



# Effect of potassium foliage application post-anthesis on grain filling of wheat under drought stress



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## ABSTRACT

Potassium foliage application (KFA) is widely used for wheat production in China. The objective of this study was to investigate the effect of KFA on grain filling of wheat under different soil moisture conditions and the underlying mechanisms. The results indicate that KFA increased the zeatin (Z), Z riboside (ZR), and abscisic acid (ABA) contents and the ethylene (ETH) evolution rate in inferior grains during the early and middle grain filling stages, which promoted sink strength. However, KFA decreased the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), as well as the soil-plant analyses development (SPDA) value and increased the malondialdehyde (MDA) content in the flag leaves. The effect of KFA on grain filling also exhibited a notable genotypic difference. In the heavy-panicle cultivar, KFA had no significant effect on grain filling under the well-watered (WW) treatment, but it decreased the rate and active period of the grain filling of inferior grains and significantly decreased the grain weight following soil-dried (SD) treatment. In the light-panicle cultivar, KFA significantly promoted the grain filling rates of inferior grains and increased the grain weight under the WW treatment. However, KFA significantly decreased the active grain filling period but increased the grain filling rate and, therefore, had no significant effect on the grain weight under the SD treatment.

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## 1. Introduction

Drought is one of the main abiotic stresses limiting wheat (*Triticum aestivum* L.) production in northern China (Jiang and Zhang, 2004; Li et al., 2000). Grain filling, an important growth stage of cereal, determines the grain weight (Yang and Zhang, 2006). In cereals such as wheat, the booting and grain filling stages are most sensitive to the water supply (Yu, 2003). Drought stress during the grain filling stage usually seriously reduces the grain weight and yield (Kobata et al., 1992; Zhang et al., 1998). Thus, reducing the inhibitory effect of drought stress on wheat grain filling is important for improving wheat production in northern China.

Potassium (K) is an important nutrient element that significantly affects the grain weight and yield of cereals (Brennan and Bolland, 2009; Kunzová and Hejman, 2010; Wani et al., 2014). Previous studies have indicated that K fertilizer promotes the grain filling of wheat in dryland (Chen et al., 2006; Hu et al., 2014). Wang et al. (2003) suggested that K significantly promoted the accumulation and transfer of sucrose in stems and increased the starch

content in wheat grain. Zou et al. (2007) suggested that the appropriate application of K fertilizer increased the net photosynthetic rate of flag leaves during the grain filling stage. In addition, K significantly affected the drought resistance of wheat (Raza et al., 2014). Wei et al. (2013) suggested that external K ameliorated drought stress in wheat while Damon et al. (2011) found that the endogenous K content of a drought-resistant variety was significantly higher than that of a drought-sensitive variety of wheat was. These results suggest that K significantly affected the grain weight and drought resistance of wheat. However, the effects of K on grain filling of wheat under drought stress conditions are unclear.

In northern China, N and P fertilizers are the main types used for wheat production. K fertilizer is rarely used in wheat production because the K level in the soil is traditionally considered sufficient for wheat production in this region (Guo et al., 2010). However, increase wheat grain yields are accompanied by increased K absorption from the soil by the wheat plants to maintain growth, which would lead to a K deficiency for subsequent wheat production if the levels in the soil are not supplemented (Chen et al., 2006). Because the price of K fertilizers has increased over the years, farmers have not been encouraged to increasing their use in wheat production. In addition, wheat roots exhibit a gradual senescence in the later growth period, which weakens their ability to absorb nutrients (Zhao and Si, 2015). For these reasons, K foliage applica-

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tion (KFA) is mainly used for wheat production in northern China because of its low cost and ease of use compared to that of soil fertilizer. However, the previously reported effects of KFA on wheat grain weight and yield differed, and some studies suggest that KFA significantly increased grain weight (Lv et al., 2011; Sun et al., 2006) while others found no significant effect on wheat yield or even an increased yield (Cao et al., 2015; Yan and Pei, 2014). Therefore, the effect of KFA on wheat grain weight is complex, and the relationship of its observed differential effects with soil moisture is unclear.

Plant hormones play an important role in regulating the grain filling of cereals including cytokinin (CTK), which is important for the endosperm cell division of wheat grains (Morris et al., 1993). The abscisic acid (ABA) levels of superior wheat grains were significantly higher than those of inferior wheat grains, while the ethylene (ETH) levels showed an opposite trend (Yang et al., 2006). Indole-3-acetic acid (IAA) levels in superior rice grains were higher than those in inferior rice grains (*Oryza sativa* L.) (Xu et al., 2007). Furthermore, the maximal grain filling rate was negatively correlated with gibberellic acids (GAs) in rice grains (Yang et al., 2001a).

Although it is well known that hormones affect grain filling in wheat, the relationship between hormonal changes and the KFA-induced grain filling is unclear. The objectives of this study were to investigate the effect of K foliage application on the grain filling of wheat under different soil moisture conditions and determine how the changes in endogenous hormones in the developing wheat grains under KFA are related to the grain filling process under drought stress conditions. Drought stress was applied during the grain filling stage of wheat and potassium chloride (KCl), the main type of KFA used for wheat production in northern China, was used as a foliage application at the anthesis stage. Furthermore, changes in IAA, ABA, zeatin (Z) plus zeatin riboside (ZR), GAs, and ETH in the wheat grain were measured during the grain filling process.

## 2. Materials and methods

### 2.1. Experimental design and treatments

The experiment was conducted in mobile waterproof sheds using 3 m × 4 m plots, which were divided by a concrete wall. The soil is Eum-Orthosols (Chinese soil taxonomy), and the readily available N, P and K concentrations were 51.23 mg kg<sup>-1</sup>, 20.01 mg kg<sup>-1</sup>, and 105.37 mg kg<sup>-1</sup>, respectively. The organic matter concentration of the 0–20 cm topsoil was 11.96 g kg<sup>-1</sup>, and the pH was 7.02. Two wheat cultivars, Zhoumai 22 and Xinong 538, were grown. The seeds were sown on October 20 and October 18 during the 2013–2014 and 2014–2015 growth years, respectively. The seedling rate was 150 kg ha<sup>-1</sup> with a row spacing of 0.20 m. The fertilizer was applied at basal levels of 150 kg ha<sup>-1</sup> each of urea and diammonium orthophosphate. The experiment was based on a 2 × 2 × 2 factorial design (two soil moisture levels, two KFA application rates, and two cultivars), with eight treatment combinations. Each treatment was applied to three plots as replicates in a complete randomized block design.

From anthesis to maturity, two levels of soil moisture were maintained, and the moisture treatments were based on those of our previous study (Liu et al., 2016). The well-watered (WW) and soil-dried (SD) treatments maintained the soil water potential of the 15–20 cm soil layer at  $-20 \pm 5$  and  $-60 \pm 5$  kilopascals (kPa), respectively. Five tension meters (SWP-100, Soil Science Research Institute, China Academy of Sciences, Nanjing, China) were installed in each plot and readings were recorded at 11:00–12:00 each day. When the reading dropped to a certain value, appropriate amounts of water were added. Before anthesis, the soil water potential was maintained at  $-20 \pm 5$  kPa.

Under each soil moisture condition at anthesis, 30 mmol L<sup>-1</sup> KCl was sprayed on the leaves using a sprayer (T1) daily for 4 days at a rate of 750 kg hm<sup>-2</sup> at each application. The same volume of deionized water was applied to the control plants (CK).

### 2.2. Measurements

Four hundred spikes that flowered on the same day in each plot were tagged and sampled from anthesis to maturity on a 4-day interval for each plot while 20 spikes were sampled at each sampling stage. Grains on a spike were divided into superior and inferior grains according to Jiang et al. (2003). One half of the sampled grains was used to measure the hormones, while the other half was dried at 70 °C to a constant weight, which was recorded. On the same sampling day, 20 flag leaves were sampled from each plot, stored at  $-40$  °C, and were used to measure the superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities, as well as malondialdehyde (MDA) content. At 8-day intervals from anthesis to maturity, the soil-plant analyses development (SPAD) value of the flag leaves was measured with an SPAD-502 chlorophyll meter model (Minolta Camera Co., Osaka, Japan).

#### 2.2.1. Grain filling process

The grain filling process was simulated using Richards's (1959) growth equation and according to Yang et al. (2006):

$$W = \frac{A}{(1 + Be^{-kt})^{\frac{1}{N}}} \quad (1)$$

The grain filling rate (G) was calculated using a derivation of Eqn. 1:

$$G = \frac{AkBe^{-kt}}{(1 + Be^{-kt})^{\frac{N+1}{N}}} \quad (2)$$

where,  $W$  is the grain weight (mg);  $A$  is the final grain weight (mg);  $t$  is the time after anthesis (d); and  $B$ ,  $k$ , and  $N$  are coefficients determined using regression.

The active grain filling period was defined as the period when  $W$  was between 5% ( $t_1$ ) and 95% ( $t_2$ ) of  $A$ . Therefore, the average grain filling rate during this period was calculated from  $t_1$  to  $t_2$  (Yang et al., 2006).

#### 2.2.2. Hormones

Approximately 0.5 g of the free weight (FW) sample was collected, and the endogenous Z, ZR, GAs ( $GA_1 + GA_4$ ), IAA, and ABA were extracted according to a previously reported method (Yang et al., 2001a). The samples were homogenized with 5 mL 80% (v/v) methanol containing 1 mmol L<sup>-1</sup> butylated hydroxytoluene (BHT). The extracting solution was passed through Chromosep C18 columns (C18 Sep-Park Cartridge, Waters Corp., Milford, MA, USA), the fractions were vacuum-dried at 40 °C, and dissolved in 1 mL phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween 20 and 0.1% (w/v) gelatin (pH 7.5) for the enzyme-linked immunosorbent assay (ELISA). The ELISA kits were manufactured by the Phytohormones Research Institute, China Agricultural University. The quantification of Z + ZR, GAs ( $GA_1 + GA_4$ ), IAA, and ABA was performed using an ELISA as previously described (Yang et al., 2001a). The recovery rates for IAA, Z + ZR, ABA, and GAs were  $85.4 \pm 4.7\%$ ,  $93.1 \pm 6.2\%$ ,  $89.5 \pm 3.2\%$ , and  $78.2 \pm 5.4\%$ , respectively.

The ETH generated by the grains was determined according to the methods of Beltrano et al. (1994) and Yang et al. (2008). The ETH was assayed using a gas chromatography (GC) system (Trace GC Ultra™, Thermo Fisher Scientific, USA) according to our previous study (Liu et al., 2016).

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