



Mitigative effect of 6-benzyladenine on photosynthetic capacity and leaf ultrastructure of maize seedlings under waterlogging stress

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Abstract

6-Benzyladenine (6-BA) is an artificial synthetic cytokinin, which plays an important role in regulating plant responses to abiotic stress. This study aimed to investigate the mitigative effect of exogenous 6-BA on photosynthetic capacities and leaf ultrastructure under waterlogging stress using two waxy corn inbred lines. The results showed that waterlogging stress disrupted the photosynthesis of waxy corn seedlings. However, exogenous 6-BA alleviated the inhibition caused by waterlogging stress. Under the waterlogging conditions, 6-BA treatment of plants helped preserve the structural integrity of the chloroplasts and retain higher contents of photosynthetic pigments. It also increased the photosynthetic capacity and promoted the openness of stomatal pores. Moreover, exogenous 6-BA promoted photosynthetic activities and the accumulation of carbohydrates. The results revealed that the detrimental effects of waterlogging stress on maize seedlings can be alleviated by 6-BA *via* modulating photosynthetic activities.

Keywords: 6-benzyladenine; photosynthetic capacity; waterlogging stress; waxy corn.

Introduction

Waterlogging stress is one of the major environmental stresses affecting crop development and represents a critical threat to crop production worldwide (Wang *et al.* 2019a, Zhang *et al.* 2019a, Misra *et al.* 2020). Maize (*Zea mays* L.) is a relatively high water-demanding crop but highly sensitive to waterlogging, which often causes yield reduction and results in significant economic losses every year, especially during the seedling stage (Guo *et al.* 2016, Ren *et al.* 2018). Waterlogging causes leaf stomata closure, increases the resistance to CO₂

diffusion inside leaves, restrains photosynthetic electron transport activities, influences the photosynthetic enzyme activity and stability, and decreases the photosynthetic rate, ultimately preventing the growth and development of crops (Zhu *et al.* 2015, Bidalia *et al.* 2018, Mollae *et al.* 2020). Pereira *et al.* (2020) reported that waterlogging stress led to stomata closure and affected stomata development, which resulted in a significant decrease in stomatal density, functionality, and index on both faces of soybean. Studies by Cotrozzi *et al.* (2021) suggested that waterlogging stress decreased the photosynthetic activity by inhibiting the photosynthetic pigment-synthesis path-

Highlights

- Two waxy corn inbred lines were used in this study
- Exogenous 6-BA improved photosynthetic capacity and leaf ultrastructure
- Exogenous 6-BA alleviated the waterlogging-induced damages of waxy corn seedlings

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Abbreviations: 6-BA – 6-benzyladenine; Chl – chlorophyll; C_i – intercellular CO₂ concentration; DM – dry mass; DMSO – dimethylsulfoxide; E – transpiration rate; EDTA – ethylenediaminetetraacetic acid; FM – fresh mass; F_v/F_m – maximum quantum yield of PSII; g_s – stomatal conductance; NPQ – nonphotochemical quenching coefficient; PEPCase – phosphoenolpyruvate carboxylase; P_N – net photosynthetic rate; q_p – photochemical quenching coefficient; SPS – sucrose phosphate synthase; SS – sucrose synthase; Φ_{PSII} – effective quantum yield of PSII photochemistry.

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way, leading to the reduction in PSII efficiency. This reduction in PSII efficiency resulted in a decrease in the light-saturated rates of CO₂ assimilation for wheat leaves. However, in C₄ plants, the assimilation of atmospheric CO₂ occurs by physically separating primary CO₂ fixation by phosphoenolpyruvate carboxylase (PEPCase) and secondary fixation by ribulose-1,5-bisphosphate carboxylase (Rubisco) (Horiguchi *et al.* 2021). Then, glucose, fructose, sucrose, starch, and other metabolites are synthesized under the action of sucrose phosphate synthase (SPS) and carbon metabolism enzyme sucrose synthase (SS) (Chen *et al.* 2019, Wang *et al.* 2019b). The effect of waterlogging stress on photosynthetic characteristics in maize was studied by Tian *et al.* (2019). The results indicated that the activities of Rubisco and PEPCase were suppressed, and the net photosynthetic capacity decreased with the increase in stress intensity, causing a decrease in the total dry matter.

Waxy corn is native to China and has significant nutritional, edible, and industrial value due to the starch in the endosperm containing nearly 100% amylopectin (Wang *et al.* 2021a). At present, the area planted and the market size of waxy corn continues to increase annually (Wang *et al.* 2021b). Global climate change has led to frequent extreme rainfall events, accentuating the risk of waxy corn suffering from waterlogging stress, especially in some low-lying areas with poor drainage (Zhang *et al.* 2019b). Therefore, improving waterlogging resistance is an important issue in waxy corn production. Considering the detrimental effects of waterlogging stress, it is imperative to seek more effective, sustained, and economical measures to improve the waterlogging tolerance of crops. As an artificial synthetic cytokinin, 6-benzyladenine (6-BA) plays an important role in regulating photosynthetic properties under environmental stress (Gong *et al.* 2022, Hu *et al.* 2022, Ma *et al.* 2022). Chen *et al.* (2020) showed that spraying 6-BA on cadmium- and uranium-contaminated soils promoted the chlorophyll (Chl) cycle and stability of pigment–protein complexes, which was beneficial to Chl synthesis and significantly increased Chl contents in *Brassica juncea* L. Wu *et al.* (2012) indicated that exogenous 6-BA significantly increased effective PSII quantum yield photochemistry (Φ_{PSII}), maximum of PSII quantum yield (F_v/F_m), and photochemical quenching coefficient (q_p), and reduced nonphotochemical quenching coefficient (NPQ) of eggplant leaves under salinity stress, indicating that 6-BA enhanced the activity of the PSII reaction center under salinity stress. According to Zhang *et al.* (2019c), spraying 6-BA increased stomatal conductance (g_s) and intercellular CO₂ concentration (C_i), and enhanced CO₂ availability, thus effectively increasing P_N and significantly enhancing the tolerance of wheat to chilling stress. However, limited studies investigated the effects and regulation of exogenous 6-BA on the photosynthetic characteristics and carbon assimilation of waxy corn leaves under waterlogging stress.

In the present study, two maize inbred lines with different waterlogging tolerance were analyzed to investigate the mitigative effect of exogenous 6-BA on the leaf ultrastructure and photosynthetic capacity of waxy

corn seedlings under waterlogging stress. Hence, the main objectives of the present study were as follows: (1) to determine whether the photosynthetic characteristics of waxy corn leaf declined under waterlogging stress and could be ameliorated by exogenous 6-BA; (2) to examine the protective role of 6-BA in waxy corn seedlings under waterlogging stress by preserving the structural integrity of the chloroplasts, which promoted the openness of stomatal pores and the activities of photosynthetic enzymes. The purpose of the present study was to explore the role of spraying 6-BA on the photosynthesis of maize seedlings under waterlogging stress and provide more theoretical evidence for increasing waterlogging tolerance of waxy corn cultivation.

Materials and methods

Experimental design and plant material: This experiment was performed at the Shenyang Agricultural University (Shenyang, China) in 2020. The waterlogging-resistant inbred line SY-MY13 and the waterlogging-sensitive inbred line SY-XT1 were provided by the Specialty Corn Institute, Shenyang Agricultural University, China. On 12 May, the seeds of SY-MY13 and SY-XT1 were sown in pots; the pots had a diameter of 10 cm at the bottom and a height of 12 cm and contained 1 kg of soil. The experimental soil was classified as brown soil, which was taken from a depth of 10–20 cm of the experimental field. The content of organic matter, alkali-hydrolyzable nitrogen, available phosphorus, and available potassium was 32.45 g kg⁻¹, 121.86 mg kg⁻¹, 9.47 mg kg⁻¹, and 114.31 mg kg⁻¹, respectively. Following seed germination, three healthy seedlings were left in each pot until the fourth leaf stage. The rain shelter was covered with a polyethylene film to preclude the effect of rainfall on the pot experiment. A completely random design was used for the experiments. 6-BA (analytically pure) was purchased from Ryon Biological Technology Co., Ltd. (Shanghai, China) and sprayed at a concentration of 0.5 mM. The processing methods were the same as those in a previous study (Wang *et al.* 2021a). The pot experiment was divided into four treatments: (1) CK: conventional watering conditions; (2) CK + 6-BA: conventional watering conditions supplemented with 0.5 mM 6-BA; (3) W: waterlogging stress conditions; and (4) W + 6-BA: waterlogging stress conditions supplemented with 0.5 mM 6-BA. On 6 June, each inbred line was sprayed with 0.5 mM 6-BA and the treated plants were placed in a plastic box (61 × 42 × 12 cm) for 24 h. The potting soil was maintained under waterlogged conditions (3 cm of water above the soil surface) during the experimentation period. A total of 40 pots were set up for each treatment. After 7 d of treatment, the seedlings were harvested and the samples from the whole plant and the fully expanded third leaves were collected to determine the various physiological and biochemical parameters.

Chlorophyll (Chl) content: Fresh, fully expanded third leaves (0.1 g) were weighed and soaked in 10 mL of dimethyl sulfoxide (DMSO) for 48 h in the dark. Then, the

absorbance was measured at 665, 649, and 480 nm against DMSO as the blank using a microplate reader (1510, Thermo Fisher, USA). The content of photosynthetic pigments [Chl *a*, Chl *b*, Chl (*a+b*), and carotenoids] was calculated using the following equations: Chl *a* [$\text{mg g}^{-1}(\text{FM})$] = $(12.19 \times \text{OD}_{665} - 3.45 \times \text{OD}_{649}) \times V / (1,000 \times W)$, Chl *b* [$\text{mg g}^{-1}(\text{FM})$] = $(21.99 \times \text{OD}_{649} - 5.32 \times \text{OD}_{665}) \times V / (1,000 \times W)$, Chl (*a+b*) [$\text{mg g}^{-1}(\text{FM})$] = Chl *a* + Chl *b*, Car [$\text{mg g}^{-1}(\text{FM})$] = $(1,000 \times \text{OD}_{480} - 2.14 \times \text{Chl } a - 70.16 \times \text{Chl } b) / 220 \times V / (1,000 \times W)$, where OD_{665} , OD_{649} , and OD_{480} are the absorbance values of the extract solution at 665, 649, and 480 nm, respectively, *V* is the total volume of the extract, *W* is the leaf fresh mass, and Car is the content of carotenoids.

Gas-exchange parameters: The third fully expanded leaves of three individual plants were selected at 10:00 and 11:00 h and measured using a portable photosynthesis system *LI-6800* (*Li-COR Inc.*, NE, USA) to evaluate the photosynthetic parameters. The leaves were placed in a controlled chamber (1×3 cm) at the CO_2 concentration of $400 \mu\text{mol mol}^{-1}$ and relative humidity of 50% under a PPFD of $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$ to obtain the values for the following parameters: net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i).

Chl fluorescence parameters: Before measuring Chl fluorescence parameters, the third fully expanded leaves of three individual plants per treatment were dark-adapted for 30 min in a chamber containing a mobile shutter. Then, the Chl fluorescence parameters were determined using *PAM-2500* (*Walz*, Germany). The minimum fluorescence (F_0) at open PSII centers was determined by measuring light [$<0.1 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$], and the maximum fluorescence (F_m) at closed PSII centers was examined after the application of a 0.7-s pulse of saturating light [$10,000 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] after 30 min of darkness, actinic light [$800 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] was applied to measure the steady-state chlorophyll fluorescence (F_s). The measured parameters included F_v/F_m , Φ_{PSII} , NPQ, and q_p .

Stomatal aperture: The leaves were sliced into 3×3 -mm fragments while avoiding the main leaf veins and then fixed with 2.5% glutaraldehyde. Images of the adaxial leaf surface stomatal apertures were obtained using a scanning electron microscope (*Regulus 8100*, *Hitachi*, Japan). The stomatal parameters including stomatal size and stomatal apertures were calculated with *ImageJ 1.4.7* software (*ImageJ*, Bethesda, MA, USA).

Chloroplast ultrastructure: The leaves were sliced into fragments of 3×1 mm while avoiding the main leaf veins and then fixed with 2.5% glutaraldehyde. Fragments sequentially underwent rinsing, dehydration, permeabilization, and embedding (embedded in *Epon 812* resin). The samples were cut with an *LKB-V* ultramicrotome (*LKB*, Bromma, Sweden) and then stained with uranyl acetate and citric acid. The images of samples

were recorded using a transmission electron microscope (*HT7700*, *Hitachi*, Japan).

Photosynthetic enzyme activity: Fresh leaves (0.5 g) were weighed, and 4 mL of pre-cooled extraction solution was added to fully grind the samples. The extraction solution consisted of 50 mM Tris-HCl (pH 7.0), 10 mM MgCl_2 , 2 mM EDTA- Na_2 , 20 mM mercaptoethanol, and 2% ethylene glycol. After centrifugation (10,000 rpm, 4°C , 30 min), the supernatant was used as the crude extract for determining enzyme activities. The enzyme activity was expressed as units of activity per minute per gram of fresh mass [$\text{U g}^{-1}(\text{FM}) \text{min}^{-1}$]. The Rubisco (EC 4.1.1.39) and PEPCase (EC 4.1.1.31) activities were calculated as proposed by [Whitehead et al. \(2014\)](#). The SS (EC 2.4.1.13) and SPS (EC 2.4.1.14) activities were determined as suggested by [Chang et al. \(2016\)](#).

Measurements of carbohydrates: The fully expanded third leaves were weighed (0.5 g) and 15 mL of 80% pre-cooled ethanol was added to the pre-cooled mortar to grind the leaves into a homogenate in the ice bath. The grinding solution was centrifuged at 7,000 rpm for 10 min, and the supernatant was used for measuring the carbohydrate content. The contents of glucose, fructose, sucrose, and starch were determined by the method proposed by [Qiu et al. \(2020\)](#). The anthrone reagent was used to determine the glucose content and expressed in $\text{mg g}^{-1}(\text{FM})$. For fructose content measurement, 1 mL of supernatant, 2 mL 0.1% resorcinol, and 1 mL H_2O were mixed and boiled for 10 min in 80°C water bath. The absorbance was measured at 480 nm using a microplate reader (1510, Thermo Fisher, USA) and then the fructose content was calculated according to the standard curve and expressed in $\text{mg g}^{-1}(\text{FM})$. For sucrose content measurement, 1 mL of supernatant was mixed with 1 mL NaOH, 5 mL HCl, and 2 mL 0.1% resorcinol, and boiled for 10 min in 80°C water bath. The absorbance was measured at 480 nm using a microplate reader (1510, Thermo Fisher, USA) and then the sucrose content was calculated according to the standard curve and expressed in $\text{mg g}^{-1}(\text{FM})$. To measure the starch content, the supernatant was incubated with amyloglucosidase in 12 mL acetate buffer for 1 h, the starch content was determined using a microplate reader (1510, Thermo Fisher, USA) according to the colorimetric method and expressed in $\text{mg g}^{-1}(\text{FM})$.

Statistical analysis: The statistical analysis of data was carried out using *SPSS 19.0* software (*SPSS, Inc.*, IL, USA) with a one-way analysis of variance (*ANOVA*). All the data were expressed as means (\pm standard deviation) of three replicates and had significant ($P < 0.05$) differences. Graphs were made using *Origin 2017* software (*OriginLab*, MA, USA).

Results

Photosynthetic pigment content: The changes in the contents of photosynthetic pigments in each treated group are shown in [Fig. 1](#). The exogenous 6-BA treatment

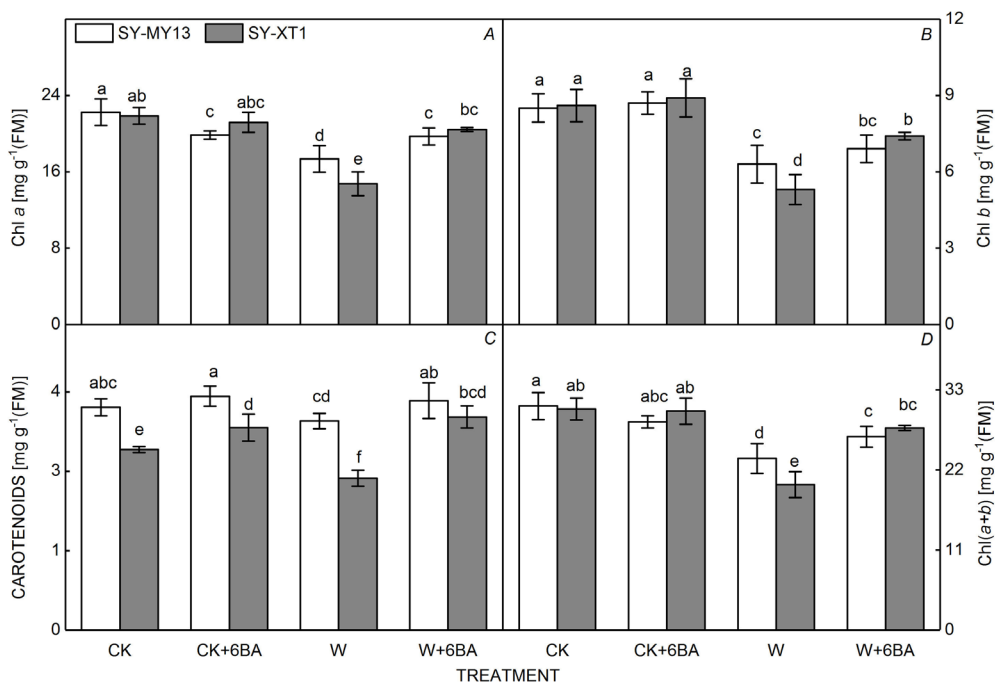


Fig. 1. Effects of 6-benzyladenine (6-BA) on (A) chlorophyll (Chl) a; (B) Chl b; (C) carotenoids; and (D) Chl (a+b) contents of two waxy corn inbred lines under different treatments. CK – conventional watering condition; CK + 6-BA – conventional watering condition supplemented with 0.5 mM 6-BA; W – waterlogging stress condition; W + 6-BA – waterlogging stress condition supplemented with 0.5 mM 6-BA. Data represent means ± SD of three replicates. For each variable, means with *different lowercase letters* are significantly different ($P < 0.05$).

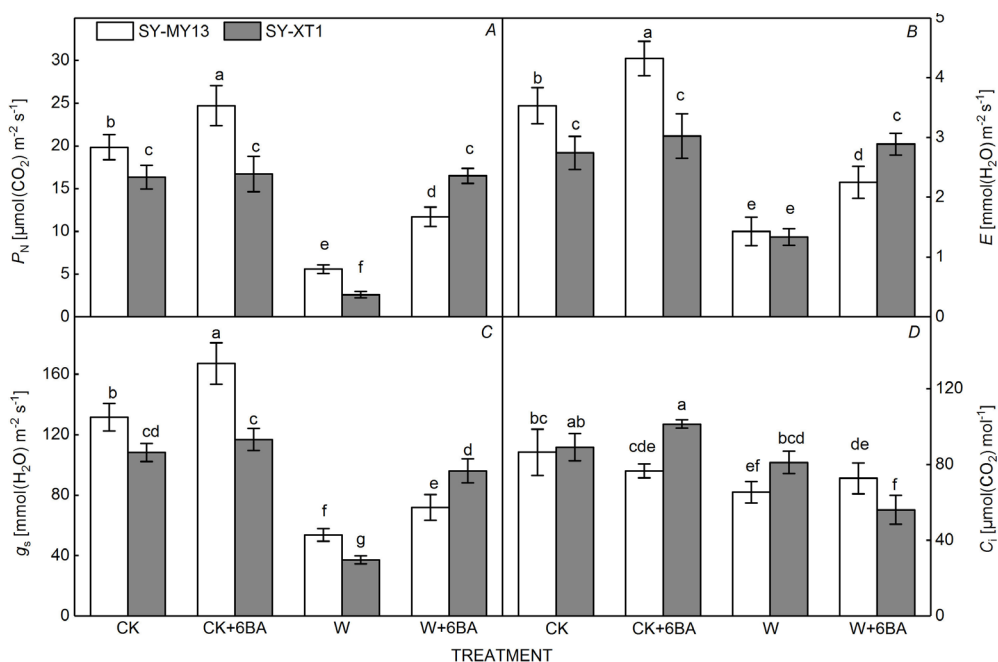


Fig. 2. Effects of 6-benzyladenine (6-BA) on (A) net photosynthetic rate (P_N); (B) transpiration rate (E); (C) stomatal conductance (g_s); and (D) intercellular CO_2 concentration (C_i) of two waxy corn inbred lines under different treatments. CK – conventional watering condition; CK + 6-BA – conventional watering condition supplemented with 0.5 mM 6-BA; W – waterlogging stress condition; W + 6-BA – waterlogging stress condition supplemented with 0.5 mM 6-BA. Data represent means ± SD of three replicates. For each variable, means with *different lowercase letters* are significantly different ($P < 0.05$).

increased the content of carotenoids (Car) of SY-XT1 significantly, despite no significant differences in the contents of Chl *b* and Chl (*a+b*) between two maize inbred lines grown under normal cultivation conditions. The contents of photosynthetic pigments were significantly reduced under the waterlogging condition for the two inbred lines, except for the content of Car in SY-MY13. The content of Chl *a*, Chl *b*, and Chl (*a+b*) in SY-MY13 was reduced by 27.6, 34.9, and 30.5%, respectively, and the content of Chl *a*, Chl *b*, Chl (*a+b*), and Car in the SY-XT1 was reduced by 49.0, 62.3, 52.0, and 18.9%, respectively. However, exogenous 6-BA increased the contents of Chl *a*, Chl *b*, Chl (*a+b*), and Car of each inbred line under the waterlogging treatment significantly, *i.e.*, by 13.9, 9.5, 12.7, and 8.8% in SY-MY13 and by 38.8, 39.6, 39.0, and 39.8% in SY-XT1.

Gas-exchange parameters: Compared with CK, 6-BA treatment increased the P_N , g_s , and E of SY-MY13 significantly (Fig. 2). The waterlogging stress treatment caused significant decreases in the P_N , g_s , C_i , and E of two waxy corn inbred lines compared with CK, which decreased by 71.8, 59.3, 24.4, and 59.5% in SY-MY13 and 84.2, 65.6, 9.2, and 51.5% in SY-XT1, respectively. This indicated that the magnitude of waterlogging effects on SY-XT1 was much greater than that on SY-MY13. The gas-exchange parameters tended to increase under exogenous 6-BA treatment with two inbred lines compared to non-sprayed lines under waterlogging stress conditions. The P_N , g_s , and E of SY-MY13 and SY-XT1 significantly

increased by 109.7, 33.9, and 56.6% and 534.6, 158.3, and 116.5%, respectively.

Chl fluorescence parameters: Under normal cultivation conditions, exogenous 6-BA had no significant effects on F_v/F_m , Φ_{PSII} , NPQ, and q_p of the two waxy corn inbred lines (Fig. 3). The waterlogging treatment significantly reduced the NPQ and q_p of SY-MY13 by 48.1 and 30.8%, respectively, compared with normal cultivation conditions, while it had no significant effect on F_v/F_m and Φ_{PSII} . The F_v/F_m of SY-XT1 also significantly decreased by 12.5% compared with normal cultivation conditions. The Φ_{PSII} , NPQ, and q_p of SY-MY13 showed an increasing trend after exogenous 6-BA treatment, compared with waterlogging treatment, and significantly increased by 26.7, 87.2, and 58.9%, respectively.

Stomatal aperture: The SEM images (Fig. 4) depicted the differences between control and treatments in each inbred line, the data are shown in Table 1. Under normal cultivation conditions, exogenous 6-BA did not affect the stomata of the two maize inbred lines. However, waterlogging stress significantly reduced stomatal length and stomatal width of SY-MY13 and SY-XT1 by 28.2 and 18.1%, and 7.5 and 16.7%, respectively. The stomatal opening length and width significantly decreased by 17.2 and 38.7%, and 25.9 and 64.3%, respectively, compared with those under normal conditions. Fortunately, the morphology of stomata was dramatically improved under the 6-BA treatment. The stomatal length and stomatal

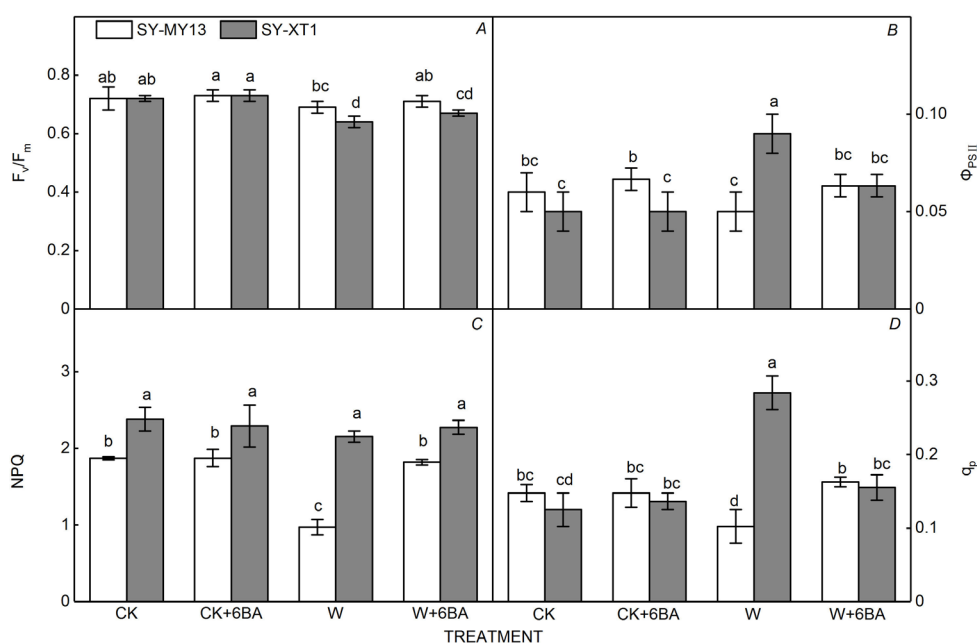


Fig. 3. Effects of 6-benzyladenine (6-BA) on (A) maximum quantum yield of PSII (F_v/F_m); (B) effective quantum yield of PSII (Φ_{PSII}); (C) nonphotochemical quenching coefficient (NPQ); and (D) photochemical quenching (q_p) of two waxy corn inbred lines under different treatments. CK – conventional watering condition; CK + 6-BA – conventional watering condition supplemented with 0.5 mM 6-BA; W – waterlogging stress condition; W + 6-BA – waterlogging stress condition supplemented with 0.5 mM 6-BA. Data represent means \pm SD of three replicates. For each variable, means with different lowercase letters are significantly different ($P < 0.05$).

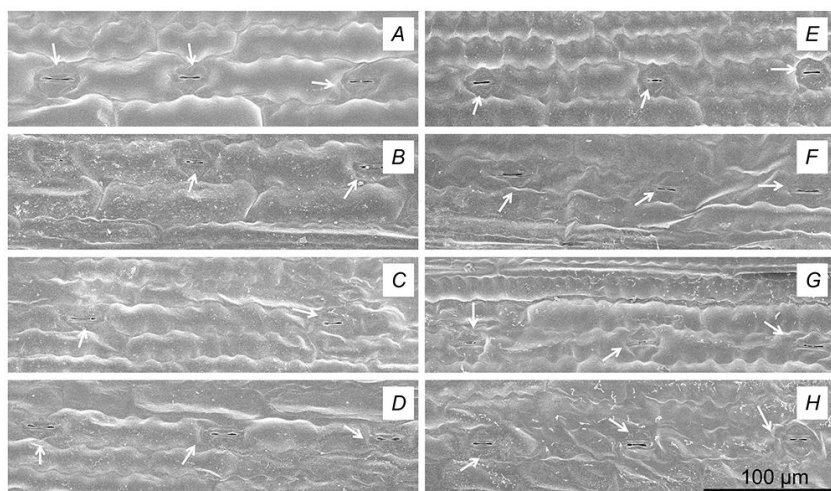


Fig. 4. SEM images of stomata in the leaves of two waxy corn inbred lines under different treatments. (A,E) Stomata in the leaves under conventional watering condition (CK); (B,F) stomata in the leaves under conventional watering condition supplemented with 0.5 mM 6-BA (CK + 6-BA); (C,G) stomata in the leaves under waterlogging stress conditions (W); and (G,H) stomata in the leaves of waterlogging stress condition supplemented with 0.5 mM 6-BA (W + 6-BA). Magnification 400 \times , scale bar 100 μ m.

Table 1. Effects of 6-benzyladenine (6-BA) on the stomatal aperture of two waxy corn inbred lines under waterlogging stress. CK – conventional watering condition; CK + 6-BA – conventional watering condition supplemented with 0.5 mM 6-BA; W – waterlogging stress condition; W + 6-BA – waterlogging stress condition supplemented with 0.5 mM 6-BA. Data represent means \pm SD of three replicates. For each variable, means with *different lowercase letters* are significantly different ($P < 0.05$).

Inbred line	Treatment	Stomatal size length [μ m]	Stomatal size width [μ m]	Stomatal apertures length [μ m]	Stomatal apertures width [μ m]
SY-MY13	CK	32.8 \pm 0.98 ^a	25.7 \pm 1.03 ^a	21.2 \pm 0.85 ^a	1.7 \pm 0.15 ^a
	CK + 6-BA	32.3 \pm 3.55 ^a	24.9 \pm 2.74 ^a	20.8 \pm 1.25 ^a	1.6 \pm 0.11 ^b
	W	23.6 \pm 1.18 ^d	21.1 \pm 1.89 ^b	17.5 \pm 1.40 ^c	1.0 \pm 0.05 ^d
	W + 6-BA	27.6 \pm 1.66 ^b	25.4 \pm 1.52 ^a	18.5 \pm 1.48 ^b	1.5 \pm 0.14 ^b
SY-XT1	CK	26.9 \pm 1.07 ^b	25.5 \pm 2.04 ^a	13.5 \pm 0.95 ^d	1.4 \pm 0.11 ^c
	CK + 6-BA	26.4 \pm 0.53 ^{bc}	25.5 \pm 0.26 ^a	13.3 \pm 1.19 ^d	1.4 \pm 0.13 ^c
	W	24.8 \pm 1.49 ^{cd}	21.3 \pm 1.49 ^b	10.1 \pm 1.03 ^f	0.5 \pm 0.04 ^e
	W + 6-BA	27.6 \pm 2.20 ^b	25.4 \pm 1.27 ^a	11.5 \pm 0.69 ^e	1.0 \pm 0.03 ^d

width of SY-MY13 and SY-XT1 significantly increased by 17.0 and 19.0%, and 12.2% and 17.6% compared with waterlogging treatment, respectively. The stomatal opening length and width also significantly increased by 5.7 and 50.0%, and 15.0 and 100.0% compared to waterlogging treatment, respectively.

Chloroplast ultrastructure: Fig. 5 shows the chloroplast structure of two waxy corn inbred lines under different treatments. Under normal cultivation conditions, the chloroplasts of both lines had normal structures. Exogenous 6-BA treatment did not significantly affect the chloroplast structure of both lines under normal cultivation conditions. After waterlogging stress, the chloroplasts showed changes in internal structure and differed significantly between the lines. The structure of the chloroplast of SY-MY13 was intact. Unlike the chloroplasts of SY-MY13, the chloroplasts of SY-XT1 had no typical chloroplast structure or thylakoid membrane system. In contrast, exogenous 6-BA ameliorated the chloroplast damage in the two waxy corn inbred lines and they exhibited normal thylakoid structure and grana. The chloroplasts had clearly defined boundaries and organized thylakoids and grana

lamellae. The chloroplast structure was integrated with lamellae arranged in an orderly manner.

Photosynthetic enzyme activities: Exogenous 6-BA had no significant effects on the activities of photosynthetic enzymes compared with CK under normal conditions (Fig. 6). However, waterlogging stress significantly reduced the photosynthetic enzyme activities. Compared with CK, the activity of Rubisco, PEPCase, SS, and SPS were significantly reduced in SY-MY13 and SY-XT1 by 23.8, 19.7, 22.6, and 29.7%, and 40.1, 28.8, 23.1, and 43.6%, respectively. Exogenous 6-BA resulted in a significant increase in the activities of Rubisco, SS, and SPS compared to waterlogging treatment. The activities of Rubisco, SS, and SPS of SY-MY13 increased by 12.8, 5.7, and 25.6%, while the activity of SY-XT1 increased by 85.9, 12.2, and 51.2%, respectively. Furthermore, the activity of PEPC of SY-XT1 also increased by 20.7%.

Accumulation of carbohydrates: Compared with normal cultivation conditions, 6-BA treatment increased the glucose content of SY-XT1 significantly. Waterlogging stress significantly decreased the glucose, fructose, sucrose,

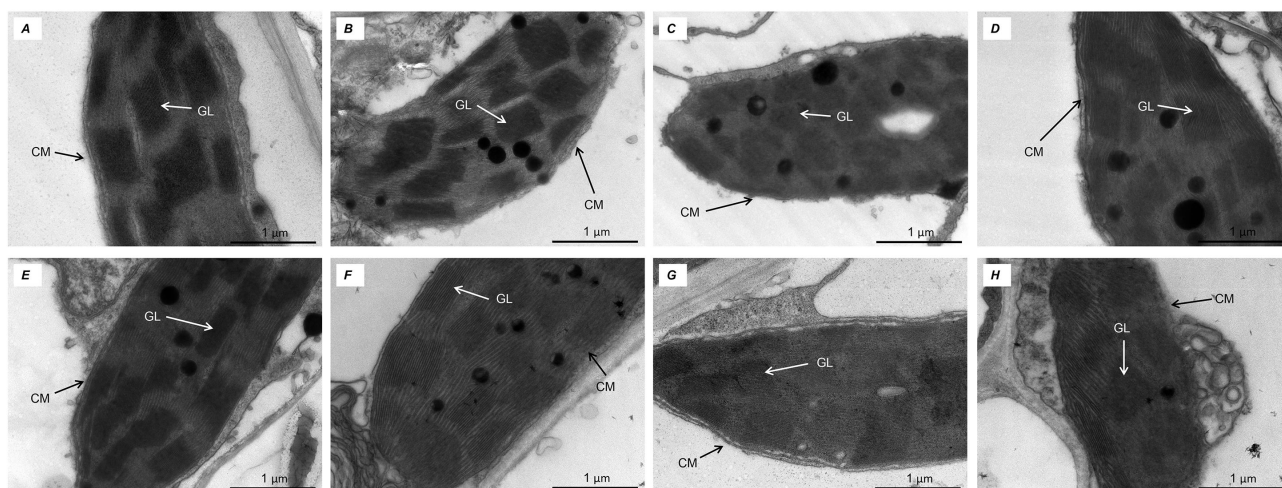


Fig. 5. Chloroplast ultrastructure images of two waxy corn inbred lines under different treatments. (A,E) Conventional watering condition (CK); (B,F) conventional watering condition supplemented with 0.5 mM 6-BA (CK + 6-BA); (C,G) waterlogging stress conditions (W); and (G,H) waterlogging stress condition supplemented with 0.5 mM 6-BA (W + 6-BA). CM – chloroplast membrane, GL – grana lamellae.

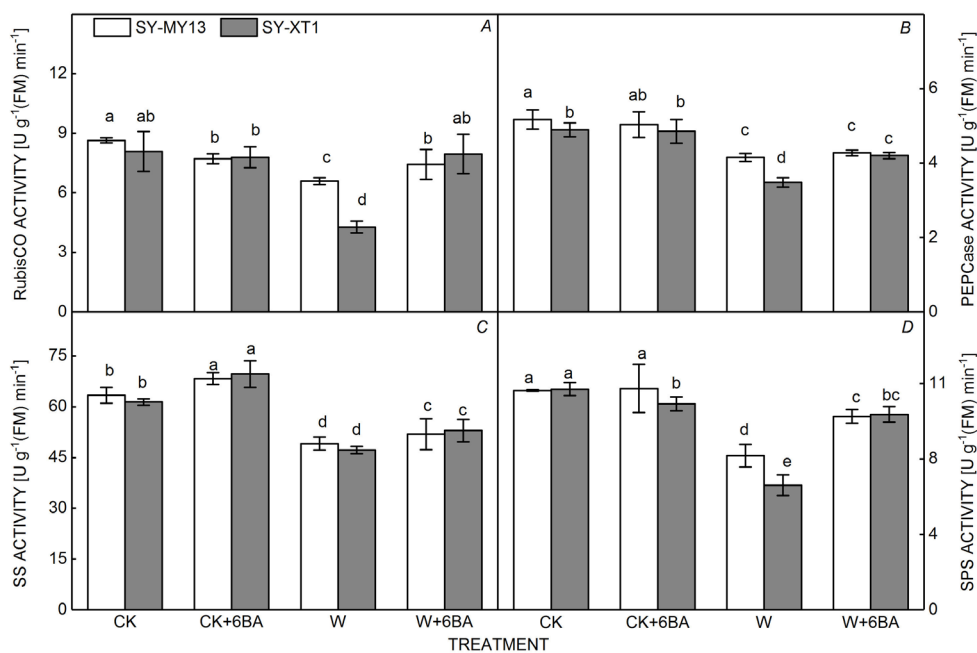


Fig. 6. Effects of 6-benzyladenine (6-BA) on the activity of (A) ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco); (B) phosphoenolpyruvate carboxylase (PEPCase); (C) sucrose synthase (SS); and (D) sucrose phosphate synthase (SPS) of two waxy corn inbred lines under different treatments. CK – conventional watering condition; CK + 6-BA – conventional watering condition supplemented with 0.5 mM 6-BA; W – waterlogging stress condition; W + 6-BA – waterlogging stress condition supplemented with 0.5 mM 6-BA. Data represent means \pm SD of three replicates. For each variable, means with *different lowercase letters* are significantly different ($P < 0.05$).

and starch contents compared with those under normal cultivation conditions (Fig. 7). However, exogenous 6-BA alleviated the damage caused by waterlogging stress on the products of photosynthesis. Exogenous 6-BA also resulted in significant increases in the contents

of the products of photosynthesis compared with the waterlogging treatment. The content of starch, sucrose, fructose, and glucose of SY-MY13 increased by 20.1, 5.0, 24.5, and 48.7%, and that of SY-XT1 by 15.9, 43.8, 24.6, and 60.9%, respectively.

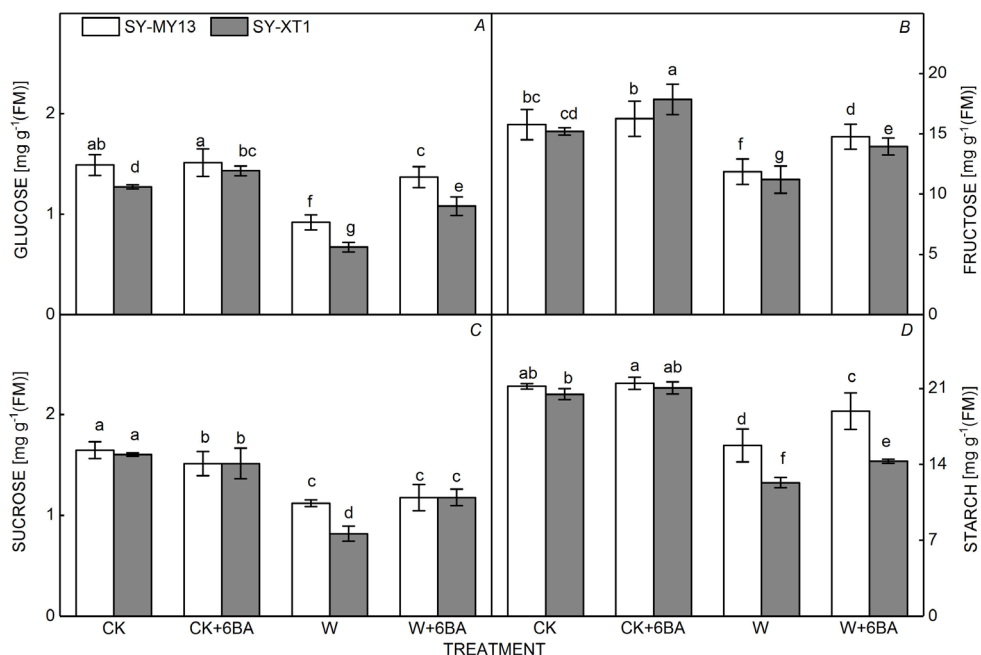


Fig. 7. Effects of 6-benzyladenine (6-BA) on the content of (A) glucose; (B) fructose; (C) sucrose; and (D) starch of two waxy corn inbred lines under different treatments. CK – conventional watering condition; CK + 6-BA – conventional watering condition supplemented with 0.5 mM 6-BA; W – waterlogging stress condition; W + 6-BA – waterlogging stress condition supplemented with 0.5 mM 6-BA. Data represent means \pm SD of three replicates. For each variable, means with *different lowercase letters* are significantly different ($P < 0.05$).

Discussion

As one of the severe abiotic stress factors, waterlogging stress negatively affected the development and growth of crops (Yu *et al.* 2020, Miricescu *et al.* 2021). 6-BA is a cytokinin and an artificial synthetic plant growth regulator, which can help crops overcome the negative effects of abiotic stresses, including waterlogging (Ghaleh *et al.* 2020, Hu *et al.* 2020). Chloroplasts are the major site of photosynthesis and one of the organelles sensitive to adversity (Tang *et al.* 2020). A decrease in the Chl content from the chloroplast leads to a reduction in the absorption, transfer, and transformation of light energy while inhibiting photosynthesis in plants (Abdallah *et al.* 2010, Hosseini *et al.* 2019). This study showed that the contents of Chl *a*, Chl *b*, and Chl (*a+b*) in waxy maize leaves significantly decreased under waterlogging stress, indicating that waterlogging stress could degrade Chl or inhibit Chl synthesis (Fig. 1). One of the functions of cytokinins is to regulate growth and delay leaf senescence (Sosnowski *et al.* 2019, Wu *et al.* 2021). Therefore, exogenous 6-BA can promote Chl synthesis or slow down its degradation rate. Studies on many plants showed that 6-BA had a favorable effect on Chl synthesis. Exogenous 6-BA application had a beneficial effect on the photosynthetic pigment contents of *Phellodendron chinense* seedlings and caused a significant increase in the contents of Chl *a*, Chl *b*, and total Chl (He *et al.* 2018). A study by Jia *et al.* (2017) showed that under oxidative damage, 6-BA application effectively inhibited Chl degradation,

improving the quality of Chinese chives. The results of the present study were consistent with the findings of the aforementioned studies, indicating that exogenous 6-BA alleviated the decline in Chl contents in leaves of maize inbred lines under waterlogging stress significantly. Besides participating in the absorption and transmission of light energy, Car also showed strong antioxidant capacity. Waterlogging reduced the Car content in waxy corn leaves, while exogenous 6-BA significantly alleviated the degradation of Car in waxy corn leaves and effectively improved the function of PSII.

The Chl fluorescence parameters are important indicators of changes in the Chl content in plants, which are related to the changes in various chemical reactions in plant photosynthesis, involved a variety of photosynthesis information, and are particularly vulnerable to abiotic stresses (Liang *et al.* 2017, He and Li 2021). Since the Chl contents also affect the light energy efficiency of PSII, abiotic stresses have substantial adverse effects on the Chl content in plants and a corresponding influence on the Chl fluorescence parameters. Multiple studies demonstrated that prolonged and severe waterlogging stress resulted in the PSII photoinhibition, PSII reaction centers were also closed (Zhu *et al.* 2016, Doupis *et al.* 2017). In this study, waterlogging treatment reduced the F_v/F_m , Φ_{PSII} , NPQ, and q_p of SY-MY13 significantly compared with normal cultivation conditions (Fig. 3). A decrease in F_v/F_m revealed the damage to the thylakoid membranes of chloroplasts and PSII reaction centers and severe inhibition of electron transport function. Also,

exogenous 6-BA could increase the F_v/F_m of both inbred lines, indicating that 6-BA could reduce the effects of waterlogging stress on the photosynthetic mechanism and PSII reaction centers of waxy corn seedling leaves, thus increasing the electron transfer rate in leaves and improving photosynthetic effect. Under waterlogging stress, exogenous 6-BA effectively maintained the higher NPQ of corn seedlings and prevented further damage to the photosynthetic apparatus. Ren *et al.* (2019) drew a similar conclusion by studying the effects of waterlogging stress on Chl fluorescence characteristics of maize. Previous studies also concluded that cytokinins could alleviate waterlogging-induced PSII damage significantly (Huynh *et al.* 2005). Therefore, 6-BA played a positive role in improving the light conversion efficiency of PSII of waxy corn under waterlogging stress.

Photosynthesis is sensitive in plants under waterlogging stress (Shao *et al.* 2013). Waterlogging stress reduced P_N , g_s , and E of waxy corn leaves significantly (Fig. 2). Waterlogging resulted in the lower E of waxy maize leaves due to reduced P_N and g_s . This might be because transpiration was affected by waterlogging stress due to partial stomatal closure. Hence, the stomatal restriction was also the reason for the decrease in the net photosynthetic rate of waxy corn seedling leaves under waterlogging stress. After spraying 6-BA, the decrease in g_s and E was significantly alleviated. Moreover, 6-BA also alleviated the decrease in P_N to varying degrees. The degree of stomatal opening and closure was caused by a variety of mechanisms. The opening stomata promoted the absorption of CO_2 and the transpiration of water (Yu *et al.* 2004). Waterlogging induced stomatal closure and prevented the absorption of CO_2 in leaves (Fig. 4). The deficiency of CO_2 was the main reason for the decrease in leaf P_N . Therefore, exogenous 6-BA increased the photosynthetic capacity of waxy corn leaves under waterlogging, which was related not only to the increase in stomatal conductance but also the fact that exogenous 6-BA was beneficial to the fixation of CO_2 in dark reactions under waterlogging.

Waterlogging stress may also inhibit the photosynthetic rate by decreasing the enzyme activity associated with the dark reaction. Kuai *et al.* (2016) explored sucrose metabolism in the leaves subtending the cotton boll after waterlogging in cotton and showed that the activities of Rubisco, SS, and SPS decreased with prolonged waterlogging. Ren *et al.* (2018) showed that waterlogging stress resulted in a significant decrease in the activities of Rubisco and PEPCase compared with normal cultivation conditions. In addition, waterlogging also resulted in a significant reduction in photosynthetic characteristics and decreased key enzyme activities of carbon metabolism (Rubisco and PEPCase) (Lukić *et al.* 2020, Xu *et al.* 2021). This study proved that waterlogging stress reduced both Rubisco and PEPCase activities, the decline in PEPCase activity was more significant than that in Rubisco activity (Fig. 6A,B). Therefore, PEPCase was more sensitive to waterlogging, which might be an important factor leading to the decline in dark reaction activity. The SPS and SS activities of the two waxy corn inbred lines also decreased

under waterlogging stress, which was consistent with the changes in photosynthetic rate (Fig. 6C,D). Hence, the decrease in the photosynthetic rate in maize under waterlogging might be related to the decrease in the PSII reaction center and dark reaction-related enzyme activities. The study also showed that waterlogging stress caused a decrease in the content of photosynthetic assimilation products in waxy corn leaves. Also, the contents of starch, sucrose, and glucose gradually decreased (Fig. 7), which was similar to previous findings (Watanabe *et al.* 2018, Orsák *et al.* 2021, Qureshi *et al.* 2021). For example, Fan *et al.* (2005) found that the contents of reducing sugar and total soluble sugar gradually decreased in wheat leaves with the prolongation of waterlogging stress. In the present study, exogenous 6-BA significantly increased the content of photosynthetic assimilation products and related enzyme activities in waxy corn leaves under waterlogging stress, which was conducive to improving photosynthetic efficiency.

Conclusions: Waterlogging stress negatively influenced the photosynthetic capacities and leaf ultrastructure of waxy corn leaves. SY-MY13 exhibited better photosynthetic ability than that of SY-XT1 under waterlogging stress. Foliar application of 6-BA at the seedling stage alleviated photosynthesis inhibition under waterlogging stress by inhibiting photosynthetic pigment decomposition, improving stomatal conductance, promoting photosynthetic efficiency, increasing photosynthetic enzyme activities and accumulation of photosynthetic assimilation products, and maintaining the structural integrity of the chloroplasts of waxy corn leaves. Therefore, pretreatment with exogenous 6-BA might be a suitable approach to improving the waterlogging stress of maize cultivation.

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