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Effects of 24-epibrassinolide on photosynthetic parameters in response to different light intensities and CO_2 concentrations in maize seedlings under NaCl stress

Z.-H. HU[+,*], C.-X. WU[+], Y.-J. WANG, and Z.-Y. GONG

College of Life Sciences, Jianghan University, Hubei Province Engineering Research Center for Legume Plants, Wuhan 430056, Hubei, P.R. China

[*]Corresponding author: E-mail: 1259769147@qq.com

Abstract

In order to explore the effect of exogenous 24-epibrassinolide (EBR, 0.1 µM) application on photosynthetic parameters in response to different light intensities and CO_2 concentrations in maize seedlings under NaCl stress (180 mM), this study investigated the light response curve, CO_2 response curve, and diurnal variation of photosynthesis of maize seedlings under NaCl stress. Maize cv. Jinlai 318 was used as the material, and the leaves were sprayed with 0.1 µM EBR. Net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) were determined. The results showed that the light response curve, CO_2 response curve, and diurnal variation trend under the four treatments (180 mM NaCl, 0.1 µM EBR, 180 mM NaCl with 0.1 µM EBR, pure water) were similar. The light saturation point of the leaves under NaCl stress was the lowest (about 1 031 mol m^{-2} s^{-1}), and that in the control group (without stress) was the highest (about 1 344 mol m^{-2} s^{-1}). When the light saturation point was reached (1 030 -1 350 mol m^{-2} s^{-1}), the maximum P_N under NaCl stress was the lowest (about 15.15 µmol m^{-2} s^{-1}), while that under EBR treatment, it was the highest (about 19.86 µmol m^{-2} s^{-1}). NaCl stress significantly reduced chlorophyll *a*, chlorophyll *b*, and carotenoid content and chlorophyll *a/b* ratio, while chlorophyll content significantly increased after EBR treatment. These results showed that exogenous EBR could effectively alleviate the damage to maize seedlings under NaCl stress, delay the light saturation phenomenon, and improve the photosynthetic capacity of maize.

Keywords: 24-epibrassinolide, CO_2 response curve, diurnal variation of photosynthesis, light response curve, *Zea mays*.

Introduction

Maize (*Zea mays* L.) is the main grain crop in China, which is an important material basis for the sustainable development of livestock industry. The growth of maize in the fields is subjected to various abiotic stresses such as soil salinity, drought, light, and temperature, thus leading to a serious decline in its yield, especially when occurs in the seedling stage, and maize seedlings are especially sensitive to salt stress (Jiang *et al*. 2017, Shahzad *et al*. 2021). Salt stress accelerates the reduction of number of stomata in leaves of maize seedlings, destroys photosynthetic pigments in cells, reduces the activities of key enzymes responsible for photosynthetic reactions,

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Abbreviations: AQE - apparent quantum efficiency; BR - brassinosteroid; Car - carotenoids; Chl *a* - chlorophyll *a*; Chl *b* - chlorophyll *b*; C_i - intercellular CO_2 concentration; *E* - transpiration rate; EBR - 24-epibrassinolide; g_s - stomatal conductance; LCP - light compensation point; LSP - light saturation point; PAR - photosynthetic active radiation; P_N - net photosynthetic rate; $P_{N,max}$ - maximum net photosynthetic rate; R^2 - coefficient of determination; RD - dark respiration efficiency; ROS - reactive oxygen species.

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[+]These authors contributed equally.

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and decreases photosynthetic capacity, thereby leading to the decrease of net photosynthetic rate, eventually causing grain yield reduction (Islam *et al*. 2022, Wu *et al*. 2022). Under salt stress, the plants' ability to absorb and assimilate soil nutrients is decreased, thus resulting in nutrient imbalance and growth inhibition, and the accumulation of free radicals and reactive oxygen species (ROS) which can destroy the cell membrane integrity. Plants develop tolerance regulation mechanisms such as regulation of ion homeostasis, accumulation of organic osmotic substances, and ROS scavenging to adapt to salt stress environment (Verma *et al*. 2022). Excessive salt stress damage beyond its regulation range will result in plant death.

Brassinosteroids (BRs) are natural brassinol sterols with the strongest physiological activity, and they play an important role in regulating plant germination, growth, metabolism, and enhancing resistance to stress (Ahammed *et al*. 2020, Barros *et al*. 2021, Li *et al*. 2022, Stachurska *et al*. 2022). 24-epibrassinolide (EBR) is the most active synthetic analogue in the BR family, and it can increase chlorophyll content, photosynthetic efficiency, antioxidant enzyme activity, and decrease malondialdehyde content (Hu *et al*. 2019, Zhong *et al*. 2020). EBR can improve plant tolerance to various stresses such as salt stress, high temperature stress, heavy metal stress, and drought stress (Otie *et al.* 2021, Xia *et al.* 2022a). In maize, EBR can reduce the effects of photoinhibition and photodestruction by protecting photosynthetic organs from the damage caused by ROS, thus improving chloroplast stability and increasing net photosynthetic rate (Li *et al.* 2021, Sun *et al*. 2022).

Photosynthesis is one of the most sensitive physiological processes to environmental factors in plants. The photosynthetic light response curve of plants reflects the characteristics of net photosynthetic rate changing with light intensities. Physiological parameters such as net photosynthetic rate, apparent quantum efficiency, dark respiration rate, light saturation point, and light compensation point obtained by fitting the light response curve are very important for understanding the light response processes of plants. These physiological parameters are the basis for determining photosynthetic capacity, photosynthetic utilization rate, and influence by environmental changes, and the operation status of plant photosynthetic apparatus (Li *et al*. 2018, Zhang *et al.* 2020, Gao *et al.* 2022). Light is the dominant factor of photosynthesis, and CO_2 is the main raw material of photosynthesis. In order to investigate the effects of light intensity and CO_2 on plant photosynthesis, it is necessary to construct the response curves of plant photosynthesis.

In recent years, numerous studies have explored the mechanism by which exogenous plant growth regulators regulate crop growth and development, thus influencing yield. The exogenous hormone application has been reported to improve the growth and physiological characteristics of plants such as stevia, radish, and soybean (Zhang *et al*. 2021, Saravi *et al.* 2022, Xia *et al.* 2022b). At present, there are some reports on the effects of exogenous EBR on the physiological characteristics of maize, but few studies have been conducted to explore the effects of EBR on the photosynthetic response curves of maize under salt stress. In this study, EBR and NaCl stress experiments were carried out on maize seedlings. The light response curve and CO_2 response curve were constructed, and diurnal variation of maize seedlings was monitored. We examined the possible alleviation of maize damage by EBR under NaCl stress by comparing diurnal variation of photosynthesis, light response curve, and CO_2 response curve. This study will provide theoretical basis for the practical application of EBR.

Materials and methods

Plants and experimental design: The experimental material maize (*Zea mays* L.) cv. Jinlai 318 was provided by *Wuhan Shuangxing Agricultural Technology Company* (Wuhan, China). The maize cv. Jinlai 318 was semi-compact plant type with moderate spike position, purple seedling leaf sheath, yellow anthers, purple filaments, cylinder-shaped fruit ear, well bracted-coated cob, and horse tooth-shaped yellow grain.

The experiment was conducted in the Laboratory of Hubei Legume Plant Engineering Technology Research Center, Jianghan University, from October 2021 to December 2021. The uniform, full, and the same-sized maize seeds of Jinlai 318 were sown in plastic pots containing matrix soil with 6 seeds per pot. Subsequently, the pots were placed into the incubator with the temperature set to 20 - 25°C and in the dark; the light was turned on after the seed germination. Seedlings were cultivated to the stage of two leaves and one heart, and then 3 seedlings with the basically same growth status were selected and planted in one pot. The temperature was 15 - 25°C, air humidity 75%, a 16-h photoperiod, and an irradiance 40 - 80 µmol m^{-2} s^{-1}. The stress treatment started when the maize seedlings grew to three leaves and one heart.

The four treatments were as follows: *1*) NaCl + EBR treatment (EN treatment): plants were treated with 180 mM NaCl and sprayed with 0.1 µM EBR; *2*) NaCl treatment (N treatment): plants were treated with 180 mM NaCl; *3*) EBR treatment (ET treatment): plants were treated with pure water and sprayed with 0.1 µM EBR; *4*) control treatment (C treatment): plants were only treated with pure water. Each treatment was repeated 3 times.

The ET treatment group was uniformly sprayed with EBR solution on both sides of leaves. Spraying with 10^{-8} M aqueous solutions of EBR regulates not only PSII but also other parts of the photosynthetic electron transport chain in maize (Rothová *et al.* 2014). The EBR (*Sigma*, St. Louis, MO, USA) was dissolved in 10 mL of absolute ethanol and the distilled water was added to yield a final concentration of 0.1 µM EBR. N treatment group was sprayed with clean water, and 180 mM NaCl was added to the matrix; it has been reported by Yan *et al.* (2016) that the growth of maize plants is impaired under this concentration. Control group was only sprayed with clean water until droplets formed. EN treatment group was sprayed with 0.1 µM EBR, and 180 mM NaCl was added into the matrix. The spray was performed continuously for two days, twice daily, once in the morning and once in the evening and the spray was immediately followed by NaCl stress treatment. The photosynthetic curve changes of maize seedlings were monitored at 6 d post stress treatments.

Construction of light response curve and CO_2 response curves: The *LI-6400XT* portable gas exchange system (*LI-COR*, Lincoln, NE, USA) was used to obtain the related data. The observation time was 9:00 ~ 11:30 h when the weather was clear. Three fully expanded leaves with the same growth status were randomly selected from each group for measurement, and the average values were taken as the measurement result. The light response curves of maize leaves were constructed based on the data from 13 gradient photosynthetic active radiations (PAR; combination of red and blue light sources) including 2 000, 1 700, 1 400, 1 100, 800, 600, 400, 200, 110, 80, 50, 20, and 0 μmol m^{-2} s^{-1}, and the leaf chamber temperature was set to 25°C (He *et al.* 2020). The CO_2 response curves were constructed based on the data of 12 CO_2 concentrations of 400, 300, 200, 100, 50, 400, 500, 700, 900, 1 200, 1 500, and 1 800 μmol mol^{-1} with the temperature in the leaf chamber set to 25°C and the PAR to 1 200 μmol m^{-2} s^{-1} (Ding *et al*. 2019). The data were automatically recorded, and the minimum time and maximum waiting times were set to 120 and 180 s, respectively.

Determination of diurnal variation of photosynthesis: The portable photosynthetic system *LI-6400XT* analyzer was also used to measure the photosynthetic characteristics of leaves at 7:00, 9:00, 11:00, 13:00, 15:00, 17:00, and 19:00 h. Three maize plants with similar leaf growth and leaf area were selected for each group, and the average value was calculated. The net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), and transpiration rate (E) of maize leaves under different treatments were measured.

Determination of chlorophyll content: The chlorophyll content was determined after leaf tissue extraction in 95% (v/v) ethanol, acetone, and water mixture (4.5:4.5:1.0, volume ratio), and the absorbance values of the extract at 663, 645 and 440 nm were measured by UV-visible spectrophotometry. The values of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoids (Car) and chlorophyll *a*/*b* (Chl *a*/*b*) were calculated according to the formulas: Chl *a* [mg g^{-1}(FM)] = (12.7 A_{663} – 2.69 A_{645}) × 10/(1 000 × W); Chl *b* [mg g^{-1}(FM)] = (22.9 A_{645} – 4.68 A_{663}) × 10/(1 000 × W); Car [mg g^{-1}(FM)] = [4.7 A_{440} – 0.27(Chl *a* + Chl *b*)] × 10/(1 000 × W), where 10 is the volume [mL] of the extract and W is the fresh weight [g] of the sample; Chl *a*/*b* = Chl *a*/Chl *b*.

Statistical analysis: The experimental data were expressed as mean of 3 replicates. *Microsoft Excel 2019* and *SPSS 26* software were used for statistical analysis and plotting. The light response curve was fitted by hyperbolic correction model using photosynthesis calculation software (Ye 2010, Ye and Kang 2012), and the maximum photosynthetic rate ($P_{N,max}$), light compensation point (LCP), light saturation point (LSP), apparent quantum efficiency (AQE), and dark respiration efficiency (RD) were obtained by this software.

Results

The dynamic light response curves under different treatments are presented in Fig. 1. With the change of light intensity, net photosynthetic rate (P_N) under the four treatments showed similar patterns. With the increase of light intensity, P_N showed a trend of first increase and then slow decrease. When photosynthetic active radiation (PAR) was less than 1 100 μmol m^{-2} s^{-1}, the P_N of maize under all treatments showed the same trend, namely, P_N was rapidly increased with the increase of PAR. The light compensation point was reached, when the PAR value was between 0 and 20 μmol m^{-2} s^{-1}. When PAR value was between 1 100 and 1 400 μmol m^{-2} s^{-1}, the light saturation point was reached, and P_N was at the maximum value. When PAR was 1 400 μmol m^{-2} s^{-1}, P_N under 180 mM NaCl treatment (N treatment) was 25.02% lower than that in control (C treatment). P_N under 180 mM NaCl with 0.1 μM EBR (EN treatment) was increased by 17.36%, compared with that under N treatment. Overall, P_N value under different treatments was ordered as ET treatment > C treatment > EN treatment > N treatment. When the light intensity continued to increase, P_N values began to decline, and photoinhibition occurred.

Stomatal conductance (g_s) firstly increased and then decreased with the increasing light intensity. When PAR was less than 1 100 μmol m^{-2} s^{-1}, g_s exhibited the same variation under different treatments, namely, it increased rapidly with the increasing PAR. The g_s reached the maximum values (0.07 - 0.11 mol m^{-2} s^{-1}), when PAR value was between 1 100 and 1 400 μmol m^{-2} s^{-1}. The g_s under N treatment was 30.87% lower than that under C treatment, and g_s under EN treatment was increased by 19.85%, compared with that under N treatment. The g_s values under different treatments were ranked as C > ET > EN > N treatment. When the light intensity continued to increase, g_s began to decrease, and g_s under C and ET treatments decreased rapidly, whereas g_s under EN treatment decreased moderately.

Light response curve showed that the variation trend of intercellular CO_2 concentration (C_i) was similar under all 4 treatments, and C_i showed a trend of first decrease and then increase with the increase in light intensity. When PAR was 0 - 200 μmol m^{-2} s^{-1}, CO_2 consumption was extremely rapid. When PAR was above 200 μmol m^{-2} s^{-1}, C_i increased and then became stable under all the 4 treatments. When PAR was 1 400 μmol m^{-2} s^{-1}, C_i under N treatment was 35.94% lower than that under C treatment, and C_i under EN treatment was 45.41% higher than that under N treatment. The C_i values under different treatments followed the order of C > EN > ET > N treatment.

Transpiration rate (E) firstly increased, and then decreased with the increase in light intensity. When the PAR was below 1 100 μmol m^{-2} s^{-1}, E exhibited the same trend under different treatments, and it increased rapidly with the increase in PAR. Transpiration rate reached

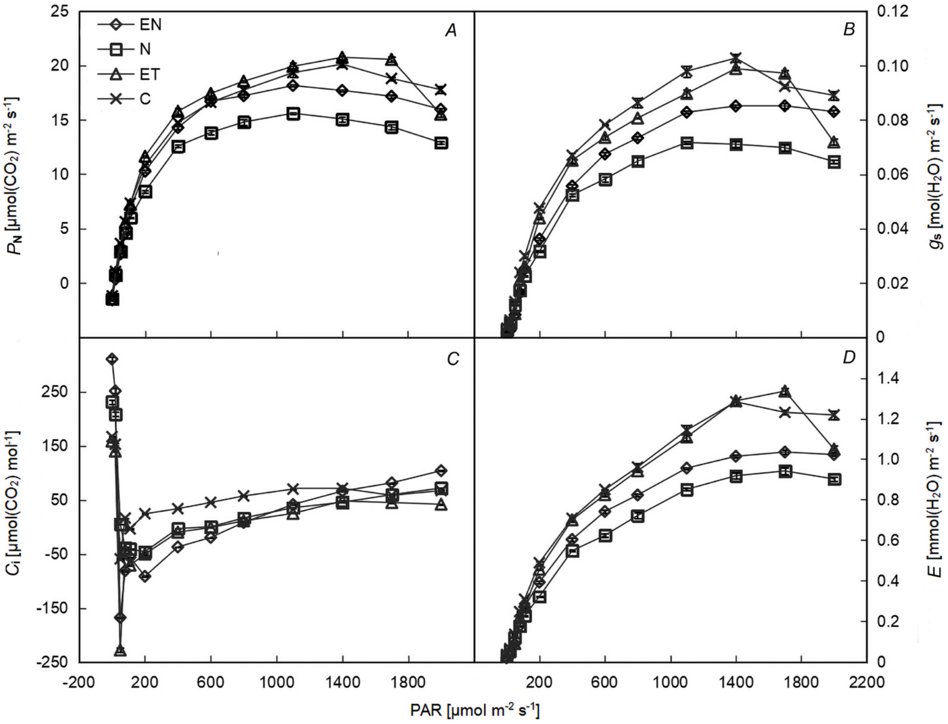

Fig. 1. Light response curves of maize leaves at 6 d after 4 treatments. The effect of EBR (0.1 μM) and NaCl (180 mM) on net photosynthetic rate (P_N, *A*), stomatal conductance (g_s, *B*), intercellular CO_2 concentration (C_i, *C*), and transpiration rate (*E*, *D*). EN - EBR + NaCl treatment, N - NaCl treatment, ET - EBR treatment, C - control. Means of three independent replicates.

its maximum (0.94 - 1.34 mmol m^{-2} s^{-1}) when PAR was between 1 400 and 1 700 μmol m^{-2} s^{-1}. Transpiration rate values under different treatments followed the order of ET > C > EN > N treatment. When PAR was 1 400 μmol m^{-2} s^{-1}, *E* under N treatment was 28.44% lower than that under C treatment, and compared with N treatment, EN treatment increased transpiration rate by 10.46%. When the light intensity continued to increase, *E* began to decline.

The hyperbolic modified model was used to fit the light response curves of each treatment, and the coefficient of determination (R^2) was all above 0.9 under all treatments (Table 1), indicating that this model could well reflect the light response process of maize under different stress conditions. The maximum photosynthetic rate of maize seedlings under N treatment was 20.18% lower than that under C treatment, and that under EN treatment was 17.95% higher than that under N treatment. These results indicated that salt stress significantly inhibited the $P_{N,max}$, and that EBR alleviated the effect of NaCl on P_N. Compared with C treatment, N treatment increased the light compensation point, and decreased the light saturation point, suggesting that under salt stress, the photosynthesis of maize was limited; the utilization rate of low light was decreased; and the adaptability to high light was also decreased.

The CO_2 response curves under four treatments showed that the change trend of P_N in response to CO_2 concentration alteration was similar (Fig. 2). Under appropriate light intensity (1 200 μmol m^{-2} s^{-1}), P_N showed a gradual upward trend with the increase in external CO_2 concentration, indicating that increasing CO_2 concentration could improve P_N of maize seedlings. Compared with C treatment, the N treatment decreased P_N, and P_N under EN treatment was higher than under N treatment.

Under the appropriate light intensity (1 200 μmol m^{-2} s^{-1}), g_s of the four treatments exhibited a similar trend with the increase in CO_2 concentration. With the increase in CO_2 concentration, g_s firstly decreased, and then became stable. When the CO_2 concentration was ≤ 300 μmol mol^{-1}, g_s decreased rapidly with the increase in CO_2 concentration. When CO_2 concentration was > 300 μmol mol^{-1}, g_s was less affected by CO_2 concentration. The g_s became stable after CO_2 concentration reached 1 200 μmol·mol^{-1}. When CO_2 concentration was 1 500 μmol mol^{-1}, g_s under N treatment was 18.05% lower than that under C treatment, and g_s under EN treatment was 16.41% higher than that under N treatment.

Under appropriate light intensity (1 200 μmol m^{-2} s^{-1}), the overall change trend of C_i of maize seedlings under treatments EN, N, ET, and C was similar. When the ambient CO_2 concentration was increasing, C_i under the four treatments gradually increased firstly, and then decreased.

Under the four treatments, the transpiration rate (*E*) exhibited basically the same change trend, decreasing with the increase in CO_2 concentration. The increase of external CO_2 concentration resulted in the decrease in g_s of maize seedlings, thus leading to the decrease in *E*, which further indicated that there was a close correlation between various indexes of maize seedlings gas exchange.

Table 1. Effects of EBR (0.1 µM) and NaCl (180 mM) on light response parameters under different light intensities in leaves of maize at 6 d post treatments. EN - EBR + NaCl treatment, N - NaCl treatment, ET - EBR treatment, C - control. Data were fitted by hyperbolic correction model. LCP - light compensation point, LSP - light saturation point, AQE - apparent quantum efficiency, RD - dark respiration efficiency.

Treatment	$P_{N,max}$ [µmol m^{-2} s^{-1}]	LCP [µmol m^{-2} s^{-1}]	LSP [µmol m^{-2} s^{-1}]	AQE	RD	R^2
EN	17.87	16.14	1 093.92	0.1018	1.55	0.9992
N	15.15	12.54	1 031.83	0.0865	1.04	0.9970
ET	19.86	9.25	1 074.38	0.0951	0.86	0.9843
C	18.98	8.99	1 344.84	0.1041	0.90	0.9961

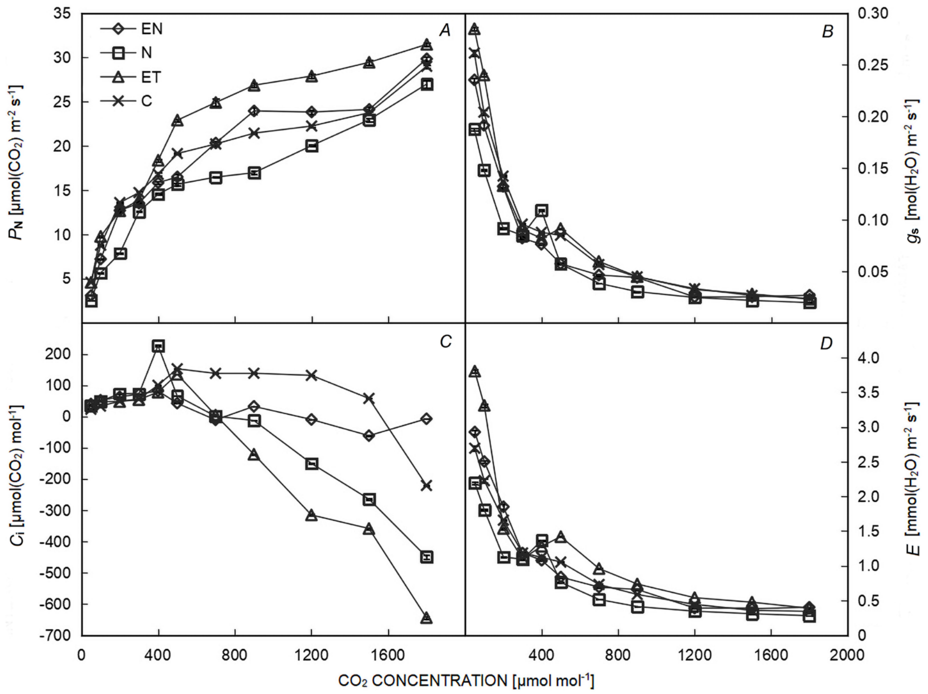

Fig. 2. Net photosynthetic rate (P_N, *A*), stomatal conductance (g_s, *B*), intercellular CO_2 concentration (C_i, *C*), and transpiration rate (*E*, *D*) in response to different CO_2 concentrations (CO_2 response curve) under EBR (0.1 µM) and/or NaCl (180 mM) in leaves of maize at 6 d after treatments. EN - EBR + NaCl treatment, N - NaCl treatment, ET - EBR treatment, C - control. Means of three independent replicates.

As shown in Fig. 3, the P_N diurnal variation of maize seedlings under 4 different treatments displayed an asymmetric bimodal curve, but the time to reach the peak was different under different treatments. Under all treatments, the P_N was lowest in the morning and evening, and P_N even had a negative value at 7:00 and 19:00 h with no significant difference in P_N among different treatments. Under all treatments, P_N was ascending from 7:00 to 9:00 h and from 11:00 to 17:00 h; it was further increased presenting double peaks, and from 17:00 to 19:00 h was the period when P_N experienced a rapid decline under each treatment with a linear decline trend. At 7:00, 9:00, 17:00, and 19:00 h, there was a slight difference in P_N among different treatments, whereas at other time points, there were greater differences. Under EN and N treatments, P_N reached its peak at 13:00 and 17:00 h, while under ET and C treatments, P_N reached its peak at 11:00 and 15:00 h. Different treatments had similar effects on diurnal variation of g_s. Under all 4 treatments, g_s diurnal variation reached the peak from 9:00 to 11:00 h; the rising period of g_s occurred from 7:00 to 9:00 h; afterwards, it maintained a high level from 9:00 to 17:00 h; after 17:00 h, g_s showed a downward trend, and at 19:00 h, it reached the lowest value. Under different treatments, the C_i diurnal variation of maize seedlings showed an obvious curve. Under EN and N treatments, the C_i diurnal variation reached its daily lowest value at 13:00 h, while under ET and C treatments, it reached its lowest value at 15:00 h. The diurnal variation of transpiration rate (*E*) under 4 different treatments exhibited a roughly same trend with that of g_s. The *E* peak value was observed from 9:00 to 11:00 h under 4 different treatments, and *E* maintained high from 9:00 to 17:00 h and exhibited a downward trend after 17:00 h.

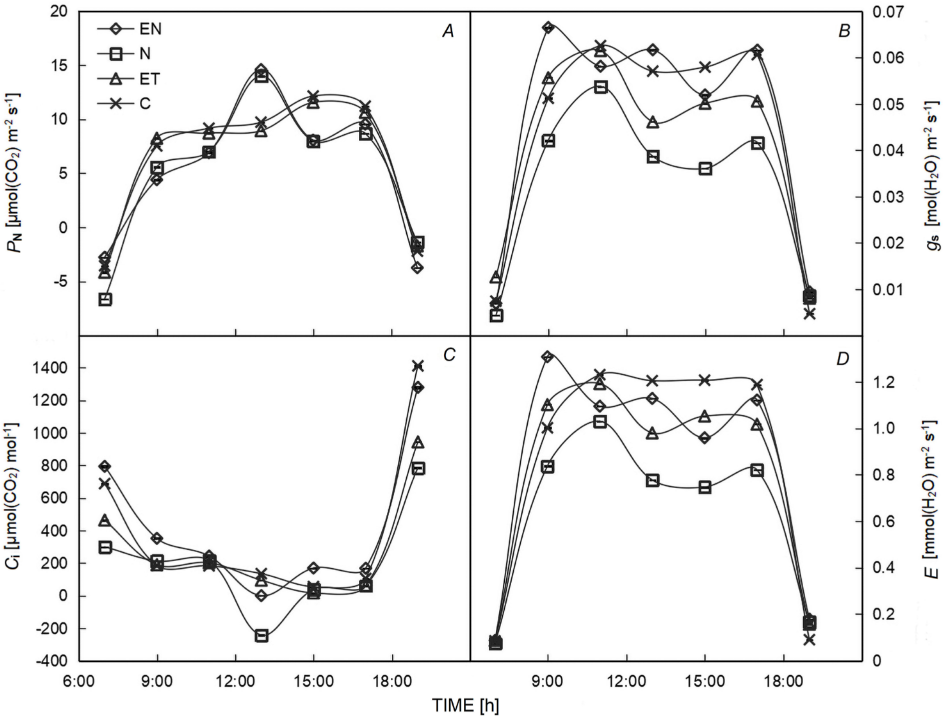

Fig. 3. Diurnal variation of net photosynthetic rate (P_N, *A*), stomatal conductance (g_s, *B*), intercellular CO_2 concentration (C_i, *C*), and transpiration rate (*E*, *D*) at different time points under EBR (0.1 μM) and/or NaCl (180 mM) concentrations in leaves of maize after 6 d of treatment. EN - EBR + NaCl treatment, N - NaCl treatment, ET - EBR treatment, C - control. Means of three independent replicates.

The chlorophyll content of maize leaves showed significant changes under NaCl stress (Fig. 4). Compared with C treatment, N treatment decreased the content of Chl *a*, Chl *b*, and Car by 40.28, 24.93, and 39.40%, respectively. Compared with N treatment, EN treatment increased the content of Chl *a*, Chl *b*, and Car by 39.65, 22.06, and 39.71%, respectively. Chlorophyll *a*/*b* ratio under N treatment was 20.40% lower than that under C treatment. Chl *a*/*b* under EN treatment was increased by 14.51%, compared with that under N treatment.

Discussion

Light response curve is an important method to understand the photochemical efficiency of plants, and it can be used to determine the operation status of photosynthesis and the utilization efficiency of light intensity by plants (Zhang 2017). Light response curve has been used to investigate the influence of environment on plant physiological processes (Li *et al.* 2017). The relationship between plant photosynthetic mechanism and environment can be determined by light response parameters. The light saturation point can reflect the ability of plants to utilize strong light, and the higher the saturation point, the greater the photosynthetic utilization range (Zhang *et al.* 2018). The light compensation point represents the light intensity when photosynthesis and respiration are balanced (Li *et al.* 2017). The maximum P_N of leaves is the maximum photosynthetic capacity of leaves during a day. Apparent quantum efficiency indicates the ability of plants to absorb, transfer, and utilize weak light under low light conditions. The dynamic response process of P_N is response to PAR. The light response curve can be divided into three stages including linear rising stage, curve rising stage, and stationary stage (He *et al.* 2020). Under various stresses, photosynthetic processes and photosynthetic capacity of plants can be reflected by light response parameters. These parameters are of great significance for understanding plant growth and development, and they are also powerful tools for determining plant photosynthetic capacity (Zhang *et al.* 2019). The utilization regularities of different light intensities during maize growth and development can be accurately and effectively reflected by light response curve. Our light response curve of maize seedlings showed that under the four treatments, P_N, g_s, and *E* firstly increased and then slowly decreased with the increase of light intensity, and that C_i showed a trend of first decrease and then increase. All the results were consistent with the three stages (linear rising stage, curve rising stage, and stationary stage) of dynamic response of plants to light intensity. The light response curve of maize under NaCl stress treatment completed these three stages in advance, and under the combined treatment of EBR and NaCl, the light saturation was delayed. The results indicated that EBR application could expand the adaptation range of maize to salt stress and enhance the photosynthetic capacity of maize. One previous study reported that under low temperature stress, the light response curve of *Panax notoginseng* moved down collectively, and that the light saturation point shifted to the left (Ding *et al.* 2020). Another study fitted the light response curves of different

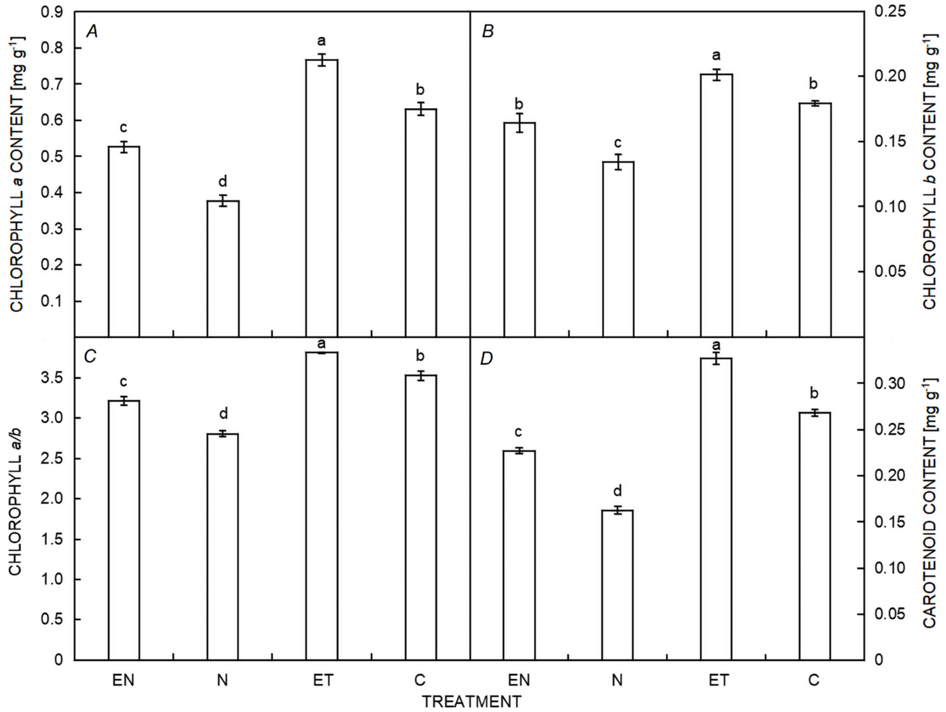

Fig. 4. Effects of EBR (0.1 μM) and NaCl (180 mM) on chlorophyll (Chl) *a* content (*A*), Chl *b* content (*B*), Chl *a*/*b* ratio (*C*), and carotenoid content (*D*) in leaves of maize after 6 d of treatment. EN - EBR + NaCl treatment, N - NaCl treatment, ET - EBR treatment, C - control. Means ± SDs, *n* = 3; bars with *different letters* are significantly different at 5% level.

drought-resistant maize varieties, and found that the AQE, P_N, and LSP of maize varieties decreased under drought stress (Liu *et al.* 2008). In the studies of cowpea, eggplant, soybean, and pepper, EBR significantly alleviated the damage of stress on the photosynthetic system (Wu *et al.* 2014, Lima and Lobato 2017, Khamsuk *et al*. 2018, Yang *et al.* 2019a, dos Santos *et al.* 2021). Our data showed that the P_N under EBR treatment was the highest, indicating that EBR application could enhance the utilization of strong light by maize. Our data also displayed that EBR treatment decreased dark respiration rate, thus reducing the consumption of photosynthates, eventually increasing the accumulation of dry matter. Compared with NaCl treatment, EBR and NaCl treatment increased the P_N and apparent quantum efficiency, indicating that EBR application could alleviate the damage to the maize photosynthetic system by NaCl stress, thereby enhancing the absorption and utilization capacity of radiation.

CO_2 response curve has been widely used for evaluating the photosynthetic capacity of plants, and it can reflect the change characteristics of photosynthetic rate in response to CO_2 concentrations (Yao *et al.* 2020). It has been reported that nitrogen application has an obvious regulatory effect on CO_2 response curve (Zhu *et al.* 2020), and that the stomatal conductance and transpiration rate of maize exhibit wavy changes with the increase in CO_2 concentration (Han 2021). Our data also showed that the inhibition effect of the $P_{N,max}$ did not appear, when CO_2 reached 1 800 μmol mol^{-1}, indicating that maize treated with EBR and/or NaCl had strong photosynthetic potential. CO_2 is one of the key factors affecting maize growth. With the continuous increase in CO_2 concentration, the $P_{N,max}$ was further enhanced so as to achieve better growth status. As one of the key factors affecting photosynthesis, C_i directly affected the P_N value. The low C_i is caused by high P_N, and thus the variation trend of C_i is opposite to that of P_N.

The diurnal variation of leaf photosynthesis is an important parameter to reflect the change of plant metabolism and substance accumulation within one day, and these parameters are important for the analyses of the influence of environmental factors on plant growth and metabolism (Li *et al.* 2020). In the process of plant growth and development, environmental factors such as light and temperature change regularly, thus resulting in the changes in photosynthetic parameters such as P_N, g_s, and E (Gao *et al.* 2021). Generally, different plants have different characteristics of diurnal variation of photosynthesis. Many studies have revealed that the diurnal variations of P_N include "single peak" type, "double peak" type, and multiple variation type (Lei and Zhang 2019, Yang *et al.* 2019b), and that *Passiflora edulis*, *Pueraria lobata*, and *Lonicera japonica* are typical "double peak" type, with an obvious "siesta" phenomenon, while *Populus euphroneum* growing in dry areas is typical of the "single peak" type (Wu *et al.* 2015). It has been reported that P_N of maize under monoculture or intercropping modes showed single peak curve at different growth stages (Su *et al.* 2022). Our data showed that the diurnal variation of P_N of maize under different treatments exhibited an asymmetric double peak curve, but the time to reach the peak varied under different treatments, indicating that the diurnal variation of P_N resulted from of long-term adaptation to climate. The change trend of C_i showed a "V" curve trend of first decrease and then increase. The diurnal variation trends of E and g_s were roughly the same, displaying a double peak variation trend with a "siesta" phenomenon.

The activity of chlorophyllase in maize leaves increased after salt stress, which led to the accelerated degradation of chlorophyll, the decrease of chlorophyll content, and the inhibition of chlorophyll synthesis (Wang *et al.* 2021). It has been reported that exogenous spermidine (Spd) treatment could significantly increase the content of chlorophyll and carotenoid in maize leaves, increase the Chl *a*/*b* ratio, and significantly alleviate the degradation of chlorophyll in maize leaves caused by drought stress (Li 2019). One previous study reported that KNO_3 combined with melatonin could increase chlorophyll content in maize under drought stress (Ahmad *et al.* 2022). Another study showed that different concentrations of EBR positively regulated the content of Chl *a* and *b* in maize leaves under salt stress (Chen *et al.* 2022). Our data showed that NaCl stress significantly reduced Chl *a*, Chl *b*, and carotenoid content and Chl *a*/*b*, while Chl content significantly increased after EBR treatment, indicating that spraying with EBR could increase Chl content of maize seedlings under NaCl stress.

In conclusion, our results indicated that the maize leaves exhibited significant changes in photosynthetic parameters and the response curves under NaCl stress, and that exogenous EBR application could alleviate the damage to maize seedlings caused by NaCl stress, improve the net photosynthetic rate, delay the light saturation phenomenon, and enhance the photosynthetic capacity of maize.

References

Ahammed G.J., Li X., Liu A., Chen S.: Brassinosteroids in plant tolerance to abiotic stress. - J. Plant Growth Regul. **39**: 1451-1464, 2020.

Ahmad S., Wang G.-Y., Muhammad I. *et al.*: Melatonin and KNO_3 application improves growth, physiological and biochemical characteristics of maize seedlings under waterlogging stress conditions. - Biology **11**: 99, 2022.

Barros Jr. U.O., Lima M.D.R., Alsahli A.A., Lobato A.K.S.: Unraveling the roles of brassinosteroids in alleviating drought stress in young *Eucalyptus urophylla* plants: Implications on redox homeostasis and photosynthetic apparatus. - Physiol. Plantarum **172**: 748-761, 2021.

Chen L.Q., Wen S.J., Zhang J.J. *et al.*: Effects of different concentrations of EBR on physiological characteristics and the expression levels of CAT enzyme and ZMPIP24 genes in maize seedlings under salt stress. - J. Maize Sci. **30**: 100-107, 2022.

Ding L.K., Kan F., Li L. *et al.*: [Photosynthetic responses models of maize to light intensity and CO_2 concentration in semi-arid area of central Gansu.] - Jiangsu Agric. Sci. **47**: 86-91, 2019. [In Chinese]

Ding Y.H., Yang Z.Q., Xu C. *et al.*: [Effect of low temperature stress on light response curve fitting of *Panax notoginseng* leaves.] - J. Northeast For. Univ. **48**: 41-49+55, 2020. [In Chinese]

dos Santos L.R., Paula L.d.S., Pereira Y.C. *et al.*: Brassinosteroids-mediated amelioration of iron deficiency in soybean plants: beneficial effects on the nutritional status, photosynthetic pigments and chlorophyll fluorescence. - J. Plant Growth Regul. **40**: 1803-1823, 2021.

Gao J.F., Zhou W., Chen S.H. *et al.*: [Diurnal variation of photosynthesis and effect of soil moisture on photosynthetic characteristics of *Kadsura coccinea*.] - South China Fruit. **50**: 80-86, 2021. [In Chinese]

Gao P., Tian Z., Lu Y. *et al.*: A decision-making model for light environment control of tomato seedlings aiming at the knee point of light-response curves. - Comput. Electron. Agr. **198**: 107103, 2022.

Han Y.X.: [Effects of regulated deficit irrigation with different CO_2 concentrations on photosynthetic characteristics and stomatal parameters of maize.] Thesis. Pp. 32-33. Hebei University of Engineering, Hebei 2021. [In Chinese]

He Z., Liu Z., Miao F.F. *et al.*: [Comparison of four light response curve models of drip irrigation maize in Ningxia Yellow River irrigation district.] - J. Maize Sci. **28**: 124-131, 2020. [In Chinese]

Hu W.H., Yan X.H., He Y., Xi R.: 24-epibrassinolide alleviate drought-induced photoinhibition in *Capsicum annuum* via up-regulation of AOX pathway. - Sci. Hortic.-Amsterdam **243**: 484-489, 2019.

Islam A.T.M.T., Koedsuk T., Ullah H. *et al.*: Salt tolerance of hybrid baby corn genotypes in relation to growth, yield, physiological, and biochemical characters. - S. Afr. J. Bot. **147**: 808-819, 2022.

Jiang C.Q., Zu C.L., Lu D.J. *et al.*: Effect of exogenous selenium supply on photosynthesis, Na^+ accumulation and antioxidative capacity of maize (*Zea mays* L.) under salinity stress. - Sci. Rep.-UK **7**: 42039, 2017.

Khamsuk O., Sonjaroon W., Suwanwong S. *et al.*: Effects of 24-epibrassinolide and the synthetic brassinosteroid mimic on chili pepper under drought. - Acta Physiol. Plant. **40**: 106, 2018.

Lei L., Zhang Y.N.: [The diurnal variation of photosynthesis and effect of drought stress on photosynthetic characteristics of *Lysimachia davurica*.] - Pratacult. Sci. **36**: 658-665, 2019. [In Chinese]

Li B.B., Fu Y.S., Li X.X. *et al.*: Brassinosteroids alleviate cadmium phytotoxicity by minimizing oxidative stress in grape seedlings: Toward regulating the ascorbate-glutathione cycle. - Sci. Hortic.-Amsterdam **299**: 111002, 2022.

Li L.J.: [Mitigation effect and regulation mechanism of exogenous spermidine (Spd) on maize under drought stress.] Thesis. Pp. 68-69. Northeast Agricultural University, Harbin 2019. [In Chinese]

Li L.Y., Li J., Tong X.J. *et al.*: [Simulation on photosynthetic light-responses of leaves of *Quercus variabilis* and *Robinia pseudoacacia* under different light conditions.] - Chin. J. Appl. Ecol. **29**: 2295-2306, 2018. [In Chinese]

Li Q.Y., Ding X.X., Jia L.F. *et al.*: [Comparative study on photosynthetic characteristics of early and late fruiting walnut.] - Southwest China J. Agric. Sci. **33**: 1417-1422, 2020. [In Chinese]

Li Y., Sun Y.P., Ma C. *et al.*: 24-epibrassinolide enhanced cold tolerance of winter turnip rape (*Brassica rapa* L.). - Biologia **76**: 2859-2877, 2021.

Li Y.B., Song H., Zhou L. *et al.*: [Modeling study on photosynthetic-light response curves of a C_4 plant, maize.] - Chin. J. Plant Ecol. **12**: 1289-1300, 2017. [In Chinese]

Lima J.V., Lobato A.K.S.: Brassinosteroids improve photosystem II efficiency, gas exchange, antioxidant enzymes and growth of cowpea plants exposed to water deficit. - Physiol. Mol. Biol. Pla. **23**: 59-72, 2017.

Liu M., Qi H., Sun S.X. *et al.*: [Effect of water stress on maize photosynthetic characteristics.] - J. Maize Sci. **4**: 86-90, 2008. [In Chinese]
Otie V., Udo I., Shao Y. *et al.*: Salinity effects on morpho-physiological and yield traits of soybean (*Glycine max* L.) as mediated by foliar spray with brassinolide. - Plants-Basel **10**: 541, 2021.
Rothová O., Holá D., Kočová M. *et al.*: 24-Epibrassinolide and 20-hydroxyecdysone affect photosynthesis differently in maize and spinach. - Steroids **85**: 44-57, 2014.
Saravi H.B., Gholami A., Pirdashti H. *et al.*: Improvement of salt tolerance in *Stevia rebaudiana* by co-application of endophytic fungi and exogenous spermidine. - Ind. Crop. Prod. **177**: 114443, 2022.
Shahzad K., Hussain S., Arfan M. *et al.*: Exogenously applied gibberellic acid enhances growth and salinity stress tolerance of maize through modulating the morpho-physiological, biochemical and molecular attributes. - Biomolecules **11**: 1005, 2021.
Stachurska J., Rys M., Pociecha E. *et al.*: Deacclimation-induced changes of photosynthetic efficiency, brassinosteroid homeostasis and *BRI1* expression in winter oilseed rape (*Brassica napus* L.) - relation to frost tolerance. - Int. J. Mol. Sci. **23**: 5224, 2022.
Su K.N., Ma Y.H., Liao C.Y. *et al.*: [Effects of walnut - Maize intercropping on photosynthetic day changes, overground part and root characteristics of plant.] - Mol. Plant Breeding **20**: 5213-5220, 2022. [In Chinese]
Sun S., Yao X., Liu X. *et al.*: Brassinolide can improve drought tolerance of maize seedlings under drought stress: By inducing the photosynthetic performance, antioxidant capacity and *ZmMYB* gene expression of maize seedlings. - J. Soil Sci. Plant Nutr. **22**: 2092-2104, 2022.
Verma S., Negi N.P., Pareek S. *et al.*: Auxin response factors in plant adaptation to drought and salinity stress. - Physiol. Plantarum **174**: e13714, 2022.
Wang Y.F., Xue Y.W., Yang K.J.: [Effects of phosphorus on lipid peroxidation and antioxidant enzyme activities in the maize seedlings under salt stress.] - J. Maize Sci. **29**: 69-74, 2021. [In Chinese]
Wu D., Long X.Q., Zhang J.L. *et al.*: [Diurnal variation of photosynthesis of four lianas in rocky desertification area of karst peak cluster depression.] - Jiangsu Agric. Sci. **43**: 254-256, 2015. [In Chinese]
Wu J.X., Nadeem M., Galagedara L. *et al.*: Effects of chilling stress on morphological, physiological, and biochemical attributes of silage corn genotypes during seedling establishment. - Plants-Basel **11**: 1217, 2022.
Wu X.X., Yao X.F., Chen J.L. *et al.*: Brassinosteroids protect photosynthesis and antioxidant system of eggplant seedlings from high-temperature stress. - Acta Physiol. Plant. **36**: 251-261, 2014.
Xia H., Liu X.L., Wang Y.M. *et al.*: 24-Epibrassinolide and nitric oxide combined to improve the drought tolerance in kiwifruit seedlings by proline pathway and nitrogen metabolism. - Sci. Hortic.-Amsterdam **297**: 110929, 2022a.
Xia M., Li J.W., Luo Z.R. *et al.*: [Research on mechanism of exogenous melatonin effects on radish growth and resistance to *Alternaria brassicae*.] - Acta Hortic. Sin. **49**: 548-560, 2022b. [In Chinese]
Yan H.P., Peng Y.L., Zhao X.Q., Lyu Y.: [Effect of exogenous 24-epibrassinolide on seed germination and seedling growth of maize under different stress.] - J. Nucl. Agric. Sci. **30**: 988-996, 2016. [In Chinese]
Yang H., Han W.D., Gao X.M.: [Diurnal photosynthetic characteristics of *Uvaria grandiflora* Roxb. and its correlation with environmental factors.] - Ecol. Sci. **38**: 92-97, 2019b. [In Chinese]
Yang P., Wang Y., Li J., Bian Z.: Effects of brassinosteroids on photosynthetic performance and nitrogen metabolism in pepper seedlings under chilling stress. - Agronomy **9**: 839, 2019a.
Yao B., Xu C., Wang J.P. *et al.*: [Light and CO_2 response characteristics of different parental bloodline maize hybrids.] - Mol. Plant Breeding **18**: 1297-1306, 2020. [In Chinese]
Ye Z.P.: [A review on modeling of responses of photosynthesis to light and CO_2.] - Chin. J. Plant Ecol. **34**: 727-740, 2010. [In Chinese]
Ye Z.P., Kang H.J.: [Study on biological significance of coefficients in modified model of photosynthesis-irradiance.] - J. Yangzhou Univ. **33**: 51-57, 2012. [In Chinese]
Zhang L.L.: [Light response curves of 5 plants and screening the optimal model.] - J. Southwest For. Coll. **37**: 20-25, 2017. [In Chinese]
Zhang M.C., He S.Y., Qin B. *et al.*: [Effects of exogenous melatonin on morphology, photosynthetic physiology, and yield of spring soybean variety Suinong 26 under drought stress.] - Acta Agron. Sin. **47**: 1791-1805, 2021. [In Chinese]
Zhang N., Su X., Zhang X.B. *et al.*: Monitoring daily variation of leaf layer photosynthesis in rice using UAV-based multi-spectral imagery and a light response curve model. - Agr. Forest Meteorol. **291**: 108098, 2020.
Zhang X.Q., Bai L.F., Lu Z.Y. *et al.*: [Light-response curves and yield of maize in grain-filling period.] - Acta Agric. Bor.-Sin. **34**: 96-103, 2019. [In Chinese]
Zhang X.W., Liu T.D., Cheng G.Z. *et al.*: [Effects of different light treatments on light response curve of maize leaves.] - Liaoning Agric. Sci. **1**: 13-16, 2018. [In Chinese]
Zhong W.X., Xie C.C., Hu D. *et al.*: Effect of 24-epibrassinolide on reactive oxygen species and antioxidative defense systems in tall fescue plants under lead stress. - Ecotox. Environ. Safe. **187**: 109831, 2020.
Zhu X.Q., Jia M., Shi P.J. *et al.*: [Fitting analysis of CO_2 response curve of tobacco under different nitrogen fertilizer levels.] - J. Southern Agric. **51**: 537-544, 2020. [In Chinese]