismutase, catalase, and peroxidase in the leaves. The overall results indicate that the co-application of Mo and Zn can increase winter wheat grain yield by improving the leaf photosynthetic efficiency and antioxidant ability.

Keywords: photosynthetic pigments and parameters; Triticum aestivum L.; antioxidant enzyme; soluble sugar

The available contents of molybdenum (Mo), an essential trace element for higher plants, animals, and microorganisms, are very low in sandy and well-drained soils (Rutkowska et al. 2017). Deficiency in available Mo in acidic soils in the wheat-growing areas of Southwestern Australia (Brennan 2006) and China (Sun et al. 2009) results in a decreased in plant biomass and seed yield. Physiologically, Mo is essential in the functioning of Mo enzymes in higher plants, such as nitrate reductase (NR), xanthine dehydrogenase and sulphite oxidase (Hille et al. 2011), which are involved in many physiological, metabolic processes, such as nitrogen assimilation in plants (Hänsch and Mendel 2009). Previous studies have shown that Mo deficiency decreases the chlorophyll concentration of winter wheat (Wu et al. 2017), while an appropriate application of Mo fertilizer could enhance the chlorophyll content and photosynthetic rate ($P_n$) of winter wheat leaves (Sun et al. 2014). Similarly, in Western Australia, the grain yield of wheat was significantly increased by the soil application of Mo (Brennan 2006). Zinc (Zn) is another essential micronutrient for higher plants, the deficiency of which leads to a decrease in both the yield and nutritional quality of cereal crop grains (Liu et al. 2018). Besides its function as an important component of many enzymes such as superoxide dismutase (SOD) and carbonic anhydrase, Zn also serves as a stabilizer and protector of proteins (Hacisalihoglu et al. 2003). Additionally, Zn plays
an essential role in the physiological processes of plants, such as in protein synthesis, carbohydrate metabolism, and the maintenance of cell membrane integrity (Broadley et al. 2007, Slosar et al. 2017). Zn was also found to increase the grain yields of winter wheat (Liu et al. 2017).

Wheat (Triticum aestivum L.) is one of the most important staple food crops in the world. Increasing wheat grain yield is necessary if the food demands of a constantly increasing global population are to be met. However, some of the wheat-growing areas in Western Australia and China are severely deficient in Mo, which restricts wheat production (Brennan 2006, Sun et al. 2009). Zn deficiency is one of the most important factors affecting wheat production globally, particularly in Turkey and China (Zhao et al. 2019). The seed yield of chickpea was increased by co-application of Zn, boron, and Mo, and there was a low significant Zn × Mo interaction on chickpea yield (Valenciano et al. 2010). The chlorophyll concentration of corn leaf was significantly increased by foliar co-application of Zn and Mo, but there was no interaction between Zn and Mo (Sun et al. 2015). A large area of winter wheat fields in Hubei province in China is deficient in both available Mo and Zn (Alloway 2009, Sun et al. 2009, Nie et al. 2015), but very few reports exist on the effects of the co-application of Mo and Zn on physiological parameters and wheat grain yield in soils deficient in Mo and Zn. Studying the effect of the combined application of Mo and Zn on physiological parameters of the wheat leaf, which was the great significance for wheat production. Therefore, the objectives of this study were to evaluate the effects of the co-application Mo and Zn on grain yield and physiological parameters of winter wheat. It was hypothesized that co-application Mo and Zn would enhance grain yield and photosynthetic efficiency of wheat leaves.

MATERIAL AND METHODS

Soils. A pot experiment was carried out at the Micro-element Research Center in Huazhong Agricultural University (30°28’26’’N, 114°20’15’’E), Wuhan, Hubei province, China from November 2014 to May 2015. The winter wheat seeds (cv. Zhengmai 7698) were sown in pots (22.0 cm H × 21.0 cm upper D, 15.5 cm bottom D) filled with 6 kg of purple sandy soil. The experimental soil was collected from Xinzhou District, Wuhan, Hubei province, China. The chemical properties of the soil were as follows: pH 8.02, organic C 2.20 g/kg, available N 13.26 mg/kg, Olsen-P 3.83 mg/kg, available K 31.41 mg/kg, Tamm reagent Mo 0.10 mg/kg, and DTPA (diethylenetriaminepentaacetic acid)-extractable Zn 0.78 mg/kg.

Experimental design. Four treatments were designed as follows: control prepared with neither Mo nor Zn (CK); Mo treatment with 0.15 mg Mo/kg soil (Mo); Zn treatment with 1 mg Zn/kg soil (Zn), and Mo – Zn treatment with 0.15 mg Mo/kg plus 1 mg Zn/kg of soil (Mo + Zn). Mo fertilizer was applied as ammonium molybdate and Zn fertilizer as zinc sulfate. Other fertilizers applied are shown in Table 1. All the fertilizers used were of analytical grade, and deionized water (18.25 MΩ·cm) was used during the experimental period. The seedlings were thinned to six uniform seedlings in each pot two weeks after emergence.

Determination of pigments, soluble protein, and sugar concentration. At the jointing stage, the concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid (CAR) in the leaves were determined as described by Wang (2006). At the tillering and jointing stages, the winter wheat leaves were collected to determine the concentrations of soluble protein and soluble sugar. The concentration of soluble protein was determined using the method of Bradford (1976), and the soluble sugar concentration in the leaf tissues was measured as described by Wang (2006).

Determination of photosynthetic parameters. The photosynthetic parameters, including the Pn', intercellular CO2 concentration (Ci), transpiration rate (T,) and stomatal limitation value (Ls) of the penultimate leaves, were measured using a portable photosynthesis system (LI-6400, Li-Cor, Inc., Lincoln, USA) from 9:30 to 10:30 AM on a sunny day at the jointing stage.

Table 1. Fertilizer amount of each pot

<table>
<thead>
<tr>
<th>Fertilizer</th>
<th>Amount</th>
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<tbody>
<tr>
<td>N</td>
<td>0.2 g/kg soil</td>
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<tr>
<td>P</td>
<td>0.065 g/kg soil</td>
</tr>
<tr>
<td>K</td>
<td>0.166 g/kg soil</td>
</tr>
<tr>
<td>MnCl2·4 H2O</td>
<td>1.81 mg/kg soil</td>
</tr>
<tr>
<td>CuSO4·5 H2O</td>
<td>0.08 mg/kg soil</td>
</tr>
<tr>
<td>H4BO4</td>
<td>2.86 mg/kg soil</td>
</tr>
</tbody>
</table>

Each pot was fertilized with N, P, and K with ammonium sulfate, potassium dihydrogen phosphate and potassium chloride used as the fertilizer sources, respectively.
Measurement of NR activity and antioxidant enzyme activity. At the jointing stage, the NR, peroxidase (POD) and catalase (CAT) activity were measured based on the method of Wang (2006). The SOD activity was determined using the method of Wu et al. (2014).

Statistical analysis. The results were expressed as the means (n = 4) and standard errors (SE). The data were statistically analyzed by SPSS 21.0 software (IBM Corp., New York, USA). The significance of the effects of the Mo and Zn treatments and their interactions on the reported traits was evaluated by two-way analysis of variance (ANOVA). Significant differences in the measured variables between treatments were assessed using Duncan's test at P < 0.05. The percentages of all the traits influenced by Mo or Zn were calculated according to the method of Hoshmand (2006).

RESULTS AND DISCUSSION

Grain yield and its components. The grain yield of winter wheat was significantly (P < 0.05) increased by 13.72% from the main effect of Mo, and which was significantly improved by 8.74% for the main effect of Zn. The grain yield was increased by the co-application of Mo and Zn (Figure 1). Recently, Mahilane and Singh (2018) demonstrated that the grain yield of summer black gram was significantly enhanced by the soil application of Mo combined with Zn. The spike number was significantly (P < 0.05) increased by the application of single Mo, and the co-application against the control. Additionally, the thousand kernel weight (TKW) was increased notably by Mo application but was not significantly increased by Zn application. The kernels per spike showed that there were no remarkable differences among the four treatments. Because co-application of Mo and Zn enhanced spike number and TKW significantly, thereby increasing the grain yield of winter wheat.

Photosynthetic pigment concentrations. The Chl a concentration of the winter wheat leaves was significantly (P < 0.05) increased by 12.53% for the main effect of Mo (Figure 2). Sun et al. (2014) found that the concentration of Chl a in the leaves was significantly enhanced by the sole Mo application. Mo deficiency changed the chloroplast structure of winter wheat leaves and inhibited the synthesis of chlorophyll, while Mo application significantly improved the chlorophyll content (Yu et al. 2005). The Chl a concentration was significantly (P < 0.05) improved by 9.04% for the main effect of Zn. Zn participates in chlorophyll formation by regulating the cytoplasmic concentrations of nutrients (Kosesakal and Unal 2009). Bharti et al. (2014) reported that the concentrations of Chl a in wheat genotypes were raised by soil Zn application. The contents of Chl a and chlorophyll a + b (Chl a + b) were increased significantly by the application of single
Mo or Zn and their combination, and their contents were significantly higher in the co-treatment than in sole Mo and Zn treatments (Figure 2). Compared with CK, the CAR concentration of winter wheat was significantly improved by the co-application of Mo + Zn (Figure 2). Photosynthetic pigments such as chlorophyll (Chl) and CAR play important roles in the photosynthetic processes of plants (Singh et al. 2017). Because co-application of Mo and Zn enhanced photosynthetic pigment concentrations, which may improve the \( P_n \) and grain yield.

**Photosynthetic parameters.** The \( P_n \) of the winter wheat leaves was significantly \((P < 0.05)\) improved by the co-application of Mo and Zn (Figure 3). Previous studies have shown that Mo application increases the \( P_n \) of plant leaves (Wu et al. 2014). Furthermore, Yu et al.
et al. (2005) also found that Mo was closely related to the photosynthesis of winter wheat leaves. Conversely, Zn plays an essential role in some biochemical and physiological processes, including photosynthesis (Broadley et al. 2007). Therefore, the highest $P_n$ was observed in the combined treatment. Despite no significant decline in single Mo or Zn treatment relative to CK, $C_i$ was significantly decreased in the co-application of Mo + Zn. Furthermore, an interaction ($F_{Mo \times Zn} = 6.76, P_{Mo \times Zn} < 0.05$) between Mo and Zn was observed, which indicated that Mo and Zn had a promoting effect on the decrease in $C_i$. The $T_r$ of the leaves was significantly ($P < 0.05$) increased by 10.92% for the main effect of Zn. Moreover, $T_r$ was higher in the co-treatment than in sole Mo or Zn application.

**Soluble sugar concentration.** At the tillering stage, the soluble sugar concentration of the leaves was significantly ($P < 0.05$) enhanced by Mo application, which was significantly increased by 22.44% for the main effect of Mo (Figure 4). At the jointing stage, the soluble sugar concentration was also significantly ($P < 0.05$) increased by Mo application, which was significantly enhanced by 9.12% for the main effect of Mo. The contents of soluble sugar in the leaves were increased significantly by the application of sole Mo and its combination with Zn. Qin et al. (2016) also found that soil Mo application increased the soluble sugar contents of *Brassica napus* leaves. Under Mo deficiency, low sugar content is mainly caused by weak photosynthesis and the non-utilization of carbohydrates for plant growth (Gupta 1997). Therefore, the increase in soluble sugar content by the co-application of Mo and Zn provided direct evidence for the improvement in the photosynthetic capacity of the winter wheat leaves.

**Soluble protein concentration.** At the tillering stage, the soluble protein concentration of the leaves was significantly ($P < 0.05$) enhanced by Mo application but was not significantly influenced by the Zn application (Figure 5). Specifically, the soluble protein content was increased by 10.20% for the main effect of Mo.

**NR activity.** The NR activity of the leaves was significantly ($P < 0.05$) increased by Mo application
but was not significantly influenced by the Zn application (Figure 6). The NR activity was increased by 16.44% for the main effect of Mo. As a constituent of NR, Mo is involved in electron-transfer reactions (Gupta 1997). The Mo co-factor directly or indirectly participates in nitrogen metabolism (Kaiser et al. 2005). Therefore, Mo plays a critical role in nitrate reduction, and its deficiency decreases NR activity, crop growth and grain yield (Mendel and Schwarz 2011). The Mo application alone and its combination with Zn significantly increased NR activity, which was the highest in the co-treatment, as observed with the grain yield. Therefore, the co-application of Mo and Zn enhanced NR activity, thereby promoting the growth of winter wheat and resulting in an increase in grain yield.

**Antioxidant enzyme activity.** The POD activity of the winter wheat leaves was significantly increased by 21.25% for the main effect of Zn, but it was not significantly influenced by Mo (Figure 7). Similarly, CAT activity was also significantly increased by 21.05% for the main effect of Zn. Wu et al. (2015) previously noted that the Zn application significantly improved the CAT activity of cotton leaves. Zhang et al. (2012) found that the CAT activity of Chinese cabbage was not significantly affected by Mo application under normal conditions, which was similar to our findings. Additionally, the SOD activity of the winter wheat leaves was significantly enhanced by 14.77% for the main effect of Zn; moreover, it was higher in the dual treatment than in the single Zn or Mo treatment.

**Correlation analysis of physiological parameters and grain yield components.** The grain yield was significantly positively correlated with Chl $a$, Chl $a + b$, $P_n$, L$_S$, NR, and CAR of the winter wheat leaves (Table 2). The increase in NR activity is associated with the enhancement of crop growth and yield (Unkles et al. 2004). The spike was significantly positively related to Chl $a$, Chl $a + b$, and $P_n$. Additionally, TKW showed a significant correlation with the Chl $a$, Chl $a + b$, NR, soluble sugar, and soluble protein of the leaves at the jointing stage. The results suggested that increased wheat grain yield was considerably related to the improved photosynthetic ability of the wheat leaves by the combined application.

Overall, the increase in wheat grain yield was remarkably related to the improved photosynthetic efficiency and antioxidant capacity of the leaves by the co-application of Mo and Zn. The contents of Chl $a$, Chl $a + b$, CAR, and $P_n$ were all enhanced by the combined application. Photosynthetic pigments promote photosynthesis in plants, enhancing the $P_n$ of the leaves, subsequently promoting the growth of winter wheat, and increasing the grain yield. The NR activity was significantly increased in the dual treatment due to the involvement of Mo in nitrogen metabolism, which subsequently promoted the growth of winter wheat. Another explanation for the grain yield increase may be that the enhanced soluble sugar content and antioxidase activities by the co-application of Mo and Zn might improve the antioxidant capacity of the leaves, thereby indirectly promoting material accumulation and resulting in an increase in grain yield.

![Figure 7. The activities of (a) peroxidase; (b) superoxide dismutase, and (c) catalase of the winter wheat leaves in four treatments. FW – fresh weight](https://doi.org/10.17221/508/2019-PSE)
Table 2. Correlation between physiological parameters and grain yield components of winter wheat

<table>
<thead>
<tr>
<th></th>
<th>Chl a</th>
<th>Chl b</th>
<th>Chl (a + b)</th>
<th>CAR</th>
<th>NR</th>
<th>P_n</th>
<th>C_i</th>
<th>T_r</th>
<th>L_s</th>
<th>Soluble protein</th>
<th>Soluble sugar</th>
<th>POD</th>
<th>SOD</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain yield</td>
<td>0.771** 0.508* 0.810**</td>
<td>0.573*</td>
<td>0.515*</td>
<td>0.674**</td>
<td>-0.738**</td>
<td>ns</td>
<td>0.747**</td>
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<td>ns</td>
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<td>ns</td>
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<tr>
<td>Spike</td>
<td>0.500* 0.566* 0.611*</td>
<td>ns</td>
<td>ns</td>
<td>0.596*</td>
<td>ns</td>
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<tr>
<td>Kernels per spike</td>
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<td>ns</td>
<td>ns</td>
<td>0.575*</td>
<td>0.551*</td>
<td>ns</td>
<td>-0.620*</td>
<td>ns</td>
<td>0.626**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>TKW</td>
<td>0.722**</td>
<td>ns</td>
<td>0.693**</td>
<td>ns</td>
<td>0.670**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<td>0.941**</td>
<td>0.826**</td>
<td>0.692**</td>
<td>0.609*</td>
<td>-0.667**</td>
<td>0.516*</td>
<td>0.644*</td>
<td>0.536*</td>
<td>0.579*</td>
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<td>Chl b</td>
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<td>0.651**</td>
<td>ns</td>
<td>0.698**</td>
<td>-0.555*</td>
<td>ns</td>
<td>0.548*</td>
<td>ns</td>
<td>ns</td>
<td>0.573**</td>
<td>ns</td>
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<tr>
<td>Chl (a + b)</td>
<td>1</td>
<td>0.700**</td>
<td>0.619*</td>
<td>0.747**</td>
<td>-0.743**</td>
<td>0.547*</td>
<td>0.722**</td>
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<td>0.599*</td>
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<tr>
<td>CAR</td>
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</table>

ns – no significance at the \(P < 0.05\); *\(P < 0.05\); **\(P < 0.01\); TKW – thousand kernel weight; Chl a – chlorophyll a; Chl b – chlorophyll b; Chl \(a + b\) – chlorophyll \(a + b\); CAR – carotenoid; NR – nitrate reductase; \(P_n\) – photosynthetic rate; \(C_i\) – intercellular \(CO_2\) concentration; \(T_r\) – transpiration rate; \(L_s\) – stomatal limitation value; POD – peroxidase; SOD – superoxide dismutase; CAT – catalase

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