

Full Length Article

Plant Growth Regulators Application Improves Spring Maize Yield by Improving Net Photosynthesis and Grain Filling Rate

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Abstract

Application of plant growth regulators can increase maize grain yield by improving its growth and development. Therefore, this study was conducted to investigate the effects of different plant growth regulators application on photosynthetic capacity, grain filling characteristics and grain yield of maize. Maize plants at six leaf stage were sprayed with two growth regulators *viz.*, duntianbao (DTB) and compound of DCPTA (2,3,4-dichlorophenoxy triethylamine) and Ethephon (2-chloroethylphosphonic acid) (KP) while distilled water spray was used as control (CK). The results disclosed that the KP and DTB treatments significantly improved the leaf area index (LAI) values in the late-growth stage and increased photosynthesis of maize. Additionally, the KP and DTB treatments increased the grain filling rate, prolonged the grain filling period and increased the grain weight, leading to an increase in grain yield of spring maize by 19.50 and 12.13%, respectively. Correlation analysis indicated that the grain yield was positively correlated with the PEPCase and RuBPCase activities, SPAD value, Pn, grain filling rate and increase in grain weight at the early-filling, middle-filling stage and late-filling stage. In conclusion, the spray of plant growth regulator KP *i.e.*, a compound of DCPTA and Ethephon accelerated the maize yield due to substantial expansion in net photosynthesis and grain filling rates. © 2019 Friends Science Publishers

Keywords: Chlorophyll contents; Grain filling rate; Maize; Photosynthesis; Yield

Introduction

Maize (*Zea mays* L.) is one of the most important food crops worldwide and has a huge yield potential. Maize is also an important ingredient of animal feed, industrial products and bio-energy, which have a significant effect on agricultural production (Shiferaw *et al.*, 2011). With population increases and climatic variability, global demand for maize has increased. In order to meet human demands, it is necessary to increase the grain yield per unit area (Shiferaw *et al.*, 2011; Ittersum and Martin, 2016; Mustafa *et al.*, 2019). The global demand for crop production is expected to double by 2050 to meet population growth, diet shifts and biofuel consumption (Ray *et al.*, 2013). Therefore, improving the grain yield of spring maize per unit area has great significance for global food security.

Crop yield is the accumulation of photoassimilates in the source and sink during grain development (Smith *et al.*, 2018). Plants accumulate photoassimilates to its kernels which are established in the early grain filling phase (Gambin *et al.*, 2006). Therefore, maize yield increases mainly due to improvements in leaf photosynthetic performance (Li *et al.*, 2015) and grain filling rates (Ordonez *et al.*, 2018).

Previous studies have shown that plant growth regulators (PGRs) could improve maize yield (Zhang *et al.*, 2014; Xu *et al.*, 2017). PGRs alter plant growth and development by regulating the biosynthesis or degradation of plant hormones and ultimately improve crop yield (Jiang and Asami, 2018; Stutts *et al.*, 2018). Exogenous spraying of PGRs could accelerate maize growth, increase the chlorophyll contents, delay leaf senescence and increase dry matter accumulation (Xie *et al.*, 2017), which provide sufficient photosynthates for yield formation. PGRs improved the nutrition utilization efficiency at the crucial stage of grain setting and the distribution of assimilation in grains improved significantly, leading to an increase in maize yield (Hutsch and Schubert, 2018).

The 2,3,4-dichlorophenoxy triethylamine (DCPTA) is a tertiary amine bioregulator that enhances leaf photosynthesis and stimulates plant growth and development. It resulted in an increase in leaf chlorophyll and carotenoids contents and enhanced C_4 -enzyme

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activities; thereby increased the photosynthetic intensity in maize (Xie *et al.*, 2017). The application of DCPTA increased sink-regulated photosynthate production in mature leaves and improved vegetative growth (Keithly and Yokoyama, 1990; Keithly *et al.*, 1990). Additionally, DCPTA increased the grain weight and grain number in maize, thus attaining higher yield (Wang *et al.*, 2016).

Ethephon significantly decreased the internode lengths, which led to a decrease in plant and ear heights. Additionally, Ethephon could increase the N, cellulose and hemicellulose contents of the basal internode (Ye *et al.*, 2016). The results indicated that Ethephon could increase stalk strength by improving the morphological and chemical characteristics of the basal internode (Ye *et al.*, 2016). Khosravi and Anderson (1991) showed that the highest maize lodging rate with Ethephon decreased by 85 and 93% in two years; however, the highest rate of reduction in yield was 6 and 2%.

Previous studies have shown that DCPTA could improve photosynthetic capacity, promote plant growth and increase the grain yield (Xie *et al.*, 2017, 2019). Ethephon could improve maize lodging resistance, while it may reduce the grain yield (Norberg *et al.*, 1988; Khosravi and Anderson, 1991). Concerning the advantages and disadvantages of these two plant growth regulators, there were few researches about the effect of the compounds derived from these PGPs on maize. Therefore, in this twoyear field study, compound of DCPTA and Ethephon (KP) along with commercially recommended PGR was applied to evaluate its effect on net photosynthesis, grain filling rates and grain yield of spring maize.

Materials and Methods

Experimental Details

This experiment was carried out at Xiangyang Experimental Farm, Northeast Agricultural University in 2016 and 2017. The experimental soil was calcareous in nature with plough layer depth of 20 cm. The soil pH was 6.85 and the contents of total nitrogen, available phosphorus, available potassium, alkali-hydrolysable nitrogen, and organic matter of soil were 1.70 g kg⁻¹, 65.34 mg kg⁻¹, 179.35 mg kg⁻¹, 118.21 mg kg⁻¹, and 25.25 g kg⁻¹, respectively. The maize variety Dongnong 253 was sown on April 25 in 2016 and 2017 at 70,000 ha⁻¹ density in 65 cm spaced rows with plant to plant distance of 22 cm. Two PGRs, the compound of DCPTA and Ethephon (KP, 10 mL L^{-1} ; the concentration of KP was chosen after a preliminary experiment) and DTB (900 mL ha⁻¹; the dose of DTB was recommended by the manufacturer (Fan et al., 2018), were evenly sprayed on the maize leaves at the sixleaf stage, and water as control (CK). This experiment was laid out as a randomized complete block design with three replicates and the net plot size was 8 m \times 6.5 m. All plots were fertilized at rates of 200, 180, and 50 kg ha⁻¹ NPK,

 Table 1: Daily precipitation and mean temperatures during the growing seasons in 2016 and 2017

| 2016 | 2017 | 2016 | | |
|-----------|--------------------------------------|---|--|--|
| 2016 2017 | | 2016 | 2017 | |
| 16.0 | 16.7 | 106.8 | 37.0 | |
| 20.1 | 19.9 | 206.1 | 215.2 | |
| 24.3 | 24.7 | 44.2 | 50.2 | |
| 23.2 | 22.1 | 31.7 | 92.2 | |
| 17.1 | 15.0 | 70.3 | 51.8 | |
| 20.1 | 19.7 | 459.1 | 446.4 | |
| | 20.1 24.3 23.2 17.1 20.1 | 20.1 19.9 24.3 24.7 23.2 22.1 17.1 15.0 20.1 19.7 | 20.1 19.9 206.1 24.3 24.7 44.2 23.2 22.1 31.7 17.1 15.0 70.3 | |

respectively. All of phosphorous, potash, and half of nitrogen were applied at sowing in the form of diammonium phosphate (DAP; 46% P_2O_5 , 18% N), sulfate of potash (SOP; 50% K₂O) and urea (46% N). The other half of nitrogen was applied at the jointing stage. No irrigation water was applied during the maize growing season. All other agronomic and plant protection practices were kept normal and uniform for all the treatments. The weather data during the maize growth period is given in Table 1.

DTB was a combination of plant growth regulators (Hetianxingnong Co., Ltd., China) containing 35-40% (2-chloroethylphosphonic Ethephon acid), 3-6% polyamino acid salts (2-chloroethyltrimethyl chloride), 3-5% trace elements, 0.6-1.2% organic acids, and other solvents (Chinese patent: ZL200610075673.9). KP was a compound of DCPTA (2-(3,4-dichlorophenoxy) triethylamine) and Ethephon (Chinese patent: ZL201310051785.0), provided by Northeast Agricultural University. This mixture contained 53-73 g L⁻¹ of PGRs (mainly DCPTA and Ethephon), 5-25 g L⁻¹ of genetic activator, 15-20 g L⁻¹ of a mixture of active agent and spreading agent, and 0.35–0.45 g L⁻¹ of a preservative; the pH value was 3–4.

Sampling and Measurements

LAI: In each plot, 5 plants were randomly selected at the jointing stage (5th July), tasseling stage (25th July), early grain filling stage (5th August), milking stage (25th August) and maturing stage (25th September) in 2016 and 2017. The LAI was calculated according to the formula LAI = leaf area / land area. The leaf area per plant was calculated by the length-width coefficient method: leaf area = length × width × 0.75 (Ren *et al.*, 2016).

SPAD value and Pn: In each plot, 3 plants were randomly selected and tagged at each growth stage in 2016 and 2017. The top unfolded leaf was sampled before the silking stage, and the ear leaf was sampled after the silking stage. The SPAD value was determined at the top, middle and base of a leaf with a CCM-200 Plus chlorophyll analyser (Opti-Sciences, Inc., Hudson, N.H., U.S.A.) at 9:00–10:00 and the average value was calculated. At the same time, the leaf Pn was measured with a CI-340 hand-held photosynthetic rate analyser.

RuBPCase and PEPCase activities: Three plants were randomly selected at each growth stage in each plot. The top unfolded leaf was sampled before the silking stage, and the ear leaf was sampled after the silking stage. Fresh leaf segments (0.5 g) were homogenized in a pre-cooled pestle and mortar with acid-washed quartz sand and 2.5 mL of extraction medium [0.1 *M* Tricine-HCl (pH 8.4), containing 10 m*M* MgCl₂, 1 m*M* EDTA, 7 m*M* b-mercaptoethanol, 5% glycerol (v/v) and 1% polyvinylpyrrolidone (PVP)] followed by centrifuging at 10000 g for 10 min below 4°C. The clear supernatant was used for the enzyme assay. The activity of RuBPCase was determined by the method of Lilley and Walker (1974). The activity of PEPCase was determined by the method of Arnozis *et al.* (1988).

Grain filling dynamics: Selected three ears that tasseled on the same day from each plot and the ears were sampled at 5-day intervals from silking to maturity. Hundred grains in the middle part of the ear were dried at 70°C to a constant weight, and the dry weight was measured. This study focused on the grain filling characteristics in 2017. The responses of the grain filling processes to the different plant growth regulators was analysed through W= K/(1+ae^b) (the logistic growth equation). The calculation of grain

^{bt}) (the logistic growth equation). The calculation of grain filling characteristic parameters refers to the method of Wang *et al.* (2014) and Gao *et al.* (2017).

Grain yield: Two central rows of each plot were hand harvested on September 25, 2016 and 2017 to record yield data following Mehboob *et al.* (2018). The number of ears, rows per ear, grain number per row and 100-grain weight were measured, and the theoretical yield was calculated (14% water content) according to Peng *et al.* (2018).

Theoretical yield = ears number per ha \times rows per ear \times grain number per row \times 100-grain weight / 100/1000 \times (1 – grain moisture content)/0.86.

Statistical Analysis

The treatments means were compared by analysis of variance (ANOVA) at the 5% significance level by the least significant difference (LSD) test. The treatment effects on the grain yield, LAI, SPAD value, and RuBPCase and PEPCase activities in the two years were analysed with one-way ANOVA using SPSS 19.0. The grain filling dynamics in 2017 were fixed by CurveExpert 1.3.

Results

Leaf Area Index (LAI)

The LAI initially increased and then decreased with the growth of maize, and the maximum value was recorded at the tasseling stage in both 2016 and 2017 (Fig. 1). At the jointing stage, there was no significant difference in the LAI between the PGR treatments and the CK in the two years. The PGRs decreased the LAI at the tasseling stage and early grain filling stage but increased the LAI at the

milking and maturing stages. Additionally, the LAI was higher with the KP treatment than with the DTB treatment at the milking stage and maturing stage (Fig. 1).

Chlorophyll Contents and Photosynthesis (Pn)

The SPAD value and Pn observed the same trend in 2016 and 2017 and their values initially increased and then decreased with the maize growth, and the maximum values were recorded at the early grain filling stage (Fig. 2). The application of PGRs improved the SPAD value and Pn in both years of study (Fig. 2). KP application enhanced the average SPAD value and Pn by 21.10–30.56% and 21.07– 27.37%, respectively. Likewise, DTB application increased the SPAD value and Pn by an average of 15.49–25.14% and 17.62–19.91% in two years compared with control (Fig. 2). Additionally, the SPAD value and Pn in the KP treatment were higher than those in the DTB treatment (Fig. 2).

RuBPCase and PEPCase Activities

The RuBPCase and PEPCase activities initially increased and then reduced with the growth of maize and the maximum values were recorded at the milking stage in 2016 and 2017 (Fig. 3). PGRs significantly increased the RuBPCase and PEPCase activities in each maize growth stage (Fig. 3). KP application enhanced the average RuBPCase and PEPCase activities by 43.10–65.98% and 49.58–66.21%, respectively. Likewise, DTB application increased the RuBPCase and PEPCase activities by an average of 28.04–39.87% and 26.42–36.26% in two years compared with control (Fig. 3). The photosynthetic enzymes activity in the KP treatment was observably higher than that in the DTB treatment (Fig. 3).

Grain Filling Characteristics

The determinant coefficients of the grain filling process equation fitting for the different treatments were all above 0.99 (Table 2), which indicates that the logistic equation is consistent with the grain filling process in the different PGR treatments in 2017. PGRs significantly increased the grain weight at the time when the maximum grain filling rate was reached (W_{max}), maximum grain filling rate (V_{max}), mean grain filling rate (V_m), active grain filling period (P) and effective grain filling time (t) (Table 2). KP application increased the W_{max}, V_{max}, V_m, P and t by 24.96, 16.67, 19.23, 7.17 and 5.21%, respectively. Likewise, DTB application increased the W_{max}, V_{max}, V_m, P and t by 16.06, 9.26, 11.54, 5.76 and 3.41%, respectively. Additionally, KP application performed better than DTB application (Table 2). PGRs had no effect on the time when the maximum grain filling rate was reached (T_{max}) (Table 2). Therefore, the PGRs prolonged the grain filling duration, accelerated the grain filling rate and created favourable conditions for a

| Table 2: Effects of PGI | ls on grain filling | characteristic | parameters in maize |
|-------------------------|---------------------|----------------|---------------------|
| | | | |

| Treatment | Determinant coefficients | Equation | on paran | neters | Grain filling parameters | | | | | | | | |
|-----------|--------------------------|----------|----------|--------|--------------------------|--|---|---|-------------|--|--|--|--|
| | | Κ | а | b | $T_{max}(d)$ | W _{max} (g 100-kernel ⁻¹) | V_{max} (g 100-kernel ⁻¹ d ⁻¹) | $V_{\rm m}$ (g 100-kernel ⁻¹ d ⁻¹) | P (d) t (d) | | | | |
| CK | 0.9933 | 26.51 | 36.44 | 0.16 | 22.15 | 13.26 | 1.08 | 0.52 | 36.96 50.45 | | | | |
| DTB | 0.9929 | 30.77 | 30.36 | 0.15 | 22.24 | 15.39 | 1.18 | 0.58 | 39.09 52.17 | | | | |
| KP | 0.9969 | 33.14 | 31.39 | 0.15 | 22.75 | 16.57 | 1.26 | 0.62 | 39.61 53.08 | | | | |

K: final grain weight; a: initial parameter; b: growth rate parameter; T_{max}: time when the maximum grain filling rate was reached; W_{max}: grain weight at the time when the maximum grain filling rate was reached; V_{max}: maximum grain filling rate; V_m: mean grain filling rate; P: active grain filling period; and t: effective grain filling time

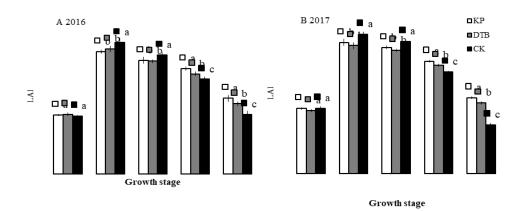


Fig. 1: Effects of PGRs (KP and DTB) on the LAI of maize during 2016 (**A**) and 2017 (**B**). Bars indicate standard errors of the means of 3 replications. The same letters on bars indicate that the differences are not significant at P < 0.05 as determined by a least significant difference (LSD) test

high yield.

According to the fitting equation, the whole grain filling stage was partitioned into three parts: the early-filling stage, middle-filling stage and late-filling stage. PGRs increased the grain filling duration and mean grain filling rate at the middle-filling and late-filling stages, thus increasing the grain weight increment in the middle-filling and late-filling stages (Table 3). The PGRs increased the mean grain filling rate and the increase in grain weight at the early-filling stage but had no effect on the grain filling duration (Table 3). The grain filling duration, mean grain filling rate and the increase in grain weight at the early, middle and late growth stages all performed in the order KP > DTB > CK in the different treatments except for the duration of the early-filling stage (Table 3).

Grain Yield

The grain yield increased significantly after applying the PGRs (Table 4). The KP and DTB treatments increased the yield by 16.43–19.50% and 9.37–12.13% in the two years, respectively (Table 4). Regarding the yield components, the PGRs had no effect on the ears per ha and rows per ear, but the grain number per row and 100-grain weight increased significantly. The KP and DTB treatments increased the grain number per row by 8.97–9.32% and 5.54–6.16% and increased the 100-grain weight by 6.30–8.31% and 3.93–4.36% in the two years, respectively (Table 4).

Correlation Analysis of the Photosynthetic Indicators, Grain Filling Parameters and Yield

The yield was positive correlated with the SPAD value, Pn, PEPCase and RuBPCase activities, mean grain filling rate at the early stage (V₁) and mean grain filling rate at the middle stage (V₂) and the correlations reached a significance level of 0.01 (Table 5). In addition, the yield was significantly positive correlated with the W_{max}, V_{max}, V_m, t, increased grain weight at the early stage (W₁), increased grain weight at the middle stage (W₂), mean grain filling rate at the late stage (V₃) and increased grain weight at the late stage (W₁), correlations reached a significance level of 0.05) (Table 5). There was a negative correlation between the grain filling duration at the early stage (T₁) and the yield, but not remarkable (Table 5).

Discussion

Application of PGRs at six expanded leaves stage increased the maize yield which was mainly due to higher grain number per row and higher 100-grain weight after the application of PGRs (Table 4). Additionally, PGRs could improve the LAI and optimizing the canopy structure of plant populations, thus improve the photosynthetic characteristics and prolong the functional period of maize leaves, which play a crucial role in improving the grain filling process and increasing the maize yield (Bian *et al.*, 2011; Ren *et al.*, 2016; Xu *et al.*, 2017).

| Table 3: Effects of the PGRs on the char | racteristic parameters of th | he three grain filling stages |
|---|------------------------------|-------------------------------|
| Tuble 5. Effects of the Forts on the enta | acteristic parameters of a | ie unee gram ming stages |

| Treatmen | | | | | | | | | | | | | | |
|----------|----------|--|------------------------|----------|--|------------------------|----------|--|--|--|--|--|--|--|
| | | Early grain filli | ng stage | | Middle grain fillir | ng stage | | Late grain filling stage | | | | | | |
| | $T_1(d)$ | $T_1(d) = V_1(g \ 100 - W_1(g \ 100 - W_1(g$ | | $T_2(d)$ | V ₂ (g 100- | W ₂ (g 100- | $T_3(d)$ | V ₃ (g 100- | W ₃ (g 100-kernel ⁻¹) | | | | | |
| | | kernel ⁻¹ d ⁻¹) | kernel ⁻¹) | | kernel ⁻¹ d ⁻¹) | kernel ⁻¹) | | kernel ⁻¹ d ⁻¹) | | | | | | |
| CK | 14.04 | 0.35 | 4.89 | 16.22 | 0.94 | 15.31 | 20.19 | 0.26 | 5.34 | | | | | |
| DTB | 13.66 | 0.40 | 5.52 | 17.16 | 1.04 | 17.77 | 21.36 | 0.29 | 6.19 | | | | | |
| KP | 14.06 | 0.43 | 5.98 | 17.39 | 1.10 | 19.13 | 21.64 | 0.31 | 6.67 | | | | | |

 T_1 : grain filling duration at the early stage; V_1 : mean grain filling rate at the early stage; W_1 : increased grain weight at the early stage; T_2 : grain filling duration at the middle stage; V_2 : mean grain filling rate at the middle stage; W_2 : increased grain weight at the middle stage; T_3 : grain filling duration at the late stage; V_3 : mean grain filling rate at the late stage; and W_3 : increased grain weight at the late stage

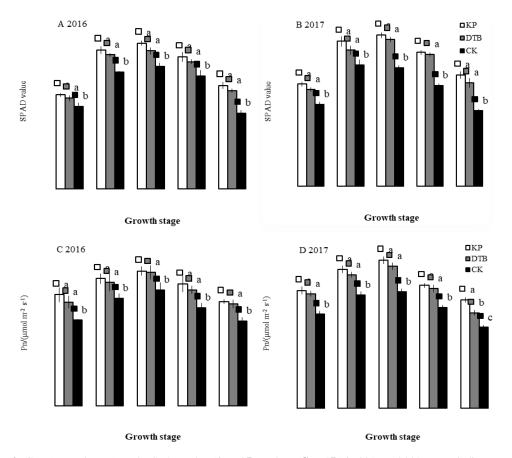


Fig. 2: Effects of PGRs (KP and DTB) on the SPAD value (**A** and **B**) and Pn (**C** and **D**) in 2016 and 2017. Bars indicate standard errors of the means of 3 replications. The same letters on bars indicate that the differences are not significant at P < 0.05 as determined by a least significant difference (LSD) test

Net photosynthesis is a key factor to determine the grain yield in maize (Mehta and Sarkar, 1992). In this study, the PGRs significantly increased Pn in maize leaves, thus effectively improve the photosynthetic capacity (Fig. 2). Chlorophyll receives and converts energy, and the chlorophyll content level affects the photosynthetic capacity of maize leaves to a certain extent (Wang *et al.*, 2017). Additionally, PEPCase and RuBPCase are key enzymes in the process of carbon assimilation in maize, and their activities are closely related to photosynthetic activity (Boyd *et al.*, 2015). Therefore, the increase in chlorophyll content, PEPCase and RuBPCase activities

after PGRs application might be the reason of the improvement in Pn by applying PGRs (Fig. 2 and 3). The SPAD value, Pn, and PEPCase and RuBPCase activities were observably positively correlated with the yield (Table 5). In addition, LAI is a dynamic index that indicates plant canopy structure and primarily associated with the population photosynthetic capacity and leaf senescence (Chen *et al.*, 2014; Gou *et al.*, 2014).

In this study, the PGRs significantly reduced the LAI values in the early-growth stage, but the LAI values increased significantly in the late-growth stage, and the duration of the high value was long (Fig. 1). Therefore, the

| Table 4: Effects of the PGRs on the | vield and vi | eld components of i | maize |
|--|--------------|----------------------|-------|
| Table 4. Effects of the Forts of the | yiciu and yn | ciu componentis or i | maize |

| Year | Treatments | Ears per ha | Rows per ear | Grain number per row | 100-grain weight (g) | Grain yield (kg ha ⁻¹) |
|------|------------|-------------|--------------|----------------------|----------------------|------------------------------------|
| 2016 | СК | 6714.73a | 14.65a | 36.27b | 31.64b | 9845.75c |
| | DTB | 6698.36a | 14.73a | 38.28ab | 33.02ab | 10768.35b |
| | KP | 6724.28a | 14.84a | 39.65a | 34.27a | 11463.64a |
| 2017 | СК | 6689.53a | 14.62a | 38.46b | 32.53b | 10025.71c |
| | DTB | 6708.34a | 14.69a | 40.83ab | 33.81ab | 11242.36b |
| | KP | 6753.92a | 14.81a | 41.91a | 34.58a | 11980.73a |

Values followed by the same letter within a column were not significantly different at P < 0.05 in each year as determined by a least significant difference (LSD) test

Table 5: Correlation analysis of the photosynthetic indicators, grain filling parameters and yield

| | Factors | | | | | | | | | | | | | | | | | | |
|--|--|--------|--------|--------|------------------|------------------|------------------|-------|------|-------|-------------|----------|-------|-----------------------|--------|-------|-----------------------|----------------|-----------------------|
| | PEPc | RuBPc | SPAD | Pn | T _{max} | W _{max} | V _{max} | Vm | Р | t | T_1 | V_1 | W_1 | T ₂ | V_2 | W_2 | T ₃ | V ₃ | W ₃ |
| Yield | 0.82** | 0.87** | 0.89** | 0.85** | 0.87 | 1.00* | 0.99* | 1.00* | 0.98 | 0.99* | -0.10 | 1.00** | 0.99* | 0.98 | 1.00** | 1.00* | 0.98 | 1.00* | 1.00* |
| PEPCase: phosphoenolpyruvate carboxylase activity; RuBPCase: Rubisco activity; SPAD: represent the chlorophyll content; Pn: photosynthetic rate; T _{max} : time when the | | | | | | | | | | | | when the | | | | | | | |
| maximum grain filling rate was reached; Wmax: grain weight at the time when the maximum grain filling rate was reached; Vmax: maximum grain filling rate; Vmax; Mmax; Mm | | | | | | | | | | | ain filling | | | | | | | | |
| rate; P: active grain filling period; t: effective grain filling time; T1: grain filling duration at the early stage; V1: mean grain filling rate at the early stage; W1: increased grain weight | | | | | | | | | | | | | | | | | | | |
| at the e | at the early stage; T2: grain filling duration at the middle stage; V2: mean grain filling rate at the middle stage; W2: increased grain weight at the middle stage; T3: grain filling | | | | | | | | | | | | | in filling | | | | | |

at the early stage; 1_2 : grain filling duration at the middle stage; V_2 : mean grain filling rate at the middle stage; W duration at the late stage; V_3 : mean grain filling rate at the late stage; and W_3 : increased grain weight at the late stage The symbols * and ** indicate significance at the 0.05 and 0.01 levels, respectively

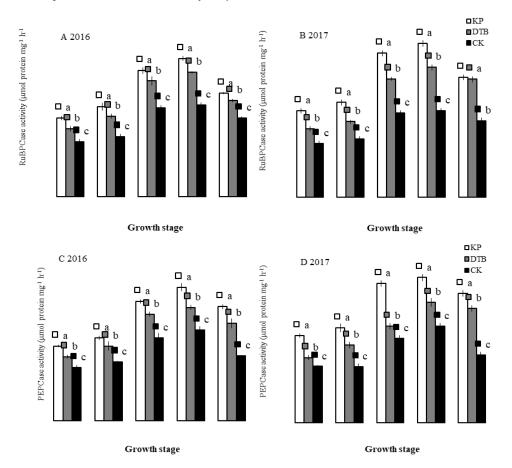


Fig. 3: Effects of the PGRs (KP and DTB) on the RuBPCase (A and B) and PEPCase (C and D) activities in 2016 and 2017. Bars indicate standard errors of the means of 3 replications. The same letters on bars indicate that the differences are not significant at P < 0.05 as determined by a least significant difference (LSD) test

application of PGRs was conducive to improving the ventilation and light transmission ability of high density populations (Xu et al., 2017) in the early growth stage, delaying leaf senescence processes in the later growth stage, increased the leaf photosynthetic activity (Dong et al., 2006) and creating conditions for the accumulation of photosynthetic matter during grain filling.

Grain filling is a complex physiological process, *i.e.*,

the materials formed by photosynthesis continuously transports into grains and converts into sugar and other storage substances (Ritchie et al., 1998). Both the grain filling rate and duration could affect the grain filling of maize (Hadi, 2004). In this study, PGRs prolonged the grain filling duration and increased the grain filling rate during the whole grain filling stage by the reason of the improvement in photosynthetic activity (Table 2). Additionally, the yield was positively correlated with the maximum grain filling rate, mean grain filling rate and effective grain filling time. Furtherly, the whole filling process can be divided into three phases: the lag phase, the effective grain filling period and the maturation drying phase (Borras and Westgate, 2006). The lag phase is a crucial phase for the establishment of the grain sink capacity. During effective grain filling period, the grain filling rate and duration directly determine the final grain weight. And during the maturation drying phase, the grain dehydrates rapidly (Johnson and Tanner, 1972; Borras and Westgate, 2006). This experiment suggested that the grain filling rate during the lag phase, the effective grain filling period and the maturation drying phase were positively correlated with the yield. PGRs increased the grain filling rate and grain weight during the lag phase, which was conducive to establishing the grain sink capacity (Gao et al., 2017). Additionally, the PGRs increased the grain filling rate and grain filling duration during the effective grain filling period and the maturation drying phase, which promoted dry matter filling into grains and increased the grain weight and yield. Therefore, the application of PGRs could improve grain filling characteristics, resulting in an increase in yield (Ahmad et al., 2018).

In this experiment, KP significantly increased the leaf photosynthesis and optimizing the canopy structure of plant populations, thus improved the grain filling characteristics and yield. The main content of KP was DCPTA and Ethephon. Ethephon significantly decreased the leaf area of a plant and regulated the plant population structure (Cicchino *et al.*, 2013). Xie *et al.* (2017) and Keithly and Yokoyama (1990) showed that DCPTA could increase the maize photosynthetic intensity and improve plant vegetative growth, which provided a foundation for increasing the grain yield. Therefore, the increase in grain yield with KP treatment mainly comes from the combined action of DCPTA and Ethephon.

Conclusion

The PGRs significantly increased the LAI values, key enzyme activities of photosynthesis and photosynthetic rate in maize, effectively increased the photosynthetic intensity of maize, and provided a basis for grain filling. In addition, the PGRs increased the grain sink capacity and grain filling rate in the early-filling stage. The PGRs stimulated the grain filling rate and grain filling duration in the middlefilling and late-filling stages. Thus, the PGRs enhanced the grain weight and yield. This experiment provided a theoretical basis for the application of PGRs to achieve high spring maize yields in Heilongjiang Province, China.

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