JARQ 50 (3), 285 - 292 (2016) http://www.jircas.affrc.go.jp

Effect of Nitrogen Application on Active Oxygen Species, Senescence, Photosynthesis, and Growth in Cucumber 'Jinchun No. 5' Seedlings

Jincai LI[1]*, Yasuyo NISHIMURA[2], Xiheng ZHAO[3] and Yasufumi FUKUMOTO[4]

[1] School of Chemical Engineering and Technology, Tianjin University (Tianjin 300072, China)
[2] Faculty of Agriculture, Kochi University (Nankoku, Kochi 783-8502, Japan)
[3] School of Environmental Science and Engineering, Tianjin University (Tianjin 300072, China)
[4] United Graduate School of Agricultural Sciences, Ehime University (Matsuyama, Ehime 790-8566, Japan)

Abstract

To gain a physiological understanding of applying nitrogen to cucumbers (*Cucumis sativus* L.), three treatment plots of 'Jinchun No. 5' seedlings were prepared for three levels of nitrogen application using low (L, 0.0 $g \cdot kg^{-1}$), medium (M, 0.4 $g \cdot kg^{-1}$), and high (H, 0.8 $g \cdot kg^{-1}$) amounts of urea. The specified amount of urea was applied to the dry culture medium of each texting group at three time points: before planting, then at 27 and 43 days after sowing. The following indicators of active oxygen species and senescence were observed in L: soluble protein was low and malondialdehyde (MDA) high in the cotyledons on the 18th day after sowing, and active oxygen species superoxide anion ($O_2^{\cdot -}$) and MDA were high, while soluble protein and chlorophyll were low in the 2nd and 3rd leaves on the 42nd and 52nd days. Therefore, no nitrogen application in L resulted in active oxygen generation and membrane lipid peroxidation, as well as accelerated senescence in the cucumber 'Jinchun No. 5' seedlings. In contrast, in H, soluble protein was high in the cotyledons on the 18th day after sowing, $O_2^{\cdot -}$, hydrogen peroxide (H_2O_2), and MDA were high on the 42nd, 52nd, and 62nd days, respectively, and soluble protein and chlorophyll were high on the 52nd and 62nd days. These results indicate that an excessive application of nitrogen resulted in active oxygen generation and membrane lipid peroxidation, but also delayed senescence at the same time. The photosynthetic properties and growth of the transpiration rate (*E*), net photosynthesis rate (P_n), plant height, number of leaves, and shoot fresh weight decreased in L, indicating that active oxygen species, senescence, and *E* were associated with decreased photosynthesis and growth in the cucumber 'Jinchun No. 5' seedlings due to a lack of nitrogen application. Conversely, in H, a decrease in stomatal conductance (g_s), *E* on the 42nd day, followed by an increase on the 62nd day, suggests that in the leaf mid-growth phase of the seedlings, active oxygen species and *E* were associated with decreased photosynthesis due to excess nitrogen application. However, delayed senescence was associated with maintaining g_s, *E*, and P_n in the late growth phase of the leaves. Moreover, plant height, the number of leaves, and shoot fresh weight showed decreases on the 42nd, 52nd, and 62nd days, respectively, showing the adverse effects of excess nitrogen application on the growth of cucumber 'Jinchun No. 5' seedlings.

Discipline: Horticulture
Additional key words: malondialdehyde, net photosynthesis rate, shoot fresh weight, superoxide anion, transpiration rate

Introduction

Plant senescence and photosynthesis are greatly affected by environmental changes. Many different varieties of cucumber (*Cucumis sativus* L.) are widely cultivated. However, given the low resistance of cucumbers to environmental changes, environmental stress (e.g. high temperature, strong light, drought) accelerates senescence and inhibits photosynthesis through active oxygen species (Li et al. 2013a, Li et al. 2014, Shang et al. 2012, Wang et al. 2001, Zhao et al. 2011).

Cucumber has large leaves, grows quickly, and is a sequentially aging plant that develops from the lowest node to the highest node (Zhao et al. 2003). Vegetative growth and reproductive growth progress concurrently, thereby entailing a high requirement for water and nutrients (Li et

*Corresponding author: e-mail lijincai@tju.edu.cn
Received 6 June 2014; accepted 4 November 2015.

al. 2000, Li et al. 2013b, Wang et al. 2005). Nitrogen is actively absorbed up to the late growth stage, and its effect on growth and yield is greater than that of other nutritional elements (Li et al. 2000, Wang et al. 2005). Moreover, cucumber has shallow roots (Roumet et al. 2006) and because it requires a lot of irrigation during the cultivation period, nitrogen in the soil easily leaches away (Miflin & Lea 1980). Therefore, varying levels of nitrogen application ranging from too much to too little are seen in cucumber production (Zhang et al. 2010).

The amount of nitrogen application is related to active oxygen metabolism, senescence, photosynthesis, and growth in such crops as spinach (Liu et al. 2007), wheat (Cai et al. 2008), and rice (Huang et al. 2004, Kumagai et al. 2009). To determine the appropriate amount of nitrogen application in cucumber, three questions need answering: (1) how does the level of active oxygen in the leaves change, (2) to what extent is senescence, which plays a prominent role in productivity, controlled and accelerated, and (3) how are the photosynthetic properties and growth, which depend on nitrogen metabolism, affected? The answers to these questions constitute basic physiological data that contribute to the cultivation of cucumber varieties resistant to nitrogen nutrient stress, and to the research and development of techniques to prevent physiological damage.

To gain a physiological understanding of cucumbers upon nitrogen application, in this study, three plots for different levels of nitrogen application (low, medium, and high) were prepared for cucumber 'Jinchun No. 5' seedlings. We examined the leaves for the active oxygen species level, the senescence process (judging from changes in the amounts of soluble protein, malondialdehyde, and chlorophyll) (Li et al. 2004), the photosynthetic properties, and the growth indicators, in order to investigate the effect of different levels of nitrogen application.

Materials and methods

1. Nitrogen application treatment

Cucumber 'Jinchun No. 5' seedlings were grown in a plastic greenhouse from September 5, 2009. Seedling pots (10 cm in diameter × 12 cm in height) contained a culture medium of soil, herbaceous peat, and vermiculite at a ratio of 3:1:1 with total nitrogen of 1.21 $g \cdot kg^{-1}$ and available nitrogen of 92.3 $mg \cdot kg^{-1}$. Three treatment plots were prepared for the nitrogen application treatment: low (L), medium (M), and high (H). Three urea treatments (0.0, 0.4, and 0.8 $g \cdot kg^{-1}$, containing 46% N) were applied to a dry culture medium three times based on the amounts used by Li et al. (2013b). In other words, one-third of the prepared urea treatment was applied in M and H (0.4 and 0.8 $g \cdot kg^{-1}$, respectively) before sowing, and on the 27th day (seedlings with two true leaves) and 43rd day (seedlings with four true leaves) after sowing.

Before the nitrogen application treatment, 30 pots (plants) were placed in each treatment plot, with 40 × 100 cm of space around each plant (i.e. 2.5 plants per m^{-2}). During the treatment period, there were 23 sunny days, 24 overcast days, and 16 rainy days. The average minimum and maximum temperatures from September 5 – 25, September 26 – October 16, and October 17 – November 6 were 17.9 and 26.5°C, 15.0 and 24.4°C, and 11.3 and 20.7°C, respectively.

2. Sampling

On the 18th day after sowing, nine plants from each treatment plot were selected and the cotyledons were sampled at about 10:30 a.m. On the 42nd, 52nd, and 62nd days after sowing, four plants from each treatment plot were selected, and the second and third true leaves were sampled at about 10:30 a.m. The seedlings in L were highly etiolated on the 62nd day, and thus were not sampled.

The sampled cotyledons from three plants from each treatment plot were quickly cut into small pieces, and after weighing out 1 g from each treatment plot, the samples were frozen in liquid nitrogen and stored at −80°C. The sampling of cotyledons from each treatment plot was repeated three times (n = 3). The second and third leaves sampled from each plant were cut into small pieces, and after weighing out 2 g from each treatment plot, the samples were frozen and stored in the same manner as the cotyledon samples. The sampling of true leaves from each treatment plot was repeated four times (n = 4). All samples were quantitatively analyzed for active oxygen and the indicators of senescence.

3. Analysis and measurement

Methods used to measure the amount of active oxygen species superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2), and the senescence indicators (i.e. amounts of soluble protein, malondialdehyde (MDA), chlorophyll) were similar to the amounts found by Li et al. (2013a).

The photosynthetic properties—stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i), transpiration rate (E), and net photosynthesis rate (P_n)—were measured with a portable photosynthesis system (LC*i*-002/B; ADC BioScientific Ltd., Hertfordshire, UK) on a leaf chamber area of 6.25 cm^2. The measurements were taken on the second and third leaves of four plants in each treatment plot at about 10:00 a.m. prior to the sampling of cotyledons and leaves (n = 4).

At the time of measurement, the light-receiving angle at the leaf chamber was 0° (horizontal), and the CO_2 concentration in the environment was 399 ± 12 $\mu mol \cdot L^{-1}$. On the 42nd, 52nd, and 62nd days after sowing, the photosynthetic photon-flux density was 661 ± 11, 627 ± 14, and 430 ± 7 μmol·m^{-1}·s^{-1}, respectively. The leaf chamber temperature was 34 ± 1.8, 28 ± 1.2, and 26 ± 0.9°C, and the H_2O partial pressure was 1.76 ± 0.15, 1.40 ± 0.16, and 1.16 ± 0.02 MPa, respectively.

At the same time when the photosynthetic properties were measured, the plant height, number of leaves, and shoot fresh weight of those four plants were also measured (n = 4).

4. Statistical analysis

The experimental data are shown as the mean ± standard error (SE). Significant differences were determined using Tukey's HSD test ($P < 0.05$), and the significance of correlation was assessed using the *t*-test.

Results

1. Cotyledon senescence

The amount of soluble protein in the cotyledons was highest in H followed by M and then L. The amount of MDA was higher in L than in M, and there was no difference in the amount of chlorophyll between the three treatment plots (Fig. 1).

2. Active oxygen species level of leaf

The amount of $O_2^{\cdot -}$ was higher in L and H than in M on the 42nd and 52nd days after sowing, and higher in H than in M on the 62nd day (Fig. 2). The amount of H_2O_2 had similar pattern as that of $O_2^{\cdot -}$ and was higher in H than in M on the 42nd, 52nd, and 62nd days.

3. Leaf senescence

The amount of soluble protein was lower in L than in M on the 42nd day, was highest in H followed by M and then L on the 52nd day, and was higher in H than in M on the 62nd day (Fig. 3). The amount of MDA was higher in L and H than in M on the 42nd and 52nd days, and was higher in H than in M on the 62nd day. The amount of chlorophyll was consistently lower in L than in M on the 42nd and 52nd days, and was lower in M than in H on the 52nd and 62nd

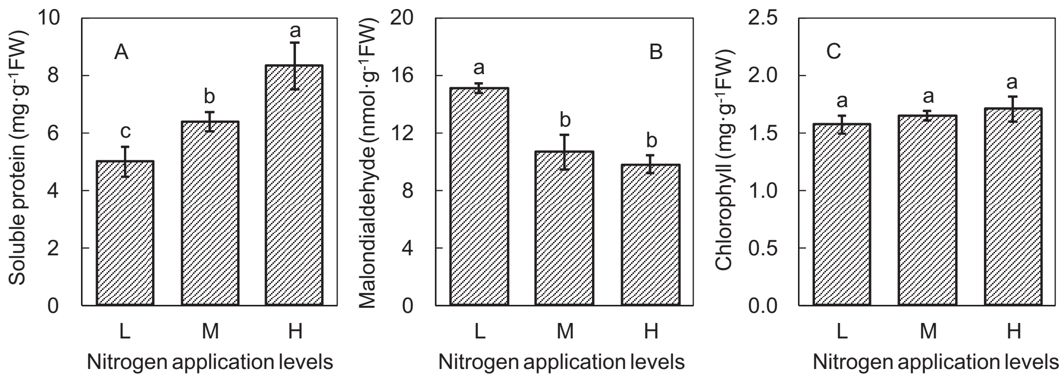

Fig. 1. Effect of nitrogen application levels on soluble protein (A), malondialdehyde (B), and chlorophyll (C) content of cotyledons in cucumber seedlings
L: low nitrogen application level, M: medium nitrogen application level, H: high nitrogen application level. Different letters denote mean separation by Tukey's HSD test, 5% level of significance. Vertical bars indicate SE (n = 3).

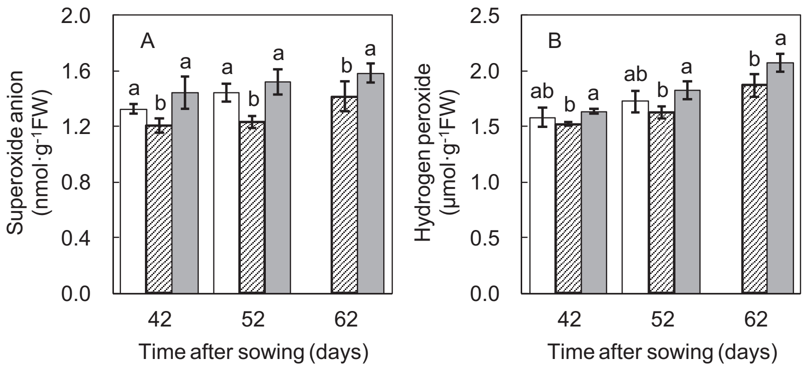

Fig. 2. Effect of nitrogen application levels on superoxide anion (A) and hydrogen peroxide (B) content of leaves in cucumber seedlings
□: low nitrogen application level (L), ▨: medium nitrogen application level (M), ■: high nitrogen application level (H). Different letters denote mean separation by Tukey's HSD test, 5% level of significance. Vertical bars indicate SE (n = 4).

days.

4. Photosynthetic properties of leaf

Regarding the photosynthetic properties of leaf, g_s was lower in H than in M on the 42nd day, lower in L and H than in M on the 52nd day, but higher in H than in M on the 62nd day (Fig. 4). C_i was higher in L than in M on the 42nd day, and was lowest in H followed by M and then L on the 52nd day. Both H and M were about the same level on the 62nd day. E was lower in L and H than in M on the 42nd and 52nd days, but was higher in H than in M on the 62nd day. P_n was lower in L and H than in M on the 42nd day, and lower in L than in M, but was higher in H than in M on the 62nd day.

5. Seedling growth

The plant height, number of leaves, and shoot fresh weight showed a similar tendency: all were lower in L and H than in M on the 42nd and 52nd days, and lower in H than in M on the 62nd day (Fig. 5).

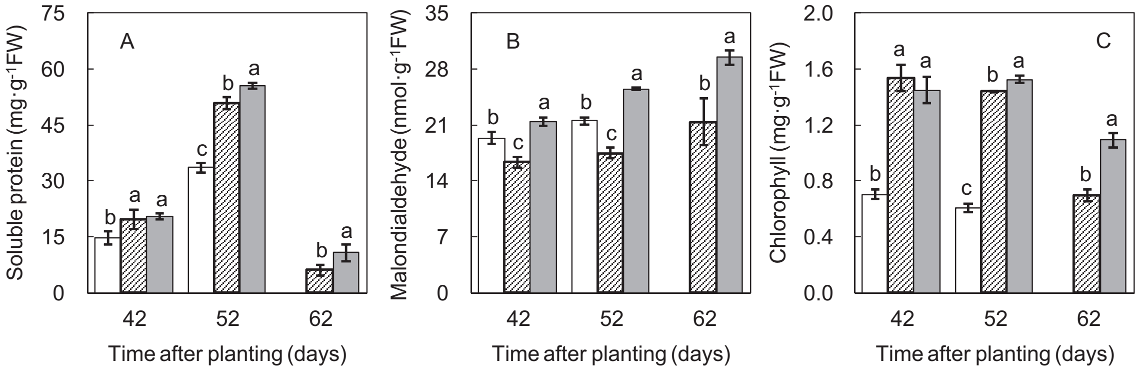

Fig. 3. Effect of nitrogen application levels on soluble protein (A), malondialdehyde (B), and chlorophyll (C) content of leaves in cucumber seedlings

□: low nitrogen application level (L), ▨: medium nitrogen application level (M), ■: high nitrogen application level (H). Different letters denote mean separation by Tukey's HSD test, 5% level of significance. Vertical bars indicate SE (n = 4).

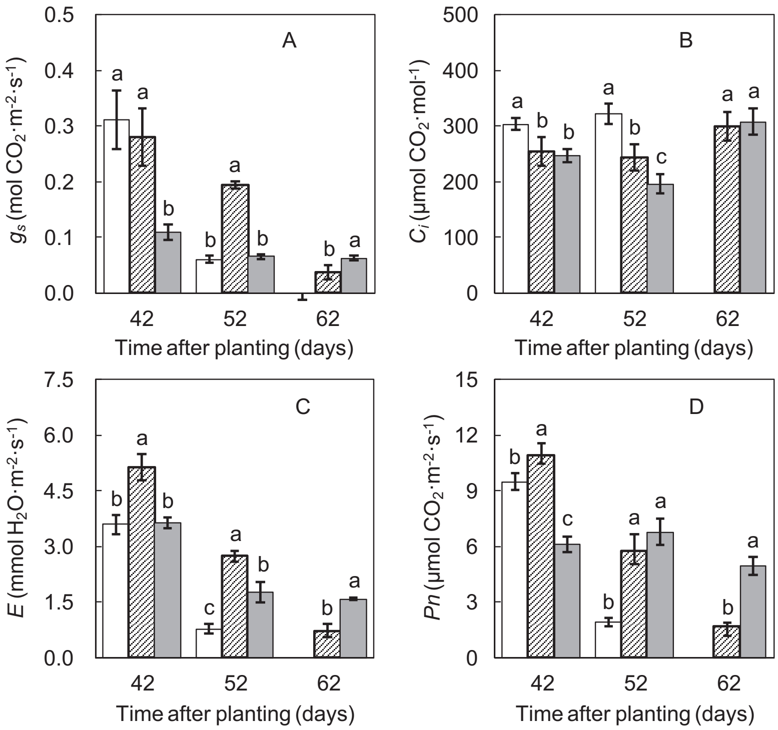

Fig. 4. Effect of nitrogen application levels on stomatal conductance (g_s) (A), sub-stomatal CO_2 concentration (C_i) (B), transpiration rate (E) (C), and net photosynthesis rate (P_n) (D) of leaves in cucumber seedlings

□: low nitrogen application level (L), ▨: medium nitrogen application level (M), ■: high nitrogen application level (H). Different letters denote mean separation by Tukey's HSD test, 5% level of significance. Vertical bars indicate SE (n = 4).

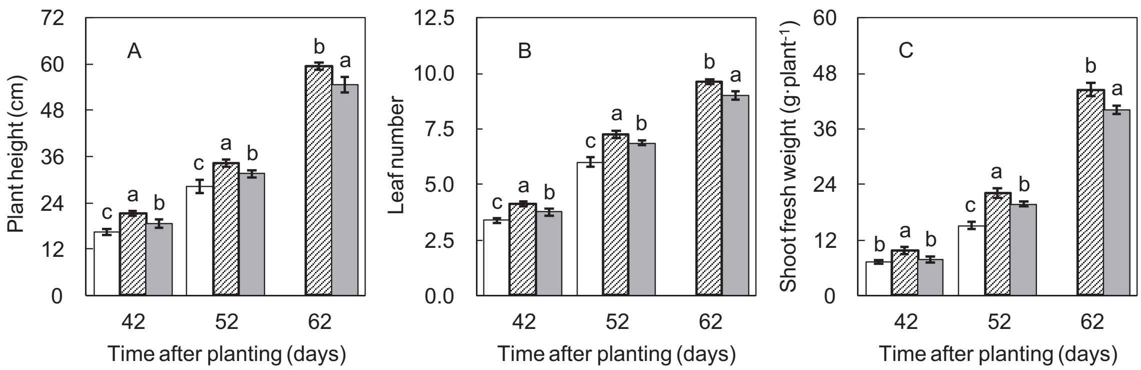

Fig. 5. Effect of nitrogen application levels on plant height (A), number of leaves (B), and shoot fresh weight (C) of cucumber seedlings

□: low nitrogen application level (L), ▨: medium nitrogen application level (M), ■: high nitrogen application level (H). Different letters denote mean separation by Tukey's HSD test, 5% level of significance. Vertical bars indicate SE (n = 4).

Discussion

As described earlier, nitrogen application in this experiment was conducted three times: before sowing, then at 27 and 43 days after sowing. One-third of the prepared urea (0.4 and 0.8 g·kg^{-1}) was applied to the dry culture medium in M and H, respectively, at those three application time points. Compared with the amount of urea used by Li et al. (2013) for nitrogen nutrient in the medium and the results of growth, it was observed that the amount of nitrogen application was insufficient in L but appropriate in M from sowing to the 62nd day after sowing. On the other hand, it was observed that a large amount of nitrogen was applied in H from sowing to the second application on the 27th day after sowing, and excess nitrogen was applied from the 27th day to the 62nd day.

The physiological leaf age of cucumber is about 55 days (Li et al. 2004) and leaf age is divided into three growth stages: early (1 – 19 days), mid (20 – 36 days), and late (37 – 55 days). In this experiment, the average ages of the second and third leaves sampled on the 42nd, 52nd, and 62nd days after sowing were 27, 37, and 47 days, respectively.

1. Cotyledon senescence

In cucumber leaves, the decreased amount of soluble protein and the increase in MDA, the end product of membrane lipid peroxidation that generally accompany senescence, are characteristic of the aging process, and the decrease in chlorophyll is a clear sign of aging (Li et al. 2004, Zhao et al. 2003). Changes in environmental parameters and physiological changes in the seedlings are most quickly reflected in the cotyledons, before the first true leaves appear. Judging from the amounts of soluble protein and MDA, senescence in the seedlings in L progressed further than in the seedlings in M (Fig. 1 A, B), showing that a lack of nitrogen application accelerated senescence.

Soluble protein in H increased as compared with M (Fig. 1 A), and a large portion of the nitrogen applied promoted the synthesis of protein. In the leaves of cucumber, membrane lipid peroxidation that accompanies senescence follows a decrease in soluble protein, and a decrease in chlorophyll follows membrane lipid peroxidation (Li et al. 2004). Thus, a lack of any difference in the amount of chlorophyll between the three treatment plots of L, M, and H (Fig. 1 C) could be because the decrease in chlorophyll due to senescence on the 18th day after planting might have just started or had yet to occur.

2. Active oxygen species of leaf

Because chlorophyll in the chloroplasts is largely a light-harvesting pigment with a light-harvesting function, it is easy to capture too much light. When electrons generated in the decomposition of water in photosystem II due to capturing excess light exceed the light energy required for the CO_2 fixing reaction, a large quantity of $O_2^{\cdot-}$ is produced by excess electrons in photosystem I. Subsequently, $O_2^{\cdot-}$ is converted to H_2O_2 by superoxide dismutase (SOD) and the H_2O_2 is eliminated by catalase (CAT) and ascorbate peroxidase (APX) (Asada 1999, Elstner 1982). The tendency for the amounts of $O_2^{\cdot-}$ and H_2O_2 in L being greater than in M (Fig. 2 A, B) is due to significant curtailing of the CO_2 fixing reaction due to a lack of nitrogen application (Fig. 4 D), and is considered related to the decrease in SOD and CAT activity due to a lack of nitrogen application as found in rice (Kumagai et al. 2009) and spinach (Liu et al. 2007).

We surmise that the main cause of the amounts of $O_2^{\cdot-}$ and H_2O_2 in H being greater than in M (Fig. 2 A, B) was significant curtailing of the CO_2 fixing reaction in the mid-growth phase of leaves on the 42nd day after sowing (Fig. 4 D) and the harvesting of excess light energy due to the abundance of chlorophyll in the late growth stage, 52 to 62 days after sowing (Fig. 3 C). It is also considered related to the decrease in leaf SOD, CAT, and APX activity due to excess nitrogen application as was found in wheat (Cai et al. 2008).

3. Leaf senescence

Judging from the amounts of soluble protein and MDA, it is understood that leaf senescence in L was accelerated by a lack of nitrogen application (Fig. 3 A, B) in the same manner as senescence in the cotyledons. Looking also at the decrease in chlorophyll, leaf senescence in L became more apparent than in M (Fig. 3 C), and the seedlings in L were observed as being highly etiolated on the 62nd day.

Soluble protein and chlorophyll in H increased during the late growth stage of leaves on the 52nd and 62nd days as compared with M, whereas MDA increased significantly (Fig. 3 A, B, C). Thus, excess nitrogen application promoted the synthesis of protein and chlorophyll, and although senescence of the cucumber seedlings was delayed, membrane lipid peroxidation was not inhibited over time. Nitrogen and chlorophyll in the leaves of wheat have also been reported to increase along with MDA due to the excessive application of nitrogen (Cai et al. 2008).

Senescence in cucumber is innately programmed, but highly subject to environmental conditions, and oxidative damage to such biological molecules as membrane lipids and chlorophyll, due to active oxygen species largely induced by environmental stress, is one of the major factors (Li et al. 2004, Li et al. 2013a, Li et al. 2014, Zhao et al. 2011). Therefore, the increase in MDA and the decrease in chlorophyll in L as compared with M, and the increase in MDA in H are thought to be associated with the large amount of active oxygen species (Fig. 2 A, B). There was high correlation between the amounts of $O_2^{\cdot-}$ and H_2O_2 with MDA, with correlation coefficients of r = 0.953** and r = 0.905** (P < 0.01), respectively (Fig. 6).

4. Photosynthetic properties of leaf

Given the fact that photosynthesis relies on CO_2 from the stomata and intercellular spaces and H_2O absorbed from transpiration, g_s, C_i, and E along with P_n are considered photosynthetic properties. Looking at the results of g_s, C_i and P_n, g_s in L were about at the same level as in M on the 42nd day, and were lower than in M on the 52nd day (Fig. 4 A). In contrast, C_i in L was higher than in M on the 42nd and 52nd days (Fig. 4 B). From these results, it is considered that g_s did not cause a drop in P_n (Fig. 4 C) in the cucumber seedlings due to a lack of nitrogen application. It has been reported that stomatal restriction is not the cause of reduced photosynthesis due to a lack of nitrogen nutrition in rice plants (Huang et al. 2004) and cucumber at harvest time (Wang et al. 2005). Subsequently, E in L was lower than in M and not in accordance with the change in g_s on the 42nd day, but was in accordance on the 52nd day (Fig. 4 A, C). From this result, it can be inferred that the reduced absorptive function of the roots is associated with a drop in E and not g_s, of the cucumber seedlings due to a lack of nitrogen application. Moreover, root activity reportedly decreases due to a lack of nitrogen application in spinach (Liu et al. 2007).

P_n in L was lower than in M as was E (Fig. 4 C, D), and E is closely associated with the decrease in P_n of the cucumber seedlings due to a lack of nitrogen application. In addition, the decrease in photosynthetic function (Fig. 2 A, B, C) that accompanies photoinhibition of the active oxygen species (Fig. 2 A, B) and accelerated senescence is believed to be associated with the decrease in P_n in the cucumber seedlings (Fig. 4 D) due to a lack of nitrogen application, as it is with high temperature, strong light, and drought stress (Li et al. 2013a, Li et al. 2014, Zhao et al. 2011).

Looking at the results of g_s, C_i, and P_n in H, g_s and P_n were lower than in M on the 42nd day whereas C_i was not, and g_s and C_i were lower than in M on the 52nd day whereas

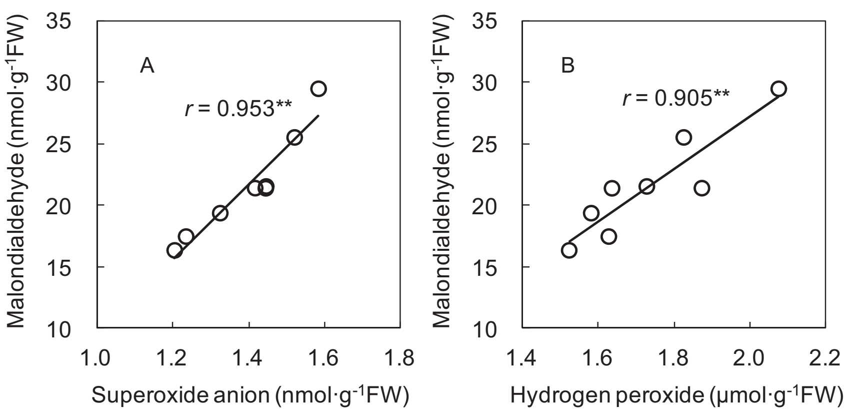

Fig. 6. Correlation between superoxide anion (A) and hydrogen peroxide (B) content, and malondialdehyde content of leaves in cucumber 'Jinchun No. 5' seedlings
** indicates significant correlation coefficients at P < 0.01 from t-test.

P_n was not (Fig. 4 A, B, D). From these results, it is considered that g_s did not cause the change in P_n of the cucumber seedlings due to excess nitrogen application. However, the change in E in H was the same as the change in g_s compared with M (Fig. 4 A, C), and thus it can be inferred that g_s played a prominent role in the change in E in the cucumber seedlings due to excess nitrogen application.

The decrease in E (Fig. 4 C, D) and the increase in $O_2^{\cdot -}$ and H_2O_2 (Fig. 2 A, B) accompanied the drop in P_n in H as compared with M in the mid-growth phase of leaves on the 42nd day, and E and the photoinhibition of active oxygen species (Fig. 2 A, B) are considered related to the drop in P_n of the cucumber seedlings due to excess nitrogen application. It has been reported that antioxidant enzyme activity (that eliminates $O_2^{\cdot -}$ and H_2O_2) and P_n decrease in wheat due to excess nitrogen application (Cai et al. 2008).

The increase in soluble protein and chlorophyll (Fig. 3 A, C) accompanied the increase in P_n in H (Fig. 4 D) as compared with M in the late growth phase of leaves from the 52nd to 62nd days, and the delayed senescence due to excess nitrogen application greatly contributed to maintaining P_n, and probably contributed to the increase in g_s and E on the 62nd day (Fig. 4 A, C).

5. Seedling growth

The findings on plant height, number of leaves and shoot fresh weight suggest that both a lack of nitrogen application and excess nitrogen application have adverse effects on the growth of seedlings (Fig. 5 A, B, C). In the study where treatment at different nitrogen levels began from two and three true leaves of seedlings, it was also shown that a lack and excess nitrogen application could cause adverse effects on seedling growth and the fruiting stages (Li et al. 2000, Li et al. 2023b).

Cucumber is a sequentially aging plant that develops from the lowest node to the highest node, where the leaf and plant growth process is inconsistent (Zhao et al. 2011). Thus, the previously discussed aging and photosynthesis of the second and third true leaves did not match the plant growth indicators (Fig. 3 B, Fig. 4 C, D, and Fig. 5). However, since plant growth mainly relies on highly photosynthetic functional leaves, the change in plant growth indicators can be considered caused by excess nitrogen application or the lack thereof on the 42nd day after sowing, and is equivalent to its mid-growth stage of the leaves active oxygen species level, MDA, E, and P_n (Fig. 2, Fig. 3 B, Fig. 4 C, D, and Fig. 5). The decline in growth caused by excess nitrogen application or the lack thereof on the 52nd and 62nd days after sowing is thus presumed to be the same.

Conclusion

The lack of nitrogen as described above resulted in the generation of active oxygen species and accelerated senescence in cucumber 'Jinchun No. 5' seedlings, and it was shown that active oxygen species, senescence, and E were involved in decreased photosynthesis and growth due to a lack of nitrogen application. On the other hand, it was shown that excess nitrogen application resulted in delaying senescence, as determined from the amounts of chlorophyll and soluble protein, along with generating active oxygen species and MDA in the cucumber seedlings. In addition, active oxygen species and E were involved in decreased photosynthesis and growth in the mid-growth stage of leaves due to excess nitrogen application, whereas the delay in senescence due to excess nitrogen application contributed to maintaining g_s, E, and P_n in the late growth stage of leaves. These physiological data can thus be used to cultivate nitrogen nutrient stress-resistant cultivars of cucumber and for research on cultivation techniques and nitrogen physiology.

References

Asada, K. (1999) The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **50**, 601-639.

Cai, R. et al. (2008) Photosynthetic characteristics and antioxidative metabolism of flag leaves in responses to nitrogen application in wheat during grain filling. *Sci. Agric. Sin.*, **41**, 53-62 [In Chinese with English abstract].

Elstner, E. F. (1982) Oxygen activation and oxygen toxicity. *Ann. Rev. Plant Physiol.*, **33**, 73-96.

Huang, Z. A. et al. (2004) Effects of nitrogen deficiency on gas exchange, chlorophyll fluorescence, and antioxidant enzymes in leaves of rice plants. *Photosynthetica*, **42**, 357-364.

Kumagai, E. et al. (2009) Effect of nitrogen-deficiency on midday photoinhibition in flag leaves of different rice (*Oryza sativa* L.) cultivars. *Photosynthetica*, **47**, 241-246.

Li, J. et al. (2000) Changes in nutrient levels in solutions used for a closed system of rockwool hydroponics of cucumber plants. *J. Soc. High Tech. Agr.*, **12(2)**, 105-111 [In Japanese with English abstract].

Li, J. et al. (2004) Changes in antioxidative levels in aging cucumber (*Cucumis sativus* L.) leaves. *J. Japan. Soc. Hort. Sci.*, **73**, 491-495 [In Japanese with English abstract].

Li, J. et al. (2013a) Ameliorated effect of shading treatment in strong light period on senescence and photosynthesis in cucumber 'Jinchun No. 5' seedlings. *JARQ*, **47**, 97-102.

Li. J. et al. (2014) Effects of drought stress on the metabolic properties of active oxygen species, nitrogen and photosynthesis in cucumber 'Jinchun No. 5' seedlings. *JARQ*, **48**, 175-181.

Li, Y. et al. (2013b) Preliminary study on the effects of excess soil N and P on soil nutrient elements and cucumber nutrient absorption. *J. Soil Water Conserv.*, **27(1)**, 98-101 [In Chinese with English abstract].

Liu, W. et al. (2007) Effect of nitrogen nutrition on physiological index of spinach senescence. *Plant Nutr. Fert. Sci.*, **13**, 1110-1115 [In Chinese with English abstract].

Miflin, B. J. & Lea, P. J. (1980) Ammonia assimilation. *In* The biochemistry of plants, ed. Miflin, B. J., Academic Press, New York, USA, 169-202.

Roumet, C. et al. (2006) Suites of root traits differ between annual and perennial species growing in the field. *New Phytol.*, **170**, 357-368.

Shang, H. et al. (2012) Effects of suboptimal temperature and photo flux density treatment on growth, photosynthetic characteristics and nitrogen content of cucumber (*Cucumis sativus* L.) seedlings. *J. Anhui Agri. Sci.*, **40**, 12419-12422 [In Chinese with English abstract].

Wang, J. et al. (2005) Effect of long-term application of inorganic NPK fertilizer on the photosynthesis and yield of cucumber. *Chin. J. Soil Sci.*, **6**, 545-548 [In Chinese with English abstract].

Wang, Y. et al. (2001) Effects of low temperature and low light intensity stress on photosynthesis in seedlings of different cucumber varieties. *Acta Hortic. Sin.*, **28**, 230-234 [In Chinese with English abstract].

Zhang, L. et al. (2010) A study on nitrate contamination of ground water sources in areas of protected vegetables-growing fields — A case study in Huimin County, Shandong Province. *Sci. Agric. Sin.*, **43**, 4427-4436 [In Chinese with English abstract].

Zhao, X. et al. (2003) Antioxidative levels in leaves at the different nodal positions of cucumber (*Cucumis sativus* L.). *J. Japan. Soc. Hort. Sci.*, **72**, 324-328 [In Japanese with English abstract].

Zhao, X. et al. (2011) Effect of high temperature on active oxygen species, senescence and photosynthetic properties in cucumber leaves. *Environ. Exp. Bot.*, **70**, 212-216.