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Effects of Drought Stress on the Metabolic Properties of Active Oxygen Species, Nitrogen and Photosynthesis in Cucumber 'Jinchun No. 5' Seedlings

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Abstract

To gain a physiological understanding of the effect of drought stress on the cucumber (*Cucumis sativus* L.), we subjected 'Jinchun No. 5' seedlings to three treatments: control (C), moderate drought stress (MDS) and severe drought stress (SDS) during exposure to high temperature and strong light. We then investigated the metabolic properties of active oxygen species, nitrogen and photosynthesis in leaves from the 3rd to 15th days of treatment. The amounts of active oxygen species superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) increased with increasing drought stress in C, MDS and SDS. The malondialdehyde (MDA) content, which reflects the excess metabolism of active oxygen was increased on the 12th and 15th days of treatment with increasing drought stress. The nitrate-reductase (NR) activity of nitrogen metabolism indicators decreased on the 3rd to 15th days of treatment, while soluble protein content increased on the 9th to 15th days of treatment with increasing drought stress. With respect to the photosynthetic properties, stomatal conductance (g_s) decreased from the 6th to 15th days of treatment with increasing drought stress, no significant difference was seen in sub-stomatal CO_2 concentration (C_i) between all three treatment plots, transpiration rate (E) was lower in SDS than C, and the net photosynthesis rate (A) decreased with increasing drought stress. A significant correlation between g_s, H_2O_2 and E with A, and secondly between MDA and NR with A was observed. These findings suggest that drought stress during periods of high temperature and strong light results in the generation and accumulation of abundant active oxygen species, and inhibition of nitrogen and photosynthesis metabolism in cucumber 'Jinchun No. 5' seedlings.

Discipline: Horticulture
Additional key words: hydrogen peroxide, malondialdehyde, net photosynthesis rate, nitrate-reductase, stomatal conductance

Introduction

The cucumber (*Cucumis sativus* L.) has shallow root distribution, its leaves are large and thin, it is fast growing, and its moisture requirements and tolerance to low light is high. Accordingly, the cucumber is affected by high temperatures, strong light and drought conditions when cultivated from spring to fall compared with cultivation in winter/spring, resulting in a considerably shortened harvest period and a substantial decline in yield and quality[10,14,30]. The threat of drought stress on plants is increasing with global warming and reduced water resources, meaning it is necessary to closely investigate the physiological damage of drought stress on crops with high water requirements such as the cucumber. Cucumber seedlings are more sensitive to water change compared to adult plants and thus heavily studied in drought stress research[4,7,13,14,16-18,23,26,30].

Water is the key requirement for plant cells. Drought stress results in the generation and accumulation of active oxygen species, the peroxidation of membrane lipids, and impairment of nitrogen metabolism, photosynthesis, growth and development, causing a substantial decline in yield and quality[4,7,13,14,16-18,23,26,30]. To what extent does the level of active oxygen species increase in the leaves of cucumber under drought stress; how much is nitrogen metabolism, which plays a prominent role in production, inhibited; and how is photosynthesis, which strongly depends on water metabolism, affected? These basic physiological data facilitate the cultivation of drought-resistant cucumber cultivars and physiological research into and the development of preventive techniques against damage

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caused by drought stress. In this study we conducted different drought stress treatments by subjecting cucumber ‘Jinchun No. 5’ seedlings to high temperature and strong light, and discussed the effects of drought stress on active oxygen species, nitrogen and photosynthesis metabolisms.

Materials and methods

1. Material under test

Cucumber ‘Jinchun No. 5’ seedlings were grown in a plastic house from July 12, 2011, and seedlings with 4 true leaves were subjected to drought stress treatment from August 1. The seedling pots (10 cm diameter by 12 cm tall) contained a soil mixture of 3:1:1 (v/v/v) soil, peat, and vermiculite.

2. Drought stress treatment

Three plots were prepared for the drought stress treatment: control (C), moderate drought stress (MDS) and severe drought stress (SDS). Thirty pots (plants) were placed in each treatment plot, with 25 × 100 cm space around each, i.e. 4 plants·m^{-2}. The seedling pots were weighed daily at 19:00 from the start of the drought stress treatment, and after calculating the average weight of the soil mixture in 5 pots from each plot (excluding the weight of the seedlings; weight was determined based on experience), we determined the relative humidity of the soil mixture based on the dry weight and field moisture capacity of the soil.

Relative humidity = moisture content ÷ field moisture capacity × 100%

Moisture content = water weight ÷ soil dry weight × 100%

Field moisture capacity = maximum water weight ÷ soil dry weight × 100%

The dry weight of the soil was the weight of the soil mixture in the pot dried at 105°C before the start of treatment, and the field moisture capacity was 43%.

As the relative humidity of an appropriate soil mixture for the growth and development of the cucumber is generally 80%–90%[29], in this study, water was given adequately when the relative humidity of the soil mixture decreased to about 80% in C, about 65% in MDS and about 45% in SDS respectively. The variation in the relative humidity of C, MDS and SDS was about 80%–100%, 65%–100% and 45%–100%, respectively.

3. Sampling

The second and third leaves from 4 plants from every treatment plot were sampled at about 10:30 on the 3rd, 6th, 9th, 12th and 15th days of drought stress treatment, and used to analyze the active oxygen species and nitrogen metabolism. The leaves were immediately finely chopped and 2 g from each plant was weighed. The samples were then frozen in liquid nitrogen and stored at −80°C. The weather on the 3rd, 12th and 15th days of treatment was fine, the 6th day was overcast and the 9th day had light rain.

4. Measurement and analysis

During the drought stress treatment period, we measured the leaf surface environmental factor photosynthetic photon-flux density (PPFD), leaf-chamber temperature and CO_2 concentration; analyzed the amounts of active oxygen species superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) in the leaves, the malondialdehyde (MDA) content, which reflects excess metabolism of active oxygen, the activity of nitrate-reductase (NR) and soluble protein content as indicators of nitrogen metabolism; as well as investigating the photosynthetic properties of stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i), transpiration rate (E) and net photosynthesis rate (A).

The leaf surface PPFD, leaf-chamber temperature and CO_2 concentration for the second and third true leaves from each of the 4 plants from each plot were measured with a portable photosynthesis system (LC*i*-002/B, ADC 6 BioScientific Ltd., Hertfordshire, U.K.) on a leaf-chamber area of 6.25 cm^2 at about 10:00 before sampling took place. During measurement, horizontal sunlight was kept in the leaf chamber.

The amounts of $O_2^{\cdot -}$, H_2O_2, soluble protein and MDA were determined in accordance with the method used by Li et al. (2013)[14].

NR activity was measured using the methods of Kaiser & Lewis (1984)[8] and Li et al. (2002)[11]. Each 2 g leaf sample was homogenized and extracted in an 8 mL 50 mM phosphate buffer (pH 7.5) containing 3 mM Na_2-EDTA and 0.5% (w/v) polyvinylpolypyrrolidone. After centrifuging for 10 min at 12,000 × g, enzyme activities were measured immediately using the supernatant as a crude enzyme solution. Each assay mixture tube contained 0.1 mL 0.5 mM potassium phosphate buffer (pH 7.5), 0.1 mL NADH (1 mg·mL^{-1}), 0.2 mL 0.1 M KNO_3 and 0.1 mL crude enzyme solution made up to a final volume of 2 mL with distilled water. After 15 min incubation at 28°C, the reaction was stopped by adding 1 mL of 20 mM sulphanilic acid in 1.5 M HCl, followed by 1 mL of 10 mM N-(1-naphthyl)ethylenediamine dihydrochloride in 1.5 M HCl. The color was allowed to develop for 15 min at 25ºC, then centrifuged at 5,000 × g for 5 min to remove suspended matter. Nitrite content was determined by measuring absorbance at 545 nm. NR activity was expressed as NO_2^- μmol·g^{-1}FW·h^{-1}.

The photosynthetic properties were determined using the photosynthesis system at the same time as PPFD, leaf-chamber temperature and CO_2 concentration were measured.

5. Statistical analysis

The data were expressed as mean ± standard errors (SE) (n = 4), significant difference using Tukey's test ($P < 0.05$), significant of correlation coefficient using *t*-test ($P <$ 0.1, 0.05 and 0.01).

Results

1. Environmental factors of leaf surface

PPFD was at the same level on the 3rd and 12th days of treatment, decreased significantly on the 6th (overcast) and 9th (light rain) days, and was somewhat low on the 15th day (Fig. 1). There was little difference in PPFD between C, MDS and SDS ($P > 0.05$). The leaf-chamber temperature was constant on the 3rd, 12th and 15th days, 2.5°C lower on the 6th day and 4.2°C lower on the 9th day respectively. The leaf-chamber temperature was 1.3°C higher in MDS than C and 1.9°C higher in SDS. There was no difference in CO_2 concentration between the three treatment plots ($P > 0.05$).

2. Metabolic property of active oxygen species

The amount of $O_2^{\cdot -}$ in MDS exceeded C on the 6th, 12th and 15th days of treatment, was greater in SDS than C from the 3rd to 15th days, and was greater in SDS than MDS on the 6th and 15th days ($P < 0.05$) (Fig. 2). The amount of H_2O_2 in MDS exceeded C on the 3rd, 12th and 15th days, was greater in SDS than C from the 3rd to 15th days, and was greater in SDS than MDS from the 6th to 15th days ($P < 0.05$).

3. Metabolic property of nitrogen

The amount of soluble protein was greater in MDS than C on the 12th and 15th days of treatment and was greater in SDS than both C and MDS from the 9th to 15th days ($P < 0.05$) (Fig. 3). The amount of MDA in MDS exceeded C on the 12th and 15th days and was greater in SDS than both C and MDS on the 12th and 15th days ($P < 0.05$). NR activity was lower in MDS and SDS compared with C from the 3rd to 15th days and lower in SDS than MDS from the 3rd to 9th days ($P < 0.05$).

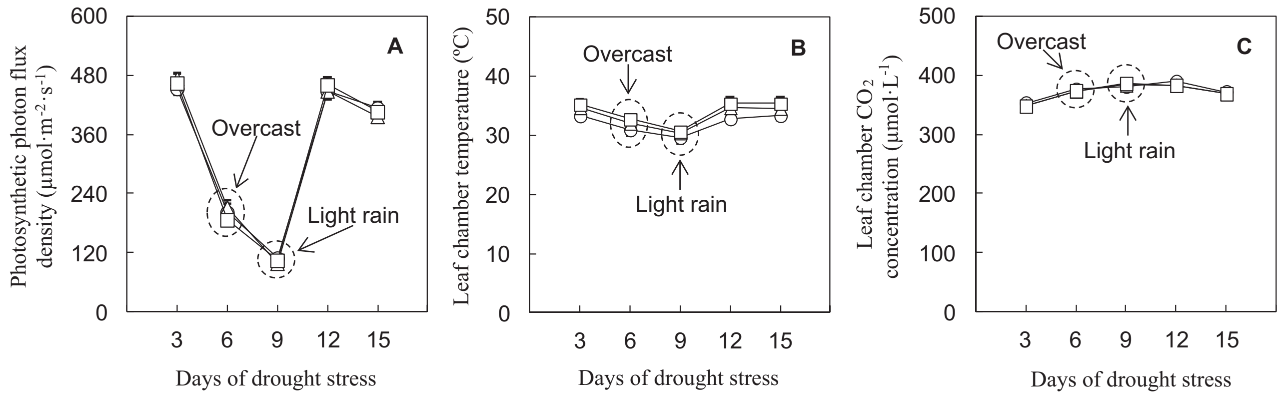

Fig. 1. Photosynthetic photon-flux density (PPFD) (A), leaf-chamber temperature (B) and CO_2 concentration (C)
○ : control (C), △ : moderate drought stress (MDS), □ : severe drought stress (SDS). Vertical bars indicate SE (n = 4).

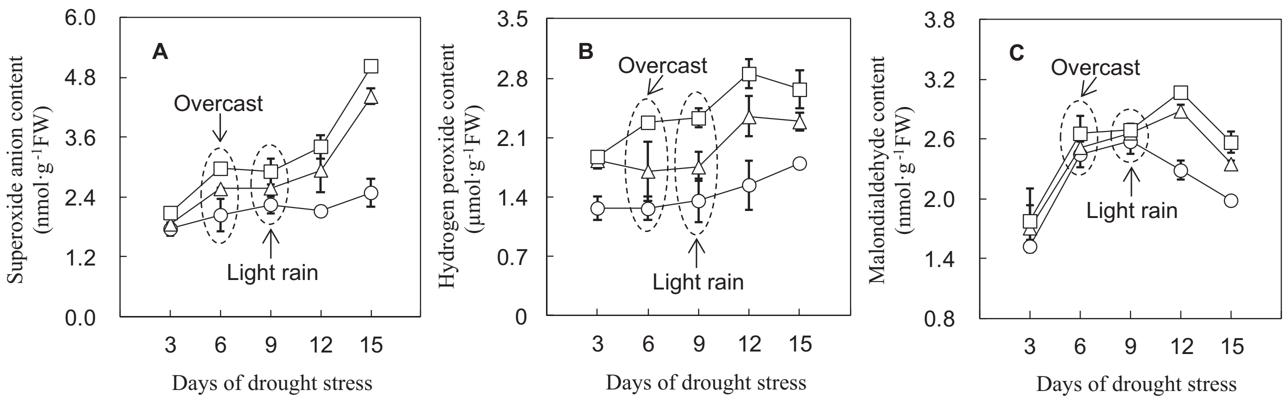

Fig. 2. Effect of drought stress on superoxide anion ($O_2^{\cdot -}$) (A), hydrogen peroxide (H_2O_2) (B) and malondialdehyde (MDA) (C) content in leaves of cucumber 'Jinchun No. 5' seedlings
○ : control (C), △ : moderate drought stress (MDS), □ : severe drought stress (SDS). Vertical bars indicate SE (n = 4).

4. Metabolic property of photosynthesis

g_s was less in MDS than C on the 12th and 15th days of treatment, was less in SDS than C from the 6th to 15th days, and less in SDS than MDS from the 9th to 15th days ($P < 0.05$) (Fig. 4). No difference in C_i was observed between C, MDS and SDS ($P < 0.05$). E in MDS was at the same level as C, was lower in SDS than C on the 6th, 9th and 15th days, and lower in SDS than MDS on the 9th and 15th days ($P < 0.05$). A was lower in MDS than C on the 3rd day and from the 9th to 15th days, was lower in SDS than C from the 3rd to 15th days, and lower in SDS than MDS on the 6th and 15th days ($P < 0.05$).

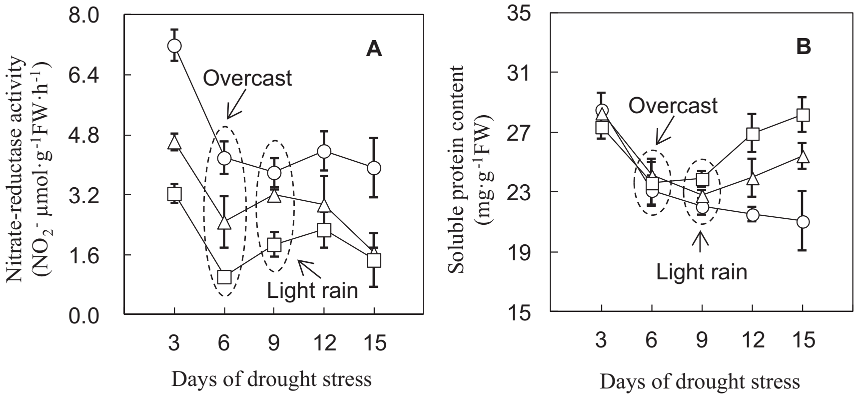

Fig. 3. Effect of drought stress on nitrate-reductase (NR) activity (A) and soluble protein content (B) in leaves of cucumber 'Jinchun No. 5' seedlings

○ : control (C), △ : moderate drought stress (MDS), □ : severe drought stress (SDS). Vertical bars indicate SE (n = 4).

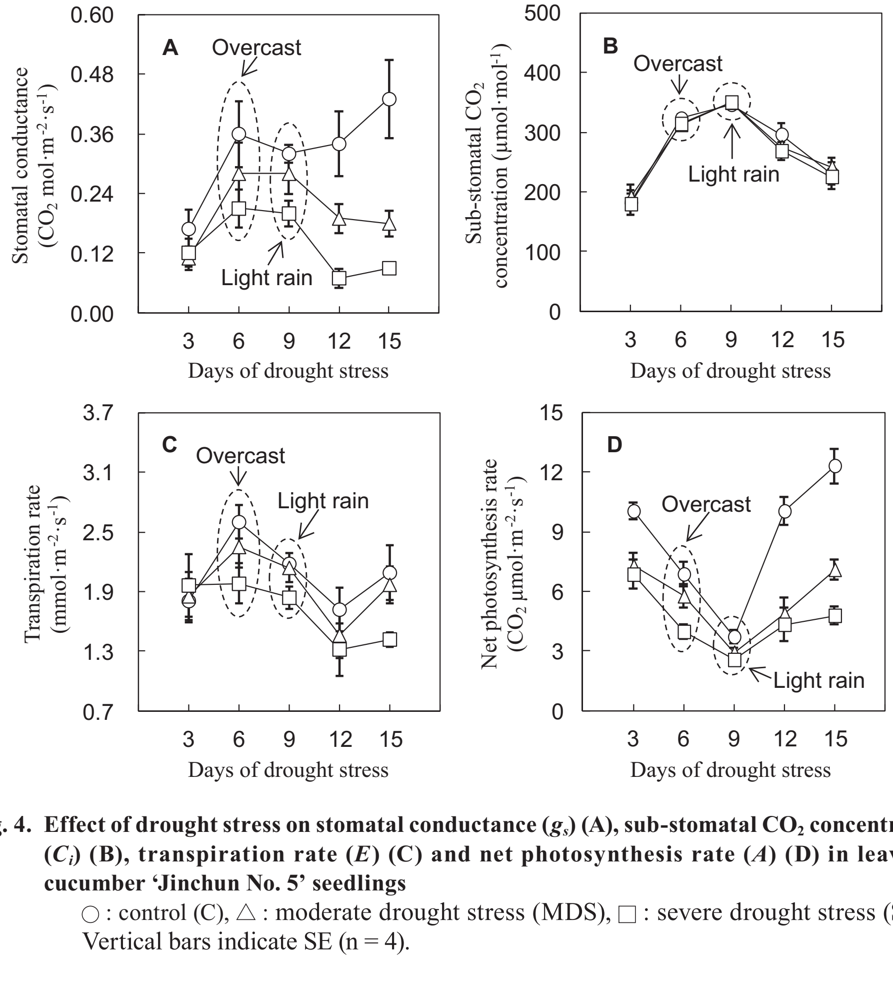

Fig. 4. Effect of drought stress on stomatal conductance (g_s) (A), sub-stomatal CO_2 concentration (C_i) (B), transpiration rate (E) (C) and net photosynthesis rate (A) (D) in leaves of cucumber 'Jinchun No. 5' seedlings

○ : control (C), △ : moderate drought stress (MDS), □ : severe drought stress (SDS). Vertical bars indicate SE (n = 4).

Discussion

1. Metabolic property of active oxygen species

Drought stress causes the excess generation and accumulation of active oxygen species in leaf cells[9,15]. In this study, the amounts of $O_2^{\cdot -}$ and H_2O_2 in cucumber 'Jinchun No. 5' seedlings increased with the level of drought stress in C, MDS and SDS (Fig. 2 A, B). Liu et al. (2009), Li et al. (2010), Li et al. (2011) and Sun et al. (2012) also reported an increase in the amounts of $O_2^{\cdot -}$ and H_2O_2 in cucumber seedlings subjected to drought stress treatment[13,16,18,26].

Most of the chlorophyll in the chloroplasts harvests light to capture light energy, but captures too much light energy when exposed to strong light. When this occurs, the excess electrons generated by photosystem II from the water splitting are not used in the CO_2 fixation reaction and reduce oxygen through photosystem I, generating more $O_2^{\cdot -}$[1,5]. Meanwhile, the H_2O_2 in the cells is converted from $O_2^{\cdot -}$ by SOD, and more H_2O_2 is generated by the oxidation reaction of glycolic acid through increased photorespiration of peroxisomes, due, in turn, to strong light and high temperature[21]. In this study, no difference was observed in $O_2^{\cdot -}$ in MDS on the 9th day of treatment (light rain) compared with C, nor in H_2O_2 on the 6th (overcast) and 9th days (Fig. 2 A, B). This finding is considered attributable to reduced generation of $O_2^{\cdot -}$ and H_2O_2 in cucumber 'Jinchun No. 5' seedlings because of reduced capture of light energy by the chloroplasts due to a reduction in strong light (Fig. 1 A) and reduced peroxisome photorespiration due to a reduction in strong light and leaf temperature (Fig. 1 A, B)[1,5,14,21].

MDA is an end product of peroxidation in plant cell membrane lipids due to active oxygen species. The increase in MDA reflects the excess metabolism of active oxygen, which is induced by high temperature, strong light and drought stress[12,14,16,18,26,30]. In this study, increase in MDA were seen together with an increase in the amounts of active oxygen species (Fig. 2 A, B, C), which shows that drought stress causes excess metabolism of active oxygen species in cucumber 'Jinchun No. 5' seedlings.

2. Metabolic property of nitrogen

NR is an important enzyme in nitrogen assimilation and the protein synthesis in plants. NR activity is induced by light and its activity is higher with more vigorous growth[15,25]. In this study, we believe NR activity, together with C, MDS and SDS, fell on the 6th and 9th days of treatment due to the weak light of overcast weather, light rain, and drought stress compared with the 3rd day, and similar declines were seen on the 12th and 15th days due to drought stress (Fig. 3 A). We also observed that NR activity in MDS was lower than C, and in SDS was lower than MDS (Fig. 3 A). This finding suggests that drought stress inhibits nitrogen assimilation and growth in cucumber 'Jinchun No. 5' seedlings. Hao et al. (2012) also reported reduced NR activity in cucumber seedlings due to drought stress treatment[7]. Similar results are also seen in wheat, in which the reduction in NR activity was significant due to drought stress for cultivars in which resistance to drought stress was low[19].

However, the amount of soluble protein increased from the 12th day in MDS and the 9th day in SDS respectively (Fig. 3 B). Wang & Zhang (2004), Li et al. (2010) and Hao et al. (2012) reported increased soluble protein in the leaves of cucumber seedlings after 7 days of drought stress treatment or after 18 days[7,16,27]. Similarly to the increase in soluble sugar, the increase in soluble protein is thought to be a positive response to drought stress[4,7,16,24,26,27].

3. Metabolic property of photosynthesis

Because photosynthetic activity depends on water absorbed from CO_2 and transpiration from stomata and sub-stomata, g_s, C_i and E are considered metabolic properties of photosynthesis and contribute directly to the photosynthesis rate. In this study, g_s and A fell with increasing drought stress in the treatment plots, while E also fell in SDS compared with C (Fig. 4 A, C, D). In fine weather, the correlation was observed between g_s, and E and A, respectively (Fig. 5). These results show that reduced CO_2 intake and water absorption linked to drought stress during periods of high temperature and strong light inhibit photosynthesis in cucumber 'Jinchun No. 5' seedlings. The effect of drought stress was also observed in C_i (Fig. 4 B), and the accompanying decrease in the consumption of CO_2 due to reduced photosynthesis is considered linked to reduced g_s.

Although the effects of active oxygen species and nitrogen metabolism on photosynthesis were indirect, a correlation was observed between the content of H_2O_2 and MDA, and NR activity with A, respectively (Fig. 6). Based on the results of this study (Figs. 2, 3, 4), we determined that the causes of reduced photosynthesis in cucumber 'Jinchun No. 5' seedlings due to such drought stress were the abundant active oxygen species that cause photosynthetic inhibition, the reduced photosynthetic function with weakened nitrogen metabolism, and the reduced CO_2 intake and water absorption[2,6,20,22,28]. This result is the same as reduced photosynthesis due to strong light stress[14], but differs from the low contribution of g_s and E to a reduction in photosynthesis due to high-temperature stress[30].

Conclusion

Drought stress during periods of high temperatures and strong light, as described above, causes excess metabolism of active oxygen species and inhibits nitrogen metabolism in cucumber 'Jinchun No. 5' seedlings. Further, reduced photosynthesis in cucumber 'Jinchun No. 5' seedlings due to drought stress is attributable to photoinhibition by active oxygen species, reduced photosynthetic function with weakened nitrogen metabolism, and a reduction in g_s and E. These physiological data help cultivate drought-resistant cucumber cultivars and the physiological research into and development of preventive techniques against damage caused by drought stress.

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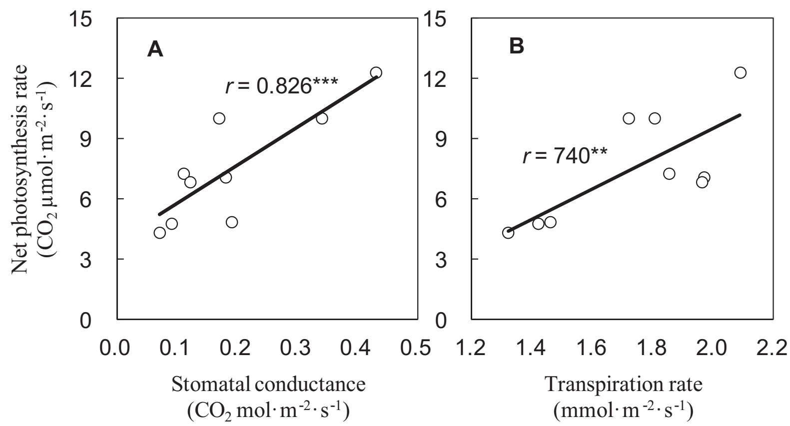

Fig. 5. Correlation between stomatal conductance (g_s) (A) and transpiration rate (*E*) (B) and net photosynthesis rate (*A*) of leaves from cucumber 'Jinchun No. 5' seedlings in fine weather
** and *** indicate significant correlation coefficients at $P = 0.05$ and 0.01 by *t*-test, respectively.

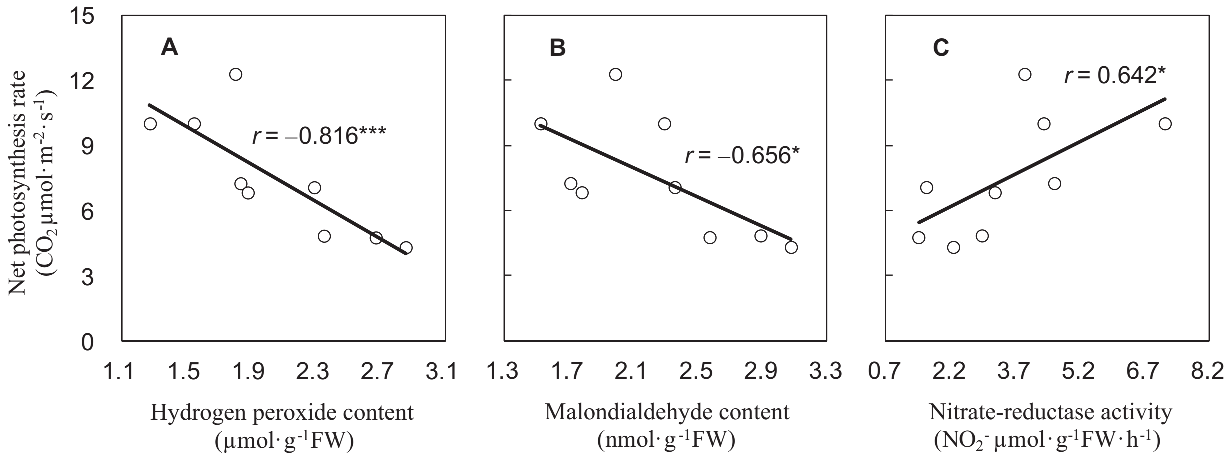

Fig. 6. Correlation between the contents of hydrogen peroxide (H_2O_2) (A), malondialdehyde (MDA) (B), nitrate-reductase activity (NR) (C) and net photosynthesis rate (*A*) of leaves from cucumber 'Jinchun No. 5' seedlings in fine weather
* and *** indicate significant correlation coefficients at $P = 0.1$ and 0.01 by *t*-test, respectively.

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