

## Exogenous diethyl aminoethyl hexanoate enhanced growth of corn and soybean seedlings through altered photosynthesis and phytohormone

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### Abstract

Diethyl aminoethyl hexanoate (DTA6) is an artificial tertiary amine with low molecular weight and high bioactive regulators. This study was conducted to investigate the effect of exogenous DTA6 on the plant growth, gas exchange, PSII photochemistry, and phytohormone in corn and soybean seedlings. To achieve this objective, a pot experiment was carried out in growth chamber to determine the response of corn and soybean plants to foliar treated with different concentrations of DTA6 at the V<sub>3</sub> stage. DTA6 treatments increased the plant height, root length and leaf areas, promoted dry matter accumulation, and improved root to shoot ratio in corn and soybean seedlings. DTA6 treated plants had greater chlorophyll content, photosynthetic rate, and Rubisco and PEPCase activity than those of control, but no difference in Chl *a/b* ratio was observed between DTA6-treated and controlled plants. DTA6 treatments increased efficiency of PS II photochemical reactions, photochemical quenching coefficient and electron transport rates, but decreased non-photochemical quenching coefficient compared with control in corn and soybean seedlings. Also, DTA6 treatments markedly enhanced IAA, zeatin riboside and GA<sub>3</sub> content, but decreased ABA content. Moreover, the greatest values of photosynthetic rate, Rubisco and PEPCase activities, and electron transport rates were shown at 9<sup>th</sup> day after DTA6 treatments in corn seedlings and at 12<sup>th</sup> day after DTA6 treatments in soybean seedlings. Optimal concentration of DTA6 sprayed on corn and soybean seedlings at V<sub>3</sub> stage were 20 mg L<sup>-1</sup> and 10 mg L<sup>-1</sup>, respectively. Our results suggest that exogenous DTA6 application can promote plant growth by enhancing photosynthesis, improving PS II photochemical reaction, increasing activities of photosynthetic key enzymes, and regulating hormonal balance.

**Keywords:** Diethyl aminoethyl hexanoate (DTA6), corn, soybean, photosynthesis, phytohormone.

**Abbreviation:** DAT<sub>day</sub> after treatment; DTA6\_Diethyl aminoethyl hexanoate; ETR<sub>electron transport rate</sub>; *F<sub>v</sub>/F<sub>m</sub>*<sub>maximal efficiency of PSII photochemistry</sub>; *NPQ*<sub>non-photochemical quenching coefficient</sub>; *P<sub>N</sub>*<sub>photosynthetic rate</sub>; *PEPCase*<sub>phosphoenol-pyruvate carboxylase</sub>; *q<sub>N</sub>*<sub>non-photochemical quenching coefficient</sub>; *q<sub>P</sub>*<sub>photochemical quenching coefficient</sub>; *RWC*<sub>relative water content</sub>; *Rubisco<sub>ribulose-1,5-bisphosphate carboxylase/oxygenase</sub>*.

### Introduction

It is widely accepted that increasing the yield potential of the crops will mostly rely on improved photosynthesis which is an important physiological process in crop development and growth (Zhu et al., 2010). Increasing canopy or leaf photosynthesis is a straight-forward way to increase yield potential, but it is difficult to improve canopy photosynthesis because most high-yielding cultivars are close to optimum canopy architecture (Jiang et al., 2002). Therefore, increasing single-leaf photosynthesis could be the only effective way to substantially enhance yield potential (Peng, 2000). Although intense selection for increased yield has been done by plant breeders, selection has not resulted in a genetic increase in photosynthesis per unit leaf area. According to Richards (2000), increased photosynthesis per unit land area has mainly been achieved by agronomic practices including irrigation and inorganic fertilizers. Furthermore, chemical regulation using plant growth regulators is one of agronomic practices and has proved to be potentially beneficial in photosynthesis of crops, such as cytokinin in rice (Ookawa et al., 2007), auxin in chickpea (Hayat et al., 2009), and GA<sub>3</sub> in mustard (Khan et al.,

2007). Tertiary amine bioregulators are effective and have been successfully applied on the crops to regulate plant growth since the late 1970s (Van Pelt and Popham, 2006). Tertiary amine bioregulators such as 2-(3,4-dichlorophenoxy) triethylamine (DCPTA) and diethyl-2-(4-methylbenzoxy) ethylamine (MBTA) have increased seed germination, root growth, and root to shoot ratio in many plants, including guayule (Hsu and Mendoza, 1992), cotton (Gausman et al., 1985), sugar beet (Keithly et al., 1990), radish (Keithly et al., 1992), paprika (Van Pelt and Popham, 2006), and tomato (Keithly et al., 1991a). In particular, tertiary amine bioregulators can promote pigment synthesis and chloroplast development, which lead to the increase of photosynthetic rates and carbohydrate concentrations in crops (Valadon and Mummery, 1982; Keithly et al., 1991b). Diethyl aminoethyl hexanoate (DTA6), also known as hexanoic acid 2-(diethylamino) ethyl ester, is an artificial tertiary amine and a structural and functional mimic of DCPTA, but is more active than DCPTA (Gausman et al., 1991). DTA6 is widely used in horticultural, forest and field crops alone or in combination with different herbicides, fungicides and fertilizers (Zhang et al.,

2008). Foliar application of DTA6 markedly regulates plant growth, increases stress tolerance, and improves quality and yield of crops (Zhang et al., 2001; Fu et al., 2011). However, several studies have shown that the mode of action for tertiary amine bioregulators is markedly different from plant hormone analogs because they affect gene expression including induction and regulation of pigment synthesis (Valadon and Chapman, 1982, Gausman et al., 1991, Keithly et al., 1992). However, little is known regarding how tertiary amine bioregulators manipulate plant processes such as photosynthesis and chlorophyll fluorescence involving hormone systems. Previous research of tertiary amine bioregulators including DTA6 mainly focused on dicotyledonous crops such as pea (Zhang et al., 2001), cotton (Gausman et al., 1985), tomato (Keithly et al., 1991a), and radish (Keithly et al., 1992). Few studies focus on monocotyledonous crops response to tertiary amine bioregulators. It is necessary to explore the difference between monocotyledonous and dicotyledonous crops response to DTA6 application for better application of DTA6. Therefore, the objective of this study was to elucidate the difference in the photosynthetic responses between corn and soybean to foliar application of DTA6. We have investigated the changes of plant biomass accumulation, photosynthesis, photosynthetic enzyme and PS II photochemical reaction in corn and soybean by DTA6 treatment, and studied the impacts of DTA6 on the changes in phytohormones. This knowledge could help to formulate strategies for application of DTA6 to monocotyledonous and dicotyledonous crops, which would be benefited to breaking the yield barrier in crop production.

## Results

### *DTA6 promoted plant growth and leaf expansion*

DTA6 enhanced plant height, root length and dry matter accumulation in corn and soybean seedling, and these effects varied with the concentrations of DTA6 (Table 1). When DTA-6 was applied to corn seedlings at concentrations increasing from 5 to 100 mg L<sup>-1</sup>, plant height was increased by 5.8 to 21.5% and root length increased by 0.4 to 7.9%, respectively. Similarly, DTA6 treatments enhanced shoot weight by 3.8 to 35.9% and root weight by 4.7 to 51.2% compared with control. Moreover, leaf areas of DTA6-treated plants were increased by 1.1 to 8.1% compared to control, and the ratio of root to shoot was increased. The greatest values of different parameters were presented at 20 mg L<sup>-1</sup> DTA6 treatment, and were markedly higher than those of control in corn seedlings. Similar results were obtained in DTA6-treated soybean seedlings, but there was difference in optimal treated concentration of DTA6 between corn and soybean. In soybean seedlings, the optimal treated concentration of DTA6 was at the level of 10 mg L<sup>-1</sup>, and plant height, root length, leaf area, biomass of shoot and root, and ratio of root to shoot were markedly increased by 19.0, 9.0, 9.1, 18.1, 57.7 and 33.1% higher than those of control, respectively.

### *DTA6 increased plant photosynthesis and chlorophyll content*

There was no remarkable difference in net photosynthetic rate ( $P_N$ ), transpiration rate ( $E$ ), and stomatal conductance ( $g_s$ ) at 1 day after treatment (DAT) between DTA6-treated and controlled corn seedlings (Fig. 1A, B and C). At 3, 6, 9 and 12 DAT, values of  $P_N$ ,  $E$  and  $g_s$  of DTA6-treated plants were markedly higher than those of control in corn seedlings. Furthermore, levels of  $P_N$ ,  $E$  and  $g_s$  at 9 DAT showed maximal values which were 26.8, 30.4 and 15.8% greater than those of

control, respectively. Similar changes of  $P_N$ ,  $E$  and  $g_s$  were observed in soybean seedling exposed to DTA6 pre-treatment (Fig. 1D, C and F). DTA6-treated plants had significantly greater in  $P_N$ ,  $E$  and  $g_s$  than control at 3, 6, 9 and 12 DAT. Values of  $P_N$ ,  $E$  and  $g_s$  in DTA6-treated plants at 12 DAT reached maximum which were 35.6, 23.6 and 10.8% greater than those in control, respectively. There was no difference in chlorophyll content between DTA6-treated and controlled corn and soybean seedlings at 1 and 3 DAT. However, at 6, 9, 12 and 15 DAT, DTA6 treatment increased chlorophyll contents by 21.7, 27.7, 22.6 and 19.6% greater than control in corn seedlings (Fig. 2A). The increase in DTA6-treated soybean seedling was 9.6, 17.5, 21.1 and 19.4% in chlorophyll contents compared with the control (Fig. 2B). Moreover, there was no difference in Chl *a/b* ratios between DTA6-treated and controlled corn or soybean seedlings (Fig. 2C, D).

### *DTA6 improved plant chlorophyll fluorescence parameters*

Compared with control plants, DTA6-treated plants exhibited significantly higher  $F_v/F_m$  values in corn and soybean seedlings (Fig. 3A, E). DTA6-treated plants presented maximum  $F_v/F_m$  values at 9 DAT in corn and 12 DAT in soybean which were 8.8 and 8.0% greater than control. At 3, 6, 9, 12 and 15 DAT, values of photochemical quenching coefficient ( $qP$ ) in DTA6-treated plants increased by 7.3, 9.1, 13.1, 10.0 and 5.8% when compared with control in corn seedlings, and 3.0, 9.0, 12.2, 15.5 and 10.8% greater than those of control in soybean seedlings, respectively (Fig. 3B, F). However, non-photochemical quenching coefficient ( $NPQ$ ) by DTA6 was markedly decreased compared with control in corn and soybean seedlings (Fig. 3C, G). For example, DTA6-treated plants decreased  $NPQ$  by 30.2, 30.6, 37.0, 13.5 and 11.3% compared to control in corn seedlings, and by 26.9, 29.2, 30.6, 33.5 and 10.9% than those of control in soybean seedlings at 3, 6, 9, 12 and 15 DAT, respectively. At 6, 9, 12 and 15 DAT, ETR values of DTA6-treated corn plants were 13.8, 18.9, 14.9 and 9.3% greater than those of control (Fig. 3D), and DTA6 treatment increased ETR by 19.2, 25.0, 28.8 and 14.8% compared with control in soybean seedlings (Fig. 3H).

### *DTA6 increased the activities of Rubisco and PEPCase*

The activities of PEPCase were significant higher in the DTA6-treated plants than controlled plants at 6, 9 and 12 DAT (Fig. 4A, C). PEPCase activity reached a maximum at 9 DAT for corn seedling and 12 DAT for soybean seedling, which were 15.9 and 24.1% higher than control. The changes of Rubisco activity were similar to PEPCase activity in corn and soybean seedlings. At 6, 9, 12 and 15 DAT, the activities of Rubisco in DTA6-treated corn plants were 26.5, 24.1, 16.0 and 14.7% higher than those of control (Fig. 4B). Also, DTA6 treatments increased Rubisco activity by 17.9, 29.2, 37.9 and 13.1% when compared with control in soybean seedlings, respectively (Fig. 4D).

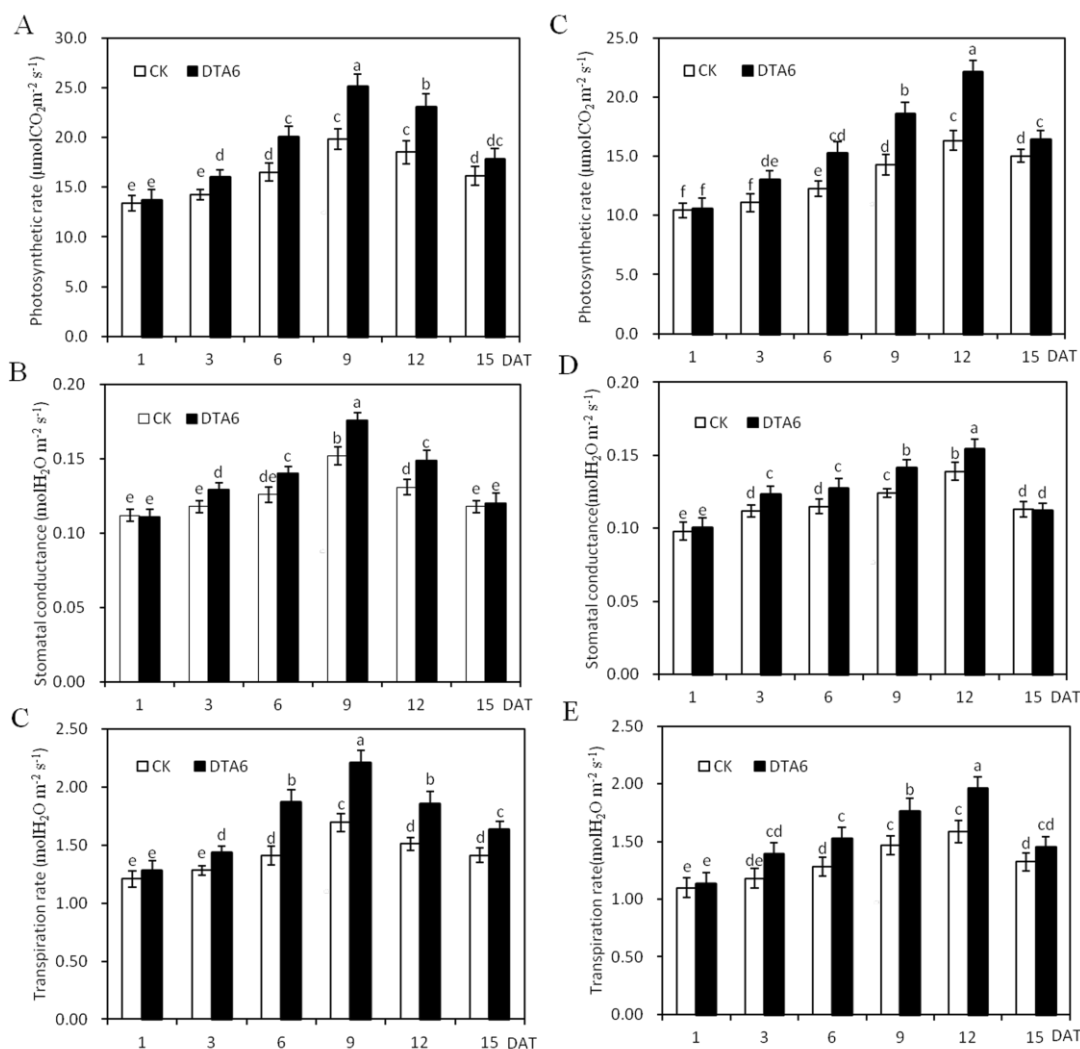
### *DTA6 regulated phytohormone content*

At 3, 6, 9, 12 and 15 DAT, IAA contents in DTA6-treated corn seedlings were 68, 42, 52, 29 and 20% higher than those of control, and maximum values were observed at 9 DAT (Fig. 5A). In soybean seedlings, DTA6 increased by 26, 52, 61, 48 and 18% compared with control at 3, 6, 9, 12 and 15 DAT, and maximum value were presented at 12 DAT (Fig. 5E). GA<sub>3</sub> contents in DTA6-treated corn and soybean seedlings showed a similar pattern to IAA (Fig. 5B, F). Similar to the changes in IAA contents, ZR contents in DTA6-treated corn plants were

**Table 1.** Effect of foliar application of different concentrations of DTA6 on plant height, leaf area, root length, dry shoot and root weight and ratio of root and shoot in corn and soybean seedlings.

Crops	DTA6 (mg.L <sup>-1</sup> )	Plant height (cm)	Leaf area (cm <sup>2</sup> .plant <sup>-1</sup> )	Root length (cm)	Dry weight (g.plant <sup>-1</sup> )		Root/shoot
					Shoot	Root	
Corn	100	39.1 bc	177 c	25.1 c	2.72 c	0.46 c	0.169 c
	40	38.2 c	180 bc	26.4 b	2.98 b	0.53 b	0.178 b
	20	43.7 a	189 a	27.2 a	3.56 a	0.65 a	0.183 a
	10	40.3 b	182 b	26.5 b	2.89 b	0.51 b	0.176 b
	5	39.0 b	178 c	25.9 bc	2.73 c	0.45 c	0.165 c
	0	36.1 d	175 c	25.2 c	2.62 d	0.43 c	0.164 c
Soybean	100	37.6 b	123 c	22.3 c	1.64 c	0.27 c	0.165 d
	40	36.6 b	125 bc	22.6 c	1.69 bc	0.29 bc	0.172 c
	20	39.1 a	129 b	23.1 b	1.72 b	0.34 b	0.198 b
	10	40.2 a	132 a	24.2 a	1.89 a	0.41 a	0.217 a
	5	37.3 b	124 c	23.5 b	1.78 b	0.32 b	0.180 c
	0	33.7 c	121 c	22.2 c	1.60 c	0.26 c	0.163 d

The data point was the mean of two independent biological experiments and each experiment comprises twelve samples. Within each column, means followed by same small letters are not significantly different according to LSD test at  $P \leq 0.05$ .



**Fig 1.** Effect of foliar application of DTA6 on net photosynthetic rate, stomatal conductance and transpiration rate in leaves of corn and soybean seedling. A, B and C, indicated net photosynthetic rate, stomatal conductance and transpiration rate in leaves of corn seedlings, respectively. D, E and F, indicated net photosynthetic rate, stomatal conductance and transpiration rate in leaves of soybean seedlings, respectively. Bars showing the same letter are not significantly different at  $P \leq 0.05$  as determined by LSD test. Each value represents the mean  $\pm$  SD ( $n = 10$ ).

22, 33, 52, 44 and 9% higher than control at 3, 6, 9, 12 and 15 DAT, respectively (Fig. 5C). Also, DTA6 treatment increased by 24, 54, 61, 32 and 7% ZR contents compared to control in soybean seedlings, respectively (Fig. 5G). In contrast to increase of IAA, GA<sub>3</sub> and ZR, ABA contents in DTA6-treated plants were lower than those of control in corn and soybean seedlings (Fig. 5D, H). ABA contents in DTA6-treated corn leaves were 16, 26, 37, 38 and 21% less than control at 3, 6, 9, 12 and 15 DAT, respectively. Similarly, ABA contents in DTA6-treated soybean leaves were reduced by 11, 25, 38, 49 and 28% compared to control at 3, 6, 9, 12 and 15 DAT, respectively.

## Discussion

### Plant morphology

DTA6 is an artificial tertiary amine and its application increased plant height, root length, leaf area, dry matter weight, and root to shoot ratio in corn and soybean seedlings. Similar results were observed in tertiary amine bioregulators such as DCPTA and MBTA. These bioregulators have increased plant productivity and crop yields (Gausman et al., 1991, Hsu and Mendoza, 1992, Van Pelt and Popham, 2006).

### Plant photosynthesis and chlorophyll fluorescence

DTA6 treatments significantly increased chlorophyll content,  $P_N$ ,  $E$ , and  $g_s$  compared with control in corn and soybean seedlings. Treatments with tertiary amine bioregulators often lead to increased pigment synthesis and larger chloroplasts which contribute to increased photosynthetic rates and carbohydrate concentrations (Valadon and Chapman, 1982; Keithly et al., 1991b). Moreover, no evident difference of Chl  $a/b$  ratio was observed in corn and soybean treated with DTA6 compared with control. These results were consistent with those by DCPTA application in spinach (Keithly et al., 1992). Chlorophyll fluorescence can reflect the changes in the thylakoid membrane organization and function. As expected, changes of PS II photochemistry induced by DTA6 were consistent with increased CO<sub>2</sub> assimilation rate in corn and soybean seedlings. DTA6-treated plants exhibited higher  $F_v/F_m$  values than control. Due to  $F_v/F_m$  used to PS II photochemistry efficiency in the photosynthetic apparatus (Baker and Rosenqvist, 2004), higher  $F_v/F_m$  showed that DTA6 treatment improved photosynthetic apparatus and led to increasing the maximum quantum efficiency of PS II photochemistry.  $qP$  can reflect the proportion of photon energy absorbed by the PS II centers (Genty et al., 1989). Thus, an increase in  $qP$  induced by DTA6 application suggested that DTA6 application led more excitation energy use for electron transport, with more ATP supplied for CO<sub>2</sub> assimilation (Harbinson et al., 1990; Zhang et al., 1996). DTA6-treated plants exhibited a rise of  $qP$  and a reduction in  $NPQ$  compared to control, which suggested that DTA6 improved thermal dissipation to preventing photodamage. Therefore, the higher  $P_N$  of the DTA6 treated plants were probably due to their higher photochemical efficiency.

### Phytohormone changes

Auxins have a stimulative effect in photosynthesis mainly on reactions of bonding to 1, 5-biphosphoribulose, synthesis of reducing sugar especially monosaccharides, photosynthetic phosphorylation and biosynthesis of assimilative pigments mainly chlorophyll (Czerpak et al., 2004). Cytokinin is known to stimulate the expression of photosynthetic enzymes like

Rubisco and development of function chloroplasts (Boonman et al., 2009). Also GA<sub>3</sub> could be found to increase the photosynthetic rate and Rubisco content and activity in leaves of plants (Yuan and Xu, 2001, Khan et al., 2007). Our results showed that DTA6 treatments markedly enhanced IAA, ZR and GA<sub>3</sub> contents in corn and soybean seedling, but decreased ABA content. Moreover, IAA, ZR and GA<sub>3</sub> contents were consistent with changed values of photosynthetic rate, chlorophyll content, and activities of RuBPCase and PEPCase in DTA6-treated corn or soybean seedling. These indicated that DTA6 treatment could regulate the IAA, ZR and GA<sub>3</sub> contents to enhance photosynthesis in corn and soybean seedlings. Otherwise, ABA content was reduced by DTA6 treatment in corn or soybean seedlings, which led to high photosynthetic rate and biomass accumulation. ABA was shown to reduce the photosynthetic capacity of a leaf through an apparent inhibition of RuBPCase activity (Chen et al., 2011). A significant reduction in grain weight with ABA treatment is associated with the reduction of net photosynthesis, chlorophyll content, and soluble protein content in flag leaves (Xie et al., 2004). It is suggested that DTA6 would regulate phytohormone to involve in regulating photosynthesis.

## Material and methods

### Plant materials

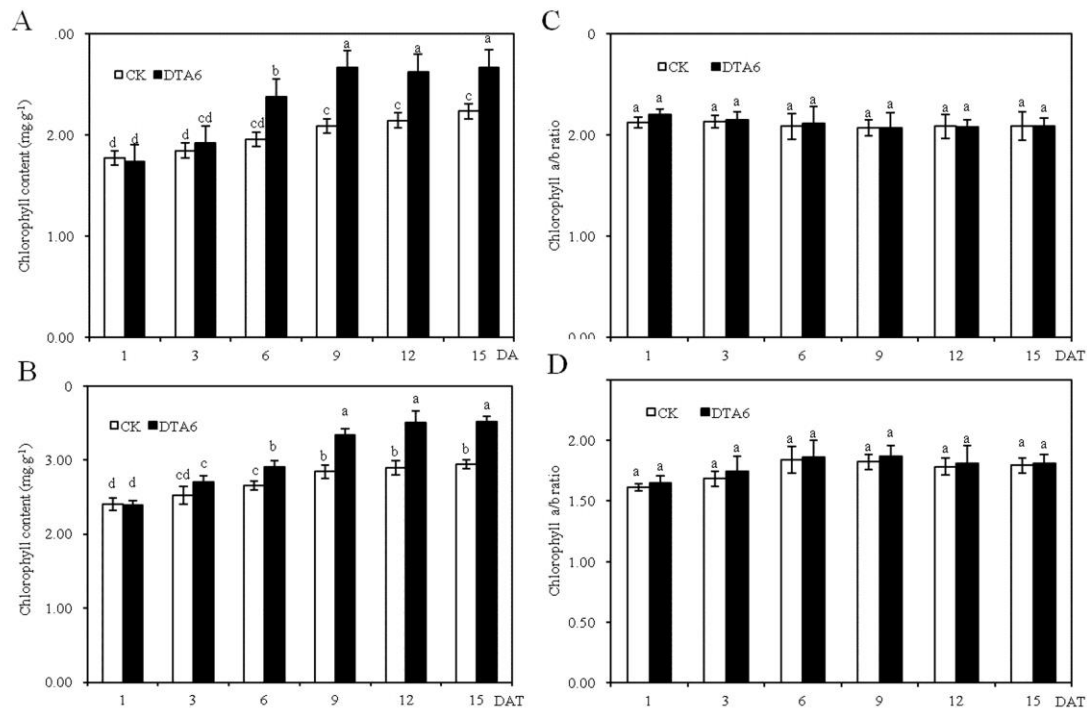
Seeds of corn (*Zea mays* L. cv. nongda108, a local elite corn cultivar from the North China plain, China) and soybean (*Glycine max* L. cv. Zhonghuang13, a local elite soybean cultivar from the North China plain, China) were sowed in the pots (15 × 15 × 20 cm deep). Each pot was filled with a mixture of vermicompost and sand (1:1; v/v) and placed in growth chamber with a 14 h photoperiod at 20/25°C night/day temperature cycle, and a relative humidity of 60%. Five seeds were sown per pot. After the seedlings reached the first true leaf stage, they were thinned to two per pot. Hoagland nutrient solution and water were supplied sufficiently throughout, and thus potential nutrients and drought stress were avoided.

### Experimental treatments

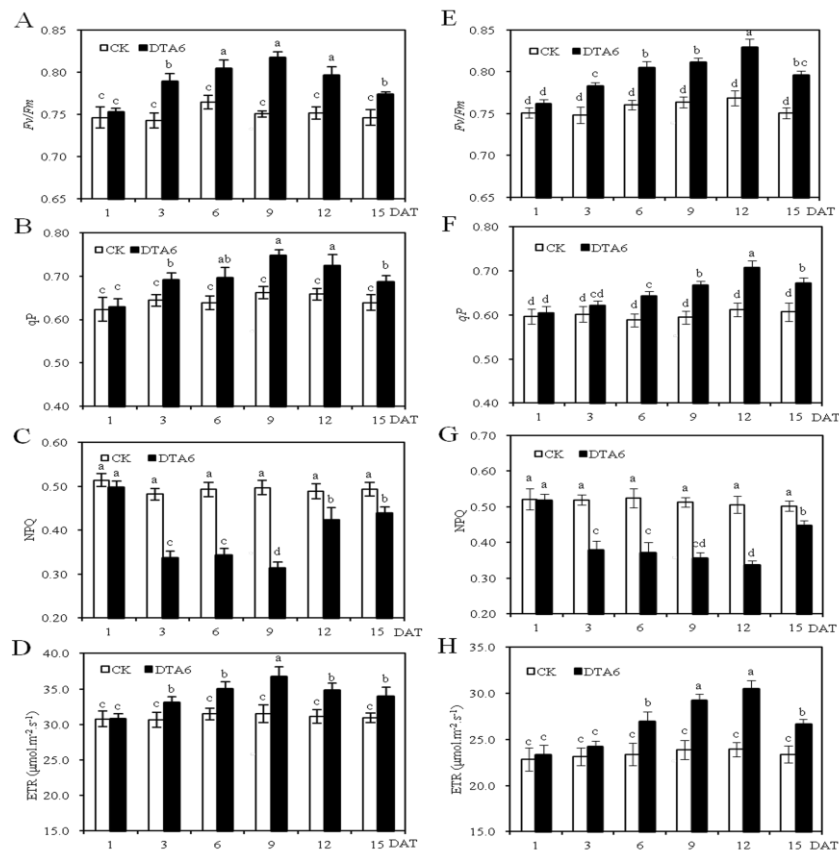
Diethyl aminoethyl hexanoate (DTA6, China Agrotech Holding Limited Company, Zhengzhou, China) were foliar applied at rates of 5, 10, 20, 40 and 100 mg L<sup>-1</sup> at the V<sub>3</sub> stage in corn and soybean seedlings. The solution was applied using a hand-held aerosol propelled sprayer to the canopy of two plants per pot until fully wetting the foliage surface. Tap water was used as control. The experiment was arranged in a completely randomized design, and each treatment had 20 pots that were replicated 3 times. The uppermost expanded leaf was labeled to sample for physiological and biochemical measurements after DTA6 applications. Samples were measured and collected at 1, 3, 6, 9, 12 and 15 days after spraying. At 24 days after spraying, the plant height, leaf area, and root length were measured, and then the plant was separated into shoots and roots. Then all samples were killed at 105 °C for 30 minutes and dried at 70 °C to determine the shoot and root dry weight. Fresh samples of all treatments were used for immediate assays or frozen in liquid nitrogen and stored at -80 °C for physiological and biochemical analysis.

### Analysis of CO<sub>2</sub> gas exchange

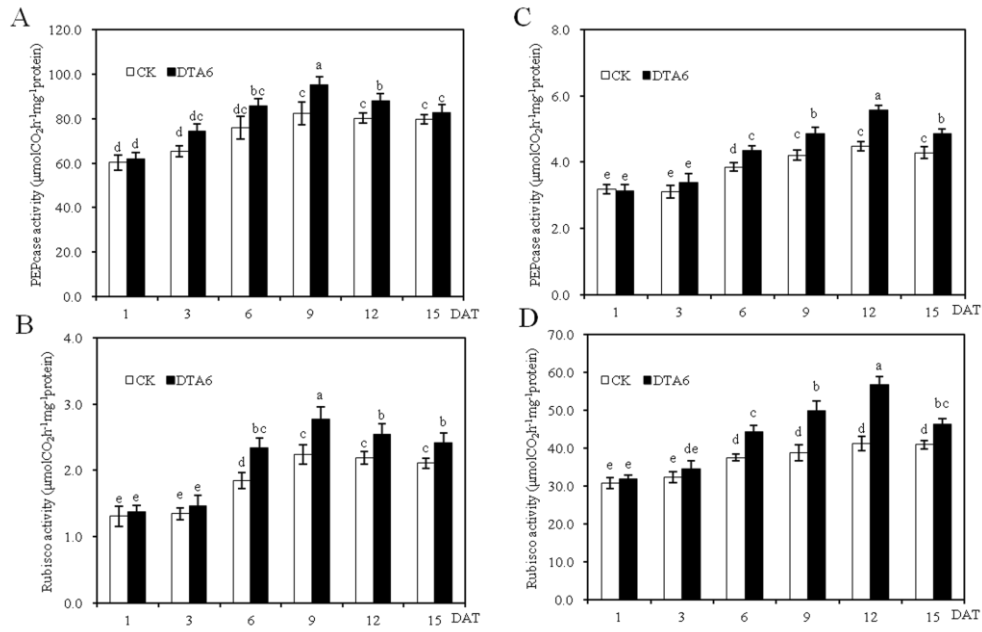
The labeled leaves of corn and soybean used for gas exchange were measured with a portable photosynthesis system (LI-6400, LI-COR, Lincoln, USA) after 1, 3, 6, 9, 12 and 15 days of



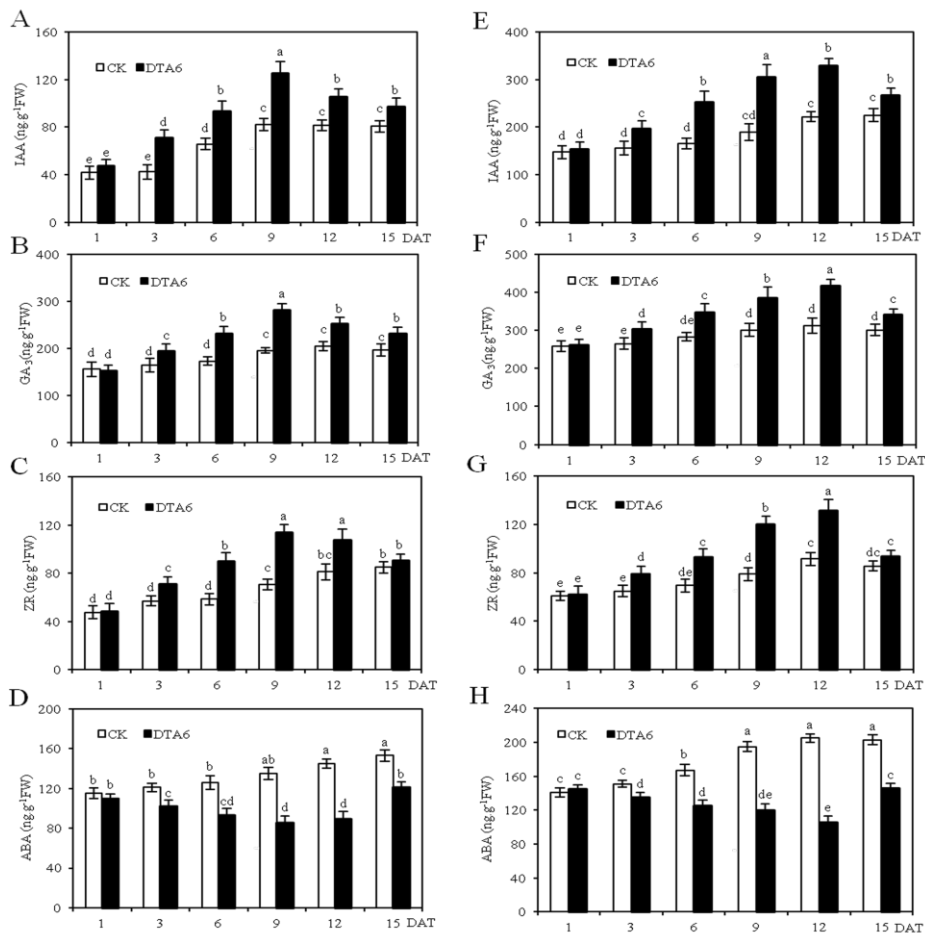
**Fig 2.** Effect of foliar application of DTA6 on chlorophyll content and chlorophyll a/b ratio in leaves of corn and soybean seedlings. A and C, chlorophyll content and chlorophyll a/b ratio in leaves of corn seedlings, respectively. B and D, chlorophyll content and chlorophyll a/b ratio in leaves, respectively. Bars showing the same letter are not significantly different at  $P \leq 0.05$  as determined by LSD test. Each value represents the mean  $\pm$  SD (n = 10).



**Fig 3.** Effect of foliar application of DTA6 on chlorophyll fluorescence parameter in leaves of corn and soybean seedlings. A and E, maximal efficiency of PS II photochemical reaction ( $F_v/F_m$ ) in corn and soybean seedlings, respectively. B and F, photochemical quenching coefficient ( $qP$ ) in corn and soybean seedlings. C and G, non-photochemical quenching coefficient ( $NPQ$ ) in corn and soybean seedlings, respectively. D and H, electron transport rate (ETR) in corn and soybean seedlings, respectively. Bars showing the same letter are not significantly different at  $P \leq 0.05$  as determined by LSD test. Each value represents the mean  $\pm$  SD (n=8).



**Fig 4.** Effect of foliar application of DTA6 on the activities of PEPCase and Rubisco in leaves of corn and soybean seedlings. A and B, the activities of PEPCase and Rubisco in leaves of corn seedlings, respectively. C and D, the activities of PEPCase and Rubisco in leaves of soybean seedlings, respectively. Bars showing the same letter are not significantly different at  $P \leq 0.05$  as determined by LSD test. Each value represents the mean  $\pm$  SD ( $n=6$ ).



**Fig 5.** Effect of foliar application of DTA6 on IAA (A, E), ZR (B, F), GA<sub>3</sub> (C, G) and ABA (D, H) contents in leaves of corn and soybean seedlings. A, B, C and D, indicate IAA, ZR, GA<sub>3</sub> and ABA contents in leaves of corn seedlings, respectively. E, F, G and H, indicate IAA, ZR, GA<sub>3</sub> and ABA contents in leaves of soybean seedlings, respectively. Bars showing the same letter are not significantly different at  $P \leq 0.05$  as determined by LSD test. Each value represents the mean  $\pm$  SD ( $n=6$ ).

treatment, respectively. All photosynthetic measurements were taken at a constant airflow rate of 500 mmol s<sup>-1</sup> and at saturation irradiance with incident photosynthetic photon flux density (PPFD) of 1000 μmol m<sup>-2</sup> s<sup>-1</sup>. The concentration of ambient CO<sub>2</sub> was approximately 400 μmol (CO<sub>2</sub>) mol<sup>-1</sup>(air) and the temperature was approximately 25 °C.

#### **Measurements of chlorophyll content and chlorophyll fluorescence**

Leaf discs were removed from the labeled leaves and extracted with 95 % ethanol in dark for 48 h until they were blanched. The concentrations of Chl *a* and Chl *b* were determined spectrophotometrically according to the methods used by Porra et al. (1989). The labeled leaves of corn and soybean used for chlorophyll fluorescence were measured by the PAM-2000 chlorophyll fluorescence system (Heinz Walz, Effeltrich, Germany). The fluorescence parameters were calculated according to Xu et al. (2011).

#### **Determination of PEPcase and Rubisco activity**

Leaf samples (1.0 g) from labeled leaves were collected and immediately immersed in liquid N<sub>2</sub>, which were used to determine the activities of phosphoenolpyruvate carboxylase (PEPcase) and ribulose-1, 5-bisphosphate carboxylase (Rubisco). PEPcase was assayed spectrophotometrically at 25 °C as described by Gonzalez et al. (1998). The extraction, purification, and determination procedures of Rubisco followed those of Makino et al. (1988) and Sawada et al. (1990).

#### **Extraction, Purification and Determination of IAA, zeatin riboside (ZR), GA<sub>3</sub> and ABA**

Leaf samples (0.5 g) from labeled leaves were used to determine the level of IAA, ZR, GA<sub>3</sub> and ABA. Extraction, purification, and determination of endogenous levels of IAA, ZR, GA<sub>3</sub> and ABA by an indirect enzyme-labeled immunosorbent assay were performed as described by Yang et al. (2001).

#### **Statistical analysis**

All physiological and biochemical measurements were made with 6 or 10 newly expanded leaves per treatment. Twelve plants of six pots per treatment were harvested for biomass and morphological parameters determination. In measuring the physiological parameters, three replicates were performed and the data were analyzed statistically according to randomized block design (RCD) using SPSS statistical software (SPSS Inc., Chicago, IL, USA). The least significant difference (LSD) was calculated for the significant data at  $P \leq 0.05$ .

#### **Conclusion**

In conclusion, DTA6 treated plants had greater biomass through improving photosynthetic rate and PSII photochemistry, increasing activity of Rubisco and PEPcase and regulating phytohormone balance in corn and soybean seedlings. Moreover, the optimal concentration of DTA6 sprayed at V<sub>3</sub> stage of the seedlings were 20 mg L<sup>-1</sup> for corn and 10 mg L<sup>-1</sup> for soybean.

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